DISSERTATION

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“Phylogenetic and phylogenomic analyses and distribution modelling of a challenging taxon – the band-winged grasshoppers”

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ABSTRACT

With more than 600 described species the bad-winged grasshoppers (Oedipodinae) are one of the most diverse subfamilies of the family Acrididae (Orthoptera). They have an almost cosmopolitan distribution with diversity hotspots in the Mediterranean region, south-west and central Asia and along the southern regions of the USA. Taxonomically the members of the group are in most cases hard to distinguish. First genetic analyses based on single and multigene phylogenies were able to divide the New and Old Word Oedipodinae, but could not differentiate most of the younger groups; one of these is the genus *Sphingonotus.* This genus has a fairly wide distribution as it is not only present in the Old World, but occurs with some few species in the New World. In my thesis, I tested the resolution of COI barcoding and a multi-gene approach to differentiate *Sphingonotus* species. While barcoding data gave almost no resolution, I was able to separate the investigated species in four larger groups using several gene fragments. As the usage of genetic analyses did not provide sufficient resolution, I employed ecological niche modelling to investigate species distributions and potential drivers which may have driven the evolution of the species *Sphingonotus rubescens.* I modelled the species distribution based on climatic variables and found a potential distribution across Northern Africa and Central Asia. Projecting the models to the past showed that the species was able to diversify in several regions due to multiple alternations of hot and cold phases. In another chapter, I used a different modelling approach to determine the potential suitable habitats of a declining grasshopper species (*Bryodemella tuberculata*). I was interested if climate change or landcover change are the reasons for decline. As all extinct locations showed high suitability for the species, I suggested that landcover change has to be the driver of the species decline. In the following chapters, I addressed more general questions on the genomic composition of grasshoppers. Chromosome number was strongly conserved, with a standard karyotype of 2n♂ = 23 (22 + X0). In contrast, genome size was quite variable and differed between 1C=5.28pg in *Locusta migratoria* up to 1C=21.96pg in *Bryodemella tuberculata* within the Oedipodinae. These large genome sizes and the young age (~35 myo) of the group make it difficult to differentiate species by using only few genes. Therefore, I used genomic analyses based on hybridization capture data to separate young taxa. I employed more than 3,000

markers to separate the Old and New World taxa and further to differentiate the groups Sphingonotini and Bryodemini from the Old World. In subsequent analyses, I focused on several convergent morphotypes which occur in the Old and New World. Using geometric morphometrics of the fore wing to quantified morphological similarity. Moreover, I modeled their potential suitable habitats and used overlap analyses to quantify the overlap in area. The data suggested that the New and Old World Oedipodinae form separate clusters, while the differentiation of the Bryodemini and Sphingonotini still was not possible. Based on my results, I hypothesize that the similar morphotypes are the result of convergent evolution in similar habitats rather than of common ancestry. With my data, I hope to provide a foundation for further systematic and evolutionary investigations of the group.

ZUSAMMENFASSUNG

Mit mehr als 600 beschriebenen Arten sind die Heuschrecken (Oedipodinae) eine der vielfältigsten Unterfamilien der Familie Acrididae (Orthoptera). Sie sind nahezu weltweit verbreitet, mit Diversitätsschwerpunkten im Mittelmeerraum, in Südwest- und Zentralasien und entlang der südlichen Regionen der USA. Taxonomisch sind die Mitglieder der Gruppe in den meisten Fällen schwer zu unterscheiden. Erste genetische Analysen auf der Grundlage von Einzel- und Multigen-Phylogenien konnten die Neu- und Altwelt-Oedipodinae aufteilen, aber die meisten jüngeren Gruppen nicht unterscheiden; eine davon ist die Gattung *Sphingonotus*. Diese Gattung hat eine recht weite Verbreitung, da sie nicht nur in der Alten Welt, sondern auch mit einigen wenigen Arten in der Neuen Welt vorkommt. In meiner Dissertation habe ich die Auflösung des COI-Barcoding Gens und eines Multi-Gen-Ansatzes zur Unterscheidung der *Sphingonotus* Arten getestet. Während die Barcodingdaten fast keine Auflösung lieferten, konnte ich die untersuchten Arten mithilfe mehrerer Genfragmente in vier größere Gruppen aufteilen. Da die Verwendung genetischer Analysen keine ausreichende Auflösung lieferte, habe ich ökologische Nischenmodelle eingesetzt, um die Verbreitung der Arten und mögliche Einflussfaktoren zu untersuchen, die die Evolution der Art *Sphingonotus rubescens* vorangetrieben haben könnten. Ich habe die Verbreitung der Art anhand von Klimavariablen modelliert und ein potenzielles Verbreitungsgebiet in Nordafrika und Zentralasien gefunden. Die Projektion der Modelle in die Vergangenheit zeigte, dass sich die Art aufgrund des mehrfachen Wechsels von Warm- und Kaltphasen in mehreren Regionen diversifizieren konnte. In einem anderen Kapitel verwendete ich einen anderen Modellierungsansatz, um die potenziell geeigneten Lebensräume einer im Rückgang begriffenen Heuschreckenart (*Bryodemella tuberculata*) zu bestimmen. Mich interessierte, ob der Klimawandel oder die Veränderung der Landschaftsstruktur die Gründe für den Rückgang sind. Da alle ausgestorbenen Standorte eine hohe Eignung für die Art aufwiesen, war erkennbar, dass die Veränderung der Landschaftsstruktur die Ursache für den Rückgang der Art sein muss. In den folgenden Kapiteln beschäftigte ich mich mit allgemeineren Fragen zur genomischen Zusammensetzung von Heuschrecken. Innerhalb der Oedipodinae war die Chromosomenzahl stark konserviert, mit einem Standard-Karyotyp von 2n♂ = 23 (22

+ X0). Im Gegensatz dazu war die Genomgröße recht variabel und reichte von 1C=5,28pg bei *Locusta migratoria* bis zu 1C=21,96pg bei *Bryodemella tuberculata*. Diese großen Genomgrößen und das junge Alter (~35 myo) der Gruppe machen es schwierig, die Arten anhand von nur wenigen Genen zu unterscheiden. Daher habe ich genomische Analysen auf der Grundlage von Hybridization Capture Daten durchgeführt, um junge Taxa zu separieren. Ich verwendete mehr als 3.000 Marker, um die Taxa der Alten und Neuen Welt zu trennen und die Gruppen Sphingonotini und Bryodemini von der Alten Welt zu unterscheiden. Bei den anschließenden Analysen konzentrierte ich mich auf mehrere konvergente Morphotypen, die in der Alten und Neuen Welt vorkommen. Mithilfe der geometrischen Morphometrie des Vorderflügels habe ich die morphologische Ähnlichkeit der Tiere analysiert. Darüber hinaus habe ich ihre potentiell geeigneten Lebensräume modelliert und mit Hilfe von Überlappungssanalysen die Überschneidung der potentiellen Lebensräume quantifiziert. Die Daten deuten darauf hin, dass die Neuwelt- und die Altwelt-Oedipodinae getrennte Gruppen bilden, während die Auflösung der Bryodemini und Sphingonotini noch nicht möglich war. Auf der Grundlage meiner Ergebnisse vermute ich, dass die ähnlichen Morphotypen das Ergebnis einer konvergenten Evolution in ähnlichen Lebensräumen und nicht auf eine gemeinsame Abstammung zurückzuführen sind. Ich hoffe, mit meinen Daten eine Grundlage für weitere systematische und evolutionäre Untersuchungen der Gruppe zu schaffen.

Chapter 1

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Introduction

Rapid species radiations are interesting model systems for evolutionary biology. In such radiations, a (often large) number of species evolved within a short time frame from a single common ancestor. Radiations can either be adaptive and have evolved in response to open niches in a newly colonized habitat, or they may be (at least superficially) non-adaptive. The first case is more often found in the literature. Rapid species radiations represent interesting examples for evolutionary studies, as often multiple stages of speciation can be found and studied within the same system at the same time. Common examples for recent radiations are butterflies of the genus *Heliconius* (Turner, 1976), the Hawaiian cricket genus *Laupala* (Shaw, 2002), or the rapidly evolving Cichlid fishes of East Africa (Olave & Meyer, 2020).

While radiations are good systems to study speciation, (Schluter, 2000Mayr, 2001; Coyne and Orr, 2004)., they also present challenges as lineage sorting is often not complete and hybridization may still occur. Hence, determining the phylogenetic relationships of radiating species may be difficult and single genes, especially nuclear gene fragments, often do not provide sufficient resolution to differentiate the investigated taxa. This missing differentiation could be due to several reasons. Hawlitschek et al. 2017 investigated the potential reasons of BIN sharing within the Orthopterans of Germany, Austria and Switzerland. One major problem they identified were potential misidentifications of the grasshoppers, which would lead to a misinterpretation of the data and is common in groups with high morphological similarity and lack of discriminating characters (e.g. Rodriguez-Flores et al. 2019; Stark et al. 2011). For example, in the cuckoo wasp genus *Chrysis* more than 100 species are known, but lack of characters for identification contribute to frequent misidentification within the genus. Here phylogenetic analyses are helpful to differentiate the taxa (Bank et al. 2021). As a further potential reason for BIN sharing Hawlitschek et al. (2017) proposed hybridization between the species, which was already found in several species of the

genus *Chorthippus.* This was already hypothesized by previous studies (Ingrisch 1995; Gottsberger 2007; Gottsberger & Mayer 2007; Hochkirch & Lemke 2011; Rohde et al.2015). As a third potential reason, Hawlitschek et al. (2017) proposed incomplete linage sorting, which means that ancestral haplotypes are shared between several species. This evolutionary phenomenon is already known from various groups of insects (Funk & Omland 2003; Hendrich et al. 2010; Hawlitschek et al.2012; Lunt et al. 1998) and may be common in young radiations, which are morphologically distinguishable, but have not yet genetically separated. A further potential reason for BIN sharing in orthopterans are non-functional copies of mitochondrial DNA (numts). Several studies (Song et al. (2008), Moulton et al. (2010), Berthier et al.(2011) show evidence that numts may influence DNA barcoding results, especially in grasshoppers. This may at least be partially related to the large genomes within the investigated taxa (Mao et al. 2020; Husemann et al. 2021; Yuan et al. 2021; Hawlitschek et al. submitted). These large genomes can limit the power of sequence assembly due to a high amount of non-functional mtDNA. Here, paralogue sequences can be falsely integrated into phylogenies and may cause incongruence. This is often the case by using the COI fragment (Francoso et al. 2019; Baeza & Fuentes, 2013; Buhay, 2009). Shared numt sequences could be not just within species groups or genera, similar copies may be even found across families. For example, Song et al. (2008) proposed that the rate of falsely identified species based on COI barcoding may be up to 3 % due to the presence of numts. As a last potential error source, Hawlitschek et al. (2017) named the infection of specimens with *Wolbachia* bacteria. Case studs on various insects showed that due to *Wolbachia* infection, the reproduction of the hosts can be modified by killing or feminizing males and they are able to induce parthenogenesis or generating cytoplasmic incompatibility (Bella et al.2010; Sarasa et al.2013, Simões et al.2011). All these factors contribute to the limited power of single gene analyses, and specifically DNA barcoding, especially in Orthoptera.

As single gene fragments are not providing sufficient resolution, multi-gene approaches appear as next step providing the best cost-benefit ratio. Even though these methods provide a better resolution for several otherwise challenging taxa (e.g. in Mustelidae (Koepfli et al. 2008), in Rosales (Zhang et al. 2011), in katydids (Mugleston et al. 2013) or

in Tytonidae barn owls (Uva et al. 2018), the resolution within young species radiations generally remains weak, e.g. in viviparous sea snakes (Sanders et al. 2013), in Caprimulgidae nighthawks and nightjars (Han et al. 2010) or in the clear-winged moth genera *Carmenta* and *Synanthedon* (Cognato et al. 2022). As an alternative and expansion to multi-gene approaches, entire mitochondrial genomes have been sequenced commonly, promising a better resolution. Here, the 15,000 to 16,000 bp of information of the entire mitochondrial genomes are used for phylogenetic reconstruction. In many cases this method is helpful to distinguish species, e.g. the Orthopteran family Scelimeninae (Li et al. 2021), in the Coleoptera suborder Adephaga (López-López & Vogler, 2017) or the species of Culicinae mosquitos (do Nascimento et al. 2021). However, mitogenomes represent only a single genetic locus, the mitochondrion, and hence suffer similar problems of hybridization and *Wolbachia* infection as the barcoding locus COI.

With the advent of Next Generation Sequencing (NGS) methods, it has become possible to generate genome-wide genetic data for non-model organisms at a reasonable price. While in the beginning of these techniques, shotgun techniques were used to relatively unspecifically generate data across the genome, which provided reasonable data only in taxa with small genomes, meanwhile several genome reduction techniques have been developed to achieve a good coverage in taxa with larger genomes. Shotgun sequencing of genomes is a comparatively cheap method; here DNA is broken up in 100 bp to 1000 bp long fragments and random parts of the genome will be sequenced. This method is mostly used for target species with small genomes (Tortoli et al. 2017), or to recover mitogenomes for phylogenomic comparison (Linard et al. 2017, Crampton-Platt et al. 2015, Choo et al. 2017). As a disadvantage shotgun sequencing is may not able to assemble repetitive sequences correctly (Staden, 1979; Anderson, 1981). As no specific markers are used, the assembly of bigger genomes can be problematic (Bankier, 2001) and the coverage across loci may be low when many specimens are sequenced.

As a method recovering more loci across a large number of individuals, double digest restriction-site associated DNA (ddRAD) using specific restriction sites for SNPs recovery can be used (Miller et al. 2007). Here, two rounds of digestions are applied based on a size selection of purified fragments. There are several variations from the

general ddRAD and RAD (Etter et al. 2012) protocol like 2b-RAD (Wang et al. 2012), SLAF-seq (Sun et al. 2013), and hyRAD (Suchan et al. 2016). These methods allow a deep coverage and high overall information content. In general the method is easy to perform in the lab and remains one of the most cost efficient next generation methods. As a disadvantage the comparability of the data can be comprised by low overlap of orthologue markers within the samples. RAD and ddRAD were successfully used in several studies like for e.g. in Neotropical *Adelpha* butterflies (Ebel et al. 2015), in an Iberian ant-eating spider (Ortiz et al. 2021) or the Mediterranean grasshopper *Dociostaurus crassiusculus* (González-Serna et al. 2018).

A further method of NGS are Ultraconserved Elements (UCEs). These are highly conserved genomic regions which are flanked by more divergent sequences (Bejerano et al. 2004). For this method the regions next to the UCEs will be sequenced, by using specific bait sets. The usage of UCEs has the advantage that the ultraconservative sites are the same across taxa, thus allowing specific reading of the flanking regions even if no genomic data is present for a target taxon. The disadvantage of this method is that these are non-coding regions and their functions are not completely understood yet (Bejerano et al. 2004). Several studies have already used UCEs for their phylogenies with successful resolution of young radiations (for e.g. in the orchid bees Euglossini (Bossert et al. 2019), in Australasian smurf-weevils (Van Dam et al. 2017) or in formicine ants (Blaimer et al. 2015)).

A further approach is Hybridization Capture (hybCap) for target sequencing. This can be used to precisely recover data for target taxa differentiation even in low quality samples. For this specific biotynalized baits have to be designed to hybridize with the regions of interest. The resolving captured fragments are overlapping and unique, because of the random shearing of the DNA during library preparation. Thus, this method allows a higher information content per locus, but on the other hand this approach permits the interrogation across divergent species for the same loci (Harvey et al. 2016). This method worked for several studies to differentiate problematic taxa like for e.g. in Cerambycidae beetles (Haddad et al. 2018), in spiders (Hamilton et al. 2016) or in the Australian *Eugongylus* group scincid lizards (Brandley et al. 2015). This method was relatively successful in the differentiation of deep and shallow relationships in most

taxa. Further, it can be applied to taxa with large genomes as only specific parts of the genome are sequenced consistently across taxa. Hence, hybridization capture represents a promising method for phylogenetic reconstruction in taxa with massive genome sizes, such as the band-winged grasshoppers.

Band-winged grasshoppers (Oedipodinae) represent a relatively young taxon with several groups that have recently radiated. With more than 797 species in 138 genera, Oedipodinae represent one of the most diverse subfamilies within the family Acrididae (Cigliano et al. 2022). Typically for this subfamily, the hind wings are often brightly marked with different shades of color (blue, red, yellow, green, pink, violet, orange, hyalin) and with or without a dark band. In stark contrast to these conspicuous hind wings, the overall color of the body provides camouflage and can vary to match the underground they are living on (pale brown to dark brown or also greenish) (Bey-Bienko & Mistshenko, 1951; Schielzeth, 2020). The band winged grasshoppers have an almost cosmopolitan distribution (except the Arctic and Antarctic continents). Most of the species diversified within the Mediterranean Region, East Asia, and in the Southern parts of North America, which are known as the Hotspot regions of the subfamily. Most species inhabit desert, semi-desert or steppe and semi-steppe areas with sparse vegetation and sandy to pebbly underground. But the species richness and young age of the taxa makes taxonomic work difficult. Husemann et al. (2011) and Song et al. (2018) estimated the age of the group at 35 to 36 million years. Song et al. (2018) assumed a single colonization event from the Old World to the New World via the Thulean route (across Greenland) or the Beringian Bridge due to widening of grass land areas. Interestingly, morphologically similar species have evolved in both regions (New and Old World). This relationship between these species was however never deeply investigated. Altogether, the wide distribution, relatively easy availability and species richness makes them a good model system to investigate the evolution of young radiations.

To get a better understanding of the genomic architecture of the subfamily, I participated in a study that measured the size of 49 grasshopper genomes using flow cytometry and

combined the dataset with literature data of 146 species. In this study we were able to find the smallest and biggest genomes of Oedipodinae (1C=5.28pg in *Locusta migratoria* (Bier and Müller, 1969) up to 1C=21.96pg in *Bryodemella tuberculata* (Hawlitschek et al. submitted)). Here we conducted an ancestral state reconstruction based on mitogenome data of 119 species of Orthoptera to investigate the origin of such genome sizes and if there was a constant evolution through time. As genome size was variable through the Oedipodinae, we decided to have a further look at the chromosome number. An ancestral reconstruction of the chromosome number based on the most recent tree showed also high variability without a clear trend of origin. Oedipodines show a high conservancy of a standard karyotype of 2n♂ = 23 (22 + X0). These large genomes can influence the effectiveness of molecular phylogenetic analyses, as they may could have several copies of a single gene or repetitive sequences are present. Even though the genome size was extremely large in some species several studies on the phylogenetic relationships within the group are published.

In a previous study (Dey, 2016) I already investigated the potential of DNA barcoding for the identification of the Old World grasshopper genus *Sphingonotus* and the New World genus *Trimerotropis.* Both genera share a superficially similar morphology and the preference of open bare grounds with sparse vegetation. 40 % of the currently described *Sphingonotus* species and 23 % of the valid species of *Trimerotropis* were integrated. My results show that *single threshold* General Mixed Yule Coalescent (*st*GMYC) algorithm was able to correctly assign 83% of the specimens of *Trimerotropis*, but for *Sphingonotus* not more than 28% of the specimens were correctly separated. The resolution of the COI gene for species delimitation was also tested for Oedipodinae of Algeria (Moussi et al. 2018). Here, I was able to differentiate several genera of the occurring Oedipodinae fauna, but also had problems within the genus *Sphingonotus*. The samples of the genus *Sphingonotus* were recovered as paraphyletic, with mixed up taxa within several species groups. However, for some other genera, e.g. *Acrotylus*, it is possible to separate the species by using only COI (Wehrt, 2019). However, in general for many genera within the Oedipodines a differentiation using only the barcoding fragment seems to be not sufficient. Hence, several studies attempted to resolve the phylogeny of groups of band-winged grasshoppers based on multi-gene phylogenies. For example Fries, Chapco and

Contreras (2007) investigated the phylogeny of the Oedipodinae and their intercontinental relationships. They included 22 species from Eurasia, 12 from North America, one from South America and two further samples from Australia and used four gene fragments (COI, COII, cytb, ND5). They were able to cluster the species in six clades. One clade included the Trimerotropini from the New World, a second clade was formed by the Sphingontini and Bryodemini from the Old World, while two further clades included several genera from Eurasia and two clades included samples from the American continents. All nodes were supported by high bootstrap values. Within the clades of the New World Trimerotropini and Old World Sphingonotini/Bryodemini, the species and genera remained admixed, while all other clades showed monophyletic relationships. In a further study, Edelmann, Lightfoot and Miller (2010) tried to place the species *Shotwellia isleta* into the phylogeny of Oedipodinae. For this phylogeny they used a sample set of only 23 New World species. Based on their limited sampling, all genera and species integrated into the study remained monophyletic using the gene fragments 12S, 16S, COII. In 2011, Chapco and Contreras published the so far largest phylogeny of Acrididae including 117 species, thereof 35 Oedipodinae, 22 Acridinae and 60 Gomphocerinae. Their phylogeny showed that all subfamilies were monophyletic, except that the Old World Acridinae genus *Duroniella* was placed in Oedipodinae. Within the Oedipodinae seven clades were recovered. These were in line with the already published phylogeny from Fries, Chapco and Contreras (2007). Species of the tribe Trimerotropini remained admixed. Based on the limited data of Old World Sphingonotini/Bryodemini, the tribes appeared to be monophyletic. In 2012, Husemann et al. investigated the relationships between the Trimerotropini, Bryodemini and Sphingonotini using the genes ND5, ITS2, H3, COI and 18S. They observed convergence of certain wing types. In this study, as in the previous ones by Chapco and Contreras (2011) and Fries, Chapco and Contreras (2007), a separation of the New and Old World species was recovered. However, Husemann et al. (2012) also could not resolve the species groups of Sphingonotini. In a further paper, the relationships of the Canary Island species of *Sphingonotus* were studied (Husemann et al. 2014). Here, a combination of the gene fragments (ITS2, 12S, ND2 and ND5) was used. In this phylogeny, two major groups of *Sphingonotus* (*S. azurescens*-group and *S. caerulans*-group) were recovered.

Within the clusters, the species remained admixed. All other used taxa remained monophyletic within the tree.

As the resolution of the single and a multi-gene approaches was not enough, methods with higher resolution must be used to get resolution within the species of the genus *Sphingonotus*. Studies on full mitogenome data are still rare. Several studies revealing full mitogenome sequences of several Oedipodinae grasshoppers have been published (for e.g. Sheffiled et al. 2010; Guan & Xu, 2019), while phylogenies on mitogenome data are mostly lacking. Up to now only three studies including Oedipodinae grasshoppers were published using phylogenetics on mitogenomes (Fenn et al. 2007; Leavitt et al. 2013; Song et al. 2018). The first research using full mitogenomes was published by Fenn et al. (2007), who did a study on Orthoptera. Here just one sample of *Locusta migratoria* was integrated into their dataset. Leavitt et al. (2013) studied a set of *Locusta migratoria, Gastrimargus marmoratus* and *Oedaleus decorus.* Both studies do not dig deeper into the complicated subfamily Oedipodinae. The only study which is shedding light on a bigger set of Oedipodinae was published by Song et al. (2018). In this publication they focus on the full Acrididae and detected four clades. The Oedipodinae remained polyphyletic. As they not primarily focus on Oedipodinae the relationships within the subfamily remain unclear. A first study on the mitochondrial gene order, also shows just low resolution within the Oedipodinae (Gaugel et al. submitted).

As full mitogenomes still do not provide sufficient resolution to resolve the problematic relationships within the Oedipodinae a further step is to use Next-Generation sequencing methods to resolve the taxa. The method is already frequently used to recover mitogenomes (e.g. Guan & Zhou, 2016; Guan & Xu, 2016; Mao et al. 2018; Shaoli et al. 2018; Liu, 2019; Dong et al. 2019; Yu & Xu, 2019). Furthermore, one study using NGS for population genetics of the grasshopper *Oedaleus decorus* was already published (Schmid et al. 2018). So far, only a single study used phylogenomic data to investigate phylogenetic relationships of grasshoppers (Song et al. 2020). As this study does not focus on shallow nodes within the Orthopteran species, it remains unclear if genomic data is able to differentiate the taxonomically problematic taxa.

Thus, in this thesis I dig deeper into the phylogenomic relationships of the Oedipodinae using a genomic approach. I used Hybridization Capture data based on 3,364 ortholog genes to conduce a stable phylogeny of Oedipodinae. This method was chosen as Next Generation Sequencing method, as it is not blindly sequencing parts of the genome as other methods. This method can deal with the huge genome size of some Oedipodinae. I was able to generate a stable phylogeny of 48 Oedipodinae species from the Old and the New World. In addition, I had a closer look at the evolutionary forces potentially leading to similar morphologies of Old and New World Oedipodinae species by using geometric morphometrics of 12 landmarks of the elytra. I used ecological niche modelling based on two different methods to compare the potential species distribution of the species pairs. First I calculated the potential species distribution using *ellipsenm*, than I analyzed the overlapping niche space using the outcomes of *ellipsenm*. After this I reconstructed the ancestral niche based on the genomic phylogeny and a UPGMA tree based on the geometric morphometric data.

Summing up, in this thesis I want to focus on the phylogenetic and phylogenomic relationships within the Oedipodinae. Here, I want to resolve the shallow nodes of the young radiations using different sequencing methods. Further, I want to focus on the genomic composition within Oedipodinae to figure out the genome size, mitogenomic arrangements and general chromosome number within the subfamily. Finally, I am using genomic data to investigate the convergent evolution of Oedipodinae from the Old and New World. Based on this several questions will be answered:

(1) What is the general genomic composition?
(2) Which method shows enough resolution to differentiate the species of Oedipodinae?
(3) Can I detect signs of convergent evolution in the Old and New World Oedipodinae?

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Chapter 2

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"Barcoding is not a solution for everything"

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Comparison of single-gene DNA barcoding and multi-gene analyses in the genus *Sphingonotus* (Acrididae: Oedipodinae)

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Vergleich von Einzel-Gen DNA Barcoding und Multi-Gen Analysen in der Gattung *Sphingonotus* (Acrididae: Oedipodinae).

Articulata, *35*, 47-59.

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ABSTRACT

With more than 143 species and a wide distributional range, the genus Sphingonotus Fieber, 1852 is the most species-rich genus within the subfamily Oedipodinae. Because of the high morphological similarity of many species, and a lack of comprehensive identification keys, it is not easy to distinguish many species of the genus. Molecular methods, specifically DNA barcoding (sequencing of the COI gene), promise to solve such problems, yet have shown to be problematic in many orthopteran groups. We used four different species delimitation tools (ABGD, stGMYC, mtGMYC und bGMYC) and two mitochondrial and one nuclear gene fragment to test for congruence with morphologically identified species. Our results show that using a single gene is not sufficient to delimit species. However, after concatenation of three gene fragments (COI, ND5, H3; total length of 1,772 bp), most species of *Sphingonotus* could be separated in different species groups and in many cases to species. The use of additional mitochondrial genes (or whole mitogenomes) and nuclear loci will further help to understand the status of many Sphingonotus species and will facilitate a strongly needed revision of the entire genus.

INTRODUCTION

With nearly 28,000 recent species, grasshoppers define a diverse order (Cigliano et al. 2019). The taxonomy within many groups of grasshoppers is unclear and the species status of many taxa also needs to be verified. Thereby taxonomists are often lacking detailed knowledge of the respective groups (Drew 2011). The search for alternative methods for objective identification and description of species has led to the development of diverse molecular and statistical tools that, at least in theory, can identify the majority of species. Modern research approaches, such as DNA barcoding, enable the study of species not only with morphological but also with molecular methods. The standard DNA barcoding gene in animals is a fragment of cytochrome oxidase 1 (COI) (about 650 base pairs (bp) long) (Hebert et al. 2003). With the help of the information content of this gene it can be theoretically used to identify all the expected animal species in the world (Moore 1995, Stockle & Hebert 2008). The use of the gene fragment has also been practiced in various taxa in the German fauna and proved to be very successful (Schmid-Egger et al. 2019, Moriniére et al. 2017, Raupach et al. 2014). However, this method also has its weaknesses, as exemplified by a study on Central European grasshopper species demonstrated (Hawlitschek et al. 2017). This study showed that 100% of Ensifera species from the Central European region could be distinguished, whereas only 59% of the Caelifera species are molecularly distinguishable from each other. In particular the Gomphocerinae are problematic. The lack of delimitation within this group can have numerous causes according to Hawlitschek et al. (2017). First, many species within this group are evolutionarily very young, so sequence differences between closely related species may be too small to distinguish them. Because statistical species delimitation methods often use percent sequence differences or the so-called barcode gap to distinguish species, the different genetic distances present a difficult hurdle to overcome. It is often the case that species with smaller sequence differences are classified as one unit (molecular operational taxonomic unit [mOTU]), or in the Barcode of Life Database (BOLD) assigned to a Barcode Index Number [BIN]). This often leads to the fact that morphologically or bioacoustically delimitable species are not genetically distinguished. However, not only the young age of some species, but also the

possible hybridization between species can be a reason for missing barcode differentiation. Several things can be a reason for barcode sharing, i.e. sharing the same COI sequence information between several species. Furthermore, pseudogenes (numts), i.e. functionless copies of genes, which occur very frequently in locusts, evolve freely (Hochkirch 2013, Song et al. 2008, Shaw 2002). Finally, infection by Wolbachia bacteria may also be a reason for poor resolution, these have an effect on the sex determination of the host and thereby also affect the inheritance of mitochondria (Hawlitschek et al. 2017). While Hawlitschek et al. (2017) could not resolve the Central European grasshoppers, the Oedipodinae were well separated using the COI gene.

However, this can also be explained by the fact that only few and phylogenetically well separated species are present in Central Europe. However, a look at a larger number of species within a genus of this subfamily, changes the picture (e.g., Husemann et al. 2014). Thus, the possibility of species differentiation with the help of DNA barcoding decreases with increasing diversity, as shown by Dey et al. (2018a) for the Biskra region in Algeria. Because of the high diversity, very similar morphology (Fig. 1), and the young age of many species within the genus *Sphingonotus* (Husemann et al. 2012), it represents an interesting model system for evolutionary biology and phylogenetic questions. With over 143 species, the genus is the most speciesrich within the Oedipodinae (Cigliano et al. 2019). Initial studies by Hochkirch & Husemann (2008) and Husemann et al. (2013, 2014) investigated the phylogeny and diversity of the genus *Sphingonotus* in the Canary Islands and the Iberian Peninsula, using sequencing of fragments of the NADH dehydrogenase subunit 5 (ND5), parts of the 16s rRNA, tRNA-Leu and of the NADH dehydrogenase subunit 1 (NDS), internal transcribed spacer 2 (ITS2) and 12S rRNA, they are able to distinguish the species, and further a new species was found in the Canary Islands, as well as two new species in the Iberian Peninsula. These studies demonstrate the utility of molecular methods for species delimitation. In this study we are now testing the power of COI, the gene most commonly used in DNA barcoding, and the combination of several genes on a large number of species of different ages with a wide distribution and different divergence within the genus *Sphingonotus*.

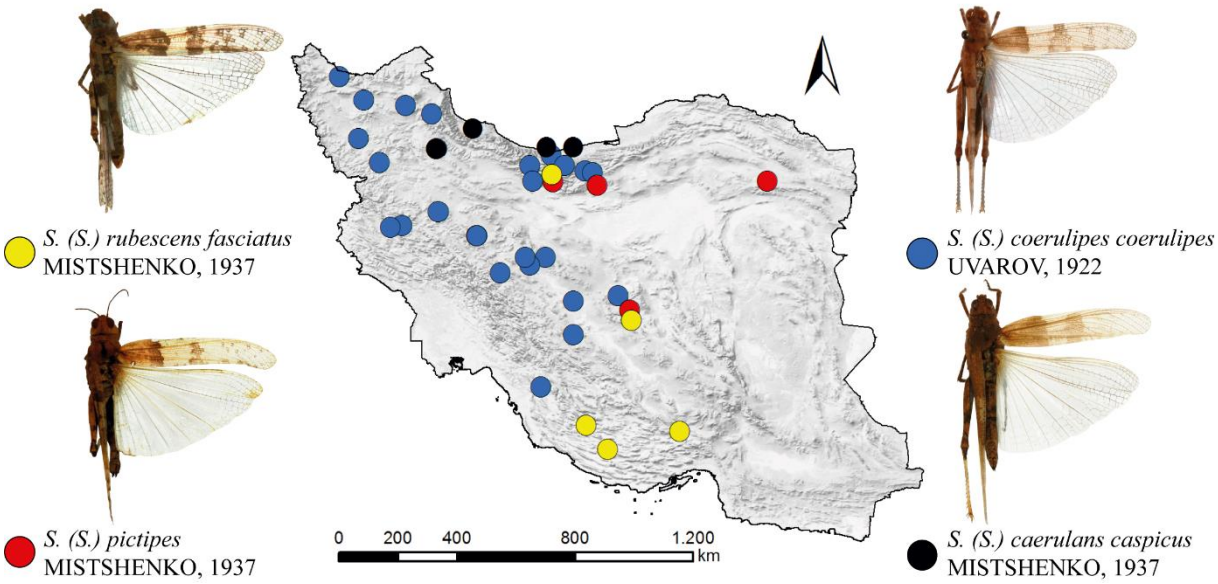

Figure 1: The graphic illustrates the similarity between some species of the genus Sphingonotus and their partly overlapping distribution. Here as an example the four (sub)species occurring in Iran *S. rubescens* fasciatus Mistshenko, 1937*; S. pictipes* Uvarov, 1952; *S. coerulipes coerulipes* Uvarov, 1922 and *S. caerulans caspicus* Mistshenko, 1937 shown with their previous known localities in Iran (modified after Dey et al. 2018).

METHODS AND RESULTS

To test the success rate of DNA barcoding in the genus *Sphingonotus*, we first compiled a dataset with as large a number of different species identified by morphological characters with a wide geographic distribution (Figure 2). Here, a fragment of COI was amplified and sequenced for 84 individuals from 29 different species (see Table 1). The sequences were compared with the databases NCBI and BOLD (Ratnasingham & Hebert 2007, Benson et al. 2012). An alignment of all sequences, including the outgroups *Trimerotropis ochraceipennis* (Blanchard, 1851) and *Thalpomena coerulescens* Uvarov, 1923, was created and phylogenies were calculated using bayesian inference and maximum likelihood methods (Ronquist et al. 2012, Tamura et al. 2013). Subsequently, four different statistical species delimitation procedures (ABGD, stGMYC, mtGMYC, and bGMYC; Pons et al. 2006, Monghan et al. 2009, Puillandre et al. 2012, Reid & Carstens 2012, Fujisawa et al. 2013, TANG et al. 2014) were applied to the dataset. While ABGD uses the barcode gap to group the mOTUs, stGMYC and mtGMYC subdivide the data

based into categorical clusters (based on a Maximum Likelihood approach). The bGMYC method, on the other hand, uses a Bayesian phylogeny. The respective calculated mOTUs were subsequently compared for congruence. Whereas bGMYC identified the individuals into 21 groups and mtGMYC into 25 groups, which was closest to the number of 29 morphologically determined species, ABGD identified 16 and stGMYC 18 mOTUs. None of the methods grouped more than 24% (bGMYC) of all analyzed individuals into the correct morphologically defined groups. In the next step, for the same individuals for which COI was sequenced, the mitochondrial gene ND5, which has already been used in previous reconstruction of phylogenies of the genus (Hochkirch & Husemann 2008), as well as the nuclear gene histone 3 (H3) (Colgan et al. 1998) were analyzed. The two additional gene fragments were analyzed individually using the same species delimitation methods as already approached for COI (ABGD, stGMYC, mtGMYC, bGMYC). It could be shown that even the two additionally used fragments did not provide sufficient resolution in the single analysis.

The analyzed ND5 dataset separated a maximum of four species correctly according to the morphological identification (ABGD), thereby the calculated number of mOTUs nearly corresponded with the respective morphologically delimited species number (21 mOTUs calculated on 29 morphological species). The H3 dataset did not show a nearly correct separation in any approximately correct cluster. The number of mOTUs was either greatly underestimated or overestimated. The results of all analyses for each gene fragment are summarized in Table 2. In addition, the three amplified gene fragments were analyzed together. The total length of the new data set was 1,772 bp. For each individual all three gene fragments were amplified and included in the analyses. Again, phylogenies were calculated using bayesian inference and maximum likelihood methods, and the resulting topologies were subsequently compared. Species delimitation methods were not used for the multi-gene trees because only few algorithms have been developed to analyze combined multi-gene datasets. However, the phylogenetic trees of the combined dataset showed a better resolution than all single used fragments. Most of the nodes of the tree were statistically well supported (> 95% posterior probability). While it is still not possible to separate all analyzed species from each other, it was at least possible to define species groups. Four large, well-separated

clusters were identified in the phylogenetic tree (Figure 3). Within these clusters some species were not clearly genetically distinguished from each other and appear in several places (e.g. *S. caerulans, S. coerulipes).* Some species, however, were clearly separable (e.g., *S. (S.) fuerteventurae, S. beybienko, S. haitensis*). Based on the concatenated phylogenetic tree, theoretically about 50% of the species can be separated. However, it must be clearly stated that this assessment was not made with the help of a species delimitation algorithm

Table 1: Overview about all in this study used species and regarding specimen numbers.

Genus	Species	Specimen number
Sphingonotus	*caerulans*	5
Sphingonotus	*canariensis*	2
Sphingonotus	*candidus*	1
Sphingonotus	*coerulipes*	6
Sphingonotus	*eurasius*	3
Sphingonotus	*finotianus*	5
Sphingonotus	*fuerteventurae*	2
Sphingonotus	*fuscoirroratus*	5
Sphingonotus	*fuscus*	4
Sphingonotus	*beybienkoi*	2
Sphingonotus	*haitensis*	5
Sphingonotus	*lucasii*	4
Sphingonotus	*obscuratus*	2
Sphingonotus	*octofasciatus*	1
Sphingonotus	*pachecoi*	2
Sphingonotus	*pilosus*	2
Sphingonotus	*rubescens*	6
Sphingonotus	*satrapes*	1
Sphingonotus	*savignyi*	8
Sphingonotus	*sublaevis*	1
Sphingonotus	*theodori*	3

Genus	Species	Specimen number
Sphingonotus	*vosseleri*	3
Sphingonotus	*azurescens*	3
Thalpomena	*coerulescens*	1
Trimerotropis	*ochraceipennis*	1
Sphingoderus	*carinatus*	1
Sphingonotus	*sp 1*	2
Sphingonotus	*cf. lucidus*	2
Sphingonotus	*sp 2*	1

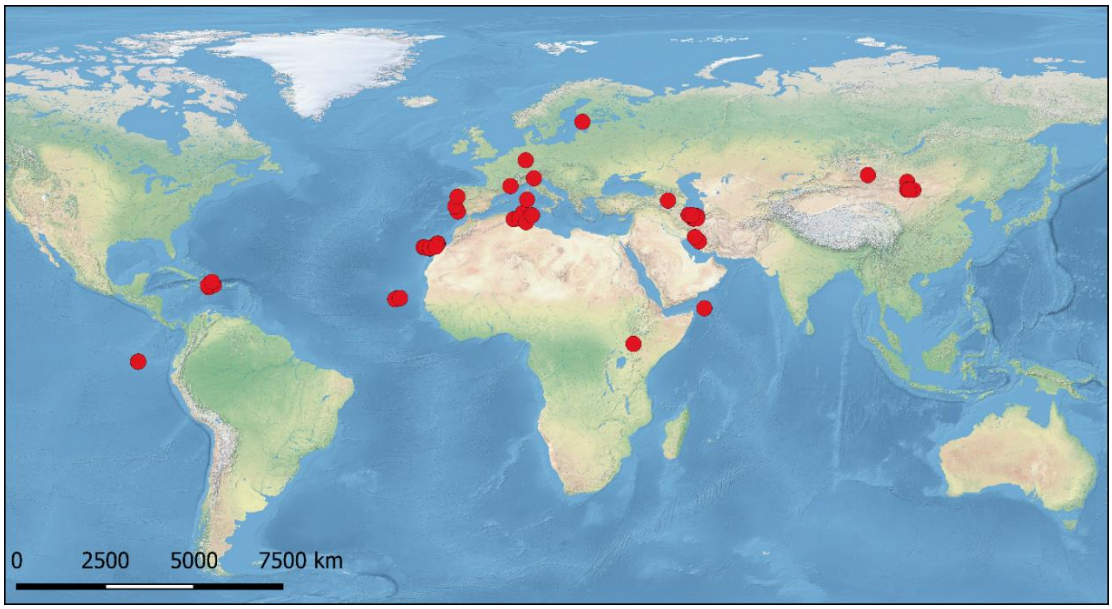

Figure 2: This map shows the origin of the samples used in this study.

DISCUSSION

In the present project, we used four different methods for molecular species differentiation based on COI, ND5 and H3 single gene fragments and the combined multi-gene dataset. To be able to examine the resolution of the gene fragments used, all markers were examined individually with the four species delimitation methods. The COI dataset showed low resolution regarding of the statistical method. Furthermore, it could be shown that also the fragments ND5 and H3 individually showed no, or only

slightly better resolution than COI. While in some species the number of mOTUs was relatively similar compared to the morphological assessment, all methods failed to correctly assign the individuals to the morphologically correctly determined group. The majority of mOTUs consisted of more than one morphologically identified species. In addition, individuals of one species sometimes occurred in different mOTUs. This pattern indicates intensive sharing of identical or closely related barcodes. This was also observed, for example, in the study of Hawlitschek et al. (2017) for Gomphocerinae, in the work of Trewick (2008) for the New Zealand grasshoppers, or also in observed in Zhou et al. (2019) for the Chinese broadleaf grasshoppers. However, not only in Orthoptera, but also in other taxa for example in the Northwest Pacific Mollusca (Li et al. 2016) or European Lepidoptera. (Huemer et al. 2014), the barcoding problems already explained occurred.

Table 2: Results of the different statistical species delimitation methods: Automatic Barcode Gap Discovery (ABGD), Single Threshold General Mixed Yule Coalescent (stGMYC), Multi Threshold General Mixed Yule Coalescent (mtGMYC) and Bayesian General Mixed Yule Coalescent (bGMYC) for each of the analyzed genes. The respective number of groups calculated can be found in column #, while % indicates the percentage of correctly assigned species after morphological species identification.

	ABGD		**stGMYC**		**mtGMYC**		**bGMYC**	
	#	%	#	%	#	%	#	%
COI	16	20	18	17	25	13	21	24
ND5	21	13	19	17	24	13	17	21
H3	38	13	2	0	2	0	3	0

In many other insect groups, such as the highly diverse bee genus *Lasioglossum*, the individual, usually even cryptic, species can be separated via DNA barcoding (Landaverde-González et al. 2017). Also in the tropical Lepidoptera, barcoding was able to distinguish 97.9% of the 521 species studied (Hajibabaei et al. 2006). Furthermore in a study by Foottit et al. (2008) more than 96% of all Canadian aphids could be differentiated. However, within the genus *Sphingonotus*, the information content of the COI fragment, as well as the other fragments ND5 and H3 used, does not appear to be

sufficient enough to differentiate species. In the analyses of the individual gene fragments, none of the statistical species delimitation methods could assign more than 24% of the individuals to the morphologically determined species. Analysis of the multigen datasets via bayesian inference and maximum likelihood phylogenies was more successful, but this method also only correctly delineated some species groups, each of which had contained several species that were not or only partially separable from each other. Some other species within the clusters, however, could be well distinguished from others. This was especially true for the basal species within the clusters. Due to the young age of many species in the genus, the wide distribution and also the morphological similarity, several aspects may play a role in the lack of resolution of the COI dataset. Potentially the most important factor for intensive BIN sharing (sharing the same COI sequence between different species) is the young age and the perceived high potential for Hybridization in young radiations.

In addition, the different evolutionary speed in individual lineages, is a major problem for statistical methods, which they can not handle (Lunt et al. 1996, Lin et al. 2004). (Lunt et al. 1996, Lin et al. 2004). This is especially true for ABGD, a Method based on the calculation of a barcode gap. Here the problem is that multiple gaps can be formed throught the origin of species, which may formed evolutionarily heterogeneous groups (Meyer & Paulay 2005). The use of additional gene fragments leads to a better resolution within the phylogeny. Compared to the results obtained from the analysis of COI sequences alone, the use of multi-gene approach made it possible to separate species into four clusters (*S. azures* separate (*S. azurescens* group; *S. caerulans* group; *S. coerulipes* group; *S. haitensis* group (Fig. 3)). Previous studies had already identified three of these groups (*S. azurescens* group; *S. caerulans* group, *S. haitensis* group; Husemann et al. 2014, 2015) and already showed the difficulty to resolve these groups with few gene fragments. The *S. coerulipes* group represents a previously unknown grouping. This group exclusively includes samples from southwest Asia. This geographic separation of individual species, some of which are endemic to this region (e.g., *S. theodori, S. fuscus*), suggests that the group split off a long time ago, formed its own independent group and has undergone its own independent radiation in this area, similar to the other two

groups, which diversified in the Mediterranean region. Furthermore, with the help of this study we were able to find a potentially new species from the region. This one is morphologically quite similar to the widely distributed *Sphingonotus (Sphingonotus) rubescens* Walker, 1870, of which several subspecies are known, which are distributed mainly in Central and Western Asia. (*S. r. afghanicus, S. r. fallax, S. r. fasciatus, S. r. subfasciatus*). Since S. rubescens however, clusters genetically within the *S. caerulans* group, we were able to distinguish the potentially new species from the widespread one. In a future study we want to investigate and describe the potentially new species in more detail.

OUTLOOK

In order to clarify the relationships within the genus *Sphingonotus*, it will be necessary to collect more individuals from as many species as possible and especially from all distribution areas. It has already been shown that data sets containing only COI sequences do not guarantee a clear separation. The so far used three gene fragments COI, H3 and ND5 seem to be variable enough in combination to distinguish species groups (and further species), but do not provide species-level resolution in all cases. Accordingly, it is necessary to expand the current database and analyze additional nuclear, as well as mitochondrial, gene fragments. The use of genomic data will, as in other recent radiations, probably allow a better resolution of the relationships. Another possibility is the use of mitogenomes in combination with nuclear markers (Song et al. 2015, 2018). Nowadays, these can be generated relatively inexpensively via next-generation sequencing platforms.

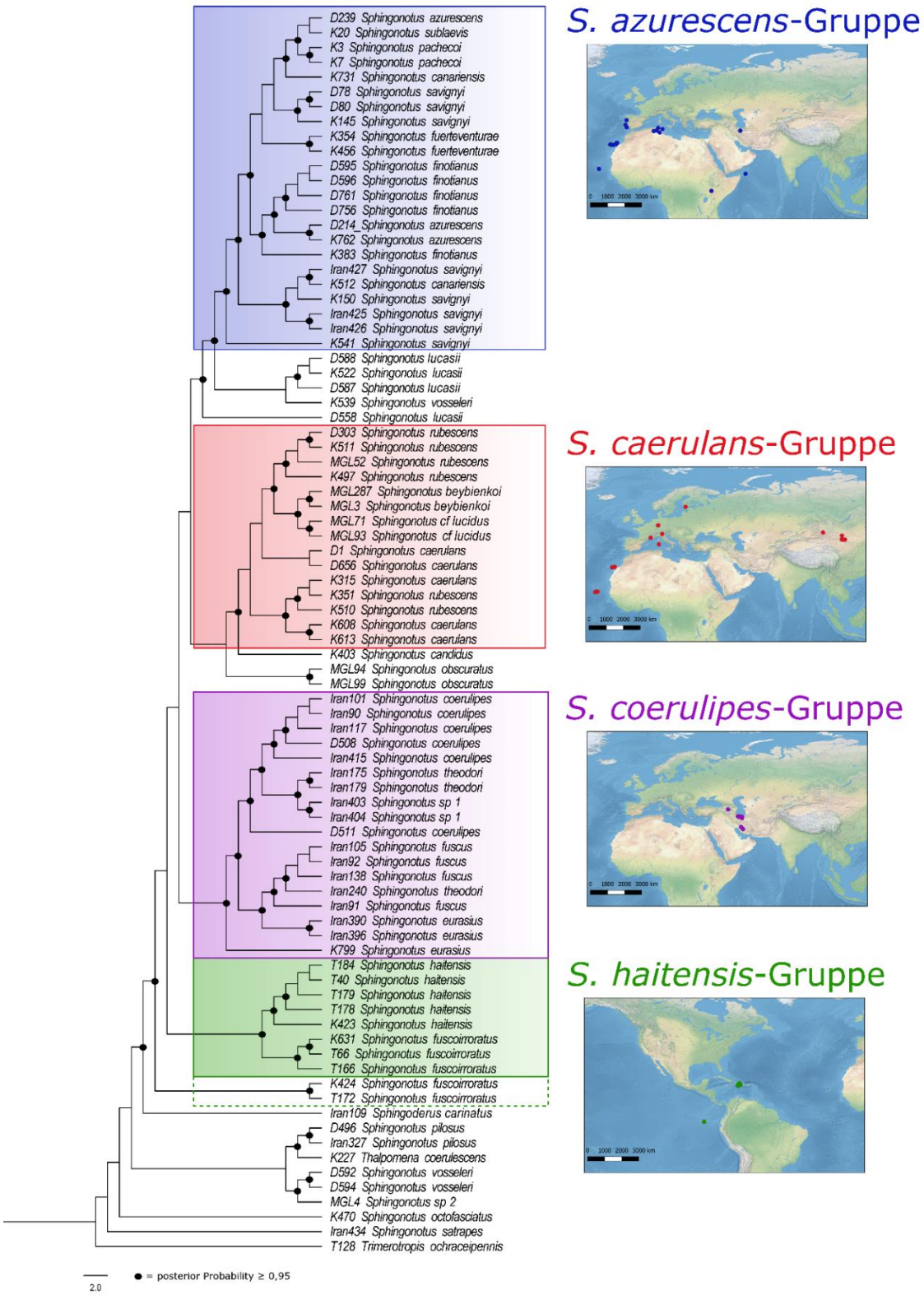

Figure 3: This Bayesian phylogeny was calculated based on the multi-gene dataset. Black nodes represent posterior probabilities above 95%. The clades defined in this study of the *S. azurescens* group (blue), the S. caerulans group (red), the *S. coerulipes* group (purple) and the *S. haitensis* group (green) are color-coded. The dashed green line shows two additional individuals that belong to the *S. haitensis* group, but do not fall directly into the clade within the tree. The respective localities of the individuals are shown on the adjacent distribution maps.

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LSD, MH designed the study; LSD collected data; LSD performed analyses; LSD wrote the original version of the manuscript; LSD, MH, AH took part in the writing process; LSD coordinated the writing process.

Hamburg, 12.01.2023 

Place/Date Sign

Chapter 3

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"Species Distribution Modelling reveals insights into wide distributions"

-

Species distribution modelling sheds light on the widespread distribution of *Sphingonotus* (*Sphingonotus*) *rubescens* (Orthoptera: Acrididae: Oedipodinae)

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ABSTRACT

Sphingonotus (Sphingonotus) rubescens (Walker, 1870) occurs from the Atlantic Islands to central and southern Asia, but its ecological preferences and the potential drivers that shaped its currently extensive distribution remain unknown. We review the known occurrence data for *S .rubescens* and reconstruct its current and palaeoclimatic niche (Last Glacial Maximum and mid-Holocene) using species distribution modelling (SDM). We examine how climatic suitability might have created potential past migratory pathways shaping its current distribution. Moreover, we test the utility of SDM approaches to flag dubious records detected in the assembled dataset. The results reveal new distributional records from four countries. Climatic models indicate high levels of overlap between current and palaeoclimatic models, with stability of large suitable areas through time. Furthermore, we observe that suitability values associated with dubious records are lower than the distribution of suitability values within the known distribution of *S. rubescens*. Climatic stability of suitable areas through time for *S. rubescens* might have aided the expansion and maintenance of its current wide distribution. Furthermore, our results support previous studies indicating the usefulness of SDM tools for the detection of doubious occurrences.

Keywords: ecological niche modelling – Last Glacial Maximum – mid-Holocene – outlier detection – Palaearctic – species distribution modelling.

INTRODUCTION

The geographical ranges of species are a result of the interplay of dispersal and colonization capacity, abiotic tolerances and biotic interactions (Soberón & Peterson, 2005; Peterson et al., 2011; Wisz et al., 2013). Understanding the factors shaping species ranges is the major question of biogeography and is central in both ecology (Lawton, 1994; Maurer & Taper, 2002) and evolutionary biology (Sexton et al., 2009). One of the major drivers of species distributions is climate (Vrba, 1992; Soberón & Nakamura, 2009). Thus, the detection of climatic variables that influence the distribution of species through time (e.g. niche conservatism; Wiens & Graham, 2005), their stability and availability, can provide insights into observed distributional patterns. *Sphingonotus (Sphingonotus) rubescens* Walker, 1870 is a very widespread grasshopper species, with a distribution ranging from the Atlantic Islands (Madeira, Canary Islands and Cape Verdes) across northern Africa and southern Europe to Central Asia (Cigliano et al., 2020; Supporting Information, Fig. S1). During the last century, new distribution records have steadily extended the known range of the species (e.g. Massa, 2009; Massa et al., 2012). Despite its high morphological uniformity across most of its range, several subspecies have been described based on variation in body size and venation patterns of the tegmina (Bey-Bienko & Mistshenko, 1951). Currently, six subspecies are recognized within the species (Cigliano et al., 2020): *S. (S.) rubescens rubescens* (Walker, 1870), spread across northern Africa to Central Asia; *S. (S.) r. subfasciatus* Bey-Bienko, 1951, distributed in Kirgizia; *S. (S.) r. afghanicus* Mistshenko, 1937, described from Afghanistan; *S. (S.) r. burri* Chopard 1936, endemic to Cape Verde; *S. (S.) r. fallax* Mistshenko, 1937, present in India; *and S. (S.) r. fasciatus* Mistshenko, 1937, recorded from Iran, Kazakhstan and Kirgizia. Moreover, *S. rubescens* is morphologically very similar to its relative *Sphingonotus (Sphingonotus) caerulans* (Linnaeus, 1767) and differs only in a few characters, i.e. strongly developed venation of the tegmina, S-shaped intercalary vein and slender body shape (Mistshenko, 1937). However, *S. rubescens* differs greatly in its melodious song from *S. caerulans* (Husemann & Hochkirch, 2007). The close genetic relationships and morphological similarity of these and several other species have led to the establishment of the *S. caerulans* species group (Hochkirch & Husemann, 2008). Within the species

group, genetic analyses based on single and multi-gene approaches have shown that *S. rubescens* has split rather recently from *S. caerulans*, which is a paraphyletic taxon (Husemann et al., 2014). Within the *S. caerulans* group, *S. (S.) r. rubescens* has the widest range, overlapping in distribution with most of its subspecies (Fig. 1). To date, no scientific evaluation of the parameters determining its wide range has been performed. Ecological niche models (ENMs) and species distribution models (SDMs) combine species occurrence data with environmental data layers to predict the spatial extent of climatically suitable areas for a species (Guisan & Thuiller, 2005; Soberón & Peterson, 2005). Ecological niche models focus on exploration of the potential distribution of a species, i.e. projections in space and time, whereas SDMs attempt to delimit objects in geographical space, referring to real distributions of species (Soberón et al., 2017). Among the many applications of ENMs, such as predicting impacts of future climate change on species distributions (Pearson & Dawson, 2003), evaluating the invasive potential of non-native species (Jiménez-Valverde et al., 2011) and conservation planning (Guisan et al., 2013), their usefulness is also related to the detection of outliers among the occurrence data (Raxworthy et al., 2007; Graham et al., 2008; Hinojosa-Díaz et al., 2009; Lash et al., 2012; Simões & Peterson, 2018). If values of environmental suitability estimates are noticeably low, they might indicate outliers in occurrence datasets, the correctness of which can then be checked (Simões & Peterson, 2018). The identification of such errors is complicated and challenging, thus an assessment of the utility of the method is of high relevance for better assessment of the quality of biodiversity data (Simões & Peterson, 2018). In this study, we examined museum material of *S. rubescens* and found new distributional records, filling collection gaps within the known species distribution range. Among these, a specimen recorded from Thailand was discovered, but with vague geographical localization to Chiang Rai or Tham Pha Thai Cave (Supporting Information, Fig. S2). Despite being clearly identifiable morphologically as *S. rubescens*, both locations are geographically distant from the known distribution limits of the species (~2500 km apart), in a region of tropical humid climate differing greatly from the subtropical arid conditions usually preferred (Khedari et al., 2002). This dubious record of occurrence in Thailand and the steady growth of literature on the distribution of *S. rubescens* inspired us to assemble occurrence records

from the literature, databases and collections as a basis for ENM/SDM analyses. More specifically, our aims were as follows: (1) to review its range and update distribution information; (2) to use SDMs to explore areas climatically analogous to its known distribution to identify collection gaps and investigate the potential distributional outliers (i.e. Thailand); and (3) to reconstruct the palaeoclimatic niche during the Last Glacial Maximum (LGM; 22 000 years ago) and mid-Holocene (MH; 6000 years ago), seeking to provide further insight on the present wide distribution of *S. (S.) rubescens*.

MATERIAL AND METHODS

Type material examined

Sphingonotus (Sphingonotus) rubescens afghanicus Mistshenko, 1937: Afghanistan, Barizendan (holotype,ZIN); *Sphingonotus (Sphingonotus) rubescens burri* Chopard, 1936: Cape Verde, Cape Verde Island, Fogo, São Filipe (syntype, MNHN); *Sphingonotus (Sphingonotus) rubescens fallax* Mistshenko, 1937: India, western Himalaya, Jammu-Kashmir, Nubra River, Panamik (holotype, ZIN); *Sphingonotus (Sphingonotus) rubescens fasciatus* Mistshenko, 1937: Kazakhstan, Nono-Voskresenovka (holotype, ZIN); *Sphingonotus (Sphingonotus) rubescens rubescens* (Walker, 1870): Egypt, Wadi Genneh (holotype, BMNH); and *Sphingonotus (Sphingonotus) rubescens subfasciatus* Bey-Bienko, 1951: Kyrgyzstan, Lake Issyk-Kul (depository of type material is unknown; topotype, SZMN). Type locations and photographs of type material examined are displayed in Figure 1.

Distribution records

In total, 809 records of all *S. (S.) rubescens* subspecies (hereafter, *S. rubescens*) were assembled: 533 extracted from the literature (Supporting Information, Tables S1 and S2); 40 records downloaded from the Global Biodiversity Facility (GBIF.org; 5 November 2019, GBIF occurrence download https://doi.org/10.15468/dl.z7kkg5); 93 records extracted from Orthoptères d'Afrique du Nord-Ouest (Louveaux et al., 2019) (Supporting Information, Table S2); 33 records extracted from Les acridiens d'Afrique

occidentale et nord-centrale (Mestre & Chiffaud, 2020); 13 records obtained from collecting trips by L.-S.D., A.H. and M.H. (Greece: one record: Kos Island, November 2014; two records: Crete, September 1999 and May 2006; Mongolia: two records: Southern Gobi desert, 19 July-15 August 2015; two records: Great Lakes Depression in western Mongolia, 1–30 July 2017; and Morocco: eight records: High Atlas Mountains, 23 March–2 April 2019).

Ninety-seven records were obtained from museum specimens deposited in the following collections: the British Museum of Natural History, London, UK (BMNH); Natural History Museum, Geneva, Switzerland (GE); Mongolian Academy of Science, Ulaan Bator, Mongolia (MAS); Muséum National d'Histoire Naturelle, Paris, France (MNHN); National Museum, Prague, Czech Republic (NM); Natural History Museum, Vienna, Austria (NMW); Department of General Biology and Ecology, Novosibirsk State University (NSU); Siberian Zoological Museum, Novosibirsk, Russia (SZMN); Zoological Institute, Russian Academy of Science, St. Petersburg, Russia (ZIN); Zoological Collection of the Center for Natural History, Hamburg, Germany (ZMH); Zoological State Collection, Munich, Germany (ZSM); and private of L.-S.D., A.H., M.H. and Rob Felix (Nijmegen, The Netherlands). Historical records lacking geographical coordinates, were georeferenced using Google Earth (https://www.google.com/earth/). Specimen data collected from museum specimens and field trips can be found in the Supporting Information (Table S3a), and further new distributional records are presented in the Supporting Information (Table S3b). Museum material was identified using identification keys (Mistshenko, 1937; Bey-Bienko & Mistshenko, 1951; Hochkirch & Husemann, 2008; Dey et al., 2018). Furthermore, specimens were compared with photographs of type material, obtained from the Orthoptera Species File (Cigliano et al., 2020). To perform ENM, 398 records were used after undergoing the data cleaning protocol described by Cobos et al. (2018).

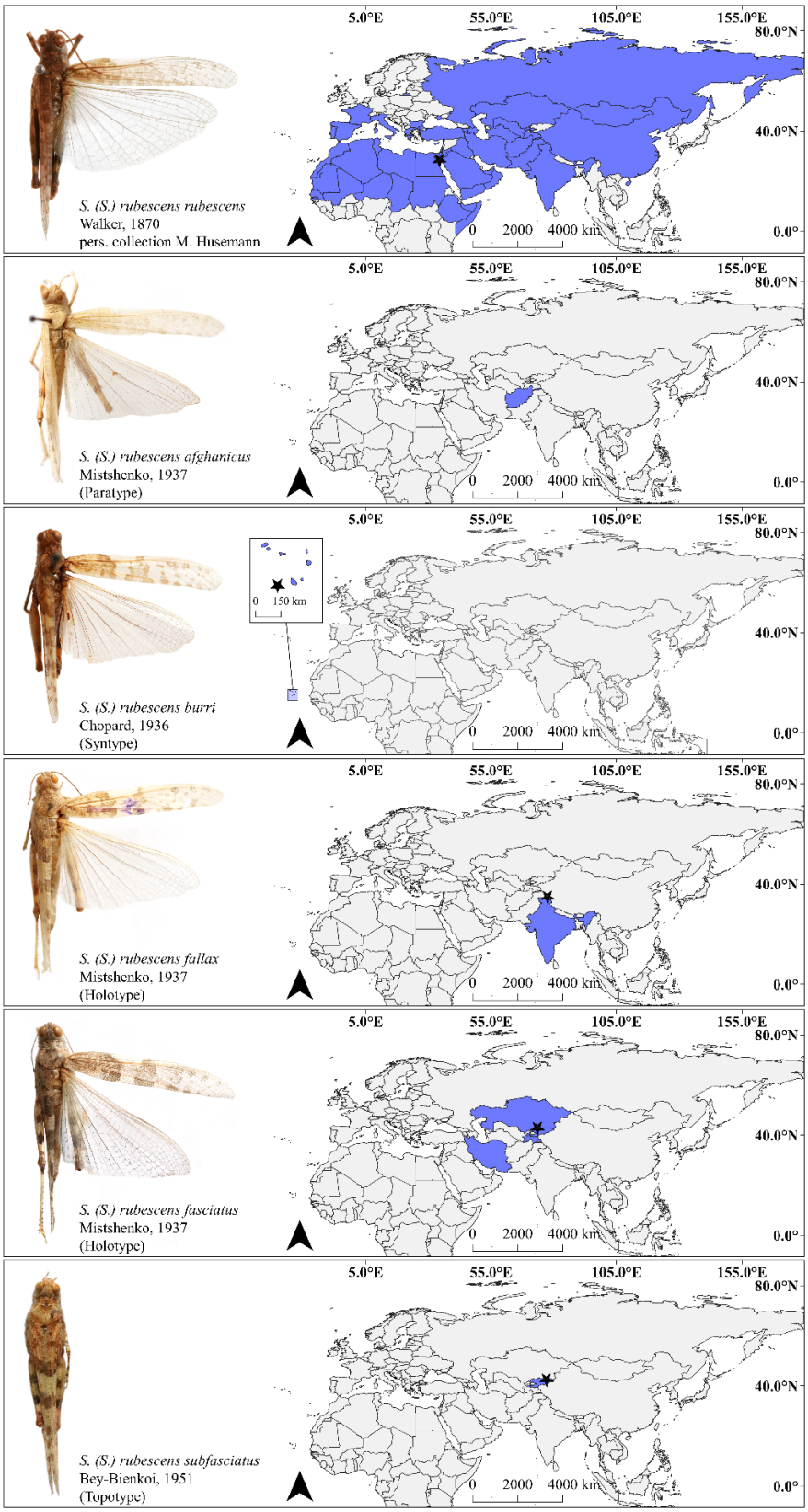

Figure 1. Distribution of subspecies of *S. (S.) rubescens*. Information on the illustrated type specimens is provided in the section 'Type material examined'. Blue indicates countries with distribution records of the respective subspecies. Black stars represent the type localities of the subspecies. Georeferencing of the type locality of *S. (S.) r. afghanicus* was not possible (Afghanistan: Barizendan).

Environmental data

Environmental data were obtained from WorldClim v.1.4 (www.worldclim.org;Hijmans et al., 2005) at 5 arc-min resolution. WorldClim is based on interpolations of weather station data (i.e. monthly precipitation and minimum and maximum temperatures) over the period 1960–1990. Of the 19 available bioclimatic variables, we excluded four (mean temperature of wettest quarter, mean temperature of driest quarter, precipitation of warmest quarter and precipitation of coldest quarter) because of known spatial artefacts between adjacent grid cells (Escobar et al., 2014; Campbell et al., 2015). To avoid overfitting and inflation of model accuracy with overly dimensional environmental space and collinearity among variables, we performed jackknife analysis to assess the relevance of variables, followed by Pearson's correlation coefficient (r) analysis to examine the cross-correlation of the variables, wherein variables with a correlation < 0.8 were excluded. Seeking to test the best set of environmental variables, models were calibrated using three environmental sets: 'set 1', containing the statistically best evaluated variables, including temperature annual range (maximum temperature of warmest month–minimum temperature of coldest month), mean temperature of coldest quarter and precipitation of driest month; 'set 2', containing the biologically and statistically most influential variables, i.e. annual mean temperature, annual precipitation, mean temperature of warmest quarter and precipitation of driest quarter; and 'set 3', containing the biologically most influential variables, such as mean temperature of warmest quarter, annual precipitation and precipitation of driest month. Projection into the past was performed using palaeoclimatic data from the LGM and MH downloaded from WorldClim (Hijmans et al., 2005), representing two general circular models (GCMs): the community climate system model (CCSM4; Gent et al., 2011) and the MIROC-ESM (Watanabe et al., 2011).

Species distribution model

Ecological niche models were generated using the maximum entropy algorithm (MaxEnt v.3.4.1; Phillips et al., 2006) and calibrated in areas included in a buffer of 50 km from each unique distributional record (Barve et al., 2011). Ecological niche modelling was used to identify areas where environmental conditions are analogous to those

within the known distribution of *S. rubescens* across Europe, Africa and Asia, aiming to identify potentially suitable areas outside its known range. Simultaneously, we aimed to explore the environmental suitability of the newly recorded distribution area in Thailand, because this is the most distant geographically from the main distributional range of *S. rubescens* and is in a distinct ecoregion (tropical and subtropical dry broadleaf forest) compared with the known range of the species. Furthermore, we reconstructed the palaeoclimatic distribution of the species to provide insights into its biogeographical history and potential past migratory pathways. To define an adequate level of complexity, different configurations of MaxEnt were evaluated by comparing the performance of 84 candidate models, resulting from parameter combinations of four regularization multipliers (0.5, 1.0, 1.5 and 2.0), seven feature classes representing combinations of linear (L), quadratic (Q) and product (P) and three sets of environmental variables. The best-fitting models were selected based on statistical significance (partial Receiver Operating Characteristic (ROC) < P-value; Peterson et al., 2008), omission rates lower than a previously defined error rate (E = 5%; Anderson et al., 2003), and the lowest value of the Akaike information criterion corrected for small sample size (AICc; Warren & Seifert, 2011), in that order. The chosen predictors were used to create final models with ten replicates by bootstrap, logistic outputs and were then applied to Europe, Africa and Asia, under current and past environmental scenarios. Model projections were created, allowing extrapolation and clamping in MaxEnt. Binary maps were derived from continuous median models from MaxEnt by applying the ten-percentile training presence value as a threshold for visualization and comparison of the extent of areas estimated as the potential distribution of *S. rubescens*. Occurrence data were randomly split into three sets: joint (100% of the data), test (25% of the data) and training (75% of the data) sets. All modelling processes were performed using the '*kuenm*' package (Cobos et al., 2019) in R v.3.6.2 (R Core Team, 2019); Quantum GIS v.3.4.2 (QGIS Development Team, 2020) was used to generate and visualize maps. The modelling protocol followed that of Simões et al. (2020). We measured model uncertainty to estimate the risk of strict extrapolation resulting from the projection to non-analogous conditions and the degree of variability resulting in the final model projections. Model variation was assessed using a variance partitioning approach, in which the variance

across the mean of all models representing each level in each source of variation is calculated, representing the amount of variance in model predictions originating in replicates and general circular models. To assess the risk of strict extrapolation, we used the mobility-oriented parity metric (MOP; Owens et al., 2013), which measures similarity between the closest 10% of the environmental conditions of the calibration area to each environmental condition in the area of transference. Areas with higher extrapolative values indicate higher uncertainty; caution is required when interpreting the likelihood of presence of the species in such areas (Alkishe et al., 2017). To reduce the risk of extrapolation on final models caused by the wide projection area, we created binary MOPs (i.e. E = 10%, where zero represents strict extrapolation and one represents non-extrapolative areas) and masked areas of extrapolation from binary final models, creating final binary models with no extrapolation (post-MOP projections; Supporting Information, Fig. S3). To test whether the two potential record locations in Thailand represent outliers, we extracted values of all known occurrence points of *S. rubescens* from its predicted environmental suitability, using the median of all the bootstrapped replicates, because the average of replicates is statistically more sensitive to extreme values. We also used a $\chi 2$ test for detection of the outliers in a vector with values from all occurrence points. For this, we used the chisq.out.test function available in the R package '*outliers'* (Komsta & Komsta, 2015). The null hypothesis for this test was that the records from Thailand (Chiang Rai or Tham Pha Thai) were part of the real distribution of *S. rubescens* (P-value > 0.05).

RESULTS

Distribution

A total of 398 unique georeferenced records were collected. Some distributional gaps were filled with new data, and the distribution range was extended by new records from Ethiopia, Iraq, Sudan, Yemen (Socotra), Uzbekistan and Thailand (see Supporting Information, Table S3b). The largest number of records was from northern Africa (N = 166) and southern to central Asia (N = 139), followed by Europe (N = 93), mostly

concentrated on the Iberian Peninsula and the Mediterranean coast of France (Fig. 1; Supporting Information, Fig. S1).

Ecological Niche Modelling

Model statistics and parameter setting

The configurations selected to produce the final model included environmental 'set 3', regularization multiplier 0.5 and a combination of linear, quadratic and product as feature classes (Supporting Information, Table S4). Partial ROC and area under the curve (AUC) values showed that the final model was significantly better than random expectations ($P < 0.000$; mean AUC = 1.062; omission rate at 5% = 0.029).

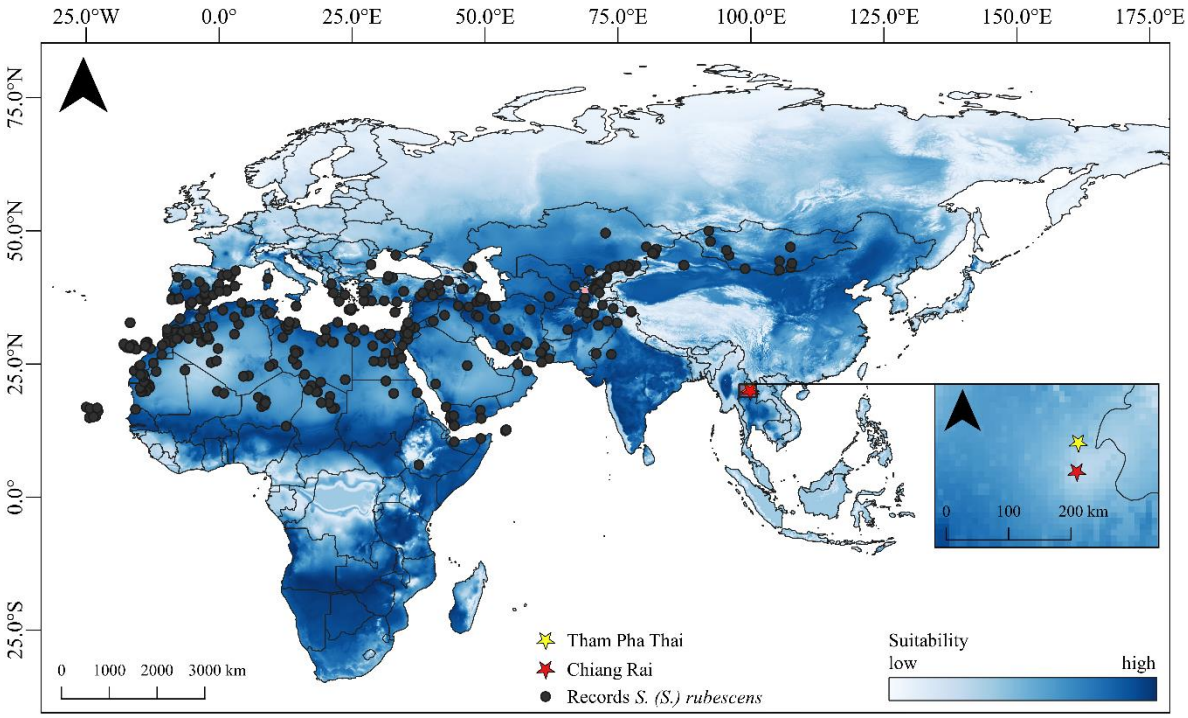

Figure 2. Map showing the current climatic suitability for *S. (S.) rubescens* in Europe, Africa and Asia. Dark blue indicates areas of high climatic suitability, whereas light blue represents areas with low climatic suitability. The distribution records used to generate the distribution model are shown as black points. Potential outliers in Thailand are represented as stars (red, Chiang Rai; yellow, Tham Pha Thai).

Model projections

High suitability was recovered within the already known distribution range of *S.rubescens* (Fig. 2), and post-MOP maps reflected the known distribution of the species

well, by excluding extrapolative areas (Fig. 3; Supporting Information, Fig. S3). Palaeoclimatic models showed congruence of suitable areas between GCMs during the LGM for the following: most of northern Asia (Russia); East Asia, restricted to north and west China and Mongolia; Central Asia, except for larger parts of Tajikistan, Kyrgyzstan and northern parts of Pakistan; small parts of north-east Afghanistan and Pakistan; and Southwest Asia. In northern Africa, suitable areas did not extend south of 10°N, except for the coasts of Eritrea, the north-east of Ethiopia and northern Somalia. In southern Africa, the suitable areas were restricted to the southern coast of Angola, south and west of Namibia, south-west Botswana and western South Africa. Areas of suitability recovered for the MH resembled patterns recovered for the LGM, but suitability was distinctly reduced in northern Asia (restricted to north-east Russia), slightly reduced in central and western Asia, and in northern Africa not extending south of 15°N. Furthermore, areas on the coasts of Eritrea, in north-east Ethiopia, Somaliland and northern Somalia had reduced suitability (Fig. 3; Supporting Information, Fig. S3).

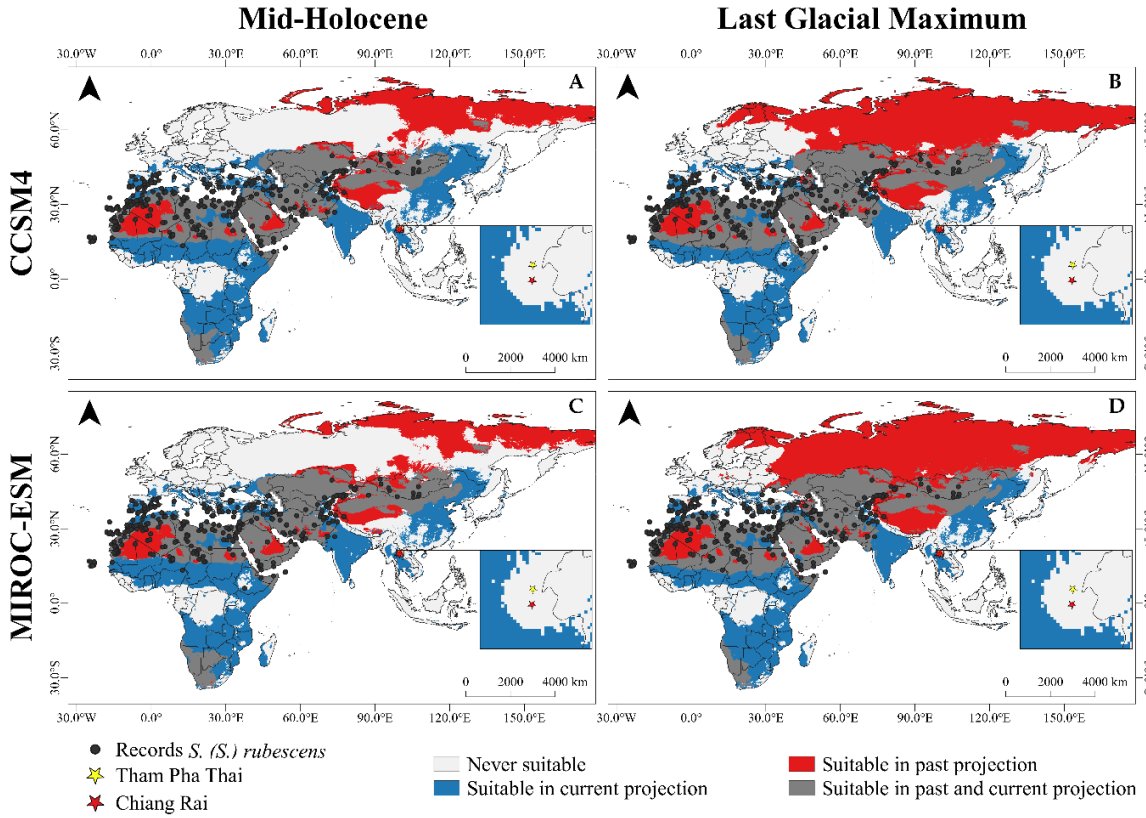

Figure 3. Binary maps displaying suitable areas in the present, mid-Holocene and Last Glacial Maximum using two distinct global climate models (GCMs), CCSM4 and MIROC-ESM. A, Mid-Holocene, GCM: CCSM4. B, Last Glacial Maximum, GCM: CCSM4. C, Mid-Holocene, GCM: MIROC-ESM. D, Last Glacial Maximum, GCM: MIROC-ESM. Regions that are never suitable are

coloured white. All currently suitable areas are shown in blue. All suitable areas in the respective palaeoclimatic past projection are shown in red. Regions recovered as suitable within all time frames are shown in grey. Records of *S. (S.) rubescens* extracted from literature, collections and field trips are shown as dark grey dots. Dubious locations in Thailand are represented by a red star for Chiang Rai and a yellow star for Tham Pha Thai.

Model exploration and variability

Areas of extrapolation of the current models were detected in high-elevation areas of Europe (i.e. Pyrenees and Alps in the north of Italy), Central Africa and Southeast Asia for the LGM (Supporting Information, Fig. S4). Areas of strict extrapolation included Europe, northern Morocco and Algeria, the south of western Africa, Central and East Africa and Southeast Asia. For the MH (Supporting Information, Fig. S4), the results were similar to LGM MOP results, except for a wider area of strict extrapolation throughout Eurasia. The variability of models coming from replicates was higher than the variability contributed by GCM scenarios (Supporting Information, Fig. S5). Higher values of variability were found in Central and southern Africa, Central and eastern Asia and Europe [LGM: Europe, Central Asia (i.e. Tajikistan, northern Afghanistan and northern India) and west China; MH: Congolese Basin north to Somalia and Ethiopia, Morocco, Europe, Central Asia (i.e. Tajikistan, northern Afghanistan and northern India), southern and western Asia and most parts of Russia; Supporting Information, Fig. S5].

Spatial error analysis

Suitability values were 0.335 for Chiang Rai and 0.366 for Tham Pha Thai. According to the χ 2 test for outliers, the location in Chiang Rai did not belong to the distribution of suitability values recovered for occurrence records describing the known distribution *of S. rubescens* (P-value: $0.00 \leq 0.05$; Fig. 4). Moreover, although both location records were slightly inside the distribution of suitability values, they were also recovered outside the suitable areas in binary map results, based on a 10% threshold (P-value: $0.00 \leq 0.05$; Fig. 4).

DISCUSSION

Sphingonotus (S.) rubescens is one of the most widely distributed Oedipodinae grasshoppers (Cigliano et al., 2020). In this study, we assembled a dataset of 398 locations sampled from museum collections, GBIF and the literature to investigate its current distribution range. Species distribution modelling based on these occurrences was used to project the known range to the whole of Eurasia and Africa, to highlight potential suitable areas (based on abiotic factors; www.worldclim.org; Hijmans et al., 2005), locate potential sampling gaps and investigate the plausibility of a dubious record from Thailand. Moreover, we used these techniques to shed light on the climatically suitable areas in the past, by projecting these data to the MH and LGM.

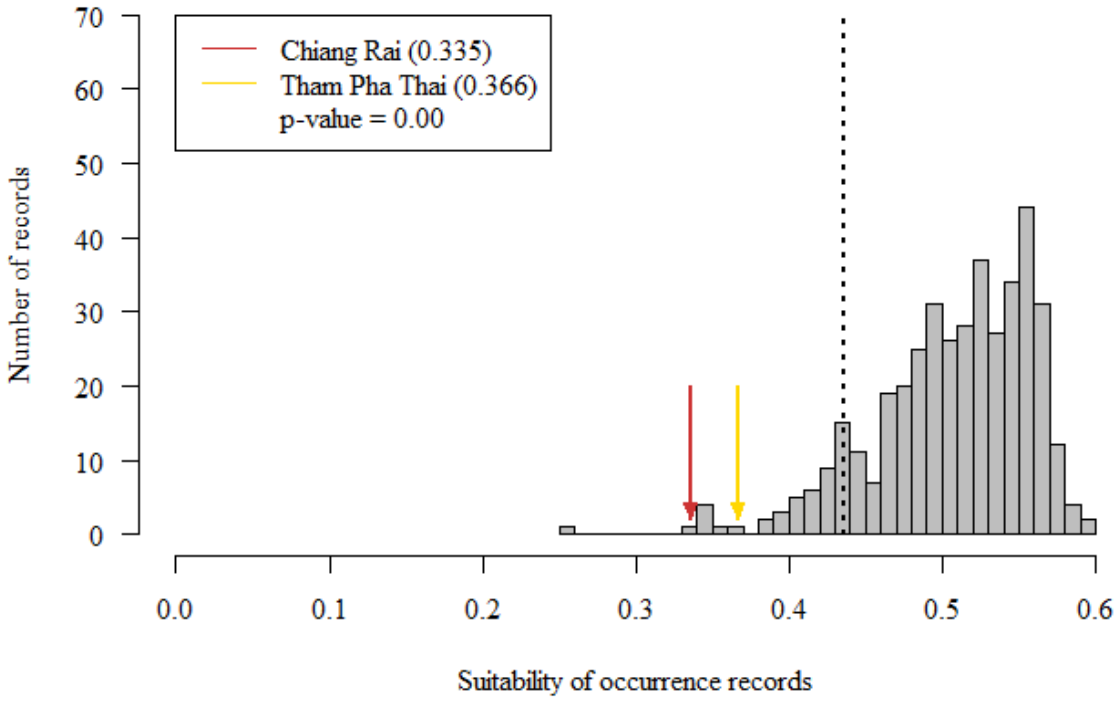

Figure 4. Histogram showing the suitability of occurrence records compared with the number of records. Grey bars show the number of records of various degrees of suitability. Tested records from Thailand are shown as arrows in the plot. The yellow arrow represents the potential locality Tham Pha Thai. The red arrow represents the potential locality in Chiang Rai. The dashed line indicates the 10% threshold. P-values for both tested localities using $\chi 2$ tests were < 0.05.

Current distribution and sampling gaps

According to the models, the potential distribution of *S. rubescens* stretches from the Canary Islands across the Mediterranean, parts of the Sahel, the Middle East and into Central and eastern Asia. The northernmost occurrences recorded during our study were found in Kazakhstan and Mongolia, where the species occurred only at low density (L.-S.D., field observations); the southern edge of the range was Ethiopia. The modelled suitable distribution area corresponds closely to our records (Fig. 3; Supporting Information, Fig. S3), with some extensions at the edges and some larger suitable areas without any records in China, Kazakhstan, India and southern Africa. The potentially suitable areas represent deserts and semi-deserts, in addition to steppe habitats with an arid climate (i.e. high temperature and low precipitation levels) with small variation, corroborating previous studies on the ecological preferences of the species (Fishpool & Popov, 1984; Launors-Luonc & Lecoq, 1988; Guendouz-Benrima et al., 2011). In its range of habitat types (Supporting Information, Fig. S1), the species mostly prefers semi-desert, desert and steppe habitats (Fig. 4), reflected in its wide distribution across northern Africa and Central Asia, which leads to the suggestion that it might benefit from current rising global temperatures and desertification, with potential further range expansions in the future (Schlesinger et al., 1990; Gonzalez, 2001). No records exist from climatically suitable areas in eastern and southern China and southern Africa. The reasons for this are likely to differ; in eastern China, the species might occur, but has not yet been documented. Given that the Chinese fauna is rich in *Sphingonotus* species, *S. rubescens* might have been misidentified or overlooked here. In southern Africa, the climatic conditions might be suitable, but biotic conditions differ strongly. Moreover, the forest–savanna mosaic in Central and eastern Africa with unsuitable environmental conditions might have acted as a geographical barrier, dividing northern and southern regions of Africa (Adams, 1998; Congo Basin Forest Partnership, 2005; Burgess et al., 2007; Fjeldså et al., 2010; Van de Perre et al., 2019).

Species distribution modelling approaches for outlier detection

Besides the gaps in the distribution, one specimen in particular raised our interest. We found one specimen deposited at the NHMW, collected in 1978 in northern Thailand,

with an imprecise geographical location in Chiang Rai or the Tham Pha Thai Cave (Supporting Information, Fig. S2). Morphologically, the specimen can be identified clearly as *S. rubescens* (Supporting Information, Fig. S2), but the record is suspicious because it is the most south-eastern locality, 2500 km distant from the closest known species occurrence in India (Supporting Information, Fig. S1). Furthermore, both locations are in areas with a tropical humid climate (Khedari et al., 2002), whereas *S. rubescens* is known to occur in subtropical arid climates (Fishpool & Popov, 1984; Launors-Luonc & Lecoq, 1988). We used SDMs to test the status of this record as a climatic outlier. Our large occurrence dataset assembled to define the potential suitable habitats of *S. rubescens* (N = 398) allowed for optimal climatic profiling (van Proosdij et al., 2016; Hallman & Robinson, 2020; Warren et al., 2020); hence, higher sensitivity of MaxEnt to differentiate between the occurrence of the species and the outlier occurrence points (P-value: 0.00 ≤ 0.05; Fig. 4; Boria et al., 2014; Varela et al., 2014; Simões & Peterson, 2018). Thus, our results corroborate previous studies that have shown the utility of MaxEnt for detection of outliers and testing the quality of biodiversity data (Graham et al., 2008; Hinojosa-Díaz et al., 2009; Lash et al., 2012; Simões & Peterson, 2018). Regarding the specimen found in Thailand, the simplest explanation is mislabelling of the specimen. Less probably, it might have reached the location by anthropogenic or natural dispersal (Rasnitsyn & Quicke, 2002). Altogether, the putative occurrence in Thailand is considered here as an unsuitable location, falling beneath the suitability threshold of 10%, outside the distribution of suitability values recovered for the known occurrences (P -value: 0.00 ≤ 0.05). Hence, we believe that the species is unlikely to have viable populations in this region.

Historical distribution and biogeographical history

We also projected the current climatic preferences to the MH (6000 years ago) and the LGM (22 000 years ago) to understand whether past climatic suitability might have contributed to the wide range of *S. rubescens*. During these periods, strong short-term climatic variations have influenced species ranges, with short cooling periods (Heinrich events) alternating with short warming periods (Dansgaard–Oeschger events) constraining or favouring speciation (Ruddiman, 2001) and range expansion. Many

species expanded their ranges northwards during warmer periods (Sommer et al., 2014) and, according to our results, this is likely to have included the ancestors of *S. rubescens* (Fig. 3; Supporting Information, Fig. S3). Uniform genetic composition across the species range supports the hypothesis of a fast dispersal of the species (M.H., A.H. and L.-S.D., unpublished data; Husemann et al., 2014). To date, no molecular phylogeny has clearly illuminated the time frame of the origin of the species; however, several studies based on *Sphingonotus* and its congeners suggest an origin in the Miocene or Pliocene, with diversification events in the Pleistocene (Fries et al., 2007; Hochkirch & Husemann, 2008; Husemann et al., 2012, 2014; Dey et al., in press). Our analyses suggest that extensive regions at northern latitudes (ranging from Sweden to Russia) might have been suitable for *S. rubescens* on both global climate models (i.e. CCSM4 and MIROC-ESM). During the LGM, most parts of eastern Asia (including Siberia and Manchuria) were not covered with ice, owing to the anticyclones produced by the European ice shield, which generated dry air masses, thus inhibiting precipitation and preventing the formation of glaciers (National Oceanic and Atmospheric Administration, 2012, Blue Marble: Sea level, ice and vegetation changes - 19,000BC - 10,000AD dataset. Science On a Sphere). Hence, most parts of central and northern Siberia and Russia were characterized by steppe and tundra habitats and might potentially have been suitable for *S. rubescens* (Ray & Adams, 2001; Pitul'ko et al., 2007; Kuzmin & Keates, 2018). Areas in central and western Russia lost suitability during the MH, although temperatures were increasing in the long term (while cold and warm periods alternated), because regional rainfall also increased (Chen et al., 2008), which promoted forestation of steppe areas during this period (Väliranta et al., 2006). Although Central Asia showed suitability for *S. rubescens* through the LGM and the MH, climatic fluctuations in this area might have influenced its intraspecific diversity (Tarasov et al., 2000; Yang et al., 2009; Leroy et al., 2013) owing to isolation of populations in refugia during cooling events (Michaux et al., 2004; Zhang et al., 2008; Cordova et al., 2013) and expansion during warmer periods (Hewitt, 2011; Schmitt & Varga, 2012). This is currently reflected in the diversity of *S. rubescens* subspecies, which are distributed mostly in south-west and Central Asia. Similar events might have promoted diversification in the Mediterranean sister species *S. (S.) caerulans* (Husemann et al., 2014). Further studies based on molecular and fossil data are needed

to reconstruct the specific time frame of the origin of *Sphingonotus* as a whole and of the *S. caerulans* group in particular. In this context, our models will help to evaluate potentially suitable areas where diversification pathways can be traced and enable the detection of the area of origin of the genus.

CONCLUSION

Our study explored the distribution of the widespread Oedipodinae grasshopper *S. rubescens.* We report new distribution records from four countries, highlighting the importance of literature-and museum-based revisions to improve our knowledge on the distributions of species. Among the new records, one specimen from Thailand was found, which was determined morphologically to be *S. rubescens*, but SDM results identified this as an outlier. This supports previous studies indicating the utility of climate-matching approaches for the detection of spatial errors and thus, assessment of biodiversity data. Whether the record is a result of mislabelling or anthropogenic transport can be resolved only by revisiting the locality. Exploration of suitable areas during LGM, MH and current environmental scenarios indicate the continuous existence of suitable areas through time and space, which might have aided the current wide distribution of *S. rubescens*. Altogether, our results show the relevance of climatic variables for shaping distribution patterns and the application of SDM for the detection of sampling gaps and distributional outliers.

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SUPPORTING INFORMATION

Figure S1. Map displaying: A, biome types; and B, all records allocated to countries, within the total current range of *S. (S.) rubescens*. Records are shown as black dots. Tested dubious location records in Thailand are shown as a red star for Chiang Rai and as a yellow star for Tham Pha Thai.

Figure S2. Images of *S. (S.) rubescens* from Thailand deposited in the Natural History Museum, Vienna, Austria (NHMV).

Figure S3. Potentially suitable areas in the past and present. Panels show results from current and palaeoclimatic models. A, mid-Holocene. Global climate model (GCM), CCSM4 projection. B, Last Glacial Maximum. GCM, CCSM4 projection. C, mid-Holocene. GCM, MIROC-ESM projection. D, Last Glacial Maximum. GCM, MIROC-ESM projection. Light grey areas are areas that were never suitable. Blue indicates areas suitable in the current projection. Red represents areas suitable in the respective palaeoclimatic past projection. Grey areas are areas suitable in the respective palaeoclimatic past and current projection.

Figure S4. Map showing the extrapolated area through time for: A, mid-Holocene; and B, Last Glacial Maximum. Blue areas represent strict extrapolation; dark grey shows extrapolative regions using MIROC-ESM; and light grey using CCSM4. Non-extrapolative areas are shown in white.

Figure S5. Calculated model variation for the mid-Holocene (MH; 6000 years ago) and the Last Glacial Maximum (LGM; 22 000 years ago), comprising the variation between the models CCSM4 and MIROC-ESM and the ten replicates for both time periods, separately. The maps show the variation: A, between the GCMS (CCSM4 and MIROC-ESM) for the MH; B, between the GCMS (CCSM4 and MIROC-ESM) for the LGM; and

C, between the replicates. The colour scheme shows variation between the models and replicates. Green indicates low variation between the projections, whereas violet indicates high variation. There is variation for the LGM between models (MIROC-ESM and CCSM4).

Table S1. Literature checked for species occurrences.

Table S2. Species records from literature and http://acrinwafrica.mnhn.fr.

Table S3. Full list of examined museum material and material from private collections, sorted by country. Collection abbreviations are as follows: GE, Natural History Museum, Geneva, Switzerland; MAS, Mongolian Academy of Science, Ulaan Bator, Mongolia; MNHS, Muséum National d'Histoire Naturelle, Paris, France; NM, National Museum, Prague, Czech Republic; NMW, Natural History Museum, Vienna, Austria; PC AH, private collection of A. Hochkirch; PC LSD, private collection of L.-S. Dey; PC MH, private collection of M. Husemann; PC RF, private collection of R. Felix; SZMN, Siberian Zoological Museum, Novosibirsk, Russia; ZMH, Zoological Collection of the Center for Natural History, Hamburg, Germany; and ZSM, Zoological State Collection, Munich, Germany.

Table S4. Performance of 63 models created during the evaluation process for S. (S.) rubescens. The table displays the set of selected variables [set 1: temperature annual range (maximum temperature of warmest month–minimum temperature of coldest month), mean temperature of coldest quarter and precipitation of driest month; set 2: annual mean temperature, annual precipitation, mean temperature of warmest quarter and precipitation of driest quarter; and set 3: mean temperature of warmest quarter, annual precipitation and precipitation of driest month]; the seven feature classes representing combinations of linear (L), quadratic (Q) and product (P); the four investigated regularization multipliers (0.5; 1.0; 1.5; 2.0); partial ROC (partial Receiver Operating Characteristic; P-values); omission rate at 5%; Akaike information criterion corrected (AICc); delta AIC [ΔAIC; score to measure the difference between the best model (smallest AIC) and each model]; Akaike information criterion weights (W AIC); and total number of parameters per setting. Selected parameters used in this study are shown in cells shaded grey.

All supporting information can be found on page 305 and following.

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LSD, MS designed the study; LSD collected data; LSD performed analyses; LSD wrote the original manuscript; LSD, MH, AH, MS took part in the writing process of the manuscript; LSD coordinated the writing process.

Hamburg, 12.01.2023

Place/Date

Sign

Chapter 4

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"Broad distribution does not mean broad differentiation."

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Analysis of geographic centrality and genetic diversity in the declining grasshopper species *Bryodemella tuberculata* (Orthoptera: Oedipodinae)

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ABSTRACT

Human-induced ecological and climatic changes have led to the decline and even local extinction of many formerly widely distributed temperate and cold-adapted species. Deter-mining the exact causes of this decline remains difficult. *Bryodemella tuberculata* was a widely distributed orthopteran species before the mid-19th century. Since then, many European populations have suffered drastic declines and are now considered extinct or critically endangered. We used ecological niche modelling based on a large dataset of extant and extinct occurrence data to investigate whether poor climatic suitability in the periphery of its global range was a possible cause of the local extinction of the European populations of *B. tuberculata*. We also used population genetics based on the COI marker to estimate and compare the genetic diversity of extant populations. We found that Europe still provides highly suitable habitats close to the climatic optimum, contradicting the assumption of climate change as major driver of this decline. Instead, changes in land-cover and other anthropogenic modifications of the habitats at the local scale seem to be the major reasons for local extinctions. Genetic analysis suggests Central Asia as center of diversity with a stable population size, whereas the effective sizes of the remaining European populations are decreasing. We found European genetic lineages nested within Central Asian lineages, suggesting a Central Asian source distribution area. Our results suggest that the declining European populations represent relics of a formerly wider distribution, which was fragmented by changes in land-use. These relics are now threatened by limited connectivity and small effective population sizes. Specific conservation actions, such as the restoration of former or potential new habitats, and translocation of individuals from extant populations to these restored sites may help slow, stall, or even revert the extinction process

Keywords Ecological modelling, Speckled buzzing grasshopper, Center-periphery hypothesis, Population decline, Insect decline

INTRODUCTION

Biodiversity decline, specifically insect decline, has been recognized as a major threat to global ecosystems (Dirzo et al. 2014; Conrad et al. 2006; Hallmann et al. 2017). Insects are key elements of most terrestrial trophic interactions, provide essential ecosystem services of global relevance, and generate significant economic and aesthetic benefits with cultural value to human society (Wagner 2019). At the same time, insect species and populations are threatened by a variety of factors, the most crucial being habitat loss, fragmentation and deterioration, followed by climate change (Franco et al. 2006; Potts et al. 2010; Vanbergen and the Insect Pollinators Initiative 2013). Species are affected differently by environmental change depending on their ecological tolerance and other species-specific life history traits, determining the likelihood of decline and local extinction (Cahill et al. 2013). In most cases, the extinction of local populations depends on the combination of the geographic location of the population within the distribution range, the ecological tolerance of the species and shifts in habitat conditions (Law-ton 1994; Cahill et al. 2013). Hutchinson's concept of the ecological niche (Hutchinson 1957) divides the multidimensional parameter space of habitat variation roughly into two regions: (1) the region in which the number of local births exceeds that of local deaths (source populations), close to the optimum niche of the species, and a source of emigration; and (2) the region in which the number of deaths exceeds that of local births (sink populations), typically far from the environmental optimum of the species, where populations are maintained by immigration from source populations (Holt 1996). Consequently, the proximity to the optimal environmental space (centroid) is expected to correspond to higher genetic diversity, growth rates and population stability (Pulliam 1988; Vanderwal et al. 2009). Known as center-periphery hypothesis, this concept has provided a baseline for many studies of population dynamics and genetic variability at species distribution limits. We know of only few studies that have explored the center-periphery hypothesis regarding genetic variability in relation to environmental suitability or niche centrality—with contrasting results. Diniz-Filho et al. (2009), for example, related genetic diversity to average ecological niche model (ENM) suitability

scores derived from multiple correlative niche modeling algorithms. Lira-Noriega and Manthey (2014) focused on testing linear regressions of genetic diversity measures (e.g., allelic richness, nucleotide diversity) in comparison with Euclidean distances between the environmental centroid of the suitable area estimated by ENM, and assumed to be the fundamental niche of the species. The correlations were tested on 40 species, including plants, birds, mammals, worms and insects. No clear relationship was recovered in any study, with the majority of cases showing negative correlations and a few cases displaying positive correlations (Lira-Noriega and Man-they 2014). The mixed results might have been a product of the lack of a direct assessment of the species environmental centroid. Hence, a possible alternative to the approaches previously used is to characterize the ecological niche from a Grinnellian perspective—a set of environmental conditions that allow a species to maintain populations for long periods of time without immigration events (Peterson et al. 2011). This concept has been successfully applied to study the relationship between geographical distances of populations to the niche centroid and their abundances (Yañez-Arenas et al. 2012; Urena-Aranda et al. 2015). However, no study has explored thus far the relationship of Grinnellian niche centroids with the geographic centroid and/or genetic diversity to explain patterns of population decline.

Due to well documented population declines within a huge part of its natural range, the speckled buzzing grasshopper *Bryodemella tuberculata* is well suited to investigate this relationship. This species occupies a wide global range across the northern Palearctic from Central Europe to eastern Asia (Bagachanova et al. 2011; Budrys et al. 2008; Budrys and Pakalniškis 2007; Fartmann et al. 2008; Reich 1991; Sergeev 1992; Srinivasan and Prabakar 2013). Up to the mid-20th century, *B. tuberculata* was still common in the European parts of its range, as documented by many specimens deposited in museum collections. It was frequently recorded from heath areas and river banks (Zacher 1919; Krauss 1883; Graber 1872). Over the course of the 20th century, many populations became extinct because their habitats (heath areas and unregulated river banks) were destroyed due to human demographic expansion, specifically a change in land-use due to intensified agriculture and shipping industry, and infrastructural developments (e.g. regulation of rivers; Laussmann et al. 2010; Bischoff 1997; Breunig and Thielmann 1992;

Quinger 2014). Today, *B. tuberculata* has become a flagship species for the conservation of its threatened habitat types, mostly due to its large size for a grasshopper, its bright reddish hindwings and its characteristic buzzing flight noise. The most recent evaluation by the IUCN in 2016 (Zuna-Kratky et al. 2016) indicated that the populations in Denmark, Latvia, Poland and Switzerland are extinct (Berg et al. 2005; Budrys et al. 2008; Budrys and Pakalniškis 2007; Liana 2004; Maas et al. 2002; Monnerat et al. 2007), and the same probably applies to those in Italy (Massa et al. 2012). Furthermore, the relic populations of most other European countries have strongly declined despite frequent monitoring and legal protection (Binot-Hafke et al. 2011; Maas et al. 2011). In Central Europe, *B. tuberculata* is now restricted to small areas in Germany and Austria along the rivers Isar and Lech. In Northern Europe it is restricted to the island Øland in Sweden (Fig. 1; Bieringer and Weißmair 2017; Pfeuffer 2004; Voith et al. 2016). At the same time, the species remains common in North and Central Asia, occupying almost all suitable areas in the region (pers. observed by the authors).

This recent and steep decline of *B. tuberculata* populations offers an optimal setting to test the center-periphery hypothesis, and in connection with analysis of the land-use change potentially providing a framework to understand the factors leading to its population decline. Hence, we calculated ellipsoid envelope models based on a large dataset of present and past occurrences of the species to characterize the climatic niche of *B. tuberculata* and test the center-periphery hypothesis by comparing relationships between genetic diversity, climatic niche, and geographic centrality between extinct and extant populations. We further evaluated landscape change in the European area of the distribution and evaluate several extinct and extant locations for their habitat structure and suitability.

We hypothesize that (1) changing climatic conditions over the last 100 years are not the reason for the decline of the species, but rather (2) changes in land-use and land-cover are responsible for the decline and local extinction. Assuming that genetic diversity and proximity to the niche centroid are related to fitness and survivability (Reed and Frankham 2003; Leimu et al. 2006), we specifically seek to understand whether the relationship between those factors could provide any insight into the susceptibility of

populations (and possibly species) to decline and extinction. Based on this, we hypothesize that (3) the genetic diversity in the Asian core populations is higher than in the European relict populations.

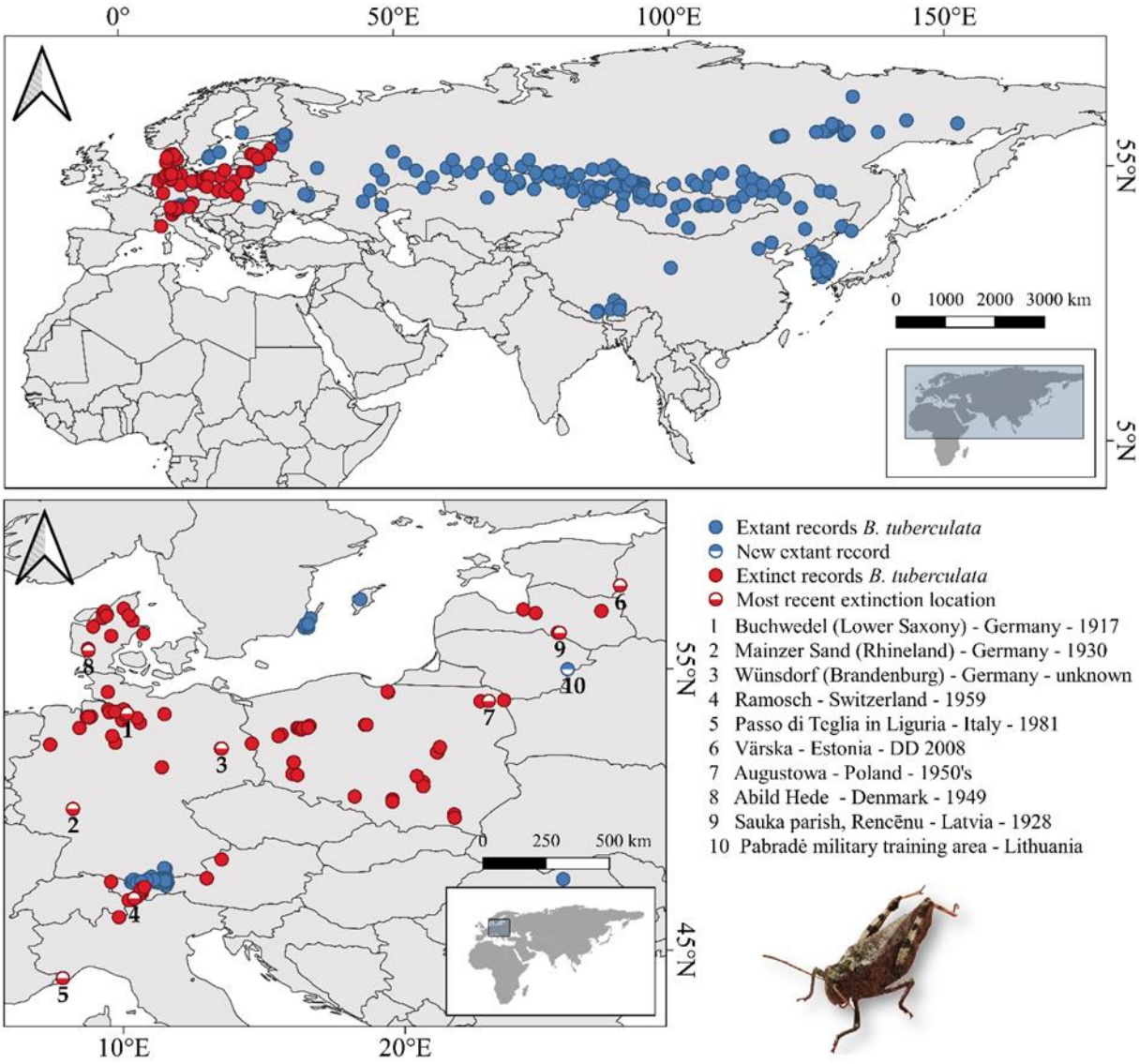

Figure 1: Distribution map based on all extinct and extant distribution records of *Bryodemella tuberculata* collected for this study from literature, museum vouchers, and private collections. Numbers 1 to 9 show the localities of the most recent records for countries (and regions of Germany) which *B. tuberculata* is now considered extinct. (Photograph of *B. tuberculata*, taken by Inci Livia Baez, Sylvenstein-Staumauer (Germany), August 2019).

MATERIAL AND METHODS

Distribution data

We assembled a set of 651 records of *B. tuberculata* obtained from fieldtrips, literature, and museum collections, complemented by data from the Global Biodiversity Information Facility (GBIF, www. gbif. org) and Naturbasen (https:// www.naturbasen.dk/art?id=7888): based on fieldtrips, a total of 93 records were collected between 1873 and 2019 from Mongolia, Russia, Kazakhstan, Germany and China (see SI 1 for further information on collected material). Further 231 records were extracted from the literature (see SI 2 for consulted references) and 114 records were obtained from public databases (111 records from GBIF Global Biodiversity Information Center; GBIF.org (14 January 2020), GBIF Occurrence Download https://doi.org/10.15468/dl.hhsegk (extant dataset); GBIF.org (14 January 2020), GBIF Occurrence Download https://doi.org/1015468/dl.pfaaea (extinct dataset); 13 records from Naturbasen (https://www.naturbasen.dk/art?id=7888)). Additionally, 213 records were obtained from museum specimens (see Supplementary Information SI1 for further information). We identified these museum specimens based on the key of Bey-Bienko (1930). Records with missing geographic coordinates were georeferenced using Google Earth (https://www.google.com/earth/) and checked for correctness following the data cleaning protocol by Cobos et al. (2018). Due to the low dispersal capacity of *B. tuberculata* (Reinhardt et al. 2005) every single record was considered as a population. If several specimens from the same location were recorded, duplicates were removed. We considered any European population as extinct if it was listed as extinct in the relevant literature (Budrys and Pakalniškis 2007; Voith et al. 2016; Ruffo 2003; De Carlini 1889; Schmidt and Lilge 1997; Baur and Museum 2006; Bakker et al. 2015; Zuna-Kratky et al. 2017; Glowacinski and Nowacki 2006) or if no findings were documented after 1999 at the sampling location or within a 10 km buffer zone around the occurrence. All other populations from Europe and all known populations from Asia were considered extant. After data cleaning and trimming, 280 extant and 100 extinct locations were used to

calculate the environmental distance to the centroid. All records used for modelling are supplied in Supplementary Information SI 3 and visualized in Fig. 1.

Environmental data

Environmental layers were obtained from Worldclim v. 1.4 (www.worldclim.org; Hijmans et al. 2005) in 2.5 arc-min resolution. WorldClim is based on interpolations of weather station data (i.e., monthly precipitation and minimum and maximum temperatures) over the period 1960–1990. From the 19 variables available, we excluded four (mean temperature of wettest quarter, mean temperature of driest quarter, precipitation of warmest quarter, precipitation of coldest quarter) a priori due to known spatial artifacts between adjacent grid cells (Escobar et al. 2014; Campbell et al. 2015). Seeking to avoid bias regarding the combination of variables used to characterize the species niche centrality, we tested three distinct environmental sets: 'set 1' included all 15 variables; 'set 2' included only temperature variables (i.e., annual mean temperature; mean diurnal range; isothermality; temperature seasonality; max. temperature of warmest month; min. temperature of coldest month; temperature annual range; mean temperature of warmest quarter and mean temperature of coldest quarter); and 'set 3' included only precipitation variables (i.e., annual precipitation; precipitation of wettest month; precipitation of driest month; precipitation seasonality; precipitation of wettest quarter; precipitation of driest quarter). To avoid overfitting, overly dimensional environmental space and collinearity among variables, we performed principal component analysis (PCA) using the function kuenm_rpca in the package 'kuenm' (Cobos et al. 2019b) in R 3.6.3 (R Core team 2020). For each set, we retained as many components as necessary to explain > 95% of the total variance in the dataset for model calibration. As a result, set 1 was reduced to five principal components (PCs) summarizing the 15 bioclimatic variables, while set 2 and 3 were reduced to three PCs, summarizing temperature and precipitation variables (see SI 6).

Ellipsoid models

To characterize the environmental niche of *B. tuberculata* we created an ellipsoid envelope model representing the niche shape assumed when multiple dimensions are considered (Jiménez et al. 2019) using the '*ellipsenm'* package (Cobos et al. 2019a). Based on these models, we calculated the niche centroid for all collected extant records of the species and extracted the Mahalanobis distances between grid cells representing local environments of study populations and environmental conditions of the optimum (ellipsoid centroid). Models were calibrated using the 95% pairwise confidence region for the ellipsoid and were evaluated as candidate models using the function '*ellipsoid_calibration*' of the '*ellipsenm'* R package (Cobos et al. 2019a). Two distinct methods were employed to construct ellipsoid models: (1) '*covmat'*, which creates ellipsoids based on the centroid and a matrix of co-variances of the variables and (2) '*mve1'*, which generates an ellipsoid that reduces the volume contained in it without losing the data contained (i.e., minimum volume ellipsoid; Van Aelst and Rousseeuw 2009). Best model selection was based on statistical significance (partial ROC; Peterson et al. 2008); the proportion of testing data known to be in suitable areas and prediction of unsuitable areas was based on omission rates (E = 5%; Anderson et al. 2003) and prevalence. To calculate the partial ROC metric, we used 500 bootstrap iterations with 50% of testing data to be used in each bootstrapped process with 5% of testing data error in the data due to uncertainty. The calibration area (i.e., region accessible to the species; Barve et al. 2011) included Eurasia, except Southeast Asia, where the species is not found.

Final parameters were selected based on best evaluated models and used to create the final models using 10 replicates with bootstrapped subsamples of 75% of the data using the function '*ellipsoid_model*' available in the '*ellipsenm'* R package (Cobos et al. 2019a). The replicates were produced by excluding one occurrence record at a time. The confidence level of a pairwise confidence region for the ellipsoid was 99%, with 1% of the occurrence data considered as potential environmental outliers, not included in the ellipsoid envelope model for the species' ecological niche.

Environmental and geographic distances

The environmental distance of each record (= population) to the ellipsoid centroid (= ecological optimum) was calculated using the Mahalanobis distance metric, which is considered well suited for non-spherically symmetric distributions and deals robustly with variables with different scales, such as the bioclimatic variables we used for characterizing niche space (De Maesschalck et al. 2000; Hijmans 2020). We generated histograms showing the Mahalanobis distance (D2) of all populations to the centroid.

To estimate the geographical distance of each population to the centroid, we created a minimum convex polygon based on all known records of *B. tuberculata* and then calculated the distances of the centroid to its edges. For that, we used the R packages '*adehabitatHR*' (Calenge 2006); '*geogrid*' (Bailey 2018); '*sp*' (Bivand et al. 2008; Pebesma and Bivand 2005); '*rgeos*' (Bivand and Rundel 2019); '*rgdal*' (Bivand et al. 2015); '*raster*' (Hijmans 2020) in R v. 3.6.3. (R Core Team 2020) under R Studio v. 1.1.463 (R Studio 2020). Histograms for both distance measures (i.e., environmental and geographic) were created with the R packages '*ggplot2*' (Wickham 2016); '*gridExtra*' (Auguie 2017); '*plyr*' (Wickham 2011); '*cowplot*' (Wilke 2019) and '*grid*' (R Core Team 2020). To test whether the distribution of Mahalanobis distances of extinct populations to the centroid are significantly distinct from the distances recovered for extant populations, we performed a non-parametric Mood's Median test, which tests if two or more independent samples originate from populations with the same median. We used this to compare the shape and scale of distributions for all groups, based on the hypothesis that the Mahalanobis distances of extant populations are lower compared to those of extinct populations (p-value > 0.05). Analyses were performed in R. Distribution maps and Mahalanobis plots were created in QGIS v. 3.10.3 (QGIS Development Team 2020).

Landcover change

Furthermore, we used the HILDA dataset v. 2 (HIstoric Land Dynamics Assessment 2—gross land-cover changes; Fuchs et al. 2013, 2014, 2015; http:// www. geo- infor matie. nl/ fuchs 003/#) to display landscape change through time from 1900 to 2000, the main

timeframe of local extinctions in Europe. Based on this, we calculated the percent land-cover change for forest, grassland, settlement and cropland area from 1900 to 2000 using the QGis plugin LecoS—Landscape Ecology Statistics v. 3.0.0 (Jung 2016).

Molecular analyses

For this study, we tested several genetic markers. Most gene fragments were highly conserved and did not show any variability; genomic data is currently not available to us and remains challenging because of the extremely large genome sizes in Orthoptera and specifically Oedipodinae (Husemann et al. 2021). In turn, DNA barcode data is known to provide meaningful estimates of genetic diversity, and additional genetic data of this marker was available from other studies in BOLD and GenBank. Hence, we decided to use a fragment of the Cytochrome Oxidase 1 for the genetic comparison of the European and Asian populations. We compiled COI sequences of 34 specimens of *B. tuberculata* from populations across its global range (Table 1). DNA was extracted from muscle tissue of a hind femur of each specimen with a high salt extraction protocol (Paxton et al. 1996). We amplified the barcoding fragment of the mitochondrial Cytochrome C Oxidase subunit I (COI) gene using the primers provided by Pan et al. (2006). For PCR reactions we used DreamTaq DNA polymerase (Thermo Fischer Scientific, Schwerte, Germany) following the manufac-turer's protocol. Cycling conditions are shown in SI 4. PCR products were visualized using 1 µl of EZ-Vision Two DNA Dye (Amresco, Darmstadt, Germany); samples were run on 1% agarose gels. We purified successfully amplified samples using the Thermo Fisher protocol for PCR product clean-up prior sequencing (Werle et al. 1994). The samples were sequenced at Macrogen Europe (Macrogen, Amsterdam, Netherlands) using the EZ-Seq service. Further, sequences from earlier projects of MH and OH (Husemann et al. 2012; Hawlitschek et al. 2017) were obtained from BOLD (see Table 1).

Analysis of genetic diversity

The MUSCLE algorithm (Edgar 2004) as implemented in Geneious v. 10.0.9 (http://www.geneious.com, Kearse et al. 2012) was used to edit, trim and align the sequences, resulting in three datasets for molecular analyses. The first dataset included all 34 sequences (total alignment length 609 bp). The second dataset only comprised samples from Europe (Germany N = 15; Sweden N = 1; Lithuania N = 1) with a total alignment length of 609 bp. A third dataset was generated comprising the samples from Central Asia (Russia N = 9; Mongolia N = 5; China N = 3; Korea N = 1) with a total alignment length of 609 bp. Geneious was used to check for internal stop codons (none were present). All sequences were blasted in NCBI GenBank and BOLD systems v. 3 (Ratnasingham and Hebert 2007) to exclude rough sequencing mistakes. ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQTree (Nguyen et al. 2015) was used to statistically select the best model of nucleotide substitution for the datasets using the Akaike information criterion (AIC) (Akaike 1973). HKY was selected as best model for all datasets. Pairwise genetic distance between the Asian (samples from Russia, Mongolia, China and Korea) and European (samples from Germany, Sweden and Lithuania) populations was calculated using Arlequin v. 3.5.2.2 (Excoffier et al. 2005). We used PopArt (Leigh and Bryant 2015) to construct a TCS haplotype network (Clement et al. 2000) of the full dataset without outgroup and with seven geographical traits (Germany, Sweden, Lithuania, Russia, Mongolia, China. Korea). To test for demographic stability we conducted Bayesian Skyline analyses using BEAST v. 2.5.1, based on the reduced datasets of Europe and Central Asia. HKY was used as substitution model. A relaxed log-normal clock using the substitution rate provided by Papadopoulou et al. (2010) was implemented as clock model to calculate a Coalescent Bayesian Skyline plot; the analysis was run for 100 million generations sampling every 1,000 generations. We ran BEAST v. 2.5.1 with automatic thread pool size and BEAGLE library (Ayres et al. 2012). Convergence was assessed with TRACER v. 1.7.1 to avoid insufficient sampling. TRACER was also used to calculate the Bayesian Skyline reconstruction (Bouckaert et al. 2014) discarding 10% of samples as burn-in.

Table 1: Specimen used for molecular analyses. Bold shaded rows indicate sequences obtained from previous studies (downloaded from BOLD); the remaining rows show individuals sequenced within this study. GenBank Accession numbers are provided in the column GB ID. Deposition: ZSM – Bavarian State Collection for Zoology; ZMH – Zoological Museum Hamburg.

Specimen ID	GB ID	Latitude	Longitude	Date	Deposition	Country	Location
1143	MW263312	-	-	05.09.2019	ZMH	Germany	Bavaria, Upper Isar River, mouth of Rißbach
1154	MW263326	47.53	11.43	12.09.2019	ZMH	Germany	Bavaria, Rißbach above Upper Isar
1155	MW263320	-	-	18.09.2019	ZMH	Germany	Bavaria, Upper Isar River below Sylvenstein
BryoJan2	MW263331	38.272	128.235	15.07.2007	ZMH	Korea	Gangwon-do Inje, Seohwa-ri
D1882	MW263325	47.6	11	28.08.2019	ZMH	Germany	Bavaria, Silvensteinstaumauer, Isar
D1883	MW263308	47.6	11	28.08.2019	ZMH	Germany	Bavaria, Silvensteinstaumauer, Isar
D1884	MW263321	55.07	25.843	03.08.2008	E. Budrys	Lithuania	Svencionys: Mazalote military training ground
FBORT257-09	GU706165	47.5	11.3	25.08.1999	ZSM	Germany	Bavaria, between Wallgau and Vorderriss
FBORT258-09	GU706166	47.5	11.3	25.08.1999	ZSM	Germany	Bavaria, between Wallgau and Vorderriss

Specimen ID	GB ID	Latitude	Longitude	Date	Deposition	Country	Location
FBORT259-09	GU706163	47.6	11	25.08.1999	ZSM	Germany	Bavaria, Silvensteinstaumauer, Isar
FBORT275-09	GU706159	47.561	11.463	05.09.2009	ZSM	Germany	Bavaria
FBORT276-09	GU706156	47.561	11.463	05.09.2009	ZSM	Germany	Bavaria
G1	MW263318	47.6	11	28.08.2019	ZMH	Germany	Bavaria, Silvensteinstaumauer, Isar
G2	MW263319	47.6	11	28.08.2019	ZMH	Germany	Bavaria, Silvensteinstaumauer, Isar
GBORT571-15	no GB ID	47.56	11.47	17.09.2014	ZSM	Germany	Bavaria, Vorderriss
GBORT745-15	no GB ID	47.561	11.463		ZSM	Germany	Bavaria
Li5	MW263332	41.211	118.736	10.07.2008	ZMH	China	Hebei Pingquan
Li6	MW263333	41.211	118.736	10.07.2008	ZMH	China	Hebei Pingquan
Li7	MW263330	41.211	118.736	10.07.2008	ZMH	China	Hebei Pingquan
MGL549	MW263327	47.692	105.812	10.07.2016	ZMH	Mongolia	Hustain Nuruu National Park
MGL6	MW263322	47.692	105.811	09.08.2015	ZMH	Mongolia	Hustain Nuruu National park
MGL639	MW263317	47.781	108.824	08.07.2019	ZMH	Mongolia	close to Tsenkhermandal,
MGL641	MW263328	47.781	108.824	09.07.2019	ZMH	Mongolia	close to Tsenkhermandal, Khentii
MGL642	MW263329	47.781	108.824	10.07.2019	ZMH	Mongolia	close to Tsenkhermandal soum, Khentii

Specimen ID	GB ID	Latitude	Longitude	Date	Deposition	Country	Location
MS1	MW263323	51.71	94.36	22.07.2003	ZMH	Russia	Central Tuva, Ulug-Khem River, 25 km W Kyzyl
S15_7	MW263310	51.567	93.388	18.07.2006	ZMH	Russia	Siberia, River Enisei
S17_2	MW263311	49.845	97.881	24.07.2006	ZMH	Russia	Siberia, South Chujskij Range, Ak-Kol River, Sofijski glacier
S17_4	MW263309	49.845	97.881	24.07.2006	ZMH	Russia	Siberia, South Chujskij Range, Ak-Kol River, Sofijski glacier
S18_1	MW263324	50.243	87.896	25.07.2006	ZMH	Russia	Siberia, Kurai Steppe
S3_2	MW263314	50.394	86.674	07.07.2006	ZMH	Russia	Siberia, near by outlet of Chuja and Katun Rivers
S3_4	MW263316	50.394	86.674	07.07.2006	ZMH	Russia	Siberia, near by outlet of Chuja and Katun Rivers
S4_4	MW263315	50.394	86.674	07.07.2006	ZMH	Russia	Siberia, near by outlet of Chuja and Katun Rivers
S5_3	MW263313	50.394	86.674	07.07.2006	ZMH	Russia	Siberia, near by outlet of (Katun Rivers
Sw	JQ513054	56.466	16.558	-	ZMH	Sweden	Øland, Stora Alvaret

RESULTS

Species distribution

We assembled 280 extant distribution records from Austria (28), China (13), Finland (1), Germany (25), Kazakhstan (19), North Korea (2), South Korea (22), Lithuania (1), Mongolia (19), Romania (1), Russia (138), Sweden (8) and Ukraine (3) and 100 records of extinct populations from Austria (11), Denmark (12), Estonia (1), Germany (34), Italy (2), Latvia (5), Poland (31) and Switzerland (5). Location files including coordinate data are provided as supplementary information SI 3. Figure 1 shows all records we used for modelling. The northernmost location was found in Siberia (Russia) at 67°N; the southernmost location was in Tibet (China) at 28°N. Most of the occurrences were recorded between 57°N and 45°N. The longitudinal extension stretches across most of Eurasia from 152°E in Russia to 12°E in Germany over a range of around 7500 km.

Ellipsoid model

The best fitting method to construct the climatic ellipsoids was '*covmat*'; mean AUC, p-value of partial ROC and omission rates were significantly better than random expectations (p-value < 0.05; Table 2). The complete report of ellipsoid characteristics (e.g., centroid, covariance matrix, semi-axes length, etc.) can be found in SI 5.

Environmental and geographic distances

The geographic prediction of Mahalanobis distance (D2) in the calibration area is shown in Fig. 2 and SI 6 and SI 7. The comparison of D2 on all environmental sets shows all extinct populations, except for one in northern Austria (Ried im Innkreis: 48.222461°N 13.484835°E) to be as close to the climatic centroid as extant populations (Table 3). The environmental set of all bioclimatic variables yielded a D2 ratio similar between the investigated locations. Statistical results of Mood's Median test show no significant

difference between the distances of extinct and extant populations to the climatic niche centroid (Mood's Median test: Set 1: p-value < 0.001; Fig. 3). Moreover, the average distances to the centroid were found to be higher for the extinct and extant European locations (distance: 38.00–66.28, Ø = 59.53 (± 6.08)) than for Asian populations, which were geographically closer to the centroid (distance: 3.86–80.42, Ø = 30.39 (± 18.27); overall p-value < 0.001; Fig. 3).

Table 2: Calibration and evaluation of ellipsoid models used to characterize the niche of *B. tuberculata*. Grey rows highlight the method selected to create final models; we provide the evaluation metrics (mean AUC, p-value partial ROC, omission rate), valid iterations and mean prevalence calculated in environmental ('Prevalence on E-space') and geographical space ('Prevalence on G-space').

Vari-bles	Me-thod	Mean AUC ratio at 5%	*p*-value (partial ROC)	Valid iterations	Omissio n rate	Preva-lence in E-space	Preva-lence in G-space
Set 1	covmat	1.331	<0.001	336.000	0.042	0.532	0.533
Set 1	mve1	1.365	<0.001	108.000	0.084	0.506	0.507
Set 2	covmat	1.355	<0.001	493.000	0.010	0.5656	0.562
Set 2	mve1	1.360	<0.001	492.000	0.010	0.537	0.544
Set 3	covmat	93.841	<0.001	488.000	0.010	0.901	0.922
Set 3	mve1	100.341	<0.001	244.000	0.052	0.867	0.910

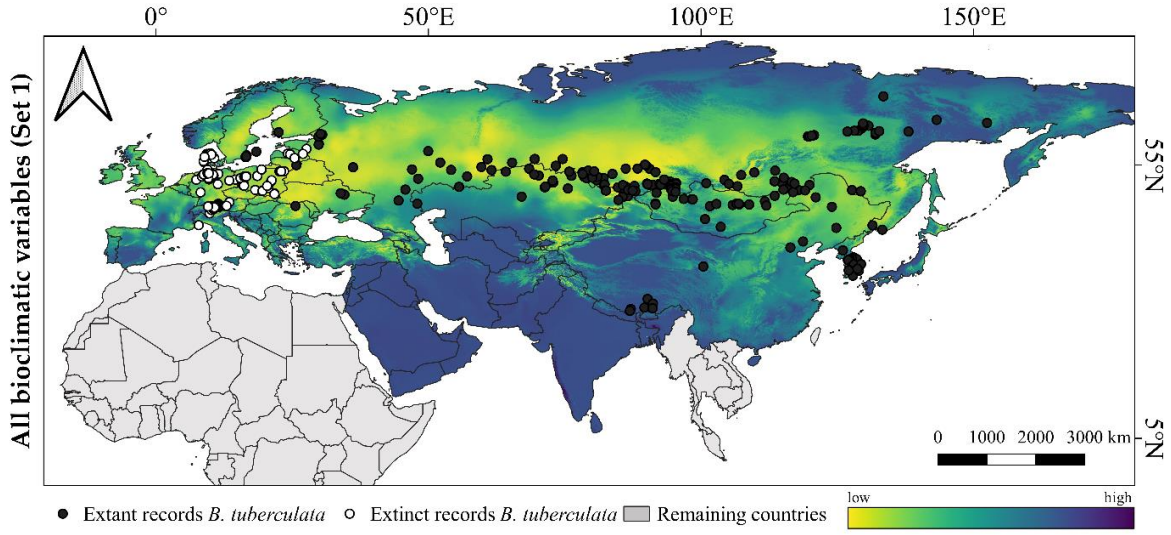

Figure 2: Geographic prediction of Mahalanobis distances in the calibration area for Set 1 including all bioclimatic variables. Colors correspond with values of Mahalanobis distances according to figure legend

Table 3: Comparison of D2 of extinct, extant, extinct outlier (Austria, Ried im Innkreis; 48.222461°N 13.484835°E) and extant outlier locations (close to the German-Austrian border in the Berchtesgaden national park)

	Mean D^2	SD ±	Min	Max
Extinct	3.37	1.53	0.61	7.61
Extant	5.62	5.34	0.41	32.61
Extinct outlier (Austria, Ried im Innkreis)	22.3	-	-	-
Extant outlier (Berchtesgaden national park)	17.14	-	-	-

Land-cover change

Using the HILDA dataset we were able to track changes in land-cover in Central Europe from 1900 to 2000 (SI 8): a massive change from cropland (1900: 33.9% to 2000: 29.3%) and grassland (1900: 34% to 2000: 27.5%) to more settlement area (1900: 2.2% to 2000: 4.2%) and forests (1900: 25% to 2000: 34.3%) can be observed.

Analysis of genetic diversity

We included 34 COI sequences of *B. tuberculata* in the analyses. These represented a total of 28 haplotypes with a haplotype diversity of 0.978 (± 0.015) and a nucleotide diversity of 0.031 (± 0.015). The pairwise distance between central (Asia) and peripheral (Europe) populations was moderate, but significant (ΦST of 0.104; p-value < 0.001).

The haplotype network (Fig. 4) shows a close relationship of all haplotypes with few evolutionary steps between them. Two main groups are recognizable, albeit with only few separating mutations. These two groups do not reflect a split between European and

Asian samples, as European samples are grouping with sequences from Central Asia. The samples from China are more closely related to those from Korea and to most of the Mongolian samples representing the first group; the other group is composed of the Russian, Mongolian and European samples (Germany, Sweden, Lithuania). Furthermore, the Russian, German, Lithuanian and one Mongolian sample share the same haplotype.

Bayesian Skyline plots tracing female effective population size through time were calculated for European and Central Asian specimens separately (Fig. 5). The analysis showed a clear trend of population decline during the last 1000 years for the European populations. The same analysis performed for the Central Asian samples yielded a 10 times higher estimate of female effective population size and demographic stability through time.

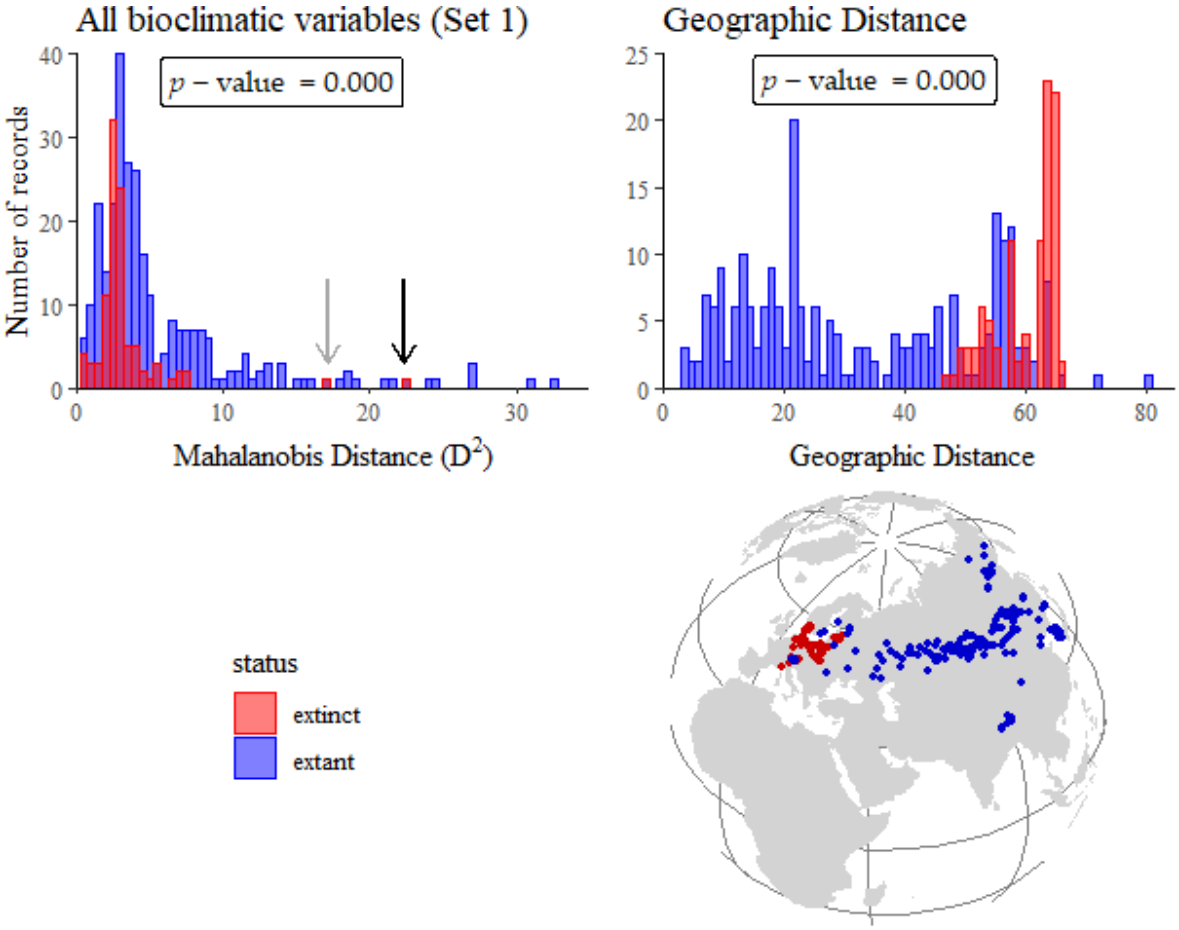

Figure 3: Histograms showing the distribution of Mahalanobis distances (D2) of extinct and extant populations. The centroid of the climatic niche was estimated using Set 1 including all bioclimatic variables. P-values included in graphs indicate results of Moods' Median test, which tests the differences between the distribution of extinct and extant distances of the different parameters. Moreover, the figure shows the geo-graphic distance from each location to the center of the distribution. Values associated with extinct populations are shown in red, while extinct are colored blue. The black arrow in the histogram "All bioclimatic variables (Set 1)" shows the outlier point in Austria close to Ried im Innkreis (D2 = 22.306), the grey arrow shows the outlier close to the German-Austrian border in the Berchtesgaden national park (D2 = 17.142).

In this study, we investigated potential reasons for the decline of the European populations of the formerly widely distributed band-winged grasshopper *B. tuberculata* using ecological niche modelling in combination with a basic population genetic assessment. Specifically, we aimed to test if (1) changing climatic conditions over the last 100 years, or (2) changes in land-use and land-cover are responsible for the decline and local extinction. For this we assessed the habitat requirements and the decline of the species and modelled the environmental niche. Furthermore, we tested if (3) the genetic diversity in the Asian core populations is higher than in the European relic populations, which may support the center-periphery hypothesis. We discuss the results in regards of conservation management of the species.

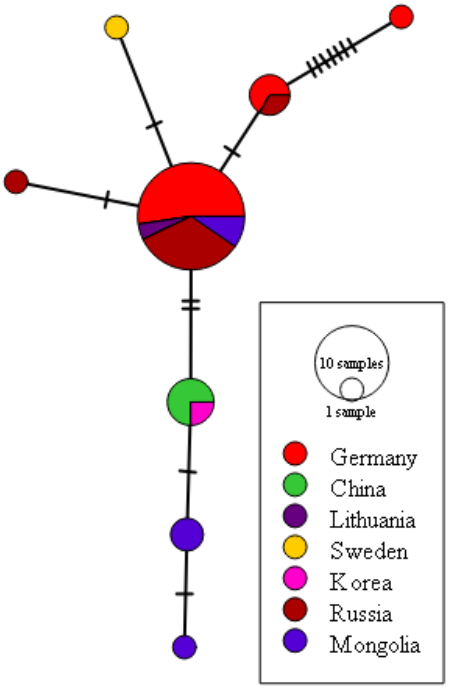

Figure 4: TCS Network created with PopArt showing the genetic relationships between all individuals. Colors represent countries, mutation steps between haplotypes are shown as marks across connections.

Climate models reveal suitable conditions in extinct locations

We used an ellipsoid envelope approach to estimate the climatic niche of *B. tuberculata* and measure the proximity of its populations to their optimum. All sets recovered low distances of the extinct populations to the climatic niche centroid, and none recovered any difference between extinct and extant populations (Figs. 2, 3; SI 6–7). Set 1 offered higher variation in distances to the centroid (range: 0.417–32.611), possibly due to the higher variation and multi-dimensionality of the principal components included (i.e., 15 variables).

While most extinct populations were located in areas with lower Mahalanobis distance (D2) to the centroid (i.e., high suitability), two extinct locations with higher values were recorded (D2 = 22.306 and 17.142; Fig. 2, Table 3). These locations represent a population close to the German-Austrian border in the Berchtesgaden national park (D2 = 17.142) and a population in Austria close to Ried im Innkreis (D2 = 22.306). The higher D2 at these two locations could have several reasons, e.g. imprecise locality data, errors in georeferencing, individuals dispersed from neighboring locations (e.g. migrating individuals; Barton and Hewitt 1982), artefacts of the bioclimatic layers used (e.g. Marchi et al. 2019; Poggio et al. 2018), or even just a high tolerance regarding abiotic conditions (e.g. Preston and Johnson 2020). The last reason is supported by data from Asia with similar D2 range. Since our models predict areas situated in the distribution gap between the European and Asian populations as climatically suitable, the destruction of natural habitats is a likely reason for the loss of stepping stones and for the extinction of any connecting populations in the area.

Habitat requirements and historical decline of **B. tuberculata**

Bryodemella tuberculata is a species of open landscapes, preferring habitats with sparse vegetation and open ground. According to our observations, all Russian collection sites in the Altay-Sayan Mountains, the Kulunda Plain (the south-eastern part of West Siberian Plain between the Irtysh and Ob Rivers) and within the south-eastern Ural Mountains (pers. obs. MGS; for further details, see SI 9) were mainly composed of dry

meadows and steppes with sparse vegetation (~ 10–30 cm height) and spots of open ground (generally, the vegetation cover was less than 80%). The ground is mostly sandy to rocky, with smaller pebbles. In most cases occurrence sites were close to water systems. The species enters transformed ecosystems, such as the steppe variants with moderate livestock grazing and the dry hayfields. This type of habitat was found at almost all visited collection sites in Central Asia and Europe (for habitat images see Supplementary Information SI 10). In Mongolia, the species is found in low abundance, but in almost all habitats with open spaces and sparse vegetation, mainly covered by shorter plant species up to 30 cm and at the edges to forests (pers. observ., LSD 2015–2019; Dey et al. in press; Supplementary Information SI 10). Pastures for grazing represent the major agricultural use in the country, hence, the landscape is characterized by bare steppe habitats with low vegetation providing good conditions for *B. tuberculata*.

All extant populations of Central Europe (Southern Germany, Austria) inhabit alpine river valleys with unmodified or little modified flood regimes; specifically zones of alluvial gravel beds with sparse or without any vegetation. Occasionally, individuals may be observed in adjacent structures similar to those described for the Asian populations (Land-mann 2017). Since the middle of the 19th century, populations of *B. tuberculata* in Europe have been declining (e.g. Reich 1991; Zuna-Kratky et al. 2017). Despite the availability of a large database, we are unable to make any statements about the exact timing of extinction of *B. tuberculata* at any particular location, but we provide the last recorded findings for several regions (Fig. 1; Supplementary Information SI 11). Altogether, our data suggest that most Central European extinction events took place between the 1920's and 1960's. We therefore suggest that each local extinction event in Central Europe may be closely connected with local landscape changes. Historically, at least in the mountainous regions, the naturally occurring annual floods following snowmelt removed most vegetation growing in the riparian habitats. Anthropogenic reductions of the flood volume, mostly due to the construction of hydroelectric dams, led to the establishment of shrub and tree vegetation in these habitats and a decline of the areas suitable for *B. tuberculata* (Reich et al. 2008; Juszczyk et al. 2020). A variety of restoration measures have been put in place over the last decades, including a reduced deduction of water for hydroelectric energy generation, mechanical removal of

vegetation, and education of the public; the mid- and long-term efficiency of these measures remains to be demonstrated (Juszczyk et al. 2020). A different type of change to the habitat structure was recorded in Denmark. There, most heathlands in the vicinity of Abild (an extinct location) have been converted to agricultural areas or were overgrown by shrubs (pers. observ. LSD; SI 10). Although the agricultural land-use decreased from 74% in 1915 to 61% in 2015, afforestation and urban expansion still led to a decline of heath areas previously typical for many parts of Denmark (Pedersen and Møllenberg 2017). The species has been listed as extinct in Denmark since the 1950s. Simi-larly, in Northern Germany, restoration of some old heath landscapes has been performed. Based on literature and museum surveys, we were able to find several records from the Lüneburger Heide heathland areas, for which the gradual conversion to arable land and forests at the end of the 19th century is historically documented (Koopmann 2000). This change in land-use was mainly triggered by a decrease in the local production of wool and honey due to external competitors, making the maintenance of large pastures uneconomic (Naturpark-Lueneburger-Heide.de; Koopmann 2000). During the early 20th century, the first areas were restored to the original heath systems. Nowadays, the Lüneburger Heide is one of the largest heath areas in Northern and Central Germany. Although most heath areas in Central Europe declined through time, some of these cultural landscapes are still intact. Many of them are now under military use and kept open by military activity, which practically equals to legal protection of the landscape. As a result, *B. tuberculata* was rediscovered on 03 August 2008 in the Pabradė military training area in Lithuania, where it had been assessed as possibly extinct until 2008 (Budrys et al. 2008; Budrys and Pakalniškis 2007). This was the first record since the first half of the 20th century (Budrys and Pakalniškis 2007); yet, the status of the population remains unknown and it will have to be monitored to check its population establishment. In Northern Europe the population on Øland (Sweden) shows similar habitat preferences for barren land. The implementation of specific conservation measures appears to keep the population relatively stable.

Bryodemella tuberculata needs larger areas with open habitat, often with frequent natural disturbance. Anthropogenic changes of the European landscape in the last centuries (Plieninger et al. 2016; Hersperger and Bürgi 2009; Antrop 2004) likely led to the current

patchy distribution of the species and will probably cause the extinction of further populations in the European range. The time series of land-cover plots (Supplementary Informa-tion SI 8) supports land-use change as a driver of the decline of *B. tuberculata* as it displays a rapid change from grassland and agricultural areas to settlement and reforestation in the middle of the 20th century and still ongoing. This change from open areas to more closed habitat types, and an increase of human pressure due to high-intensity land-use, may have led to the extinction of *B. tuberculata* at many Central European locations. Even though some habitats of extinct populations have now been restored, they are highly fragmented, and the dispersal capabilities of *B. tuberculata* appear insufficient to allow for quick colonization of these habitats without support. Similar scenarios of decline have been described for many other European species, e.g., carabid beetles with different ecological preferences in Belgium, Denmark and the Netherlands (Kotze and O'Hara 2003); common and widespread butterflies in the Netherlands (Van Dyck et al. 2009); or the ground nesting Black Grouse populations in Lower Saxony, Germany (Ludwig et al. 2009). In turn, the availability of large stretches of natural habitats suitable for *B. tuberculata* currently remains much higher in the Asian range. However, vast areas of habitat are also being destroyed in Russia, especially in the European part of the country, due to urbanization, agriculture and mining (Smelansky and Tishkov 2012). This process is much slower due to lower human activity in these areas and simply a larger area, but can also be expected to fragment populations and impact genetic diversity in the near future.

Distribution of genetic diversity and historical demography

While our genetic data is limited, also due to the rareness of the species, it suggests that the majority of genetic diversity is found in Central Asia. The European populations show lower diversity and are nested within the Asian populations. Hence, based on our COI data, European populations only represent a subset of the species' genetic diversity, as expected for relic populations, which may have gone through a population bottleneck (e.g. Chen et al. 2016; Gaublomme et al. 2013; Hájková et al. 2007; Lucchini et al. 2004). We did not find any evidence supporting the status of these populations as distinct

genetic lineages. This may suggest a previously more continuous, potentially panmictic distribution across much of Europe and Asia and a relatively recent fragmentation and decline in Central Europe, as also suggested by our historical distribution data.

The genetic patterns do not support the center-periphery hypothesis, i.e., low genetic variability in areas of low environmental suitability, as low genetic variability was also detected in areas of high environmental suitability. However, due to strong contemporary restrictions on migration, gene flow is likely completely interrupted and the European populations are likely under a drift regime, rendering them prone to extinction. On the other hand, Bayesian Skyline plots provide some evidence that supports the center-periphery hypothesis driven by geographic distance. Our results show that female effective population size is larger in Central Asia and also has been more stable in the last 10 ka (Fig. 5), while the European populations have smaller estimated population sizes and are in decline. The results we obtained match other taxa with similar distributions, e.g., the leaf-beetle *Cheilotoma musciformis*, which has some relict populations in Poland. These populations are declining, probably because habitat destruction caused a disjunction from the main distribution, resulting in an observed genetic bottleneck (Kajtoch et al. 2016). Such patterns of loss of genetic diversity associated with habitat destruction, which have led to the disjunction of populations, can also be seen in the decline of the Danish population of the otter *Lutra lutra* (Pertoldi et al. 2001), among others (e.g. Cremene et al. 2005; Kotze and O'Hara 2003).

Insufficient genetic samples and lack of more fine-scaled genome-wide markers inhibited the direct assessment of a relationship between genetic diversity and niche centrality. Nevertheless, our results support the findings of Lira-Noriega and Manthey (2014), who equally failed to detect any clear relationship between genetic diversity, and geographic and environmental distance to the geographic centroid. However, they did find a strong relationship between genetic diversity and climatic niche centrality. In some taxa, Lira-Noriega and Manthey (2014) describe a negative tendency for the relationship of genetic diversity and geographical distance to the source population, reflecting environmental impact on the population dynamics, rather than a fundamental ecological relationship. In case of *B. tuberculata*, we suggest a similar effect of habitat destruction in the periphery of the distribution supported by decreasing female effective

population size in the European populations (geographically distant from the main distribution; source) in contrast to the stable demography in the Asian populations (geographically close to the source). A more detailed study of other habitat characteristics, especially land-use patterns, may shed light on the reasons for the local decline and could help to manage the conservation activities to save the remaining European populations from decline, which may also facilitate the recolonization of former locations.

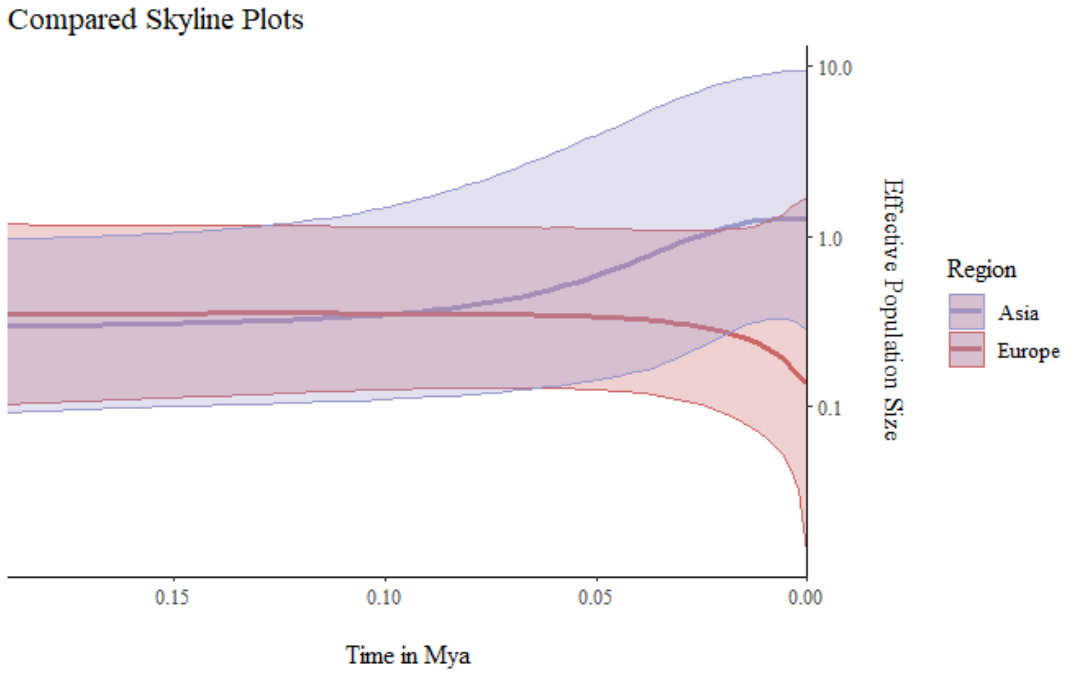

Figure 5: Skyline plot created with BEAST v.2 tracing effective female population size over time (last 120,000 years) for European and Central Asian populations.

Aspects of conservation management

Local conservation efforts for species with isolated and declining populations may often be seen as unsustainable, because these populations may be threatened by the effects of climate change, which cannot be counteracted by localized efforts (e.g. Sinervo et al. 2010, Malcom et al. 2006). Our results show that extinct or threatened European populations of *B. tuberculata* are in areas of optimal climatic conditions for this species, whereas some Asian populations appear to be thriving also in areas of poor climatic

suitability. This suggests that *B. tuberculata* is most likely resilient to the purely climatic effects of global warming in the Central European part of its range. If our results hold true, this means that threatened populations may survive the next decades of changing climate as long as their habitats are locally protected, and that these local efforts can be considered sustainable. This includes not only threatened extant populations, but also means that reintroduction efforts into the localities of extinct populations may be a promising option as long as the habitat structures necessary for *B. tuberculata* have been restored and longterm management measures are in place. We suggest that monitoring of extant and potentially translocated populations should be accompanied by genetic studies using deeper population-level sequencing methods, such as microsatellites or RAD sequencing. Once a high-quality whole genome becomes available, genome sequencing may become a viable option.

The speckled buzzing grasshopper is just one species of an assembly of taxa sharing a habitat that is highly threatened by anthropogenic land-cover change. We believe that our study reinforces that status of *B. tuberculata* as a flagship and umbrella species for this organism community, and we hope that our results help making a case for increased efforts to their protection.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https:// doi. org/ 10. 1007/ s10531- 021- 02221-8.

All supporting information can be found on page 347 and following.

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Hamburg, 12.01.2023

Place/Date

Sign

Chapter 5

-

"Does (genome) size matter?"

-

Chapter 5.1

-

New genome size estimates for band-winged and slant-faced grasshoppers (Orthoptera:Acrididae: Oedipodinae, Gomphocerinae) reveal the so far largest measured insect genome

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ABSTRACT

Grasshoppers, specifically those of the family Acrididae are known to have the largest genomes of all insects. However, less than 100 species of Orthoptera have their genome size estimated so far. In the present study, we measured the genome size of five acridid species belonging to the two subfamilies Oedipodinae and Gomphocerinae. All of the genomes measured are large and range between 1C = 11.31 pg in the female of *Chorthippus dorsatus* and 1C = 18.48 pg in the female of *Stethophyma grossum*. The latter represents the so far largest measured insect genome. We further provide a summary of genome size estimates available for Orthoptera.

Keywords:C-value, flow cytometry, *Stethopyhma, Oedipoda, Sphingonotus, Chorthippus.*

INTRODUCTION

The genome has become one of the most important targets of interest for biologists. In times of high throughput sequencing, projects like i5k generate data of entire genomes are at a daily base (Robinson et al. 2011; Li et al. 2019). However, we still have little data and a limited understanding of the variance in genome size across organisms. Especially for insects, the most diverse group of organisms on earth, data of only about 1,300 of the expected diversity of several million species are available (Sadílek et al. 2019a; Gregory 2020). Generating new data on genome sizes is important, e.g., for choosing the adequate NGS applications for genomic sequencing (Rodríguez et al. 2017). Yet, genome size can also be a taxonomic feature and can be used for species determination (Sadílek et al. 2019b). For many applications taxa with specifically large genomes still remain a difficult target, especially if no complete genome sequence is available. Further, in order to understand why some species or species groups have specifically large genomes, whereas others are rather small requires comprehensive data across a large range of taxa. While the so far largest genome of any organism was estimated in a plant, the monocot *Paris japonica* Franchet with 1C = 152.23 pg (Pellicer et al. 2010), the largest genome sizes in insects have been measured in Orthoptera, specifically Caelifera, with 1C values of 16.93 pg in *Podisma pedestris* (Linnaeus, 1758) (Podisminae) and 16.34 pg in *Stauroderus scalaris* (Fischer von Waldheim, 1846) (Gomphocerinae) (Gregory 2020 for a list). However, there is also a lot of variation within Orthoptera with genome sizes as small as 1C = 1.55 pg found in the cricket *Hadenoecus subterraneus* (Scudder, 1861) (Rasch and Rasch 1981). Nevertheless, a clear trend for larger genomes in the short-horned grasshoppers is observed, and specifically in the family Acrididae. In the present study, we were able to locate only 85 published genome size estimates from all Orthoptera (e.g. Gregory 2020).

To better understand the evolution of genome size in Orthoptera, especially the huge genomes of grasshoppers of the Acrididae family, it is obligatory to generate additional information. Hence, we provide new genome size information for members of the Acrididae family, i.e. three species of the subfamily Oedipodinae and two species of the Gomphocerinae. We present, to our knowledge, the so far largest genome size of any insect and summarize the knowledge on genome sizes in Orthoptera.

MATERIAL AND METHODS

Sampling

Eight specimens from five species (Table 1), all of the family Acrididae, were collected for our analyses in September 2019 in Hamburg, Georgswerder (Germany, 53.5097°N 10.0301°E). Specimens were collected by hand and kept alive until further processing. We included two species of the subfamily Gomphocerinae: *Chorthippus dorsatus* (Zetterstedt, 1821) and a species of the *Chorthippus biguttulus* (Linnaeus, 1758) group (a group of three species *C. biguttulus, C. brunneus* (Thunberg, 1815), *C. mollis* (Charpentier, 1825), which can only be identified with certainty by male song patterns; our specimen is a female, but according to morphological traits most likely represents *C. biguttulus*), as well as three species of the subfamily Oedipodinae: *Oedipoda caerulescens* (Linnaeus, 1758), *Sphingonotus caerulans* (Linnaeus, 1767), and *Stethophyma grossum* (Linnaeus,1758) (Table 1, 2).

Reference specimens are deposited in the Zoological Museum Hamburg (ZMH), part of the Center of Natural History (CeNak) under the accession ZMH 2019/21.

Genome size analysis

Nuclear DNA content (2C) was measured by the flow cytometry method (FCM) as in Sadílek et al. (2019a, b) at the Department of botany of Charles University, Prague. The muscle tissue of one hind femur was used for FCM analysis against the plant-internal standard *Pisum sativum* L. "Ctirad" (Fabaceae) with 2C = 9.09 pg (Doležel et al. 1998; Doležel and Greilhuber 2010). Fresh tissue was homogenized and mixed with a leaf of the standard in 500 µl of 4°C cold Otto buffer I.

The suspension of released cells was then filtered through a 42 µm nylon mesh and divided in two parts. One part was stained with 1,000 µl DAPI solution (stock: 25 ml Otto buffer II, 1 ml DAPI (0.1 mg/ml), 25 µl 2mercaptoethanol (2 µl/ml)); the second part was stained with 1,000 µl propidiumiodide (PI) solution (stock: 25 ml Otto buffer II, 1 ml RNase (1 mg/ml), 1 ml PI (1 mg/ml), 25 µl 2-mercaptoethanol) (Doležel et al. 2007).

For DAPI analysis, the Partec CyFlow instrument (Partec GmbH, Münster, Germany) with UV LED chip and for PI analysis the Partec SL instrument with a green solidstate

laser (Cobolt Samba, 532 nm, 100 mW) were used. Each sample was stained for several minutes before measurement, and 3,500 to 5,000 particles were recorded in each FCM analysis. FCM data were analyzed with the Partec FloMax v. 2.52 software (Partec GmbH, Münster, Germany). Combined DAPI and PI measurement results of the same sample express the AT/GC ratio of the genome of the species, the GC content (e.g. Šmarda etal. 2008; Sadílek et al. 2019a, b). The GC content of P. sativum is 38.50% (e.g. Barrow and Meister 2002; Šmarda et al. 2008) and the GC content of the analyzed samples was calculated with the Microsoft Excel macro from Šmarda et al. (2008).

Table 1. Diploid chromosome number, 2C genome size, sample/standard ratio of both DAPI- and PI-stained samples and GC content of grasshopper species studied. Samples were measured against *P. sativum* standard with 2C = 9.09 pg. F = female, M = male, 2n = male diploid chromosome number, 2C = nuclear DNA content for nuclei with diploid chromosome number, CV = average coefficient of variation for each stain used.

Species	**2n**	**Sex**	**2C (pg)**	**Sample/ standard DAPI ratio**	**Sample/ standard ratio**	**GC content PI(%)**	**Sample CV DAPI - PI**
S. caerulans	22+XX	F	26.63	2.424	2.930	42.14	2.70 - 2.95
S. caerulans	22+X0	M	25.12	2.321	2.764	41.87	2.71 - 2.81
O.caerulescens	22+XX	F	28.39	2.621	3.123	41.88	3.71 - 5.62
C. dorsatus	16+XX	F	24.14	2.359	2.656	40.82	2.58 - 2.64
C. biguttulus	16+XX	F	22.62	2.149	2.488	41.35	2.50 - 4.07
S.grossum	22+XX	F	36.95	3.326	4.065	42.35	3.41 - 4.23
S.grossum	22+X0	M	34.72	3.172	3.820	42.08	2.19 - 2.84

Table 2. Genome sizes of Orthoptera so far measured. The template of the table was extracted from Gregory (2020); it was complemented with original references and additional studies. References with an * indicate that the original reference could not be accessed and data are extracted only from Gregory (2020). [1]relative genome size - measured with the DAPI - from Morgan-Richards (2005). M= male, F = female, 2C = genome size of the diploid cell, 2n = diploid chromosome number (if sex is not determined, karyotype of the male is presented; in all species the sex determining system is XX/X0, only males of *Podisma pedestris* can be variable with XY/X0), n.a. = not available; FD = Feulgen densitometry, FCM = flow cytometry method; AN = antenna, BR = brain, HE = haemocytes, MS = muscle, OV = ovaries, S = sperm, TS = testes; AC = *Allium cepa* (1C = 16.50 pg), BO = *Bos taurus* (1C = 3.70 pg), BP = *Bellis perennis* (1C = 1.76 pg), DM = *Drosophila melanogaster* (1C = 0.18 pg), DV = *Drosophila virilis* (1C = 0.34 pg), GD = *Gallus domesticus* (1C = 1.25 pg), HS = *Homo sapiens* (1C = 3.50 pg), LM = *Locusta migratoria* (1C = 5.50 pg), MD = *Musca domestica* (1C = 0.90 pg), MM = *Mus musculus* (1C = 3.30 pg), OM = *Oncorhynchus mykiss* (1C = 2.60 pg), PA = *Periplaneta americana* (1C = 3.41 pg), PS = *Pisum sativum* (1C = 4.55 pg), SG = *Schistocerca gregaria* (1C = 8.70 pg)

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Suborder: Caelifera									
Acrididae	Acridinae	*Acrida conica*	n.a.	12.55	23	FD	HE	GD, OM	Rasch 1985*
Acrididae	Acridinae	*Acrida conica*	M	10.82	23	FD	TS	GD	Rees et al. 1978
Acrididae	Acridinae	*Caledia captiva*	M	10.9	23	FD	TS	GD	Rees et al. 1978
Acrididae	Acridinae	*Cryptobothrus chrysophorus*	M	9.37	23	FD	TS	GD	Rees et al. 1978

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Acridinae	*Schizobothrus flavovittatus*	M	7.5	n.a.	FD	TS	GD	Rees et al. 1978
Acrididae	Catantopinae	*Macrotona australis*	M	8.49	23	FD	TS	GD	Rees et al. 1978
Acrididae	Catantopinae	*Peakesia hospita*	M	10.47	23	FD	TS	GD	Rees et al. 1978
Acrididae	Catantopinae	*Phaulacridium vittatum*	M	10.73	23	FD	TS	GD	Rees et al. 1978
Acrididae	Cyrtacanthacridinae	*Schistocerca cancellata*	M	9.49	23	FD	TS	LM	John and Hewitt 1966
Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	n.a.	8.96	23	FD	V	MM	Fox 1970*
Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	M	8.71	23	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	M	8.55	23	FD	TS	LM	John and Hewitt 1966
Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	M	8.74	23	FD	S	n.a.	Camacho et al. 2015

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Cyrtacanthacridinae	*Schistocerca paranensis*	M	8.63	23	FD	TS	LM	John and Hewitt 1966
Acrididae	Cyrtacanthacridinae	*Valanga irregularis*	M	9.44	23	FD	TS	GD	Rees et al. 1978
Acrididae	Eyprepocnemidinae	*Eyprepocnemis plorans*	M	9.7	23	FD	S	LM	Ruiz-Ruano et al. 2011
Acrididae	Eyprepocnemidinae	*Heteracris adspersus*	M	6.34	23	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Gomphocerus sibiricus*	M	8.95	17	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Chorthippus apicalis*	n.a.	12.61	17	FD	TS	GD	Belda et al. 1991*
Acrididae	Gomphocerinae	*Chorthippus biguttulus*	F	11.31	18	FCM	MS	PS	**this study**
Acrididae	Gomphocerinae	*Chorthippus binotatus*	n.a.	10.91	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus cf. binotatus*	n.a.	10.35	17	FD	TS	GD	Belda et al. 1991

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Gomphocerinae	*Chorthippus brunneus*	M	10.15	17	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Chorthippus brunneus*	M	9.46	17	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Gomphocerinae	*Chorthippus brunneus*	M	8.55	17	FD	TS	LM	John and Hewitt 1966
Acrididae	Gomphocerinae	*Chorthippus dorsatus*	n.a.	8.34	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus dorsatus*	F	12.07	18	FCM	MS	PS	**this study**
Acrididae	Gomphocerinae	*Chorthippus jacobsi*	n.a.	10.84	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus jucundus*	n.a.	11.88	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus longicornis*	M	8.58	17	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Chorthippus nevadensis*	n.a.	11.53	17	FD	TS	GD	Belda et al. 1991

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	n.a.	14.72	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	n.a.	13.83	17	n.a.	n.a.	n.a.	Petitpierre 1996
Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	M	13.36	17	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	M	12.31	17	FD	TS	LM	John and Hewitt 1966
Acrididae	Gomphocerinae	*Chorthippus scalaris*	n.a.	14.72	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus vagans*	M	8.68	17	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Chorthippus vagans*	n.a.	8.64	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*	n.a.	13.38	17	n.a.	n.a.	n.a.	Petitpierre 1996
Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*	M	12.66	17	FD	TS	MM	Wilmore and Brown 1975

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*	M	12.14	17	FD	TS	LM	John and Hewitt 1966
Acrididae	Gomphocerinae	*Omocestus viridulus*	M	13.16	17	FD	TS	LM	John and Hewitt 1966
Acrididae	Gomphocerinae	*Stauroderus scalaris*	n.a.	16.34	17	n.a.	n.a.	n.a.	Petitpierre 1996
Acrididae	Melanoplinae	*Campylacantha olivacea*	F	6.98	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Acrididae	Melanoplinae	*Campylacantha olivacea*	M	6.15	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Acrididae	Melanoplinae	*Melanoplus differentialis*	M	6.79	23	FCM	BR	PA	Hanrahan and Johnston 2011
Acrididae	Melanoplinae	*Melanoplus differentialis*	n.a.	6.23	23	FD	HE	GD, OM	Rasch unpubl. *
Acrididae	Melanoplinae	*Melanoplus differentialis*	n.a.	3.84	23	FD	OV, TS	BO	Swift and Kleinfeld 1953*
Acrididae	Melanoplinae	*Melanoplus differentialis*	F	7.26	24	FCM	BR	PA	Hanrahan and Johnston 2011

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Melanoplinae	*Melanoplus sanguinipes*	n.a.	5.83	23	FD	HE	GD, OM	Rasch unpubl. *
Acrididae	Melanoplinae	*Podisma pedestris*	M	16.93	23/24	FD	S	SG	Westermann et al. 1987
Acrididae	Oedipodinae	*Ailopus thalassinus*	M	6.68	23	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Austroicetes pusilla*	M	6.29	23	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Gastrimargus musicus*	M	9.01	n.a.	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Humbe tenuicornis*	M	8.21	23	FD	TS	LM	John and Hewitt 1966
Acrididae	Oedipodinae	*Chortoicetes terminifera*	M	7.22	23	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Oedipodinae	*Chortoicetes terminifera*	M	5.99	23	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Locusta migratoria*	F	6.44	24	FCM	n.a.	MM	Wang et al. 2014

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Oedipodinae	*Locusta migratoria*	n.a.	6.35	23	FD	HE	GD, OM	Rasch 1985
Acrididae	Oedipodinae	*Locusta migratoria*	n.a.	6.27	23	FD	V	MM	Fox 1970
Acrididae	Oedipodinae	*Locusta migratoria*	M	6.09	23	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Oedipodinae	*Locusta migratoria*	M	5.47	23	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Locusta migratoria*	n.a.	5.28	23	FD	S	MD	Bier and Müller 1969*
Acrididae	Oedipodinae	*Oedipoda caerulescens*	F	14.2	24	FCM	MS	PS	**this study**
Acrididae	Oedipodinae	*Sphingonotus caerulans*	M	12.56	23	FCM	MS	PS	**this study**
Acrididae	Oedipodinae	*Sphingonotus caerulans*	F	13.32	24	FCM	MS	PS	**this study**
Acrididae	Oedipodinae	*Stethophyma grossum*	M	17.36	23	FCM	MS	PS	**this study**

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Oedipodinae	*Stethophyma grossum*	F	18.48	24	FCM	MS	PS	**this study**
Morabidae	Morabinae	*Warramaba virgo*	n.a.	4	15	FD	BR	GD	White and Webb 1968
Morabidae	Morabinae	*Warramaba virgo*	n.a.	3.75	15	n.a.	n.a.	n.a.	Petitpierre 1996
Suborder: Ensifera									
Anostostomatidae	Deinacridinae	*Hemideina crassidens* [1]	M	5.4	15	FCM	AN	BP	Morgan-Richards 2005
Anostostomatidae	Deinacridinae	*Hemideina crassidens* [1]	F	6.01	16	FCM	AN	BP	Morgan-Richards 2005
Anostostomatidae	Deinacridinae	*Hemideina thoracica* [1]	M	5.95	15	FCM	AN	BP	Morgan-Richards 2005
Anostostomatidae	Deinacridinae	*Hemideina thoracica* [1]	F	6.53	16	FCM	AN	BP	Morgan-Richards 2005
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2.38	11	FIA	HE	DM	Koshikawa et al. 2008

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2	11	FD	HE	GD, OM	Rasch 1985
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2	11	FD	OV, TS	MM, HS	Lima-de-Faria et al. 1973
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2	11	FCM	BR	DM	Gregory unpubl.
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2	11	FIA	HE	GD	Gregory unpubl.
Gryllidae	Gryllinae	*Gryllus pennsylvanicus*	n.a.	2.68	11	n.a.	n.a.	n.a.	Petitpierre 1996
Gryllidae	Gryllinae	*Gryllus pennsylvanicus*	n.a.	2.06	21	FD	S	MD	Bier and Müller 1969
Gryllidae	Gryllinae	*Gryllus pennsylvanicus*	n.a.	2	21	FD	HE	GD, OM	Rasch 1985
Gryllidae	Oecanthinae	*Oecanthus niveus*	n.a.	1.71	n.a.	FCM	BR	DV	Hanrahan and Johnston 2011
Gryllotalpidae	Gryllotalpinae	*Neoscapteriscus borellii*	n.a.	3.41	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Rhaphidophoridae	Ceuthophilinae	*Ceuthophilus stygius*	n.a.	9.55	n.a.	FD	HE	GD, OM	Rasch and Rasch 1981
Rhaphidophoridae	Ceuthophilinae	*Hadenoecus subterraneus*	n.a.	1.55	n.a.	FD	HE	GD, OM	Rasch and Rasch 1981
Tettigoniidae	Conocephalinae	*Conocephalus sp.*	M	2.65	33	FCM	BR	GD	Hanrahan and Johnston 2011
Tettigoniidae	Conocephalinae	*Conocephalus sp.*	F	3.03	34	FCM	BR	GD	Hanrahan and Johnston 2011
Tettigoniidae	Conocephalinae	*Neoconocephalus triops*	M	7.29	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Tettigoniidae	Conocephalinae	*Neoconocephalus triops*	F	7.93	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Tridactylidae	n.a.	unknown sp.	n.a.	2.63	n.a.	FCM	BR	DV	Hanrahan and Johnston 2011
Trigoniidae	Trigonidiinae	*Laupala cerasina*	n.a.	1.93	n.a.	FCM	BR	GD	Petrov et al. 2000

RESULTS

DAPI-stained samples yielded a lower coefficient of variation (CV) than PI-stained samples, on average CV = 2.83% and 3.59% respectively. All the analysed species of Oedipodinae reached higher genome size values than the analysed species of Gomphocerinae. We were able to measure the genome size of both sexes only in two species (*S. caerulans* and *S. grossum*). There, the female/male genome size values clearly reflected the XX/X0 sex determination system differences. Due to this sex determination system it is generally preferred to report genome size in 2C values rather than the commonly used 1C value. However, to allow for better comparability, we here report both values.

All analysed species of Oedipodinae had distinct genome size (Table 1). The male of *S. caerulans* had 2C = 25.12 pg (1C = 12.56 pg); the female had 2C = 26.63 pg (1C = 13.32 pg). The female specimen of *O. caerulescens* exhibited a 2C value of 28.39 pg (1C = 14.20 pg). The largest genome size was recorded in S. grossum, where the male reached 2C = 34.72 pg (1C = 17.36 pg) and the female 2C = 36.95 pg (18.48 pg). Both closely related Gomphocerinae species showed very similar genome size es (Table 1): 2C = 22.62 pg (1C = 11.31 pg) in the *C. cf. biguttulus* female and 2C = 24.14 pg (1C = 12.07) in the female of *C. dorsatus*. The sample/standard ratio of samples stained with PI was always higher than in DAPI-stained samples of the same specimen, ranging from 11% difference in the female of *C. dorsatus* to 18% difference in the female of *S. grossum*. This trend is observable also in the GC content, where *C. dorsatus* had only 40.82% and the female of *S. grossum* had 42.35% (Table 1). However, the GC content differences among all species analysed were minimal.

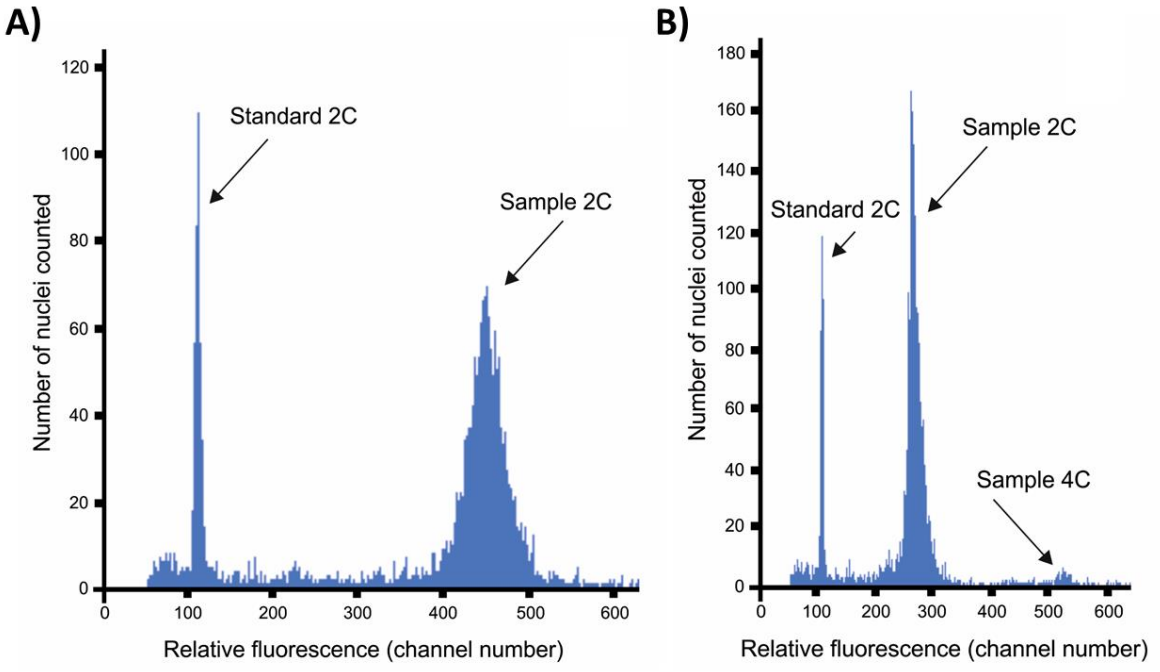

Figure 1. Relative fluorescence histograms for samples stained with PI. 2C peaks represent diploid cells and 4C peaks represent cells in the G2 phase of the cell cycle. with replicated DNA. Standard used: P. sativum 2C = 9.09 pg. (A) *S. grossum* female with 2C = 36.95 pg. (B) *C. biguttulus* female with 2C = 22.62 pg.

DISCUSSION

We present new genome size estimates for five species of Acrididae, one of which represents the largest genome of all insects measured so far, the genome of the female of *Stethophyma grossum* with 2C = 36.95 pg (1C = 18.48 pg). We also measured a female of *C. dorsatus* with 2C = 24.14 pg (1C = 12.07 pg). This species was measured before using the Feulgen densitometry method with 1C = 8.34 pg (Belda et al. 1991). However, the more recent method of flow cytometry we used is considered more accurate for genome size estimations (e.g. Doležel and Greilhuber 2010). Furthermore, we collected all previous estimates from Gregory (2020) and added few additional resources to provide some basic visualization of the genome size variation in the different subfamilies of Orthoptera (Fig. 1).

In total, we gathered 92 (our new data included) estimates of genome sizes belonging to 54 species (Table 2, Fig. 1). These data included 68 estimates for Caelifera (43 species) and 17 for Ensifera (11 species). They ranged from 1C = 3.75 pg for *Warramaba virgo* (Key, 1963) (Morabidae) (Petitpierre 1996) to 1C = 18.48 pg for *Stethophyma grossum* (Oedipodinae, present study) in Caelifera and from 1C = 1.55 pg for *Hadenoecus subterraneus to* 1C = 9.55 pg for *Ceuthophilus stygius* (Scudder,1861) (both cave Rhaphidophoridae) in Ensifera (Rasch and Rasch 1981). Average 1C values in Ensifera and Caelifera are 3.16 pg (± 2.18 pg) and 9.83 pg (± 3.32 pg) respectively. Further analyses at the family and subfamily level are difficult, as most data comes from Acrididae with 66 measurements (78%). The average genome size in Acrididae is 10.01 pg (± 3.19 pg). Within Acrididae, most estimates came from 26 measurements of Gomphocerinae and 17 of Oedipodinae with average genome sizes of 1C = 11.52 pg (± 2.17 pg) and 9.13 pg (± 4.20 pg) respectively (Table 2, Fig. 1).

Generally, the short-horned grasshoppers (Caelifera) appear to have larger genomes compared to the longhorned grasshoppers (Ensifera). However, this is not correlated with the number of chromosomes. Despite their relatively low male number of chromosomes of 2n = 17 (most of other Acrididae have 2n = 23; e.g. Sylvester et al. 2019), Gomphocerinae have some of the largest genome sizes. Their average genome size is 1C = 11.52 pg ranging from 1C = 8.34 pg in *C. dorsatus* (Belda et al. 1991) to 16.34 pg in *Stauroderus scalaris* (Petitpierre 1996; Gregory 2020). Moreover, they show large intraspecific variation in genome size evident from different studies (Table 2), for example: 1C = 12.31 pg to 14.72 pg for *Pseudochorthippus parallelus* (Zetterstedt, 1821) (John and Hewitt 1966; Wilmore and Brown 1975; Belda et al. 1991; Petitpierre 1996) or 1C = 8.55 to 10.15 pg for *C. brunneus* (John and Hewitt 1966; Wilmore and Brown 1975; Gosalvez et al. 1980). All studies of the two species mentioned above share the method of Feulgen densitometry and used testes to measure genome size. Hence it remains unclear whether this variation is natural or the result of methodological differences. However, it is more likely that the large intraspecific differences are a result of a combination of multiple factors: different populations analysed, lack of chromosome observations, various standards used and also different instrumentation could play some role.

The variation in genome size is even higher in Oedipodinae with a minimum of 1C = 5.28 pg for *Locusta migratoria* (Linnaeus, 1758) (Bier and Müller 1969) and a maximum of 1C = 18.48 pg in Stethophyma grossum. Hence, *S. grossum* represents the so far largest measured confirmed insect genome. A study by Schielzeth et al. (2014) measured much larger genome sizes for the Gomphocerinae species *C. biguttulus* with 1C up to 236.05pg.

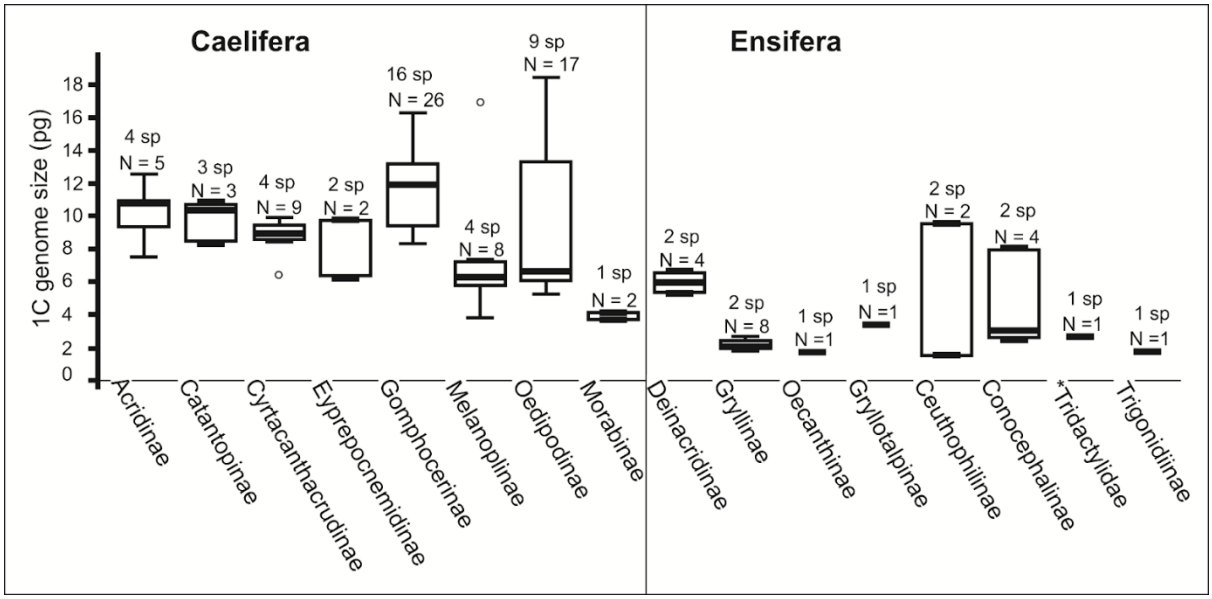

Figure 2. Genome Size variation in the different subfamilies of Orthoptera visualized as a boxplot. Provided is the number of measurements (N) and the number of species (sp) these measurements were derived of (some of the species were measured repeatedly by different authors). Most of the data excerpted from database Gregory (2020) completed with another original data comprehended in Table 2. *unknown species genome size was analysed, determined only on family level.

Due to the enormous variation of the estimates in the study and critical methodological issues, Camacho (2016) suggested that these estimates cannot be considered reliable. Hence, we consider our estimate of the *S. grossum* genome size as the current upper size of insect genomes. Since only very few species have been measured so far, it is expected that this is not the upper bound for genome sizes in grasshoppers or for insects in general.

The reasons for the large size of Caelifera genomes remain largely unknown. However, a recent paper by Shah et al. (2020) suggests that repetitive DNA and especially the expansion of satellite DNA may be a main reason for the large genomes in Orthoptera.

The most likely causes are genome duplications at the basis of the Acrididae, which would also explain their specifically high rates in nuclear mitochondrial pseudogenes (numts Bensasson et al. 2000; Song et al. 2008) posing difficulties to species identification using DNA barcoding and to phylogenetic reconstruction (Hawlitschek et al. 2017, Song et al. 2018). It may also explain why only a single incomplete genome is available to date (Wang et al. 2014). Grasshopper genome sizes remain a major obstacle to genomic research, and many further studies will be required to understand genome size variation and evolution in Orthoptera.

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DATA AVAILABILITY STATEMENT

All data generated and used in this article is included as tables and figures.

GEOLOCATION INFORMATION

All sampling for this study was performed 2019 in Hamburg, Georgswerder (Germany, 53.5097°N 10.0301°E).

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The candidate took part in the data collection and writing process of the manuscript.

Hamburg, 12.01.2023

Place/Date | Sign

Chapter 5.2

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New estimates of genome size in Orthoptera and their evolutionary implications

submitted to PLoS one

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ABSTRACT

Animal genomes vary widely in size, and much of their architecture and content remains poorly understood. Even among related groups, such as orders of insects, genomes may vary in size by orders of magnitude – for reasons unknown. The largest known insect genomes were repeatedly found in Orthoptera, e.g., *Podisma pedestris* (1C = 16.93 pg), *Stethophyma grossum* (1C = 18.48 pg) and *Bryodemella holdereri* (1C = 18.64 pg). While all these species belong to the suborder of Caelifera, the ensiferan *Deracantha onos* (1C = 19.60 pg) was recently found to have the largest genome. Here, we present new genome size estimates of 50 further species of Ensifera (superfamilies Gryllidea, Tettigoniidea) and Caelifera (Acrididae, Tetrigidae) based on flow cytometric measurements. We found that *Bryodemella tuberculata* (Caelifera: Acrididae) has the so far largest measured genome of all insects with 1C = 21.96 pg (21.48 gBp). Species with 2n = 16 and 2n = 22 chromosomes have significantly larger genomes than species with other chromosome counts. Gryllidea genomes vary between 1C = 0.95 and 2.88 pg, and Tetrigidae between 1C = 2.18 and 2.41, while the genomes of all other studied Orthoptera range in size from 1C = 1.37 to 21.96 pg. Reconstructing ancestral genome sizes based on a phylogenetic tree of mitochondrial genomic data, we found genome size values of >15.84 pg only for the nodes of *Bryodemella holdereri* / *B. tuberculata* and *Chrysochraon dispar* / *Euthystira brachyptera*. The predicted values of ancestral genome sizes are 6.19 pg for Orthoptera, 5.37 pg for Ensifera, and 7.28 pg for Caelifera. The reasons for the large genomes in Orthoptera remain largely unknown, but a duplication seems unlikely as chromosome numbers do not differ. Sequence-based genomic studies may shed light on the underlying evolutionary mechanisms.

Key words: Orthoptera, Caelifera, Ensifera, chromosomes, genome size

INTRODUCTION

Despite the enormous advances in sequencing technology, much of the structures and functions of genomes remain poorly understood. One of these is the 'C-value enigma' or 'C-value paradox' (1), which relates to the issue that different species have highly variable contents of non-coding DNA despite similar amounts of coding DNA. Large amounts of non-coding DNA and, consequently, large genomes pose problems to genomic sequencing and genome assembly. Even genetic studies based on single-read sequencing (i.e., Sanger) may become complicated due to the high prevalence of paralogs (2). Knowledge of at least the rough size of its genome is therefore a prerequisite for genomic studies on any organism. Unfortunately, the genome sizes of just relatively few species are known. The Animal Genome Size Database (3) holds records of 6,222 species as of 30 June 2022, representing less than 0.37% of the know 1.7 million species. Out of the more than 1 million described species of insects, the most diverse class of organisms, only 1,164 species have a total of 1,345 records in the Animal Genome Size Database. While some species with small genome sizes are well-known as model organisms, e.g., *Drosophila melanogaster* with 1C = 0.18 pg (4) and *Tenebrio molitor* with 1C = 0.52 pg (5), many have much larger genomes. One order with exceptionally large genomes is Orthoptera.

For several years, grasshoppers of the family Acrididae have held the records for the largest insect genomes. These were *Podisma pedestris* (1C = 16.93 pg; (6)), *Bryodemella holdereri* (1C = 18.64 pg; (7)) and *Stethophyma grossum* (1C = 18.48 pg; (8)). Satellite DNAs and transposable elements have been suggested as potential explanations for the large sizes (9,10). Complete genome duplications may be less likely, as there is a lack of correlation of chromosome number and genome size. Despite their mostly higher chromosome numbers, ensiferans typically have smaller genomes than caeliferans (11). Remarkably in this context, the most recent record holder for genome size in Orthoptera is the ensiferan *Deracantha onos* (1C = 19.60 pg; (12)). These studies, as reviewed by Gregory (3), show that genome sizes vary widely in grasshoppers and probably also all other groups of Orthoptera, warranting further investigation.

To obtain a better understanding of genome size variation in Orthoptera and its underlying evolutionary mechanisms, we generated estimates of genome size for 50 species of Orthoptera and used a mitogenomic phylogeny to track genome size across the evolution of the group. The main goals of this study were: 1) To provide measurements of the genome size of further species of Orthoptera and thus improve our knowledge on the range and variation of this character. 2) To track the evolution of genome size along the phylogenetic tree of Orthoptera. 3) To compare genome size data with chromosome numbers and, in the light of the XX/X0 sex determination system, discuss their implications for future studies.

MATERIALS AND METHODS

Sampling

We collected specimens at eight sites across Germany and one in Austria: Meadows around Motzen, Brandenburg, 52.2013 13.5892; Meadows around Pevestorf, Lower Saxony, 53.0610 11.4578; Railway parking area Munich Allach (Rangierbahnhof München Nord), Bavaria, 48.1902 11.5318; Eglinger Filz bogs near Wolfratshausen, Bavaria, 47.9016 11.5060; Upper Isar river near Sylvenstein, Bavaria, 47.5631 11.4746; Fröttmaninger Heide meadows North of Munich, Bavaria, 48.2128 11.6153; Alpiner Steig rock outcrops near Nittendorf, Bavaria, 49.0037 11.9680; Sudelfeld alpine meadows, Bavaria, 47.6835 12.0371; Rofanspitze alpine meadows, Tyrol, Austria, 47.4531 11.7892. We furthermore obtained specimens of some species that do not naturally occur in Germany from the pet trade. The identification of freshly collected species was based on morphological and bioacoustic characters (13,14). We follow the nomenclature of Cigliano et al. (15). Voucher specimens were deposited in the Zoological Museum Hamburg (ZMH), part of the Leibniz Institute for the Analysis of Biodiversity Change (LIB) under the accession ZMH 2019/21. A detailed list of all specimens and samples with individual accession numbers is given in Supplementary Table 1.

Genome size measurements

We measured the nuclear DNA content (2C) of samples using the flow cytometry method (FCM) as described in Sadilek et al. (16,17) (see also (8)). For every sample, we extracted the muscle tissue of one hind femur and homogenized it with a leaf of the internal plant standard *Pisum sativum* L. "Ctirad" (Fabaceae) with 2C = 9.09 pg (18,19) in 500 µl of Otto buffer I at 4°C. We then filtered the cell suspension through a 42 µm nylon mesh and split it in two halves. One half was stained with 1,000 µl DAPI solution (stock: 25 ml Otto buffer II, 1 ml DAPI, 25 µl 2-mercaptoethanol) and the second half with 1,000 µl propidium iodide (PI) solution (stock: 25 ml Otto buffer II, 1 ml RNase, 1 ml PI, 25 µl 2-mercaptoethanol), for several minutes. We conducted the analysis of the DAPI-stained sample in a Partec CyFlow instrument with an UV LED chip and the analysis of the PI-stained sample in a Partec SL instrument with a green solid-state laser (Cobolt Samba, 532 nm, 100 mW; Partec GmbH, Münster, Germany). 3,500 to 5,000 particles were recorded in each FCM analysis. We analyzed the output data with the Partec FloMax v. 2.52 software (Partec GmbH, Münster, Germany). Median coefficients of variation are 2.31 for DAPI and 3.81 for PI. All measurements and analyses were conducted at the Institute of Botany of Czech Academy of Sciences, Prague.

Combined DAPI and PI measurement results of the same specimen express the AT/GC ratio of the genome of the species, the GC content (e.g.: (16,17,20)). The GC content of *P. sativum* is 38.50% (21) and the GC content of the analysed samples was calculated with an Microsoft Excel macro (20). Measurements in pg were converted to base pairs $*10^9$ (Gbp) using the formula 1 pg = 0.978 Gbp (22).

Analyses of genome size data

We assembled a dataset of newly measured species and Orthoptera genome size measurements from previous studies, based on the Animal Genome Size Database (3) complemented with further recent studies. We then plotted the male genome sizes against the number of chromosomes (11) for all species for which both values were available; we used male genome size because more male measurements are available from the literature combined with our new data. We tested for statistical significance

using a Kruskal-Wallis test and pairwise Mann-Whitney tests (Bonferroni corrected) of all chromosome numbers with more than one record in PAST 4.03 (23). Finally, we used our data to calculate the difference between mean male and female genome size for each species where both sexes were available and tested for correlation of size differences between sexes and male genome size with Pearson's r.

We also tested for correlation between genome size and GC content among our new measurements, independently for female and male specimens. We then checked if GC content was generally different between females and males using a t-test. As this test resulted non-significant (Results), we used an ANOVA and pairwise Mann-Whitney tests (Bonferroni corrected) to test for differences in GC content between families.

The evolution of genome size in Orthoptera

In order to track the development of genome size along the evolutionary history of Orthoptera, we plotted the known genome sizes on a phylogenetic tree. We assembled a dataset of complete and partial mitochondrial genomes for tree reconstruction with our new measurements combined with data from GenBank and BOLD (24) (Supplementary Table 1). Out of the 146 species with known genome sizes, we found complete mitochondrial genomes available for 86 individuals belonging to 70 species. Under the rationale that all species included in the dataset should at least be represented in the mitochondrial genes COI, CytB, COII or ND5, we added 49 further species for which at least one of these additional mitochondrial markers was available. We aligned the dataset using MUSCLE (25) integrated in Geneious v.10.0.9 (26) and KALIGN (27). Since many regions of the mitogenome were represented in only relatively few specimens, we reduced the dataset to the genes Cytochrome C Oxidase I and II, Cytochrome B, and NADH Dehydrogenase 5.

We then reconstructed a Maximum Likelihood tree using the IQtree web server (28,29) with automatic substitution model selection, 1,000 Bootstrap alignments, and 1,000 iterations under a minimum correlation coefficient of 0.99. The single branch test was performed after 1,000 replicates, a perturbation strength of 0.5, and an internal IQ-Tree stopping rule of 100. Based on this tree, we reconstructed ancestral states of genome size

in the R v.3.6.3. environment (30) using the packages '*phytools'* (31) and '*ape*' (32). In species for which more than one measurement was available, we used the mean value.

RESULTS

Genome size variation

We provide newly measured genome size data for 103 individuals assigned to 50 species of the families Acrididae, Tetrigidae, Gryllidae, and Tettigoniidae, of which 38 species were measured for the first time (Table 1). The largest genome measured in this study is 1C = 21.96 pg (21.48 Gbp) and belongs to the speckled buzzing grasshopper *Bryodemella tuberculata* (Acrididae: Oedipodinae). The second largest genome belongs to *Chrysochraon dispar* (Acrididae: Gomphocerinae; 1C = 19.43 pg, 19.00 Gbp), followed by *Stethophyma grossum* (Acrididae: Oedipodinae; 1C = 18.51 pg, 18.10 Gbp).

Table 1: A list of genomic and cytogenetical data on all 50 species measured for this study. See Supplementary Table 1 for individual details. **N** = number of females and males analyzed, **1C** = average haploid genome size for females and males, **GC** = average content of GC basis in the genome of the species, **2n** = male diploid chromosome number, * = chromosome number available only for another species of the same genera.

Species	**N**	**1C F [pg]**	**1C M [pg]**	**GC [%]**	**2n**
Acrididae					
Bryodemella tuberculata	2F	21.92	-	42.05	22+X0
Calliptamus italicus	2F,1M	11.68	10.91	42.66	22+X0*
Euthystira brachyptera	2F	17.95	-	41.51	16+X0
Gomphocerippus rufus	2F	13.18	-	41.53	16+X0

Chorthippus albomarginatus	1F,1M	11.88	11.79	41.20	16+X0
Chorthippus apricarius	2F,1M	12.52	11.92	40.84	16+X0*
Chorthippus biguttulus	1M	-	10.99	41.80	16+X0
Chorthippus brunneus	1M		10.47	41.17	16+X0
Chorthippus dorsatus	2F,1M	12.59	12.80	41.39	16+X0
Chorthippus mollis	1M	-	11.58	41.50	16+X0*
Chorthippus pullus	1F	13.44	-	41.32	16+X0*
Chorthippus vagans	2F	11.11	-	41.30	16+X0
Chrysochraon dispar	1F,1M	19.43	18.76	41.43	16+X0
Locusta migratoria	1M	-	7.62	41.52	22+X0
Myrmeleotettix maculatus	2F	11.83	-	41.40	16+X0
Oedipoda caerulescens	2F	14.13	-	42.13	22+X0
Omocestus haemorrhoidalis	1F,1M	12.83	12.14	41.00	16+X0
Omocestus viridulus	1F,1M	14.03	13.28	41.11	16+X0
Pseudochorthippus montanus	1F,1M	13.12	12.42	41.22	16+X0*
Pseudochorthippus parallelus	2F,1M	13.14	12.67	41.83	16+X0
Psophus stridulus	1M	-	16.44	41.95	22+X0
Schistocerca gregaria	1F,1M	10.68	10.36	43.40	22+X0
Stenobothrus lineatus	1F,1M	14.00	13.63	41.25	16+X0
Stenobothrus nigromaculatus	1F,1M	13.18	12.48	41.05	16+X0*
Stenobothrus stigmaticus	1F,1M	11.91	11.21	41.24	16+X0*
Stethophyma grossum	1F	18.51	-	42.56	22+X0
Gryllidae					
Acheta domesticus	1F,1M	2.88	2.63	39.13	10+X0
Gryllus assimilis	1F,1M	2.24	2.09	38.58	28+X0
Gryllus bimaculatus	1F,1M	2.22	1.98	38.83	28+X0
Gryllus campestris	1F,1M	2.23	2.08	39.02	28+X0
Nemobius sylvestris	1F	2.56	-	36.41	16+X0
Oecanthus pellucens	1F,1M	1.44	1.37	39.97	18+XY

Tetrigidae					
Tetrix subulata	1M	-	2.22	35.62	12+X0
Tetrix tuerki	2F	2.37	-	36.08	12+X0*
Tetrix undulata	1F,1M	2.36	2.18	35.84	12+X0
Tettigoniidae					
Bicolorana bicolor	1F,1M	8.05	6.99	39.68	30+X0
Conocephalus dorsalis	1M	-	3.52	39.32	32+X0
Conocephalus fuscus	1F,1M	4.42	3.79	39.57	32+X0*
Decticus verrucivorus	2F,2M	8.21	7.34	41.01	30+X0
Leptophyes punctatissima	1F,3M	7.98	6.81	41.03	30+X0
Meconema meridionale	1F,1M	10.69	9.90	41.23	26+X0*
Meconema thalassinum	2F	12.72	-	40.85	26+X0
Metrioptera brachyptera	1F,1M	8.78	7.97	39.93	30+X0
Phaneroptera falcata	1F,1M	7.25	6.08	38.78	26+X0
Pholidoptera griseoaptera	2F,2M	7.11	6.30	40.73	30+X0
Pholidoptera littoralis	1F	7.69	-	40.20	30+X0*
Platycleis albopunctata	2F,2M	6.54	5.74	39.51	30+X0*
Roeseliana roeselii	1F,3M	8.30	7.70	40.30	30+X0
Tettigonia cantans	1F,1M	7.16	6.34	40.89	28+X0
Tettigonia viridissima	2M	-	5.69	42.88	28+X0

Table 2: An overview comparison of approximate genome sizes (1C) of Orthoptera families and subfamilies analyzed so far. Males and females of each taxon are analyzed together. See Supplementary Table 1 for individual details. Data for Deinacridinae are given in relative genome size as the samples were measured with DAPI stain. DAPI measurements are influenced by AT/GC ratio in the genome and are considered less accurate than the PI stain used in all other FCM values listed here.

	Species	MIN	MAX	Average	male 2n
CAELIFERA	**79**	**2.18**	**21.92**	**5.45**	**13,15,17,19,21,23**
Acrididae	**75**	**5.83**	**21.92**	**10.17**	**17,19,21,23**
Acridinae	5	7.50	12.55	10.43	23
Calliptaminae	3	9.64	11.68	10.41	23
Catantopinae	3	8.49	10.73	9.90	23
Cyrtacanthacridinae	4	8.63	10.68	9.28	23
Eyprepocnemidinae	3	6.34	9.70	7.42	23
Gomphocerinae	32	8.58	19.43	12.39	17,23
Melanoplinae	7	5.83	16.93	8.98	21,23
Oedipodinae	16	5.99	21.92	11.04	23
Pyrgomorphinae	1	7.55	8.21	7.88	19
Thrinchinae	2	13.51	14.45	13.96	19
Morabidae	**1**	**3.75**	**4.00**	**3.88**	**15**
Morabinae	1	3.75	4.00	3.88	15
Tetrigidae	**3**	**2.18**	**2.41**	**2.30**	**13**
Tetriginae	3	2.18	2.41	2.30	13
ENSIFERA	**63**	**0.95**	**19.14**	**4.92**	**11,13,15,17,20,21,23, 27,29,31,33,35,37**
Anostostomatidae	**2**	**5.40**	**6.53**	**5.97**	**15**
Deinacridinae	2	5.40	6.53	5.97	15
Gryllacrididae	**1**	**9.45**	**9.45**	**9.45**	**unknown**
Gryllacridinae	1	9.45	9.45	9.45	unknown
Gryllidae	**14**	**0.95**	**2.88**	**2.12**	**11,13,17,20,21,27,29**
Eneopterinae	1	2.09	2.35	2.22	unknown
Gryllinae	8	1.98	2.88	2.29	11,13,21,27,29

	Species	MIN	MAX	Average	male 2n
Nemobiinae	1	2.56	2.56	2.56	17
Oecanthinae	3	0.95	1.71	1.31	20
Podoscirtinae	1	2.23	2.23	2.23	unknown
Gryllotalpidae	**2**	**3.41**	**4.21**	**3.81**	**unknown**
Gryllotalpinae	2	3.41	4.21	3.81	unknown
Mogoplistidae	**1**	**3.08**	**3.48**	**3.28**	**unknown**
Mogoplistinae	1	3.08	3.48	3.28	unknown
Rhaphidophoridae	**3**	**1.55**	**9.55**	**5.44**	**29,35,37**
Aemodogryllinae	1	5.15	5.48	5.32	29
Ceuthophilinae	2	1.55	9.55	5.55	35,37
Tettigoniidae	**38**	**2.65**	**19.14**	**9.63**	**21,23,27,29,31,33**
Bradyporinae	2	12.71	19.14	15.80	29,31
Conocephalinae	9	2.65	10.05	6.09	21,23,33
Hexacentrinae	1	12.80	14.01	13.41	31,33
Meconematinae	3	4.38	12.44	8.96	27
Mecopodinae	1	13.45	14.58	14.02	27,29
Phaneropterinae	7	5.09	10.58	7.10	27,29,31
Pseudophyllinae	2	3.47	5.91	4.69	unknown
Tettigoniinae	13	5.34	8.78	6.97	29,31
Tridactylidae	**1**	**2,63**	**2,63**	**2,63**	**11,13**
Trigonidiidae	**1**	**1.93**	**1.93**	**1.93**	**15**
Trigonidiinae	1	1.93	1.93	1.93	15

Plotting genome size vs. chromosome numbers (Fig. 1) suggests correlations for some chromosome counts. The Kruskal-Wallis test of correlation between genome size and chromosome number was highly significant with $p = 1.196E^{-07}$. Mann-Whitney tests were significant ($p \leq 0.05$) or highly significant ($p \leq 0.001$, in the case of 2n = 22 and 2n = 32) for all pairwise comparisons of chromosome numbers of 2n = 16 with any other chromosome numbers except 2n = 14 and 2n = 18, and for most pairwise comparisons of 2n = 22 (Table 3).

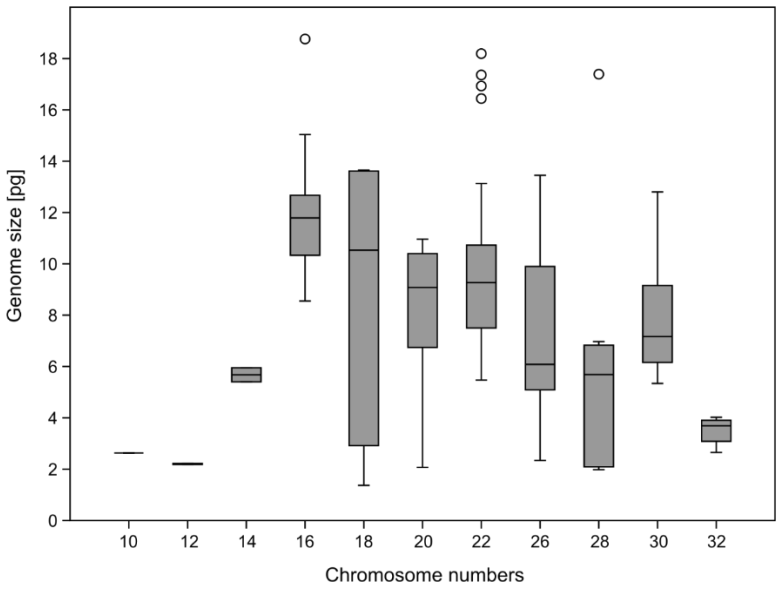

Figure 1: Box plot of number of chromosomes in male Orthoptera vs. genome size (1C [pg]).

Table 3: Mann-Whitney tests (Bonferroni corrected) for all pairwise comparisons of chromosome numbers of 2n = 16 and 2n = 22 with any other chromosome numbers except 2n = 18 and 2n = 20. * = significant ($p \leq 0.05$), ** = highly significant ($p \leq 0.001$). Kruskal-Wallis: Chi^2 49.76, $p = 1.196E^{-7}$

	12	14	16	18	20	22	26	28	30
14	0.2453								
16	0.0214*	0.0214							
18	0.4875	0.4875	0.8155						
20	0.2433	0.2433	0.0061*	0.9151					
22	0.0192*	0.0223*	0.0001**	0.9241	0.772				
26	0.057	0.6605	0.0084*	0.5083	0.5203	0.0962			
28	0.4521	0.9145	0.0002*	0.358	0.0927	0.0006**	0.3539		
30	0.0413*	0.1626	0.0013*	0.4367	0.3028	0.0577	0.6605	0.0452*	
32	0.0814	0.0814	0.0004**	0.2703	0.0828	0.0003**	0.0513	0.2446	0.0027*

The genome size differences between sexes are given in Table 4, including 83 species. The highest genome size differences were detected within four species of Acrididae (all 2n = 16+X0) – with maximum of 2.52 pg in *Gomphocerippus rufus* (1C (male) = 10.66 pg), followed by *Chorthippus vagans* (2.43 / 8.68 pg), *Pseudochorthippus parallelus* (2.25 / 10.89 pg), and *Schistocerca gregaria* (2.13 / 8.55 pg). The next largest differences in the genome size were found in Tettigoniidae – with maximum of 1.75 pg in *Deracantha onos* (1C (male) = 17.39 pg, 2n = 28+X0). Expectably, we found a significant correlation between larger genome size differences between sexes and larger male genome sizes (p = 0.005*). The difference in genome size between female and male (XX/X0) can be interpreted also as the size of the sex chromosome X. Negative size differences, i.e., larger genome sizes of males than of females, were detected in *Myrmeleotettix maculatus* (Acrididae; 1C (male) = 12.14 pg, 2n = 16+X0) with -0.31 pg and *Chorthippus dorsatus* (Acrididae; 1C (male) = 12.80 pg, 2n = 16+X0) with -0.21 pg.

Table 4: The genome size difference [pg] between the male and female measured by FCM, given for each species of completed dataset if data for both sexes were available (species from a single study was preferred). See Supplementary Table 1 for individual details. Data for Anostostomatidae are given in relative genome size due to the samples were measured with DAPI stain which is AT specific.

* = difference calculated between specimens from diverse studies, both measured by FCM method

** = difference calculated between specimens from diverse studies, one measured by FCM and second by FD method

Acrididae

Acrida cinerea	0.60	*Melanoplus differentialis*	0.47
Atractomorpha sinensis	0.66	*Myrmeleotettix maculatus*	-0.31**
Bryodemella holdereri	0.45	*Oedaleus asiaticus*	0.59
Calliptamus abbreviatus	0.39	*Oedaleus infernalis*	0.56
Calliptamus barbarus	0.41	*Omocestus haemorrhoidalis*	0.69
Calliptamus italicus	0.77	*Omocestus viridulus*	0.87
Campylacantha olivacea	0.83	*Pararcyptera microptera meridionalis*	0.75
Chorthippus albomarginatus	0.09	*Pedopodisma tsinlingensis*	0.88

Chorthippus apricarius	0.60	*Pseudochorthippus montanus*	0.70
Chorthippus biguttulus	0.32*	*Pseudochorthippus parallelus*	2.25
Chorthippus dorsatus	-0.21	*Schistocerca gregaria*	2.13*
Chorthippus vagans	2.43**	*Shirakiacris shirakii*	0.49
Chrysochraon dispar	0.67	*Sinopodisma qinlingensis*	0.39
Epacromius coerulipes	0.41	*Sphingonotus caerulans*	0.76
Euchorthippus unicolor	0.87	*Stenobothrus lineatus*	0.37
Filchnerella rubimargina	0.70	*Stenobothrus nigromaculatus*	0.70
Fruhstorferiola huayinensis	0.32	*Stenobothrus stigmaticus*	0.70
Gomphocerippus rufus	2.52*	*Stethophyma grossum*	1.15*
Haplotropis brunneriana	0.80	*Trilophidia annulata*	0.69
Locusta migratoria	0.97**		
Anostostomatidae			
Hemideina crassidens	0.61	*Hemideina thoracica*	0.58
Gryllidae			
Acheta domesticus	0.25	*Oecanthus pellucens*	0.07
Gryllodes sigillatus	0.20	*Oecanthus sinensis*	0.13
Gryllus assimilis	0.15	*Teleogryllus emma*	0.27
Gryllus bimaculatus	0.24	*Xenogryllus marmoratus*	0.26
Gryllus campestris	0.15		
Mogoplistidae		**Rhaphidophoridae**	
Ornebius kanetataki	0.40	*Diestrammena* sp.	0.33
Tetrigidae			
Tetrix undulata	0.18		
Tettigoniidae			
Atlanticus sinensis	0.35	*Metrioptera bonneti*	0.56
Bicolorana bicolor	1.06	*Metrioptera brachyptera*	0.81
Conocephalus fuscus	0.63	*Microconema clavata*	0.34
Conocephalus gladiatus	0.52	*Neoconocephalus triops*	0.64
Conocephalus maculatus	0.30	*Phaneroptera falcata*	1.17
Conocephalus sp.	0.38	*Phaneroptera gracilis*	1.02
Decticus verrucivorus	0.87	*Pholidoptera griseoaptera*	0.81
Deracantha onos	1.75	*Platycleis albopunctata*	0.81
Ducetia japonica	0.87	*Pseudorhynchus crassiceps*	1.28

Elimaea berezovskii	0.74	*Roeseliana roeselii*	0.59
Hexacentrus unicolor	1.21	*Ruidocollaris sinensis*	0.97
Kuwayamaea brachyptera	1.53	*Ruspolia dubia*	0.61
Leptophyes punctatissima	1.17	*Ruspolia lineosa*	0.74
Meconema meridionale	0.79	*Tettigonia cantans*	0.82
Mecopoda elongata	1.13	*Zichya tenggerensis*	1.24

We found genome size and GC content highly correlated in both females ($p = 5.924E^{-12}$) and males ($p = 9.597E^{-06}$), with smaller genomes mostly having lower GC content. The GC content range was 35.86%-43.17% (N = 57, mean = 40.63±1.61%) for females and 35.58%-44.21% (N = 46, mean = 40.56±1.59%) for males. Female and male GC content did not differ significantly (p = 0.826). The ANOVA test of differences of GC content among the families Acrididae, Tetrigidae, Gryllidae, and Tettigoniidae was highly significant ($p = 5.965E^{-29}$), and all pairwise Mann-Whitney tests (Bonferroni corrected) among these families were significant or highly significant, except between Tetrigidae and Gryllidae (Table 5).

Table 5: A comparison of GC content between genomes of the families Acrididae, Tetrigidae, Gryllidae, and Tettigoniidae. Mean is given ± standard deviation. Mann-Whitney (MV) tests (Bonferroni corrected) are highlighted as * = significant ($p \leq 0.05$) or ** = highly significant ($p \leq 0.001$).

Family	**N**	**Max**	**Min**	**Mean**	**MV Tetri-gidae**	**MV Gryllidae**	**MV Tettigoniidae**
Acrdidae	49	43.62	40.61	41.58±0.64	0.016*	1.65E-06**	4.43E-09**
Tetrigidae	5	36.29	35.58	35.89±0.31		0.131	0.002*
Gryllidae	11	40.21	36.41	38.86±0.98			4.17E-04**
Tettigoniidae	38	44.21	38.7	40.46±1.00			

Evolutionary analysis

The phylogenetic tree based on mitochondrial genomes generated with IQtree is given as partial trees of Ensifera (Fig. 2) and Caelifera (Fig. 3) (see also Supplementary File 1 for the complete tree). The tree is overall well supported with bootstrap values of >95. Caelifera and Ensifera are found monophyletic, as are Gryllidea and Tettigoniidea within Ensifera. Caelifera is only represented by Acrididea, since no members of Tridactylidea were included. The genus *Meconema* is found as the sister group to all other Tettigoniidea, making Tettigoniidae paraphyletic with respect to all other families of this infraorder. All subfamilies of Tettigoniidae included are retrieved as monophyletic. The only genera of this group found paraphyletic are *Ruspolia* (with respect to *Neoconocephalus*) and *Metrioptera* (with respect to *Bicolorana*). Within Caelifera, both Tetrigidae and Acrididae, as well as all subfamilies of Acrididae included by us, were monophyletic. The genus *Chorthippus* is found polyphyletic: *Chorthippus pullus* is placed in a group containing *Myrmeleotettix*, *Omocestus*, and *Stenobothrus*. The remainder of *Chorthippus* is paraphyletic with respect to *Gomphocerippus*, *Gomphocerus*, and *Stauroderus*.

Plotting genome sizes on the tree shows consistent values of mean genome size in different clades of the Orthoptera tree (Fig. 4). The sizes of all Gryllidea genomes are between 1C = 0.91 pg (*Oecanthus sinensis*) and 2.88 pg (*Acheta domesticus*), with the exceptions of *Neoscapteriscus borellii* (3.41), *Ornebius kanetataki* (3.28) and *Gryllotalpa orientalis* (4.21). The values in Tetrigidae range between 2.22 and 2.36. On the other hand, all members of Tettigoniidea and Acrididae show values between 4.01 and 14.15. The exceptions are *Hadenoecus subterraneus* (1.55) at the lower end and *Stauroderus scalaris* (15.04 / 16.34), *Psophus stridulus* (16.44), *Podisma pedestris* (16.93), *Eusthystira brachyptera* (17.94), *Stethophyma grossum* (18.11), *Deracantha onos* (18.26), *Bryodemella holdereri* (18.41), *Chrysochraon dispar* (19.09), and *B. tuberculata* (21.92).

The ancestral state reconstruction found genome size values of >15.84 pg only for the nodes of *Bryodemella holdereri* / *B. tuberculata* and *Chrysochraon dispar* / *Euthystira brachyptera* (Supplementary File 1). The predicted genome size values are 1C = 6.19 pg for Orthoptera, 5.37 pg for Ensifera, and 7.28 pg for Caelifera.

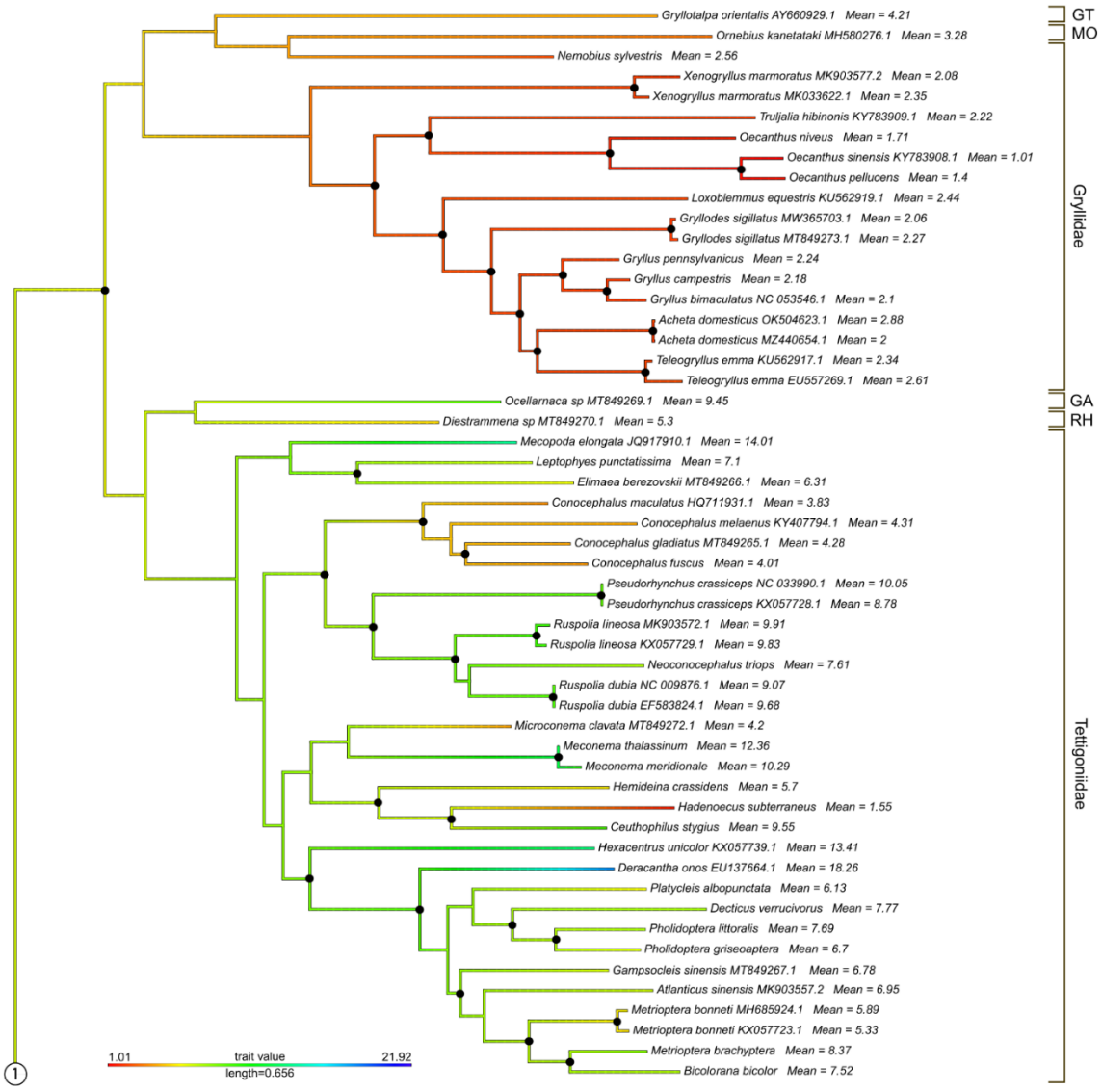

Figure 2: Maximum likelihood phylogenetic tree of the mitochondrial dataset of Orthoptera. Black dots on nodes represent Bootstrap support values of >95%. Branch colors code genome size (see legend). Mean genome size values are given for all tips. The circled connector "1" links this tree to the tree in Fig. 3. Higher taxa are abbreviated: GT = Gryllotalpidae, MO = Mogoplistidae, GA = Gryllacrididae, RH = Rhaphidophoridae.

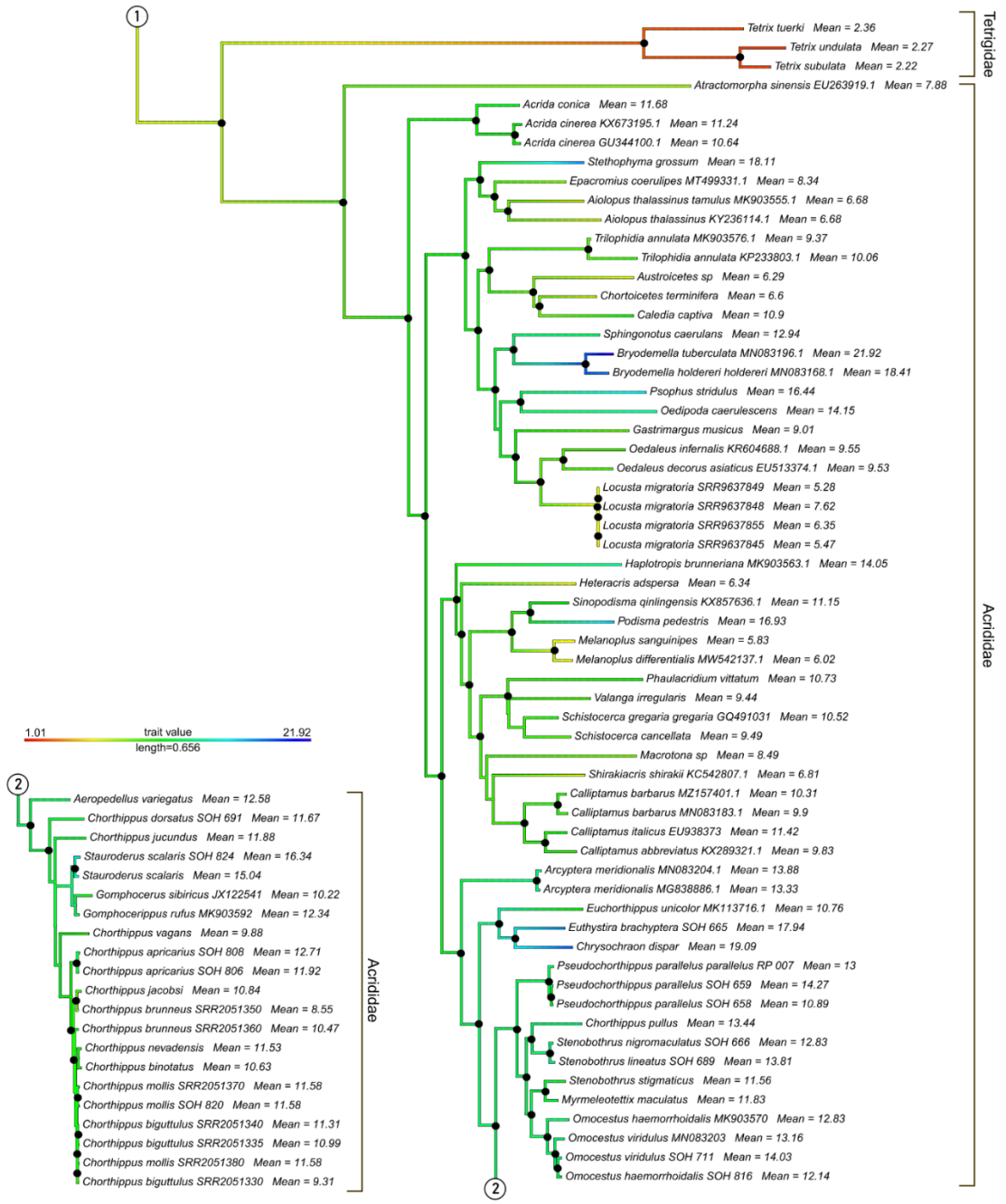

Figure 3: Maximum likelihood phylogenetic tree of the mitochondrial dataset of Orthoptera. Black dots on nodes represent Bootstrap support values of >95%. Branch colors code genome size (see legend). Mean genome size values are given for all tips. The circled connector "1" links this tree to the tree in Fig. 2. The circled connector "2" links parts of the tree shown in this figure.

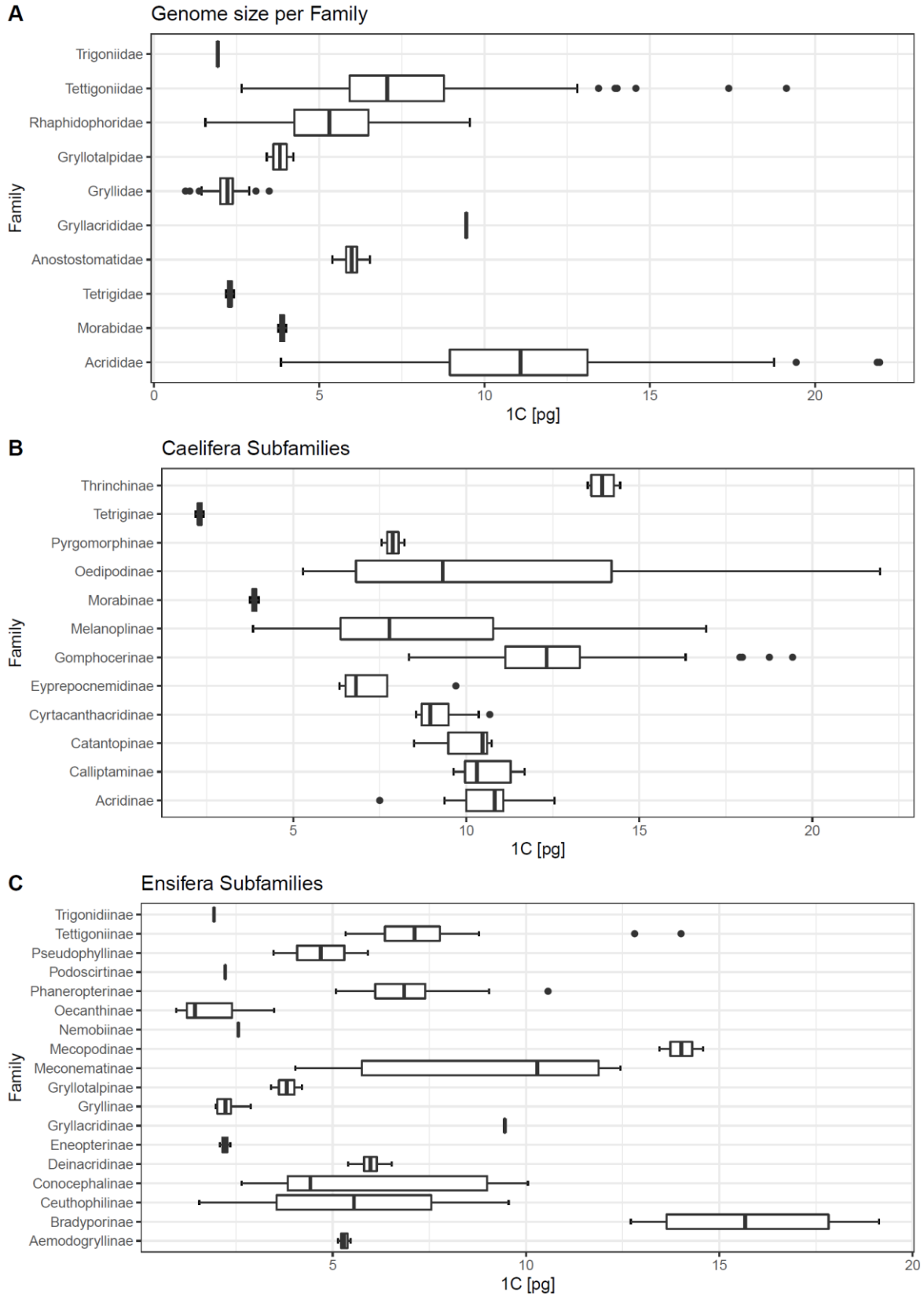

Figure 4: Box plots of genome size in families of Orthoptera (A), in subfamilies of Caelifera (B) and Ensifera (C).

DISCUSSION

The diversity in genome size is very likely an important parameter in organismal diversity research, yet it remains poorly studied. This is particularly true for insects, where genome size is known for less than 0.5 % of the described species (3). So far, the largest genomes of all insects have been detected among members of Orthoptera, exceeding the human genome by the factor seven. Nevertheless, data is available for only a small selection of species. We contributed measurement of 50 species, 38 measured for the first time, and added these to the known set of species. The variation in genome sizes was even larger than expected but does not follow a clear pattern.

Genome size variation in Orthoptera

The newly measured size of 1C = 21.96 pg of the genome *Bryodemella tuberculata* surpasses the previous records held by 1C = 19.60 pg in *Deracantha onos* (12), 1C = 18.48 pg in *B. holdereri* (7), 1C = 18.48 pg in *Stethophyma grossum* (8), and 1C = 16.93 pg in *Podisma pedestris* (6). We are not aware of any insect with a larger genome published in the meantime. Therefore, *B. tuberculata* holds the record for the largest known insect genome size. The genome size of *Chrysochraon dispar* (1C (female) = 19.43) also surpasses that of all previously known genomes of Caelifera. Furthermore, the size of the largest genome measured of *Stethophyma grossum* slightly exceeds the value Husemann et al. (8) found in that species. This intraspecific or measurement variation is within the ranges detected in other similar studies (7,12).

Schielzeth et al. (33) provided a measurement of the genome size of *Chorthippus biguttulus* (Acrididae) of 1C = 236.05 pg, which would exceed our measurement of *B. tuberculata* by an order of magnitude. We measured the genome size of *Ch. biguttulus* as 1C = 10.99 pg, which is in line with the measurements of Shah et al. (9) (1C = 9.31 pg) and Husemann et al. (8) (1C = 11.31 pg). This suggests that, as commented by Camacho (34), the measurement of Schielzeth et al. (33) was indeed unreliable and does not represent a true value.

Within Acrididae, the largest genomes belong to representatives of the subfamilies Oedipodinae (maximum: *Bryodemella tuberculata*, 2n = 22+XX, 1C = 21.96 pg), Gomphocerinae (*Chrysochraon dispar*, 2n = 16+XX, 1C = 19.43 pg), and Melanoplinae (*Podisma pedestris*, 2n = 22+X0, 1C = 16.93 pg). Fig. 1 shows that species with male chromosome counts of 2n = 16+X0, followed by 2n = 22+X0, have the largest genomes (11). All these species belong to the family Acrididae. The representatives of other families of Caelifera, Tetrigidae, and Morabidae have far smaller recorded genomes. Overall, the genomes measured in Orthoptera so far span a large size range from less than 1 GB in some crickets to more than 20 GB as measured here for Oedipodinae. This suggests complex evolutionary processes underlying the evolution of genomes in Orthoptera, which will have to be explored in the future. So far, few Orthoptera genomes have been sequenced (35) owing to their large size, but comparative genomic analyses across genomes of different sizes will be necessary to understand the genome gigantism in this group.

Our study adds to other recent works (7,8,12), in providing new records of the largest genome size in Orthoptera and at the same time in all insects by studying just a comparatively limited number of species. We consider it very likely that future studies will discover even larger genomes in Orthoptera or among members of another insect order.

The evolution of genome size

In order to study the evolution of genome size in Orthoptera, we plotted the known measurements on a phylogenetic tree based on mitochondrial data. We selected mitochondrial data because it was available for a large number of species included in our genome size dataset (110 out of 146 = 75.3%). The tree obtained by Maximum Likelihood reconstruction is largely congruent with other trees available for Orthoptera so far (36–40). However, it does not resolve the positions of Gryllotalpidae (*Gryllotalpa*), Mogoplistidae (*Ornebius*), and Trigoniidae (*Nemobius*), which have been placed as hierarchical sister groups to Gryllidae in other studies (40,41). We found Tettigoniidae paraphyletic with respect to a clade consisting of Anostostomatidae (*Hemideina*) +

Rhaphidophoridae (*Ceuthophilus* + *Hadenoecus*) that is placed as sister group to Meconematinae, albeit with poor support of 70% Bootstrap (Supplementary File 1). Note that *Hemideina* measurements were generated with the DAPI stain, which is influenced by AT/GC ratio in the genome and therefore considered less accurate than the PI stain used in all other FCM values listed here. *Acrida* was retrieved as the sister group to all other Acrididae, which contrasts with previous hypotheses (37,40).

The reconstructed paraphyly of genera *Ruspolia* and *Metrioptera* most likely reflects the urgent need of revising their taxonomy. The genus *Chorthippus* is notoriously complicated, and a revision will have to be based on more comprehensive data, as mitochondrial datasets have been shown to yield phylogenetic results incongruent to those based on larger genomic datasets (42).

Our phylogenetic tree also shows that exceptionally large genome sizes (more than 1C = 16 pg) are attained only in isolated clades. This result is certainly restricted by our dataset, which includes only few representatives of many clades. Nevertheless, it shows that large genome sizes are characteristic of only single genera (*Bryodemella*, *Deracantha*, *Stethophyma*) or closely related genera, e.g. *Chrysochraon* + *Euthystira*. Husemann et al. (43) estimated the split of *Bryodemella* from *Sphingonotus* to about 31.8 million years ago (ma) Song et al. (38). The split of *Euthystira* from *Euchorthippus* was estimated to 12.88 [15.69–9.96] ma by Hawlitschek et al. (42). There are no estimates for the split of *Stethophyma* from *Epacromius* + *Aiolopus*. Estimating the age of the lineage of *Deracantha* is difficult due to its contradictory placing in phylogenetic trees by Mugleston et al. (36) and Yuan et al. (12), but lineages in Ensifera are typically older than in Caelifera. The splits from related clades with smaller genome sizes (e.g., *Phaneroptera*) have been dated to around 100 ma in these studies. Based on these estimates, some large genomes may be of comparatively old evolutionary age. However, the increase in genome size is not necessarily related to the splitting of lineages we were able to detect. Duplications may have played a role for speciation with subsequent merging of chromosomes to the original number, but due to lack of evidence this remains speculation. Much finer phylogenetic resolution at the genus and species level will be required to track the evolution of genome size in individual clades more reliably.

The relationship of genome size with life history and cytogenetic traits

No previous studies have been able to answer the question as to why some species of grasshoppers have such large genomes. Some of the earlier record keepers, *Podisma pedestris* and *Stauroderus scalaris*, as well as *Bryodemella tuberculata*, are species of montane habitats in Central Europe today. However, this does not hold true for their current global and historical European distributions (15). Our sampling for this study was restricted to central Europe, but more species of other regions need to be studied to detect possible correlations between genome size and ecological or geographic variables.

Hypotheses on a correlation between life history traits and genome size have also been raised, e.g., body size and the ability to fly (44–46). Larger body size was hypothesized to correlate with larger genomes, whereas the genomes of flying species were hypothesized to be smaller than those of flightless species. Yuan et al. (12) tested for any correlation of these traits with genome size in their dataset of tettigoniid ensiferans but found none. We did not test this in our dataset because it covers a wide phylogenetic range at rather coarse taxonomic resolution, and a finer scale will probably be necessary to detect any such correlation. Caeliferan species with particularly large genomes, such as *Chrysochraon dispar* and *Podisma pedestris* are flightless (with the exception of rare long-winged individuals), whereas *Bryodemella tuberculata* and *Stethophyma grossum* are good fliers (15). Among the Ensifera, *Deracantha onos* is large-bodied and flightless, as is *Hemideina crassidens*, whose genome is substantially smaller (1C = 5.7 pg). No other large flightless ensiferan genome has been analyzed, which makes the search for correlation between these traits and genome sized difficult.

Our dataset includes two cave-dwelling species, *Ceuthophilus stygius* and *Hadenoecus subterraneus* (both Gryllidea – Rhaphidophoridae – Ceuthophilinae). While both species have very similar chromosome counts (36+X0 vs. 34+X0), the genome of *C. stygius* is almost the five-fold size of that of *H. subterraneus* (9.55 pg vs. 1.55 pg). It is therefore difficult to speculate if the adaptation to the cave environment has any consequences for genome size. Notably, Gryllidea are overall very heterogeneous regarding genome size (0.95 pg to 9.55 pg) and chromosome count (10+X0 to 36+X0).

Other than life history and ecology, genome size has been hypothesized to correlate with traits of cytogenetics and genome architecture. Several studies found large genomes, including those of orthopterans, rich in satellite DNA, long terminal repeats and

transposons, including helitrons, and transposons, and mariner like elements (9,10,47–49). Whole genome duplications are another presumably common reason for large genome size (e.g. in fish (50)), going along with a polyploidization and a large number of chromosomes. However, such a relation was not found in Orthoptera. Intuitively, taxa with more chromosomes might be expected to also have larger genome sizes. Our analysis does not suggest any positive correlation of chromosome number and genome size. Some taxa with especially small numbers of chromosomes, such as European Gomphocerinae, have some of the largest genomes (8). This suggests that the chromosome number reduction is associated with fusions rather than the actual loss of chromosomes.

On the other hand, we found, despite an overall rather narrow range of GC content, a correlation between genome size and GC content. Larger genomes had a generally higher GC content. There was no indication of difference in GC content between sexes, but the families studied here differed significantly. Acrididae and Tettigoniidae (with large genomes) were found to have genomes with higher GC content compared to Tetrigidae and Gryllidae (with small genomes). The general implications of GC content on animal genomes are not well studied. Low GC content may be an indicator of the presence of bacterial endosymbionts (51), whereas high GC content may be a sign of low chromatin condensation (52). How these phenomena affect insects has not been studied (53).

As the majority of Orthoptera investigated, most species included in our study follow an XX/X0 sex determination system, implying that female genomes should be larger than male genomes just due to the additional sex chromosome X. The same can be assumed for species with XX/XY (here in *Oecanthus*), as neo-Y chromosomes should be smaller than X chromosomes (54). We find this reflected in the difference between female and male genomes of most species. However, the differences are minuscule in some species and even inverted (with male genomes larger than female genomes) in *Chorthippus dorsatus* and *Myrmeleotettix maculatus* (both Acrididae). In the case of *M. maculatus*, the inversion can most likely be attributed to different methods used to measure genome size (Feulgen densitometry vs. Flow cytometry) of males and females in different studies. Conversely, the specimens of *Ch. dorsatus* were from the same locality and

measured in the same workflow, suggesting real intraspecific variability. The presence of B chromosomes might offer an explanation for the larger genome size of males than females, but no such phenomena have been reported for this species (55,56) and chromosomes of specimens were not analyzed in the present study. As larger genome sizes in males than females of XX/X0 species contradicts the presence of the female X chromosome, conclusions drawn on intraspecific difference in genome size will have to be backed by much larger sample sizes. However, we uphold that the comparison of our present genome size measurements with the same species reported by previous studies show sufficient overall congruence to allow for interspecific comparisons and the tracking of genome size evolution.

Finally, genomic sequence data will be necessary to investigate the reasons behind the huge genomes of Orthoptera. Currently, better transcriptome and genome assemblies are on the way which may help to better understand the reasons for the large sizes of Orthoptera genomes (35,57).

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SUPPLEMENTARY MATERIAL

Supplementary Table 1: Complete table of all genome size measurements of Orthoptera reviewed for this study.

Supplementary File 1: The Maximum Likelihood phylogenetic tree reconstructed in IQtree.

All supporting information can be found on page 380 and following.

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Chapter 5.3

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Evolution of chromosome number in grasshoppers (Orthoptera: Caelifera: Acrididae)

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ABSTRACT

Orthoptera have some of the largest genomes of all insects. At the same time, the architecture of their genomes remains poorly understood. Comparative cytological data across a wide range of taxa, even for basic parameters such as chromo- some number, may provide important insights into the evolution of these genomes and help answer the question of why some species attained such large genome sizes. We collected and compiled more than 1,000 records of chromosome numbers of 339 genera (13.8% of 2,452 known genera) and 769 species (6.2% of 12,250 known species) of Caelifera, the suborder of Orthoptera that includes those taxa with short antennae. Within the family Acrididae, most of the records come from the subfamilies Oedipodinae (N = 325), Melanoplinae (N = 192) and Gomphocerinae (N = 254). Out of the 621 investigated species of Acrididae, 459 (73.9%) shared a chromosome number of 2n♂ = 23. Chromosome numbers of 2n♂ = 17 (12.2%) and 2n♂ = 21 (9.9%) were less common. The remaining 4.0% of species exhibited different chromosome numbers between 2n♂ = 8 (6 + XY) and 2n♂ = 27. Plotted on a phylogenetic tree, our results confirm that chromosome numbers, especially in the largest grasshopper family Acrididae, are highly conserved with a basic count of 2n♂ = 23 (22 + X0), sometimes reduced to, e.g., 2n♂ = 17 (16 + X0) in some genera of the slant-faced grasshopper subfamily Gomphocerinae. Species with divergent chromosome numbers occur in many of the groups we studied, but are not a systematic trait and have evolved multiple times independently. Our study supports the view that chromosome numbers are much more stable across the investigated Caelifera compared to Ensifera, the second suborder of Orthoptera that includes the long antennae bush crickets and crickets. Our results significantly extend our knowledge on the diversity of this character in Caelifera.

Keywords Chromosomal evolution, Cytogenetics, Genome architecture, Karyotype

INTRODUCTION

Prior to the genomic era, cytogenetic studies provided the foundation for our understanding of animal genome organization (Bugrov, 1988, 1996; Bugrov & Vysotskaya, 1981; Confalonieri et al., 1998; Gokhman & Kuznetsova, 2006; King, 1995; Kirkpatrick, 2010; Vandergast et al., 2017). While genetic and genomic sequencing have become far more popular fields of research, cytogenetic studies still provide important information about the genomic organization of a species and clues to the evolution of whole groups of taxa (White, 1973). They have been used to address a variety of systematic, evolutionary and phylogenetic questions in plants and animals and have helped to improve our understanding of speciation (Charlesworth, 2004; Charlesworth & Charlesworth, 2005; Grzywacz et al., 2019; Navarro & Barton, 2003). Comparative cytogenetics implements relatively simple studies of chromosome numbers and morphology, but it may also include more complex analyses of various banding patterns or highly specified gene probes with fluorescent staining (White & Solt, 1978; Zhong et al., 1996; Gokhman & Kuznetsova, 2006; Bishop, 2010). While these complex methods allow fine-scale analyses on the level of populations, comparative studies of chromosome numbers may give us insight into the higher levels of evolutionary processes.

Grasshoppers of the family Acrididae (Orthoptera: Caelifera) have been the target of intense cytogenetic studies (Cigliano et al., 2021). This group has been suggested to be relatively uniform in their chromosome number, with some exceptions (Hewitt, 1979; John & Hewitt, 1966). While a diploid chromosome number of 2n♂ = 23 (22 + X0) is considered the basic plan for Acrididae (Hewitt, 1979), different kinds of rearrangements, especially Robertsonian fusions, led to a reduction in chromosome number in some groups of Caelifera (e.g., many Eurasian Gomphocerinae have 2n♂ = 17 (16 + X0) chromosomes). McClung (1917) considered this variation in the number of chromosomes to be a matter of rearrangements of chromatin rather than a result of the loss or gain of individual chromosomes. Besides this, some variation in the sex determining system has led to variation in chromosome number. In general, loss of the

Y chromosome led to the highly conserved sex chromosome pattern of X0♂/XX♀ found in most species. Due to several chromosome rearrangements (autosomes and sex chromosomes), some species evolved several alternative sex determining systems, e.g., neo-XY♂/XX♀ or even neo-X1X2Y♂/ neo-X1X1X2X2♀ or X1X20♂/X1X1X2X2♀ (Palacios-Gimenez et al., 2013, 2018; Castillo et al., 2010; Hewitt, 1979; White, 1973) leading to some variation in chromosome number and providing a possible basis for reproductive isolation in some species groups.

Despite some exceptions, in comparison with its sister group, Ensifera (katydids, crickets and allies), the variation in chromosome number is relatively lower in Caelifera. Also, in general, the chromosome number appears to be lower in Caelifera compared to most Ensifera, as Warchałowska-Śliwa (1998) reported a basic number of 2n♂ = 31 (30 + XO) chromosomes in males of most of the investigated subfamilies of the family Tettigoniidae. Interestingly, genome sizes are, regardless of the chromosome number, much smaller in Ensifera compared to Caelifera, which may suggest some duplication events at the advent of the diversification of Caelifera (Husemann et al., 2021; Mao et al., 2020). A recent meta analysis of Polyneoptera showed that many interacting factors underlie chromosome variation (Sylvester et al., 2020). Warchałowska-Śliwa (1998) summarized the cytogenetic information of about 400 species of Tettigoniidae with the aim of tracing the evolution of chromosome number in that ensiferan family. Such a systematic review and analysis of chromosome number and evolution is lacking for the diverse caeliferan family Acrididae. Hence, with the aim of closing this gap, we provide new karyotype data of 36 species (and additional estimates of 8 previously investigated species) of Acrididae and assembled a dataset of 1,284 records of chromosome numbers for Caelifera representing 339 genera (13.8% of 2,452 known genera) and 769 species (6.2% of 12,250 known species), including 1,108 records of Acrididae. We provide an overview of the variability of karyotypes for several subfamilies of Acrididae and map chromosome numbers on the most recent phylogeny of Caelifera (Song et al., 2018) in order to get an insight into the evolution of chromosome number in this diverse group of grasshoppers.

MATERIAL AND METHODS

Material examined

We collected male grasshoppers belonging to 16 species of Oedipodinae by sweep net sampling on field trips between 2014 and 2016 (SI 1). Voucher specimens were deposited at the entomological collection of the Zoological Museum Hamburg, Germany (ZMH). David B. Weissman and David Lightfoot have collected and analyzed western US grasshoppers over the years and we included 28 unreported results herein (DBW, unpubl. Data).

Cytogenetic analyses

We dissected and fixed testes of the collected specimens in the field in a solution consisting of three parts of ethanol– acetic acid (3:1, v/v). Specimens were fixed in 99.9% ethanol after dissection. The samples were subsequently stored in a freezer at -20 °C until further processing. NU conducted chromosome analysis: Testes were stained with an alcohol–carmine solution for several hours, before being transferred to glass slides for squash preparation, chromosome counts and microscopic imaging (see Lightfoot et al., 2011; Ueshima & Rentz, 1979). DBW material was analyzed as in Rentz & Weissman (1984).

Chromosome mapping and ancestral state reconstruction

We added our newly generated data to a large dataset based on previously published data: We screened the literature and additionally included all unique records from two online databases: www. bchrom. csic. es and www. coleo guy. github. io/ karyo types. In total, we gathered 1,284 records of chromosome numbers of Caelifera, including the 1,108 records of Acrididae used in our analyses. Throughout the manuscript, we only

show the male chromosome numbers if the normal X0 sex determining system is realized. In cases of deviating sex chromosome configurations, these are noted.

We visualized the distribution of male chromosome numbers of Acrididae as a histogram in R using the packages *ggplot2* (Wickham, 2016), ggpubr (Kassambara, 2020), *scales* (Wickham & Seidel, 2020) and *cowplot* (Wilke, 2019). All subfamilies were colored to display differences in numbers between taxa.

We mapped male chromosome numbers on the most recent phylogeny of the group (Song et al., 2018) using the R packages *BiocManager* (Morgan, 2019), *phytools* (Revell, 2012), *vctrs* (Wickham et al., 2020), *ggplot2* (Wickham, 2016), *ggtree* (Yu et al., 2018, 2017), *gtable* (Wickham & Pedersen, 2019), *grid* (R Core Team, 2020), *ggstance* (Henry et al., 2020) and *tidyverse* (Wickham et al., 2019). A parsimony-based ancestral state reconstruction of chromosome number was done in Mesquite v. 3.51 (The Mesquite Project Team., 2019) based on a character matrix approach. We chose this reconstruction method due to missing data of several groups within the tree and several chromosome number configurations occurring only single times throughout the evaluated taxa. Parsimony approaches for ancestral state reconstruction are known to be as accurate in state reconstruction of deep and shallow nodes as likelihood approaches (Holland et al., 2020). We performed all R analyses in R version 3.6.3 (R Core Team, 2020).

RESULTS

Cytogenetic analyses

All species newly analyzed here had a karyotype of 2n♂ = 23 chromosomes with no variation or heteromorphism in any of the specimens studied (Fig. 1, SI 1) with the exception of *Teicophrys californiae* Descamps, 1977, which had 2n♂ = 17. All chromosomes were acrocentric or telocentric.

Review of chromosome numbers in Caelifera

We assembled records of chromosome numbers for 1,284 records of Caelifera, including 1,108 records for Acrididae. The data include 769 species in 339 genera of Caelifera (SI 1). We found multiple records for many species, some of which documented variation in the chromosome count of some species. Specifically, we found documented differences for *Miramella alpina* (Kollar, 1833), *Bucephalacris bohlsii* (Giglio-Tos, 1898), *Circotettix coconino* Rehn, 1921, *C. crotalum* Rehn, 1921, *C. undulatus thalassinus* Saussure, 1884, *Trimerotropis cyaneipennis* Bruner, 1889, T. *gracilis gracilis* (Thomas, 1872), *T. ochraceipennis* (Blanchard, 1851), *T. sparsa* (Thomas, 1875) and *Podisma pedestris* (Linnaeus, 1758) with 2n♂ = 21 and 2n♂ = 23 individuals reported; *Scyllinula humilis* (Blanchard, 1851), *Dichroplus maculipennis* (Blanchard, 1851) and *Leiotettix sanguineus* Bruner, 1906 with 2n♂ = 22 (20 + XY) and 2n♂ = 23*; Orphulella punctata* (De Geer, 1773) and *Chortoicetes terminifera* (Walker, 1870) with 2n♂ = 17 and 2n♂ = 23; *Leiotettix politus* Rehn, 1913 with 2n♂ = 13 and 2n♂ = 14 (12 + XY); *Dichroplus pratensis* Bruner, 1900 with 2n♂ = 18 (16 + XY) and 2n♂ = 22 (20 + XY); *Oedipoda schochii* Brunner von Wattenwyl, 1884 2n♂ = 23 and 2n♂ = 25; *Gomphocerus sibiricus* (Linnaeus, 1767) 2n♂ = 17 and 2n♂ = 19; *Dichroplus vittatus* Bruner, 1900 2n♂ = 18 (16 + XY) and 2n♂ = 20 (18 + XY); and *Dichroplus fuscus* (Thunberg, 1815) 2n♂ = 19 and 2n♂ = 23. Such discrepancies within a species should be reinvestigated as these differences, if reconfirmed, potentially represent cryptic species situations.

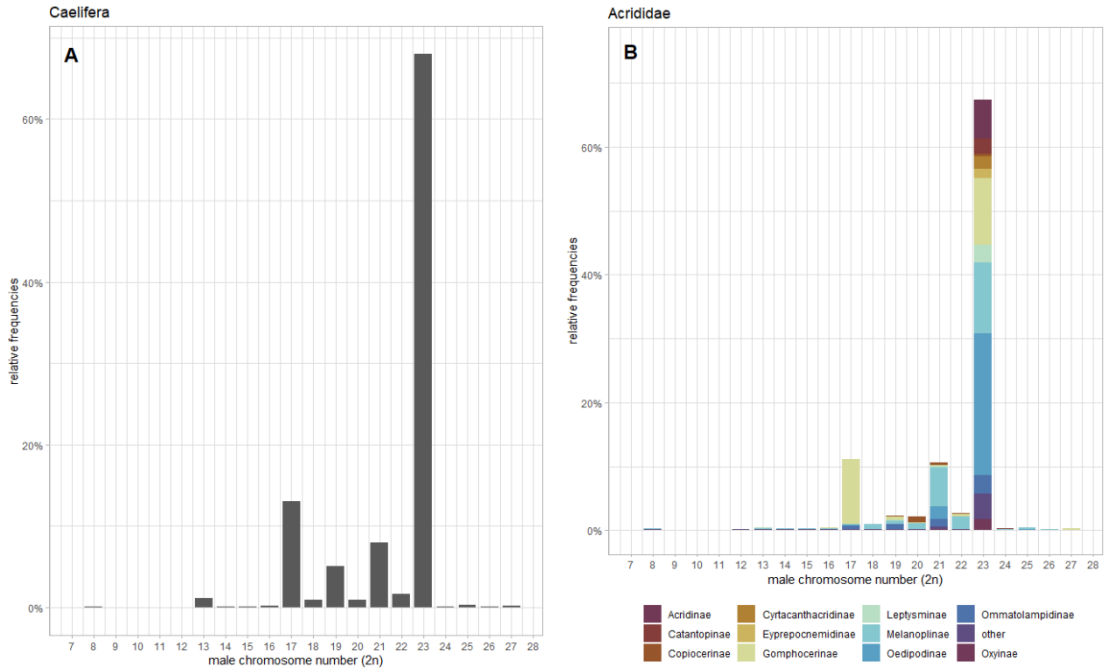


Figure 1: Histogram displaying A: the distribution of chromosome numbers across Caelifera and B: chromosome numbers across the different subfamilies of Acrididae. Chromosome numbers are shown as relative frequencies in percent; here, just subfamilies with more than ten records are shown as separated units. Subfamilies with lower sample sizes are aggregated in the unit other (Calliptaminae, Coptacrinae, Euryphyminae, Hemiacridinae, Marelliinae, Paulinііnae, Pezotettiginae, Proctolabinae, Rhytidochrotinae, Spathosterninae and Tropidopolinae).

For mapping and ancestral state reconstruction, we also included some more general records at the family or subfamily level with missing species identification based on the data from White (1973). Within the family Acrididae, most of the records come from the subfamilies Oedipodinae (N = 325), Melanoplinae (N = 192) and Gomphocerinae (N = 254). Out of the 621 investigated species of Acrididae, 459 (73.9%) shared a chromosome number of 2n♂ = 23. Chromosome numbers of 2n♂ = 17 (12.2%) and 2n♂ = 21 (9.9%) were less common. The remaining 4.0% of species exhibited different chromosome numbers between 2n♂ = 8 (6 + XY) and 2n♂ = 27. A chromosome number of 2n♂ = 17 was found mostly in Gomphocerinae (Stenobothrini, Gomphocerini and European Chrysochraontini), while 2n♂ = 21 was found mostly in Melanoplinae and some Oedipodinae (*Trimerotropis* Stål, 1873 and *Circotettix* Scudder, 1876). The lowest number of chromosomes of all Caelifera studied so far was found in *Dichroplus silveiraguidoi*

Liebermann, 1956 with 2n♂ = 8 (6 + XY) (Mesa et al., 1982). A high number with 2n♂ = 25 was found in *Oedipoda schochii* Brunner von Wattenwyl, 1884 (Türkoglu & Koca, 2002) and *Conometopus sulcaticollis* (Blanchard, 1851) (Mesa et al., 1982). The highest number with 2n♂ = 27 was found in *Dichroplus intermedius* Ronderos, 1976 (Türkoglu & Koca, 2002). Caelifera show more deviation from the typical 2n♂ = 23 than Acrididae alone. The number of 2n♂ = 19 is common within Pamphagidae (e.g., *Melanotmethis* Uvarov, 1943, *Pezotmethis* Uvarov, 1943, *Strumiger* Zubovski, 1896) and several Eumastacidae genera (e.g., *Phytomastax* Bey-Bienko, 1949, *Gomphomastax* Brunner von Wattenwyl, 1898, *Clinomastax* Bey-Bienko, 1949) (e.g., Bugrov, 1986, 1988, 1996; Bugrov et al., 1991; Vysotskaya, 1983; White, 1968). Interestingly the Tetriginae genera are known to be more variable in their chromosome number configuration. Here, several species of the genus *Tetrix* have a common chromosome number of 2n♂ = 13 (Bugrov, 1996).

Mapping and ancestral state reconstruction

We mapped the chromosome number on the phylogeny provided by Song et al. (2018) (Fig. 2) and performed ancestral state reconstruction. In their phylogeny, Song et al. (2018) showed that Acrididae roughly form four monophyletic groups (Clades A to D). Our analysis shows that three of these groups (A to C) show different degrees of polymorphism in chromosome number, whereas the fourth group (Clade D) appears monomorphic with a consistent chromosome number of 2n♂ = 23. However, we were not able to obtain chromosome numbers for all taxa included in the phylogeny.

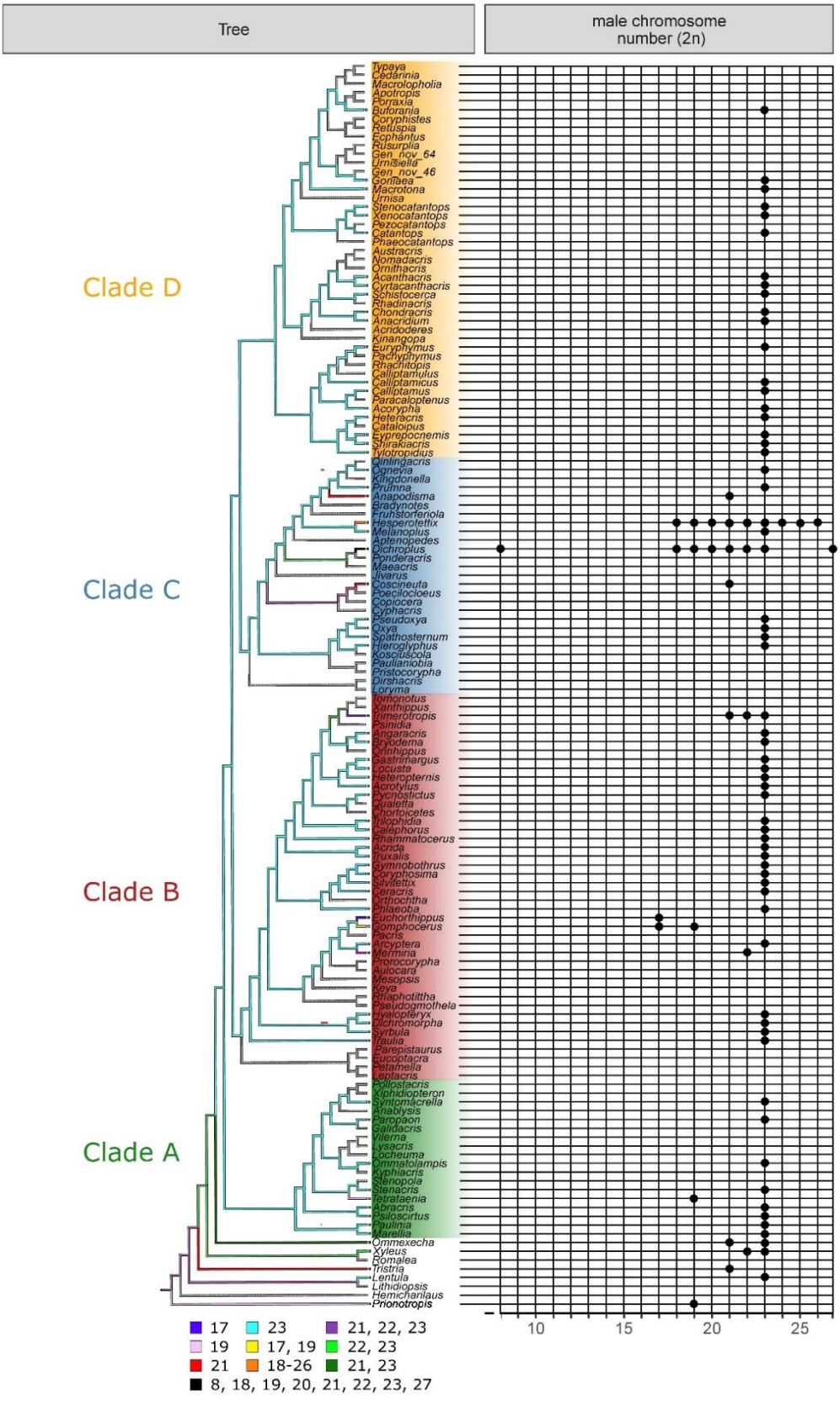
Clade A comprises Marelliinae, Pauliniinae, Lepty-sminae, Ommatolampidinae (polyphyletic) and Rhytidochrotinae. Leptysminae most commonly showed the typical 2n♂ = 23, but chromosome numbers also comprised 2n♂ = 19 in the genus *Tetrataenia* Stål, 1873, 2n♂ = 13 or 21 in *Leptysma* Stål, 1873 and 2n♂ = 21 in *Stenopola* Stål, 1873. In Ommatolampidinae, chromosome numbers varied among the tribes Abracrini (2n♂ = 19: *Jodacris* Giglio-Tos, 1897, *Sitalces* Stål, 1878; 2n♂ = 21: *Eujivarus* Bruner, 1911, *Abracris* Walker, 1870, *Omalotettix* Bruner, 1906), Pycnosarcini (2n♂ = 17: *Pycnosarcus* Bolívar,

1906, *Lagidacris* Amédégnato & Descamps, 1979) and Clematodini (2n♂ = 21: *Bucephalacris* Giglio-Tos, 1894).

Clade B comprises mostly paraphyletic subfamilies: Hemiacridinae, Tropidopolinae, Oedipodinae, Coptacrinae (monophyletic), Gomphocerinae and Acridinae. Chromosome number deviation from 2n♂ = 23 was only recorded for Oedipodinae (*Trimerotropis*, *Circotettix*: 2n♂ = 21; *Oedipoda*: 2n♂ = 25; *Machaerocera* Saussure, 1859 2n♂ = 16 (14 + XY); *Chortoicetes* Brunner von Wattenwyl, 1893: 2n♂ = 17) and Gomphocerinae (*Gomphocerus* Thunberg, 1815, *Neopodismopsis* Bey-Bienko, 1932 syn. *Chloealtis* Harris, 1841: 2n♂ = 19 *Euchorthippus* Tarbinsky, 1926, *Euthystira* Fieber, 1852, *Eclipophleps* Tarbinsky, 1927, *Chorthippus* Fieber, 1852, *Gomphocerus*, *Mongolotettix* Rehn, 1928, *Myrmeleotettix* Bolívar, 1914, *Omocestus* Bolívar, 1878, *Chloealtis* Harris, 1841, *Podismopsis* Zubovski, 1900, *Stenobothrus* Fischer, 1853: 2n♂ = 17; *Mermiria* Stål, 1873, *Scyllinula* Carbonell, 1995 2n♂ = 22 (20 + XY)).

Clade C represents the most variable group within the dataset. The group contains several genera of the paraphyletic subfamilies Hemiacridinae, Oxyinae, Copiocerinae and the monophyletic Melanoplinae, Proctolabinae and Spathosterninae. Chromosome number varied between 2n♂ = 17 and 2n♂ = 25 in the Melanoplinae genus *Hesperotettix* Scudder, 1876 and between 2n♂ = 8 (6 + XY) and 2n♂ = 23 within the genus *Dichroplus* Stål, 1873. Further variation within the subfamily was recorded for several genera of *Dichroplini* (2n♂ = 8 (6 + XY), 2n♂ = 13 – 16 (14 + XY), 2n♂ = 18 (16 + XY) – 23, 2n♂ = 22 (20 + XY), 2n♂ = 27) and Podismini (2n♂ = 21 – 23, 2n♂ = 25). Except for the genera *Anapodisma* and *Coscineuta* Stål, 1873 (2n♂ = 21) and some genera of the Copiocerinae (*Aleuas* Stål, 1878: 2n♂ = 20 (18 + XY), 2n♂ = 22 (20 + XY), 2n♂ = 19; *Bucephalacris*: 2n♂ = 21; *Zygoclistron* Rehn, 1905: 2n♂ = 20 (18 + XY), all remaining subfamilies showed the common chromosome number of 2n♂ = 23.

Ancestral state reconstruction (Fig. 2) suggests an ancestral chromosome number of 2n♂ = 23 for Acrididae. Changes in chromosome number across the phylogeny in most cases represent single species.



= 13

Figure 2: Male chromosome numbers mapped on the phylogeny of Acrididae constructed by Song et al. (2018). Mapping and ancestral state reconstruction of chromosome number with Mesquite.

DISCUSSION

Based on our dataset, we confirm a high stability of chromosome number in Acrididae with almost three quarters (73.9%) of all records reporting a number of 2n♂ = 23. This is in line with previous findings of White (1973), who suggested that two-thirds of all species have this karyotype, and findings of Aswathanarayana & Ashwath (2006) who even suggested that 90% of Acrididae share this configuration. Our study therefore confirmed the traditional view of Acrididae as a prime example of karyotypic conservatism (White, 1973), but provides a more comprehensive analysis.

Due to the rather monomorphic chromosome number configuration, many studies investigate additional chromosomal characteristics like the number of chromosome arms (e.g., Vysotskaya, 1993; Bugrov & Vysotskaya, 1981), C-banding patterns (e.g., Souza & Melo, 2007; Bugrov et al., 1991; Vysotskaya & Bugrov, 1987) or even chiasmata frequency (e.g., Gusachenko et al., 1992; Cano et al., 1986; Riva et al., 1984). However, the additional characteristics of the chromosomes were out of the scope of this study and we focused on the numbers alone.

Nevertheless, despite high degree of conservation of the chromosome number configuration of 2n♂ = 23, some groups exhibited deviations from this typical number: Several tribes of Gomphocerinae (i.e., Stenobothrini, Gomphocerini and Chrysochraontini) share a number of 2n♂ = 17, while several Tetriginae species show a configuration of 2n♂ (Bugrov, 1996). Many Pamphagidae genera show a general chromosome number of 2n♂ = 19 (e.g., Bugrov, 1986, 1996). Coleman (1948) suggested that this reduction in the chromosome number was the result of centric fusions, also known as Robertsonian translocations (Cabrero & Camacho, 1986). It is difficult to assess whether the event of chromosome number reduction occurred a single time or gradually in multiple events because the currently available phylogenetic data include only few taxa with this reduced chromosome number. As no intermediate forms have been found in any closely related groups, it may be possible that the reduction occurred in a single

step as, for example, also suggested in Oxyopidae spiders (Stávale et al., 2011). However, intermediates may also be of meiotic disadvantage potentially explaining their absence.

A reduction to 2n♂ = 21 is fairly widespread in some genera of Melanoplinae, e.g., *Hesperotettix* and *Dichroplus*, and the Oedipodinae *Trimerotropis* and *Circotettix*. The North American tribe Trimerotropini was the subject of intense cytogenetic studies and showed variation in chromosome number between 2n♂ = 21 and 2n♂ = 23. Within this tribe, species of *Trimerotropis* and *Circotettix* show geographic variation in chromosome number, and several evolutionary scenarios have been developed, potentially explaining these differing chromosome numbers (Confalonieri et al., 1998; Confalonieri & Bidau, 1986; Evans, 1954; White, 1949; Coleman, 1948). White (1949) suggested that the ancestral state is the typical 2n♂ = 23 and proposed that the fusion of two acrocentric chromosomes to a metacentric chromosome has produced the decreased karyotype of 2n♂ = 21 in species of *Circotettix* and *Trimerotropis*. The metacentric chromosomes in other Trimerotropini genera originated probably by pericentric inversions (Evans, 1954), rather than translocations as suggested for the Gomphocerinae (Coleman, 1948). The effect of this chromosomal polymorphism for reproductive isolation remains debated: natural hybrids with 2n♂ = 22 have been observed in several crosses; yet, sperm quality strongly suffered in many cases suggesting some degree of hybrid sterility (Shaw et al., 1998; John et al., 1983; John & Weissman, 1977; Evans, 1954).

While deviations in chromosome numbers across most of the investigated taxa seem rather species-specific and hence have little systematic value, this appears different in some European Gomphocerinae, which share in general the reduced number of 2n♂ = 17 (except for e.g., *Eremippus mistshenkoi* Stebaev, 1965 2n♂ = 19 (Bugrov et al., 1993); *Chorthippus hammarstroemi* (Miram, 1907) 2n♂ = 21 (Kiknadze & Vyotskaya, 1970) or *Stenobothrus eurasius* 2n♂ = 16 (XY) (Bugrov et al., 1991)), and in the American Trimerotropini. In the latter, species have even been divided into three cytogenetically distinct groups (Sections A to C; Weissman & Rentz, 1980; White, 1949, 1951) differing in their chromosome number and morphology. It has been suggested that these differences may contribute to reproductive isolation and, therefore, speciation (e.g., Shaw et al., 1998). The two main chromosomal Sections A and B were also recovered in

phylogenetic reconstructions using mitochondrial and nuclear genes (Husemann et al., 2012) and hence represent a useful systematic character. This still has to be evaluated in the Gomphocerinae.

In turn, some genera are particularly diverse in their chromosome constitutions, foremost the Melanoplinae genera *Dichroplus* and *Hesperotettix*. Chromosome numbers vary between 2n♂ = 18 (16 + XY) and 26 (24 + XY) within the genus *Hesperotettix* (McClung, 1917) and between 2n♂ = 8 (6 + XY) and 2n♂ = 27 in *Dichroplus* (Castillo et al., 2017; Mesa et al., 1982). Interestingly, there have been several studies performed on the chromosome number variation of the species *Podisma sapporensis* and *Podisma pedestris* in hybridization zones (e.g., Warchałowska-Śliwa et al., 2008; Bella et al., 1991). These studies show that reproductive isolation systems exist in hybrids, but the variation is most likely based on Robertsonian translocations between a sex chromosome and an autosome, and several chromosome rearrangements. Further, they show a clear differentiation into X0 and neo-XY chromosome races and complex chromosomal polymorphism in contact zones, which could permit the differentiation of several chromosomal races (Warchałowska-Śliwa et al., 2008).

CONCLUSION

Overall, a basic chromosome number of 2n♂ = 23 was observed across the whole Acrididae phylogeny and hence in all four clades described by Song et al. (2018). No subfamily with a number consistently diverging from the standard 2n♂ = 23 was recovered in the tree; but, some taxon-specific chromosome number variation appears to be present in Gomphocerinae and Trimerotropini. We conclude that the chromosome number in Caelifera, and specifically in Acrididae, is rather constant and phylogenetically less informative compared to several groups of Ensifera, which show more variation (e.g., Eneopterinae with range from 2n♂ = 9 (6 + XXY) (Palacios-Gimenez

et al., 2017) up to 2n♂ = 57 in Rhaphidophoridae (Vandergast et al., 2017 and references therein)). The reasons for this need to be further explored in the future.

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SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https:// doi. org/ 10. 1007/ s13127- 022- 00543-1.

All supporting information can be found on page 423 and following.

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Sign

Chapter 5.4

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Evolution of mitogenomic gene order in Orthoptera

Submitted to Insect Molecular Biology

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ABSTRACT

Mitochondrial gene order has contributed to the elucidation of evolutionary relationships in several animal groups. It generally has found is application as a phylogenetic marker for deep nodes. Yet, in Orthoptera limited research has been performed on the gene order, although the group represents one of the oldest insect orders. We performed a comprehensive study on mitochondrial genome rearrangements (MTRs) within Orthoptera in the context of mitogenomic sequence-based phylogeny. We used 280 published mitogenome sequences from 257 species including three outgroup species to reconstruct a molecular phylogeny. Using a heuristic approach, we assigned MTR scenarios to the edges of the phylogenetic tree and reconstructed ancestral gene orders to identify possible synapomorphies in Orthoptera. Most of the suggested MTRs were in single and unrelated species. Out of five MTRs which were unique in subgroups of Orthoptera, we suggest four of them to be synapomorphies. Those were in the infraorder Acrididea, in the tribe Holochlorini, in the subfamily Pseudophyllinae, and in the two families Phalangopsidae and Gryllidae or their common ancestor (leading to the relationship ((Phalangopsidae + Gryllidae) + Trigonidiidae)). However, similar MTRs have been found in distant insect lineages. Our findings suggest convergent evolution of specific mitochondrial gene orders in several species, deviant from the evolution of the mitogenome DNA sequence. As most MTRs were detected at terminal nodes, a phylogenetic inference of deeper nodes based on MTRs is not supported. Hence, the marker does not seem to be a promising candidate to aid resolving the phylogeny of Orthoptera but adds further evidence for the complex evolution of the group as a whole, especially at the genetic and genomic levels. The results indicate a high demand for more research on patterns and underlying mechanisms of MTR events in Orthoptera.

Key words: Crickets, grasshoppers, inversions, katydids, mitochondrial genome rearrangement, phylogeny, transpositions, *TreeREx*

INTRODUCTION

Orthoptera has been suggested to be one of the oldest extant lineages of insects with the earliest fossils dating back to the upper Carboniferous (290 million years ago; Grimaldi and Engel, 2005) and an estimated age of 300-355 mya (Song et al., 2015, Song et al., 2020). Its 29,365 species (Cigliano et al., 2022) are divided into two suborders – the Ensifera and Caelifera (Flook et al., 1999, Song et al., 2015). Their high age, but also their large and complex genomes (e.g. Husemann et al. 2021, 2022) have made assessing their phylogeny difficult, but also make Orthoptera an interesting target to study the evolution of the mitochondrial genome (in the following mitogenome) and to test its potential as a reliable marker for phylogenetic reconstructions (Fenn et al., 2008).

In insects, the mitogenome is usually 15-18 kb in size (Boore, 1999, Cameron, 2014). The mitogenome is generally haploid due to the typical maternal mode of inheritance (Hayashi et al., 1978). However, alterations of this mode, e.g. via heteroplasmy has been suggested for example in dipterans (Wolff et al., 2013), fishes (Nesbø et al., 1998), and bats (Wilkinson and Chapman, 1991). The mitogenome is organized in a circular structure and contains 13 protein coding genes (PCGs; *nad1-6*, *nad4L*, *cox1-3*, *cob*, and *atp6/8*), two ribosomal RNA genes (rRNA: *rrnL, rrnS*), and 22 genes for transfer RNAs (tRNAs; see Table S2 for gene names; Boore, 1999)). Furthermore, there is a large non-coding region, also called the adenine and thymine-rich region (AT-rich region) or control region (CR), which contains the origin of replication and transcription and is highly variable among insects (Lewis et al., 1995). Due to their high variability, mitochondrial sequences and whole mitogenomes are frequently used in phylogeny and phylogeography. However, this high evolutionary rates also make mitochondrial sequences more prone to saturation effects and hence make them less suited for resolving deeper phylogenetic relationships (Allio et al., 2017, Ballard and Whitlock, 2004). However, not only the sequence of the mitochondrial DNA, but also the order of genes can be used as a phylogenetic marker (Boore, 1999). The mitochondrial gene order generally evolves at slow rates and is much more conserved than amino acid or nucleotide sequences in metazoans (Boore and Brown, 1998). Hence, the frequency and type of changes in the gene order, also called mitochondrial genome rearrangements

(MTRs), may be a target for the investigation of deeper phylogenetic relationships (Rokas and Holland, 2000). Due to selective constraints, MTRs of tRNA genes have been suggested to occur at a higher rate than MTRs involving protein coding genes (Dowton et al., 2009). In studies across all insects and in some specific orders, e.g. Hymenoptera, mitogenome gene order proved to be informative and provided new insights into the evolution of these groups (e.g. Cameron, 2014, Gotzek et al., 2010, Silvestre et al., 2008).

For Orthoptera, an unambiguous phylogeny based on DNA sequences still remains a challenge (Song et al. 2015, 2018, 2020). This is partially due to their large genome sizes and complex genomic architecture, leading to inconsistencies between mitochondrial and nuclear genomic data, as demonstrated e.g. for the subfamily Gomphocerinae (Hawlitschek et al., 2022). Gene order as a conservative marker, may provide some additional information improving phylogenetic resolution, especially at deeper nodes. However, so far limited research has been performed to investigate mitochondrial gene order information content in Orthoptera, making it crucial to examine the information content of gene order for phylogenetic research in this insect order. So far, research on MTRs in Orthoptera has only addressed specific subgroups with novel MTRs representing relatively shallow branches, e.g. two families of Grylloidea (Ma and Li, 2018) and Holochlorini (Liu et al., 2013). One noteworthy exception is a major tRNA gene rearrangement supporting a closer relationship between Tetrigoidea and Acridomorpha (Song et al., 2015). Apart from that, evolutionary patterns of MTRs are largely unknown in Orthoptera.

Therefore, we here reconstructed a molecular phylogeny of 277 published mitogenomes of Orthoptera (and three additional outgroup sequences) as a base for estimating the ancestral gene order and to identify MTRs. In this work, we provide the first comprehensive analysis of MTRs of all accessible orthopteran mitogenomes (up to July 2021) with the aim of 1) detecting patterns of MTRs in Orthoptera and 2) understanding the evolution of these MTRs. Our goal was to obtain information on the scale and direction of the divergence of mitochondrial gene order within Orthoptera. We hypothesize that (a) MTR events occur at a higher rates for tRNA genes rather than for protein coding genes and (b) MTRs are infrequent genomic changes which may help to elucidate deep branches of Orthoptera.

MATERIAL AND METHODS

We obtained 280 mitogenomes from 257 species (including three outgroups) for the analyses (Table S1). Out of these, we downloaded 239 Caelifera and Ensifera mitogenomes and three outgroup species mitogenomes (*Mantis religiosa*, *Euborellia arcanum*, and *Musca domestica*) from the NCBI GenBank Organelle Research database (assessed May-July 2021; Sayers et al., 2021). Additionally, 38 Acrididae mitogenomes were annotated and provided by Hawlitschek et al. (2022). The dataset consisted of taxa from 30 orthopteran families: Phalangopsidae, Trigonidiidae, Mogoplistidae, Gryllotalpidae, Myrmecophilidae, Tettigoniidae, Rhaphidophoridae, Stenopelmatidae, Anostostomatidae, Prophalangopsidae, Gryallacrididae, Schizodactylidae, Tridactylidae, Ripipterygidae, Tetrigidae, Episactidae, Thericleidae, Tanaoceridae, Pneumoridae, Pyrgomorphidae, Pyrgacrididae, Pamphagidae, Lentulidae, Lithidiidae, Tristiridae, Romaleidae, Ommexechidae, Dericorythidae, and Acrididae. From this dataset, 193 mitogenomes were caeliferans, representing 17 families. The majority (157 specimens) belonged to the family Acrididae (dominantly representing the subfamilies Gomphocerinae, Oedipodinae, and Melanoplinae), Pamphagidae (11 sequences), and Tetrigidae (7 sequences). The suborder Ensifera was represented by 84 sequences from 13 families. Out of these, 44 mitogenomes were assigned to Tettigoniidae (dominantly from Phaneroptinae, Conocephalinae, and Meconematinae), and 11 mitogenomes were from Gryllidae (mainly from the subfamily Gryllinae). Moreover, we included the mitogenomes of *Mantis religiosa* (Mantodea: Mantidae) and *Euborellia arcanum* (Dermaptera: Anisolabididae) as outgroups (GenBank). It is noteworthy that the mitogenome from the species labelled *Dasyhippus barbipes* actually belonged to the housefly *Musca domestica* (Diptera: Brachycera: Muscidae; based on blasting of COI; Altschul et al., 1990). As this species has been labelled incorrectly by the provider, it should be tagged in the database. *Musca domestica* was subsequently used as additional more distant outgroup. We followed the current taxonomy outlined in the Orthoptera

Species File (Cigliano et al., 2022). As part of the data collection, we provide a documentation of the gene order as deposited in GenBank (accessed between May and July, 2021). We included supplementary information, such as the total genome length, reference, Accession ID (if obtained from GenBank), suborder, family, subfamily, and tribe (see Table S1).

Backbone phylogeny

For the backbone phylogeny, we processed the GenBank files in Geneious Prime 2021.1.1 (Kearse et al., 2012) as follows: all 13 PCGs (*nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad5*, *nad4*, *nad4L*, *nad6*, *cob*, *nad1*; for explanations see Table S2) and the two ribosomal RNAs (*rrnL*, *rrnS*) were extracted from the whole mitogenomes. The tRNA sequences and control region (CR; AT-rich region, D-Loop) were excluded. We subsequently generated an alignment of each individual genes (MAFFT v7.450; Katoh et al., 2002, Katoh and Standley, 2013) with the default settings (Auto algorithm, Scoring Matrix: 200 PAM / k=2, gap open penalty: 1.53, offset value: 0.123). Finally, we concatenated the sequences in *Geneious* in the order mentioned above. Since one species (*Trigonidium sjostedti*) showed an extremely long extension of the *rrnS* gene, we cropped this part in *Geneious* resulting in an overall complete (ungapped) length of the concatenated sequences of 15,808 base pairs (bp).

Before starting the actual phylogenetic analysis, we defined the best partitioning scheme with PartitionFinder 2.1.1 (Lanfear et al., 2017), for which we predefined a data block for the configuration file. We performed this step considering codon positions individually. The parameters were set as follows: branch lengths = linked, model selection = AICc (Hurvich and Tsai, 1989), search = rcluster (Lanfear et al., 2014). We used RAxML (Stamatakis, 2014) to test three substitution models: GTR, GTR+G, and GTR+I+G. Subsequently, we applied the best partitioning scheme, resulting in 37 partitions based on the 37 subsets for BI and ML analysis.

We then used the concatenated sequences to reconstruct a phylogeny using BI and ML. We used MrBayes 3.2.2 (Altekar et al., 2004, Huelsenbeck and Ronquist, 2001, Ronquist and Huelsenbeck, 2003) on XSEDE (Towns et al., 2014) in the CIPRES gateway (Miller et al., 2010) with the models determined by PartitionFinder, 30,000,000 generations, and 25 % burn-in, sampling every 3,000 generations. The MCMC output files (parameter files) from MrBayes were subjected to statistical analysis in Tracer v. 1.7.1 (Rambaut et al., 2018). MrBayes failed to reach good statistical support (ESS < 200), therefore we corrected the evolutionary model and data partitioning as follows: instead of 37 partitions, we condensed the 37 subsets to two partitions (PartitionFinder found only two different best substitution models for the dataset: rates = invgamma: invariant sites and gamma distributed rate variation among sites; rates = gamma: gamma distributed rate variation among sites). Furthermore, we set the number of substitution types (nst) to the more flexible mixed (reversible jump model) model instead of the model parameter nst = 6 (allows all substitution rates to be different, subject to the constraint of time-reversibility). This option can result in a better statistical support and higher ESS, since all possible reversible substitution models are considered (Forster et al., 2012).

Using the IQTree Web Server (Trifinopoulos et al., 2016), we inferred a phylogeny based on ML. We performed the analysis with the best partitioning scheme and otherwise default settings, i.e. with 1,000 iterations of ultrafast bootstrapping (UFBoot; Minh et al., 2013), a minimum correlation coefficient of 0.99, and Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT) with 1,000 replicates (Guindon et al., 2010, Minh et al., 2013). After calculating the phylogenies, we used Figtree v 1.4.4. for visualization and rooted the trees with *Musca domestica* as outgroup. Additionally, we added the information of major clades according to the Orthoptera Species file (Cigliano et al., 2022) for each taxon in Inkscape 1.1.1.

TreeREx analysis

We used the topology resulting from BI as input for *TreeREx* (Bernt et al., 2008) in combination with the gene order of each taxon to reconstruct rearrangement scenarios

of the mitogenome gene order. We directly obtained the gene order information from GenBank and saved it in a .txt file (see Appendix S1). *TreeREx* was then used to reconstruct gene orders of deeper nodes based on the gene order of the terminal nodes of the tree. We applied the *TreeREx* algorithm on 269 of the 280 taxa in our dataset. Eleven taxa with gene duplications, deletions, and missing data had to be excluded (see Table S3). Their gene orders were replaced by the ancestral insect gene order (Boore, 1999, Boore et al., 1998) in the input gene order file. We studied the set of 13 PCGs, 22 tRNAs, and two rRNA units based on the common insect ancestor (Boore et al., 1998). Position and number of CRs, *ori*s, occurrence of pseudo-genes, and other non-coding regions were not considered to create a consistent input dataset. We ran *TreeREx* with the default parameters: strong consistency method (-s), weak consistency method (-w), and parsimonious weak consistency method (-W). These methods use algorithms with different stringencies and were applied to make sure that every node of the phylogenetic tree gets assigned a gene order regardless of the confidence level (Bernt et al., 2008). We added alternative scenarios from patterns in breakpoints (-o) and the default number of inversions/input permutations per binary tree (-m = 2) to the algorithm. Subsequently, we converted the output .dot-file to a graphic svg file with Graphviz (Gansner and North, 2000) to visualize the combined tree consisting of the phylogenetic information and the MTR events (see Appendix S2 for original *TreeREx* output data).

Editing and visualization of TreeREx results

Finally, we mapped the MTR events on the complete BI tree and displayed a gene order map generated with Microsoft Excel v. 16.0 (2019). Before mapping, we edited the phylogenetic trees and gene order maps with Figtree and Inkscape for better clarity. Although novel CRs, gene duplications, and deletions could not be integrated in *TreeREx*, they complemented the mapped tree. For visualization of a TDRL event in the mapped tree, *CREx* (Bernt et al., 2007) was used to show the subsequent rearrangement in the genome (Fig. S2 of supporting information).

RESULTS

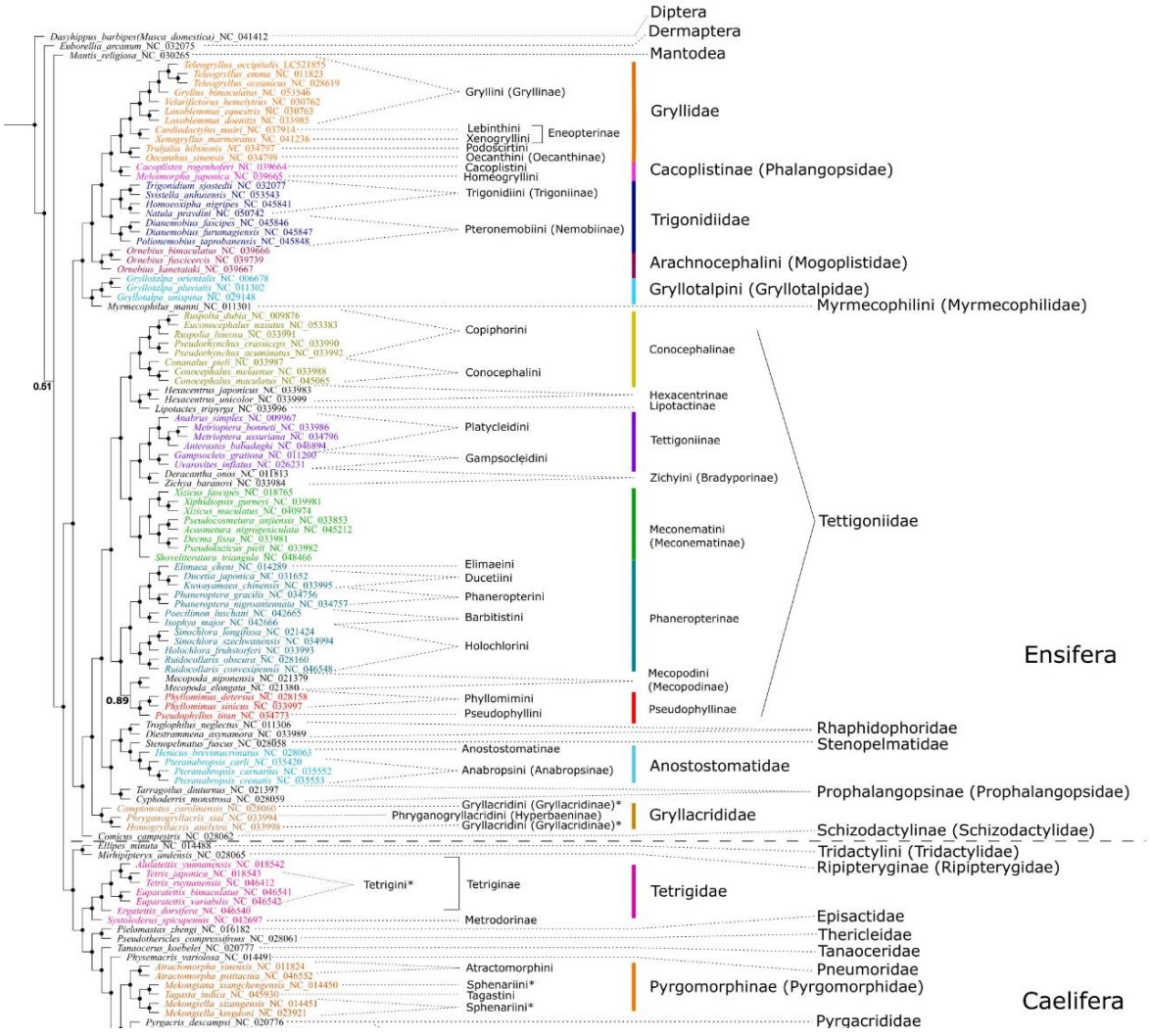
Molecular phylogeny

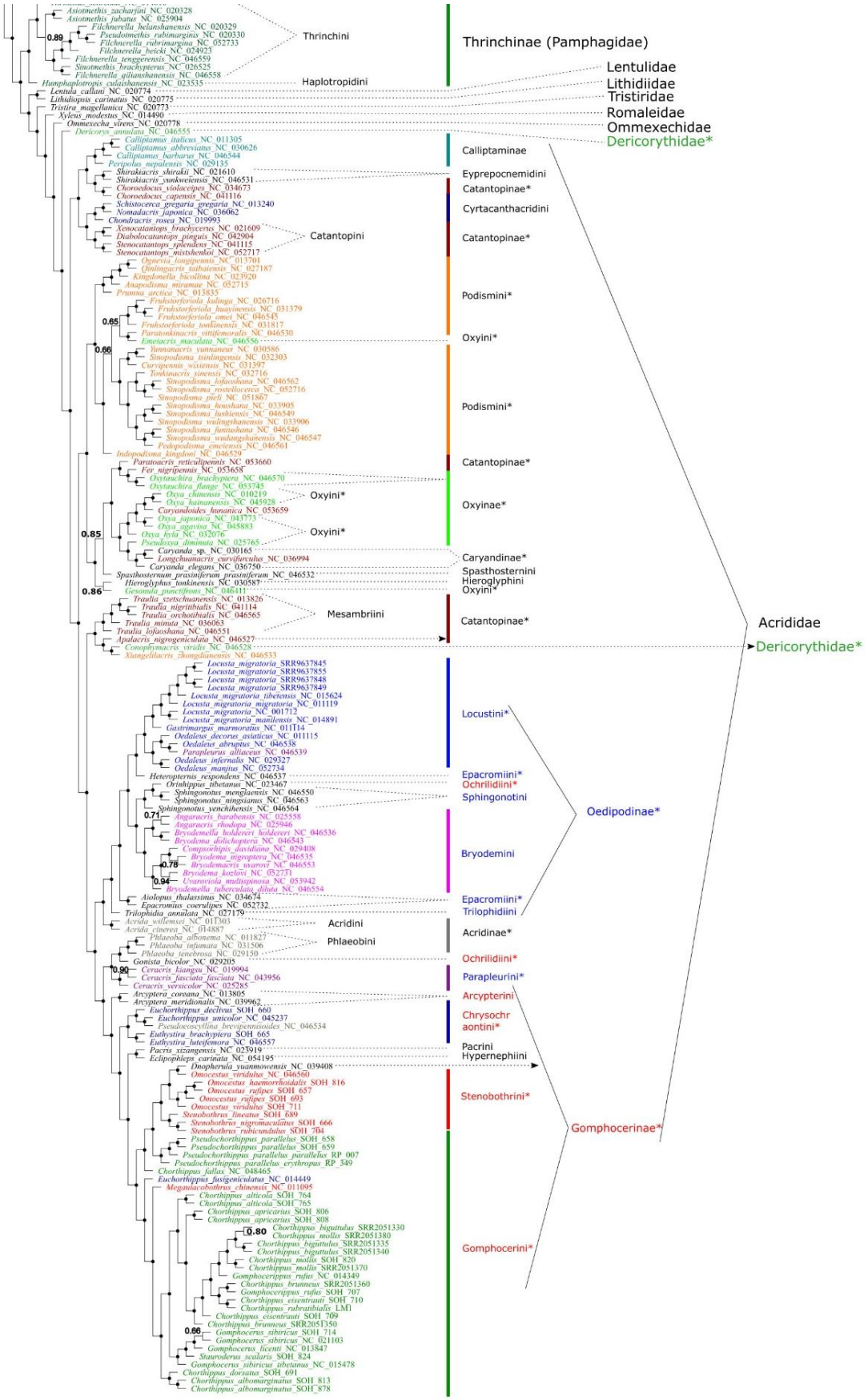
The final dataset contained 280 mitogenome sequences (277 ingroup sequences and three outgroups) of 257 species. The final alignment used for phylogenetic inference had a cropped length of 15,808 bp. The overall GC content was 26.6% with no significant difference between Ensifera (N = 84; 28.5%) and Caelifera (N = 193; 25.8%). The results of Bayesian inference (BI) and Maximum Likelihood (ML) were largely consistent within Ensifera with one exception. The ML topology grouped Phaneropterinae with Mecopodinae and Pseudophyllinae, albeit with low support (bootstrap = 77%). The BI analysis instead grouped Mecopodinae as sister group of Pseudophyllinae together with Phaneropterinae (posterior probability value = 0.86, Figure 1). More inconsistencies between the BI and ML trees were observed in Caelifera (see Fig. 1): according to BI Calliptaminae, Catantopinae, Cyrtacanthacridini, and Eyprepocnemidini form a highly supported (pp > 0.95) branch, distinct and divergent from the rest of the Acrididae. ML placed this clade within the Acrididae. Most other branches which differed between the BI and ML were poorly supported (a phylogeny based on ML is given as Fig. S1).

Gene order inference

Eleven taxa which contained deletions or duplications of genes (see Table S3) were not considered for *TreeREx* analysis, but still included in the phylogenetic analyses. We used the BI tree as input for reconstructing MTR events and assigning them to the nodes of the tree in *TreeREx*. We decided for the BI tree because it received overall better support values compared to the ML tree (in the following, all numerals and letters marked by brackets represent MTR events as shown in Fig. 2 and 3; all tRNA gene abbreviations are according to the IUPAC IUB nomenclature for amino acids (see also Table S2; Cornishbowden, 1984). The hyphens in '(-)' indicate genes which are encoded on the light strand).

Figure 1. Phylogenetic Tree by BI inferred with MrBayes. Numbers near the nodes indicate posterior probabilities (pp). Dots on nodes indicate a pp > 0.95. The taxon labels are highlighted in different colors assigned to major groups based on the currently accepted taxonomy (obtained from Orthoptera Species File; Cigliano et al., 2021) which are shown on the right. The '*'-sign is added to all clades which are considered either paraphyletic or polyphyletic by the present results. No assigned tribe/subfamily implies that the taxon in question is currently not classified into any of the groups within a family. Editing was performed with Inkscape and Figtree software.




Thrinchinae (Pamphagidae)
Lentulidae
Lithidiidae
Tristiridae
Romaleidae
Ommexechidae
Dericorythidae*
Acrididae
Dericorythidae*
Thrinchini
Haplotropidini
Calliptaminae
Eyprepocnemidini
Catantopinae*
Cyrtacanthacridini
Catantopinae*
Catantopini
Podismini*
Oxyini*
Podismini*
Catantopinae*
Oxyinae*
Oxyini*
Oxyini*
Caryandinae*
Spasthosternini
Hieroglyphini
Oxyini*
Catantopinae*
Mesambriini
Locustini*
Epacromiini*
Ochrilidiini*
Sphingonotini
Oedipodinae*
Bryodemini
Epacromiini*
Trilophidiini
Acridinae*
Acridini
Phlaeobini
Ochrilidiini*
Parapleurini*
Arcypterini
Chrysochraontini*
Pacrini
Hypernephiini
Gomphocerinae*
Stenobothrini*
Gomphocerini*

According to our analysis, all four types of MTR events were found in Orthoptera: inversions, transpositions, inverse transpositions, and tandem-duplication/random loss events (TDRL). We found six transpositions, 26 inversions (eight branches had multiple inversions), one TDRL event, and one inverse transposition. For each event we provide the confidence level (C-value), indicating the support for the inferred rearrangements and reflecting the consistency of the gene order information with the phylogeny (Bernt et al., 2008, Nei and Kumar, 2000). The rearrangement scenarios were overall highly consistent with the results obtained by BI, since the C-value was equal to 1 in most cases. The analysis led to a k-consistent result (consistency with a relaxed consistency definition) only in the case of *Fruhstorferiola tonkinensis* and *Paratonkinacris vittifemoralis* with a C-value of 0.5 (a complete tree of MTR scenarios is given in Appendix S2). With few exceptions, only tRNA genes and rRNA genes were affected. The majority of MTR events were found in single species, whereas some were shared by unrelated species (convergent events). Inversions were the most common MTR events.

All Caelifera species of the infraorder Acrididea shared a transposition of *trnK-trnD* → *trnD-trnK* (Fig. 2; MTR event '(0)'). In the Tridactylids *Mirhipipteryx andensis* and *Ellipes minuta*, this MTR did not occur. Furthermore, in the Gryllidae, Phalangopsidae, Trigonidiidae, and their common ancestor, the most parsimonious scenario was an inversion of the *trnN-trnS1-trnE* block (Fig. 2; MTR event '(2)'). We reconstructed the transposition *trnV-rrnS* → *rrnS-trnV* for Trigonidiidae and for *Ornebius bimaculatus*, suggesting the independent evolution of this MTR in the two branches (Fig. 2; MTR event '(3)'). Within the tribe Holochlorini, only *Holochlora fruhstorferi* and *Sinochlora* spp. shared the transposition *trnI-trnQ-trnM-nad2* → *trnI-trnM-nad2-trnQ* (Fig. 2; MTR event '(8)'). On the other hand, *Ruidocollaris obscura* and *R. convexipennis*, representing the sister group of *Sinochlora* and *Holochlora* in our phylogenetic reconstruction, did not show this MTR. The mitogenome of *Sinochlora szechwanensis* furthermore included an inversion of *rrnL* (Fig. 2; MTR event '(9)'). A transposition event could be assigned to Pseudophyllinae: *trnI-trnQ-trnM* → *trnM-trnI-trnQ* (Fig. 2; MTR event '(10)').

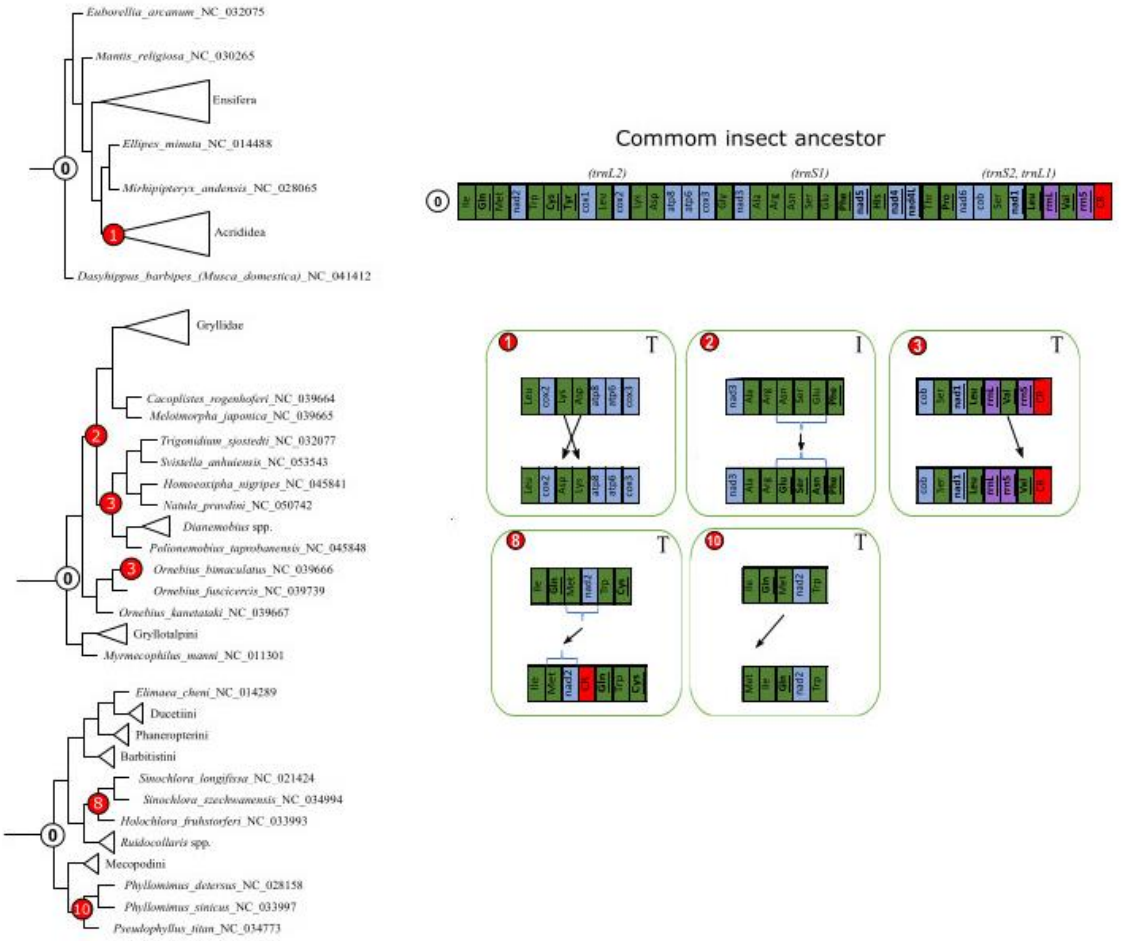


Figure 2. Mitochondrial genome rearrangement (MTR) events shared between related species mapped on the phylogenetic reconstruction based on the mitogenome (BI). Numerals in red circles are assigned to taxa with MTR events, while '0' is the ancestral insect gene order. Letters 'T' and 'I' refer to transversion and inversion events, respectively. Collapsed branches are indicated by triangles. All taxa of clade (2) have an inversion of glutamic acid, asparagine, and serine tRNA genes. *Ornebius bimaculatus* and Trigonidiidae (3) show a transposition of the valine tRNA gene. Moreover, the Holochlorini (8) share the transversion of the *trnM* and *nad1* block. The node with the numeral (1) refers to the clade Acrididea, in which the transversion of *trnK* and *trnD* was found. All species of the clade Pseudophyllinae in our dataset (10) shared the transversion of *trnM* and *trnQ* genes. Note that the MTR scenarios of *Euborellia arcanum* are not shown despite its multiple modifications, since it was excluded in the TreeREx analysis.

The only species with a TDRL event was *Lipotactes tripyrga*, in which the most parsimonious MTR scenario was an inverse transposition of *trnG-nad3-trnA-trnR-trnN-trnS1* → *(-)trnS1-(-)trnN-(-)trnR-(-)trnA-trnG-nad3*, followed by an inversion of the *trnA-trnR-trnN-trnS1*-block to the original strand (Fig. S2; MTR event '(7)'). A transposition was assigned to *Cyphoderris monstrosa* (*nad3-trnA-trnR*→ *nad3-trnR-trnA;* see Fig. S2; MTR event '(11)').

All other MTR events were inversions which appear exclusively as single or in unrelated species, based on the mitogenome-based phylogeny (see Fig. 3). In Ensifera these were *Trigonidium sjostedti* (*trnY*, and *trnE* + *trnS;* Fig. 3 a); MTR event '(4)'), *Loxoblemmus doenitzi* (*trnS1*; Fig. 3 e); '(5)'), *Pseudorhynchus acuminatus* (*trnP*; Fig. 3 f); '(6)'), *Sinochlora szechwanensis* (*rrnL*; Fig. 2; '(9)'), *Pseudokuzicus pieli* (*rrnL* and *rrnS*; Fig. 3 g); '(17)'), and *Phyllomimus sinicus* (*trnC* and *trnV*; Fig. 3 c), d); MTR event '(23)'). Moreover, the inversion of *Hexacentrus unicolor* (*trnL1*) was shared with the unrelated *Gryllus bimaculatus* (Fig. 3 i); MTR event '(22)').

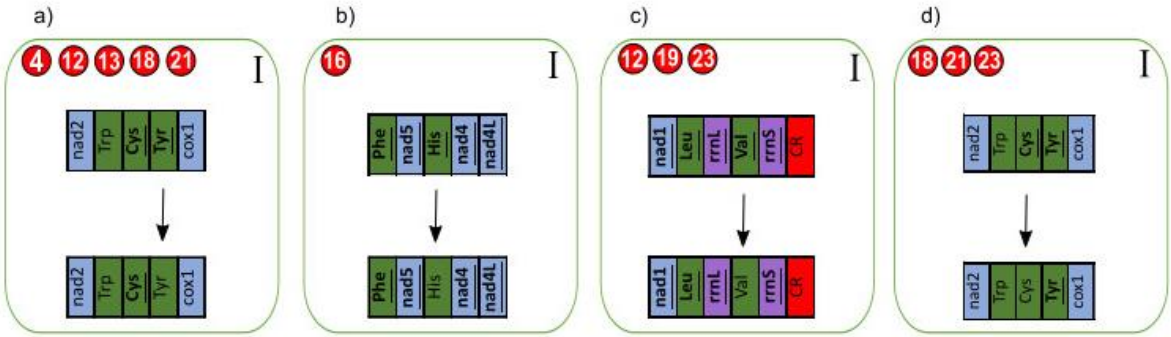


Figure 3. Mitochondrial genome rearrangement (MTR) events of many unrelated species mapped on the phylogenetic reconstruction based on the mitogenome (BI). Numerals in red circles are assigned to taxa with MTR events. 'I' refers to an inversion event. a) Inversion event of *trnY*. b) Inversion of *trnH* (16). c) Inversion of *trnV*. d) Reconstruction for *trnC* inversion scenarios. 4: *T. sjostedti*, 12: *T. indica*, 13: *M. xiangchengensis*, 16: *G. bicolor*, *H. tonkinensis*, *T. sinensis*, *P. vittifemoralis*, *F. tonkinensis*, and *L. callani*, 18: *O. infernalis*, 19: *E. unicolor*, 21: *M. chinensis*, 23: *P. sinicus*

In Caelifera, inversions were assigned to *Tagasta indica* (*trnY* and *trnV;* MTR event '(12)'), *Euchorthippus unicolor* (*trnV* and *trnT*; '(19)'), and *Mekongiella xizangensis* (*trnY;* '(13)'), but not to the congeneric *M. kingdoni* (Fig. 3 a) and c)). For the *trnY* inversion, there was an overlap between Caelifera and Ensifera species, accordingly (Fig. 3 a; MTR events '(4)', '(12)', '(13)', '(18)', '(21)'). Moreover, *Filchnerella beicki* had a mitogenome which contained a *rrnL* inversion (Fig. 3 j; MTR event '(14)'). Interestingly, the inversion of *trnV* found in *T. indica* (Pyrgomorphidae) also occurred in *Euchorthippus unicolor* (Acrididae) and in *P. sinicus* (Ensifera, Phyllomimini) (Fig. 3 c); MTR events '(12)', '(19)', and '(23)'). Furthermore, *Lentula callani* (Lentulidae), *Fruhstorferiola tonkinensis*, *Paratonkinacris vittifemoralis*, *Tonkinacris sinensis* (all Podismini), *Gonista bicolor* (Ochrilidiini), and *Hieroglyphus tonkinensis* (Hieroglyphini) had a shared gene order with a *trnH* inversion (Fig. 3 b; MTR event '(16)'). *Oxya japonica* (Oxyinae), unlike its congeneric species *O. agavisa*, had an inversion of the two rRNA subunits (*rrnL* and *rrnS)*, similar to *Chorthippus fallax* (Gomphocerinae) and to the ensiferan *P. pieli* (Fig. 3 g; MTR events '(17)' and '(20)'). Additionally, TreeREx assigned a *trnQ* inversion to *C. fallax*, which was shared with *Oedaleus infernalis* (Oedipodinae) (Fig. 3 h; MTR event '(18)'). In turn, the mitogenome of *O. infernalis* contained two additional *trnC* and *trnY* inversions shared with *Megaulacobothrus chinensis* (Stenobothrini), which was found absent in *Oedaleus manjius*, the closest relative to *O. infernalis* (Fig. 3 a), d); MTR events '(18)' and '(21)'). Again, we found a similar *trnC* inversion event in an ensiferan species: *P. sinicus* (Fig. 3 d; MTR event '(23)').

As 11 species did not fulfill the requirements of *TreeREx* in terms of a consistent gene set, they were excluded from the analysis. However, they are shown in Fig. S2 and evaluated for complementation. The noncoding control region (CR) was excluded from the gene order inference for all taxa, but the differences are nevertheless observable: *Polionemobius taprobanensis* was predicted to have, in addition to the initial CR, another CR between *trnK* and *trnD* (Fig. S2; event '(a)'). In *Holochlora* and *Sinochlora* spp., the CR is placed between *nad2* and *trnQ*, instead of the ancestral state (Fig. 2; MTR event '(8)'). A second CR between *trnS* and *nad1* was predicted in the two *Sinochlora* mitogenomes (Fig. S2; event '(b)'). The two *Ruidocollaris* species ('(c)'), on the other hand, had one CR at the

ancestral position and one CR between *trnY* and *cox1*. The mitogenomes of *Xenogryllus marmoratus* and *Troglophilus neglectus* contained duplications of *trnH* and *trnL2* (MTR events '(24)' and '(25)'), respectively. The outgroup, *Mantis religiosa*, showed a duplication of the *trnR* gene (Fig. S2; MTR event '(26)').

DISCUSSION

Gene order inference

This study represents the first comprehensive attempt to reconstruct mitochondrial gene order evolution for the order of Orthoptera based on all published mitogenomes (up to the end of data collection in July 2021). We aimed at testing if MTR events occur at a higher rates for tRNA genes rather than for protein coding genes and if MTRs represent potential alternative markers to elucidate deep branches of Orthoptera. While we found support for the first hypothesis, we had to reject the second one, as our results showed a low overall occurrence in deep branches, but not in shallow branches. In the following we discuss these results in more details.

MTR bias towards tRNAs

Generally, most of our MTRs were detected for tRNAs, rather than for PCGs, a pattern commonly observed. A study of hymenopteran gene order (Dowton et al., 2009) suggested selective constraints for the position of protein coding genes (PCGs) due to size differences. According to this study, MTRs are more likely lethal or selectively disadvantageous, e.g. through incomplete replication, if they concern larger genes. As the function of the transcription machinery can be affected, MTRs can lead to a loss of function of the genes. In addition, the movement of PCGs between strands can severely be restricted by strand compositional skews (Foster et al., 1997, Min and Hickey, 2007). Thus, MTRs including only tRNA genes are generally expected to be more frequent. Our

results support this, because tRNA gene inversions are most abundant in the context of our dataset. Only in three species, PCGs were involved in MTR events: the species *Holochlora fruhstorferi*, *Sinochlora longifissa,* and *Sinochlora szechwanensis* shared the transposition *trnI-trnQ-trnM-nad2* → *trnI-trnM-nad2-trnQ*, which involves the movement of the *nad2* gene (Fig. 2; MTR event '(8)'). All other MTRs are associated only with the movement of tRNA and rRNA genes, which is consistent with our hypothesis.

MTRs as potential phylogenetic marker in Orthoptera

Mitochondrial gene order has been used successfully as phylogenetic marker in other insect lineages (Cameron, 2014, Cao et al., 2012, Timmermans and Vogler, 2012), hence, we determined possible synapomorphies in MTRs for deep branches in Orthoptera to assess the phylogenetic signal. Because MTRs are rather infrequent events in many lineages, the saturation effect and the evolutionary rate are expected to be low, which makes gene order a valuable phylogenetic marker for old branches; accordingly, homoplasy levels will be low as well (Rokas and Holland, 2000). Regarding the major clades, we found only five different MTR events for deeper nodes, from which only four events are considered synapomorphic. In Caelifera, all species assigned to the infraorder Acrididea shared the transposition of *trnK-trnD* → *trnD-trnK*, while the two species of the Tridactylidea, *Mirhipipteryx andensis* and *Ellipes minuta*, did not show this MTR (Fig. 2; '(1)'). Hence, it can be regarded as synapomorphic for Acrididea. This observation, which was suggested to be a synapomorphy also by Song et al. (2015), can be obscured due to the independent occurrence of this MTR in the hymenopteran families Apidae, Stephanidae, Braconidae, Formicidae, and Scelionidae (Cameron, 2014). Thus, this MTR is considered homoplastic when applied in a deeper phylogeny and hence dependent on the phylogenetic scale. Within Orthoptera it can be seen as synapomorphy for Acrididea.

Furthermore, for the Gryllidae, Phalangopsidae, and Trigonidiidae, we also found an inversion of the *trnN-trnS1-trnE* block (Fig. 2; MTR event '(2)'). Since it was exclusively found in these groups, this MTR is considered synapomorphic. Likewise, Song et al.

(2015) found similar gene orders in the three families, but based on a smaller sample size. Consequently, a larger taxonomic sampling is needed to assess the phylogenetic signal for this feature. Two other studies concluded that the inversion of *trnN-trnS1-trnE* block appeared in the common ancestor of Gryllidae, Phalangopsidae, and Trigonidiidae, consistent with their phylogenetic reconstructions (Ma and Li, 2018, Sanno et al., 2021). Yet, there is no formal higher taxon unifying the clade "((Phalangopsidae + Gryllidae) + Trigonidiidae)" at the moment. Furthermore, *Holochlora fruhstorferi* and *Sinochlora* spp. share the transposition *trnI-trnQ-trnM-nad2* → *trnI-trnM-nad2-trnQ* (Fig. 2; MTR event '(8)'). This leads to the hypothesis that this MTR is synapomorphic for Holochlorini based on the dataset. Support for a possible synapomorphy is given by a mitogenome analysis of Liu et al. (2013), in which the gene orders of *S. longifissa* and *S. retrolateralis* were compared to other Orthopterans. Both species shared the MTR event *trnI-trnQ-trnM-nad2* → *trnI-trnM-nad2-trnQ* and a novel position of the CR. We found the same MTR in *S. szechwanensis* and in *H. fruhstorferi*, but the novel control region was absent in *H. fruhstorferi* (Fig. 2). An association between the shift of the CR and the MTR event, as Liu et al. (2013) assumed, is thus not clear.

Interestingly, we could assign a transposition event exclusive to *Phyllomimus* spp. and *Pseudophyllus titan*: *trnI-trnQ-trnM* → *trnM-trnI-trnQ* (Fig. 2; MTR event '(10)'). Different studies revealed this MTR for *Phyllomimus detersus* (Yang et al., 2016) and *P. titan* (Li et al., 2019), but an evolutionary conclusion has not yet been drawn. A mitogenomic analysis reported a similar MTR event in Lepidoptera and Hymenoptera (Wu et al., 2014), which leads to the hypothesis that the MTR "*trnI-trnQ-trnM* → *trnM-trnI-trnQ*" is plesiomorphic when considering insects as a whole. Yang et al. (2016) suggested that the MTR is based on a TDRL event. In contrast, the transposition *trnV-rrnS* → *rrnS-trnV*, reconstructed for Trigonidiidae, but revealed as well for *Ornebius bimaculatus*, as an unrelated species, implies homoplasy (Fig. 2; MTR event '(3)'). This is also supported by the results of Sanno et al. (2021) and Ma and Li (2018), who found the transposition *trnV-rrnS* → *rrnS-trnV* occurring two times independently in Grylloidea. Based on our data we suggest that the evolution of mitochondrial gene order does not follow a linear pattern. This is deviant from previous assumptions, that a high rate of nucleotide

substitutions is correlated with a high rate of gene rearrangements in insects (Shao et al., 2003). More research is needed to understand the evolution of the mitogenome in Orthoptera since its characteristics seem to differ strongly from other insects.

Despite the evolution of independent MTR events observed for the inversions of *trnY* ('(4)', '(12)', '(13)', '(18)', and '(21)'), *trnV* ('(12)', '(19)' and '(23)'), *rrnL* ('(9)', '(14)', '(17)', and '(20)'), *rrnS* ('(17)' and '(20)'), *trnC* ('(18)' and '(21)'), and *trnQ* ('(18)' and '(20)') (see Fig. 3 and Fig. S2), there are MTRs which we suggest being apomorphic for single species in the context of our dataset. Due to limited taxon sampling, the occurrence in other related species, however, remains largely uncertain. For example, the transposition of *nad3-trnA-trnR* → *nad3-trnR-trnA* was found in *Cyphoderris monstrosa* only (MTR event '(11)'). Other MTR events at the species level likely represent plesiomorphies. One prime example in our study is the inversion of the *trnH* gene, which occurred six times independently in *Lentula callani*, *Fruhstorferiola tonkinensis*, *Paratonkinacris vittifemoralis*, *Tonkinacris sinensis*, *Hieroglyphus tonkinensis*, and *Gonista bicolor* (Fig. 3; MTR event '(16)'). Based on our phylogeny, these species are unrelated. Hence, we suggest that this MTR has evolved convergently in these species. A similar pattern was observed by a study that analyzed the mitogenomes of Hymenoptera, in which an MTR occurred independently in at least five different families (Cameron, 2014).

Convergence of MTR events is not unusual in insects, even at larger taxonomic scales, as previously already reported by the similar gene orders in hymenopterans and Lepidoptera (Babbucci et al., 2014). That convergence of different traits appears to be common in Orthoptera has previously already been suggested, e.g. for morphological traits (Husemann et al., 2012), or for chromosome formation (e.g. Palacios-Gimenez et al., 2018) and number (Husemann et al., 2022). At least for chromosome number, a similar pattern, as found here for mitochondrial genome rearrangement has been found: variation occurred largely at terminal nodes rather than as synapomorphies of deeper branches (Husemann et al. 2022). In general, much remains to be learned about the evolution of gene order and similar traits in Orthoptera. However, we can conclude from

our analyses, that mitochondrial gene order has only limited power as a phylogenetic marker in Orthoptera.

Our results generally have to be treated with caution as it remains likely, that not all mitogenomes available in the databases are correctly assembled and such errors may lead to wrong conclusions. Yet, analyzing such potential errors is beyond the scope of our study. However, it remains likely, that wrongly assembled mitogenomes would rather lead to additional artificial variation and mitogenomic gene order may be even more conserved than observed in our analyses. More large scale sequencing efforts will likely shed more light on this in the future.

Comparison of phylogenetic patterns with previous analyses

We here used published mitogenomic data to reconstruct a phylogeny of Orthoptera, similar to previously used approaches (e.g. Song et al. 2015, 2018). The phylogenetic reconstruction by BI and ML (Fig. 1 anf Fig. S1) enabled a subsequent analysis of MTR events based on the information of the gene orders in terminal nodes. As the validity of our results rely on a robust and accurate phylogeny, we discuss our present phylogenetic tree in the light to the most recent and comprehensive phylogeny of Orthoptera (i.e. Song et al., 2020). Overall, our results are largely consistent with this phylogeny, which was based on transcriptomic and whole mitogenome data. This supports the idea that mitochondrial genomes are expected to recover intraordinal phylogenetic relationships concordant with other sources of data with high nodal support (Fenn et al., 2008). However, some differences were observed, which however, may be expected considering differences in the species composition of the datasets and the different marker systems used (mitogenomes vs. transcriptomes).

In Ensifera, Song et al. (2020) suggested Myrmecophilidae to be the sister group of (Trigoniidae (Phalangopsidae + Gryllidae)). In our phylogeny, Myrmecophylidae (only represented by a single species, *Myrmecophilus manni*) forms a sister taxon to

Gryllotalpidae (Fig. 1 and Fig. S1). In turn, *Ornebius* (Mogoplistidae) forms the sister taxon of (Trigoniidae (Phalangopsidae + Gryllidae)). However, Song et al. (2020) did not include any genera from the family Mogoplistidae, which explains the different topologies. For clarity in these families, we suggest including more taxa of Mogoplistidae and Myrmecophilidae in future phylogenetic studies. Moreover, in the tree by Song et al. (2020), Rhaphidophoridae was the sister group to the rest of the Tettigoniidea, however, without bootstrap support. In our present phylogeny, Rhaphidophoridae form the sister group to Anostostomatidae and *Stenopelmatus fuscus* (Stenopelmatidae) with high nodal support (Fig. 1 and Fig. S1). Moreover, (Rhaphidophoridae (Anostostomatidae + Stenopelmatidae)) form the sister group to Prophalangopsidae only in our BI tree; in the ML tree they group as follows: (Rhaphidophoridae (Prophalangopsidae (Anostostomatidae + Stenopelmatidae))) (Fig. S1). However, Song et al. (2020) suggest a closer relationship of Prophalangopsidae to Tettigoniidae. In contrast, *Comicus campestris* (Schizodactylidae) is the sister taxon of the remaining Tettigoniidea based on our analysis and does not represent the sister taxon to the grylloids like in the analysis of Song et al (2020). Gryllacrididae split off from the Tettigoniidea after *Comicus campestris* in our trees, but cluster with Stenopelmatidae and Anostostomatidae in the phylogeny of Song et al. (2020). This was also supported by an earlier analysis using four nuclear loci combined with mitogenomic data (Song et al., 2015). However, they placed *Comicus campestris* to the non-grylloid ensiferans in the phylogeny from 2015, similar to our results (Fig. 1 and Fig. S1). Overall, the placement of taxa within Tettigonidae is similar in both phylogenies, but no support is given for the monophyly of some subfamilies, e.g. Tettigoniinae and Conocephalinae (Song et al., 2020). All subfamilies in Ensifera, except Pseudophyllinae and Mecopodinae, are well-supported in our tree (pp > 0.95; Fig. 1).

In Caelifera, the topologies of all families are consistent with the phylogeny of Song et al. (2020). However, some subgroups are in need of revision. Our tree suggests paraphyly for the tribe Tetrigini, as the genera *Tetrix* and *Euparettix* do not form a clade (Fig. 1 and Fig. S1). These genera were not included in the study of Song et al. (2020) and can thus not be evaluated. Furthermore, within Pyrgomorphidae *Mekongiana* and

Mekongiella form a clade in the study by Song et al. (2020), but *Tagasta indica* was not included. Its inclusion in our (BI) phylogeny resulted in paraphyly of Sphenariini with high support by pp (> 0.95). Within Acrididae, the phylogeny by Song et al. (2020) and our study show some minor differences. The genus *Sinopodisma* is not monophyletic in our reconstruction. Song et al. (2020) in contrast, only included a single species *Sinopodisma tsinlingensis* not allowing an evaluation of the monophyly of the genus. The same is true for *Oxya, Euchorthippus*. *Chorthippus, Euthystira* and *Pseudochorthippus,* which were not included in the study by Song et al. (2020). Moreover, *Gonista bicolor* is considered as sister taxon to *Phlaeoba* spp. in our analysis, but forms a sister taxon to *Ceracris* spp. in Song et al. (2020).

In contrast to the analyses by Song et al. (2020), where the tribe Podismini is sister group to the rest of Acrididae, in our study a cluster consisting of Calliptaminae, *Shirakiacris*, *Choroedocus*, Cyrtacanthacridini and Catantopini forms the sister group to the remaining Acrididae. Next, a clade including the genera *Oxya, Caryanda, Spasthosternum*, *Pseudoxya, and Hieroglyphus* splits off after Podismini according to Song et al. (2020), but in our tree, these form the sister clade to Podismini, suggesting a closer relationship (Fig. 1). Acrididae is considered monophyletic by Song et al. (2020), but paraphyletic when using four nuclear loci and mitogenomic data combined, as Romaleidae clustered with Acrididae (Song et al., 2015). In our analyses, Derycorythidae clustered within Acrididae suggesting paraphyly as well (Fig. 1 and Fig. S1). As we only included a single species of Romaleidae, the monophyly of this family is yet to be resolved. The remaining Acrididae are not discussed in detail here, as the group is in strong need of revision and the systematics remain unclear.

However, as both our BI and ML trees have overall high nodal support and are largely consistent in the topology with the so far largest phylogenetic study by Song et al. (2020), the discussed inconsistencies do not diminish the validity of our findings on MTR evolution in Orthoptera.

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DATA AVAILABILITY STATEMENT

All data used in this study was obtained from NCBI Genbank and is available therefrom. The output from *TreeREx* is provided in the supporting information.

FUNDING STATEMENT

No funding was obtained for this study.

CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

ETHICS APPROVAL STATEMENT

This study involved no animal or human experiments requiring ethics approval.

SUPPORTING INFORMATION

Figure S1. Phylogenetic tree based on mt genome DNA by ML inferred with IQTree. Numbers near the nodes indicate bootstrap values (BS). Red dots on nodes indicate a BS value > 90. Black dots near the root indicate unknown BS values. The taxon labels are highlighted in different colors assigned to major groups based on the currently accepted taxonomy (obtained from Orthoptera Species File; Cigliano et al., 2021) which are shown on the right. The '*' -sign is added to all clades which are considered either paraphyletic or polyphyletic by the present results. No assigned tribe/subfamily implies that the taxon in question is currently not classified into any of the groups within a family. Tribes in blue letters: classified into Oedipodinae, tribes in red letters: classified into Gomphocerinae. Editing was performed with Inkscape and Figtree software.

Figure S2. Complete phylogenetic reconstruction (Bayesian Inference) of Orthoptera from mt genome data (left). MTR events predicted by TreeREx mapped on the tree (right). Numerals refer to rearrangement events of taxa; red circles: inversions, transpositions, inverse transpositions or TDRLs; blue circles: duplication; yellow circles: deletions or missing information; see Table S3 for those excluded taxa. letters in white circles: novel control region position. Collapsed branches are indicated by triangles. Dots on nodes indicate a consistency index (CI) of 1. An output scheme inferred with CrEx is shown for visualization of multiple MTR events of Lipotactes tripyrga. Monophyletic genera occur as “Genus spp.” monophyletic species as "ssp.” Note that the MTR scenarios of Euborellia arcanum are not shown despite its multiple modifications, since it was excluded from the TreeREx analysis.

Table S1. List of taxa used in the study for phylogenetic inference and gene order analyses with additional taxonomic information according to the Orthoptera species file (Cigliano et al., 2021) and genome length. “GB” = GenBank, “OH” = O. Hawlitschek, “Prepr.” = preprint.

Table S2. Abbreviation list

Table S3. List of taxa with mt genomes containing gene duplications, deletions or *missing gene information, e.g. due to incomplete assembling. These taxa were not used in the TreeREx analysis

Appendix S1. Gene order file (as .txt file) uploaded to https://github.com/laradey/mitogenome_gene_order.

Appendix S2. Original TreeREx output (Bayesian inference tree as .svg file) with consistency indices (CI) indicated in green and yellow boxes. "I": inversion, "T": transposition, "iT": inverse transposition, TDRL: tandem-duplication/random loss uploaded to https://github.com/laradey/mitogenome_gene_order.

All supporting information can be found on page 514 and following.

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SG, OH, MH designed the study; SG collected data and carried out analyses; SG, MH, OH prepared the original draft; SG, OH, MH, LSD took part in the writing process of the manuscript.

Hamburg, 12.01.2023 

Place/Date Sign

Chapter 6

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"Same same but different"

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Genomic data, morphometrics and ecological modelling confirm large scale convergence of wing morphology in band winged grasshoppers (Orthoptera: Oedipodinae)

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ABSTRACT

Convergent evolving traits are a frequently observed evolutionary mechanism: similar traits are evolving without common ancestry. This mechanism was investigated in a large number of species that show similar ecological or morphological traits despite geographical separation. Species of the grasshoper subfamily which show similar morphological traits at different continents that lack common ancestry are the Oedipodinae grasshoppers. More than 797 species are recorded with an almost cosmopolitan distribution. In this study we use Hybridization capture sequencing approach to gain suitable markers to build a stable phylogeny of the group. We used eleven groups of morphological similar species analyzed these using geometrical morphometrics of 12 landmarks. We tested the similarity of the climatic niche via niche overlap analyses and reconstruction of potential suitable habitats on the counterpart continent and calculated the ancestral niche based on a morphological and phylogeny traits. Based on hundreds of single-copy protein coding molecular markers. Phylogenomic tree reconstruction shows a separation of Old and New world Oedipodinae. The morphometrical analyses clustered the species into four groups, which all contain individuals from the Old and New world, without a separation of the geographical regions. We found niche overlap in eight species pairs. Ancestral niche reconstruction showed less evolutionary steps in the morphometrical than in the phylogeny based on molecular markers. Our analyses show a clear trend of convergent evolved species and it is most likely that a similar niche lead to similar morphology despite geographical separation.

Key words

Convergent evolution, Oedipodinae, Hybridization Capture, phylogenomics, geometric morphometrics, ecological niche modelling, ellipsenm, nichevol

INTRODUCTION

Convergent evolution produces similar phenotypes, which do not origin from common ancestry, but are the result from similar selective forces, which may drive the evolution of similar morphological traits without close ancestry (Gavrilets & Losos, 2009; Schulter, 2000). Such convergent traits can evolve at almost any scale. From the evolution of similar patterns across orders (e.g. flight morphology and foraging niche of bats and birds; Norberg, 1986' body shape specialized anatomy of sharks, dolphins and tuna; Lingham-Soliar, 2016), to the evolution at genus or species level such as flower coloration of fly pollinated plants (Garcia et al. 2022). Convergent adaptation is frequently observed in various model organisms like the anoles lizards, where several similar ecomorphs evolved on different islands of the Greater Antilles (Losos et al. 1998, 2003). Even though these similarities could have resulted from a single origin, followed by vicariance, dispersal and extinction events, the outcome of the phylogenetic analyses show that the ecomorphs are the result of independent origins. Another example of convergent evolution are stickleback fishes which repeatedly move between freshwater and marine environments. These fishes have convergently evolved to adapt and re-adapt to these environments in different species (Schluter et al. 1995; Rundle et al. 2000; Jones et al. 2012). A lot of research in the past decades has also been done in the cichlid fishes. When Kocher et al. (1993) started working on the cichlid populations from six morphologically similar fish species of several genera from Lake Tanganyika and Lake Malawi, they investigated the genetic relationship between the species. Using phylogenetics they found two radiations. One radiation in Lake Tanganyika, which is suggested to be the older one, and a second radiation in Lake Malawi. In adaption to similar ecological and trophic niches the species in different lakes evolved similar morphological features. Further studies do not only show convergent morphology, but also in the behavior for e.g different cichlid fishes in different species flocks (Colombo et al. 2013).

One insect taxon showing large scale patterns of morphological similarities with a likely lack of common ancestry are Oedipodinae grasshoppers. They are distributed across the

whole world, except the Arctic and Antarctic regions. More than 797 species are currently described (Cigliano et al. 2022). Recent studies on this group dated the ancestors to a relatively young age (Paleogene; Chapco & Contreras, 2011; Husemann et al. 2011; Song et al. 2018;). Song et al. (2018) used phylogenomic data to investigate phylogenetic relationships within Acrididae and dated phylogeny to trace back their potential migratory pathways. Their results suggested that the ancestor of Orthoptera originated in South America, although most studies supported an African origin. They proposed at least three colonization events in the taxon's history. In the Cretaceous the ancestors of the Acrididae were present across the connected South American and African continents. After the split off of these two continents, the species evolved separately. They hypothesized that a first colonization wave happened transatlantic from the Old World (OW) to the New World (NW) thus, common ancestors of Orthoptera must have recolonized the NW. Within the second wave of recolonization the Acridinae-Gomphocerinae-Oedipodinae complex - which has an OW origin - conquered the NW during the Eocene from Eurasia to North America through the Thulean route (across Greenland) or the Beringian Bridge. This might have been possible due to expanding grasslands within this time. The third wave of colonization occurred only in the subfamily Cyrtacanthacridinae, potentially as a result of long distance flight from the OW to the NW. Summing up, the study hypothesizes a single colonization event of the Oedipodinae from the OW to the NW around 36 Million years ago (Mya). Husemann et al. (2011) investigated the colonization of the Bryodemini and Sphingonotini of the Old World (OW) and New World (NW) on a limited taxon set (17 NW and 22 OW species). Here, the NW and the OW taxa each formed a clade, based on a multi-gene phylogeny. The study also suggested that the species have colonized the NW regions by a single colonization event and had an origin at about 35 Mya. But based on the very limited lineage representation, further statements about the relationships within the Oedipodinae were not possible.

With their intercontinental studies based on mitogenome and multi-gene phylogenies Song et al. (2018) and Husemann et al. (2011) provided first insights on the morphologically similar taxa from the OW and NW based on a more extensive sampling,

as the sampling of previous studies had a very limited taxon set (e.g. Chapco et al. 1997; Fries & Chapco, 2007; Chapco & Contreras, 2011). Both regions harbor a large number of species which are morphologically very similar in body and wing shape, but also in behavior and ecology. The proposed single colonization event leads to the question on how these similarities have evolved over time.

In this study, we reconstruct for the first time a phylogeny of Oedipodinae based on a large representation of the genome using the hybridization capture approach (Horn, 2012) that results in hundreds of molecular markers used for phylogenomics. We discuss the usefulness of this approach for the taxon Oedipodinae and investigate whether or not similar morphological traits of the NW and OW clades emerged due to convergent evolution. We therefore use eleven pairs of species from the two regions which are subjectively similar in body and wing shape. The pairs are analyzed by geometric morphometrics of the elytra. Additionally, we investigate their ecological niche evolution, hypothesizing that species with morphological similarities occur in very similar environments in the OW and NW. This could have led to morphological convergences, resulting from climatic-driven adaptations to similar environmental niches. On the top, we analyze the overlap of lineages in climatic space and reconstruction of their climatic niches to receive further insights into potential drivers of morphological convergent traits.

MATERIAL AND METHODS

Taxon sampling

Altogether 97 specimens of 56 species were sampled for the molecular analyses, thereof 77 specimens of 48 species were analyzed on Amino acid level. Furthermore, 475 specimens of 11 species were photographed for geometrical morphometrics, thereof 342 photos were used for analysis.

For comparative investigation of the morphological similarity of Oedipodinae species, we designed two datasets. The first dataset comprised several NW and OW species for genomic analyses and the second dataset only comprised 11 species pairs of similar looking species to check for convergent evolution. The 11 pairs consisted of **A** - *Mioscirtus wagneri* (Eversmann, 1859; OW) and *Trachyrhachis kiowa* (Thomas, 1872; NW); **B** - *Bryodemella tuberculata* (Fabricius, 1775 ; OW) and *Circotettix undulatus* (Thomas, 1872; NW); **C** - *Sphingonotus nebulosus* (Fischer von Waldheim, 1846; OW) and *Trimerotropis latifasciata* Scudder, 1880 (NW); **D** - *Trilophidia annulata* (Thunberg, 1815; OW) and *Chortophaga viridifasciata* (De Geer, 1773; NW); **F** - *Sphingonotus pilosus* Saussure, 1884 (OW) and *Trimerotropis sparsa* (Thomas, 1875; NW); **G** - *Scintharista notabilis* (Walker, 1870; OW) and *Arphia simplex* Scudder, 1875 (NW); **J** - *Acrotylus patruelis* (Herrich-Schäffer, 1838; OW) and *Heliastus sumichrasti* (Saussure, 1861; NW); **K** - *Psophus stridulus* (Linnaeus, 1758; OW) and *Arphia pseudonietana* (Thomas, 1870; NW); **M** - *Oedipoda aurea* Uvarov, 1923 (OW) and *Lactista azteca* (Saussure, 1861; NW); **N** - *Bryodema luctuosum* (Stoll, 1813; OW) and *Circotettix carlinianus* (Thomas, 1870; NW); **O** - *Morphacris fasciata* (Thunberg, 1815; OW) and *Conozoa sulcifrons* (Scudder, 1876; NW). Pictures of all species are provided in Figure 1 and 5. As no genetic material was available for *Heliastus sumichrasti*, a sample of the rather similar looking congeneric *Heliastus subroseus* Caudell, 1904 was used for genomic analyses. Within the geometric morphometric analyses *Circotettix rabula* Rehn & Hebard, 1906 was used instead of the congeneric *Circotettix undulatus.*

Data generation and Phylogenetic analyses based on Molecular markers

DNA extraction

Genomic DNA (gDNA) was extracted for 96 samples (Table 1) following the High Salt Extraction Protocol by Paxton et al. (1996). DNA was eluted in 100ul ddH2O for Quality checking. Quality checking was performed using Nanodrop 2000 (Thermo Fisher, Waltham, Massachusetts, USA), Qbit 3 (Thermo Fisher, Waltham, Massachusetts, USA) and Tapestation 4200 (Agilent Technologies, Santa Clara, California, USA). Only

samples with DNA quality (UV ratio 260:280 of 1.7 to 1.94) and quantity (concentration of 4.5 to 116 (ng/ul) were included into the study. DdH2O was removed from all samples using a Concentrator 5301 (Eppendorf, Hamburg, Germany) and sent for Hybridization Capture to Daicel Arbor Biosciences (Ann Arbor, Michigan, USA).

Bait design

Baits were originally designed by Song et al. (in prep.) for two separate sets. The used combined set, kindly provided by Song et al. (in prep.) included 1,270 single copy protein coding genes in two sets (set1: 331 and set2: 939) based on OrthoDB 7 (Kriventseva et al. 2007) .

Sequencing

All individuals were sequenced at for Hybridization Capture to Daicel Arbor Biosciences (Ann Arbor, Michigan, USA) on a NovaSeq S4 PE150 platform with paired-end sequencing and a sequencing depth of 2400-3000Gb.

Hybridization capture data quality check

Upon receiving the raw sequence data, we performed initial quality checks on a local LINUX distribution using FastQC v.0.11.9 (Andrews, 2010) on Anaconda3 (Anaconda Software distribution, 2020) followed by summarizing the FastQC outputs using MultiQC v.1.12 (Ewels et al. 2016). Adapters were then trimmed and duplicates removed using fastp (Chen et al. 2018) under paired-end function. Furthermore, AfterQC v.0.9.7 (Chen et al. 2017; paired-end) was used for a final check of sequence quality.

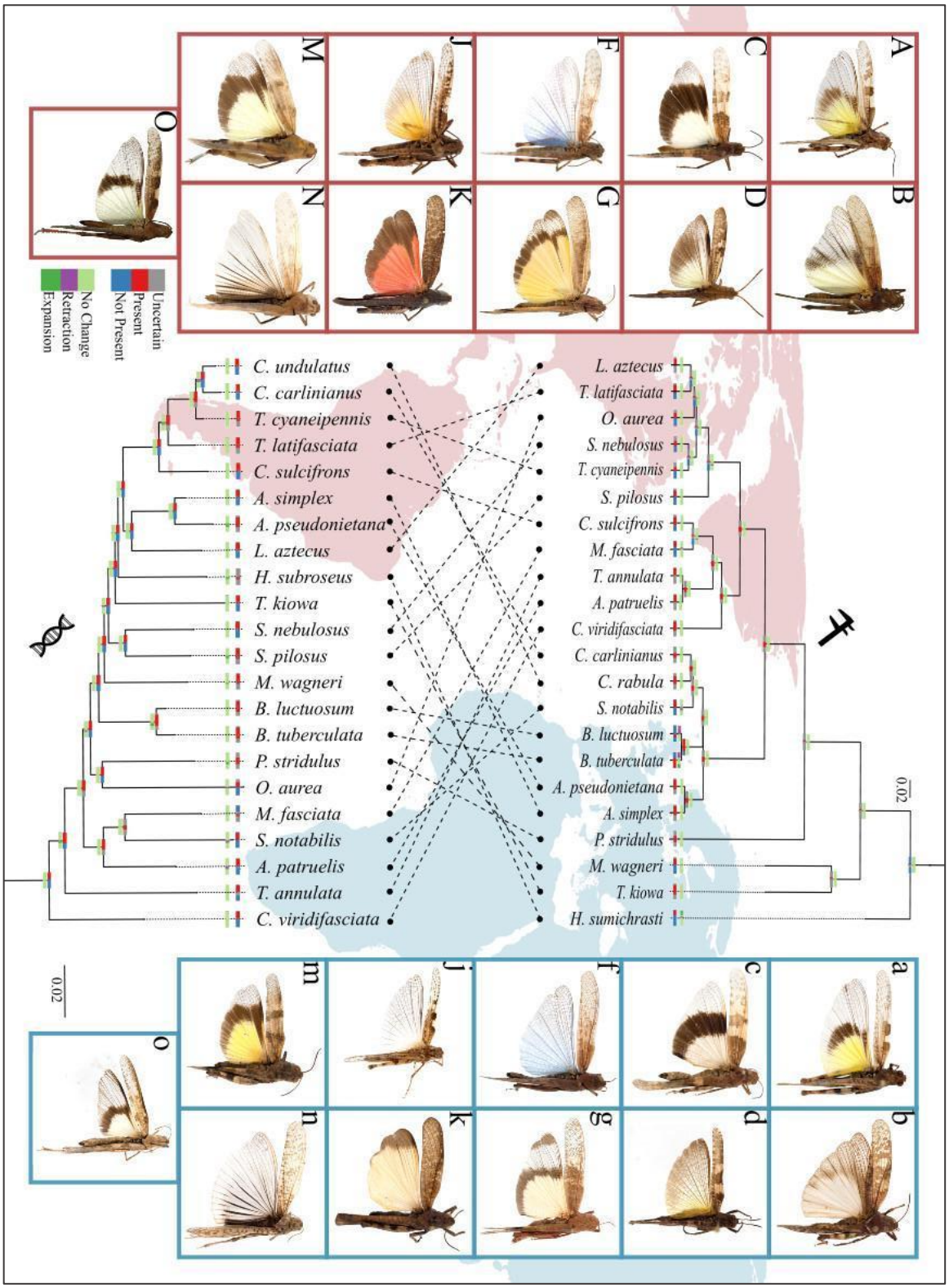

A
B
C
D
F
G
J
K
M
N
O
Uncertain
Present
Not Present
No Change
Retraction
Expansion
C. undulatus
C. carlinianus
T. cyaneipennis
T. latifasciata
C. sulcifrons
A. simplex
A. pseudonietana
L. aztecus
H. subroseus
T. kiowa
S. nebulosus
S. pilosus
M. wagneri
B. luctuosum
B. tuberculata
P. stridulus
O. aurea
M. fasciata
S. notabilis
A. patruelis
T. annulata
C. viridifasciata
L. aztecus
T. latifasciata
O. aurea
S. nebulosus
T. cyaneipennis
S. pilosus
C. sulcifrons
M. fasciata
T. annulata
A. patruelis
C. viridifasciata
C. carlinianus
C. rabula
S. notabilis
B. luctuosum
B. tuberculata
A. pseudonietana
A. simplex
P. stridulus
M. wagneri
T. kiowa
H. sumichrasti
0.02
0.02
a
b
c
d
f
g
j
k
m
n
o

Figure 1: Combining analysis of UPGMA tree created with geometrical morphometric data, genomic data and ancestral distribution reconstruction. All included NW species are shown in red boxes – group representation is shown in the upper left corner as capital letter. All OW species are shown in blue panels – group representation is shown in the upper left corner as lowercase letter. UPGMA tree based on geometric morphometric data is shown in the top and marked with a digital caliper symbol, while the genomic tree is shown in the bottom and marked with a DNA symbol. Needed evolutionary steps of the ancestral niche reconstructed are plotted on the trees. Abbreviations stand for: **A** - *Mioscirtus wagneri;* **a** - *Trachyrhachis kiowa*; **B** - *Bryodemella tuberculata*; **b** - *Circotettix undulatus*; **C** - *Sphingonotus nebulosus;* **c** - *Trimerotropis latifasciata*; **D** - *Trilophidia annulate*; **d** *Chortophaga viridifasciata*; **F** - *Sphingonotus pilosus*; **f** - *Trimerotropis sparsa;* **G** - *Scintharista notabilis*; **g** - and *Arphia simplex*; **J** - *Acrotylus patruelis;* **j** - *Heliastus sumichrasti*; **K** - *Psophus stridulus*; **k** - *Arphia pseudonietana*; **M** - *Oedipoda aurea*; **m** - *Lactista azteca*; **N** - *Bryodema luctuosum*; **n** - *Circotettix carlinianus*; **O** - *Morphacris fasciata*; **o** - *Conozoa sulcifrons.*

Data assembly and Orthology prediction

All further steps were carried out on the HUMMEL HPC Cluster of Hamburg University, Genome DeNovo assembly was performed using Trinity v. 2.11.0 using default options (Haas et al. 2013). We followed the general analysis strategy and scripts published or kindly provided by the 1KITE consortium (e.g. Misof et al. 2014, Peters et al 2017, Bank et al. 2017, Wipfler et al. 2019, Evangelista et al. 2019, Song et al. 2020). Orthograph v.0.7.1 (Petersen et al., 2017) was used for orthology prediction of the hybrid capture data: Orthograph required a file with the clusters of orthologous genes (COG) listed. For this the original COG file was used (5,414; Song et al. in prep.) of selected reference taxa: *Acyrthosiphon pisum* official gene set version 2.0 (APISU; Davis & International Aphid Genomics Consortium, 2010), *Pediculus humanus* official gene set version 1.0 (PHUMA, Schoch et al. 2020), *Rhodnius prolixus* official gene set version 1.2 (RPROL; Mesquita et al. 2015) and *Zootermopsis nevadensis* official gene set version 2.2 (ZNEVA; Terrapon et al. 2014). It furthermore requires the full official gene set (OGS) of these reference taxa for identification of the best reciprocal hit. From these Orthograph used an internal SQLite database (version 3.33; http://www.sqlite.org/) to identify

potential candidates and afterwards the best reciprocal hits from the hybrid capture sequences form each sample.

A config file for each sample (here taxon) was created including the programs mafft-linsi v.7.273 (Katoh et al. 2013), hmmbuild function of HMMER v.3.1b1 (Eddy, 2009), makeblastdb v.2.2.28+ (Camacho et al. 2009), fastatranslate (Slater & Birney, 2005), hmmsearch function of HMMER v.3.1b1 (Eddy, 2009), blastp (Altschul et al. 1990) and exonerate v.2.2.0 (Slater & Birney, 2005). The max-blast-searches was set to 15, blast-max-hits to 15, num-threads to 1, brh-only to 1, orf-overlap-minimum to 0.5, strict-search was activated, as well as U's were substituted to X. The Orthograph-manager ran all taxa integrating the peptide files of the reference taxa and subsequently Orthograph-analyzer was executed. Orthograph-reporter was used to fetch the search results assign ortholog relations by clustering best reciprocal hits. All results were summarized using the summarize_orthograph_results v.2016-01-14 script (Peters et al 2017). All outcomes (3,637 EOGs) were converted to fasta format using orthograph2hamstrad script provided with Orthograph. Orthograph statistics were generated. Results were checked with the calculate_statistics_orthograph_results v.2017 script., also included in the Orthoraph package

Alignment preparation

Summarized results ("Hamstrad" format) were used as input files for multiple sequence alignments per locus, and saved in separate directories (amino acid level, in the following aa and nucleotide level, in the following nt). MAFFT with the algorithm L-INS-I (v.7.123; Katoh et al. 2013) was used to align the aa sequences for each orthologous group (OG) separately. All files were then converted to non-interleaved format using the fastasingleline script provided by 1KITE which changes the interleaved fasta files to non-interleaved format.

Outlier check

All alignments on aa level were checked for outlier sequences (see e.g. Misof et al 2014; Peters et al 2017) with same settings using the scripts checker_complete 1.3.2.1 and checker_complete 1.3.2.2 (Karmeinski et al. 2021). All output files were checked for outlier sequences, none were identified, so all OGs and all sequences were kept for further analyses.

Removal of reference taxa and gaps within the aa alignment

For removal of reference taxa of the original ortholog set (i.e. APISU, PHUMA, ZNEVA and RPROL) the script RemoveCoreTaxa_DB (Misof et al. 2014) was executed. Only new files with the taxa of interest were kept in the dataset. Afterwards the script selectSites.pl (Bagley, 2020) was used to remove columns with gaps only from the alignments. Files were again converted to non-interleaved format.

Alignment masking of OGs on aa level

For Alignment creation using Aliscore (Misof & Misof, 2009) headers of all files were shortened to the specimen ID. Aliscore v.2015-06-22 was run to filter ambiguous or randomly similar sites within the alignments using the default settings. A new directory including all not processed genes was written. Afterwards Alicut was executed using the Aliscore outputs to remove ambiguously aligned positions from the sequence alignment using the default settings. Altogether 3,364 EOGs were left after Alicut analyses, 273 files were not processed by Aliscore.

Design of supermatrix datasets

To generate a supermatrix of all multi-sequence alignments FasConCAT-G v.1.02 (Kück & Longo, 2014) using the options Rename Sequence Names(-k), Print PHYLIP Output (Strict) (-p) and Start ProtTest for RAxML Partition File (-m) was executed. For this, a

text file including all previous and new specimen names, including specimen ID and species identification was written for renaming.

A charset file was written manually including the Eukaryote Orthologues Gene (EOG) name, starting and end position of each gene. Output files (supermatrix and charset file) were processed with MARE v. 0.1.2 (Misof et al. 2013) to identify and remove partitions without information content. Alistat v.1.2.1 (Wong et al. 2020) was used to check the information content (presence absence) of each specimen in the initial alignment Using a heat map of sequence pairs scores for evaluation. Further Symtest v.2.0.47 (Jermiin & Ott, 2017; https://github.com/ottmi/symtest) was used to calculate the pairwise similarity between all specimens (SI 1). A final charset and supermatrix file was used in further analyses. To process subsets of the full dataset, partitions without any information content were again screened and removed using the remove_IC0.1.1 v.2013-04-16 script (Misof et al. 2014) provided by 1KITE. Files including subsets of the full dataset were written and processed running the script fasta2hypo.v.1.5.2 (Misof et al. 2014).

Two datasets were created: (1) a full dataset containing all analyzed specimens (77 individuals of 48 species– Table 1) following called "*full dataset*" and (2) a dataset containing only the convergent taxa (40 specimens of 22 species– Table 1) in the following called the "*convergence dataset*". Both included a sample of Gomphocerinae as outgroup. Several taxa were excluded from both the "*full dataset*" as after filtering no usable data remained: Iran243 *Pseudoceles persa*, WW481 *Anconia integra,* JB01 *Arphia pseudonietana*, D1305 *Scintharista notabilis*, D830 *Psophus stridulus*, D321 *Oedipoda aurea*, Iran247 *Pseudoceles persa* and more than 50% gaps/ambiguity: MS *Bryodema orientale simulans*. Furthermore JB01 *Arphia pseudonietana,* D830 *Psophus stridulus* and D321 *Oedipoda aurea* were excluded from the "*convergence dataset*". All following steps were executed for each dataset.

Phylogenetic reconstruction

As a first step the alignments were evaluated for best models to run the analyses. This step was performed using Modelfinder (Kalyaanamoorthy et al. 2017) integrated in the IQ-TREE v. 2.2.0 package (Minh et al. 2019) allowing to search for all model parameters including LG4X, LG4M.

Best model schemes for each dataset were used to calculate 20 trees for each alignment using IQ-TREE, 10 trees with a parsimony start tree (default) and 10 trees with a random start tree. Log-Likelihood scores of each tree were checked manually. The tree with the best log-Likelihood score was defined as best tree. For the "*full dataset*" 750 bootstrap replicates were calculated and separated into fifteen partitions of each 50 bootstraps using randomized start trees. For each dataset, all bootstrap trees were written into a file and mapped onto the best ML tree. Whether or not we conducted sufficient bootstrap replicates (convergence) which were tested in RAxML v. 8.2.12 (Stamatakis, 2014) using the bootstop criterion of 100, parsimony random seed, GTRGAMMA as substitution model, threshold for bootstrapping criteria of 0.01 and autMRE (extended majority-rule consensus tree criteria). Bootstrap convergence was confirmed after 750 bootstrap replicates for the "*full dataset*". For the "*convergence dataset*" only 100 bootstrap replicates were calculated. Convergence was checked. Afterwards bootstrap values were plotted onto the best ML tree for the "*full dataset*" and the reduced "*convergence dataset*" using IQ-TREE with the option -st AA (sequence type) -gmedian (median approximation for Gamma rate heterogeneity) and -safe (avoiding numerical underflow for large datasets). The final trees including bootstrap support were exported and further processed for tree visualization.

Seaview was used for rooting of the tree, afterwards trees were processed with FigTree and InkScape for visualization.

Table 1: All specimens and information regarding Specimen ID, Taxon, collection date, collector, collection country and collection location. Abbreviations in column collector are the following – MH; Martin Husemann; LSD: Lara-Sophie Dey; OH: Oliver Hawlitschek.

Specimen ID	Taxon	date	collector	Country	Location
19	*Compsorhipis orientalis*	22.07.2019	LSD	Mongolia	45.238959°N 109.878633°E, 896m
Bal27	*Arphia simplex*	8/27/2011	MH	USA	Texas, Singleton Road, half mile south of gloated bend recreation area
Bal28	*Arphia simplex*	8/27/2011	MH	USA	Texas, Singleton Road, half mile south of gloated bend recreation area
D1074	*Trilophidia annulata*	17.10.2018	MH	Japan	Kamo river, Sukyo, Kyoto
D1076	*Trilophidia annulata*	18.10.2018	MH	Japan	Mt. Yoshida, Sukyo, Kyoto
D11	*Trimerotropis sparsa*	31.07.2011	D.Ferguson	USA	New Mexico, Window Rock, New Mexico side of state line
D12	*Trimerotropis sparsa*	31.07.2011	D.Ferguson	USA	New Mexico, Window Rock, New Mexico side of state line
D122	*Thalpomena viridipennis*	11.02.2014	MH	Morocco	5 km hinter Aknaul von Taza aus
D1255	*Sphingonotus insularis*	11.02.2010	R. Felix	Yemen, Socotra	Wadi Shilliyin, N12.52643 E54.24531
D13	*Conzoa sulcifrons*	30.08.2013	D.Ferguson	USA	New Mexico, Rio Grande Botanic Garden, Alberquerque
D1309	*Morphacris fasciata*	3/24/2019	LSD & MH	Morocco	30.597349N 09.275689W

Specimen ID	Taxon	date	collector	Country	Location
D1313	*Acrotylus patruelis*	3/30/2019	LSD & MH	Morocco	30.769562N 69.145271W
D1328	*Neosphingonotus azurescens*	3/24/2019	LSD & MH	Morocco	30.571324N 09.283294W
D14	*Conozoa sulcifrons*	30.08.2013	D.Ferguson	USA	New Mexico, Rio Grande Botanic Garden, Alberquerque
D1447	*Scintharista notabilis*	31.03.2019	LSD & MH	Morocco	31.410931°N 08.121467°W
D1463	*Sphingonotus rubescens*	3/31/2019	LSD & MH	Morocco	31.410931°N 08.121467°W
D1565	*Acrotylus patruelis*	3/30/2019	LSD & MH	Morocco	30.769562N 09.145271W
D1596	*Egnatiella major*	3/28/2019	LSD & MH	Morocco	29.974158°N 09.593866°W, 44m
D1609	*Morphacris fasciata*	3/27/2019	LSD & MH	Morocco	30.396019°N 9.595755°W
D165	*Thalpomena viridipennis*	13.02.2014	MH	Morocco	Close to Bab Taza
D1746	*Sphingonotus savignyi*	04.05.2004	Hochkirch	Spain	La Gomera, San Sebastian
D1748	*Sphingonotus savignyi*	2/13/2004	A. Hochkirch	Spain	Tenerife, Palm Mar
D1882	*Bryodemella tuberculata*	28.08.2020	LSD	Germany	Silvensteinspeicher
D1884	*Bryodemella tuberculata*	03.08.2008	E. Budrys	Lithuiana	Svencionys: Mazalote military training ground

Specimen ID	Taxon	date	collector	Country	Location
D1932x	*Circotettix carlinianus*	05.08.2008	R.D. Scott	USA	Mc Cone Mt. N 47 17.683 W 105 48.360
D264	*Aiolopus simulatrix*	13.06.2006		Marocco	Asmaa
D320	*Oedipoda aurea*	04. Nov 14	MH	Greece	Kos, Mountains close to Zia, highest point
D499	*Sphingonotus pilosus*	20.09.2014	MH	Iran	Shemshak roadnext to phosphate mine of Girood
D501	*Sphingonotus pilosus*	20.09.2014	MH	Iran	Shemshak roadnext to phosphate mine of Girood
D518	*Sphingonotus savignyi*	5/19/2012		Algeria	Bir Naam
D59	*Heliastus subroseus*	04.09.2013	MH	USA	Texas, Galveston Island SP
D753	*Sphingonotus savignyi*	4/14/2009	A. Moussi	Algeria	A Benoui
D78	*Sphingonotus savignyi*	2013	C. Hemp	Kenya	Lake Turkana
G3	*Psophus stridulus*	28.08.2020	LSD	Germany	Silvensteinspeicher
HC21	*Lactista aztecus*		MH	USA	Texas, Hill Country, Double R Ranch
InIl_09	*Chorthippus brunneus / eisentrauti*	13.08.2016	OH	Austria	Hermagor, Presseggen, north of Seeblickfelsen
Iran135	*Mioscirtus wagneri*	Aug.-Sep. 2012	Hodjat	Iran	Yasnj, Bakti Ary

Specimen ID	Taxon	date	collector	Country	Location
Iran193	*Mioscirtus wagneri*	Aug.-Sep. 2012	Hodjat	Iran	Broojen & Shahr-Kord, Chahar-Mahal Bakhtiari, Yasuj, Jahrom
Iran244	*Pseudoceles persa*	20. Sep 14	MH	Iran	Shemshak roadnext to phosphate mine of Girood
Iran348	*Heliopteryx humeralis*	18.09.2015	MH	Iran	
Iran349	*Heliopteryx humeralis*	18.09.2015	MH	Iran	
Iran435	*Sphingonotus satrapes*	28.08.2017	MH	Iran	N36°26.306' E055°09.142', 1308m
Iran457	*Leptopternis gracilis*	26.08.2017	MH	Iran	Ianat Abad, N35°21.106' E51°59.297'
Iran471	*Leptopternis gracilis*	8/28/2017	MH	Iran	N36°26.306' E55°09.142'
Li7	*Bryodemella tuberculata*	-	L. Xinjiang	China	China, Hebei Pingquan
M3	*Parasphingonotus radioserratus*	-	MH	Morocco	Tarda, 10 km westlich Errachidia (Oase)
MGL204	*Bryodema gebleri mongolica*	07.05.2017	LSD	Mongolia	Semi-desert region 46.136433N, 99.177505E, 1718m
MGL475	*Bryodemella holdereri*	7/22/2017	LSD	Mongolei	47.71684 N 105.021805 E; H: 821m
MGL49	*Sphingonotus nebulosus*	30.07.2015	LSD	Mongolia	Südabdachung Church-uul, 101m Höhe
MGL629	*Angaracris barabensis*	25.07.2019	LSD	Mongolia	45.779485°N 107.252248°E, 1442m

Specimen ID	Taxon	date	collector	Country	Location
MGL677	*Bryodemella tuberculata*	09.07.2019	LSD	Mongolia	48.382807°N 111.707577°E, 1261m
MGL699	*Bryodema luctuosum*	13.07.2019	LSD	Mongolia	47.876445°N 117.883861°E
MGL729	*Bryodema luctuosum*	08.07.2019	LSD	Mongolia	48.273358°N 111.712596°E, 1127m
MGL758	*Oedaleus decorus*	7/21/2019	LSD	Mongolia	45.622964°N 112.616956°E, 830m
MGL8	*Sphingonotus nebulosus*	30.07.2015	LSD	Mongolia	southside of Church-uul
MGL94	*Sphingonotus obscuratus*	7/25/2015	LSD	Mongolia	Khanbogd
MS_1	*Bryodema zaisanicum zaisanicum*	-	M. Sergeev	Russia	(MS_15)
MS	*Bryodema orientale simulans*	-	M. Sergeev	Russia	(MS_3)
MS11	*Bryodemella holdereri occidentale*		M. Sergeev	Russia	
S17_2	*Bryodemella tuberculata*		MH	Russia	Siberia, South Tschuense, Ak-Kol river, Sofijski glacier
Sw	*Bryodemella tuberculata*		MH	Sweden	Öland, Stora Alvaret

Specimen ID	Taxon	date	collector	Country	Location
T107	*Circotettix carlinianus*	*09.08.2006*	R.D. Scott	USA	Montana, Philips Co. Mt. N47 38.509 W 108 34.200
T109	*Trimerotopis latifasciata*	23.07.2008	R.D. Scott	USA	Montana, Blain Co. MT, Missouri Breaks, N 47 44.727 W 109 23.093
T111	*Trimerotopis latifasciata*	23.07.2008	R.D. Scott	USA	Montana, Blain Co. MT, Missouri Breaks, N 47 44.727 W 109 23.093
T148	*Anconia integra*	27.08.2010	D. Ferguson	USA	
T17	*Trimerotropis cyaneipennis*	10.01.2010	D. Ferguson	USA	New Mexico, unnamed canyon, west base of Manzano Mountains, Tierra Grande, se. Valencia County, New Mexico
T170	*Sphingonotus fuscoirroratus*			Equador	Galapagos, Santa Cruz, Tortuga Bay
T23	*Circotettix undulatus*	20.09.2009	D. Ferguson	USA	California, East of summit, Tioga Pass, Mono County, California
T24	*Circotettix undulatus*	20.09.2009	D. Ferguson	USA	California, East of summit, Tioga Pass, Mono County, California
T4	*Trimerotropis cyaneipennis*	04.10.2009	D. Ferguson	USA	Arizona,Top of Hualapai Mountain, Mountain Road, south of Kingman, Mojave County, Arizona
WW288	*Chortophaga viridifasciata*	23.06.2012	MH	USA	Texas, Brazos River in Waco
WW308	*Heliastus subroseus*	18.08.2012	MH	USA	Texas, Padre Island National Seashore, 8 mls down the beach

Specimen ID	Taxon	date	collector	Country	Location
WW362	*Arphia pseudonietana*	29.03.2013	MH	USA	Texas, Rest area on I35S, close to exit 123
WW527	*Heliaula rufa*	19.09.2010	D. Ferguson	USA	New Mexico, Tierra Grande, SE corner Valencia Co.
WW55	*Trachyrhachys kiowa*		MH	USA	Texas
WW603	*Chortophaga viridifasciata*	09.07.2013	MH	USA	Texas, Baylor Campus
WW96	*Lactista aztecus*		MH	USA	Texas, Cameron Park

Geometric morphometrics

Landmark acquisition

Wings of all individuals were fixed between two flattened glass slides and photographed from the same angle (180°) using a Canon Eos D200 with 100mm macro-lens. A tpsdig input file was written using tpsUtil (Rohlf, 2004). All pictures of wings were integrated into tpsDig (Rohlf, 2004) afterwards. First a scale of each object was set, afterwards 12 landmarks (SI 2) were digitized for each individual. Landmark coordinate data was saved as tps file (SI 3) and opened in MorphoJ (Klingenberg, 2011). In MorphoJ a new project was created, Procrustes analyses were run and a covariant matrix was written. This analysis is a multivariate technique which involves transformations (i.e., translation, rotation, reflection, isotropic rescaling) of the landmarks data matrix. Further outlier specimens were discarded from the final dataset. Three classifier groups were defined for the dataset ("*species*", "*region*" and "*morphological group*").

A consensus matrix was then applied, including only the average of each species. The resulting average matrix was then loaded into Past3 to calculate a UPGMA Tree based on Euclidean distances. The resulting tree matrix was then modified in Figtree and Inkscape. ANOVA test was applied to the dataset. Furthermore, Past3 was used to calculate a PCA and PCoA of the non-consensus Generalized Procrustes dataset.

As a last tool for geometric morphometric analyses, the R package "geomorph" (Baken et al. 2021; Adams et al. 2021) was used. Here the raw tps dataset was uploaded, and a Generalized Procrustes analysis was performed. PCAs for the three classifiers: *species*, *region* and *morphological group* were calculated. This was done to test if there is any bias or correlation between the sets.

Ecological Niche Modelling

Data acquisition

Occurrences of all 22 selected species and one outgroup taxon, were obtained from GBIF (SI 4). Confirmation of records identification was carried out based on available images. For species with less than 20 georeferenced occurrences available in GBIF database, their dataset was complemented with information regarding distribution available in literature and museum collection specimens from Naturalis Biodiversity Center, Leiden. Locations lacking geographic coordinates, were georeferenced using Google Maps in decimal degrees, duplicates and outliers were eliminated following the protocol in Simões et al. (2020), in R environment (Cobos et al. 2019). Final occurrence dataset (SI 4 and all occurrences are available on github: https://github.com/laradey/Convergence_Oedipodinae) was then split into test (25%) and training (75%) sets on further analysis. Environmental data was obtained at 2.5 min resolution from WorldClim (version 1, http://www.worldclim.org (accessed on 30.04.2022). From the total of 19 variables available, four were excluded a priori (i.e., mean temperature of wettest quarter, mean temperature of driest quarter, precipitation of warmest quarter and precipitation of coldest quarter), based on known spatial artefacts between adjacent grid cells (Campbell et al. 2015, Escobar et al. 2014), and the remaining 15 bioclimatic layers were retained for the next step of analyses. To visualize the most influencing variables, MaxEnt v.3.4.1 (Phillips et al., 2006) was used to perform a Jackknife analysis, followed by Pearson Correlation Coefficient (*r*) analysis to examine the cross-correlation of the variables, where variables with correlation above 0.8 were excluded. Finally, three sets of three bioclimatic variables were selected to calibrate models created (Table 2) in the R package "*ellipsenm*" (Cobos et al. 2020) analyses. Calibration area (Barve et al. 2011) for each species was defined using a 20 km buffer, following previous studies (i.e., Dey et al. 2021). Models were projected to the extent of the whole world. All outputs were checked with QGIS v.3.4.2 (QGIS Development team, 2020).

Table 2: Calibration results of ellipsenm per species, showing selected set and model.

					EllipsENM	
Genus	**Species**	**set_1**	**set_2**	**set_3**	**set**	**model**
Arphia	*pseudo-nietana*	bio_1, bio_12, bio_14	bio_3, bio_12, bio_15	bio_1, bio_12, bio_15	set2	mve1
Arphia	*simplex*	bio_1, bio_2, bio_12	bio_1, Bio_12, bio_15	bio_2, bio_12, bio_15	set_3	covmat
Chortophaga	*viridifasciata*	bio_2, bio_7, bio_17	bio_1, bio_7, bio_17	bio_7, bio_15, bio_17	set_3	covmat
Circotettix	*carlinianus*	bio_2, bio_14, bio_16	bio_1, bio_14, bio_16	bio_2, bio_3, bio_14	set_2	mve1
Conozoa	*sulcifrons*	bio_2, bio_3, bio_10	bio_1, bio_2, bio_10	bio_2, bio_10, bio_16	set_2	covmat
Circotettix	*undulatua*	bio_1, bio_12, bio_13	bio_1, bio_12, bio_14	bio_12, bio_13, bio_14	set_2	covmat
Lactista	*azteca*	bio_2, bio_12, bio_17	bio_2, bio_12, bio_14	bio_2, bio_12, bio_13	set_2	mve1
Trachyrachis	*kiowa*	bio_1, bio_6, bio_13	bio_3, bio_6, bio_14	bio_6, bio_14, bio_16	set_1	covmat
Trimerotropis	*latifasciata*	bio_2, bio_12, bio_13	bio_2, bio_12, bio_17	bio_12, bio_13, bio_17	set_2	covmat

Genus	Species	set_1	set_2	set_3	EllipsENM set	EllipsENM model
Trimerotropis	*sparsa*	bio_3, bio_10, bio_15	bio_1, bio_10, bio_15	bio_2, bio_3, bio_10	set3	mve1
Heliastus	*sumichrasti*	bio_5, bio_12, bio_16	bio_3, bio_12, bio_17	bio_5, bio_12, bio_17	set_1	mve1
Acrotylus	*patruelis*	bio_1, bio_10, bio_12	bio_3, bio_10, bio_12	bio_10, bio_12, bio_16	set_3	covmat
Bryodema	*luctuosum*	bio_1, bio_6, bio_16	bio_1, bio_3, bio_6	bio_1, bio_6, bio_7	set_3	covmat
Bryodemella	*tuberculata*	bio_6, bio_13, bio_17	bio_6, bio_13, bio_14	bio_4, bio_6, bio_14	set_1	mve1
Mioscirtus	*wagneri*	bio_3, bio_5, bio_14	bio_2, bio_5, bio_14	Bio_3, bio_4, bio_15	set_1	covmat
Morphacris	*fasciata*	bio_2, bio_12, bio_17	bio_3, bio_12, bio_17	bio_2, bio_12, bio_16	set_1	mve1
Oedipoda	*aurea*	bio_2, bio_6, bio_14	bio_2, bio_7, bio_17	bio_2, bio_10, bio_17	set_2	covmat
Psophus	*stridulus*	bio_5, bio_11, bio_15	bio_2, bio_5, bio_11	bio_2, bio_6, bio_10	set_3	covmat

Genus	Species	set_1	set_2	set_3	EllipsENM set	EllipsENM model
Scintharista	*notabilis*	bio_3, bio_7, bio_17	bio_7, bio_12, bio_16	bio_3, bio_7, bio_14	set_2	mve1
Sphingonotus	*nebulosus*	bio_1, bio_7, bio_11	bio_3, bio_7, bio_11	bio_7, bio_11, bio_15	set_1	mve1
Trilophidia	*annulata*	bio_6, bio_11, bio_15,	bio_7, bio_11, bio_15	bio_1, bio_7, bio_15	set_1	covmat
Sphingonotus	*pilosus*	bio_2, bio_4, bio_13	bio_2, bio_5, bio_13	bio_1, bio_5, bio_13	set_3	mve1

Climatic niche estimation and overlap

To estimate the climatic niche of the 22 species, the R package "*ellipsenm*" (Cobos et al. 2020) was used. Model calibration was performed using '*covmat'* (i.e., creating ellipsoids based on the centroid and a matrix of co-variances of all variables) and '*mve1'* (i.e., generating an ellipsoid which is reducing the volume, without losing the contained data). Best model selection was based on partial ROC (Peterson et al. 2008), omission rates (E = 5%; Anderson et al. 2003) and prevalence. To calculate the partial ROC metric, 500 bootstrap iterations with 50% of testing data were applied in each bootstrapped process with 5% of testing data error in the data due to uncertainty. Final models were created using 10 replicates with bootstrapped subsamples of 75% of the data. Further, to test whether climatic niche overlap might have driven the convergence of independent lineages to similar morphotypes, the niche overlap was estimated between the 11 species pairs showing similar morphology, using the R package "*ellipsenm*" (Cobos et al. 2020).

Seeking to avoid bias regarding the combination of variables used to characterize the species niche, three distinct environmental sets were tested: 'set 1' included all 15 variables; 'set 2' included only temperature variables (i.e., annual mean temperature; mean diurnal range; isothermality; temperature seasonality; max. temperature of warmest month; min. temperature of coldest month; temperature annual range; mean temperature of warmest quarter and mean temperature of coldest quarter); and 'set 3' included only precipitation variables (i.e., annual precipitation; precipitation of wettest month; precipitation of driest month; precipitation seasonality; precipitation of wettest quarter; precipitation of driest quarter) and the union overlap was calculated.

Climatic Niche Reconstruction

To reconstruct the ancestral climatic niche within a phylogeny, to find out how many retraction and expansion events are present in the morpho and genomic tree the package "*nichevol*" (Cobos et al. 2019) was used. For this it required the M areas of each species and environmental layers. These were calculated following Cobos et al. 2021 using a 20km buffer around each occurrence. In contrast to the other method, here just one layer at a time could be visualized. As a first step the needed stats for all variables were calculated using the options mean, sd, median and quantile. After this, histograms were created and bins selected. Smoothing of the data was implied to avoid gaps in the distribution values. Finally the calculated bins were plotted on the genomic and morphometric phylogeny for the eleven groups and a further outgroup taxon, which was needed to root the morphological and genomic trees and therefore was also needed in the "*nichevol*" analysis to leave no taxon without information content. Reconstruction was processed for 15 bioclimatic variables.

RESULTS

Phylogenomic tree reconstruction

In total we analyzed 3,364 orthologous genes from 74 individuals of Oedipodinae grasshoppers from the NW and OW regions (Table 1). Three individuals of Gomphocerinae grasshoppers (two from the OW - *Chorthippus sp*. and *Egnatiella major* - and one individual from the NW - *Heliaula rufa*) were included as outgroup taxa. Phylogenomic analyses were separately done for the "*full dataset*" (Figure 2) and the reduced so called "*convergence dataset*" (Figure 3), to analyze the phylogenetic relationships based on molecular loci and to analyze comparatively the relationship between the morphologically similar taxa from the OW and NW.

Within both datasets, a clear separation of OW and NW taxa was possible except for the species *Sphingonotus fuscoirroratus* which is distributed in the NW but clusters within the OW taxa. Furthermore the specimens of the taxon *Chortophaga viridifasciata* was grouping as sister clade to the Oedipodinae.

The NW dataset (full dataset) comprising 21 specimens of 14 species showed all tribes monophyletic supported by bootstrap values above 95% at most splits. Individuals of the genera *Trimerotropis, Circotettix* and *Conozoa* were clustering into one group (Trimerotropini), whereby individuals of *Trimerotropis* are mixed up within the clade. All other NW genera were monophyletic. The same relationships were recovered in the reconstruction of the phylogenomic tree of the reduced "*convergence dataset*" supported with bootstrap values above 95%.

The OW clade was remarkably larger including 51 individuals of 32 species. The tribes Sphingonotini and Oedipodini were reconstructed being paraphyletic. The bigger clade

of Sphingonotini also included the currently tribless genus *Leptopternis* and the Oedipodini genus *Mioscirtus*. The genus *Sphingonotus* was not monophyletic. Most splits within the Sphingonotini clade were supported by bootstrap valued above 95%. All members of the tribe Bryodemini formed a separate sister clade to Sphingonotini. Only some splits in this clade were supported by bootstrap values above 95%. Within this clade the genera and species remain mixed up. The genera *Oedipoda, Pseudoceles* and *Psophus* form a sister clade to Bryodemini. A further clade comprised individuals of the genera *Morphacris, Oedaleus, Scintharista* and *Acrotylus,* Here all splits were supported by bootstrap values above 95%. The members of this clade were placed in several tribes. The different tribes remained monophyletic. Finally, *Trilophidia* and *Aiolopus* which belong to the tribes Trilophidiini and Epacromiini formed one clade.

Geometric morphometrics

Altogether, 342 specimens were used for geometrical morphometric analyses, 115 were discarded due to missing venation or broken parts within the forewings.

PCA analyses showed roughly the same picture within the two analyses. Results of the PCA analyses using "*geomorph"* (SI 6) presented a larger differentiation into point clouds, results of the PCA using PAST3 (SI 5) showed the points more equally spread. Nevertheless, results of both analyses show no differentiation within the "*region dataset*". Within the "*morphological group dataset*" an admixture of closely and wider convex hulls were shown. PCoA resulted in a similar picture compared with the regular PCA lot (SI 5,6).

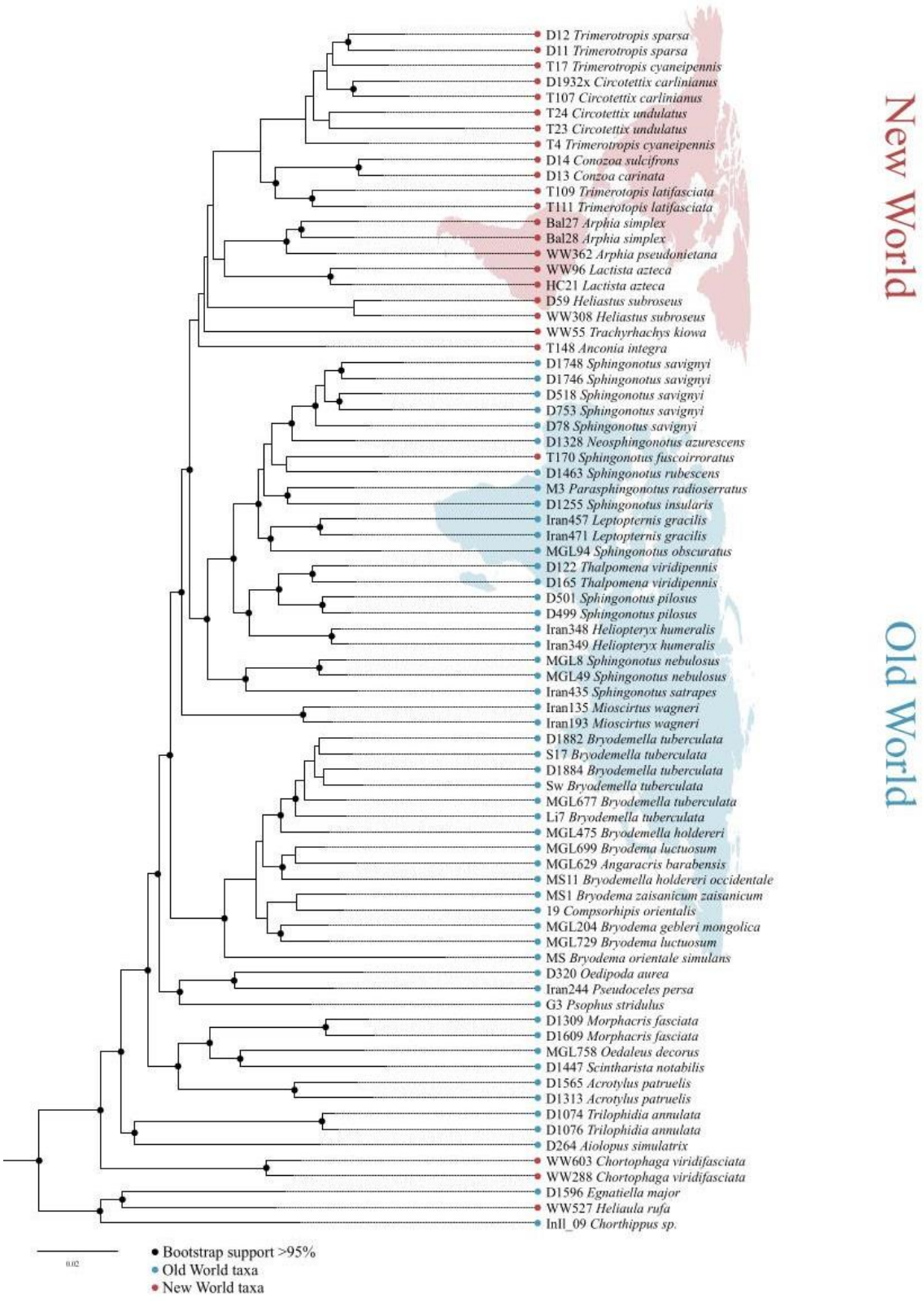


Figure 2: Full genomic tree generated with Hybridization Capture data, showing in read specimens from the New World and individuals in blue from the Old World. Bootstrap support above 95% is shown as black dots.

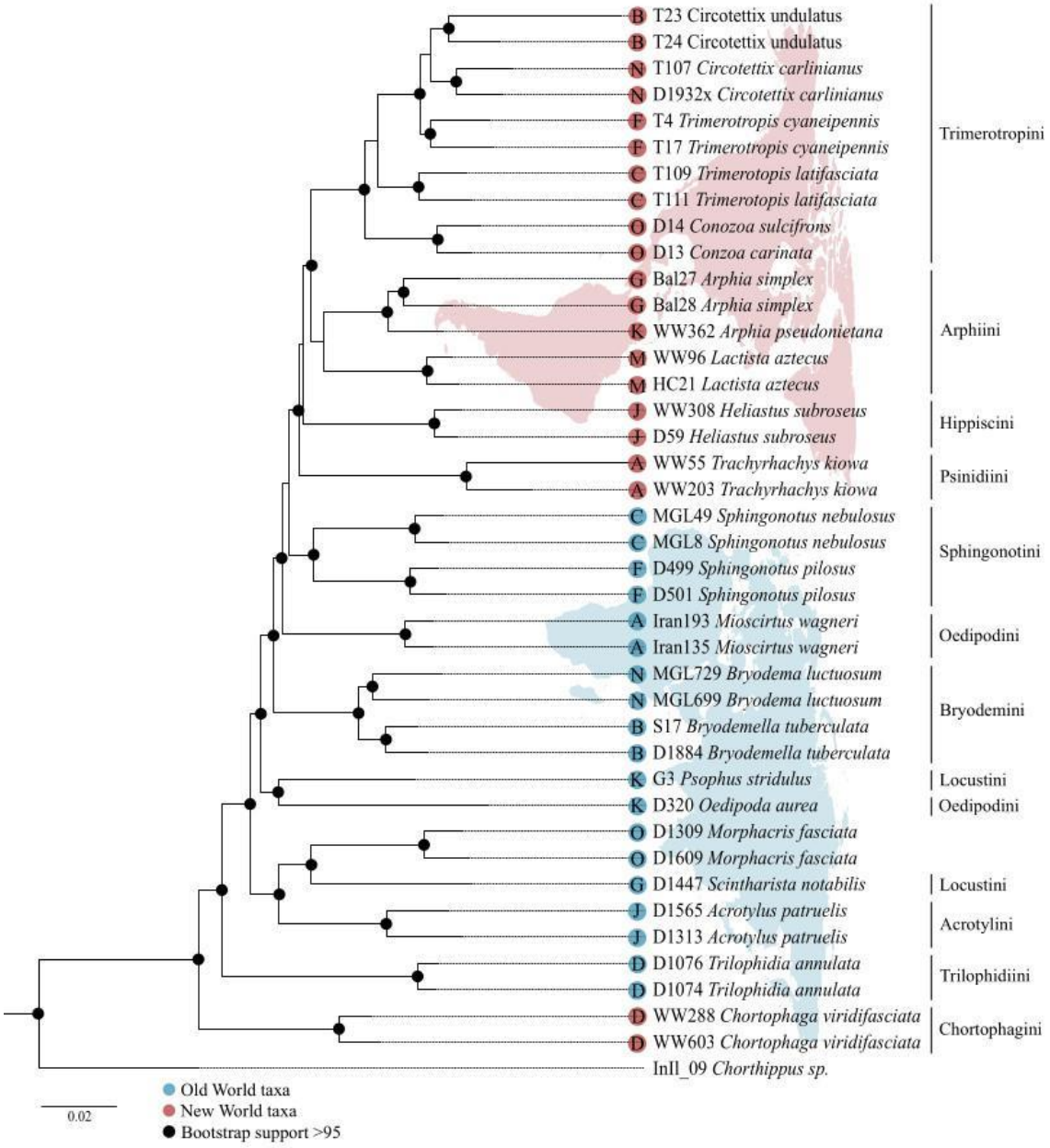


Figure 3: Reduced genomic tree for convergence analysis. Red dots represent specimens from the New World, blue dots show individuals from the Old World. Black dots represent bootstrap values above 95%. Group information is shown in the red and blue dots.

The UPGMA tree showed no clustering based on *regions* (Figure 4). The tree was structured into four major clades. Clade 1 comprised both, species of group C and M and *S. pilosus* (group F). Clade 2 includes both, members of group O and D and *A. patruelis* (group J). The third clade was composed of both individuals of group N, B and G. The species *A. pseudonietana* (group K) was also grouping into this cluster. Cluster 4 includes only the two species *T. kiowa* and *M. wagneri* of group A. *Psophus stridulus* was clustering as sister branch to clade 1 to 3, while

Ecological Niche Modelling (ENM)

The ENM resulted in suitable areas of all species which were in line with the current knowledge of species distribution. All resulting native regions, modeled suitable areas and extrapolated suitable areas are shown in Table 3. Results of all modeled groups are presented in Figure 5. Niche overlap estimation (Table 4) revealed a high overlap (>60%) for the following groups F and O, mean overlap (>30%) for B, C, J and M, and low overlap (<20%) for group A and N. No overlap was found for groups D, G and K. All overlap values can be found in Table 3.

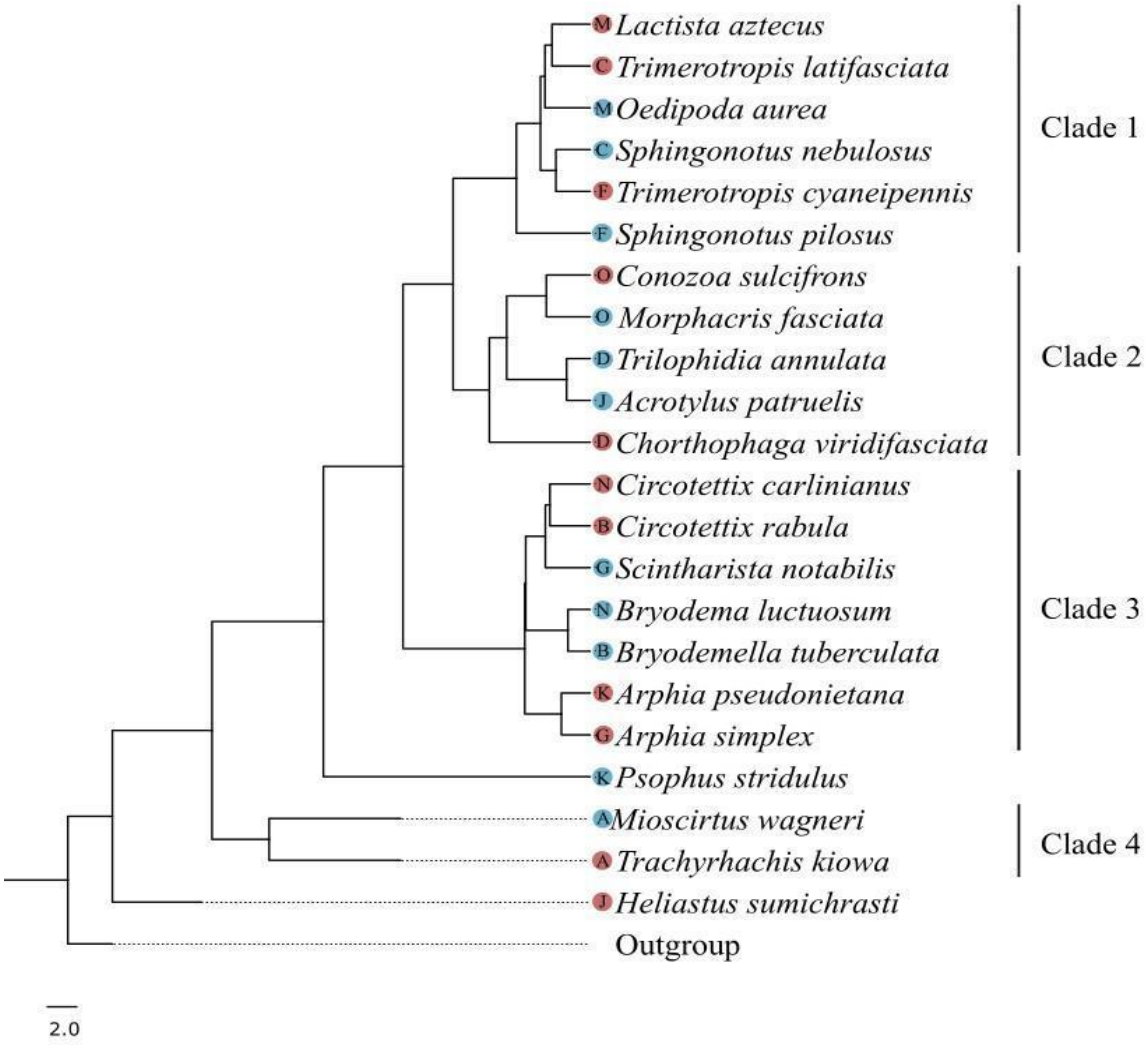


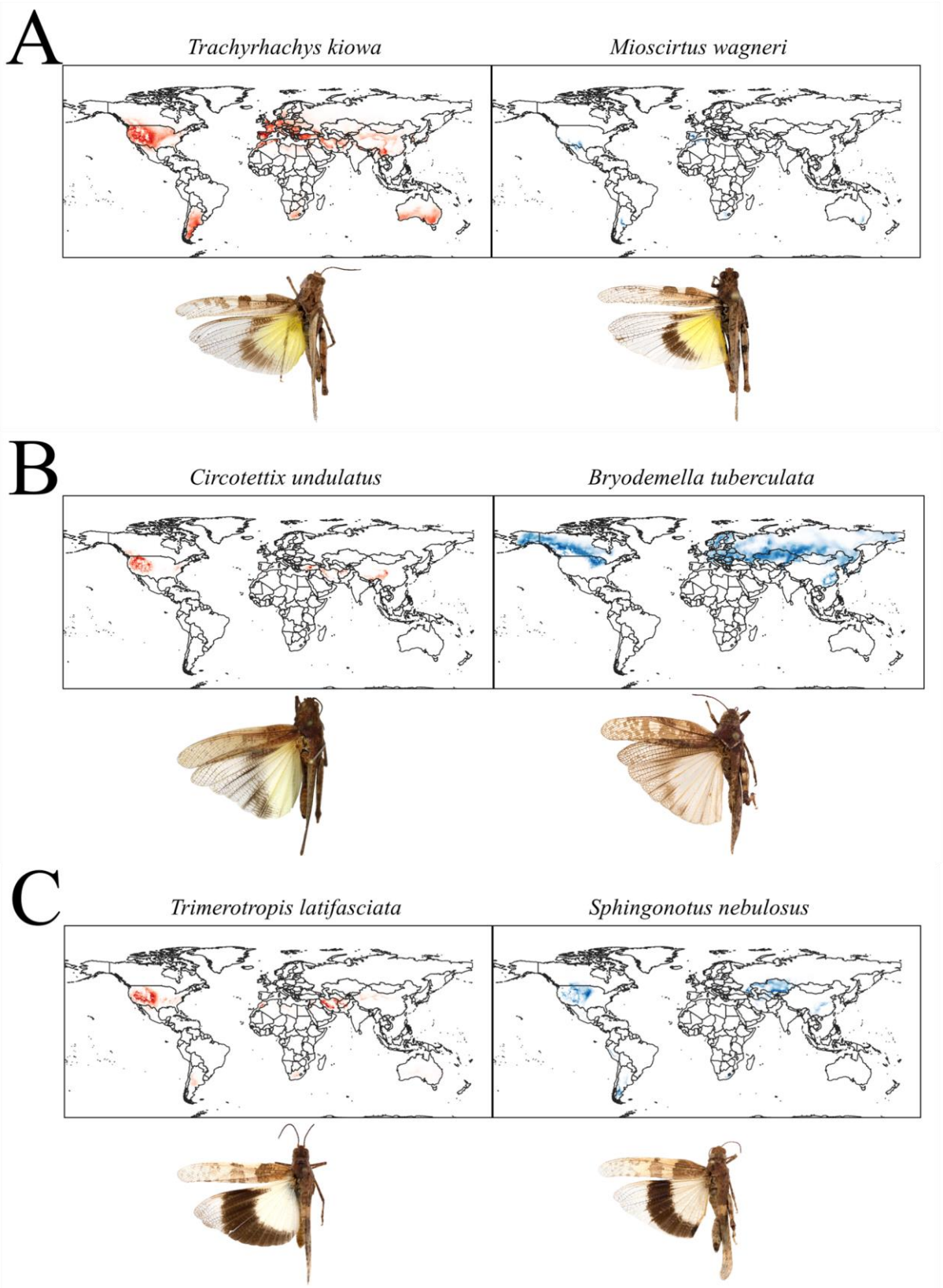
Figure 4: Geometric Morphometry UPGMA tree showing in red individuals from the New World and in blue specimens from the Old World. Group information is shown in the dots. Clade information is represented in the side panel.

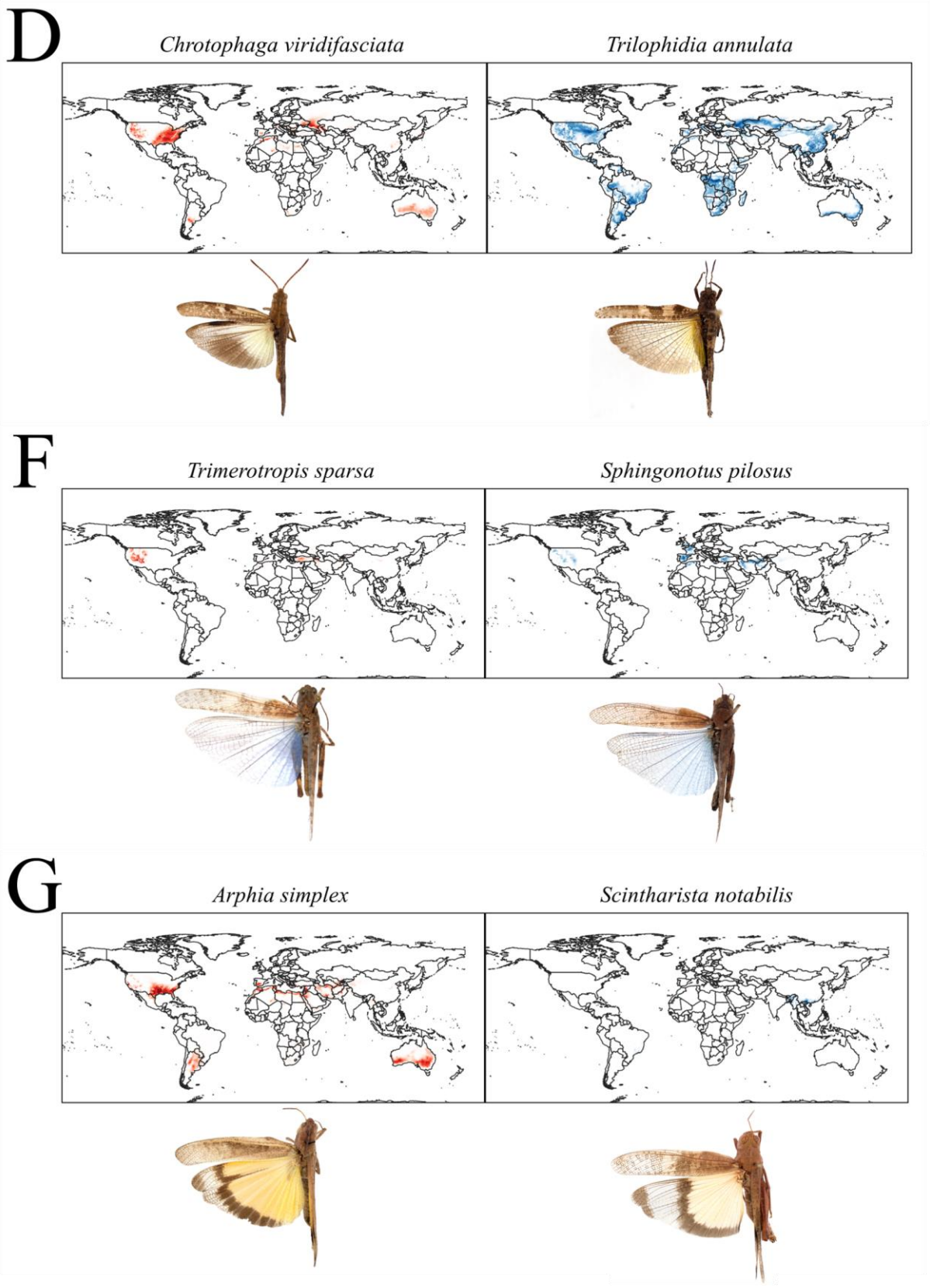
Table 3: Potential species distribution per species modelled with ellipsenm.

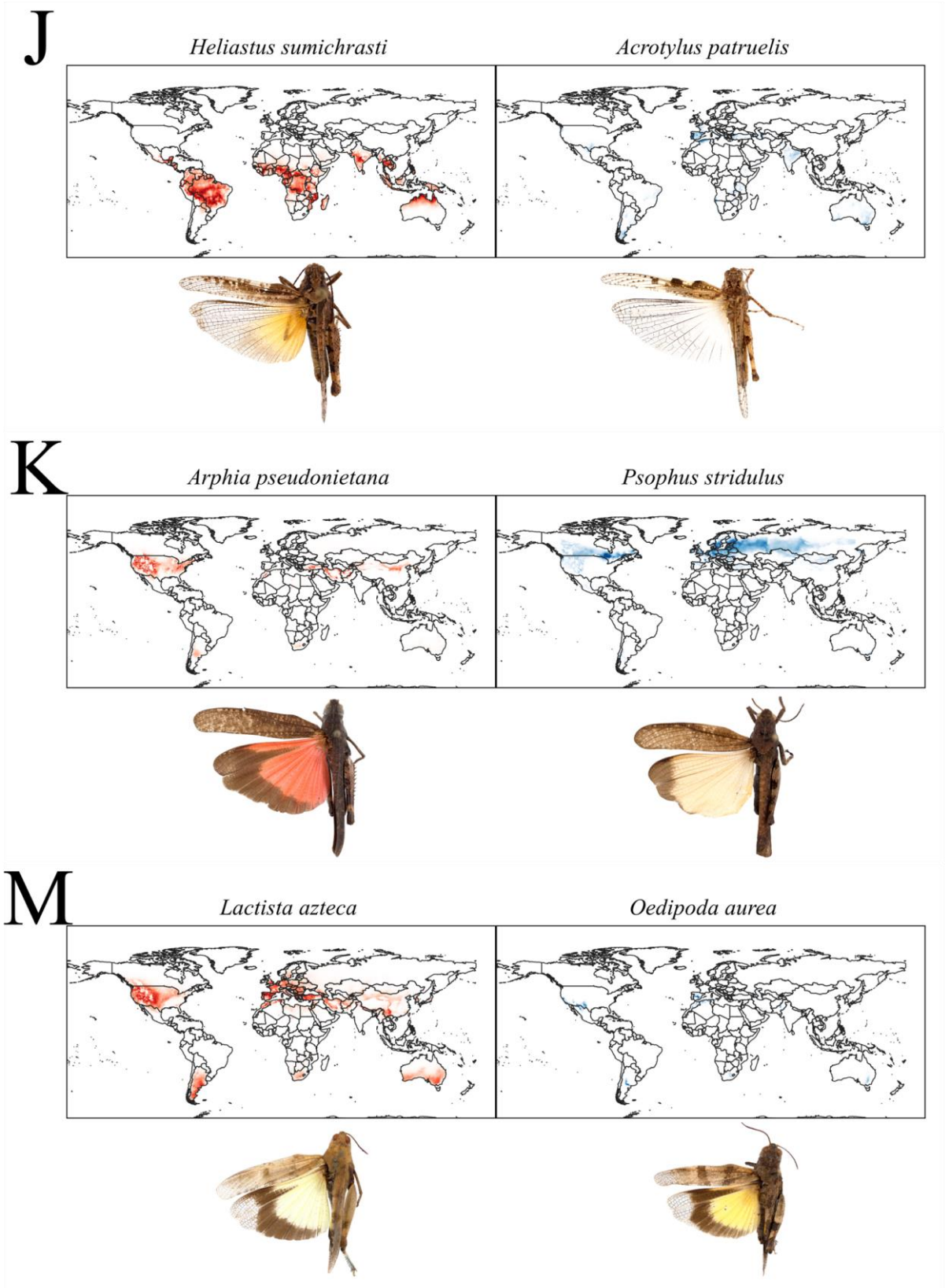
Group	Species	Native range	Modelled range	Extrapolated range
A	*Mioscirtus wagneri*	Coastline of Southern Europe/ Northern Africa to Eastern Asia	Native rang plus spots in South Africa and Australia	Across southern USA and regions in Argentina
A	*Trachyrhachis kiowa*	Across US and Mexico	Native range plus bigger parts of Argentina	Europe to Central and Eastern Asia, North African Coast line, parts of South Africa
B	*Bryodemella tuberculata*	Central Europe to Eastern Asia	Native range plus bigger parts in South-East China	Alaska and Canada
B	*Circotettix undulatus*	Western US	Native range plus spots in Eastern US and Argentina	Western to Central Asia
C	*Sphingonotus nebulosus*	Western and Central Asia	Native range plus spots in Southern Africa and Eastern China	Central US, Southern Argentina
C	*Trimerotropis latifasciata*	Central US and Southern Mexico	Native range plus spots in Argentina	Western Asia, Northern Africa, southern Africa
D	*Trilophidia annulata*	South West Asia: India, China, Thailand, Malaysia, Indonesia, Vietnam, Philippines	Native range plus Kazakhstan, Southern Mongolia, South to Central Africa, Coastline of Mediterranean Sea, Coastline of Southern Australia	US, Mexico, Guatemala, Honduras, Nicaragua, Costa Rica, Panama, Cuba, Dominican Republic; big parts of Brazil and Argentina
D	*Chorthophaga viridifasciata*	Eastern to Central US, Mexico	Native range plus spots in Argentina	Western Europe, Spots in Southern Europe and North Africa
F	*Sphingonotus pilosus*	Western Asia: Turkey, Iran,	Native range plus Spain, France and Algeria	Eastern to Central US

F	*Trimerotropis sparsa*	Eastern to Central US	Native range	Turkey, Iran
G	*Scintharista notabilis*	Canary Islands, Morocco, Turkey, Yemen, India, Pakistan, Israel	Native range plus coastline of South China and Vietnam	none
G	*Arphia simplex*	Eastern Coast line to Central US, Mexico	Native range plus big parts of Argentina	Coast line of North Africa, Iran, peninsula Sinai, Southern parts of Australia
J	*Acrotylus patruelis*	Across Central Africa, Southern Europe, Northern African Coast line up to Iran	Native range plus big parts of India	Southern Parts of South America, Texas
J	*Heliastus sumichrasti*	Mexico to Colombia	Native range plus Northern and Central Parts of South America, Cuba, Dominican Republic	Central Africa, India, Burma, Thailand, Malaysia, Indonesia, Philippines, Cambodia, Vietnam
K	*Psophus stridulus*	Central Europe to Central Asia	Native range plus marginal areas in western Africa	Big parts of Canada plus western US, small parts in Southern Chile and Argentina
K	*Arphia pseudonietana*	US and Mexico	Native range plus parts of Argentina	Western to Central Asia
M	*Oedipoda aurea*	Weestern Asia: Greece, Turkey, Syria, Lebanon	Native range plus big parts of Southern Africa, small spots in Central China, Coast line of Libya and Egypt	Parts of Mexico and Argentina
M	*Lactista azteca*	Southern US, Mexico	Native range plus parts of Argentina	Coast line of Northern Africa, across peninsula Sinai, Iran up to India

N	*Bryodema luctuosum*	Central Asia: Mongolia, Southern China and Tibet	Native range plus some spots in Northern China	none
N	*Circotettix carlinianus*	Western and Central US	Native range plus parts at the Eastern US Coastline	Turkey, Iran, Azerbaijan, Central parts of China, Japan
O	*Morphacris fasciata*	Spain, Morocco, South and Central Africa	Native range plus Yemen, South West India, Burma, Thailand, Vietnam, Cambodia, Laos,	Mexico, Central and Northern South America
O	*Conozoa sulcifrons*	Western US to Mexico	Native range plus Southern Argentina	Southern European Coastline, North African Coastline across Turkey, Iran up to Western China, parts of South Africa


A
Trachyrhachys kiowa
Mioscirtus wagneri
B
Circotettix undulatus
Bryodemella tuberculata
C
Trimerotropis latifasciata
Sphingonotus nebulosus


D
Chrotophaga viridifasciata
Trilophidia annulata
F
Trimerotropis sparsa
Sphingonotus pilosus
G
Arphia simplex
Scintharista notabilis


J
Heliastus sumichrasti
Acrotylus patruelis
K
Arphia pseudonietana
Psophus stridulus
M
Lactista azteca
Oedipoda aurea

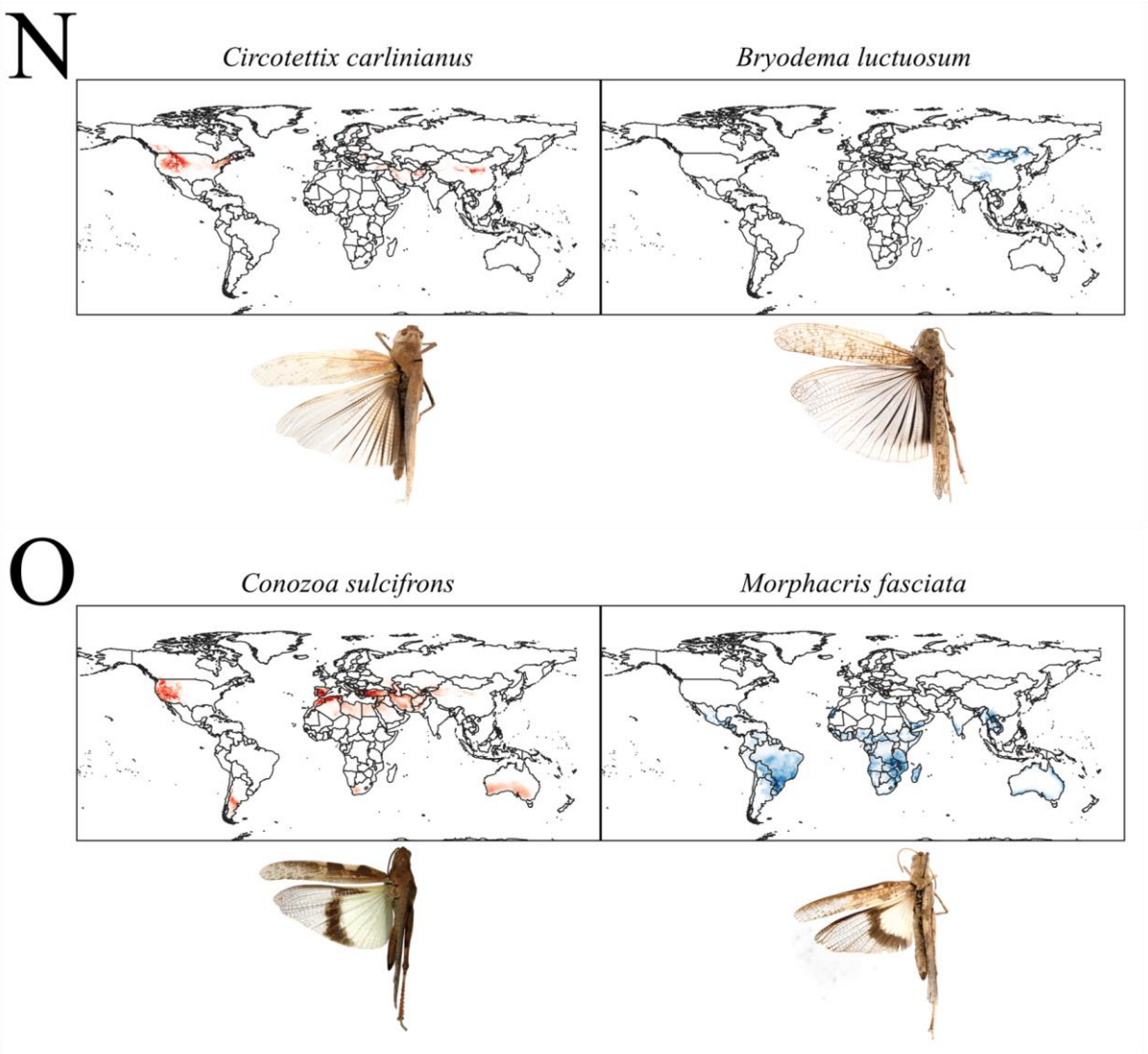


Figure 5: Species Distribution Model of all groups created with *ellipsenm*.

Reconstructing ecological niche evolution relayed on the bio_1 layer (Annual Mean Temperature), which was chosen to be the most relevant layer to all species (Figure 1). Results of all further reconstructions are attached to the supplementary (SI 7). We reconstructed the ecological niche based on the phylogenomic and the morphological tree. Taking the phylogenomic tree as basis, retraction events were found at the tips of *S. notabilis* and *A. patruelis*. Expansion events were proposed in *A. pseudonietana, H. subroseus, S. pilosus, M. wagneri* and *P. stridulus*. Within the earlier splits of the tree more

retraction events were found. A single expansion event at the split between *T. annulata* and all other species.

With the morphological tree as basis, less retraction and expansion events were reconstructed; retraction events at the tips of the tree were only inferred for *S. notabilis, B. luctuosum, B. tuberculata, M. wagneri* and *H. sumichrasti* while expansion events were present in *H. sumichrasti* and *B. tuberculata*. Within the clades only the split between *C. sulcifrons* and *M. fasciata* as well as *S. pilosus* and the remaining taxa of the clade showed potential retraction events.

Table 4: Generated union overlap between the members of each group using ellipsenm.

Group	**set**	**union overlap**	**p-value union overlap**
A	1	0.0892	0.18
	2	0.103	0.14
	3	0.192	0.12
B	1	0.296	0.35
	2	0.57	0.99
	3	0.1	0.26
C	1	0.406	0.74
	2	0.457	0.73
	3	0.121	0.76
D	1	0.62	0
	2	0.604	0.04
	3	0.693	0
F	1	0.83	0.69
	2	0.523	0.53

Group	set	union overlap	p-value union overlap
	3	0.666	0.66
G	1	0.577	0.01
	2	0.268	0.02
	3	0.618	0
J	1	0.109	0.05
	2	0.363	0.16
	3	0.184	0.02
K	1	0.396	0
	2	0.572	0
	3	0.486	0
M	1	0.426	0.07
	2	0.457	0.15
	3	0.185	0.1
N	1	0	0.82
	2	0	0.99
	3	0.0037	0.34
O	1	0.381	0.21
	2	0.625	0.23
	3	0.193	0.83

DISCUSSION

In this study, we focused on the Oedipodinae grasshoppers from the NW and OW. We wanted to explore the origin of morphologically similar taxa in both regions with the aim to test if the similarities are based on convergence or common ancestry. For this, we used a combination of phylogenomics, ecological niche modelling and morphometrics. By using genomic data, we built a stable phylogeny of the subfamily and investigated their similarity by using geometric morphometrics of the elytra. We also used ecological niche modelling to (1) determine the potential suitable area of all species pairs, (2) investigate the overlap in niche space between the pairs and (3) use ancestral reconstruction of the ecological niche to find out if genetic or morphological similarity influences ancestral reconstruction. Altogether we found evidence of convergent evolution within the morphological data and niche space suggesting that a similar niche leads to similar morphological features despite lack of direct relatedness. In the following, we discuss the different aspects of our analyses and their implications in detail.

Evolution of convergent morphological traits is forced by similar ecological niches

Convergent evolution is a frequently observed mechanism. Here, similar traits are evolving despite lack of near ancestry. In our study, we investigated the relatedness of eleven pairs of Oedipodinae species with similar wing color patterns and shape. Similarity in morpho-space was quantified using geometric morphometrics. Our analyses have shown that there is no morphological differentiation between the OW and NW taxa. By calculating a UPGMA tree, we found four morphological clades, each including several groups of genera (clade 1: M, C, F; clade 2: O, D, J; clade 3: N, B, G, K; clade 4: A). This suggests there is more similarity within the species of one clade than within the species of a geographical region, thus supporting the hypothesis of convergent evolution of similar morphological traits of these taxa.

Furthermore, we used ecological niche modelling to investigate whether climatic niche similarity might be a selective force contributing to convergent phenotypes. An overlap of ecological niches was found in eight of the eleven pairs. Overlap varied between the three used sets (i.e., set 1: all environmental variables, set 2: temperature variables, set 3: precipitation variables). In average, high overlap (>60%) was recorded for groups F and O; mean overlap (>30%) for groups B, C, J, M, N, and low overlap (<20%) for group: A and N. No overlap was found for groups D, G and K. When we calculated species distribution overlap with set 1, the highest overlap was of 83% for group F, in which both species (*S. pilosus* and *T. cyaneipennis*) are defined by a small distribution range and are more bound to specific habitats. While *T. cyaneipennis* is only present in Nevada, Utah, California, Arizona and New Mexico at the rocky slopes of mountains, the counterpart species *S. pilosus* is endemic to mountainous areas with pebbly underground in Iran and the surrounding countries.

Group O showed an overlap of more than 62% (set 2); both species in the group have a wide distribution range. *Conozoa sulcifrons* is present across most of the western USA (i.e. Otte, 1985), while the distribution of *M. fasciata* stretches across large parts of Africa, especially the northern coast up to India (Cigliano et al. 2022). An overlap of 45% (set 2) was found in group M: here *L. azteca* is present in the southern parts of North America (Texas, Arizona, New Mexico, Mexico), especially at the coast lines of Mexico and Texas, where it prefers open bare grounds with sparse vegetation with a wide distribution range across North America. Similar to *L. azteca*, *O. aurea* prefers open semi-dry to dry habitats which may be sand dunes, river beds or even mountain slopes (Willemse, Kleukers, & Odé, 2018). *Oedipoda aurea* has a smaller distribution range across the coast line of Turkey and the Arabic peninsula. Further, 45% overlap was found in group C (set 2), where the species show different patterns in distribution. *Trimerotropis latifasciata* is very widespread in the western and central USA up to Canada, while *S. nebulosus* is only found at relatively small isolated locations from Turkey to central Asia. At least both species prefer the central parts of the continents. *Sphingonotus nebulosus* can be found at

smaller slopes with pebbly underground. *Trimerotropis latifasciata* prefers a similar habitat with mountainous sides and stony underground, as well as desert grasslands.

To provide insight into the biogeography and diversification of lineages, the ancestral climatic niches of all species were reconstructed. We suggest that in species with a similar ecological niche, less range decline and expansion events are needed to reconstruct the ancestral niche. Species with close ancestry show limited niche overlap, while species with no common ancestry inhabit similar niches (e.g. Silva et al. 2014). Based on our knowledge of the habitat preference of Oedipodinae (bare grounds with sparse vegetation), we already suggested less retraction and expansion events than in taxa with quite different habitat preferences. The reconstruction of the ancestral niche using the R package *nichevol* showed just single retraction and expansion events in both trees (genomic and morphometric; Figure 1) confirming this hypothesis. Similar findings were recovered for the White-edged Oriole (*Icterus graceannae*; Owens et al. 2020) or the Eurasian snowfinches (Cobos et al. 2021). In these studies the power of ancestral niche reconstruction to reconstruct the biogeographical pathways was demonstrated. Due to the usage of the reduced trees in our study, such reconstructions were not useful, as too many taxa were missing. In our genomic tree, we found altogether thirteen range expansion and retraction events. In contrast, the morphometric tree only showed nine changing events. Following, the morphometrical tree needed fewer changing events to reconstruct the ancestral niche suggesting a more similar niche within the clusters. Hence, species with similar morphometrical features share a more similar niche than species with close genetic ancestry. This is supported by the general idea of niche theory, which states that niche overlap between species increases with relatedness (Sydenham et al. 2018).

Phylogenomic relationships

The phylogenetic tree based on capture data proposed in this study is in general in line with earlier published phylogenies (Chapco & Contreras, 2011; Husemann et al. 2012;

Song et al. 2018). Hybridization capture was chosen as method to reconstruct the phylogenomic relationships as it reduces the complexity of the genome and hence, increases coverage and consistency of sampling of the captured loci. Based on the sometimes huge size of the genomes of Oedipodinae (Husemann et al. 2020; Yuan et al. 2021; Hawlitschek et al. submitted), random sequencing of parts of the genome likely would not yield sufficient loci sampled across all taxa.

The resulting tree was generally well resolved. Within the tree, all splits were supported by high bootstrap values; even most shallow splits were well supported. In contrast to early published phylogenies, our study included more Oedipodinae genera and species. The results remained similar to previous studies: NW and OW taxa were clearly differentiated in two distinct clades, rather than a clustering by wing shape and pattern. Following, the morphological similar species evolved independently showing not only morphological similarities, but also similar ecological traits. Similar relationships were already described for Tettigoniidae species (Mugleston et al. 2018), where several biogeographical groups were found having convergent ecomorphs. Moreover, the impact of specific ecological pressures (altitude) was already tested on two wētā species (*Hemideina crassidens, H. thoracica*). Both species adapted independently, but in a similar way to ecological pressures by faster growth rates and larger adults supporting the hypothesis of convergent evolution despite close ancestry (Minards et al. 2014). Also, in the family of Pyrgomorphidae similar morphological traits evolved despite near ancestry (Zahid et al. 2021). Hence, this pattern appears common in Orthoptera.

The split of NW and OW taxa supported the findings of Husemann et al. 2012. Only the sample from *S. fuscoriroratus* from Galapagos was placed in the OW clade. Furthermore, we found that the status of the genus *Chortophaga* has to be re-evaluated. Based on our findings the individuals of the genus clustered outside the NW and OW Oedipodinae and hence likely should be assigned to a different subfamily. This clustering was already found in Husemann et al. 2011. Within the NW clade *Trimerotropis, Circotettix* and

Conozoa formed one cluster, while the other genera from the NW form grouped at the genus level. A similar clustering of *Trimerotropis, Circotettix* and *Conozoa* was already shown in earlier publications (Fries, Chapco & Contreras, 2007; Chapco & Contreras, 2011). Interestingly the members of these genera formed two clusters of overall chromosome number (cluster 1: 2n=21(20+X) and 2n=23(22+X), cluster 2: 2n=23(22+X)). These chromosomal groups are also reflected in the tree and hence a taxonomic revision is required to account for these evolutionary patterns.

In the OW clade, the genus *Sphingonotus* clustered at several points within a bigger cluster of *Sphingonotus, Heliopteryx, Leptopternis* and *Thalpomena*. These genera all belong to the tribe Sphingonotini and showed a clustering into three main groups: a clade combining the genera *Sphingonotus* and *Leptopternis;* a second clade of *Sphingonotus pilosus, Thalpomena* and *Heliopteryx,* which was already found in Dey et al. (2022); and a third clade of *Sphingonotus nebulosus* and *Sphingonotus satrapes.* Similar, to the NW clade, also here taxonomic changes are required. However, in the OW group there is also a lack of resolution in some taxa, which may be the result of the putative young age of the tribe as many species likely evolved during the Pleistocene.

The next cluster included only genera from the tribe Bryodemini. Within this tribe no separation even at the genus level was possible. The genera *Bryodema, Bryodemella, Angaracris* and *Compsorhipis* are all mixed up. The same had already been shown in Dey et al. (2021), just with fewer markers. It is questionable why up to now no method was able to differentiate the morphologically clearly differentiated taxa. Based on morphology the species and genera are easily distinguishable. One possibility may be the young age of the clade and the likely still happening low rates of hybridization. However, it may also be a problem of the genetic architecture of the taxa. First studies on the genome size of *Bryodemella tuberculata* showed very high genome sizes of 1C = 21.96 pg (Hawlitschek et al. in submitted); the same is true for some other species of the tribe (e.g. *Bryodemella holdereri* 1C = 18.41 pg; Vyotskaya, 1986). The reason for such large

genome sizes were highly discussed by for e.g. Commoner (1964), Bennett (1972) or Cavalier-Smith (1978). Due to the large genome size, there is a high potential that repetitive elements and hence non-homologues loci may have been included in the dataset distorting the topology. In Orthoptera, the occurrence of large genome sizes are known throughout the whole order.

By using hybridization capture data, we were able to reconstruct deep and shallow splits in the Oedipodinae phylogeny with overall high bootstrap support. Our study showed the up to now biggest published Oedipodinae phylogeny including 48 species. In contrast to already published studies, we showed that in most groups taxonomy is not in line with the current phylogeny and hence supports the re-evaluation of the group. Further, we found that morphological and climatic niche similarities are present, despite near ancestry within the Oedipodinae, suggesting convergent evolution of morphological features driven by climatic factors.

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SUPPORTING INFORMATION

All supporting information can be found on page 564 and following.

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Candidate contribution to the chapter:

LSD, MH designed the study; LSD, MH collected data; LSD performed analyses; LSD wrote the original version of the manuscript; LSD, MH, MS, KM, OH took part in the writing process; LSD coordinated the writing process.

Hamburg, 12.01.2023	
Place/Date	Sign

Chapter 7

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"Discussion"

Young radiations of rapidly evolving clades can be found in almost all groups of organisms. This includes many groups within Orthoptera, for which rapid diversification events have already been investigated (e.g. Melanoplinae in North America (Knowles & Otte, 2000), or *Laupala* crickets on Hawaii (Shaw, 2002)). In this work, I focused on band-winged grasshoppers (Oedipodinae). Due to their high number of described species (797 species, Cigliano et al. 2022) and their estimated young age (~35-36 myo; Husemann et al. 2012; Song et al. 2018), this group of grasshoppers is a perfect model system for the study of the genomic composition, marker resolution, ecological niche and convergent evolution.

Marker resolution within the band-winged grasshoppers

DNA barcoding has been established as a method to distinguish species of several groups, e.g., tropical butterflies (Hajibabaei et al. 2006), Japanese click-beetles (Oba et al. 2015) or in Australian stick insects (Velona et al. 2015). The barcoding region has been used with varying success in Orthopterans. The marker worked well in the Oedipodinae genus *Acrotylus* (Wehrt, 2019). Here, Wehrt (2019) was able to differentiate the morphologically rather similar species of the Oedipodinae genus *Acrotylus* into several BINs. Within a study of grasshoppers from Germany, Austria and Switzerland, Hawlitschek et al. (2017) were able to differentiate 78.4% of 127 Orthoptera taxa by using DNA barcoding data only. Also Trewick (2008) discusses the controversial results for some New Zealand grasshopper species. Here, he found high genetic distances within species with geographical partitioning. Only paraphyletic relationships were found in the phylogenetic tree (Trewick, 2008). For other Oedipodinae grasshoppers for e.g. the genus *Sphingonotus* (Dey, 2016), *Bryodemella*, *Bryodema*, *Angaracris*, *Compsorhipis* (Dey &

Husemann, 2021), *Thalpomena* (Moussi et al 2018; Dey et al. 2022) and others, the barcoding region is not sufficient to separate species.

Several reasons for the bad resolution of the fragment have been discussed in the past and in Chapter 2. The problem behind the usage of the barcoding fragment of COI is the BIN sharing (sharing of the same COI sequence), which might be caused by misidentification of species (Lehmann et al. 2017; Ingrisch 1995;), hybridization between species (Gottsberger 2007; Gottsberger & Mayer 2007; Hochkirch & Lemke 2011; Rohde et al. 2015) or incomplete linage sorting, which is known to be present in rather young species groups (Song et al. 2008; Moulton et al. 2010; Berthier et al. 2011). A further reason could be nuclear copies of mitochondrial genes (numts), which can vary between taxon groups. For grasshoppers several studies have addressed the presence of numts within Orthoptera (e.g. Bensasson et al. 2000; Pereira et al. 2021). Altogether, the COI barcoding fragment seems to not provide sufficient resolution to differentiate most shallow nodes within the Oedipodinae phylogeny.

As a further method, the usage of several concatenated genes for phylogenetic reconstruction has been put forward, as it provides more genetic information than just barcoding itself. Several multi-gene phylogenies of Oedipodinae have already been published. Chapco et al. (1997) published a study on the North American band-winged grasshoppers based on two mitochondrial markers (16S and cytb). They were able to differentiate most of the genera, while they had no resolution within the genera *Trimerotropis*, *Conozoa* and *Circotettix.* The later published phylogenies by Contreras & Chapco (2006), Chapco & Contreras (2007), Fries et al. 2007 and Chapco & Contreras 2011 were not able to improve the resolution of the three genera. Studies comparing OW and NW Oedipodinae are mostly lacking. Only Husemann et al. (2012, 2014) integrated a larger number of OW Oedipodinae in their research. These published phylogenies are in line with findings of earlier publications: even though Husemann et al. (2012, 2014) were able to differentiate the New World (NW) and OW Oedipodinae, they had less resolution within the Sphingonotini and Bryodemini.

In this work, I used mitochondrial and nuclear fragments of a total length of 1,772 bp to test the resolution within the genus *Sphingonotus* (Chapter 2). Even though I was not able

to differentiate all species, I found four species groups, which are also geographically separated or have been recognized taxonomically before. My results confirmed the suggestion of Husemann et al. (2012, 2014). Here, they already proposed three of these groups within the genus *Sphingonotus* by also using a multigene approach. Interestingly, an Iranian species group (*S. coerulipes* group) was discovered in my work for the first time. Unfortunately, it remains unclear how and when this species group evolved.

As the previously described phylogenetic marker systems did not provide a good resolution for many taxa, i.e. the Sphingonotini, Trimerotropini and Bryodemini, the next step to get better resolved phylogeny of the Oedipodinae was to implement next generation sequencing data. Specifically, I used hybridization capture data of 3,364 designed baits for Orthoptera (Song et al. unpublished; Chapter 6). Using this approach, I was able to separate the OW from the NW taxa. This had already been achieved in the studies by Song et al. (2018), Husemann et al. (2012, 2014), Chapco et al. (1997), Chapco & Contreras (2006, 2011) and Fries et al. (2007). In contrast to our study, Song et al. (2018) only relied mitogenome data for their phylogeny and merged it with single or few gene data from additional taxa. With this approach they received relatively good resolution across the tree, but the band-winged grasshoppers remained polyphyletic. As a general remark, Oedipodinae were underrepresented in the study, especially those from the OW. In my dataset several genera remained paraphyletic in the tree. This may suggest the data do not provide sufficient resolution to separate the taxonomical hard to distinguish taxa like *Trimerotropis, Sphingonotus* and the genera of the tribe Bryodemini. This problem of lacking resolution in the single and multi-gene phylogenies might be a result of questionable taxonomic relationships and rather genomic resolution, as my hybridization capture data showed a similar picture. Using this method, we got high resolution for all species, except for the genera *Trimerotropis, Circotettix, Conozoa, Sphingonotus* and the genera of Bryodemini. We suggest that the lacking resolution in some species groups could be not a problem of marker resolution, but a problem of the current taxonomy for e.g. in *Trimerotropis, Circotettix* and *Conozoa,* while the missing resolution could be also a problem of ILS like suggested for the genus *Sphingonotus*. Taxonomic revisions are needed to shed light on the species relationships.

Convergent evolution of the band-winged grasshoppers

Besides the phylogenic relationship and resolution of markers itself, I was especially interested in the evolution of morphologically similar taxa from the NW and OW (Chapter 6). I used geometric morphometrics of the elytra to formally investigate superficially similar taxa. Altogether four clades were found. These clusters comprised each several groups, but in most cases both target species from the OW and NW were included. Within the cluster the species remained mixed up. Nevertheless this analysis showed evidence of similar morphological features shared between the NW and OW target species. I further calculated the potential suitable distribution areas based on 15 bioclimatic layers of all target species and did overlap analyses for all pairs. I found overlap in eight of eleven groups. In a last step, I traced back the ancestral climatic niche based on the genomic and morphometric tree and found evidence that the reconstruction based on the morphological tree shows a lower number of range decline and expansion events than in the genomic tree. This suggests that species with similar morphology inhabit similar climatic niches. Following this means ecological factors may have promoted similar morphological features despite distant phylogenetic relationships and would, therefore, promote the hypothesis of convergent evolution of the species.

Genomic composition

To further understand the genomic composition of grasshoppers, we studied their genome size. Within a first study (Chapter 5.1) we measured five Acrididae species and showed that genome size varied between 1C = 11.31 pg in a female *Chorthippus dorsatus* and 1C = 18.48 pg in a female *Stethophyma grossum* (Husemann et al. 2021). In a further study (Chapter 5.2), my colleagues and I measured 49 additional species and included data from all available genome size measurements of Orthoptera. We mapped the genome sizes on a phylogeny based on mitochondrial markers and full mitogenomes to investigate the relationship between the taxa and to trace back the evolution of genome size across the tree. In general, there was a trend to similar genome sizes within a

taxonomic unit, but at least in most of the investigated groups there were outliers with higher or lower values. Our finding was in line with Yuan et al. (2021), who discovered similar patterns regarding the ancestral genome size. In this study, the scientists investigated the genome sizes of 32 Ensifera species. They showed a range from 1C = 0.952 pg in *Oecanthus sinensis* to 1C = 19.135 pg in *Deracantha onos* and concluded that the evolution of genome size seems to be complicated in Ensifera. In our study, we focused on the Acrididae, which appear to be the most diverse group with genome sizes from 1C =10.68 pg in *Schistocerca gregaria* to 1C =21.96 pg in *B. tuberculata.* Thus, *B. tuberculata* currently represents the largest known insect genome. Our study suggests that larger genomes are more common in Caelifera than in Ensifera, which is in line with the current literature (Yuan et al. 2021; Mao et al. 2020; Hanrahan et al. 2011). Kraaijveld (2010) investigated the relationship between the size of a genome and speciation rates. He suggested that larger genomes are more prone to constrained speciation rates, increased extinction rates or both.

Despite the large genome sizes in Acrididae, previous studies have reported a relatively constant number of chromosomes (John & Hewitt, 1966; Confalonieri & Bidau, 1986; Castillo et al. 2010). However, no systematic review has been performed. Hence, we assembled a dataset of chromosome number counts of 769 species and plotted them on the phylogeny provided by Song et al. (2018) (Chapter 5.3). Altogether, the most common chromosome number overall was N=23, followed by N=17 and N=21. In Oedipodinae the commonly found chromosome numbers were N=21 and N=23 which is not in line with the huge genome sizes of some of the taxa. Similarly, Gomphocerinae have a common chromosome number of N=17 and very large genome sizes (*Chrysochraon dispar* 1C= 19.43pg).

Furthermore, we had a look at the mitochondrial gene order in Orthoptera (Chapter 5.4). For this, 277 published mitogenomes were analyzed and a phylogeny based on this data was reconstructed. Ensifera (N = 84; 28.5%) and Caelifera (N = 193; 25.8%) did not differ significantly in GC content. All four potential gene rearrangement types (inversions, transpositions, inverse transpositions, and tandem-duplication/random loss events (TDRL)) were present within the order Orthoptera. Altogether, within the phylogenetic

tree the results showed a low overall occurrence of rearrangements in deep branches, but not in shallow branches, with a high level of mitochondrial gene rearrangement (MTR) events at the species level.

In comparison the three phylogenies tracing back the ancestral genome size, chromosome number and MTR, most Oedipodinae taxa showed a constant chromosome number of 2n♂ = 23 (22 + X0), only the genus *Trimerotropis* appeared with 2n♂ = 21 (20 + X0). Mitochondrial gene rearrangements were only present in the genus *Oedaleus* (inversions, transpositions, inverse transpositions or TDRLs), genome size appeared to be random across the subfamily without any sign of correlation to chromosome number or MTR. Other groups, like the sister clade Gomphocerinae appeared differently. While the chromosome count showed a dominant number of 2n♂ = 17 (16 + X0) and a more equally distributed genome size of 1C=10.47pg (*Chorthippus brunneus*) to 1C=14.72pg (*Pseudochorthippus parallelus*) with an outlier of *Chrysochraon dispar* of 1C=19.43, the group shows several MTR events in the reconstruction. Here multiple inversions, transpositions, inverse transpositions, TDRLs or deletions are present. These events cannot be correlated with any changes in genome size. Summing up, neither in Oedipodinae nor in Gomphocerinae any correlation of genome size, chromosome number or MTR events are detectable.

Ecological Niche Estimation

The previous presented work is manly based on the phylogenetic and phylogenomic relationships within the Oedipodinae. But as my results and previous work of Contreras & Chapco (2006), Chapco & Contreras (2007, 2011), Fries et al. (2007), Husemann et al. (2012, 2014), Chapco et al. (1997) and Song et al. (2018) show, a separation of the species groups of Sphingonotini and Bryodemini is mostly lacking. This could be a reason of their overall young age and the possibility of BIN sharing due to ongoing speciation events. Missing differentiation leads to unclear phylogenetic relationships. As a further tool for species delimitation, ecological niche modelling can be applied. Here the species

will be separated based on their climatic niche. This tool has the power to highlight regions of potential suitable habitats based on known distribution data of a species. I used this approach in two different species as case studies to demonstrate its usefulness.

In this thesis I modelled the climatic niche of *Sphingonotus rubescens* (Chapter 3). This species has a large distribution range from the Atlantic Islands to central and southern Asia and a huge number of distribution records is available. Altogether the species has six subspecies which are defined by morphological characters. Next to the revision of the potential current distribution of the species I tried to trace back the ancestral pathways. Oedipodinae are known to be a rather young group (~ 35 myo to 36 myo; Song et al. 2018; Husemann et al. 2012), but the genus *Sphingonotus* seems to be much younger (Husemann et al. 2014). In this study, I calculated the potential suitable area based on climate data and projected the current niche of the species to the Last Glacial Maximum and the Mid Holocene. My results support the hypothesis that the taxon has a huge range of potentially suitable areas, but geographic barriers may prevent the expansion of its distribution. *Sphingonotus rubescens* has a broad climatic niche allowing for the large distribution range. These territories, which represent the nowadays potential suitable areas, also seemed to be present in the paleoclimatic models, based on high levels of overlap between the projections. Even though the species may have reached larger parts of Northern Asia during the Last Glacial Maximum and Mid Holocene, it must have retreated back to warmer areas, when temperatures were falling. This suggests that during these timeframes strong short-term climatic variations likely influenced species' range. Shorter cooling periods (Heinrich events) alternated with short warming periods (Dansgaard–Oeschger events) what favored the speciation of the ancestors of *S. rubescens* and caused a range expansion (Ruddiman, 2001). Such a scenario has already been proposed for other species, which expanded their ranges to the north during warmer periods (Sommer et al., 2014). These climatic models show how the species was able to expand its range through time. Continuous expansion and retraction may have caused the species to have a very diverse gene pool. Similar events might have happened in the Mediterranean region, where species originated as result of alternating warm and cold phases. Here, they needed to retract back into warmer regions

in colder times. For the Mediterranean region a huge number of studies particularly mentioning this evolutionary process promoting speciation are published (e.g. Dey et al. 2022; Klesser et al. 2021; Recuero et al. 2007; Stöck et al. 2008; Habel et al. 2008; Perera & Harris, 2010).

Within a further study, I used a similar approach to investigate the potential niche of *B. tuberculata* (Chapter 4). *Bryodemella tuberculata*, is a band-winged grasshopper which has strongly declined during the last century, but had an originally large distribution; yet, more northern than *S. rubescens*. In this study, I was interested in the forces which may have led to the extinction of the species in central Europe, while the species is still widespread in Asia. Only few central European populations are still present (e.g. Reich 1991; Zuna-Kratky et al. 2017). I integrated genetic data to trace back the timeframe of extinction. Skyline plots were used to reconstruct female effective population size for the different regions. The result was decreasing population size within the European populations, but stable populations in Asia. This was in line with the findings of our literature and museum sample survey. I was able to trace back the last findings of several Central European populations to the mid of the 19th century. Interestingly, almost all populations went extinct during the same timeframe. But contrary the habitats remained climatically suitable for almost all extinct distributions. To test the potential suitability of the extinct locations I used the Asian locations to calibrate the model. Then I extrapolated the potential suitable habitats to the European distributions. I found similar Mahalanobis distances for the extinct and extant locations, suggesting equal climatic suitability for *B. tuberculata*. As the modeling data only relies on climatic factors, I concluded that the reason for the extinction of the populations was not climate change. Further, I calculated the distance of each distribution to the ecological center and found out that the distance to the core is much higher for the extinct locations compared to the most extant distributions. This may mean that the populations being disjunct from the main distribution experienced a genetic bottleneck (Kajtoch et al. 2016). Similar patterns were also found for the Danish population of the otter *Lutra lutra* (Pertoldi et al. 2001, Cremene et al. 2005; Kotze and O'Hara 2003). At this point I had a closer look at the

history of some extinct locations. For example in the Lüneburger Heide, I noticed that the species was not found in the literature and local collections in the 19th century. At the end of the last glacial maximum, the landscape was dominated by forest areas (Naturpark-Lueneburger-Heide.de; Koopmann 2000). These areas were deforested in the neolithic and in the following, heaths were able to grow. During this time, I suggest *B. tuberculata* was expanding its range and colonized the Lüneburger Heide. The people in the Lüneburger Heide during this time were performing traditional heath and sheep agriculture. In the middle of the 18th century imported products became cheaper than the traditional heaths economy products and the farmers decided to switch to traditional agriculture with little success (Naturpark-Lueneburger-Heide.de; Koopmann 2000). However, the landscape transformation and the accompanying loss of suitable habitats led to the extinction of *B. tuberculata*. Later on, the landscape changed again back to the traditional heath systems, but the species was not able to recolonize the areas and remained extinct. At many other extinct locations changing of the riversides, which are one common habitat type of the species, also erased the populations. Nowadays, only few populations remain extant with a trend towards extinction in Central Europe. Interestingly, despite the huge distance to the Asian core populations, the species was found again at the Padbrade Military area in 2008 (Budrys et al. 2008; Budrys and Pakalniškis 2007). This finding may show the potential of recolonization of still suitable habitats in Central Europe like for e.g. the Lüneburger Heide or several Military areas around Europe. I suggest that the only barrier that hinders the recolonization from Asia to Europe is the large distance between the remaining extant Asian and the extinct European populations. Maybe recolonization aided by humans would be an option to reestablish some populations in Europe.

Summing up, using ENM methods I was able to trace back the ancestral suitable areas for *S. rubescens* in the Mid Holocene and Last Glacial Maximum, highlighting that this method provides us new insights into the history of the young species group. Furthermore, we were able to determine that the existing extinction processes of *B. tuberculata* are not due to climate change. Based on my results the method shows the potential for species differentiation based on their climatic niche. This tool could

especially be helpful in groups with low or no phylogenetic signal, like in Sphingonotini and Bryodemini.

Conclusion

Summing up, in this thesis I worked on mitogenome arrangements, chromosome numbers and genome sizes of Orthopterans to find out the general genomic composition. Here, my colleagues and I found a general chromosome number of N=21 and N=23 in Oedipodinae, while the genome size varied between 1C=5.28pg in *Locusta migratoria* and 1C=21.96pg in *B. tuberculata.* Oedipodinae seem to all have the same mitogenome gene order. No correlation between chromosome number, genome size and MTR is possible.

I further investigated the resolution of different markers and methods to differentiate species of band-winged grasshoppers. Here I found almost no resolution within the taxonomically difficult *Sphingonotus* species by using only the COI fragment. Using a multi-gene approach allowed the separation into four groups (*S. caerulans, S. azuresens*, *S. coerulipes* and *S. haitensis* group). But not even genomic analyses based on hybridization capture were able to separate the species according to the taxonomic classification. This may suggest that the taxonomic classification needs to be updated, as some genera still appear para- and polyphyletic in the tree.

Further, I investigated the appearance of morphologically similar species from the OW and NW. Here, I used several methods to compare the species in morphological and ecological space. My analyses show evidence of convergent evolution within the selected Oedipodinae pairs. This suggests that similar morphology may have evolved in response to comparable climatic niches, and not due to common ancestry.

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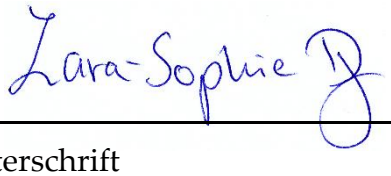
EIDESSTATTLICHE ERKLÄRUNG

zur Dissertation

"Phylogenetic and phylogenomic analyses and distribution modelling of a challenging taxon – the band-winged grasshoppers"

vorgelegt von Lara-Sophie Dey, geb. 23.04.1995

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Ich versichere weiterhin, dass die vorliegende Arbeit noch nicht als Abschlussarbeit an anderer Stelle eingereicht wurde.

Hamburg, 12.01.2023 

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SUPPLEMENTARY INFORMATION

DISSERTATION

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"Phylogenetic and phylogenomic analyses and distribution modelling of a challenging taxon – the band-winged grasshoppers"

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Dissertation zur Erlangung des Doktorgrades

An der Fakultät für Mathematik, Informatik und Naturwissenschaften

Fachbereich Biologie

der Universität Hamburg

-

In Kooperation mit dem

Leibniz Institut zur Analyse des Biodiversitätswandels, Standort Hamburg

-

vorgelegt von Lara-Sophie Dey, geb. 23.04.1995

-

Hamburg 2023

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SI CHAPTER 3

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SPECIES DISTRIBUTION MODELLING REVEALS INSIGHTS INTO WIDE DISTRIBUTIONS

Shedding light on the widespread distribution of *Sphingonotus (Sphingonotus) rubescens* (Acrididae: Oedipodinae)

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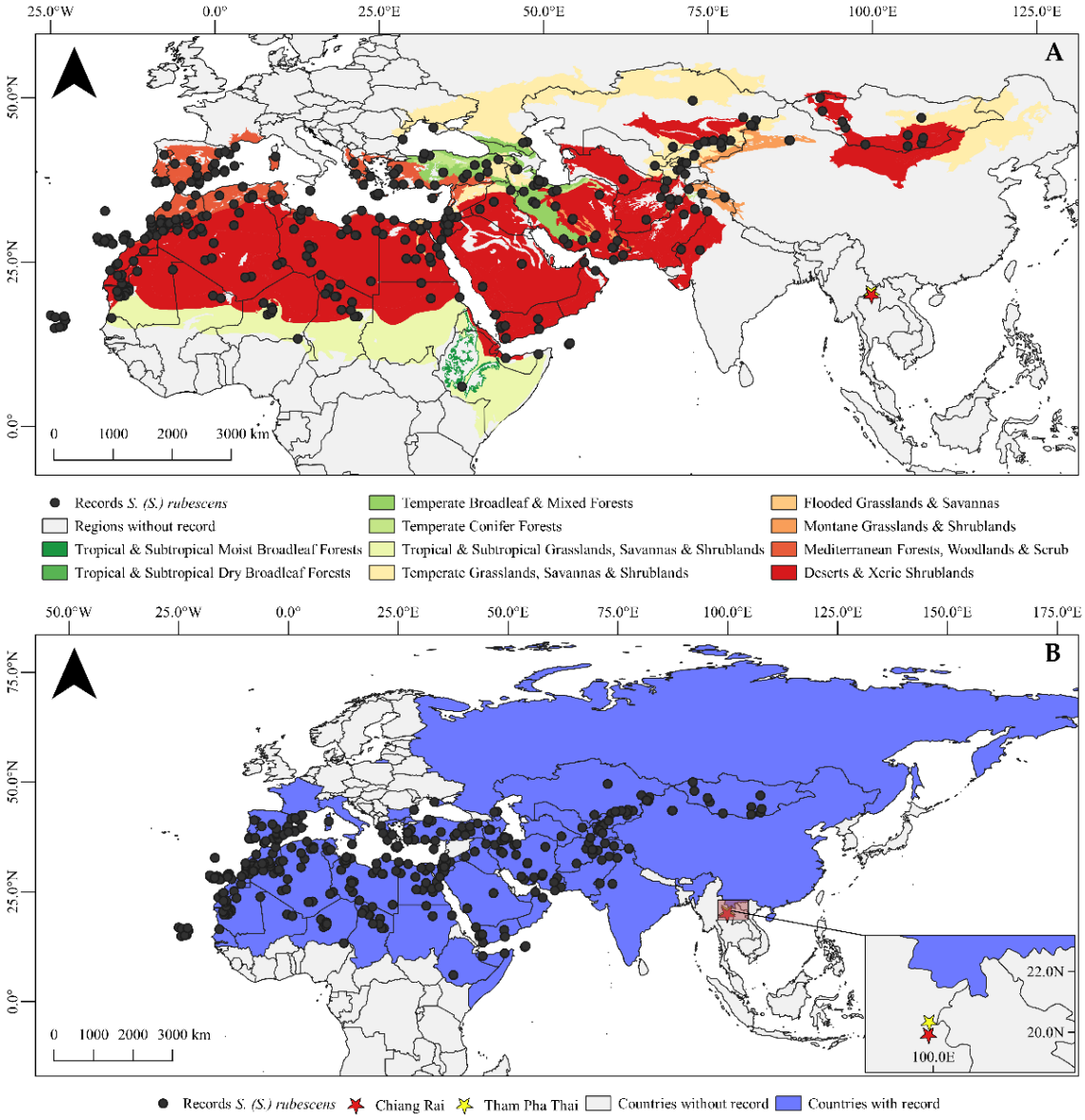
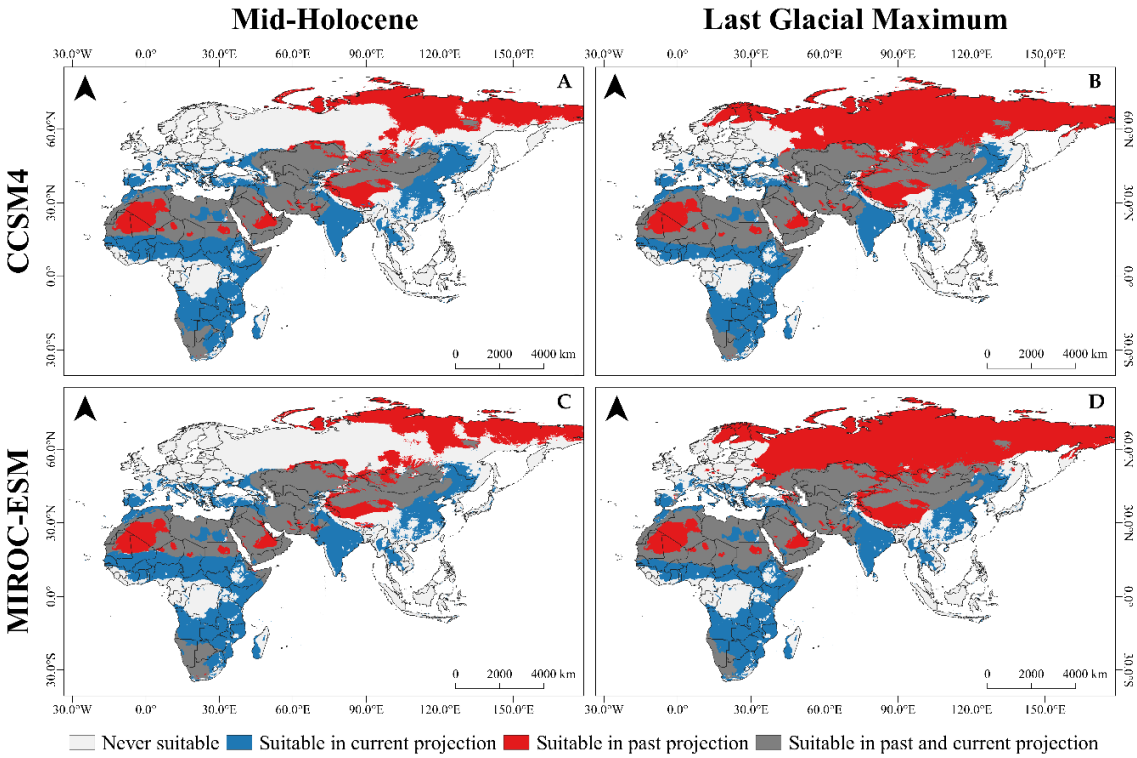
Supporting Information Figure S1: Map panel displaying A: biome types; and B: all records allocated to countries, within the total current range of *S. (S.) rubescens*. Records are shown as black dots. Tested dubious location records in Thailand are shown as a red star for Chiang Rai and as a yellow star for Tham Pha Thai.

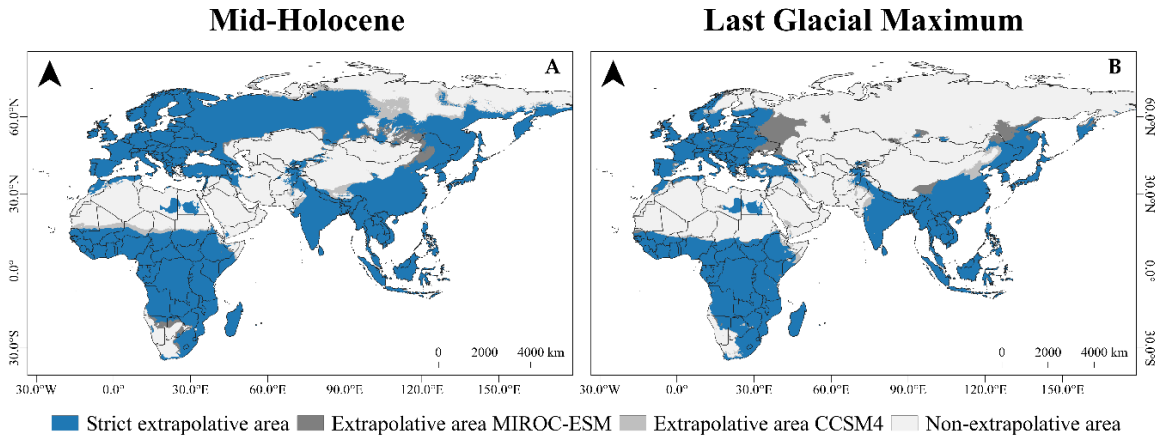


Supporting Information Figure S2: Images of *S. (S.) rubescens* from Thailand deposited in the Natural History Museum Vienna, Austria (NHM).

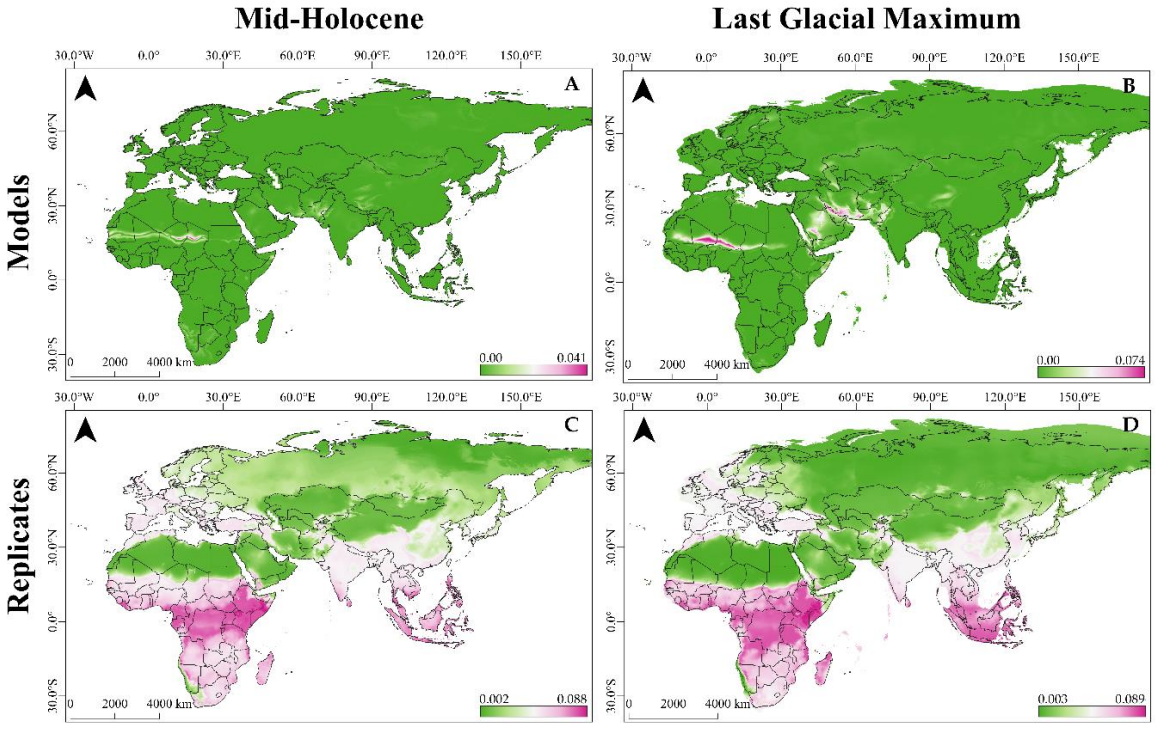
Supporting Information Figure S3: Potentially suitable areas in the past and present. Panels show results from current and paleo-climatic models. A: mid-Holocene, GCM: CCSM4 projection; B: Last Glacial Maximum, GCM: CCSM4 projection; C: mid-Holocene, GCM: MIROC-ESM projection; D: Last Glacial Maximum, GCM: MIROC-ESM projection. Light grey are areas which were never suitable; blue indicates areas suitable in the current projection; red represents areas suitable in the respective paleo-climatic past projection; grey are areas suitable in respective paleo-climatic past and current projection.



Supporting Information Figure S4: Map panel showing the extrapolated area through time for A: Mid-Holocene; and B: Last Glacial Maximum. Blue areas represent strict extrapolation; dark grey shows extrapolative regions using MIROC-ESM, light grey using CCSM4. Non-extrapolative areas are shown in white.



Supporting Information Figure S5: Calculated model variation for mid-Holocene (MH: 6,000 ya) and Last Glacial Maximum (LGM: 22,000 ya), comprising the variation between the models CCSM4 and MIROC-ESM and the 10 replicates for both time periods, separately. Map panel shows the variation for A: between the GCMS (CCSM4 and MIROC-ESM) for mid-Holocene; B: between the GCMS (CCSM4 and MIROC-ESM) for Last Glacial Maximum; C: between the replicates, the color scheme shows variation between the models and replicates. Green indicates low variation between the projections, violet indicates high variation. Model variation for LGM between models (MIROC-ESM and CCSM4).



Supporting Information Table S1: Literature checked for species occurrences.

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Supporting Information Table S2: Species records from literature and http://acrinwafrica.mnhn.fr.

Subspecies	Ref. No.	Country	Location
afghanicus	89	Afghanistan	Kabul
afghanicus	89	Afghanistan	Rabatak
afghanicus	89	Afghanistan	Dschelalabad
afghanicus	89	Afghanistan	Bamian
afghanicus	89	Afghanistan	Kandagarsk Province
burri	13	Cape Verde	S. Vincente, Mindelo
burri	13	Cape Verde	Boavista, Sal Rei
burri	33	Cape Verde	Maio
burri	33	Cape Verde	Fogo
burri	33	Cape Verde	Brava
burri	33	Cape Verde	St. Lucia
burri	33	Cape Verde	Sal
fallax	79	India	Karakoram ridge, mouth of river Nubra
fallax	79	India	Panamik, on r. Nubra 11.000 ft
fallax	88	India	Jammu
fasciatus	45	Iran	Fars Province, Safe Abad (to Lar)
fasciatus	45	Iran	Hormozgan Province, Near Haji Abad
fasciatus	45	Iran	Fars Province, near Mahlakeh
fasciatus	45	Iran	Fars Province, 30 km NW of Qir
fasciatus	45	Iran	Yazd Province, 2 km W of Zeyn-od-Din (to Mehriz), 1600 m
fasciatus	89	Iran	Borudsched, Lorestan
fasciatus	89	Iran	Zachbechar, Mekran

Subspecies	Ref. No.	Country	Location
fasciatus	89	Iran	Dschiroft, Darsin
fasciatus	89	Iran	Iranschechr, Beludschistan
fasciatus	89	Iran	Bempur, Beludschistan
fasciatus	79	Kazakstan	Novo-Voskresenovka
fasciatus	79	Kazakstan	Sugarty Gorge, Alma-Ata District
fasciatus	79	Kazakstan	station Tastakh, r. Bol. Almatinka
fasciatus	79	Kazakstan	valley of r. Chilik
fasciatus	79	Kazakstan	Kurdai near Alma-Ata
fasciatus	79	Kirghiz Republic	Kukumeren, Frunse District
fasciatus	79	Kirghiz Republic	south of station Belorodosk, stony river bed Ak-su
rubescens	16	Algeria	Beni-Abbes
rubescens	16	Algeria	Tabelbala
rubescens	16	Algeria	Dj. Antar
rubescens	16	Algeria	Zerhamra
rubescens	16	Algeria	Dj. D'Ougarta, Ouschtat
rubescens	16	Algeria	Reggan
rubescens	25	Algeria	Djurdjura, H. Maurel
rubescens	80	Algeria	Tindouf (Territoire des Con f ins Algéro-Marocains)
rubescens	103	Algeria	Beni Ounif [Bechar] (MNHN)
rubescens	103	Algeria	Meknassy = Maknassy
rubescens	103	Algeria	Takhemaret
rubescens	103	Algeria	Tamanar [Essaouira], Agadir (MNHN)
rubescens	103	Algeria	Tamanrasset
rubescens	103	Algeria	Tazenakht, Tinghir =Tinerhir
rubescens	103	Algeria	Laghouat

Subspecies	Ref. No.	Country	Location
rubescens	103	Algeria	El Menia
rubescens	103	Algeria	Reggane
rubescens	103	Algeria	Béni-Abbès
rubescens	103	Algeria	Silet
rubescens	103	Algeria	Adrar
rubescens	89	Azerbaijan	Abrakunis, Kasanzi, Nachizevansk, ASSR
rubescens	89	Azerbaijan	Ailis (u Ordubada)
rubescens	1	Egypt	Costal Stripe, Mersa Matrouh
rubescens	1	Egypt	Delta and lower Nile Valley, Giza
rubescens	1	Egypt	Upper Nile Valley, Abutig
rubescens	1	Egypt	Upper Nile Valley, Dahshour
rubescens	1	Egypt	Upper Nile Valley, Edfou
rubescens	1	Egypt	Upper Nile Valley, Luxor
rubescens	1	Egypt	Eastern Desert, Hurguda
rubescens	1	Egypt	Eastern Desert, Ismailiya
rubescens	1	Egypt	Eastern Desert, Kosseir
rubescens	1	Egypt	Eastern Desert, Wadi Digla
rubescens	1	Egypt	Eastern Desert, Wadi Hoff
rubescens	1	Egypt	Eastern Desert, Baharia Oasis
rubescens	1	Egypt	Eastern Desert, Dakhla Oasis
rubescens	1	Egypt	Eastern Desert, Kharga Oasis
rubescens	1	Egypt	Eastern Desert, Siwa Oasis
rubescens	1	Egypt	Eastern Desert, Wadi El Natroun
rubescens	87	Egypt	Khargeh
rubescens	69	France	Port-Vendres, Cap de Biarra et a Cerbere, Cap de Cerbere

Subspecies	Ref. No.	Country	Location
rubescens	64	India	Rajasthan
rubescens	64	India	Jaisalmer
rubescens	31	Iran	Sistan and Baluchistan Province, Gusheh Khash
rubescens	31	Iran	Sistan and Baluchistan Province, 80 km S Jiroft
rubescens	36	Iran	Kerman, near Lahidschan
rubescens	36	Iran	Sabazawara
rubescens	36	Iran	Deh Bakri
rubescens	45	Iran	Kurdistan Province, Zagros Mts.
rubescens	45	Iran	Zanjan Province, W Elburs: Tarom Valley, 20 km NE of Zanjan, 2350 m
rubescens	45	Iran	Yazd Province, Kuh-e Madvar
rubescens	45	Iran	Guilan Province, Talesh Mts., 12 km E of Gilvan, 300 m
rubescens	45	Iran	Zanjan Province, W Elburs: Tarom Valley, 20 km NE of Zanjan, 2350 m
rubescens	45	Iran	Isfahan Province, 7 km NW of Natanz
rubescens	45	Iran	Hamadan Province, Zagros Mts., 25 km SE of Nehavand, 2000 m
rubescens	48	Iran	Guilan province, Sowme'eh Sara
rubescens	79	Iran	Kashmir
rubescens	98	Iran	Tehran
rubescens	98	Iraq	Rutba
rubescens	70	Israel	Arad
rubescens	*	Israel	Lower Galilee
rubescens	*	Israel	Judean Hills
rubescens	*	Israel	Negev

Subspecies	Ref. No.	Country	Location
rubescens	2	Italy	Lampedusa
rubescens	72	Italy	Isola di Lampedusa
rubescens	55	Yemen	Al Hudaydah, Jebel Burra, 25km SE Bajil, 600m
rubescens	70	Jordan	Wadi Rum
rubescens	70	Jordan	Aqaba
rubescens	70	Jordan	Wadi Rum (Aqaba) Qa Disi
rubescens	70	Jordan	Amman
rubescens	70	Jordan	Wadi al Wala
rubescens	70	Jordan	Quasr Amra
rubescens	70	Jordan	Azrak
rubescens	70	Jordan	Dana Reserve, Desert area
rubescens	70	Jordan	Wadi Araba
rubescens	70	Jordan	Wadi Finan [Feynan]
rubescens	70	Jordan	Wadi Dana
rubescens	70	Jordan	Dana Reserve. Acacia area
rubescens	*	Jordan	Arava Valley
rubescens	39	Libya	Hamada el-Hamra
rubescens	65	Libya	Garián
rubescens	65	Libya	Mízda
rubescens	73	Libya	Scegga, Giarabub
rubescens	73	Libya	Tibesti
rubescens	73	Libya	Augila
rubescens	73	Libya	15km E Ain Dona (Auenat), Ain Dona, Arkenu
rubescens	73	Libya	Sebha, Uadi Tanezzuft
rubescens	73	Libya	Tripoli, Barka

Subspecies	Ref. No.	Country	Location
rubescens	73	Libya	Mizda, U. Sofeggin
rubescens	73	Libya	Garian, Gheria esh-Scerghia, Hamada el-Hamra
rubescens	73	Libya	Jabal as Sawdā' [Gebel es Soda]
rubescens	73	Libya	Garian
rubescens	73	Libya	Ain Zara
rubescens	73	Libya	Jadu [Giado]
rubescens	73	Libya	Munizip Ghadames [Ghadames]
rubescens	73	Libya	Um El Abib,
rubescens	73	Libya	Carcura,
rubescens	73	Libya	Marada,
rubescens	73	Libya	Homs
rubescens	73	Libya	Jefren, Uadi
rubescens	73	Libya	Agedabia
rubescens	73	Libya	Gialo
rubescens	73	Libya	Port Bardia
rubescens	73	Libya	Ghat
rubescens	73	Libya	Al Qatrun
rubescens	73	Libya	Sebha
rubescens	73	Libya	Samnu
rubescens	73	Libya	Brack
rubescens	79	Libya	Tripoli
rubescens	8	Mongolia	Us Nuur, 50km Ulangom
rubescens	49	Mongolia	Gobi Altai, 20km S Schiene-Dschinst
rubescens	49	Mongolia	Gobi Altai, 30km S Schiene-Dschinst
rubescens	49	Mongolia	Gobi Tjan-Schan, 180km SSW Schiene-Dschinst

Subspecies	Ref. No.	Country	Location
rubescens	49	Mongolia	80km S Schiene-Dschinst, Echin-Gol
rubescens	15	Morocco	M'Hamid
rubescens	15	Morocco	Oued el Maleh
rubescens	15	Morocco	Taouz
rubescens	15	Morocco	Kelaá des M'Gouna
rubescens	25	Morocco	Haute Moulouya
rubescens	25	Morocco	Haouz
rubescens	25	Morocco	Vallée du Dadès
rubescens	25	Morocco	Vallée du Draa
rubescens	25	Morocco	Bassin de Tarfaya
rubescens	102	Morocco	Mogador
rubescens	103	Morocco	Agadir Tissint [Tata]
rubescens	103	Morocco	Amerzgane [Ouarzazate]
rubescens	103	Morocco	Anmid [Ouarzazate] (legs Louveaux MNHN)
rubescens	103	Morocco	Aousserd,
rubescens	103	Morocco	Biskra (MNHN)
rubescens	103	Morocco	Dakhla ex Villa Cisneros [Oued Ed-Dahab]
rubescens	103	Morocco	Djanet
rubescens	103	Morocco	El Guettar [Gafsa] (MNHN).
rubescens	103	Morocco	Erfoud = Arfoud
rubescens	103	Morocco	Foum el Hassan
rubescens	103	Morocco	Oued Ternit [Es Smara]
rubescens	103	Morocco	Pozo Tuf (puit)
rubescens	103	Morocco	Skoura des Ahl el Oust

Subspecies	Ref. No.	Country	Location
rubescens	103	Morocco	Tagoundaft [Al Haouz], Pays Goundafa, Taouz
rubescens	103	Morocco	Errachidia
rubescens	103	Morocco	Tinghir =Tinerhir
rubescens	103	Morocco	Zagora
rubescens	103	Morocco	Ouarzazate
rubescens	103	Morocco	El Aïoun du Drâa
rubescens	103	Morocco	Akka Izem
rubescens	103	Morocco	Tata
rubescens	103	Morocco	El Kantara
rubescens	52	Morocco	El Feid
rubescens	79	Oman	Barka
rubescens	63	Oran	Beni-Ounif
rubescens	18	Portugal	Canical
rubescens	18	Portugal	Pico Juliana
rubescens	38	Portugal	Selvagem Grande
rubescens	46	Portugal	Sao Lourenco
rubescens	46	Portugal	Desertas
rubescens	46	Portugal	Canical
rubescens	46	Portugal	Deserta Grande
rubescens	50	Portugal	Portimao
rubescens	68	Portugal	la Sierra de los Filabres, at 1865m altitude (Bacares, Almería)
rubescens	68	Portugal	Sierra del Segura, at 1910m (Albacete)
rubescens	70	Saudi Arabia	Wadi Jizan [Jazan]
rubescens	25	Spain	Province de Murcia, D.Morin
rubescens	25	Spain	Almeria

Subspecies	Ref. No.	Country	Location
rubescens	25	Spain	Jaén
rubescens	25	Spain	Valencia
rubescens	25	Spain	Lerida
rubescens	25	Spain	Barcelona
rubescens	25	Spain	Granada
rubescens	41	Spain	Mallorca
rubescens	41	Spain	Ibiza
rubescens	41	Spain	Formentera
rubescens	43	Spain	Canary Islands, Bajamar
rubescens	43	Spain	Canary Islands, Candelaria
rubescens	43	Spain	Canary Islands, Los Christianos
rubescens	43	Spain	Canary Islands, Las Galletas
rubescens	43	Spain	Canary Islands, Guimar
rubescens	43	Spain	Canary Islands, Icod, Pinar de
rubescens	43	Spain	Canary Islands, Igueste de S. Andres, Carretera
rubescens	43	Spain	Canary Islands, Barranco de la Lena
rubescens	43	Spain	Canary Islands, Barranco de Martianez
rubescens	43	Spain	Canary Islands, Medano=El Medano
rubescens	43	Spain	Canary Islands, Mesas=Monte de las Mesas
rubescens	43	Spain	Canary Islands, Puerto de la Cruz
rubescens	43	Spain	Canary Islands, Punta del Hidalgo
rubescens	43	Spain	Canary Islands, Barranco de San Andreas
rubescens	43	Spain	Canary Islands, Santa Cruz

Subspecies	Ref. No.	Country	Location
rubescens	43	Spain	Canary Islands, Barranco de Santos = Barranco Santos
rubescens	43	Spain	Canary Islands , Barranco de Seco = Barranco Seco
rubescens	43	Spain	Canary Islands, Monte de los Silos = Monte del Agua
rubescens	43	Spain	Canary Islands, Barranco de Tahodio = Barranco Tahodio
rubescens	43	Spain	Canary Islands, Vilaflor
rubescens	47	Spain	Las Palmas, Maspalomas
rubescens	50	Spain	southern coast, Castellon
rubescens	52	Spain	Lanzarote, Charco del Palo
rubescens	52	Spain	Gran Canaria, San Nicolás
rubescens	52	Spain	Tenerife, Punta de Teno
rubescens	52	Spain	La Gomera, Casas de Langrerc
rubescens	52	Spain	La Palma, La Fajana
rubescens	52	Spain	Extremadura, Monfrague
rubescens	69	Spain	Sierra de los Filabres (Almeria)
rubescens	69	Spain	Sierra de Segura (Albacete)
rubescens	91	Turkey	Erzincan
rubescens	91	Turkey	Erzurum
rubescens	91	Turkey	Kars
rubescens	91	Turkey	Manisa
rubescens	92	Turkey	Işıklar, 900m
rubescens	92	Turkey	İzmir
rubescens	92	Turkey	Malatya
rubescens	92	Turkey	Muş

Subspecies	Ref. No.	Country	Location
rubescens	92	Turkey	Nevşehir
rubescens	93	Turkey	Bolu: Mengen, Pazarköy 4. km
rubescens	93	Turkey	Zonguldak: Dirgine, Akçabey, 10.8.2000
rubescens	93	Turkey	Karabük: Yenice, Karakavuz Köyü, 230m, 17.8.2001
rubescens	89	Turkmenistan	Bairam-Ali
rubescens	80	Western Sahara	El Aiun
rubescens	80	Western Sahara	Guelta del Zemur
rubescens	103	Western Sahara	Ghardaïa
rubescens	103	Western Sahara	Guelmin (MNHN)
rubescens	103	Western Sahara	Gueltat Zemmour = Galtat Zemmour [Boujdour]
rubescens	55	Yemen	25km NNE Al Mukalla, Al Ain, 20km NNW Ar Rayyan
rubescens	55	Yemen	Sana'a, mountains SW Sana'a, Jabal'Ayban, Bait Na'ama, 2700-2750m
rubescens	55	Yemen	Al-Mahwit, 5km N Kamis Bani Sa'd, 600m
rubescens	55	Yemen	Ta'izz, Wadi Warazan, 5km NW Ar Rahidah, 1100m
rubescens	55	Yemen	Ta'izz, Wadi Warazan, 5km NW Ar Rahidah, 1080m
rubescens	55	Yemen	Hadramaut, 25km NNE Al Mukalla, Al Ain, 20km NNW Ar Rayyan, 100m
rubescens	104	Somalia	42°50'E 10°25'N
rubescens	104	Somalia	foot of Mt. Elmis (80km W of Berbera
rubescens	104	Somalia	Tug Hodma E of Karin
subfasciatus	3	Kyrgyzstan	Lake Issyk-Kul

Supporting Information Table S3: Full list of examined museum and private collection material sorted by country. Collection abbreviations are used as follows: Mongolian Academy of Science Ulaan Bator, Mongolia (MAS), Muséum National d'Histoire Naturelle Paris, France (MNHN), National Museum Prague, Czech Republic (NM), Natural History Museum Geneva, Switzerland (GE), Natural History Museum Vienna, Austria (NMW), Siberian Zoological Museum Novosibirsk, Russia (SZMN), Zoological Collection of the Center for Natural History Hamburg, Germany (ZMH), Zoological State Collection Munich, Germany (ZSM), private collection of A. Hochkirch (PC AH), private collection of M. Husemann (PC MH), private collection of L.-S. Dey (PC LSD), private collection of R. Felix (PC RF).

SI Tab. S3a: Museum material examined.

Country	Location	Coordinates	Collector	Date	Depository
Afghanistan	Ali Khel (Gardez)	33.6061°N 69.2413°E	F. Letellier	August 1970	MNHN
	Kabul	34.6138°N 69.3219°E	F. Heydemann	20.09.1953	ZMH
Algeria	Bir Naam	34.7535°N 5.1084°E	A. Moussi	07.05.2009	PC MH
	Biskra	34.8511°N 5.7285°E	A. Moussi	04.04.2009	PC MH
	El Kantara	35.2231°N 5.7092°E	A. Moussi	06.08.2015	PC MH
	Foghala	34.7583°N 5.2997°E	A. Moussi	30.04.2009	PC MH
	Oasis Biskra	34.8705°N 5.6917°E	H. Strümpel	30.08.1968	ZMH
Armenia	Ararat	39.9418°N 44.9849°E	L. Darimont & H. Seeboth	August 2014	PC AH
Cape Verde	Ile de Boa Vista	16.1267°N -22.7714°E	-	06.10.– 13.11.1980	MNHN

Country	Location	Coordinates	Collector	Date	Depository
	Ile de San Nicolau [Ilha de São Nicolau]	16.6039°N -24.2976°E	-	06.10.–13.11.1980	MNHN
	Ile de Santiago	15.0820°N -23.6200°E	-	October 1979	MNHN
China	Urumchi River	43.52°N 87.32°E	M. Sergeev	12.08.2014	SZMN
	S Urumchi	43.53°N 87.45°E	M. Sergeev	12.08.2014	SZMN
Cyprus	Akrotiri Bay	34.7074°N 33.0742°E	-	-	GE
Egypt	W Hurghada	27.2552°N 33.8017°E	A. Michalik	20.10.2006	PC AH
Ethiopia	Gemu-Gofa, Arba Minch, 1200–1400 m	6.0489°N 37.5475°E	-	03.11–07.11.1978	MNHN
Greece	Hellos, 3km E Gythio/Peloponnese	36.7642°N 22.5604°E	R. Kinzelbach	20.09.1973	GE
	Crete, Tymbaki	35.0761°N 24.7670°E	H. Eckerlein	29.07–31.07.1958	GE
	Kos Island, Mountains close to Zia	36.8458°N 27.2046°E	M. Husemann	04.11.2014	PC MH
	Evyros, Evinochori, pebbly river bed	38.3646°N 21.5395°E	L. Willemse, J. Tumbrick, R. Kleukers & B. Odé	05.09.2011	PC AH
	Smixi	40.0666°N 21.1166°E	K.G. Heller	26.07.2003	PC AH
	Krete, E Plakias near Anthos	35.1874°N 24.4007°E	U. Blaschke	26.05.2005	PC AH
	Krete, Ahlada	35.4049°N, -24.9872°E	A. Hochkirch	20.05.2006	PC AH
	Krete, Plakias, Anthos	35.1892°N 24.3996°E	R. Kleukers	03.08.2012	PC AH
	Krete, Agia-Salini [Ayía Galíni]	35.1018°N 24.6874°E	A. Hochkirch	28.09.1999	PC AH
Italy	Sardinia, 6 km W Luogosanto	41.0501°N 9.2050°E	Y. Görzig	28.08.2006	PC AH
Kazakhstan	Ai	47.02°N 80.35°E	-	07.07.1975	SZMN

Country	Location	Coordinates	Collector	Date	Depository
	SE Makanchi	46.58°N 82.22°E	-	-	SZMN
	Jamanty River	45.85°N 81.48°E	I. Stebaev & E. Moiseeva	12.07–14.07.1975	SZMN
	Rgaity River	45.68°N 81.9°E	I. Stebaev & E. Moiseeva	15.07.1975	SZMN
	Jamanty River	45.88°N 81.58°E	E. Moiseeva	-	SZMN
	S Kopa	43.48°N 75.8°E	-	11.08.1981	SZMN
	Zhety-Zhol Mts., N Kordaj (Georgievka)	43.32°N 74.42°E	-	12.08.1981	SZMN
Kyrgyzstan	Alay Range, SE Khaidarkan	39.95°N 71.3°E	M. Sergeev	11.08.1986	SZMN
	Alay Range, Shohimardon River	40.08°N 71.72°E	M. Sergeev	18.08.1986	SZMN
	Alay Range, Shohimardon River	40.1°N 71.72°E	M. Sergeev	19.08.1986	SZMN
	Alay Range, Shohimardon River	40.1°N 71.73°E	M. Sergeev	19.08.1986	SZMN
	Alay Range, Shohimardon River	40.08°N 71.7°E	M. Sergeev	19.08.1986	SZMN
	Fergan Range, Arslanbob	41.35°N 72.93°E	M. Sergeev	23.08.1986	SZMN
	Fergan Range, NE Bazar-Korgon, Kara-Unkiur River	41.13°N 72.87°E	M. Sergeev	31.08.1986	SZMN
	Sokh Ravine, near Ak-Turpak vill.	40.2080°N 71.0286°E	D. Milko	18.07.2005	PC AH
Libya	Tripolitania, 23 km S Ariziza	32.8582°N 13.1803°E	H. Eckerlein	28.04.1965	GE
Mongolia	Char Us Nuur	47.9846°N 92.3919°E	L.-S. Dey	10.07.2017	PC LSD
	close to Bayantsagaan	46.9510°N 107.4386°E	L.-S. Dey	30.07.2015	PC LSD

Country	Location	Coordinates	Collector	Date	Depository
	close to Sharga	46.3579°N 95.4095°E	L.-S. Dey	07.07.2017	PC LSD
	Khanbogd	43.0861°N 107.4851°E	-	17.08.2013	MAS
	Nomgon	42.6418°N 105.3442°E	-	-	MAS
	Sutegijn Bajan-Gol	43.9055°N 107.7281°E	L.-S. Dey	23.07.2015	PC LSD
	Tseel	45.4292°N 95.8886°E	-	25.08.2012	MAS
	Tsogt-Ovoo	44.3203°N 105.3778°E	-	15.08.2013	MAS
Morocco	Al Haouz	31.4335°N -8.1303°E	L.-S. Dey, O. Hawlitschek & M. Husemann	31.03.2019	PC MH
	Iguisse Izder	30.5973°N -9.2756°E	L.-S. Dey, O. Hawlitschek & M. Husemann	24.03.2019	PC MH
	near Assaka Ou Blagh	29.6917°N -9.5218°E	L.-S. Dey, O. Hawlitschek & M. Husemann	29.03.2019	PC MH
	Nzala	31.4985°N -8.1738°E	L.-S. Dey, O. Hawlitschek & M. Husemann	31.03.2019	PC MH
	Oumnass	31.4109°N -8.1214°E	L.-S. Dey, O. Hawlitschek & M. Husemann	31.03.2019	PC MH
	Ouzina	30.7695°N -4.1452°E	L.-S. Dey, O. Hawlitschek & M. Husemann	30.03.2019	PC MH
	Route de a Tafraout Tiznit	29.5485°N -9.3392°E	L.-S. Dey, O. Hawlitschek & M. Husemann	29.03.2019	PC MH
	Taghazout	30.5348°N -9.6941°E	L.-S. Dey, O. Hawlitschek	30.03.2019	PC MH

Country	Location	Coordinates	Collector	Date	Depository
			& M. Husemann		
Niger	Mt. Air, Akerebres, 40 km E de Timia	18.0580°N 8.6593°E	A. Foulary	27.10.1989	MNHN
	Arlit	18.7444°N 7.3809°E	T. McNary	10.03.1985	PC AH
	Tiffa	13.3152°N 12.6043°E	T. McNary	20.10.1985	PC AH
Pakistan	northern Areas, Chilas, 1075 m	35.4310°N 74.1002°E	K. Schoenitzer	07.06.2007	ZSM
Russia	Sulak River, near Sulak	43.27°N 47.48°E	M. Sergeev	06.08.1987	SZMN
	coastal plain of Caspian Sea, E Sulak	43.32°N 47.55°E	M. Sergeev	09.08.1987	SZMN
	SW Sulak	43.25°N 47.47°E	M. Sergeev	08.08.1987	SZMN
	NW Sulak	43.3°N 47.43°E	M. Sergeev	09.08.1987	SZMN
	S Kizilyurt	43.15°N 46.87°E	M. Sergeev	12.08.1987	SZMN
	N Kizilyurt	43.22°N 46.85°E	M. Sergeev	13.08.1987	SZMN
	Dagestan, Caspian Depression, Sulak River near Sulak	43.2726°N 47.5068°E	M. Sergeev	07.08.1987	PC AH
Saudi Arabia	215 km N Riyadh	24.7135°N 46.6752°E	M. Donskoff	10.05.1993	MNHN
	30 km E Taif, route Al Suddayrah, zone surpaturee, 1400 m	21.2461°N 40.7013°E	M. Donskoff	20.06.1992	MNHN
Spain	Canary Islands, Gran Canary, Aguele	27.7741°N -15.5320°E	A. Hochkirch	-	PC AH
Tajikistan	E Dusti	37.35°N 68.77°E	M. Sergeev	02.06–19.06.1983	SZMN

Country	Location	Coordinates	Collector	Date	Depository
	E Dusti	37.32°N 68.77°E	M. Sergeev & A. Bugrov	10.06–13.07.1983	SZMN
	Gissar Range, Varzob River, Chorbog	38.67°N 68.77°E	M. Sergeev	28.07.1983	SZMN
	E Dusti	37.32°N 68.77°E	M. Sergeev, I. Kazakova & N. Sobolev	13.06–25.06.1984	SZMN
	Surkhob River, Garm	39.02°N 70.37°E	M. Sergeev	04.07.1984	SZMN
	Peter the Great Range, S Tojikobod	39.05°N 70.83°E	M. Sergeev	July 1984	SZMN
	Pamirs, Darvaz Range, E Vanj (Vanch)	38.38°N 71.5°E	A. Pokivajlov	15.08.1991	SZMN
Tunisia	Djebel Chambi	35.2063°N 8.6810°E	H. Strümpel	20.08.1968	ZMH
Turkey	Bornova	38.4710°N 27.2201°E	K. Harz	16.07.1961	GE
	Izmir	38.4094°N 27.2009°E	K. Harz	15.05.1963	GE
	Mersin, Taurus, 4 km NW Mut, 300 m	36.6613°N 33.4231°E	H. Hacker & G. Derra	04.09.1983	GE
	Iztuzu, Dalyan, Provinz Mugla, SW Anatolia	36.8266°N 28.6393°E	T. Kordges	05.07.2005	PC AH
	5 km W Belek near Antalya	36.8651°N 31.0628°E	J. Habel	05.09.2005	PC AH
United Arab Emirates	Sharja Desert Park	25.2822°N 55.6962°E	D. Llucia Pomares	25.02.2012	PC AH

SI Tab. S3b: New distributional records.

New distribution records					
Country	**Location**	**Coordinates**	**Collector**	**Date**	**Depository**
Iraq	Dokan	35.9296°N 44.9789°E	C. Kosswig	22.04.1958	ZMH

	Haditha	34.1489°N 42.4063°E	C. Kosswig	10.04.1958	ZMH
Sudan	Port Sudan, ca. 15 km N Suokia	19.6192°N 37.2117°E	H. Kriegbaum	25.12.1985	ZSM
	Wadi Halfa	21.8049°N 31.3680°E	R. Remane	7.08.–13.08.1964	MNHN
Thailand	Tham Pha Thai / Chiang Rai, doubtful location information	20.3296°N 99.8618°E / 19.9108°N 99.8408°E	H. Kusch & I. Staber	05.04.1978	NMW
Uzbekistan	Vuadil	40.15°N 71.73°E	M. Sergeev	16.06.1986	SZMN
	Samarkand	39.6532°N 66.9012°E	W. H. Muche	27.06.–30.6.1972	GE
Yemen	Hadramaut, Qabr. Hud-Terim	16.3179°N 49.1294°E	H. Wissmann	May to June 1931	ZMH
	Socotra, Hadiboh	12.6353°N 54.0054°E	R. Felix	07.11.2010	PC RF
	Socotra, near Aden	12.4634°N 53.8237°E	W. Wranik	01.11.1988	NM
	Socotra, Samha	12.1525°N 53.0526°E	W. Wranik	December 1982	NM

Supporting Information Table S4: Performance of 63 models created during the evaluation process for *S. (S.) rubescens*. The table displays the Set of selected variables (Set 1: Temperature Annual Range (Max Temperature of Warmest Month - Min Temperature of Coldest Month), Mean Temperature of Coldest Quarter and Precipitation of Driest Month; Set 2: Annual Mean Temperature, Annual Precipitation, Mean Temperature of Warmest Quarter and Precipitation of Driest Quarter; Set 3: Mean Temperature of Warmest Quarter, Annual Precipitation, and Precipitation of Driest Month); the seven feature classes representing combinations of linear (L), quadratic (Q) and product (P); the four investigated regularization Multipliers (0.5; 1.0; 1.5; 2.0); partial ROC (*p-values*); omission rate at 5%; Aikaike Information Criterion corrected (AICc); delta AIC (ΔAIC; score to measure the difference between the best model (smallest AIC) and each model); Aikaike Information Criterion weights (W AIC) and total number of parameters per setting. Selected parameters used in this study are shown underlaid with grey.

Set	Feature classes	Reg. Multi-plier	Mean AUC	partial ROC (*p-value*)	Omission rate at 5%	AICc	ΔAIC	W AIC	Number of parameters
1	l	0.5	1.02092	0.048	0.0686	8161.356	37.437	8.95E-11	3
1	q	0.5	1.02095	0.256	0.049	8188.505	64.586	1.18E-16	3
1	p	0.5	1.03097	0.04	0.0686	8227.427	103.51	4.34E-25	3
1	lq	0.5	1.04004	0	0.0588	8123.919	0	0.01333	5
1	lp	0.5	1.01688	0.246	0.0784	8159.319	35.399	2.86E-10	6
1	qp	0.5	1.01795	0.224	0.098	8178.911	54.992	1.66E-14	4
1	lqp	0.5	1.0413	0	0.0686	8124.97	1.0505	0.00888	7
1	l	1	1.02342	0.058	0.0588	8162.048	38.128	8.26E-11	3
1	q	1	1.02229	0.14	0.049	8189.117	65.197	1.15E-16	3
1	p	1	1.02846	0.078	0.0784	8227.752	103.83	4.93E-25	3
1	lq	1	1.03864	0	0.0392	8127.74	3.8206	0.0027	5
1	lp	1	1.01547	0.218	0.098	8159.8	35.881	3.12E-10	3
1	qp	1	1.01661	0.218	0.0882	8179.708	55.789	1.58E-14	4

Set	Feature classes	Reg. Multi-plier	Mean AUC	partial ROC (*p-value*)	Omission rate at 5%	AICc	ΔAIC	W AIC	Number of parameters
1	lqp	1	1.03854	0	0.0588	8130.564	6.6449	0.00079	7
1	l	1.5	1.02572	0.052	0.0294	8162.851	38.932	8.22E-11	3
1	q	1.5	1.02441	0.086	0.049	8189.822	65.903	1.23E-16	3
1	p	1.5	1.03217	0.024	0.0784	8228.145	104.23	6.34E-25	3
1	lq	1.5	1.03427	0.062	0.049	8133.525	9.6061	0.00024	5
1	lp	1.5	1.01431	0.206	0.0784	8160.385	36.465	3.92E-10	3
1	qp	1.5	1.01734	0.176	0.0882	8180.621	56.702	1.75E-14	4
1	lqp	1.5	1.0342	0.076	0.049	8135.504	11.585	0.00012	6
1	l	2	1.02567	0.052	0.0392	8163.763	39.844	1.02E-10	3
1	q	2	1.02506	0.126	0.049	8190.616	66.696	1.75E-16	3
1	p	2	1.02989	0.03	0.0686	8228.604	104.68	1.17E-24	3
1	lq	2	1.03244	0.052	0.0588	8138.225	14.306	6.12E-05	4
1	lp	2	1.01575	0.166	0.0784	8161.05	37.131	8.84E-10	3
1	qp	2	1.01869	0.162	0.0882	8181.648	57.729	4.29E-14	4
1	lqp	2	1.0286	0.106	0.0392	8140.868	16.948	5.51E-05	5
2	l	0.5	1.01895	0.124	0.0392	8196.877	72.958	1.75E-18	4
2	q	0.5	1.0079	0.372	0.0686	8220.877	96.957	1.12E-23	4
2	p	0.5	1.04543	0.038	0.0392	8178.043	54.124	2.32E-14	6
2	lq	0.5	1.03907	0	0.049	8180.63	56.711	6.55E-15	6
2	lp	0.5	1.04362	0	0.0392	8173.896	49.977	1.98E-13	9
2	qp	0.5	1.03745	0.034	0.049	8180.493	56.573	7.63E-15	9
2	lqp	0.5	1.04395	0	0.0392	8178.016	54.096	2.73E-14	11
2	l	1	1.02047	0.092	0.0392	8198.123	74.203	1.23E-18	4
2	q	1	1.00924	0.338	0.0784	8219.585	95.666	2.83E-23	3
2	p	1	1.04379	0.022	0.0392	8186.58	62.66	4.37E-16	4
2	lq	1	1.03603	0	0.049	8183.267	59.347	2.41E-15	6
2	lp	1	1.04447	0.036	0.049	8175.751	51.831	1.10E-13	7
2	qp	1	1.03779	0.028	0.0392	8185.066	61.147	1.10E-15	8
2	lqp	1	1.0403	0	0.049	8179.181	55.262	2.23E-14	9
2	l	1.5	1.01989	0.074	0.0392	8199.589	75.67	8.86E-19	4
2	q	1.5	1.01438	0.262	0.0784	8220.379	96.46	2.92E-23	3
2	p	1.5	1.04278	0.022	0.049	8192.889	68.97	2.95E-17	4
2	lq	1.5	1.03517	0.018	0.049	8186.544	62.624	7.68E-16	6
2	lp	1.5	1.04197	0.026	0.0392	8176.816	52.897	1.10E-13	6
2	qp	1.5	1.03134	0.046	0.0588	8187.976	64.057	4.60E-16	6
2	lqp	1.5	1.03883	0	0.0392	8178.397	54.478	6.22E-14	7
2	l	2	1.01948	0.12	0.0392	8201.284	77.364	7.63E-19	4
2	q	2	1.01625	0.216	0.0686	8221.312	97.393	3.99E-23	3
2	p	2	1.0422	0	0.049	8200.88	76.961	1.31E-18	4
2	lq	2	1.03129	0.028	0.0588	8190.312	66.393	3.25E-16	6
2	lp	2	1.03908	0.046	0.0392	8178.434	54.514	1.65E-13	5

Set	Feature classes	Reg. Multi-plier	Mean AUC	partial ROC (*p-value*)	Omission rate at 5%	AICc	ΔAIC	W AIC	Number of parameters
2	qp	2	1.03127	0.066	0.0588	8191.086	67.167	4.49E-16	6
2	lqp	2	1.03691	0	0.0392	8181.776	57.857	9.80E-14	7
3	l	0.5	1.01397	0.256	0.0392	8228.42	104.5	2.51E-25	3
3	q	0.5	1.01875	0.224	0.049	8235.966	112.05	5.99E-27	3
3	p	0.5	1.02073	0.17	0.049	8222.981	99.062	4.11E-24	2
3	lq	0.5	1.06046	0	0.0098	8209.488	85.569	3.59E-21	6
3	lp	0.5	1.03185	0.074	0.0784	8224.62	100.7	1.94E-24	6
3	qp	0.5	1.02656	0.15	0.049	8223.58	99.66	3.41E-24	6
3	lqp	0.5	1.06261	0	0.0294	8209.82	85.901	3.44E-21	7
3	l	1	1.01509	0.266	0.0392	8228.761	104.84	2.78E-25	3
3	q	1	1.01959	0.234	0.049	8236.233	112.31	6.98E-27	3
3	p	1	1.0178	0.178	0.049	8223.426	99.507	4.44E-24	2
3	lq	1	1.03994	0.054	0.0294	8214.974	91.055	3.20E-22	6
3	lp	1	1.02249	0.178	0.049	8228.557	104.64	3.82E-25	6
3	qp	1	1.02076	0.202	0.049	8222.575	98.655	8.08E-24	4
3	lqp	1	1.04383	0.056	0.0294	8213.28	89.36	9.00E-22	6
3	l	1.5	1.0156	0.25	0.0392	8229.179	105.26	3.41E-25	3
3	q	1.5	1.02023	0.244	0.049	8232.508	108.59	6.97E-26	1
3	p	1.5	1.02007	0.158	0.049	8223.961	100.04	5.43E-24	2
3	lq	1.5	1.03064	0.158	0.0392	8220.714	96.794	3.01E-23	5
3	lp	1.5	1.01791	0.208	0.0392	8227.187	103.27	1.31E-24	4
3	qp	1.5	1.01631	0.244	0.0392	8226.366	102.45	2.20E-24	5
3	lqp	1.5	1.03113	0.13	0.0294	8219.523	95.604	7.63E-23	6
3	l	2	1.01469	0.288	0.0392	8227.639	103.72	1.52E-24	2
3	q	2	1.01699	0.256	0.049	8232.753	108.83	1.39E-25	1
3	p	2	1.01806	0.214	0.0392	8224.584	100.66	1.00E-23	2
3	lq	2	1.02062	0.182	0.0392	8223.553	99.634	2.15E-23	4
3	lp	2	1.01956	0.194	0.0392	8226.064	102.14	8.48E-24	3
3	qp	2	1.0166	0.192	0.049	8226.639	102.72	1.03E-23	4
3	lqp	2	1.02109	0.184	0.0392	8223.047	99.128	1.67E-22	5

SI CHAPTER 4

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BROAD DISTRIBUTION DOES NOT MEAN BROAD DIFFERENTIATION

Analysis of genetic diversity and geographic centrality in the declining grasshopper species *Bryodemella tuberculata* (Fabricius, 1775) (Orthoptera: Oedipodinae)

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SI 1: Number of distribution data obtained from field trips of the authors to Germany, Russia, Mongolia, China and Kazakhstan and Museum collections.

No. of records	Reference
21	Fieldtrips of LSD to Mongolia from 2015 to 2019
20	Alexander Koenig Research Museum Bonn, Germany (ZFMK)
31	British Museum of Natural History London, England (BMNH)
21	Center for Natural History Hamburg, Germany (ZMH)
3	Fieldtrip of MH to Russia in 2006
1	Fieldtrip of SQX to China in 2008
66	Fieldtrips of MS to China, Kazakhstan & Russia from 1978 to 2019
2	Fieldtrips of OH to Germany (Bavaria) in 2019
3	Finnish Museum of Natural History Luomus; Helsinki, Finland (MZH)
5	National Museum of Natural History Naturalis, Leiden, Netherlands (NMNL)
1	Natural History Museum of Central Finland, Jyväskylä, Finland (JYU)
23	Natural History Museum of Geneva, Switzerland (MHNG)
5	Natural History Museum Vienna, Austria (NHM)
15	Novosibirsk State University (NSTU)
13	Siberian Zoological Museum (SZMN)
4	State Museum of Natural History Stuttgart, Germany (SMNS)

24	Swedish Museum of Natural History Stockholm, Sweden (NRM)
5	University of Tartu, Estonia (UT)
33	Zentralmagazin Naturwissenschaftlicher Sammlungen Halle (Saale), Germany (ZNS)
2	Zoological Institute, Russian Academy of Science, St. Petersburg, Russia (ZIN)
8	Zoologische Staatssammlung, Munich, Germany (ZSM)

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SI 2.2: Distribution records obtained from literature.

Reference	country	location	collection date
1	Russia	Rep. Tatarstan, Akhmetievo	-
2	Germany	Frankfurt Oder	-
2	Germany	Porstluch	-
2	Germany	Wünstdorf	-
2	Germany	Kutzdorf in der Neumark	-
2	Germany	Hannover	-
2	Germany	Wilsede	-
2	Germany	Buchwald	-
2	Germany	Bevensen	-
2	Germany	Maschen	-
2	Germany	Lüneburg	-
2	Germany	Riestedt	-
2	Germany	Hambergen	-
2	Germany	Wilderhausen	-
2	Germany	Vesbeck	-
2	Germany	Oldenbüttel	-
2	Germany	Spreddig	-
2	Germany	Heisenbüttel	-
2	Germany	Myle	-
2	Germany	Feißenbüttel	-
2	Germany	Garlstedt	-
2	Germany	Elm	-
2	Germany	Ohlenstedt	-
2	Germany	Hülseberg	-
2	Germany	Hagen bei Stade	-
2	Germany	Harsfeld	-
2	Germany	Buxtehude	-
2	Estonia	Varska	-
2	Latvia	Garciems	-
2	Latvia	Sauka (Ges. Runceni)	-
2	Latvia	Lazdogas ezers	-
2	Latvia	Ropazi	-
3	Swiss	Swiss, Resgia	-
3	Swiss	Swiss, Ardez	-
3	Swizz	Swizz, Martina	-
3	Swizz	Urgen	-
3	Swizz	Ried	-
3	Swizz	Pfunds	-

Reference	country	location	collection date
3	Swizz	Weißenbach am Lech	-
3	Swizz	Alpenpark Karwendel	-
3	Swizz	Tiroler Isar im Hinterauta	-
3	Swizz	Stanzach	-
3	Swizz	Torsäulenbach Plansee	-
3	Swizz	Forchach am Lech	-
4	Poland	Olkusz	1914
4	Poland	Toruń	-
4	Poland	Kielce/Dyminy	-
4	Poland	Stara Miłosna	1917
4	Poland	Głogów na Śląsku	-
4	Poland	Głogów (okolice)	-
4	Poland	Skwierzyna n Wartą	-
4	Poland	Wieleń	-
4	Poland	Wrzeszczyna, Cz	-
4	Poland	Biała, Cz	-
4	Poland	Gulcz, Cz	-
4	Poland	Czarnków	-
4	Poland	Elbląga	-
4	Poland	Lipiny koło Augustowa	-
4	Poland	Augustowa	-
4	Poland	Nizina Wielko-polsko-Kujawska	-
4	Poland	Nizina Mazowiecka: okolice Warszawy	-
4	Poland	Śląsk Dolny: okolice Legnicy	-
4	Poland	Śląsk Górny: Przyborów kołoKoźla	-
4	Poland	Wyżyna Krakowsko-Wieluńska: Olkusz (	-
4	Poland	Wyżyna Małopolska: Końskie	-
4	Poland	Góry Świętokrzyskie	-
4	Poland	Beskid Wschodni: Krosno	-
9	Russia	Nolinsk, Viatka province	27.07.1899
9	Russia	Korsinsk district, Simbirsk province	10.07.1864
9	Ukraine	Jareski, Mirgorod district, Poltava province	26.07.1924
9	Russia	Sarepta, on the river Volga	-
9	Russia	the river Irgizla, Orenburg province	July 1899
9	Kazakhstan	Kazakhstan, Ak-Bulak, Turgai province	-
9	Kazakhstan	Arganaty Mountains, Kushanai province	16.07.1900
9	Kazakhstan	Nura River, S Nursultan (=Astana. Akmolinsk)	16.07.1900
9	Russia	Altai mountains, Ongudai	02.07.1898
9	Russia	upper Uimon	-
9	Russia	between Katanda and upper Uimon	10-18.08.1897
9		river Tumuliuk, Tshulyshman Mountains	21-22.07.1912

Reference	country	location	collection date
9	Kazakhstan	Tshernovaia on the river Bukhtarma	7.8.1897
9		river Topolevka, basin of the river Argut	22.8.1897
9	Kazakhstan	valley of the river Bukhtarma near the village Artshaty	24.08.1926
9	Russia	Belebei district. Ufa province	-
9	Kazakhstan	village Borovoi, Kustanai province	September 1898
9	Russia	Ak-Bulag, Turgai province	-
9	Kazakhstan	river Nura, South from Akmolinsk, Akmolinsk province	16.07.1900
9	Kazakhstan	Akmolinsk	25.07.1925
9	Russia	Altai mountains, Ongudai	June to July 1898
9	Russia	between Katanda and upper Uimon	10-18.8.1897
9		river Tshulyshman	16-18.8.1901
9	Russia	village Inia	24.08.1925
9	China	Gyangtse, 3962m	June 1904
9	China	Lhassa	August-September 1904
9	China	Shekar, 4419m	09.08.1924
9	China	Mt. Everest Expedition up to 5638m	July 1921
9	China	Peking	1908
9	China	Peking	22.05.1907
9	China	Kingiang-fan, Eastern Gan-su province	-
9	China	Pung-tung, Kang-Wöndo	1884
9	China	Guntshulin, South from Kuantshen	Aug 05
9	Mongolia	Urga	05.07.1926
9	Mongolia	near Urga	June 1909
9	Mongolia	Ulandaban, East from Urga	31.07.1897
9	Mongolia	river Tshikoi, near Russian boarder	July 1925
9	Mongolia	Noin-bogdo Mountains, Orok-nuur, Central Gobi	26.09-10.10.1926
9	Mongolia	middle course of the river Tuin-gol	28.07.1926
9	Mongolia	river Dzurche, Tzagangol system	28.07.1898
9	Mongolia	Nikolskoye, Urianchai country	July 1916
9	Russia	South-Ussuri region	-
9	Russia	Katshikatsky nasleg, Jakutsk province	12.07.1925
9	Russia	Jakutsk	15.07.1925
9	Russia	Sergeliak near Jarkutsk	15.07.1925
9	Russia	five versts above Tshekurskaya, East from Olekminsk, jakutsk province	04.09.1925
9	Russia	st. Solianka, Olekminsk district, Jakutsk province	10.07.1902

Reference	country	location	collection date
9	Russia	Hatyngytördö on the river Amga, Jakutsk province	07.08.1925
9	Russia	st. Bertiach, between Jakutsk and Amiginskoye	19.08.1925
9	Russia	Amiginskoye, Jakutsk province	10.08.1925
9	Russia	Urulga railway station	-
9	Russia	lake Keedy, near river Amga, Jakutsk province	15.07.1925
9	Russia	river Amga, Jasemkonsky nasleg	18-20.7.1902
9	Russia	river Ya, basin of the river Zeya, Transbaikalia railway	june to july 1910
9	Russia	river Tytun, system of the river Onon	14.7.1894
9	Russia	river Borcha, system of the river Onon, Transbaikalia	16.7.1894
9	Russia	Tshita, Transbaikalia	June to July 1866
9	Russia	isle Olhon, Baikal lake	15.07.1915
9	Russia	North Baikal	-
9	Russia	Irkutsk, Irkutsk province	1903
9	Russia	valley of the river Irkut, Irkutsk province	30.06.1916
9	Russia	village Osnatshennaia, Minusinsk country, Yenisei province	
9	Russia	Minusinsk, Yemisei province	-
9	Russia	upper Uimon	14-16.7.1897
9	Russia	Ust-Bashkaus, South from Teletzkoye lake	16-18.7.1912
9	Russia	Ust-Kann, 1500m	13.07.1920
9	Kazakhstan	valley of the river Bukhtarma	14.7.1899
9	Kazakhstan	village Berezovka	11.8.1897
9	Kazakhstan	Katon Karagai	01.07.1906
9	Russia	Kuraiskaya steppe	22.07.1925
9	Russia	lake Tashany, Barabinskaya steppe	2.7.1868
9	Russia	Omsk	July to August 1925-1927
9	Russia	village Bolsheretshinskoye between Omsk and Tara	Aug 26
9	Russia	village Parkovskoye, Tulkalinsk district	July 1903
9	Russia	Ishim	11.07.1905
9	Kazakhstan	Javlenka near Petropavlovsk	07-08 1926
9	Kazakhstan	Mountain Boiartshikha, Ust-Kamenogorsk district, Semipalatinsk province	July 1924
9	Kazakhstan	river Sekisovka, Ust-Kamenogorsk district, Semipalatinsk province	Aug 24
9	Kazakhstan	Arefievskij khutor, Semipalatinsk district	1921
9	Kazakhstan	Borovoje, Koktshetav Mountains, Akmolinsk province	06.08.1926

Reference	country	location	collection date
9	Kazakhstan	Javlenka, Petropavlovsk district, Akmolinsk province	Aug 26
9	Germany	Berchtesgarden, Eisgraben	Aug 25
11	Lithuania	Pabradė military training area	03.08.2008
13	Mongolia	33km SE Chalch-gol	19.07.1975
13	Mongolia	Chigai, Numragi-Gol	23.07.1975
15	Russia	Onon near Nischnevo Tschasutschea do Mosta	01.07.1996
15	Russia	SE Onon near Nischnevo Tschasutschea	24.08.1995
19	Poland	50.357°N 19.548°E	-
19	Poland	50.969°N 20.64°E	-
19	Poland	49.821°N 21.74°E	-
19	Poland	52.025°N 21.146°E	-
19	Poland	52.031°N 21.126°E	-
19	Poland	53.842°N 22.674°E	-
19	Poland	52.663°N 15.609°E	-
19	Poland	52.97°N 16.605°E	-
19	Poland	51.253°N 16.001°E	-
19	Poland	50.454°N 18.21°E	-
22	Korea	Korea, Gensan	04.06.1900
22	Korea	Ponchje to Huadin	26.06.1900
22	Korea	Selminchakory to Sonu	27.06.1900
22	Korea	Palmuk to Singes	30.06.1900
22	Korea	Choanso	03.07.1900
22	Korea	Kosön to Mengne	09.07.1900
22	Korea	Kosön	12.07.1900
22	Korea	Naxans	15.07.1900
22	Korea	Monasterium Olchons	20.07.1900
22	Korea	Olchons to Chovun	21.07.1900
22	Korea	Shuokara to Nodjama	29.07.1900
23	Korea	the river Karim 10km NEE from Bochonbo 1100m	27.07.1975
23	Korea	Unsan about 65 km NE from Pyongyang along the river Tedong	12.06.1970
23	Korea	Mt. Daesongsan 10 km NE of Pyongyang	11.09.1979
23	Korea	Yanggu	04.06.1967
23	Korea	Mt. Odaesan	27.07.1958
23	Korea	Mt. Chiaksan, Wonseong	30.07.1975
23	Korea	Yuchon Doam, Pyeongchang	02.07.1985
23	Korea	Ganpyeongri, Jinbu Pyeongchang	29.06.1985
23	Korea	Dongsan-ri Jinbu Pyeongchang	02.07.1985
23	Korea	Gangchon Chuncheon	23.06.1973

Reference	country	location	collection date
23	Korea	Mt. Bagyonsan near Sanchonri in the San river valley about 22 km from Kaesong	07.06.1970
23	Korea	Mt. Godongsan Gapyeong	23.08.1977
23	Korea	Gwangneung	07.06.1968
23	Korea	Gwangju	17.07.1972
23	Korea	Maseok Namyangju	11.06.1966
23	Korea	Paldang Namyangju	03.10.1963
23	Korea	Mt. Cheonmasan	23-25.7.1961
23	Korea	Sangsangok-dong Hanam	10.06.1977
23	Korea	Gupabal Seoul	11.06.1961
23	Korea	Jeongneung Seoul	05.06.1959
23	Korea	Silim Jecheon	11.07.1988
23	Korea	Songmyeonri Cheongcheon-myeon Goesan	23.06.1989
23	Korea	Mt. Songnisan	06.06.1970
23	Korea	Mungyeongsaejae	11.07.1977
23	Korea	Mt. Bohyeonsan Yeongcheon	19.07.1968
23	Korea	Uljin	27-30.06.1972
23	Korea	Temple Unmunsa Mt. Unmunsan Sinwon-ri Unmun-myeon Cheongdo	18.06.2001
23	Korea	Jeonju	12.06.1983
23	Korea	Mt. Moaksan Jeonju	30.07.1984
24	Korea	River Karim 10 km NEE from Bochonbo 1100 m	27.07.1975
24	Korea	Unsan about 65 km NE from Pyongyang along the River Tedong	12.06.1970
24	Korea	Pyongyang city Mt. Daesongsan 10 km NE of Pyongyang	11.09.1979
24	Korea	Bagyonsan near Sanchonri in the San river valley about 22 km from Kaesong	07.06.1970
24	Korea	Bagyonsan near Sanchontong about 22 km SE from Kaesong	08.06.1970
24	Korea	Bagyonsan near Sanchontong about 10 km from Kaesong	08.06.1970
26	Austria	Plansee bei Reute	-
26	Austria	Hindelang, bayrisches Allgäu	-
27	Italy	Valtellina (Lombardia)	1889
27	Italy	Passo di Teglia (Liguria)	1981
28	Mongolia	Steppe vlidizi r. Terildscha at Nalaichi	25.06.1967
28	Mongolia	Kamenistaja steppe, SE Ulaan Bator	01.07.1967
28	Mongolia	Steppe Gurvan-Saichan, 40km S Somon Bulgan	28-19-7-1967
28	Mongolia	Steppe Arz-Bogd, 20km S Chovd	13.08.1967
29	Korea	Korea, Polgong San, 4000 ft	27.07.1968
31	Poland	Poland, Elblag	-

Reference	country	location	collection date
31	Poland	Poland, Torun	-
31	Rumenia	Rumenia, Cimpulung Moldovenesc	-
32	India	India, Himachal Pradesh, Himalaya	-
35	Korea	Khanka Lake	-
36	Russia	N of Volgograd Area, Kamyshin Region, env. Shcherbakovka Village	13.07.2005
36	Russia	Buryatia, the valley of Irkut River 3-4km W of Mondy village (80km W of Kyren)	29-30.6.2007
36	Russia	Buryatia, Selenga River valley 5km N of Novoselenginsk Town	10.07.2007
36	Russia	Buryatia, Barguzin Valley, Ina River in the env. Of Ina Village (50km NE of Barguzin town)	19.07.2007
36	Russia	SE of Chita area, Klichkinskiy Mtn. Ridge at the crossing with Urulyunguy river (15km W of Klichka town)	23.07.2003
38	China	China, Lasa (Lhasa), Xizang (Tibet), 4000m	30.06.2002
39	Germany	Schotter am Ufer des Eisgrabens bei der St. Johann- und Paul-Kapelle	08.08.1917

SI 3: Full coordinate dataset used for modelling.

status	longitude	latitude
extant	10.983	47.450
extant	11.083	47.500
extant	124.003	47.353
extant	11.250	47.433
extant	11.467	47.467
extant	11.283	47.516
extant	10.833	47.483
extant	10.817	47.478
extant	11.497	47.889
extant	11.541	47.578
extant	16.558	56.466
extant	10.323	47.389
extant	48.106	52.501
extant	11.111	47.418
extant	10.270	47.415
extant	106.494	47.924
extant	107.007	47.815
extant	102.807	47.878
extant	91.816	49.927
extant	98.477	48.725
extant	91.633	47.975
extant	98.270	48.771
extant	101.621	47.471
extant	47.939	47.939
extant	105.812	47.693
extant	108.824	47.781
extant	113.508	49.385
extant	111.708	48.383
extant	94.790	49.212
extant	96.822	48.804
extant	10.336	47.389
extant	11.452	47.912
extant	78.250	53.667
extant	79.317	53.983
extant	82.183	51.333
extant	78.550	52.850
extant	82.133	52.633
extant	82.317	51.450
extant	90.583	54.300
extant	89.900	54.933
extant	80.833	52.233
extant	80.967	52.867

status	longitude	latitude
extant	79.050	52.567
extant	75.583	50.717
extant	72.883	51.800
extant	72.783	52.167
extant	76.233	53.033
extant	86.500	50.500
extant	80.050	52.683
extant	116.300	51.000
extant	116.600	51.900
extant	127.683	50.433
extant	86.167	51.083
extant	59.717	54.650
extant	90.200	30.500
extant	90.200	51.500
extant	90.083	51.617
extant	90.467	51.400
extant	90.450	51.400
extant	91.067	51.417
extant	90.200	51.517
extant	94.300	51.983
extant	95.433	51.733
extant	92.383	51.567
extant	92.750	51.450
extant	92.733	51.467
extant	92.750	51.467
extant	94.167	51.483
extant	94.167	51.567
extant	94.067	51.367
extant	95.350	51.533
extant	94.533	51.133
extant	95.267	50.950
extant	95.283	50.783
extant	92.619	51.407
extant	94.567	50.750
extant	95.317	50.683
extant	95.333	50.700
extant	95.233	50.617
extant	95.183	50.617
extant	95.183	50.633
extant	94.550	50.717
extant	94.517	50.533
extant	94.517	50.550
extant	95.317	50.633
extant	95.200	50.183

status	longitude	latitude
extant	94.750	50.233
extant	95.350	50.083
extant	93.217	52.350
extant	16.645	56.787
extant	10.402	47.481
extant	33.917	49.845
extant	16.427	56.460
extant	16.559	56.467
extant	16.517	56.533
extant	44.509	48.520
extant	100.413	36.441
extant	16.461	56.576
extant	16.559	56.590
extant	18.414	57.463
extant	83.733	53.383
extant	34.581	49.588
extant	11.436	47.561
extant	121.033	49.100
extant	29.897	58.857
extant	29.859	58.743
extant	36.176	54.592
extant	49.950	57.559
extant	46.981	54.199
extant	33.909	49.836
extant	44.520	48.512
extant	57.090	52.919
extant	55.619	51.006
extant	67.081	49.219
extant	71.328	50.931
extant	86.132	50.755
extant	85.810	50.269
extant	86.005	50.206
extant	85.879	49.225
extant	86.562	49.290
extant	54.153	54.118
extant	64.184	53.785
extant	71.454	51.086
extant	86.656	50.452
extant	89.604	28.917
extant	91.172	29.652
extant	87.124	28.662
extant	86.948	28.316
extant	116.383	39.917
extant	124.809	43.504

status	longitude	latitude
extant	106.917	47.920
extant	100.735	45.119
extant	128.944	61.320
extant	129.744	62.021
extant	120.724	60.475
extant	131.981	60.597
extant	114.796	51.770
extant	113.465	52.024
extant	107.363	53.188
extant	104.278	52.303
extant	91.646	53.712
extant	87.766	51.183
extant	84.752	50.933
extant	85.601	49.177
extant	73.364	54.873
extant	74.616	56.105
extant	69.473	56.102
extant	68.459	54.344
extant	82.495	50.350
extant	82.588	50.328
extant	70.237	53.077
extant	118.702	41.018
extant	126.027	39.390
extant	127.981	38.108
extant	128.543	37.798
extant	128.051	37.382
extant	128.593	37.673
extant	128.564	37.771
extant	127.708	37.814
extant	127.166	37.755
extant	127.311	37.653
extant	127.244	37.548
extant	127.273	37.681
extant	127.232	37.492
extant	126.918	37.637
extant	127.000	37.615
extant	127.811	36.628
extant	127.900	36.534
extant	128.062	36.811
extant	128.974	36.162
extant	129.360	36.888
extant	128.960	35.661
extant	127.108	35.828
extant	127.084	35.730

status	longitude	latitude
extant	126.019	39.390
extant	10.365	47.503
extant	128.694	36.017
extant	45.761	50.512
extant	100.959	51.681
extant	106.670	51.130
extant	109.774	53.647
extant	117.881	50.423
extant	91.100	28.800
extant	115.121	50.527
extant	103.617	43.701
extant	91.655	47.792
extant	134.597	43.321
extant	88.605	50.751
extant	84.194	51.686
extant	29.830	60.196
extant	30.517	60.567
extant	22.519	61.019
extant	30.213	60.698
extant	129.302	50.211
extant	131.453	43.968
extant	133.246	43.168
extant	87.943	50.263
extant	87.947	50.264
extant	87.953	50.205
extant	85.656	51.331
extant	84.847	48.613
extant	75.821	53.801
extant	78.281	53.345
extant	120.433	60.383
extant	120.724	60.471
extant	138.046	61.133
extant	143.213	63.265
extant	133.453	67.581
extant	129.428	61.674
extant	130.714	62.192
extant	128.766	61.265
extant	129.732	62.027
extant	129.818	62.281
extant	128.109	61.270
extant	129.713	62.629
extant	126.836	61.131
extant	131.981	60.897
extant	132.681	61.214

status	longitude	latitude
extant	119.603	60.254
extant	119.673	60.258
extant	119.885	60.265
extant	120.301	60.398
extant	52.162	55.462
extant	152.469	62.723
extant	113.589	53.983
extant	105.115	51.554
extant	89.698	55.081
extant	86.632	50.449
extant	87.453	50.341
extant	111.975	47.578
extant	119.900	51.353
extant	116.893	50.040
extant	113.500	52.033
extant	115.183	52.683
extant	115.124	50.508
extant	119.080	50.360
extant	117.156	51.611
extant	61.567	54.083
extant	60.834	56.069
extant	65.341	55.441
extant	69.500	56.117
extant	69.392	53.292
extant	80.407	53.855
extant	86.192	54.343
extant	88.358	54.354
extant	25.612	47.517
extant	10.558	47.385
extant	10.840	47.496
extant	10.596	47.430
extant	25.768	54.981
extant	11.000	47.600
extant	11.300	47.500
extant	11.500	47.500
extant	11.470	47.561
extant	11.392	47.544
extant	11.158	47.496
extant	10.580	47.408
extant	11.328	47.377
extant	11.279	47.382
extant	11.506	47.280
extant	11.361	47.374
extant	11.498	47.463

status	longitude	latitude
extant	11.490	47.463
extant	10.644	47.440
extant	10.921	47.550
extant	11.567	47.407
extant	10.505	47.372
extant	10.472	47.405
extant	11.538	47.438
extant	11.438	47.505
extant	11.505	47.455
extant	11.488	47.455
extant	11.090	47.498
extant	10.572	47.422
extant	10.855	47.472
extant	11.472	47.472
extant	10.805	47.472
extant	10.638	47.438
extant	10.830	47.480
extant	10.641	47.439
extinct	10.258	46.777
extinct	10.305	46.794
extinct	10.385	46.828
extinct	10.487	46.890
extinct	10.608	47.018
extinct	9.842	53.458
extinct	10.036	53.532
extinct	9.824	53.465
extinct	9.961	53.165
extinct	11.457	53.383
extinct	9.845	46.163
extinct	7.848	44.000
extinct	8.827	53.287
extinct	10.131	53.408
extinct	8.209	50.016
extinct	10.189	46.771
extinct	10.603	47.119
extinct	13.485	48.222
extinct	10.542	46.970
extinct	9.903	53.473
extinct	9.897	53.452
extinct	10.000	53.550
extinct	13.485	52.163
extinct	19.394	54.166
extinct	18.580	53.005
extinct	16.036	51.673

status	longitude	latitude
extinct	19.548	50.357
extinct	20.640	50.969
extinct	21.740	49.821
extinct	21.146	52.025
extinct	21.126	52.031
extinct	22.674	53.842
extinct	15.609	52.663
extinct	16.605	52.970
extinct	16.001	51.253
extinct	18.210	50.454
extinct	10.655	47.188
extinct	10.655	47.038
extinct	10.572	46.988
extinct	10.605	47.122
extinct	10.738	47.238
extinct	10.740	47.240
extinct	9.561	47.426
extinct	7.400	52.300
extinct	14.560	52.338
extinct	13.461	52.162
extinct	9.716	52.371
extinct	9.964	53.168
extinct	10.584	53.077
extinct	10.034	53.374
extinct	10.480	53.244
extinct	11.363	51.496
extinct	8.809	53.303
extinct	8.441	52.894
extinct	9.608	52.607
extinct	9.446	54.171
extinct	8.849	53.303
extinct	8.790	53.262
extinct	8.695	53.281
extinct	8.752	53.304
extinct	8.760	53.275
extinct	9.462	53.549
extinct	9.506	53.451
extinct	9.700	53.472
extinct	27.628	57.962
extinct	24.195	57.104
extinct	25.483	56.274
extinct	26.966	57.052
extinct	24.629	56.977
extinct	8.756	55.648

status	longitude	latitude
extinct	9.271	56.966
extinct	9.269	56.803
extinct	9.410	56.923
extinct	9.565	56.164
extinct	9.336	57.037
extinct	8.745	55.707
extinct	9.400	56.880
extinct	10.011	57.133
extinct	10.191	56.891
extinct	8.923	56.494
extinct	25.421	56.281
extinct	19.550	50.279
extinct	18.626	53.006
extinct	20.642	50.820
extinct	21.230	52.211
extinct	16.049	51.674
extinct	15.505	52.604
extinct	16.173	52.896
extinct	16.237	52.881
extinct	16.308	52.830
extinct	16.371	52.879
extinct	16.553	52.903
extinct	19.374	54.191
extinct	23.511	53.877
extinct	22.970	53.853
extinct	16.174	51.218
extinct	19.548	50.273
extinct	20.414	51.183
extinct	12.958	47.546
extinct	21.756	49.701

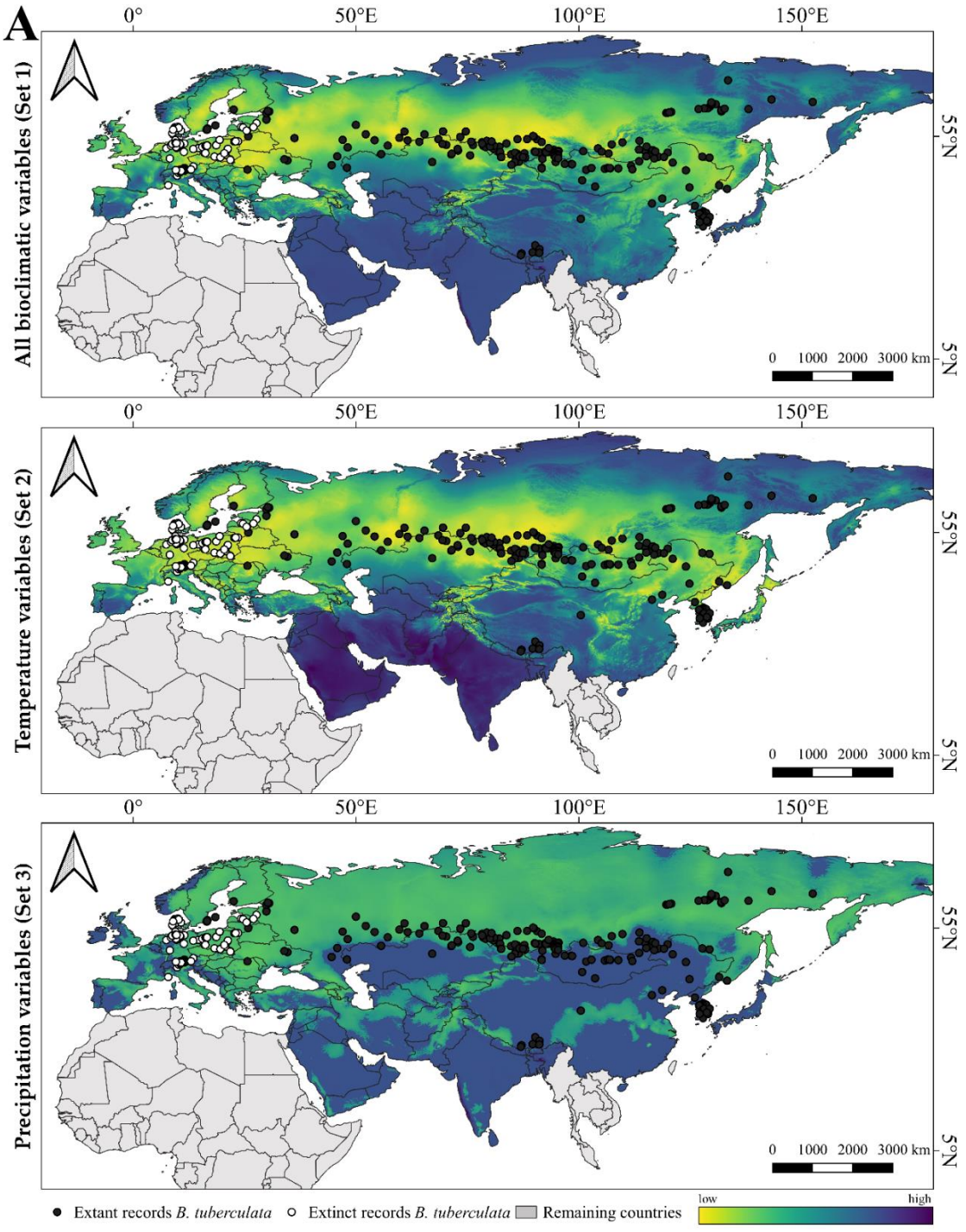
SI 4: PCR cycle conditions for the gene fragments amplified in this study.

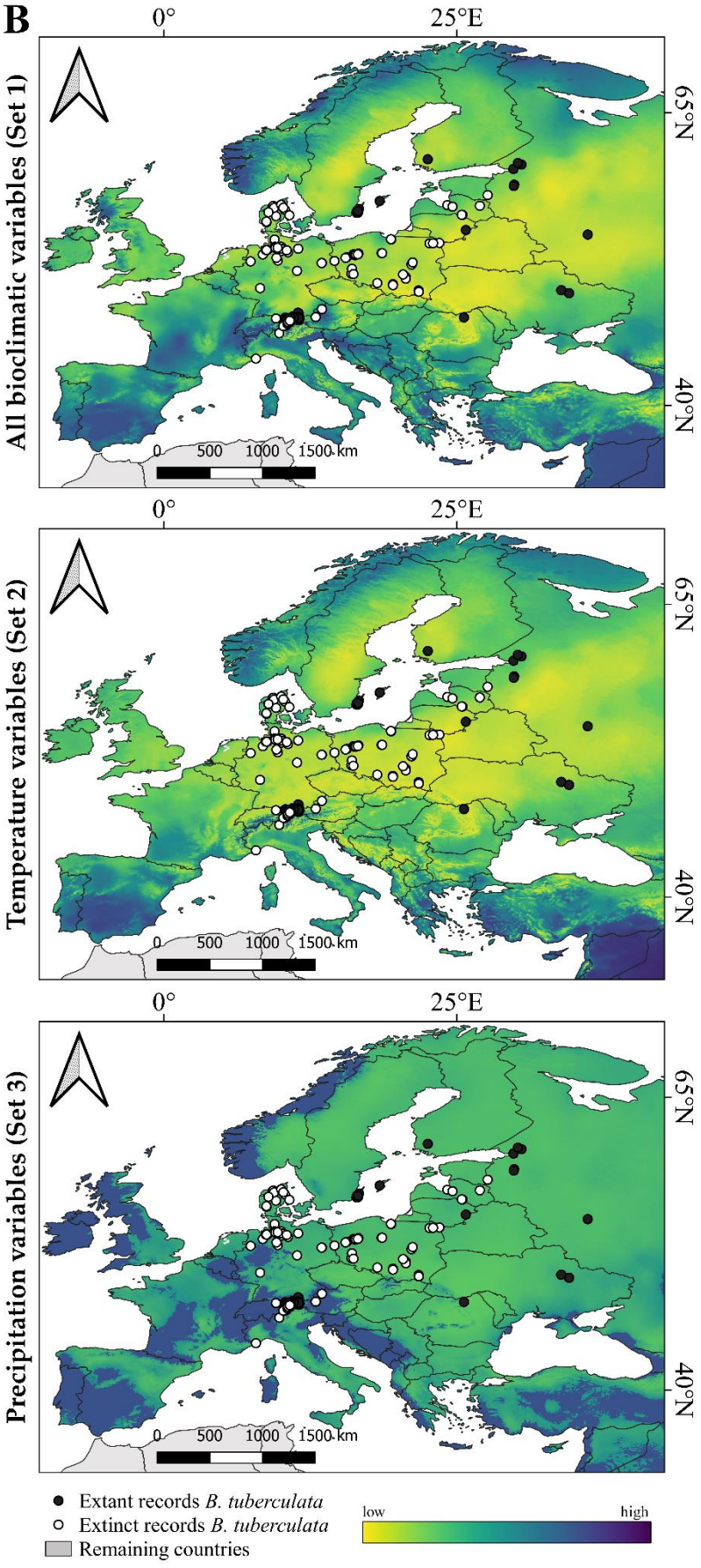
	COI		Times
Initial Denaturation	95 °C		10 min
			1 min
Denaturation	95 °C		
			1 min
Annealing	50 °C	35 x	
			1 min
Elongation	72 °C		
Initial Elongation	72 °C		8 min
Break	4 °C		∞

SI 5: Complete report of ellipsoids' characteristics using an extracted covariance matrix (covmat). The table displays the mean, min and max ellipsoid volume of all three sets (*Set 1* - Temperature and precipitation variables; *Set 2* - Temperature variables; *Set 3* - Precipitation variables).

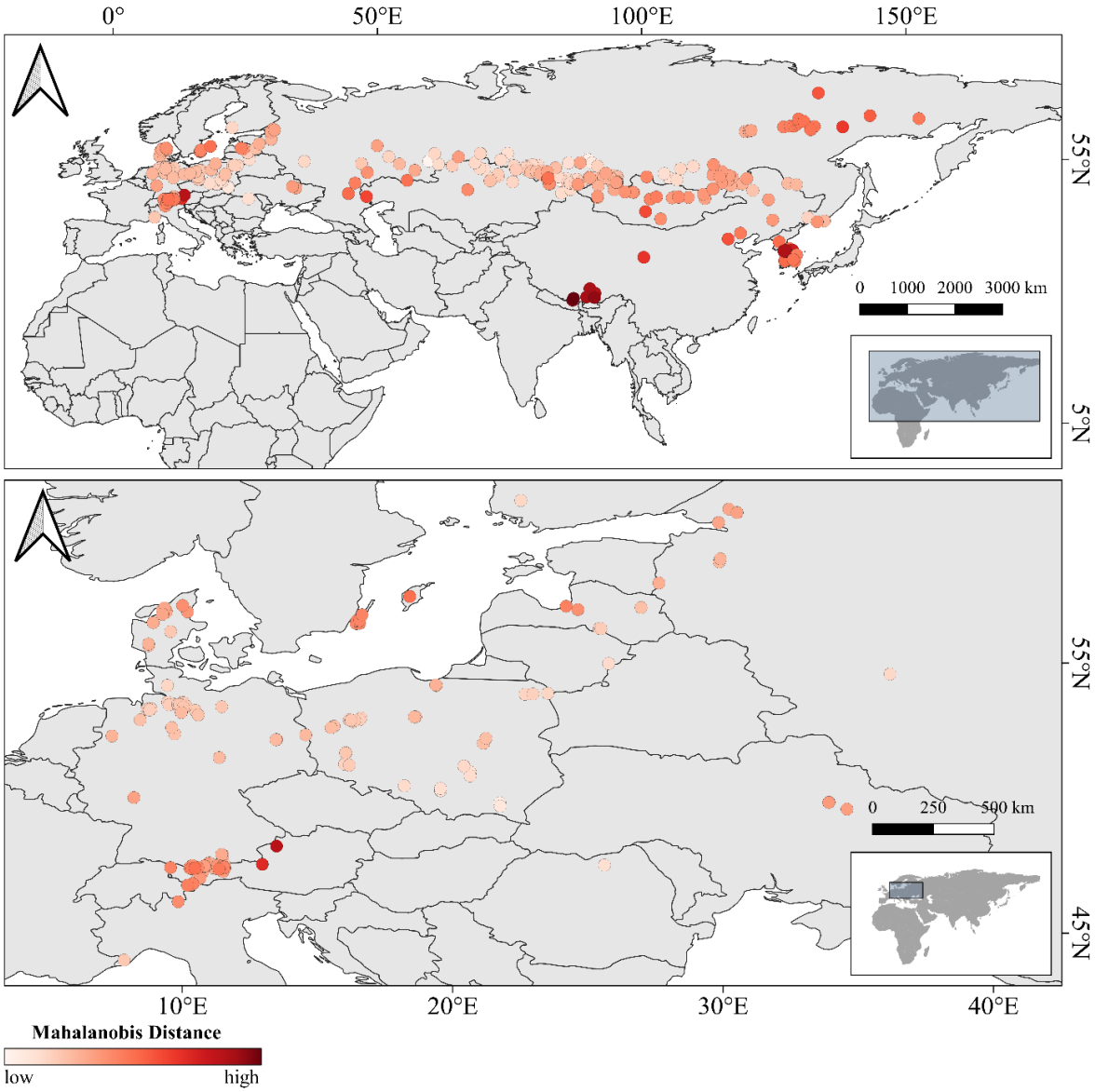
Set 1 - Temperature and precipitation variables			
	mean_ellipsoid	min_ellipsoid	max_ellipsoid
Method	covmat	covmat	covmat
Level	99	99	99
Volume	4173.78	3791	4817.11
Set 2 - Temperature variables			
	mean_ellipsoid	min_ellipsoid	max_ellipsoid
Method	covmat	covmat	covmat
Level	99	99	99
Volume	124.46	115.07	138.05
Set 3 - Precipitation variables			
	mean_ellipsoid	min_ellipsoid	max_ellipsoid
Method	covmat	covmat	covmat
Level	99	99	99
Volume	158.29	142.7	172.16

SI 6: A geographic prediction of Mahalanobis distances (D^2) within Eurasia (A) and Europe and adjacent areas (B). Yellow correspond to lower distances to the centroid, while blue represents higher Mahalanobis distances. All countries not included in the model are shown in grey. Occurrences of extinct populations are shown as white circles, while extant once are represented in black.

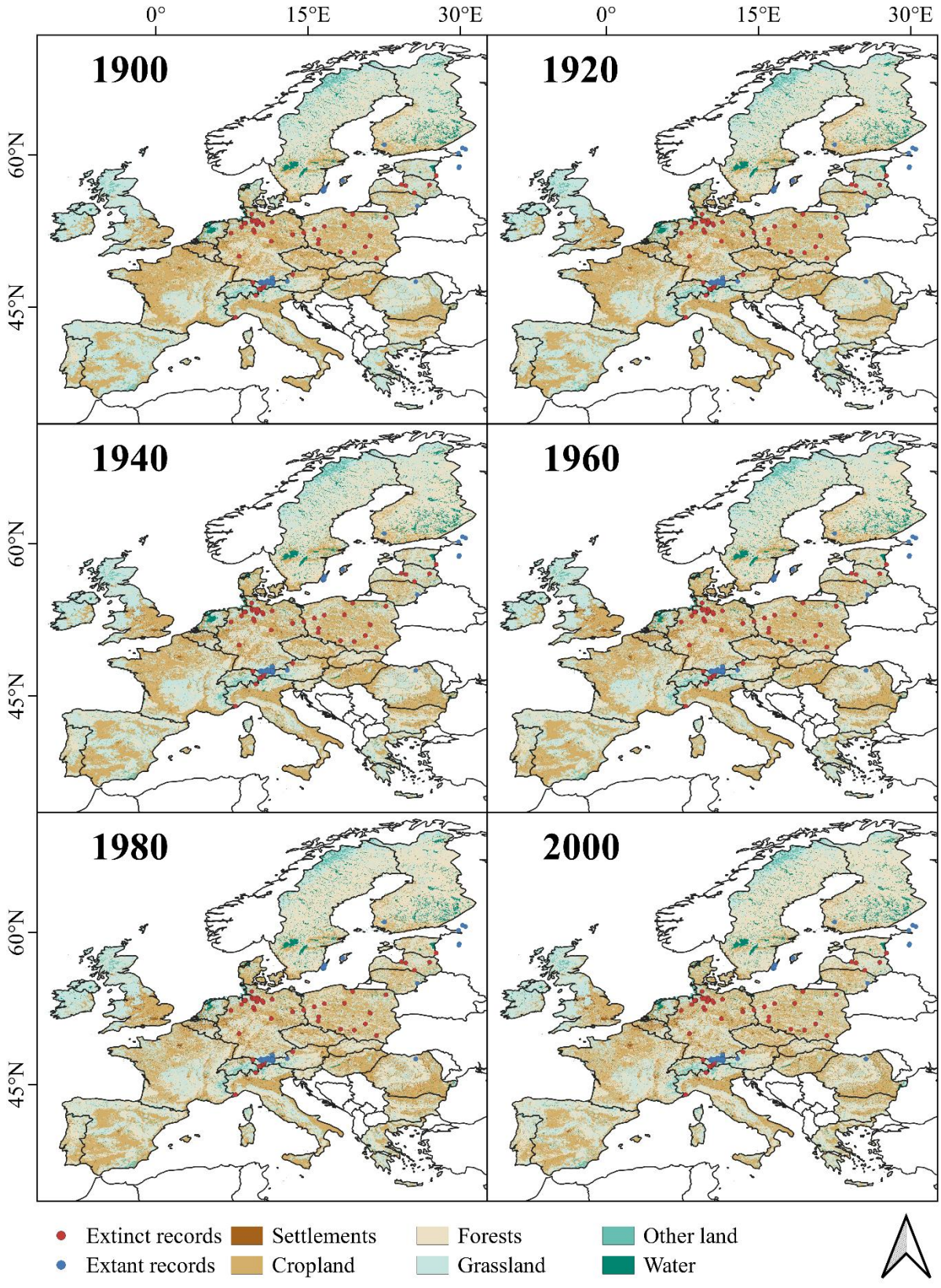



B
All bioclimatic variables (Set 1)
Temperature variables (Set 2)
Precipitation variables (Set 3)
0°
25°E
65°N
40°N
0 500 1000 1500 km
Extant records B. tuberculata
Extinct records B. tuberculata
Remaining countries
low
high

SI 7: Distribution map of *B. tuberculata* showing Mahalanobis distance per occurrence record. Color gradient representing higher Mahalanobis Distance in dark red, while lower values are shown in light red.



SI 8: Landscape change through time based on HILDA dataset from 1900 to 2000 dosplaying every 20 years. Detailed change thorugh time can be found online: http://www.geo-informatie.nl/fuchs003/#.



SI 9: Further information regarding the Russian sampling locations.

Bryodemella tuberculata was and is widely distributed over the semi-arid parts of the Altay-Sayan Mountains, especially across the central parts of the Altay Mts. and Tuva (Tyva Republic) (pers. obs. MGS 2014, 2016–2018; SI 10). It can be found across the stony steppe plots on the southern mountain slopes and the stony flood-plains and terraces above the steppe and semi-desert altitudinal belts, but never above 1,500m. The species could be found in almost all suitable habitats in the Tuva region, but always in low abundances. The observed levels of maximum density are about 0.3-0.5 adults per sqm, as observed during all field trips of MGS from 1977 until 2019 (Sergeev et al., 2020).

The situation of the Kulunda Plain, i.e., the south-eastern part of West Siberian Plain between the Irtysh and Ob Rivers, is very similar to that of the Altay-Sayan Mountains (pers. obs. MGS 2015–2019; SI 10). We observed that *B. tuberculata* avoids meadows with vegetation of more than 30 cm height and without patches of open soil. It also enters some modified ecosystems, such as pastures with moderate livestock grazing and the dry forest belts with sparse grass vegetation The general level of its abundance is relatively low: with several adults per hour captures in typical habitats. The populations of *B. tuberculata* in the Kulunda Plain appear to be scattered: the species could not be recorded at all apparently suitable habitats. No observations were made during an expedition in 2019 (pers. obs. MGS.

A survey of the south-eastern part of the Ural Mountains in June 2019 (SI 8) yielded several adults and larvae of *B. tuberculata* in the dry steppes on the upper terraces and piedmont plains, but none were recorded in the steppes on the southern mountain slopes.

SI 10: Pictures of extant and extinct locations. Extant: Russia (A, B), Mongolia (C, D), Lithuania (E), Sweden (F), Germany – Upper Isar river bench (G, H). Extinct: Germany – Lüneburger Heide heathland area (I, J), Denmark (K, L). Source information: LSD – Lara-Sophie Dey; MGS – Michael G. Sergeev ; OH – Oliver Hawlitschek.

A - Russia, Siberia, Central Kulunda Plain, 53.667°N 78.2545°E
(source: MGS, 2019)

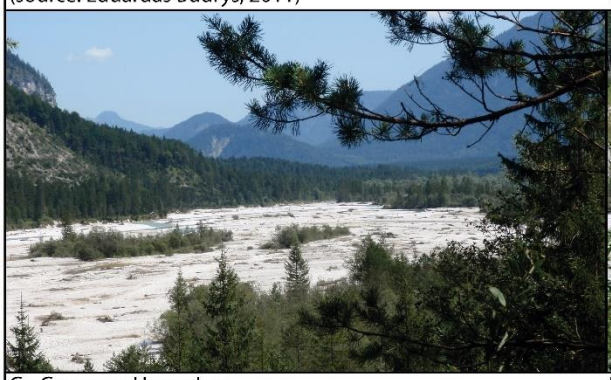
B - Russia, NE Tuva, 51.4133°N 91.0608°E
(source: MGS, 2018)

C - Mongolia, Husteinuuru National Park, 47.9625°N 105.8118°E
(source: LSD, 2019)

D - Mongolia, Batnorov, 48.3828°N 111.7075°E
(source: LSD, 2019)

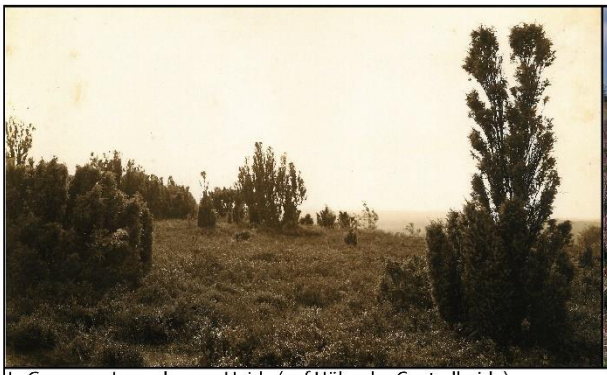
E - Lithuania, Pabradé military training area
(source: Eduardas Budrys, 2011)

F - Sweden, Øland Stora Alvaret
(source: https://www.alltpaoland.se/artiklar/stora-alvaret/)



G - Germany, Upper Isar
(source: OH, 2009)

H - Germany, Upper Isar
(source: I. Baetz, 2020)



I - Germany, Lueneburger Heide (auf Höhe der Centralheide)
(source: Bildarchiv der Alfred Toepfer Stiftung, O. Kofahl, 1906)

J - Germany, Lueneburger Heide, Schneverdingen
(source: Bildarchiv der Alfred Toepfer Stiftung, D. Blume-Winkler, 2013)

K - Denmark, close to Abild Ribe Landevej
(source: LSD, 2019)

L - Denmark, heath close to Henne Strand
(source: LSD, 2019)

SI 11: Last records of now extinct populations in different European countries.

country	location	date	collector	reference
Denmark	Abild Hede (Varde, South Denmark)	1949	-	Naturbasen
Northern Germany	Hamburg, Buchwedel (Lower Saxony)	16.07.1917	F. Borchmann	BNHM London
Western Germany	Mainzer Sand (Rhineland-Palatinate)	23.08.1930	Zeuner	BNHM London
Eastern Germany	Wünsdorf (Brandenburg)		-	NGM Stockholm
Eastern Germany	Frankfurt (Oder) and near Fangschleuse	1911	-	Ramme 1911
Poland	Augustowa	1950s	-	Bazyluk & Liana, 2000
Switzerland	Ramosch	1959	-	Monnerat *et al.*, 2007; Nadig *et al.*, 1986)
Italy	Passo di Teglia in Liguria in the Alps	1981	-	Ruffo, 2003
Estonia	Värska	DD 2008	-	Red Data Book of Estonia, 2008
Latvia	Sauka parish, Rencēnu meadow	1928	-	NHML Riga

SI CHAPTER 5.2

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DOES (GENOME) SIZE MATTER?

New estimates of genome size in Orthoptera and their evolutionary implications

Oliver Hawlitschek, David Sadílek, Lara-Sophie Dey, Katharina Buchholz, Sajad Noori, Inci Livia Baez, Timo Wehrt, Jason Brozio, Pavel Trávníček, Matthias Seidel, Martin Husemann

SI 1: Complete table of all genome size measurements of Orthoptera reviewed for this study.

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Caelifera	Acrididae	Acridinae	*Acrida cinerea*		F	11,24	22+X0*	FCM	BR	LM	Mao et al. 2020	Inoue 1985
Caelifera	Acrididae	Acridinae	*Acrida cinerea*		M	10,64	22+X0*	FCM	BR	LM	Mao et al. 2020	Inoue 1985
Caelifera	Acrididae	Acridinae	*Acrida conica*		M	10,82	22+X0	FD	TS	GD	Rees et al. 1978	King & John 1980
Caelifera	Acrididae	Acridinae	*Acrida conica*		n. a.	12,55	22+X0	FD	HE	GD, OM	Rasch 1985	King & John 1980
Caelifera	Acrididae	Acridinae	*Caledia captiva*		M	10,90	22+X0	FD	TS	GD	Rees et al. 1978	Shaw 1976
Caelifera	Acrididae	Acridinae	*Cryptobothrus chrysophorus*		M	9,37	22+X0	FD	TS	GD	Rees et al. 1978	Sharman 1952
Caelifera	Acrididae	Acridinae	*Schizobothrus flavovittatus*		M	7,50	22+X0	FD	TS	GD	Rees et al. 1978	Rees et al. 1978
Caelifera	Acrididae	Calliptaminae	*Calliptamus abbreviatus*		F	10,03	22+X0*	FCM	BR	LM	Mao et al. 2020	Camacho & Cabrero 1982
Caelifera	Acrididae	Calliptaminae	*Calliptamus abbreviatus*		M	9,64	22+X0*	FCM	BR	LM	Mao et al. 2020	Camacho & Cabrero 1982
Caelifera	Acrididae	Calliptaminae	*Calliptamus barbarus*		F	10,31	22+X0	FCM	BR	LM	Mao et al. 2020	Camacho & Cabrero 1982
Caelifera	Acrididae	Calliptaminae	*Calliptamus barbarus*		M	9,90	22+X0	FCM	BR	LM	Mao et al. 2020	Camacho & Cabrero 1982
Caelifera	Acrididae	Calliptaminae	*Calliptamus italicus*	ZMH862079	F	11,66	22+X0*	FCM	MS	PS	this study	Camacho & Cabrero 1982
Caelifera	Acrididae	Calliptaminae	*Calliptamus italicus*		F	11,69	22+X0*	FCM	MS	PS	this study	Camacho & Cabrero 1982

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Caelifera	Acrididae	Calliptaminae	*Calliptamus italicus*	ZMH862056	M	10,91	22+X0*	FCM	MS	PS	this study	Camacho & Cabrero 1982
Caelifera	Acrididae	Catantopinae	*Macrotona australis*		M	8,49	22+X0	FD	TS	GD	Rees et al. 1978	Rees et al. 1978
Caelifera	Acrididae	Catantopinae	*Peakesia hospita*		M	10,47	22+X0	FD	TS	GD	Rees et al. 1978	King & John 1980
Caelifera	Acrididae	Catantopinae	*Phaulacridium vittatum*		M	10,73	22+X0	FD	TS	GD	Rees et al. 1978	Jackson & Cheung 1967
Caelifera	Acrididae	Cyrtacanthacridinae	*Schistocerca cancellata*		M	9,49	22+X0	FD	TS	LM	John & Hewitt 1966	John & Hewitt 1966
Caelifera	Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	ZMH862103	F	10,68	22+X0	FCM	MS	PS	this study	John & Hewitt 1966
Caelifera	Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*		M	8,74	22+X0	FD	S	n.a.	Camacho et al. 2015	John & Hewitt 1966
Caelifera	Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*		M	8,55	22+X0	FD	TS	LM	John & Hewitt 1966	John & Hewitt 1966
Caelifera	Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	ZMH862102	M	10,36	22+X0	FCM	MS	PS	this study	John & Hewitt 1966
Caelifera	Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*		M	8,71	22+X0	FD	TS	MM	Wilmore & Brown 1975	John & Hewitt 1966
Caelifera	Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*		n.a.	8,96	22+X0	FD	V	MM	Fox 1970	John & Hewitt 1966
Caelifera	Acrididae	Cyrtacanthacridinae	*Schistocerca paranensis*		M	8,63	22+X0	FD	TS	LM	John & Hewitt 1966	John & Hewitt 1966
Caelifera	Acrididae	Cyrtacanthacridinae	*Valanga irregularis*		M	9,44	22+X0	FD	TS	GD	Rees et al. 1978	King & John 1980
Caelifera	Acrididae	Eyprepocnemidinae	*Eyprepocnemis plorans*		M	9,70	22+X0	FD	S	LM	Ruiz-Ruano et al. 2011	Gosálvez et al. 1980
Caelifera	Acrididae	Eyprepocnemidinae	*Heteracris adspersus*		M	6,34	22+X0	FD	TS	AC	Gosálvez et al. 1980	Gosálvez et al. 1980
Caelifera	Acrididae	Eyprepocnemidinae	*Shirakiacris shirakii*		F	7,06	22+X0	FCM	BR	LM	Mao et al. 2020	Inoue 1985

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Caelifera	Acrididae	Eyprepocnemidinae	*Shirakiacris shirakii*		M	6,57	22+X0	FCM	BR	PA	Mao et al. 2020	Inoue 1985
Caelifera	Acrididae	Gomphocerinae	*Aeropedellus variegatus*		M	12,58	22+X0*	FCM	BR	AD	Shah et al. 2020	Hamrick & Hamrick 1989
Caelifera	Acrididae	Gomphocerinae	*Chorthippus albomarginatus*	ZMH862082	F	11,88	16+X0	FCM	MS	PS	this study	Lin et al. 2015
Caelifera	Acrididae	Gomphocerinae	*Chorthippus albomarginatus*	ZMH862002	M	11,79	16+X0	FCM	MS	PS	this study	Lin et al. 2015
Caelifera	Acrididae	Gomphocerinae	*Chorthippus apicalis*		n. a.	12,61	16+X0	FD	TS	GD	Belda et al. 1991	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus apricarius*	ZMH862003	F	12,71	16+X0*	FCM	MS	PS	this study	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus apricarius*	ZMH862041	F	12,32	16+X0*	FCM	MS	PS	this study	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus apricarius*	ZMH862048	M	11,92	16+X0*	FCM	MS	PS	this study	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus biguttulus*	ZMH862009	M	10,99	16+X0	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Chorthippus biguttulus*		M	9,31	16+X0	FCM	BR	AD	Shah et al. 2020	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Chorthippus biguttulus*		F	11,31	16+X0	FCM	MS	PS	Husemann et al. 2021	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Chorthippus binotatus*		n. a.	10,91	16+X0	FD	TS	GD	Belda et al. 1991	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Chorthippus brunneus*		M	10,15	16+X0	FD	TS	AC	Gosálvez et al. 1980	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus brunneus*		M	8,55	16+X0	FD	TS	LM	John & Hewitt 1966	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus brunneus*	ZMH862021	M	10,47	16+X0	FCM	MS	PS	this study	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus brunneus*		M	9,46	16+X0	FD	TS	MM	Wilmore & Brown 1975	Cabrero & Camacho 1986

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Caelifera	Acrididae	Gomphocerinae	*Chorthippus dorsatus*		F	12,07	16+X0	FCM	MS	PS	Husemann et al. 2021	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus dorsatus*	ZMH862008	F	12,40	16+X0	FCM	MS	PS	this study	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus dorsatus*	ZMH862023	F	12,77	16+X0	FCM	MS	PS	this study	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus dorsatus*	ZMH862005	M	12,80	16+X0	FCM	MS	PS	this study	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus dorsatus*		n. a.	8,34	16+X0	FD	TS	GD	Belda et al. 1991	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus jacobsi*		n. a.	10,84	16+X0	FD	TS	GD	Belda et al. 1991	Bridle et al. 2002
Caelifera	Acrididae	Gomphocerinae	*Chorthippus jucundus*		n. a.	11,88	16+X0	FD	TS	GD	Belda et al. 1991	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Chorthippus longicornis*		M	8,58	16+X0	FD	TS	AC	Gosálvez et al. 1980	Gosálvez et al. 1980
Caelifera	Acrididae	Gomphocerinae	*Chorthippus mollis*	ZMH862017	M	11,58	16+X0*	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Chorthippus nevadensis*		n. a.	11,53	16+X0	FD	TS	GD	Belda et al. 1991	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Chorthippus pullus*	ZMH862066	F	13,44	16+X0*	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Chorthippus scalaris*		n. a.	14,72	16+X0	FD	TS	GD	Belda et al. 1991	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Chorthippus vagans*	ZMH862076	F	11,13	16+X0	FCM	MS	PS	this study	Gosálvez et al. 1980
Caelifera	Acrididae	Gomphocerinae	*Chorthippus vagans*	ZMH862074	F	11,08	16+X0	FCM	MS	PS	this study	Gosálvez et al. 1980
Caelifera	Acrididae	Gomphocerinae	*Chorthippus vagans*		M	8,68	16+X0	FD	TS	AC	Gosálvez et al. 1980	Gosálvez et al. 1980
Caelifera	Acrididae	Gomphocerinae	*Chorthippus vagans*		n. a.	8,64	16+X0	FD	TS	GD	Belda et al. 1991	Gosálvez et al. 1980

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Caelifera	Acrididae	Gomphocerinae	*Chrysochraon dispar*	ZMH862039	F	19,43	16+X0	FCM	MS	PS	this study	Fletcher & Hewitt 1980
Caelifera	Acrididae	Gomphocerinae	*Chrysochraon dispar*	ZMH862022	M	18,76	16+X0	FCM	MS	PS	this study	Fletcher & Hewitt 1980
Caelifera	Acrididae	Gomphocerinae	*Euchorthippus unicolor*		F	11,20	16+X0*	FCM	BR	LM	Mao et al. 2020	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Euchorthippus unicolor*		M	10,33	16+X0*	FCM	BR	LM	Mao et al. 2020	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Euthystira brachyptera*	ZMH862067	F	17,91	16+X0	FCM	MS	PS	this study	Fletcher & Hewitt 1980
Caelifera	Acrididae	Gomphocerinae	*Euthystira brachyptera*	ZMH862019	F	17,98	16+X0	FCM	MS	PS	this study	Fletcher & Hewitt 1980
Caelifera	Acrididae	Gomphocerinae	*Gomphocerippus rufus*	ZMH862096	F	13,36	16+X0	FCM	MS	PS	this study	Shu-juan 2016
Caelifera	Acrididae	Gomphocerinae	*Gomphocerippus rufus*	ZMH862081	F	13,00	16+X0	FCM	MS	PS	this study	Shu-juan 2016
Caelifera	Acrididae	Gomphocerinae	*Gomphocerippus rufus*		M	10,66	16+X0	FCM	BR	AD	Shah et al. 2020	Shu-juan 2016
Caelifera	Acrididae	Gomphocerinae	*Gomphocerus sibiricus*		M	8,95	16+X0	FD	TS	AC	Gosálvez et al. 1980	Gosálvez et al. 1980
Caelifera	Acrididae	Gomphocerinae	*Gomphocerus sibiricus*		M	11,52	16+X0	FCM	BR	AD	Shah et al. 2020	Gosálvez et al. 1980
Caelifera	Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*	ZMH862012	F	11,81	16+X0	FCM	MS	PS	this study	Barker 1960
Caelifera	Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*		M	12,14	16+X0	FD	TS	LM	John & Hewitt 1966	Barker 1960
Caelifera	Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*	ZMH862007	M	11,85	16+X0	FCM	MS	PS	this study	Barker 1960
Caelifera	Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*		M	12,66	16+X0	FD	TS	MM	Wilmore & Brown 1975	Barker 1960
Caelifera	Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*		n.a.	13,38	16+X0	n.a.	n.a.	n.a.	Petitpierre 1996	Barker 1960

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Caelifera	Acrididae	Gomphocerinae	*Omocestus haemorrhoidalis*	ZMH862071	F	12,83	16+X0	FCM	MS	PS	this study	John & Hewitt 1966
Caelifera	Acrididae	Gomphocerinae	*Omocestus haemorrhoidalis*	ZMH862004	M	12,14	16+X0	FCM	MS	PS	this study	John & Hewitt 1966
Caelifera	Acrididae	Gomphocerinae	*Omocestus viridulus*	ZMH862085	F	14,03	16+X0	FCM	MS	PS	this study	John & Hewitt 1966
Caelifera	Acrididae	Gomphocerinae	*Omocestus viridulus*		M	13,16	16+X0	FD	TS	LM	John & Hewitt 1966	John & Hewitt 1966
Caelifera	Acrididae	Gomphocerinae	*Omocestus viridulus*	ZMH862090	M	13,28	16+X0	FCM	MS	PS	this study	John & Hewitt 1966
Caelifera	Acrididae	Gomphocerinae	*Pararcyptera microptera meridionalis*		F	13,88	22+X0	FCM	BR	LM	Mao et al. 2020	John & Hewitt 1968
Caelifera	Acrididae	Gomphocerinae	*Pararcyptera microptera meridionalis*		M	13,13	22+X0	FCM	BR	LM	Mao et al. 2020	John & Hewitt 1968
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus montanus*	ZMH862063	F	13,12	16+X0*	FCM	MS	PS	this study	Bugrov & Vysotskaya 1981
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus montanus*	ZMH862094	M	12,42	16+X0*	FCM	MS	PS	this study	Bugrov & Vysotskaya 1981
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	ZMH862001	F	12,67	16+X0	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	ZMH862029	F	13,28	16+X0	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*		M	12,31	16+X0	FD	TS	LM	John & Hewitt 1966	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*		M	13,00	16+X0	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*		M	10,89	16+X0	FCM	BR	AD	Shah et al. 2020	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*		M	13,36	16+X0	FD	TS	MM	Wilmore & Brown 1975	Santos et al. 1983

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Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*		n.a.	14,72	16+X0	FD	TS	GD	Belda et al. 1991	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*		n.a.	13,83	16+X0	n.a.	n.a.	n.a.	Petitpierre 1996	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Stauroderus scalaris*		M	15,04	16+X0	FCM	BR	AD	Shah et al. 2020	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Stauroderus scalaris*		n.a.	16,34	16+X0	n.a.	n.a.	n.a.	Petitpierre 1996	Cabrero & Camacho 1986
Caelifera	Acrididae	Gomphocerinae	*Stenobothrus lineatus*	ZMH862070	M	13,63	16+X0	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Stenobothrus lineatus*		F	14,00	16+X0	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Stenobothrus nigromaculatus*	ZMH862065	F	13,18	16+X0*	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Stenobothrus nigromaculatus*	ZMH862064	M	12,48	16+X0*	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Stenobothrus stigmaticus*	ZMH862068	F	11,91	16+X0*	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Gomphocerinae	*Stenobothrus stigmaticus*	ZMH862069	M	11,21	16+X0*	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Melanoplinae	*Campylacantha olivacea*		F	6,98	n.a.	FCM	BR	GD	Hanrahan & Johnston 2011	
Caelifera	Acrididae	Melanoplinae	*Campylacantha olivacea*		M	6,15	n.a.	FCM	BR	GD	Hanrahan & Johnston 2011	
Caelifera	Acrididae	Melanoplinae	*Fruhstorferiola huayinensis*		F	8,62	20+X0*	FCM	BR	LM	Mao et al. 2020	Bugrov et al. 2000
Caelifera	Acrididae	Melanoplinae	*Fruhstorferiola huayinensis*		M	8,30	20+X0*	FCM	BR	LM	Mao et al. 2020	Bugrov et al. 2000
Caelifera	Acrididae	Melanoplinae	*Melanoplus differentialis*		F	7,26	22+X0	FCM	BR	PA	Hanrahan & Johnston 2011	Abdel-Hameed 1983
Caelifera	Acrididae	Melanoplinae	*Melanoplus differentialis*		M	6,79	22+X0	FCM	BR	PA	Hanrahan & Johnston 2011	Abdel-Hameed 1983

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Caelifera	Acrididae	Melanoplinae	*Melanoplus differentialis*		n. a.	6,23	22+X0	FD	HE	GD, OM	Rasch unpubl.	Abdel-Hameed 1983
Caelifera	Acrididae	Melanoplinae	*Melanoplus differentialis*		n. a.	3,84	22+X0	FD	OV, TS	BO	Swift & Kleinfeld 1953	Abdel-Hameed 1983
Caelifera	Acrididae	Melanoplinae	*Melanoplus sanguinipes*		n. a.	5,83	22+X0	FD	HE	GD, OM	Rasch unpubl.	White 1973
Caelifera	Acrididae	Melanoplinae	*Pedopodisma tsinlingensis*		F	11,09	20+X0	FCM	BR	LM	Mao et al. 2020	Ma & Guo 2001
Caelifera	Acrididae	Melanoplinae	*Pedopodisma tsinlingensis*		M	10,21	20+X0	FCM	BR	PA	Mao et al. 2020	Ma & Guo 2001
Caelifera	Acrididae	Melanoplinae	*Podisma pedestris*		M	16,93	22+X0	FD	S	SG	Westermann et al. 1987	Hewitt & John 1972
Caelifera	Acrididae	Melanoplinae	*Sinopodisma qinlingensis*		F	11,35	20+X0*	FCM	BR	LM	Mao et al. 2020	Bugrov et al. 2000
Caelifera	Acrididae	Melanoplinae	*Sinopodisma qinlingensis*		M	10,96	20+X0*	FCM	BR	LM	Mao et al. 2020	Bugrov et al. 2000
Caelifera	Acrididae	Oedipodinae	*Aiolopus thalassinus*		M	6,68	22+X0	FD	TS	GD	Rees et al. 1978	King & John 1980
Caelifera	Acrididae	Oedipodinae	*Austroicetes pusilla*		M	6,29	22+X0	FD	TS	GD	Rees et al. 1978	White & Key 1957
Caelifera	Acrididae	Oedipodinae	*Bryodemella holdereri*		F	18,64	22+X0	FCM	BR	LM	Mao et al. 2020	Vyotskaya 1986
Caelifera	Acrididae	Oedipodinae	*Bryodemella holdereri*		M	18,19	22+X0	FCM	BR	LM	Mao et al. 2020	Vyotskaya 1986
Caelifera	Acrididae	Oedipodinae	*Bryodemella tuberculata*	ZMH862087	F	21,88	22+X0	FCM	MS	PS	this study	Bugrov 1996
Caelifera	Acrididae	Oedipodinae	*Bryodemella tuberculata*	ZMH862086	F	21,96	22+X0	FCM	MS	PS	this study	Bugrov 1996
Caelifera	Acrididae	Oedipodinae	*Chortoicetes terminifera*		M	5,99	22+X0	FD	TS	GD	Rees et al. 1978	Webb & Neuhaus 1979
Caelifera	Acrididae	Oedipodinae	*Chortoicetes terminifera*		M	7,22	22+X0	FD	TS	MM	Wilmore & Brown 1975	Webb & Neuhaus 1979

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Caelifera	Acrididae	Oedipodinae	*Epacromius coerulipes*		F	8,55	22+X0	FCM	BR	LM	Mao et al. 2020	Husemann et al. 2022
Caelifera	Acrididae	Oedipodinae	*Epacromius coerulipes*		M	8,14	22+X0	FCM	BR	LM	Mao et al. 2020	Husemann et al. 2022
Caelifera	Acrididae	Oedipodinae	*Gastrimargus musicus*		M	9,01	22+X0	FD	TS	GD	Rees et al. 1978	King & John 1980
Caelifera	Acrididae	Oedipodinae	*Humbe tenuicornis*		M	8,21	22+X0	FD	TS	LM	John & Hewitt 1966	John and Hewitt, 1966
Caelifera	Acrididae	Oedipodinae	*Locusta migratoria*		F	6,44	22+X0	FCM	n.a.	MM	Wang et al. 2014	Bier & Müller 1969
Caelifera	Acrididae	Oedipodinae	*Locusta migratoria*	ZMH862104	M	7,62	22+X0	FCM	MS	PS	this study	Bier & Müller 1969
Caelifera	Acrididae	Oedipodinae	*Locusta migratoria*		M	5,47	22+X0	FD	TS	GD	Rees et al. 1978	Bier & Müller 1969
Caelifera	Acrididae	Oedipodinae	*Locusta migratoria*		M	6,09	22+X0	FD	TS	MM	Wilmore & Brown 1975	Bier & Müller 1969
Caelifera	Acrididae	Oedipodinae	*Locusta migratoria*		n.a.	5,28	22+X0	FD	S	MD	Bier & Müller 1969	Bier & Müller 1969
Caelifera	Acrididae	Oedipodinae	*Locusta migratoria*		n.a.	6,27	22+X0	FD	V	MM	Fox 1970	Bier & Müller 1969
Caelifera	Acrididae	Oedipodinae	*Locusta migratoria*		n.a.	6,35	22+X0	FD	HE	GD, OM	Rasch 1985	Bier & Müller 1969
Caelifera	Acrididae	Oedipodinae	*Oedaleus asiaticus*		F	9,83	22+X0*	FCM	BR	PA	Mao et al. 2020	Takizawa & Narasawa 1971
Caelifera	Acrididae	Oedipodinae	*Oedaleus asiaticus*		M	9,24	22+X0*	FCM	BR	LM	Mao et al. 2020	Takizawa & Narasawa 1971
Caelifera	Acrididae	Oedipodinae	*Oedaleus infernalis*		F	9,83	22+X0	FCM	BR	LM	Mao et al. 2020	Takizawa & Narasawa 1971
Caelifera	Acrididae	Oedipodinae	*Oedaleus infernalis*		M	9,27	22+X0	FCM	BR	LM	Mao et al. 2020	Takizawa & Narasawa 1971
Caelifera	Acrididae	Oedipodinae	*Oedipoda caerulescens*		F	14,20	22+X0	FCM	MS	PS	Husemann et al. 2021	Santos et al. 1983

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Caelifera	Acrididae	Oedipodinae	*Oedipoda caerulescens*	ZMH862080	F	14,05	22+X0	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Oedipodinae	*Oedipoda caerulescens*	ZMH862020	F	14,20	22+X0	FCM	MS	PS	this study	Santos et al. 1983
Caelifera	Acrididae	Oedipodinae	*Psophus stridulus*	ZMH862095	M	16,44	22+X0	FCM	MS	PS	this study	Suja et al. 1986
Caelifera	Acrididae	Oedipodinae	*Sphingonotus caerulans*		F	13,32	22+X0	FCM	MS	PS	Husemann et al. 2021	Santos et al. 1983
Caelifera	Acrididae	Oedipodinae	*Sphingonotus caerulans*		M	12,56	22+X0	FCM	MS	PS	Husemann et al. 2021	Santos et al. 1983
Caelifera	Acrididae	Oedipodinae	*Stethophyma grossum*		F	18,48	22+X0	FCM	MS	PS	Husemann et al. 2021	Callan 1941
Caelifera	Acrididae	Oedipodinae	*Stethophyma grossum*	ZMH862015	F	18,51	22+X0	FCM	MS	PS	this study	Callan 1941
Caelifera	Acrididae	Oedipodinae	*Stethophyma grossum*		M	17,36	22+X0	FCM	MS	PS	Husemann et al. 2021	Callan 1941
Caelifera	Acrididae	Oedipodinae	*Trilophidia annulata*		F	10,06	22+X0	FCM	BR	LM	Mao et al. 2020	Inoue 1985
Caelifera	Acrididae	Oedipodinae	*Trilophidia annulata*		M	9,37	22+X0	FCM	BR	LM	Mao et al. 2020	Inoue 1985
Caelifera	Acrididae	Pyrgomorphinae	*Atractomorpha sinensis*		F	8,21	18+X0	FCM	BR	LM	Mao et al. 2020	Inoue 1985
Caelifera	Acrididae	Pyrgomorphinae	*Atractomorpha sinensis*		M	7,55	18+X0	FCM	BR	LM	Mao et al. 2020	Inoue 1985
Caelifera	Acrididae	Thrinchinae	*Filchnerella rubimargina*		F	14,21	18+X0	FCM	BR	LM	Mao et al. 2020	Peng 1991
Caelifera	Acrididae	Thrinchinae	*Filchnerella rubimargina*		M	13,51	18+X0	FCM	BR	LM	Mao et al. 2020	Peng 1991
Caelifera	Acrididae	Thrinchinae	*Haplotropis brunneriana*		F	14,45	18+X0	FCM	BR	LM	Mao et al. 2020	Chen 1937
Caelifera	Acrididae	Thrinchinae	*Haplotropis brunneriana*		M	13,65	18+X0	FCM	BR	LM	Mao et al. 2020	Chen 1937

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Caelifera	Morabidae	Morabinae	*Warramaba virgo*		n.a.	3,75	14+X0	n.a.	n.a.	n.a.	Petitpierre 1996	White et al. 1977
Caelifera	Morabidae	Morabinae	*Warramaba virgo*		n.a.	4,00	14+X0	FD	BR	GD	White & Webb 1968	White et al. 1977
Caelifera	Tetrigidae	Tetriginae	*Tetrix subulata*	ZMH862034	M	2,22	12+X0	FCM	MS	PS	this study	Henderson 1961
Caelifera	Tetrigidae	Tetriginae	*Tetrix tuerki*	ZMH862073	F	2,32	12+X0*	FCM	MS	PS	this study	Henderson 1961
Caelifera	Tetrigidae	Tetriginae	*Tetrix tuerki*	ZMH862072	F	2,41	12+X0*	FCM	MS	PS	this study	Henderson 1961
Caelifera	Tetrigidae	Tetriginae	*Tetrix undulata*	ZMH862033	F	2,36	12+X0	FCM	MS	PS	this study	Henderson 1961
Caelifera	Tetrigidae	Tetriginae	*Tetrix undulata*	ZMH862052	M	2,18	12+X0	FCM	MS	PS	this study	Henderson 1961
Ensifera	Anostostomatidae	Deinacridinae	*Hemideina crassidens*		F	6,01 (DAPI)	14+X0	FCM	AN	BP	Morgan-Richards 2005	Morgan-Richards 1997
Ensifera	Anostostomatidae	Deinacridinae	*Hemideina crassidens*		M	5,40 (DAPI)	14+X0	FCM	AN	BP	Morgan-Richards 2005	Morgan-Richards 1997
Ensifera	Anostostomatidae	Deinacridinae	*Hemideina thoracica*		F	6,53 (DAPI)	14+X0	FCM	AN	BP	Morgan-Richards 2005	Morgan-Richards 1997
Ensifera	Anostostomatidae	Deinacridinae	*Hemideina thoracica*		M	5,95 (DAPI)	14+X0	FCM	AN	BP	Morgan-Richards 2005	Morgan-Richards 1997
Ensifera	Gryllacrididae	Gryllacridinae	*Ocellarnaca sp.*		F	9,31	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllacrididae	Gryllacridinae	*Ocellarnaca sp.*		F	9,50	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllacrididae	Gryllacridinae	*Ocellarnaca sp.*		F	9,60	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllacrididae	Gryllacridinae	*Ocellarnaca sp.*		F	9,37	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllacrididae	Gryllacridinae	*Ocellarnaca sp.*		F	9,47	n.a.	FCM	HD	GD	Yuan et al. 2021	

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Ensifera	Gryllidae	Eneopterinae	*Xenogryllus marmoratus*		F	2,36	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Eneopterinae	*Xenogryllus marmoratus*		F	2,34	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Eneopterinae	*Xenogryllus marmoratus*		M	2,00	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Eneopterinae	*Xenogryllus marmoratus*		M	2,02	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Eneopterinae	*Xenogryllus marmoratus*		M	2,06	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Eneopterinae	*Xenogryllus marmoratus*		M	2,16	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Gryllidae	Eneopterinae	*Xenogryllus marmoratus*		M	2,15	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Gryllidae	Eneopterinae	*Xenogryllus marmoratus*		M	2,14	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Gryllidae	Gryllinae	*Acheta domesticus*	ZMH862084	F	2,88	10+X0	FCM	MS	PS	this study	Cave & Allen 1969
Ensifera	Gryllidae	Gryllinae	*Acheta domesticus*	ZMH862083	M	2,63	10+X0	FCM	MS	PS	this study	Cave & Allen 1969
Ensifera	Gryllidae	Gryllinae	*Acheta domesticus*		n.a.	2,00	10+X0	FCM	BR	DM	Gregory unpubl.	Cave & Allen 1969
Ensifera	Gryllidae	Gryllinae	*Acheta domesticus*		n.a.	2,00	10+X0	FD	HE	GD	Gregory unpubl.	Cave & Allen 1969
Ensifera	Gryllidae	Gryllinae	*Acheta domesticus*		n.a.	2,38	10+X0	FD	HE	DM	Koshikawa et al. 2008	Cave & Allen 1969
Ensifera	Gryllidae	Gryllinae	*Acheta domesticus*		n.a.	2,00	10+X0	FD	OV, TS	MM, HS	Lima-de-Fariaet al. 1973	Cave & Allen 1969
Ensifera	Gryllidae	Gryllinae	*Acheta domesticus*		n.a.	2,00	10+X0	FD	HE	GD, OM	Rasch 1985	Cave & Allen 1969
Ensifera	Gryllidae	Gryllinae	*Gryllodes sigillatus*		F	2,29	20+X0	FCM	HD	PA	Yuan et al. 2021	Ohmachi 1927

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Ensifera	Gryllidae	Gryllinae	*Gryllodes sigillatus*		F	2,27	20+X0	FCM	HD	PA	Yuan et al. 2021	Ohmachi 1927
Ensifera	Gryllidae	Gryllinae	*Gryllodes sigillatus*		F	2,28	20+X0	FCM	HD	PA	Yuan et al. 2021	Ohmachi 1927
Ensifera	Gryllidae	Gryllinae	*Gryllodes sigillatus*		F	2,25	20+X0	FCM	HD	PA	Yuan et al. 2021	Ohmachi 1927
Ensifera	Gryllidae	Gryllinae	*Gryllodes sigillatus*		M	2,03	20+X0	FCM	HD	PA	Yuan et al. 2021	Ohmachi 1927
Ensifera	Gryllidae	Gryllinae	*Gryllodes sigillatus*		M	2,08	20+X0	FCM	HD	PA	Yuan et al. 2021	Ohmachi 1927
Ensifera	Gryllidae	Gryllinae	*Gryllodes sigillatus*		M	2,09	20+X0	FCM	HD	PA	Yuan et al. 2021	Ohmachi 1927
Ensifera	Gryllidae	Gryllinae	*Gryllus assimilis*	ZMH862100	F	2,24	28+X0	FCM	MS	PS	this study	Honda 1926
Ensifera	Gryllidae	Gryllinae	*Gryllus assimilis*	ZMH862101	M	2,09	28+X0	FCM	MS	PS	this study	Honda 1926
Ensifera	Gryllidae	Gryllinae	*Gryllus bimaculatus*	ZMH862098	F	2,22	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1979
Ensifera	Gryllidae	Gryllinae	*Gryllus bimaculatus*	ZMH862099	M	1,98	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1979
Ensifera	Gryllidae	Gryllinae	*Gryllus campestris*	ZMH862093	M	2,08	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1979
Ensifera	Gryllidae	Gryllinae	*Gryllus campestris*	ZMH862057	M	2,23	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1979
Ensifera	Gryllidae	Gryllinae	*Gryllus campestris*		M	2,23	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1979
Ensifera	Gryllidae	Gryllinae	*Gryllus pennsylvanicus*		n.a.	2,06	20+X0	FD	S	MD	Bier & Müller 1969	Randell 1960
Ensifera	Gryllidae	Gryllinae	*Gryllus pennsylvanicus*		n.a.	2,68	20+X0	n.a.	n.a.	n.a.	Petitpierre 1996	Randell 1960
Ensifera	Gryllidae	Gryllinae	*Gryllus pennsylvanicus*		n.a.	2,00	20+X0	FD	HE	GD, OM	Rasch 1985	Randell 1960

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Gryllidae	Gryllinae	*Loxoblemmus equestris*		F	2,47	12+X0	FCM	HD	PA	Yuan et al. 2021	Manna & Bhattacharjee 1970
Ensifera	Gryllidae	Gryllinae	*Loxoblemmus equestris*		F	2,38	12+X0	FCM	HD	PA	Yuan et al. 2021	Manna & Bhattacharjee 1970
Ensifera	Gryllidae	Gryllinae	*Loxoblemmus equestris*		F	2,50	12+X0	FCM	HD	PA	Yuan et al. 2021	Manna & Bhattacharjee 1970
Ensifera	Gryllidae	Gryllinae	*Loxoblemmus equestris*		F	2,44	12+X0	FCM	HD	PA	Yuan et al. 2021	Manna & Bhattacharjee 1970
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,57	26+X0	FCM	HD	PA	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,60	26+X0	FCM	HD	PA	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,57	26+X0	FCM	HD	PA	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,59	26+X0	FCM	HD	PA	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,61	26+X0	FCM	HD	GD	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,55	26+X0	FCM	HD	GD	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,60	26+X0	FCM	HD	GD	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,78	26+X0	FCM	HD	GD	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		F	2,63	26+X0	FCM	HD	GD	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		M	2,40	26+X0	FCM	HD	PA	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		M	2,36	26+X0	FCM	HD	PA	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		M	2,28	26+X0	FCM	HD	PA	Yuan et al. 2021	You et al. 2007

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		M	2,32	26+X0	FCM	HD	PA	Yuan et al. 2021	You et al. 2007
Ensifera	Gryllidae	Nemobiinae	*Nemobius sylvestris*	ZMH862097	F	2,56	16+X0	FCM	MS	PS	this study	Favrelle 1936
Ensifera	Gryllidae	Oecanthinae	*Oecanthus niveus*		n.a.	1,71	n.a.	FCM	BR	DV	Hanrahan & Johnston 2011	
Ensifera	Gryllidae	Oecanthinae	*Oecanthus pellucens*	ZMH862077	F	1,44	18+XY	FCM	MS	PS	this study	Ohmachi 1927
Ensifera	Gryllidae	Oecanthinae	*Oecanthus pellucens*	ZMH862078	M	1,37	18+XY	FCM	MS	PS	this study	Ohmachi 1927
Ensifera	Gryllidae	Oecanthinae	*Oecanthus sinensis*		F	1,09	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Oecanthinae	*Oecanthus sinensis*		F	1,07	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Oecanthinae	*Oecanthus sinensis*		M	0,98	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Oecanthinae	*Oecanthus sinensis*		M	0,97	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Oecanthinae	*Oecanthus sinensis*		M	0,91	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Podoscirtinae	*Truljalia hibinonis*		M	2,18	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Podoscirtinae	*Truljalia hibinonis*		M	2,17	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Podoscirtinae	*Truljalia hibinonis*		M	2,30	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllidae	Podoscirtinae	*Truljalia hibinonis*		M	2,27	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllotalpidae	Gryllotalpinae	*Gryllotalpa orientalis*		F	4,34	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllotalpidae	Gryllotalpinae	*Gryllotalpa orientalis*		F	4,33	n.a.	FCM	HD	PA	Yuan et al. 2021	

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Gryllotalpidae	Gryllotalpinae	*Gryllotalpa orientalis*		F	4,06	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllotalpidae	Gryllotalpinae	*Gryllotalpa orientalis*		F	4,12	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Gryllotalpidae	Gryllotalpinae	*Gryllotalpa orientalis*		F	4,23	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Gryllotalpidae	Gryllotalpinae	*Gryllotalpa orientalis*		F	4,16	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Gryllotalpidae	Gryllotalpinae	*Neoscapteriscus borellii*		n.a.	3,41	n.a.	FCM	BR	GD	Hanrahan & Johnston 2011	
Ensifera	Mogoplistidae	Mogoplistinae	*Ornebius kanetataki*		F	3,47	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Mogoplistidae	Mogoplistinae	*Ornebius kanetataki*		F	3,47	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Mogoplistidae	Mogoplistinae	*Ornebius kanetataki*		F	3,51	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Mogoplistidae	Mogoplistinae	*Ornebius kanetataki*		M	3,17	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Mogoplistidae	Mogoplistinae	*Ornebius kanetataki*		M	3,05	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Mogoplistidae	Mogoplistinae	*Ornebius kanetataki*		M	3,03	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Rhaphidophoridae	Aemodogryllinae	*Diestrammena sp.*		F	5,47	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1993
Ensifera	Rhaphidophoridae	Aemodogryllinae	*Diestrammena sp.*		F	5,48	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1993
Ensifera	Rhaphidophoridae	Aemodogryllinae	*Diestrammena sp.*		M	5,11	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1993
Ensifera	Rhaphidophoridae	Aemodogryllinae	*Diestrammena sp.*		M	5,18	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1993
Ensifera	Rhaphidophoridae	Ceuthophilinae	*Ceuthophilus stygius*		n.a.	9,55	36+X0	FD	HE	GD, OM	Rasch & Rasch 1981	Stevens 1912

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Ensifera	Rhaphidophoridae	Ceuthophilinae	*Hadenoecus subterraneus*		n. a.	1,55	34+X0	FD	HE	GD, OM	Rasch & Rasch 1981	Lamb 1975
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		F	19,00	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		F	18,54	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		F	19,00	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		F	19,18	28+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		F	19,49	28+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		F	19,60	28+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		M	17,55	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		M	17,45	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		M	17,70	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		M	17,08	28+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Deracantha onos*		M	17,18	28+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		F	14,28	30+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		F	13,81	30+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		F	13,99	30+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		F	13,72	30+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997

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Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		M	12,51	30+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		M	12,26	30+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		M	13,07	30+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		M	12,91	30+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Bradyporinae	*Zichya tenggerensis*		M	12,78	30+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa & Bugrov 1997
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus dorsalis*	ZMH862047	M	3,52	32+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus fuscus*	ZMH862024	F	4,42	32+X0*	FCM	MS	PS	this study	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus fuscus*	ZMH862053	M	3,79	32+X0*	FCM	MS	PS	this study	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,43	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,40	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,41	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,42	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,59	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,47	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,60	32+X0	FCM	HD	GD	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,69	32+X0	FCM	HD	GD	Yuan et al. 2021	Douk Hoon et al. 1987

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Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,54	32+X0	FCM	HD	GD	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		F	4,86	32+X0	FCM	HD	GD	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		M	3,96	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		M	4,02	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		M	4,00	32+X0	FCM	HD	PA	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		M	3,99	32+X0	FCM	HD	GD	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		M	4,04	32+X0	FCM	HD	GD	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus gladiatus*		M	4,13	32+X0	FCM	HD	GD	Yuan et al. 2021	Douk Hoon et al. 1987
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus maculatus*		F	4,08	32+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus maculatus*		F	3,89	32+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus maculatus*		M	3,71	32+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus maculatus*		M	3,67	32+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus melaenus*		F	4,21	32+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus melaenus*		F	4,34	32+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus melaenus*		F	4,22	32+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus melaenus*		F	4,34	32+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa 1984a

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus melaenus*		F	4,39	32+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus sp.*		F	3,03	32+X0	FCM	BR	GD	Hanrahan & Johnston 2011	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Conocephalus sp.*		M	2,65	32+X0	FCM	BR	GD	Hanrahan & Johnston 2011	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Conocephalinae	*Neoconocephalus triops*		F	7,93	22+X0	FCM	BR	GD	Hanrahan & Johnston 2011	White 1941
Ensifera	Tettigoniidae	Conocephalinae	*Neoconocephalus triops*		M	7,29	22+X0	FCM	BR	GD	Hanrahan & Johnston 2011	White 1941
Ensifera	Tettigoniidae	Conocephalinae	*Pseudorhynchus crassiceps*		F	10,06	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Conocephalinae	*Pseudorhynchus crassiceps*		F	10,05	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Conocephalinae	*Pseudorhynchus crassiceps*		F	10,04	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Conocephalinae	*Pseudorhynchus crassiceps*		M	8,91	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Conocephalinae	*Pseudorhynchus crassiceps*		M	8,57	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Conocephalinae	*Pseudorhynchus crassiceps*		M	8,83	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		F	9,60	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		F	9,55	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		F	9,77	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		F	10,11	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		F	9,80	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		F	9,77	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		F	9,42	20+X0*	FCM	HD	GD	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		F	9,41	20+X0*	FCM	HD	GD	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia dubia*		M	9,07	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		F	9,70	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		F	9,70	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		F	10,27	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		F	10,22	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		F	9,53	20+X0*	FCM	HD	GD	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		F	9,55	20+X0*	FCM	HD	GD	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		M	8,81	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		M	9,00	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		M	9,04	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		M	9,33	20+X0*	FCM	HD	PA	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		M	8,94	20+X0*	FCM	HD	GD	Yuan et al. 2021	Torelli 1940
Ensifera	Tettigoniidae	Conocephalinae	*Ruspolia lineosa*		M	9,41	20+X0*	FCM	HD	GD	Yuan et al. 2021	Torelli 1940

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Hexacentrinae	*Hexacentrus unicolor*		F	13,86	30/32+X*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Hexacentrinae	*Hexacentrus unicolor*		F	14,15	30/32+X*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Hexacentrinae	*Hexacentrus unicolor*		M	12,84	30/32+X*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Hexacentrinae	*Hexacentrus unicolor*		M	12,14	30/32+X*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Hexacentrinae	*Hexacentrus unicolor*		M	12,63	30/32+X*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Hexacentrinae	*Hexacentrus unicolor*		M	12,67	30/32+X*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Hexacentrinae	*Hexacentrus unicolor*		M	13,67	30/32+X*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Hexacentrinae	*Hexacentrus unicolor*		M	12,87	30/32+X*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Meconematinae	*Meconema meridionale*	ZMH862060	F	10,69	26+X0*	FCM	MS	PS	this study	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Meconematinae	*Meconema meridionale*	ZMH862031	M	9,90	26+X0*	FCM	MS	PS	this study	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Meconematinae	*Meconema thalassinum*	ZMH862038	F	12,28	26+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Meconematinae	*Meconema thalassinum*	ZMH862030	F	12,44	26+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984a
Ensifera	Tettigoniidae	Meconematinae	*Microconema clavata*		F	4,41	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Meconematinae	*Microconema clavata*		F	4,34	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Meconematinae	*Microconema clavata*		M	4,07	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Meconematinae	*Microconema clavata*		M	4,01	n.a.	FCM	HD	GD	Yuan et al. 2021	

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		F	14,37	26/28+X	FCM	HD	PA	Yuan et al. 2021	Bugrov et al. 2004
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		F	14,33	26/28+X	FCM	HD	PA	Yuan et al. 2021	Bugrov et al. 2005
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		F	13,72	26/28+X	FCM	HD	PA	Yuan et al. 2021	Bugrov et al. 2006
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		F	15,00	26/28+X	FCM	HD	GD	Yuan et al. 2021	Bugrov et al. 2007
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		F	14,58	26/28+X	FCM	HD	GD	Yuan et al. 2021	Bugrov et al. 2008
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		F	15,48	26/28+X	FCM	HD	GD	Yuan et al. 2021	Bugrov et al. 2009
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		M	13,43	26/28+X	FCM	HD	PA	Yuan et al. 2021	Bugrov et al. 2010
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		M	13,53	26/28+X	FCM	HD	PA	Yuan et al. 2021	Bugrov et al. 2011
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		M	13,17	26/28+X	FCM	HD	PA	Yuan et al. 2021	Bugrov et al. 2012
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		M	13,36	26/28+X	FCM	HD	PA	Yuan et al. 2021	Bugrov et al. 2013
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		M	13,04	26/28+X	FCM	HD	PA	Yuan et al. 2021	Bugrov et al. 2014
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		M	13,98	26/28+X	FCM	HD	GD	Yuan et al. 2021	Bugrov et al. 2015
Ensifera	Tettigoniidae	Mecopodinae	*Mecopoda elongata*		M	13,66	26/28+X	FCM	HD	GD	Yuan et al. 2021	Bugrov et al. 2016
Ensifera	Tettigoniidae	Phaneropterinae	*Ducetia japonica*		F	7,90	28+X0	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Ducetia japonica*		F	8,13	28+X0	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Ducetia japonica*		F	7,66	28+X0	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Phaneropterinae	*Ducetia japonica*		F	7,67	28+X0	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Ducetia japonica*		M	6,80	28+X0	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Ducetia japonica*		M	6,87	28+X0	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Ducetia japonica*		M	6,94	28+X0	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Ducetia japonica*		M	7,28	28+X0	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		F	6,62	26+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		F	6,69	26+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		F	6,45	26+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		F	6,62	26+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		F	6,99	26+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		F	6,71	26+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		M	5,77	26+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		M	5,78	26+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		M	5,77	26+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		M	6,08	26+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Phaneropterinae	*Elimaea berezovskii*		M	6,31	26+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		F	10,31	26t/28+X0*	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		F	10,38	26t/28+X0*	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		F	10,07	26t/28+X0*	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		F	11,09	26t/28+X0*	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		F	11,14	26t/28+X0*	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		F	10,91	26t/28+X0*	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		F	10,15	26t/28+X0*	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	9,13	26t/28+X0*	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	9,00	26t/28+X0*	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	8,88	26t/28+X0*	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	8,95	26t/28+X0*	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	9,22	26t/28+X0*	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	9,19	26t/28+X0*	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	9,02	26t/28+X0*	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	9,18	26t/28+X0*	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Kuwayamaea brachyptera*		M	8,91	26t/28+X0*	FCM	HD	GD	Yuan et al. 2021	

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Phaneropterinae	*Leptophyes punctatissima*	ZMH862040	F	7,98	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa & Heller 1998
Ensifera	Tettigoniidae	Phaneropterinae	*Leptophyes punctatissima*	ZMH862011	M	6,73	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa & Heller 1998
Ensifera	Tettigoniidae	Phaneropterinae	*Leptophyes punctatissima*	ZMH862050	M	6,84	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa & Heller 1998
Ensifera	Tettigoniidae	Phaneropterinae	*Leptophyes punctatissima*	ZMH862043	M	6,86	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa & Heller 1998
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera falcata*	ZMH862014	F	7,25	26+X0	FCM	MS	PS	this study	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera falcata*	ZMH862061	M	6,08	26+X0	FCM	MS	PS	this study	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		F	6,15	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		F	6,17	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		F	6,02	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		F	6,06	26+X0	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		F	6,17	26+X0	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	4,96	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	5,22	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	5,28	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	5,13	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	5,13	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	5,05	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	5,03	26+X0	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	5,11	26+X0	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Phaneroptera gracilis*		M	4,94	26+X0	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa et al. 1996
Ensifera	Tettigoniidae	Phaneropterinae	*Ruidocollaris sinensis*		F	7,03	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Ruidocollaris sinensis*		F	6,97	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Ruidocollaris sinensis*		M	5,99	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Phaneropterinae	*Ruidocollaris sinensis*		M	6,06	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Pseudophyllinae	*Phyllomimus sinicus*		F	5,87	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Pseudophyllinae	*Phyllomimus sinicus*		F	5,91	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Pseudophyllinae	*Phyllomimus sinicus*		F	5,95	n.a.	FCM	HD	PA	Yuan et al. 2021	
Ensifera	Tettigoniidae	Pseudophyllinae	*Tegra novaehollandiae viridinotata*		F	3,43	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Pseudophyllinae	*Tegra novaehollandiae viridinotata*		F	3,62	n.a.	FCM	HD	GD	Yuan et al. 2021	
Ensifera	Tettigoniidae	Pseudophyllinae	*Tegra novaehollandiae viridinotata*		F	3,36	n.a.	FCM	HD	GD	Yuan et al. 2021	

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Tettigoniinae	*Atlanticus sinensis*		F	7,12	28+X0	FCM	HD	PA	Yuan et al. 2021	Chang & Lian 2002
Ensifera	Tettigoniidae	Tettigoniinae	*Atlanticus sinensis*		F	7,23	28+X0	FCM	HD	PA	Yuan et al. 2021	Chang & Lian 2002
Ensifera	Tettigoniidae	Tettigoniinae	*Atlanticus sinensis*		F	7,04	28+X0	FCM	HD	PA	Yuan et al. 2021	Chang & Lian 2002
Ensifera	Tettigoniidae	Tettigoniinae	*Atlanticus sinensis*		M	6,81	28+X0	FCM	HD	PA	Yuan et al. 2021	Chang & Lian 2002
Ensifera	Tettigoniidae	Tettigoniinae	*Atlanticus sinensis*		M	6,86	28+X0	FCM	HD	PA	Yuan et al. 2021	Chang & Lian 2002
Ensifera	Tettigoniidae	Tettigoniinae	*Atlanticus sinensis*		M	6,92	28+X0	FCM	HD	PA	Yuan et al. 2021	Chang & Lian 2002
Ensifera	Tettigoniidae	Tettigoniinae	*Atlanticus sinensis*		M	6,77	28+X0	FCM	HD	GD	Yuan et al. 2021	Chang & Lian 2002
Ensifera	Tettigoniidae	Tettigoniinae	*Atlanticus sinensis*		M	6,53	28+X0	FCM	HD	GD	Yuan et al. 2021	Chang & Lian 2002
Ensifera	Tettigoniidae	Tettigoniinae	*Bicolorana bicolor*	ZMH862027	F	8,05	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa et al. 1987
Ensifera	Tettigoniidae	Tettigoniinae	*Bicolorana bicolor*	ZMH862055	M	6,99	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa et al. 1987
Ensifera	Tettigoniidae	Tettigoniinae	*Decticus verrucivorus*	ZMH862045	F	8,11	30+X0	FCM	MS	PS	this study	Mohr 1919
Ensifera	Tettigoniidae	Tettigoniinae	*Decticus verrucivorus*	ZMH862046	F	8,30	30+X0	FCM	MS	PS	this study	Mohr 1919
Ensifera	Tettigoniidae	Tettigoniinae	*Decticus verrucivorus*	ZMH862044	M	7,34	30+X0	FCM	MS	PS	this study	Mohr 1919
Ensifera	Tettigoniidae	Tettigoniinae	*Decticus verrucivorus*		M	7,34	30+X0	FCM	MS	PS	this study	Mohr 1919
Ensifera	Tettigoniidae	Tettigoniinae	*Gampsocleis sinensis*		F	6,68	30+X0*	FCM	HD	PA	Yuan et al. 2021	Kociński 2018
Ensifera	Tettigoniidae	Tettigoniinae	*Gampsocleis sinensis*		F	6,84	30+X0*	FCM	HD	PA	Yuan et al. 2021	Kociński 2018

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Tettigoniinae	*Gampsocleis sinensis*		F	6,87	30+X0*	FCM	HD	GD	Yuan et al. 2021	Kociński 2018
Ensifera	Tettigoniidae	Tettigoniinae	*Gampsocleis sinensis*		F	6,74	30+X0*	FCM	HD	GD	Yuan et al. 2021	Kociński 2018
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		F	5,91	30+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		F	5,83	30+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		F	5,84	30+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		F	5,62	30+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		F	5,80	30+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		F	5,88	30+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		F	6,14	30+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		F	6,15	30+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		M	5,36	30+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		M	5,16	30+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		M	5,23	30+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		M	5,26	30+X0*	FCM	HD	PA	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		M	5,53	30+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		M	5,35	30+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		M	5,50	30+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera bonneti*		M	5,31	30+X0*	FCM	HD	GD	Yuan et al. 2021	Asana et al. 1938
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera brachyptera*	ZMH862092	F	8,78	30+X0	FCM	MS	PS	this study	White 1941
Ensifera	Tettigoniidae	Tettigoniinae	*Metrioptera brachyptera*	ZMH862091	M	7,97	30+X0	FCM	MS	PS	this study	White 1941
Ensifera	Tettigoniidae	Tettigoniinae	*Pholidoptera griseoaptera*	ZMH862010	F	7,06	30+X0	FCM	MS	PS	this study	Henderson 1961
Ensifera	Tettigoniidae	Tettigoniinae	*Pholidoptera griseoaptera*	ZMH862037	F	7,16	30+X0	FCM	MS	PS	this study	Henderson 1961
Ensifera	Tettigoniidae	Tettigoniinae	*Pholidoptera griseoaptera*	ZMH862051	M	6,24	30+X0	FCM	MS	PS	this study	Henderson 1961
Ensifera	Tettigoniidae	Tettigoniinae	*Pholidoptera griseoaptera*	ZMH862016	M	6,35	30+X0	FCM	MS	PS	this study	Henderson 1961
Ensifera	Tettigoniidae	Tettigoniinae	*Pholidoptera littoralis*	ZMH862062	F	7,69	30+X0*	FCM	MS	PS	this study	Henderson 1961
Ensifera	Tettigoniidae	Tettigoniinae	*Platycleis albopunctata*	ZMH862054	F	6,41	30+X0*	FCM	MS	PS	this study	Camacho et al. 1981
Ensifera	Tettigoniidae	Tettigoniinae	*Platycleis albopunctata*	ZMH862049	F	6,67	30+X0*	FCM	MS	PS	this study	Camacho et al. 1981
Ensifera	Tettigoniidae	Tettigoniinae	*Platycleis albopunctata*	ZMH862013	M	5,73	30+X0*	FCM	MS	PS	this study	Camacho et al. 1981
Ensifera	Tettigoniidae	Tettigoniinae	*Platycleis albopunctata*	ZMH862042	M	5,74	30+X0*	FCM	MS	PS	this study	Camacho et al. 1981
Ensifera	Tettigoniidae	Tettigoniinae	*Roeseliana roeselii*	ZMH862059	F	8,30	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Roeseliana roeselii*	ZMH862028	M	7,63	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Roeseliana roeselii*	ZMH862058	M	7,71	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984b

Suborder	Family	Subfamily	Species	ZMH Number	Sex	Genome size 1C [pg]	Chromosome count (2n)	Method	Cell Type	Standard	Reference genome size	Reference chromosome count
Ensifera	Tettigoniidae	Tettigoniinae	*Roeseliana roeselii*	ZMH862035	M	7,77	30+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia cantans*	ZMH862089	F	7,16	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia cantans*	ZMH862088	M	6,34	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia chinensis*		F	6,46	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia chinensis*		F	6,44	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia chinensis*		F	6,55	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia chinensis*		F	6,52	28+X0*	FCM	HD	PA	Yuan et al. 2021	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia chinensis*		F	7,00	28+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia chinensis*		F	7,00	28+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia chinensis*		F	6,80	28+X0*	FCM	HD	GD	Yuan et al. 2021	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia viridissima*	ZMH862036	M	5,58	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984b
Ensifera	Tettigoniidae	Tettigoniinae	*Tettigonia viridissima*	ZMH862025	M	5,79	28+X0	FCM	MS	PS	this study	Warchalowska-Sliwa 1984b
Ensifera	Tridactylidae	n.a.	unknown *sp.*		n.a.	2,63	10/12+X*	FCM	BR	DV	Hanrahan & Johnston 2011	John & Rentz 1986
Ensifera	Trigoniidae	Trigonidiinae	*Laupala cerasina*		n.a.	1,93	14+X0*	FCM	BR	GD	Petrov 2000	Parsons & Shaw 2002

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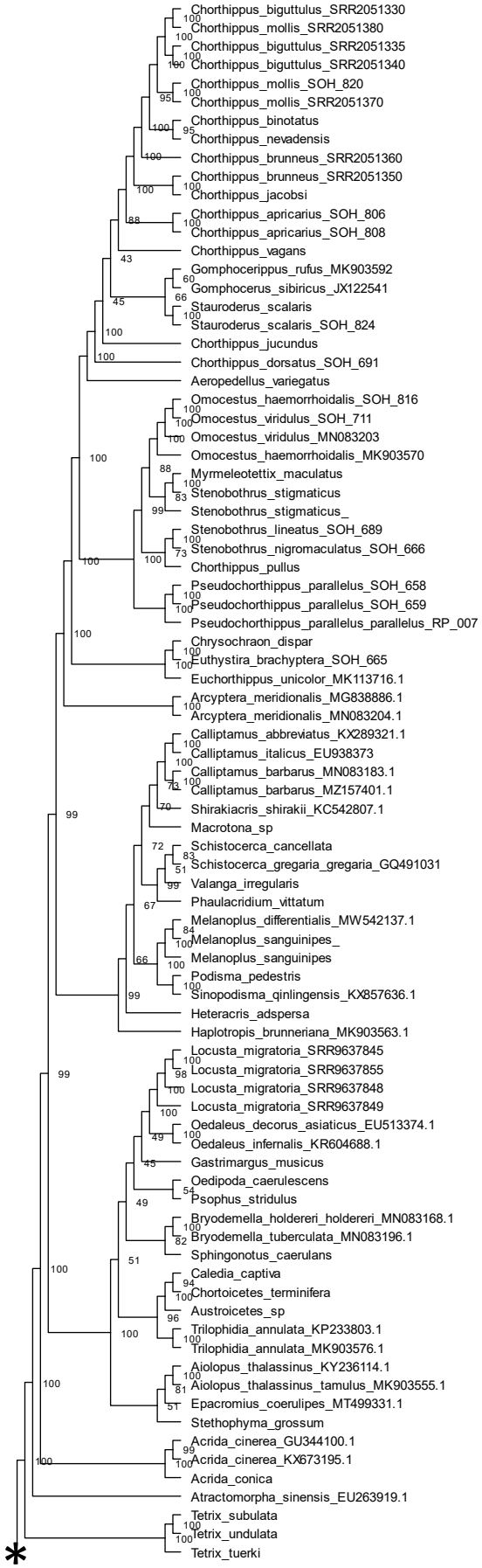

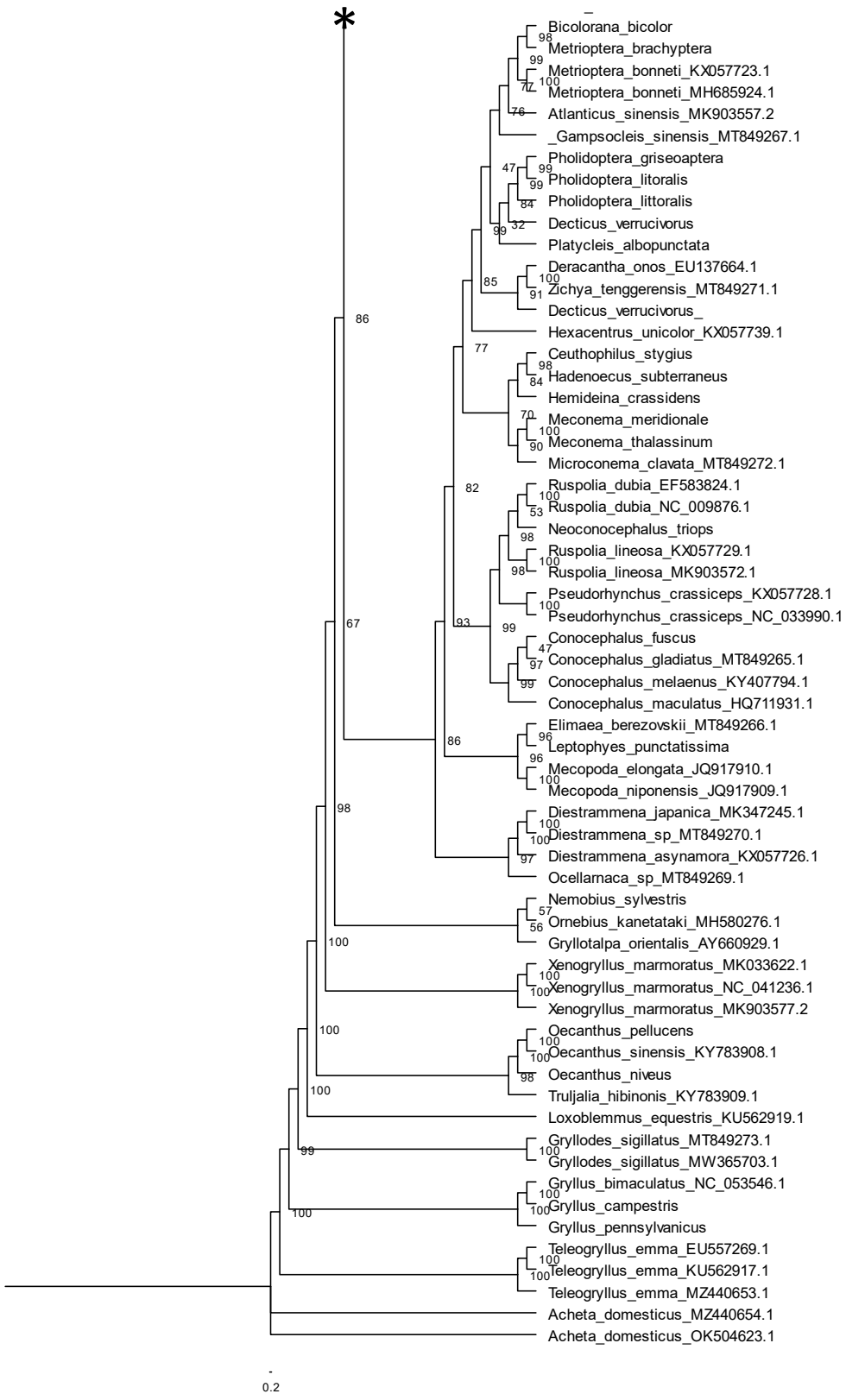
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Oecanthus_niveus
Truljalia_hibinonis_KY783909.1
Loxoblemmus_equestris_KU562919.1
Gryllodes_sigillatus_MT849273.1
Gryllodes_sigillatus_MW365703.1
Gryllus_bimaculatus_NC_053546.1
Gryllus_campestris
Gryllus_pennsylvanicus
Teleogryllus_emma_EU557269.1
Teleogryllus_emma_KU562917.1
Teleogryllus_emma_MZ440653.1
Acheta_domesticus_MZ440654.1
Acheta_domesticus_OK504623.1
0.2

SI CHAPTER 5.3

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DOES (GENOME) SIZE MATTER?

Evolution of chromosome number in grasshoppers (Orthoptera: Caelifera: Acrididae)

Martin Husemann, Lara-Sophie Dey, David Sadílek, Norihiro Ueshima, Oliver Hawlitschek, Hojun Song & David B. Weissman

Supporting Information Table S1 – chromosome numbers of Orthopterans from literature survey and own analyses.

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Acridinae	Acridini	*Acrida*	*cinerea*	2n=23(22+X)	Inoue, 1985
Acrididae	Acridinae	Acridini	*Acrida*	*conica*	2n=23(22+X)	King and John, 1980
Acrididae	Acridinae	Acridini	*Acrida*	*exaltata*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Acridinae	Acridini	*Acrida*	*exaltata*	2n=23(22+X)	Koli et al., 2013
Acrididae	Acridinae	Acridini	*Acrida*	*exaltata*	2n=23(22+X)	Singh, 2006
Acrididae	Acridinae	Acridini	*Acrida*	*lata*	2n=23(22+X)	Kayano et al., 1960
Acrididae	Acridinae	Acridini	*Acrida*	*lata*	2n=23(22+X)	Kayano et al., 1970
Acrididae	Acridinae	Acridini	*Acrida*	*lata*	2n=23(22+X)	Sannomiya, 1963
Acrididae	Acridinae	Acridini	*Acrida*	*lata*	2n=23(22+X)	Sannomiya and Kayano, 1968
Acrididae	Acridinae	Acridini	*Acrida*	*lata*	2n=23(22+X)	Sannomiya and Kayano, 1969
Acrididae	Acridinae	Acridini	*Acrida*	*lata*	2n=23(22+X)	Takizawa and Narasawa, 1971
Acrididae	Acridinae	Acridini	*Acrida*	*oxycephala*	2n=23(22+X)	Bugrov and Vysotskaya 1981
Acrididae	Acridinae	Acridini	*Acrida*	*sp.*	2n=23(22+X)	Berhanu et al., 2012
Acrididae	Acridinae	Acridini	*Acrida*	*sp.*	2n=23(22+X)	Santos et al., 1983
Acrididae	Acridinae	Acridini	*Acrida*	*turrita*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Acridinae	Acridini	*Acrida*	*turrita*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Acridinae	Acridini	*Acrida*	*turrita*	2n=23(22+X)	Momma, 1943
Acrididae	Acridinae	Acridini	*Acrida*	*turrita*	2n=23(22+X)	Seino and Akongnui, 2010
Acrididae	Acridinae	Acridini	*Acrida*	*turrita*	2n=23(22+X)	Seino et al., 2013
Acrididae	Acridinae	Acridini	*Acrida*	*exaltata*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Acridinae	Acridini	*Caledia*	*captiva*	2n=23(22+X)	Marchant and Shaw, 1993
Acrididae	Acridinae	Acridini	*Caledia*	*captiva*	2n=23(22+X)	Shaw, 1976
Acrididae	Acridinae	Acridini	*Cryptobothrus*	*chrysophorus*	2n=23(22+X)	John and King, 1977
Acrididae	Acridinae	Acridini	*Cryptobothrus*	*chrysophorus*	2n=23(22+X)	Sharman, 1952

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Acridinae	Acridini	*Cryptobothrus*	*chrysophorus*	2n=23(22+X)	White, 1973
Acrididae	Acridinae	Acridini	*Cryptobothrus*	*chrysophorus*	2n=23(22+X)	Rees et al., 1978
Acrididae	Acridinae	Acridini	*Perala*	*viridis*	2n=23(22+X)	King and John, 1980
Acrididae	Acridinae	Calephorini	*Calephorus*	*compressicornis*	2n=23(22+X)	Santos et al., 1983
Acrididae	Acridinae	Calephorini	*Calephorus*	*viridis*	2n=23(22+X)	King and John, 1980
Acrididae	Acridinae	Gymnobothrini	*Chirista*	*compta*	2n=23(22+X)	Seino et al., 2014
Acrididae	Acridinae	Gymnobothrini	*Coryphosima*	*stenoptera*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Acridinae	Gymnobothrini	*Coryphosima*	*stenoptera producta*	2n=23(22+X)	Seino and Dongmo, 2015
Acrididae	Acridinae	Gymnobothrini	*Gymnobothrus*	*cruciatus*	2n=23(22+X)	Fossey and Liebenberg, 1990
Acrididae	Acridinae	Hyalopterygini	*Allotruxalis*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Allotruxalis*	*striata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Allotruxalis*	*strigata*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Acridinae	Hyalopterygini	*Cocytotettix*	*argentina*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Cocytotettix*	*intermedia*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Cocytotettix*	*pulchripennis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Cocytotettix*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Eutryxalis*	*filata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Eutryxalis*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Hyalopteryx*	*rufipennis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Metaleptea*	*brevicornis*	2n=23(22+X)	Bidau, 1986
Acrididae	Acridinae	Hyalopterygini	*Metaleptea*	*brevicornis*	2n=23(22+X)	Grieco and Bidau, 2000
Acrididae	Acridinae	Hyalopterygini	*Metaleptea*	*brevicornis*	2n=23(22+X)	Grieco and Bidau, 1999
Acrididae	Acridinae	Hyalopterygini	*Metaleptea*	*brevicornis adspersa*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Acridinae	Hyalopterygini	*Metaleptea*	*brevicornis brevicornis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Orphula*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	Hyalopterygini	*Parorphula*	*graminea*	2n=23(22+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Acridinae	n.a.	*Covasacris*	*albitarsis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Acridinae	n.a.	*Covasacris*	*sp.*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Acridinae	Pargaini	*Machaeridia*	*bilineata*	2n=23(22+X)	Fossey and Liebenberg, 1990
Acrididae	Acridinae	Pargaini	*Parga*	*xanthoptera*	2n=23(22+X)	Fossey and Liebenberg, 1990
Acrididae	Acridinae	Phlaeobini	*Cannula*	*gracilis*	2n=23(22+X)	Fossey and Liebenberg, 1990
Acrididae	Acridinae	Phlaeobini	*Duroniella*	*gracilis*	2n=23(22+X)	Bugrov, 1988
Acrididae	Acridinae	Phlaeobini	*Duroniella*	*kalmyka*	2n=23(22+X)	Bugrov, 1988
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*antennata*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*antennata*	2n=23(22+X)	Singh, 2006
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*formosana*	2n=23(22+X)	Momma, 1943
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*galeata*	2n=23(22+X)	Ho and Lee, 1971
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*infumata*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*infumata*	2n=23(22+X)	Ho and Lee, 1971
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*infumata*	2n=23(22+X)	Liu et al., 1962
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*infumata*	2n=23(22+X)	Momma, 1943
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*infumata*	2n=23(22+X)	Singh, 2006
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*antennata*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*antennata*	2n=23(22+X)	Koli et al., 2013
Acrididae	Acridinae	Phlaeobini	*Phlaeoba*	*infumata*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Acridinae	Phlaeobini	*Sikkimiana*	*darjeelingensis*	2n=23(22+X)	Singh, 2006
Acrididae	Acridinae	Truxalini	*Truxalis*	*eximia*	2n=23(22+X)	Bugrov, 1988
Acrididae	Acridinae	Truxalini	*Truxalis*	*nasuta*	2n=23(22+X)	Cabrero and Camacho, 1986
Acrididae	Acridinae	Truxalini	*Truxalis*	*nasuta*	2n=23(22+X)	Minouchi, 1934
Acrididae	Calliptaminae	n.a.	*Acorypha*	*glaucopsis*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Calliptaminae	n.a.	*Calliptamus*	*barbarus*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Calliptaminae	n.a.	*Calliptamus*	*barbarus*	2n=23(22+X)	Santos et al., 1983

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Calliptaminae	n.a.	*Calliptamus*	*italicus*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Calliptaminae	n.a.	*Calliptamus*	*palaestinensis*	2n=23(22+X)	Nur, 1963
Acrididae	Calliptaminae	n.a.	*Calliptamus*	*sp.*	2n=23(22+X)	This study
Acrididae	Calliptaminae	n.a.	*Calliptamus*	*wattenwylianus*	2n=23(22+X)	Santos et al., 1983
Acrididae	Calliptaminae	n.a.	*Peripolus*	*pedarius*	2n=23(22+X)	Singh, 2006
Acrididae	Catantopinae	Catantopini	*Buforania*	*crassa*	2n=23(22+X)	John and Weissman, 1977
Acrididae	Catantopinae	Catantopini	*Catantops*	*innotabilis*	2n=23(22+X)	Singh, 2006
Acrididae	Catantopinae	Catantopini	*Catantops*	*pinguis innotabilis*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Catantopinae	Catantopini	*Fipurga*	*crassa*	2n=23(22+X)	King and John, 1980
Acrididae	Catantopinae	Catantopini	*Goniaea*	*australasie*	2n=23(22+X)	Peacock, 1970
Acrididae	Catantopinae	Catantopini	*Macrotona*	*australis*	2n=23(22+X)	King and John, 1980
Acrididae	Catantopinae	Catantopini	*Macrotona*	*australis*	2n=23(22+X)	Hubert Rees et al., 1978
Acrididae	Catantopinae	Catantopini	*Peakesia*	*hospita*	2n=23(22+X)	King and John, 1980
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*marginale*	2n=23(22+X)	Martin, 1970
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*marginale*	2n=23(22+X)	Westerman, 1974
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*marginale*	2n=23(22+X)	Westerman, 1975a
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*marginale*	2n=23(22+X)	Westerman, 1975b
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*marginale*	2n=23(22+X)	Westerman, 1977
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*marginale*	2n=23(22+X)	Westerman, 1983
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*marginale*	2n=23(22+X)	Westerman and Fontana, 1973
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*marginale*	2n=23(22+X)	Westerman and Ritchie, 1984
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*otagense*	2n=23(22+X)	Westerman and Ritchie, 1984
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*vitattum*	2n=23(22+X)	Jackson and Cheung, 1967
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*vittatum*	2n=23(22+X)	John and Freeman, 1974
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*vittatum*	2n=23(22+X)	John and Freeman, 1975
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*vittatum*	2n=23(22+X)	Rowe and Westerman, 1974

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*vittatum*	2n=23(22+X)	Webb and Westerman, 1978
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*vittatum*	2n=23(22+X)	Westerman and Dempsey, 1977
Acrididae	Catantopinae	Catantopini	*Phaulacridium*	*vittatum*	2n=23(22+X)	Rees et al., 1978
Acrididae	Catantopinae	Catantopini	*Rectitropis*	*sp.*	2n=23(22+X)	King and John, 1980
Acrididae	Catantopinae	Catantopini	*Stenocatantops*	*splendens*	2n=23(22+X)	Singh, 2006
Acrididae	Catantopinae	Catantopini	*Xenocatantops*	*humilis*	2n=23(22+X)	Singh, 2006
Acrididae	Catantopinae	Mesambriini	*Traulia*	*ornata*	2n=23(22+X)	Momma, 1943
Acrididae	Catantopinae	n.a.	*Choroedocus*	*robustus*	2n=23(22+X)	Singh, 2006
Acrididae	Catantopinae	n.a.	*Cryptocatantops*	*simlae*	2n=23(22+X)	Singh, 2006
Acrididae	Catantopinae	n.a.	*Oxycatantops*	*spissus*	2n=23(22+X)	Seino and Dongmo, 2013
Acrididae	Catantopinae	n.a.	*Oxycatantops*	*spissus*	2n=23(22+X)	Seino et al., 2008
Acrididae	Catantopinae	n.a.	*Sygrus*	*rehni*	2n=24(23+X)	White, 1967
Acrididae	Catantopinae	n.a.	*Sygrus*	*sp.*	2n=23(22+X)	White, 1967
Acrididae	Copiocerinae	Aleuasini	*Aleuas*	*gracilis*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Copiocerinae	Aleuasini	*Aleuas*	*lineatus*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Copiocerinae	Aleuasini	*Aleuas*	*sp. 1*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Copiocerinae	Aleuasini	*Aleuas*	*sp. 2*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Copiocerinae	Aleuasini	*Aleuas*	*sp. 3*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Copiocerinae	Aleuasini	*Aleuas*	*vitticollis*	2n=19(18+X)	Mesa et al., 1982
Acrididae	Copiocerinae	Aleuasini	*Zygoclistron*	*falconium*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Copiocerinae	Aleuasini	*Zygoclistron*	*nasicum*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Copiocerinae	Aleuasini	*Zygoclistron*	*trachystictum*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Copiocerinae	Clematodini	*Bucephalacris*	*bohlsii*	2n=21(20+X)	Sylvester and Blackmon, 2019
Acrididae	Copiocerinae	Clematodini	*Bucephalacris*	*bohlsii*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Copiocerinae	Clematodini	*Bucephalacris*	*bohlsii*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Copiocerinae	Clematodini	*Bucephalacris*	*sp.*	2n=21(20+X)	da Costa et al., 2014

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Copiocerinae	Copiocerini	*Adimantus*	*cubiceps*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Copiocerinae	Copiocerini	*Adimantus*	*ornatissimus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Coptacrinae	n.a.	*Coptacra*	*foetada*	2n=23(22+X)	Momma, 1943
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Anacridium*	*aegyptium*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Anacridium*	*aegyptium*	2n=23(22+X)	Abdel-Haleem et al., 2009
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Anacridium*	*aegyptium*	2n=23(22+X)	Santos et al., 1983
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Chondracris*	*rosea*	2n=23(22+X)	Singh, 2006
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Cyrtacanthacris*	*tatarica*	2n=23(22+X)	Koli et al., 2013
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Cyrtacanthacris*	*tatarica*	2n=23(22+X)	Singh, 2006
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Patanga*	*japonica*	2n=23(22+X)	Inoue, 1985
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Patanga*	*japonica*	2n=23(22+X)	Sannomiya, 1962
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Patanga*	*japonica*	2n=23(22+X)	Sannomiya, 1964
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Patanga*	*succincta*	2n=23(22+X)	Singh, 2006
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*americana*	2n=23(22+X)	Perez-Gelabert, 1988
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*cancellata*	2n=23(22+X)	John and Hewitt, 1966
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*cancellata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*cancellata*	2n=23(22+X)	Sylvester and Blackmon, 2019

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*flavofasciata*	2n=23(22+X)	de Souza and Melo, 2007
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*flavofasciata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*flavofasciata*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*gregaria*	2n=23(22+X)	Camacho et al., 2015
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*gregaria*	2n=23(22+X)	John and Hewitt, 1966
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*nitens nitens*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*pallens*	2n=23(22+X)	de Souza and Melo, 2007
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*pallens*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*paranensis*	2n=23(22+X)	John and Hewitt, 1966
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*paranensis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*quisqueya*	2n=23(22+X)	Perez-Gelabert, 1988
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*gregaria*	2n=23(22+X)	Fox, 1970
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca*	*gregaria*	2n=23(22+X)	Wilmore and Brown, 1975
Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Valanga*	*irregularis*	2n=23(22+X)	King and John, 1980
Acrididae	Cyrtacanthacridinae	n.a.	*Acanthacris*	*sp.*	2n=23(22+X)	Berhanu et al., 2012

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Egnatiinae	Egnatiini	*Egnatius*	*apicalis*	2n=23(22+X)	Bugrov, 1988
Acrididae	Egnatiinae	Egnatiini	*Ferganacris*	*mushketovi*	2n=23(22+X)	Sergeev and Bugrov, 1988
Acrididae	Euryphyminae	n.a.	*Amblyphymus*	*adspersus*	2n=23(22+X)	Fossey, 1991
Acrididae	Euryphyminae	n.a.	*Amblyphymus*	*roseus*	2n=23(22+X)	Fossey, 1991
Acrididae	Euryphyminae	n.a.	*Aneuryphymus*	*erythropus*	2n=23(22+X)	Fossey, 1991
Acrididae	Euryphyminae	n.a.	*Calliptamicus*	*semiroseus*	2n=23(22+X)	Fossey, 1991
Acrididae	Euryphyminae	n.a.	*Calliptamus*	*abbreviatus*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Euryphyminae	n.a.	*Euryphymus*	*tuberculatus*	2n=23(22+X)	Fossey, 1991
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*alacris*	2n=23(22+X)	Singh, 2006
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Abdelaziz et al., 2007
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Bakkali et al., 1999
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Bakkali et al., 2010
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Bakkali et al., 2002
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Cabrero et al., 1987
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Cabrero et al., 2003
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Cabrero et al., 1996
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Cabrero et al., 2014
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Cabrero et al., 2009
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Camacho, 2016

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Camacho et al., 2003
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Camacho et al., 1980
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Camacho et al., 2011
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Camacho et al., 2015
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Camacho et al., 1997
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Cano et al., 1986
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Cano et al., 1987
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Clemente et al., 2004
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Dziubenko et al., 2006
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Gonsalvez et al., 1980a
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Henriques-Gil and Arana, 1990
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Henriques-Gil et al., 1983
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Henriques-Gil et al., 1984
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Henriques-Gil et al., 1989
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Henriques-Gil et al., 1984
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Henriques-Gil et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Henriques-Gil et al., 1983
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Herrera et al., 1996
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-Fernández et al., 1992
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-León et al., 1991
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Lopez-Leon et al., 1991
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-León et al., 1995
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-León et al., 1996
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-León et al., 2008
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-León et al., 1993
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-León et al., 1994
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-León et al., 1992
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	López-León et al., 1992
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Manrique-Poyato et al., 2015
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Manrique-Poyato et al., 2013
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Manrique-Poyato et al., 2006
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Montiel et al., 2014

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Montiel et al., 2015
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Munoz et al., 1998
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Munoz-Pajares et al., 2011
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Perfectti et al., 2004
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Rebollo et al., 1998
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Riera et al., 2004
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Ruíz-Estévez et al., 2013
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Rufas et al., 1989
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Ruiz-Estévez et al., 2014
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Ruiz-Estevez et al., 2012
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Ruiz-Estévez et al., 2015
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Ruiz-Ruano et al., 2011
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Santos et al. 1983
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Suja et al., 1989
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Teruel et al., 2007
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Teruel et al., 2009a

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*rosea*	2n=23(22+X)	Singh, 2006
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*roseus*	2n=23(22+X)	Chatterjee et al., 1971
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*plorans*	2n=23(22+X)	Ruiz-Ruano et al., 2011
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Eyprepocnemis*	*unicolor*	2n=23(22+X)	Bugrov, 1988
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Heteracris*	*littoralis*	2n=23(22+X)	Cano and Santos, 1988
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Heteracris*	*littoralis*	2n=23(22+X)	Cano and Santos, 1989
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Heteracris*	*littoralis*	2n=23(22+X)	Rebollo et al., 1998
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Heteracris*	*littoralis*	2n=23(22+X)	Santos et al., 1983
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Heteracris*	*adspersus*	2n=23(22+X)	Gosalvez et al., 1980
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Shirakiacris*	*shirakii*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Shirakiacris*	*shirakii*	2n=23(22+X)	Inoue, 1985
Acrididae	Eyprepocnemidinae	n.a.	*Tylotropidius*	*varicornis*	2n=23(22+X)	Singh, 2006
Acrididae	Eyprepocnemidinae	n.a.	*Tylotropidus*	*voricornis*	2n=23(22+X)	Goswami et al., 1982
Acrididae	Gomphocerinae	Acrolophitini	*Acrolophitus*	*hirtipes*	2n=23(22+X)	DBW unpublished
Acrididae	Gomphocerinae	Acrolophitini	*Acrolophitus*	*maculipennis*	2n=23(22+X)	DBW unpublished
Acrididae	Gomphocerinae	Acrolophitini	*Bootettix*	*argentatus*	2n=23(22+X)	DBW unpublished
Acrididae	Gomphocerinae	Acrolophitini	*Bootettix*	*joerni*	2n=23(22+X)	DBW unpublished

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Amblytropidiini	*Amblytropidia*	*australis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Amblytropidiini	*Amblytropidia*	*australis*	2n=23(22+X)	Remis, 1989
Acrididae	Gomphocerinae	Amblytropidiini	*Apolobamba*	*prope pulchra*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Gomphocerinae	Amblytropidiini	*Apolobamba*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Amblytropidiini	*Fenestra*	*bohlsii*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Amblytropidiini	*Peruvia*	*nigromarginata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Amblytropidiini	*Sinipta*	*acuta*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Amblytropidiini	*Sinipta*	*dalmani*	2n=23(22+X)	Bidau, 1988
Acrididae	Gomphocerinae	Amblytropidiini	*Sinipta*	*dalmani*	2n=23(22+X)	Colombo and Remis, 1997
Acrididae	Gomphocerinae	Amblytropidiini	*Sinipta*	*dalmani*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Amblytropidiini	*Sinipta*	*dalmani*	2n=23(22+X)	Remis, 2008
Acrididae	Gomphocerinae	Amblytropidiini	*Sinipta*	*maldonai*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Amblytropidiini	*Syrbula*	*admirabilis*	2n=23(22+X)	Robertson, 1908
Acrididae	Gomphocerinae	Arcypterini	*Arcyptera*	*fusca*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Arcypterini	*Arcyptera*	*fusca*	2n=23(22+X)	Gosalvez et al., 1980a
Acrididae	Gomphocerinae	Arcypterini	*Arcyptera*	*fusca*	2n=23(22+X)	Gosálvez and López-Fernández, 1984
Acrididae	Gomphocerinae	Arcypterini	*Arcyptera*	*fusca*	2n=23(22+X)	Lopez-Fernandez and Gosalvez, 1983
Acrididae	Gomphocerinae	Arcypterini	*Arcyptera*	*fusca*	2n=23(22+X)	López-Fernández and García de la Vega, 1983

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Arcypterini	*Arcyptera*	*fusca*	2n=23(22+X)	Suja et al., 1991
Acrididae	Gomphocerinae	Arcypterini	*Arcyptera*	*microptera*	2n=23(22+X)	Bugrov, 1996
Acrididae	Gomphocerinae	Arcypterini	*Arcyptera*	*tornosi*	2n=23(22+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Aulocarini	*Psoloessa*	*thamnogaea*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Gomphocerinae	Chrysochraotini	*Chloealtis*	*gracilis*	2n=17(16+X)	Rentz and Weissman, 1981
Acrididae	Gomphocerinae	Chrysochraotini	*Chrysochraon*	*dispar*	2n=17(16+X)	Kiknadze and Vyotskaya, 1970
Acrididae	Gomphocerinae	Chrysochraotini	*Chrysochraon*	*dispar*	2n=17(16+X)	Fletcher and Hewitt, 1980
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*albolineatus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*chopardi*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*chopardi*	2n=17(16+X)	Cano and Santos, 1990
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*elegantulus gallicus*	2n=17(16+X)	Riva et al., 1984
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*pulvinatus*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*pulvinatus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*pulvinatus*	2n=17(16+X)	Cano and Santos, 1990
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*pulvinatus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*unicolor*	2n=17(16+X)	Lin et al., 2015
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*unicolor*	2n=17(16+X)	Bugrov, 1996

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Chrysochraotini	*Euchorthippus*	*dahingalingensis*	2n=17(16+X)	Yang et al., 2012
Acrididae	Gomphocerinae	Chrysochraotini	*Euthystira*	*brachyptera*	2n=17(16+X)	Fletcher and Hewitt, 1980
Acrididae	Gomphocerinae	Chrysochraotini	*Euthystira*	*brachyptera*	2n=17(16+X)	Kiknadze and Vyotskaya, 1970
Acrididae	Gomphocerinae	Chrysochraotini	*Euthystira*	*brachyptera*	2n=17(16+X)	Fletcher and Hewitt, 1988
Acrididae	Gomphocerinae	Chrysochraotini	*Mongolotettix*	*japonicus*	2n=17(16+X)	Inoue, 1985
Acrididae	Gomphocerinae	Chrysochraotini	*Mongolotettix*	*japonicus*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Chrysochraotini	*Neopodismopsis*	*abdominalis*	2n=19(18+X)	Rothfels, 1950
Acrididae	Gomphocerinae	Chrysochraotini	*Neopodismopsis*	*abdominalis*	2n=19(18+X)	Rothfels and Procunier, 1975
Acrididae	Gomphocerinae	Chrysochraotini	*Podismopsis*	*altaica*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Chrysochraotini	*Podismopsis*	*genicularibus*	2n=17(16+X)	Inoue, 1985
Acrididae	Gomphocerinae	Chrysochraotini	*Podismopsis*	*jacuta*	2n=17(16+X)	Bugrov et al., 1987
Acrididae	Gomphocerinae	Chrysochraotini	*Podismopsis*	*poppiusi*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Chrysochraotini	*Podismopsis*	*ussuriensis*	2n=17(16+X)	Bugrov et al., 1987
Acrididae	Gomphocerinae	Cibolacrini	*Cibolacris*	*parviceps*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Gomphocerinae	Cibolacrini	*Cibolacris*	*weissmani*	2n=23(22+X)	DBW unpublished
Acrididae	Gomphocerinae	Compsacrini	*Notopomala*	*glaucipes*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Compsacrini	*Silvitettix*	*concolor*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Compsacrini	*Staurorhectus*	*longicornis*	2n=23(22+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*brevicollis*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*crassiusculus*	2n=23(22+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*genei*	2n=23(22+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*genei*	2n=23(22+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*hispanicus*	2n=23(22+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*kraussi*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*maroccanus*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*maroccanus*	2n=23(22+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*plotnikovi*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Dociostaurini	*Dociostaurus*	*tartarus*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Dociostaurini	*Eremippus*	*comatus*	2n=17(16+X)	Bugrov et al., 1993
Acrididae	Gomphocerinae	Dociostaurini	*Eremippus*	*foveolatus*	2n=17(16+X)	Sergeev and Bugrov, 1988
Acrididae	Gomphocerinae	Dociostaurini	*Eremippus*	*miramae*	2n=17(16+X)	Bugrov et al., 1993
Acrididae	Gomphocerinae	Dociostaurini	*Eremippus*	*mistshenkoi*	2n=19(18+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Dociostaurini	*Eremippus*	*onerosus*	2n=17(16+X)	Bugrov et al., 1993
Acrididae	Gomphocerinae	Dociostaurini	*Eremippus*	*simplex*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Dociostaurini	*Eremippus*	*sobolevi*	2n=19(18+X)	Sergeev and Bugrov, 1990
Acrididae	Gomphocerinae	Dociostaurini	*Mizonocara*	*kusnetzovae*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Dociostaurini	*Mizonocara*	*saksinae*	2n=23(22+X)	Bugrov, 1996
Acrididae	Gomphocerinae	Dociostaurini	*Mizonocara*	*uvarovi*	2n=20(XY)	Bugrov, 1988
Acrididae	Gomphocerinae	Dociostaurini	*Notostaurus*	*albicornis*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Dociostaurini	*Notostaurus*	*popovi*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Eritettigini	*Amphitornus*	*coloradus ornatus*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Gomphocerinae	Eritettigini	*Opeia*	*obscura*	2n=23(22+X)	DBW unpublished
Acrididae	Gomphocerinae	Gomphocerini	*Aeropedellus*	*baliolus*	2n=23(22+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Aeropedellus*	*clavatus*	2n=23(22+X)	Hamrick and Hamrick, 1989

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Gomphocerini	*Aeropedellus*	*variegatus*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*albomarginatus*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*albomarginatus*	2n=17(16+X)	Gusachenko et al., 1992
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*albomarginatus*	2n=17(16+X)	Lin et al., 2015
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*angulatus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*apicalis*	2n=17(16+X)	Belda et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*apicalis*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*apicalis*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*bicolor*	2n=17(16+X)	Momma, 1943
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*biguttulus*	2n=17(16+X)	This study
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*biguttulus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*biguttulus*	2n=17(16+X)	Kiknadze and Vyotskaya, 1970
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*binotatus*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*binotatus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*binotatus binotatus*	2n=17(16+X)	Belda et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*bornhalmi*	2n=17(16+X)	Çakmak and Koca, 2014
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*brunneus*	2n=17(16+X)	Bridle et al., 2002
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*brunneus*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*brunneus*	2n=17(16+X)	Gonsalvez et al., 1980a
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*brunneus*	2n=17(16+X)	Inoue, 1985
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*brunneus*	2n=17(16+X)	John and Hewitt, 1966
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*brunneus*	2n=17(16+X)	Wilmore and Brown, 1975
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*dichrous*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*dorsatus*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*dorsatus*	2n=17(16+X)	This study
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*fallax*	2n=17(16+X)	Bugrov et al., 1987

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*ferghanensis*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*fumatus*	2n=17(16+X)	Inoue, 1985
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*hammarstroemi*	2n=21(20+X)	Kiknadze and Vyotskaya, 1970
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*indus*	2n=17(16+X)	Singh, 2003
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*indus*	2n=17(16+X)	Singh, 2006
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*intermedius*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*jacobsi*	2n=17(16+X)	Bridle et al., 2002
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*jacobsoni*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*jucundus*	2n=17(16+X)	Belda et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*jucundus*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*jucundus*	2n=17(16+X)	Cano and Santos, 1990
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*jucundus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*latipennis*	2n=17(16+X)	Inoue, 1985
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*longicornis*	2n=17(16+X)	Gonsalvez et al., 1980a
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*loratus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*macrocerus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*montanus*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*nevadensis*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*parallelus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*parallelus*	2n=17(16+X)	Belda et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*parallelus*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*parallelus*	2n=17(16+X)	Cano and Santos, 1990
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*parallelus*	2n=17(16+X)	John and Hewitt, 1966
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*parallelus*	2n=17(16+X)	Petitpierre, 1996
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*parallelus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*parallelus*	2n=17(16+X)	Wilmore and Brown, 1975

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*saxatilis*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*vagans*	2n=17(16+X)	Belda et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*vagans*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*vagans*	2n=17(16+X)	Cano and Santos, 1990
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*vagans*	2n=17(16+X)	Gosalvez et al., 1980
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*vagans*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus*	*vicinus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Dasyhippus*	*barbipes*	2n=23(22+X)	Bugrov et al., 1987
Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerippus*	*rufus*	2n=17(16+X)	Bugrov et al., 1987
Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus*	*sibiricus*	2n=17(16+X)	Gusachenko et al., 1993
Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus*	*sibiricus*	2n=17(16+X)	Gonsalvez et al., 1980a
Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus*	*sibiricus*	2n=17(16+X)	Gosalvez and Lopez-Fernandez, 1981
Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus*	*sibiricus*	2n=17(16+X)	López-Fernández et al., 1986
Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus*	*sibiricus*	2n=17(16+X)	Gosalvez et al., 1980
Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus*	*sibiricus*	2n=19(18+X)	Yang et al., 2012
Acrididae	Gomphocerinae	Gomphocerini	*Mesasippus*	*kozhevnikovi*	2n=23(22+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Mesasippus*	*tarbagataicus*	2n=23(22+X)	Sergeev and Bugrov, 1988
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Barker, 1960
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Barker, 1966
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Bull and Hewitt, 1979
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Camacho et al., 2011
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Dover and Henderson, 1976
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Fletcher and Hewitt, 1988
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Fox et al., 1974
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Gallagher et al., 1972

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Gibson, 1973
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Gibson and Hewitt, 1970
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Gibson and Hewitt, 1972
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Harvey and Hewitt, 1979
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt, 1973
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt, 1972
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt, 1976
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt and Brown, 1970
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt and East, 1978
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt and John, 1967
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt and John, 1970
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt et al., 1987
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Hewitt and Ruscoe, 1971
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	John and Hewitt, 1966
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	John and Hewitt, 1965
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Ramel 1969
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Ramel, 1980
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Robinson and Hewitt, 1976
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Shaw et al., 1983
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Shaw, 1983
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Shaw, 1984
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Shaw and Hewitt, 1984
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Shaw and Hewitt, 1985
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Shaw et al., 1985
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Southern, 1967

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Wilmore and Brown, 1975
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Petitpierre, 1996
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*maculatus*	2n=17(16+X)	Wilmore and Brown, 1975
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*pallidus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Myrmeleotettix*	*palpalis*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Gomphocerini	*Pezohippus*	*callosus*	2n=23(22+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Schmidtiacris*	*schmidti*	2n=23(22+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Gomphocerini	*Stauroderus*	*scalaris*	2n=17(16+X)	Cabrero and Camacho, 1985
Acrididae	Gomphocerinae	Gomphocerini	*Stauroderus*	*scalaris*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Gomphocerini	*Stauroderus*	*scalaris*	2n=17(16+X)	Vysotskaya, 1979
Acrididae	Gomphocerinae	Gomphocerini	*Stauroderus*	*scalaris*	2n=17(16+X)	Corey, 1933
Acrididae	Gomphocerinae	Hypernephiini	*Eclipophleps*	*bogdanovi*	2n=17(16+X)	Bold et al., 2016
Acrididae	Gomphocerinae	Hypernephiini	*Eclipophleps*	*glacialis*	2n=17(16+X)	Bugrov, 1994
Acrididae	Gomphocerinae	Mermiriini	*Mermiria*	*bivittata*	2n=22(21+X)	McClung, 1917
Acrididae	Gomphocerinae	n.a.	*Brachycrotaphus*	*tryxalicerus*	2n=23(22+X)	Santos et al., 1983
Acrididae	Gomphocerinae	n.a.	*Dnopherula*	*sp.*	2n=23(22+X)	Singh, 2006
Acrididae	Gomphocerinae	Ochrilidini	*Gonista*	*bicolor*	2n=23(22+X)	Inoue, 1985
Acrididae	Gomphocerinae	Ochrilidini	*Gonista*	*bicolor*	2n=23(22+X)	Sannomiya, 1973
Acrididae	Gomphocerinae	Ochrilidini	*Gonista*	*sagitta*	2n=23(22+X)	Bugrov, 1996
Acrididae	Gomphocerinae	Ochrilidini	*Ochrilidia*	*hebetata*	2n=23(22+X)	Bugrov, 1996
Acrididae	Gomphocerinae	Orphulellini	*Isonyx*	*paraguayensis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Orphulellini	*Isonyx*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Orphulellini	*Laplatacris*	*dispar*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Orphulellini	*Orphulella*	*concinnula*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Orphulellini	*Orphulella*	*punctata*	2n=17(16+X)	Sáez, 1930
Acrididae	Gomphocerinae	Orphulellini	*Orphulella*	*punctata*	2n=23(22+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Orphulellini	*Orphulella*	*punctata*	2n=23(22+X)	Perez-Gelabert, 1988
Acrididae	Gomphocerinae	Orphulellini	*Orphulella*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Orphulellini	*Orphulina*	*pulchella*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Orpulellini	*Dichromorpha*	*australis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Ramburiellini	*Ramburiella*	*bolivari*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Ramburiellini	*Ramburiella*	*foveolata*	2n=23(22+X)	Bugrov, 1988
Acrididae	Gomphocerinae	Ramburiellini	*Ramburiella*	*hispanica*	2n=23(22+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Ramburiellini	*Ramburiella*	*hispanica*	2n=23(22+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Ramburiellini	*Ramburiella*	*turcomana*	2n=23(22+X)	Bugrov, 1996
Acrididae	Gomphocerinae	Scyllinini	*Euplectrotettix*	*conspersus*	2n=23(22+X)	Vilardi, 1986b
Acrididae	Gomphocerinae	Scyllinini	*Euplectrotettix*	*shultzi*	2n=23(22+X)	Vilardi, 1986b
Acrididae	Gomphocerinae	Scyllinini	*Euplectrotettix*	*sp.*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Gomphocerinae	Scyllinini	*Euplectrotettix*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Euplectrotettix*	*sp. 2*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Euplectrotettix*	*sp. 3*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Meloscirtus*	*montanus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Meloscirtus*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Parapellopedon*	*instabilis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Parapellopedon*	*sp.*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Gomphocerinae	Scyllinini	*Pellopedon*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Rhammatocerus*	*brasiliensis*	2n=23(22+X)	Loreto et al., 2008
Acrididae	Gomphocerinae	Scyllinini	*Rhammatocerus*	*cyanipes*	2n=23(22+X)	Perez-Gelabert, 1988
Acrididae	Gomphocerinae	Scyllinini	*Rhammatocerus*	*brasiliensis*	2n=23(22+X)	Oliveira et al., 2011
Acrididae	Gomphocerinae	Scyllinini	*Scyllinops*	*brunneri*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Scyllinops*	*pallida*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Scyllinops*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Scyllinini	*Scyllinops*	*sp. 2*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Scyllinula*	*humilis*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Scyllinula*	*humilis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Scyllinula*	*signatipennis*	2n=23(22+X)	Bidau, 1984
Acrididae	Gomphocerinae	Scyllinini	*Scyllinula*	*signatipennis*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Scyllinula*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Scyllinini	*Stereotettix*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*bolivari*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*bolivari*	2n=17(16+X)	Santos et al. 1983
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*bolivari*	2n=17(16+X)	Viseras and Camacho, 1985
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*burri*	2n=17(16+X)	Rebollo et al., 1998
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*burri*	2n=17(16+X)	Santos et al., 1993
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*haemmorrhoidalis*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*llorenteae*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*minutissimus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*panteli*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*panteli*	2n=17(16+X)	Cano and Santos, 1990
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*panteli*	2n=17(16+X)	Santos et al., 1983
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*petraeus*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*raymondi*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*viridulus*	2n=17(16+X)	John and Hewitt, 1966
Acrididae	Gomphocerinae	Stenobothrini	*Omocestus*	*viridulus*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*carbonarius*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*eurasius*	2n=16(XY)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*festivus*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*festivus*	2n=17(16+X)	Santos et al., 1983

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*fischeri*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*grammicus*	2n=17(16+X)	Cabrero and Camacho, 1986
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*lineatus*	2n=17(16+X)	Bugrov and Vysotskaya, 1981
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*newskii*	2n=17(16+X)	Bugrov et al., 1991
Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus*	*stigmaticus*	2n=17(16+X)	Santos et al., 1983
Acrididae	Hemiacridinae	Hieroglyphini	*Hieroglyphus*	*banian*	2n=23(22+X)	Koli et al., 2013
Acrididae	Hemiacridinae	Hieroglyphini	*Parahieroglyphus*	*biliniatus*	2n=23(22+X)	Koli et al., 2013
Acrididae	Hemiacridinae	Hieroglyphini	*Parahieroglyphus*	*biliniatus*	2n=23(22+X)	Yaday and Yaday, 1987
Acrididae	Leptysminae	Leptysmini	*Belosacris*	*coccineipes*	2n=23(22+X)	Mesa and Fontanetti, 1983
Acrididae	Leptysminae	Leptysmini	*Belosacris*	*coccineipes*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Leptysmini	*Carbonellacris*	*grossa*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Leptysmini	*Cylindrotettix*	*obscurus*	2n=23(22+X)	Colombo, 1989
Acrididae	Leptysminae	Leptysmini	*Cylindrotettix*	*obscurus*	2n=23(22+X)	Confalonieri and Bidau, 1986
Acrididae	Leptysminae	Leptysmini	*Cylindrotettix*	*santarosae*	2n=23(22+X)	Confalonieri and Bidau, 1986
Acrididae	Leptysminae	Leptysmini	*Leptysma*	*argentina*	2n=21(20+X)	Bidau and Hasson, 1984
Acrididae	Leptysminae	Leptysmini	*Leptysma*	*argentina*	2n=21(20+X)	Colombo, 1989
Acrididae	Leptysminae	Leptysmini	*Leptysma*	*argentina*	2n=21(20+X)	Colombo and Remis, 1997
Acrididae	Leptysminae	Leptysmini	*Leptysma*	*dorsalis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Leptysmini	*Leptysma*	*sp.*	2n=13(12+X)	Colombo and Remis, 1997
Acrididae	Leptysminae	Leptysmini	*Leptysmina*	*pallida*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Leptysmini	*Leptysmina*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Leptysmini	*Stenacris*	*megacephala*	2n=23(22+X)	Mesa and Fontanetti, 1983
Acrididae	Leptysminae	Tetrataeniini	*Cornop*	*aquaticum*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Cornop*	*frenatum*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Eumastusia*	*koebelei*	2n=23(22+X)	Anjos et al., 2016
Acrididae	Leptysminae	Tetrataeniini	*Eumastusia*	*koebelei*	2n=23(22+X)	Mesa and Fontanetti, 1983

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Leptysminae	Tetrataeniini	*Haroldgrantia*	*ligenosa*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Mastusia*	*quadricarinata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Oxyblepta*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Oxybleptella*	*sagitta*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Stenopola*	*bohlsii*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Stenopola*	*boliviana*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Stenopola*	*dorsalis*	2n=23(22+X)	Mesa and Fontanetti, 1983
Acrididae	Leptysminae	Tetrataeniini	*Stenopola*	*dorsalis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Stenopola*	*pallida*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Stenopola*	*rubrifons*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Leptysminae	Tetrataeniini	*Stenopola*	*rubrifrons rubrifrons*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Leptysminae	Tetrataeniini	*Tetrataenia*	*surinama*	2n=19(18+X)	Mesa et al., 1982
Acrididae	Marelliinae	n.a.	*Marellia*	*remipes*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Marelliinae	n.a.	*Marellia*	*remipes*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dactylotini	*Dactylotum*	*sp.*	2n=17(16+X)	Helwig, 1942
Acrididae	Melanoplinae	Dactylotini	*Perixerus*	*squamipennis*	2n=21(20+X)	Helwig, 1942
Acrididae	Melanoplinae	Dichroplini	*Apacris*	*rubrithorax*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Apacris*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Atrachelacris*	*olivaceus*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Atrachelacris*	*unicolor*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Baeacris*	*punctulatus*	2n=21(20+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dichroplini	*Chlorus*	*bolivanus*	2n=19(18+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Chlorus*	*borellii*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Chlorus*	*sp. 1*	2n=19(18+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Chlorus*	*vittatus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichromatos*	*corupa*	2n=21(20+X)	Sylvester and Blackmon, 2019

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Melanoplinae	Dichroplini	*Dichromatos*	*lilloanus*	2n=21(20+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dichroplini	*Dichromatos*	*montanus*	2n=21(20+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dichroplini	*Dichromatos*	*schrottkyi*	2n=21(20+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*alejomesai*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*auriventris*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*bergi*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*conspersus*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*democraticus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*dubius*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Remis et al., 2004
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Loray et al., 1991
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Remis and Vilardi, 1986
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Remis et al., 2004
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Remis et al., 1998
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Rosetti and Remis, 2013
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Rosetti et al., 2010
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Rosetti et al., 2007
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Rosetti et al., 2008
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Sequeira et al., 1995
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*elongatus*	2n=23(22+X)	Vilardi, 1985
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*exilis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*fuscus*	2n=19(18+X)	Mesa *et al.*, 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*fuscus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*fuscus*	2n=23(22+X)	Taffarel et al., 2015
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*maculeipennis*	2n=22(20+XY)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*maculeipennis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*mantiqueirae*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*misionensis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*obscurus*	2n=18(16+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*paraelongatus*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*paraguayensis*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*paralongatus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*patruelis*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*piceomaculatus*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*porteri*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*pratensis*	2n=18(16+XY)	Bidau, 1986
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*pratensis*	2n=18(16+XY)	Bidau, 1987
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*pratensis*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*pseudopunctatus*	2n=23(22+X)	Bidau, 1988
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*pseudopunctulatus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*punctulatus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*robustulus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*robustus*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*schulzi*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*silveiraguidoi*	2n=8(6+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*sp. 11*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*sp. 12*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*sp. 13*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*sp. 14*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*sp. 15*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*sp. 16*	2n=22(20+XY)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*vittatus*	2n=18(16+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*vittatus*	2n=20(18+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*vittigerum*	2n=18(16+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*exilis*	2n=23(22+X)	Castillo et al., 2017
Acrididae	Melanoplinae	Dichroplini	*Dichroplus*	*intermedius*	2n=23(22+X)/2n=27(26+X)	Castillo et al., 2017
Acrididae	Melanoplinae	Dichroplini	*Eurotettix*	*lilloanus*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Eurotettix*	*minor*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Eurotettix*	*schrottkyi*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Eurotettix*	*sp. 1*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Eurotettix*	*sp. 2*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*flavipes*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*politus*	2n=13(12+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*politus*	2n=14(12+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*pulcher*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*sanguineus*	2n=22(20+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*sanguineus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*sp. 1*	2n=15(14+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*sp. 2*	2n=18(16+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*sp. 3*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Leiotettix*	*viridis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Neopedies*	*brunneri*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Neopedies*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Neopedies*	*sp. 2*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Neopedies*	*sp. 3*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Neopedies*	*sp. 4*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*nigrigena*	2n=23(22+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*sp. 2*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*sp. 3*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*sp. 4*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*sp. 5*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*sp. 6*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*sp. 7*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Pseudoscopas*	*sp. 8*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Ronderosia*	*dubius*	2n=21(20+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dichroplini	*Ronderosia*	*robustus*	2n=21(20+X)	Sylvester and Blackmon, 2019
Acrididae	Melanoplinae	Dichroplini	*Ronderosia*	*ommexechoides*	2n=20+neoXY	Carbonell and Mesa, 2006
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*cliens*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*daguerrei*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*delicatula*	2n=16(14+XY)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*deliculata*	2n=16(14+XY)	Mesa, 1964
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*impudica*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*lemiscata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*lemniscata*	2n=23(22+X)	Saez and Solari, 1959
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*liebermanni*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*liebermanni*	2n=21(20+X)	Mesa and Zolessi, 1968
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*sp.*	2n=21(20+X)	Mesa, 1960
Acrididae	Melanoplinae	Dichroplini	*Scotussa*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Jivarini	*Nahuelia*	*rubriventris*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Melanoplini	*Hesperotettix*	*brevipennis*	2n=23(22+X)	McClung, 1917
Acrididae	Melanoplinae	Melanoplini	*Hesperotettix*	*festivus*	2n=23(22+X)	McClung, 1917
Acrididae	Melanoplinae	Melanoplini	*Hesperotettix*	*pratensis*	2n=23(22+X)	McClung, 1917

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Melanoplinae	Melanoplini	*Hesperotettix*	*speciosus*	2n=23(22+X)	McClung, 1917
Acrididae	Melanoplinae	Melanoplini	*Hesperotettix*	*viridis*	2n=18-26	McClung, 1917
Acrididae	Melanoplinae	Melanoplini	*Hesperotettix*	*pacificus pacificus*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*borealis*	2n=23(22+X)	White, 1973
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*devastator*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*differentialis*	2n=23(22+X)	Abdel-Hameed et al., 1970
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*differentialis*	2n=23(22+X)	White, 1973
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*femur-rubrum*	2n=23(22+X)	Klein, 1975
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*femur-rubrum*	2n=23(22+X)	Lucov and Nur, 1973
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*femur-rubrum*	2n=23(22+X)	Nur, 1977
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*femur-rubrum*	2n=23(22+X)	Nur, 1978
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*femur-rubrum*	2n=23(22+X)	Stephens and Bregman, 1972
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*fricki*	2n=23(22+X)	Weissman and Rentz, 1978
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*frigidus*	2n=23(22+X)	Gonsalvez et al., 1980a
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*frigidus*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*frigidus*	2n=23(22+X)	Gosalvez et al., 1980b
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*obespsolus*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*sanquinipes*	2n=23(22+X)	White, 1973
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*bivittatus*	2n=23(22+X)	Nowlin, 1908
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*cyaneus paficicus*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*differentialis*	2n=23(22+X)	Hanrahan and Johnston, 2011
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*differentialis*	2n=23(22+X)	Rasch unpubl.
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*differentialis*	2n=23(22+X)	Swift and Kleinfeld, 1953
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*frigidus*	2n=23(22+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Melanoplini	*Melanoplus*	*sanguinipes*	2n=23(22+X)	Zhan et al., 1984
Acrididae	Melanoplinae	Melanoplini	*Oedaleonotus*	*borcki*	2n=23(22+X)	Weissman and Rentz, 1978

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Melanoplinae	Melanoplini	*Oedaleonotus*	*enigma*	2n=21(20+X)	Hewitt and Schroeter, 1968
Acrididae	Melanoplinae	Melanoplini	*Oedaleonotus*	*enigma*	2n=21(20+X)	Schroeter and Hewitt, 1972
Acrididae	Melanoplinae	Melanoplini	*Oedaleonotus*	*phryneicus*	2n=23(22+X)	Schroeter and Hewitt, 1974
Acrididae	Melanoplinae	Melanoplini	*Oedaleonotus*	*tenuipennis*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Melanoplinae	n.a.	*Parascopas*	*exertus*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Parascopas*	*obesus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Parascopas*	*sanguineus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Parascopas*	*similis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Pedies*	*andeanus*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Pedies*	*sp. 1*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Pedies*	*sp. 2*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Pedies*	*sp. 3*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Pedies*	*sp. 4*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Propedies*	*bilobus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Propedies*	*bipunctatus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Propedies*	*fusiformis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Propedies*	*olivaceus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Propedies*	*sanguineus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	n.a.	*Propedies*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Melanoplinae	Podismini	*Anapodisma*	*miramae*	2n=21(20+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Booneacris*	*glacialis*	2n=21(20+X)	Fontana and Vickery, 1976
Acrididae	Melanoplinae	Podismini	*Booneacris*	*variegata*	2n=21(20+X)	Fontana and Vickery, 1976
Acrididae	Melanoplinae	Podismini	*Dendrotettix*	*quercus*	2n=23(22+X)	Fontana and Vickery, 1976
Acrididae	Melanoplinae	Podismini	*Eirenephilus*	*longipennis*	2n=23(22+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Indopodisma*	*spp*	2n=21(20+X)	White, 1973
Acrididae	Melanoplinae	Podismini	*Miramella*	*alpina*	2n=21(20+X)	Bugrov et al., 1994

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Melanoplinae	Podismini	*Miramella*	*alpina*	2n=23(22+X)	Hewitt and John, 1972
Acrididae	Melanoplinae	Podismini	*Miramella*	*dairisama*	2n=23(22+X)	Momma, 1943
Acrididae	Melanoplinae	Podismini	*Miramella*	*sapporoensis*	2n=23(22+X)	Inoue, 1985
Acrididae	Melanoplinae	Podismini	*Miramella*	*solitaria*	2n=21(20+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Miramella*	*spp*	2n=21(20+X)	White, 1973
Acrididae	Melanoplinae	Podismini	*Niitakacris*	*spp*	2n=21(20+X)	White, 1973
Acrididae	Melanoplinae	Podismini	*Ognevia*	*longipennis*	2n=23(22+X)	Inoue, 1985
Acrididae	Melanoplinae	Podismini	*Ognevia*	*longipennis*	2n=23(22+X)	Momma, 1943
Acrididae	Melanoplinae	Podismini	*Ognevia*	*longipennis*	2n=23(22+X)	Kiknadze and Vyotskaya, 1970
Acrididae	Melanoplinae	Podismini	*Parapodisma*	*fauriei*	2n=21(20+X)	Inoue, 1985
Acrididae	Melanoplinae	Podismini	*Parapodisma*	*mikado*	2n=21(20+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Parapodisma*	*mikado*	2n=21(20+X)	Inoue, 1985
Acrididae	Melanoplinae	Podismini	*Parapodisma*	*mikado*	2n=21(20+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Parapodisma*	*setouchiensis*	2n=21(20+X)	Inoue, 1985
Acrididae	Melanoplinae	Podismini	*Parapodisma*	*subaptera*	2n=21(20+X)	Inoue, 1985
Acrididae	Melanoplinae	Podismini	*Podisma*	*carpentana ignatii*	2n=23(22+X)	Gonsalvez et al., 1980a
Acrididae	Melanoplinae	Podismini	*Podisma*	*kanoi*	2n=23(22+X)	Bugrov et al., 2007
Acrididae	Melanoplinae	Podismini	*Podisma*	*motodomariensis*	2n=21(20+X)	Helwig, 1942
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=21(20+X)	Hewitt, 1975
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Bella et al., 1991
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=21(20+X)	Hewitt and John, 1972
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Gonsalvez et al., 1980a
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Gonsalvez et al., 1980a
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Helwig, 1942
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Hewitt, 1975

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Hewitt and John, 1972
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Westerman and Hewitt, 1985
Acrididae	Melanoplinae	Podismini	*Podisma*	*sapporensis*	2n=23(22+X)	Helwig, 1942
Acrididae	Melanoplinae	Podismini	*Podisma*	*sapporensis*	2n=20(XY)	Bugrov, 1995
Acrididae	Melanoplinae	Podismini	*Podisma*	*sapporensis*	2n=23(22+X)	Bugrov, 1995
Acrididae	Melanoplinae	Podismini	*Podisma*	*sapporensis*	2n=23(22+X)	Natori, 1932
Acrididae	Melanoplinae	Podismini	*Podisma*	*sapporensis*	2n=23(22+X)	Warchalowska-Sliwa et al., 2008
Acrididae	Melanoplinae	Podismini	*Podisma*	*sapporoensis*	2n=23(22+X)	Momma, 1943
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=22/23	Westerman et al., 1987
Acrididae	Melanoplinae	Podismini	*Podisma*	*pedestris*	2n=23(22+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Prumna*	*hayachinensis*	2n=23(22+X)	Inoue, 1985
Acrididae	Melanoplinae	Podismini	*Prumna*	*littoralis*	2n=23(22+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Prumna*	*plana*	2n=23(22+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Prumna*	*primnoides*	2n=23(22+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Prumna*	*primnoides*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Melanoplinae	Podismini	*Prumna*	*primonoa*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Melanoplinae	Podismini	*Prumna*	*ussuriensis*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Melanoplinae	Podismini	*Pseudoparapodisma*	*niihamensis*	2n=21(20+X)	Inoue, 1985
Acrididae	Melanoplinae	Podismini	*Zubovskya*	*koeppeni koeppeni*	2n=21(20+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Zubovskya*	*koeppeni parvula*	2n=21(20+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Zubovskya*	*koreana*	2n=21(20+X)	Bugrov et al., 1994
Acrididae	Oedipodinae	Acrotylini	*Acrotylus*	*fischeri*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Acrotylini	*Acrotylus*	*humbertianus*	2n=23(22+X)	Manna, 1954
Acrididae	Oedipodinae	Acrotylini	*Acrotylus*	*inficita*	2n=23(22+X)	Singh, 2006
Acrididae	Oedipodinae	Acrotylini	*Acrotylus*	*insubricus*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Acrotylini	*Acrotylus*	*insubricus*	2n=23(22+X)	Türkoglu and Koca, 2002

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Acrotylini	*Acrotylus*	*insubricus*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Acrotylini	*Acrotylus*	*humbertianus*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Oedipodinae	Acrotylini	*Acrotylus*	*insubricus*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Arphiini	*Arphia*	*conspersa ramona*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Arphiini	*Lactista*	*gibbosus*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Arphiini	*Leuronotina*	*orizabae*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Bryodemini	*Angaracris*	*barabensis*	2n=23(22+X)	Vysotskaya and Bugrov, 1987
Acrididae	Oedipodinae	Bryodemini	*Angaracris*	*barabensis*	2n=23(22+X)	Yang et al., 2012
Acrididae	Oedipodinae	Bryodemini	*Bryodema*	*gebleri*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Bryodemini	*Bryodema*	*heptapotamicum*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Bryodemini	*Bryodema*	*luctuosum*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Bryodemini	*Bryodemella*	*holdereri*	2n=23(22+X)	Vysotskaya and Bugrov, 1987
Acrididae	Oedipodinae	Bryodemini	*Bryodemella*	*holdereri*	2n=23(22+X)	Vyotskaya, 1986
Acrididae	Oedipodinae	Bryodemini	*Bryodemella*	*orientalis*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Bryodemini	*Bryodemella*	*semenovi*	2n=23(22+X)	White, 1954
Acrididae	Oedipodinae	Bryodemini	*Bryodemella*	*tuberculata*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Chortophagini	*Chimarocephala*	*pacifica*	2n=23(22+X)	Schroeter and Hewitt, 1974
Acrididae	Oedipodinae	Chortophagini	*Chimarocephala*	*pacifica*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Chortophagini	*Encoptolophus*	*pallidus*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Chortophagini	*Encoptolophus*	*robustus*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Chortophagini	*Shotwellia*	*isleta*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Epacromiini	*Ailopus*	*thalassinus*	2n=23(22+X)	Hubert Rees et al., 1978
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*japonicus*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*sp.*	2n=23(22+X)	Manna, 1954
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*sp.*	2n=23(22+X)	Ray-Chaudhuri and Guha, 1955
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*sp.*	2n=23(22+X)	Ray-Chaudhuri and Manna, 1951

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*strepens*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*strepens*	2n=23(22+X)	Suja et al., 1987
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*tamulus*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*tamulus*	2n=23(22+X)	Momma, 1943
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*tamulus*	2n=23(22+X)	Singh, 2006
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*thalassinus*	2n=23(22+X)	Bugrov, 1988
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*thalassinus*	2n=23(22+X)	King and John, 1980
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*thalassinus*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*thalassinus*	2n=23(22+X)	Viseras et al., 1991
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*thalassinus*	2n=23(22+X)	Yaday and Yaday, 1987
Acrididae	Oedipodinae	Epacromiini	*Aiolopus*	*sp.*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Oedipodinae	Epacromiini	*Epacromius*	*tergestinus*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Epacromiini	*Heteropternis*	*respondens*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Oedipodinae	Epacromiini	*Heteropternis*	*respondens*	2n=23(22+X)	Singh, 2006
Acrididae	Oedipodinae	Epacromiini	*Heteropternis*	*respondens*	2n=23(22+X)	Yaday and Yaday, 1987
Acrididae	Oedipodinae	Epacromiini	*Heteropternis*	*obscurella*	2n=23(22+X)	King and John, 1980
Acrididae	Oedipodinae	Epacromiini	*Paracinema*	*luculenta*	2n=23(22+X)	Seino and Akongnui, 2010
Acrididae	Oedipodinae	Epacromiini	*Paracinema*	*luculenta*	2n=23(22+X)	Seino and Dongmo, 2013
Acrididae	Oedipodinae	Epacromiini	*Paracinema*	*tricolor*	2n=23(22+X)	Berhanu et al., 2012
Acrididae	Oedipodinae	Epacromiini	*Paracinema*	*tricolor*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Hippiscini	*Agymnastus*	*ingens*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Hippiscini	*Camnula*	*pellucida*	2n=23(22+X)	Carroll, 1920
Acrididae	Oedipodinae	Hippiscini	*Camnula*	*pellucida*	2n=23(22+X)	Nur, 1969
Acrididae	Oedipodinae	Hippiscini	*Camnula*	*pellucida*	2n=23(22+X)	Schroeter and Hewitt, 1974
Acrididae	Oedipodinae	Hippiscini	*Camnula*	*pellucida*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Hippiscini	*Cratypedes*	*neglectus*	2n=23(22+X)	DBW unpublished

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Hippiscini	*Hadrotettix*	*trifasciata*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Hippiscini	*Heliastus*	*subroseus*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Hippiscini	*Leprus*	*intermedius*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Hippiscini	*Sticthippus*	*californicus*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Hippiscini	*Xanthippus*	*corallipes*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Locustini	*Gastrimargus*	*africanus africanus*	2n=23(22+X)	Koli et al., 2013
Acrididae	Oedipodinae	Locustini	*Gastrimargus*	*africanus orientalis*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Oedipodinae	Locustini	*Gastrimargus*	*marmoratus*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Locustini	*Gastrimargus*	*marmoratus*	2n=23(22+X)	Momma, 1943
Acrididae	Oedipodinae	Locustini	*Gastrimargus*	*musicus*	2n=23(22+X)	King and John, 1980
Acrididae	Oedipodinae	Locustini	*Gastrimargus*	*sp.*	2n=23(22+X)	Berhanu et al., 2012
Acrididae	Oedipodinae	Locustini	*Gastrimargus*	*transversus*	2n=23(22+X)	Yaday and Yaday, 1987
Acrididae	Oedipodinae	Locustini	*Gastrimargus*	*wahlbergi*	2n=23(22+X)	Fossey and Liebenberg, 1991
Acrididae	Oedipodinae	Locustini	*Gonista*	*bicolor*	2n=23(22+X)	Sannomiya, 1974
Acrididae	Oedipodinae	Locustini	*Locusta*	*danica*	2n=23(22+X)	Itoh, 1934
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Bier & Müller 1969
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Cabrero et al., 1984
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Cabrero et al., 1985
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Camacho et al., 2004
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Castro et al., 1998
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Dearn, 1974
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Fox, 1970
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Itoh, 1938
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	John and Hewitt, 1966

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Hiroshi Kayano, 1971
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	King and John, 1980
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Lespinasse, 1973
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Lespinasse, 1977
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Lespinasse, 1981
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Lespinasse and Nicolas, 1975
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Nolte, 1967
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Nur, 1969
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Pardo et al., 1995
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Rasch, 1985
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Rebollo et al., 1998
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Rees et al., 1978
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Rees and Jamieson, 1954
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Ruiz-Ruano et al., 2015
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Ruiz-Ruano et al., 2011
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Salcedo et al., 1988
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Henriques-Gil et al., 1983
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Teruel et al., 2009b
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Teruel et al., 2010
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Viseras et al., 1990
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Viseras et al., 1988
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Wang et al. 2014
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Wilmore and Brown, 1975
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Bergerard and Seugé, 1959
Acrididae	Oedipodinae	Locustini	*Locusta*	*migratoria*	2n=23(22+X)	Bergerard et al., 1972
Acrididae	Oedipodinae	Locustini	*Oedaleus*	*abruptus*	2n=23(22+X)	Aswathanarayana, 2006

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Locustini	*Oedaleus*	*abruptus*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Oedipodinae	Locustini	*Oedaleus*	*australis*	2n=23(22+X)	King and John, 1980
Acrididae	Oedipodinae	Locustini	*Oedaleus*	*infernalis*	2n=23(22+X)	Takizawa and Narasawa, 1971
Acrididae	Oedipodinae	Locustini	*Oedalus*	*abruptus*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Oedipodinae	Locustini	*Oedalus*	*abruptus*	2n=23(22+X)	Yaday and Yaday, 1987
Acrididae	Oedipodinae	Locustini	*Oedalus*	*decorus*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Locustini	*Oedalus*	*decorus*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Locustini	*Oedalus*	*decorus*	2n=23(22+X)	Viseras et al., 1991
Acrididae	Oedipodinae	Locustini	*Oedalus*	*infernalis*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Locustini	*Psophus*	*stridulus*	2n=23(22+X)	Suja et al., 1986
Acrididae	Oedipodinae	Locustini	*Psophus*	*stridulus*	2n=23(22+X)	Kiknadze and Vyotskaya, 1970
Acrididae	Oedipodinae	Locustini	*Pyrgodera*	*armata*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Machaerocini	*Machaerocera*	*mexicana*	2n=16(14+XY)	Helwig, 1942
Acrididae	Oedipodinae	n.a.	*Chortoicetes*	*terminifera*	2n=17(16+X)	Fletcher and Hewitt, 1980
Acrididae	Oedipodinae	n.a.	*Chortoicetes*	*terminifera*	2n=23(22+X)	Gregg et al., 1984
Acrididae	Oedipodinae	n.a.	*Chortoicetes*	*terminifera*	2n=23(22+X)	Godfrey, 1971
Acrididae	Oedipodinae	n.a.	*Chortoicetes*	*terminifera*	2n=23(22+X)	Webb, 1976
Acrididae	Oedipodinae	n.a.	*Chortoicetes*	*terminifera*	2n=23(22+X)	Webb and Neuhaus, 1979
Acrididae	Oedipodinae	n.a.	*Humbe*	*tenuicornis*	2n=23(22+X)	John and Hewitt, 1966
Acrididae	Oedipodinae	n.a.	*Morphacris*	*fasciata*	2n=23(22+X)	da Costa et al., 2014
Acrididae	Oedipodinae	n.a.	*Morphacris*	*fasciata*	2n=23(22+X)	Seino et al., 2013
Acrididae	Oedipodinae	n.a.	*Pycnostictus*	*seriatus*	2n=23(22+X)	King and John, 1980
Acrididae	Oedipodinae	Oedipodini	*Celes*	*skalozubov*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Oedipodini	*Celes*	*skalozubovi akitanus*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Oedipodini	*Celes*	*variabilis*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Oedipodini	*Mioscirtus*	*wagneri*	2n=23(22+X)	Bugrov, 1996

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*caerulescens*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*caerulescens*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*caerulescens*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*caerulescens*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*charpentieri*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*germanica*	2n=23(22+X)	López-Fernández et al., 1989
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*germanica*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*miniata*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*miniata*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*schochi*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*schochi*	2n=25(24+X)	Türkoglu and Koca, 2002
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*fuscocincta*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Oedipodini	*Oedipoda*	*caerulescens*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Parapleurini	*Ceracris*	*nigricornis*	2n=23(22+X)	Manna, 1954
Acrididae	Oedipodinae	Parapleurini	*Mecostethus*	*alliaceus*	2n=23(22+X)	Fletcher and Hewitt, 1980
Acrididae	Oedipodinae	Parapleurini	*Mecostethus*	*alliaceus*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Parapleurini	*Mecostethus*	*alliaceus fastigiatus*	2n=23(22+X)	Momma, 1943
Acrididae	Oedipodinae	Parapleurini	*Mecostethus*	*magister*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Parapleurini	*Mecostethus*	*parapleurus*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Parapleurini	*Stethophyma*	*grossum*	2n=23(22+X)	Callan, 1941
Acrididae	Oedipodinae	Parapleurini	*Stethophyma*	*grossum*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Parapleurini	*Stethophyma*	*grossum*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Parapleurini	*Stethophyma*	*grossum*	2n=23(22+X)	Jones, 1971
Acrididae	Oedipodinae	Parapleurini	*Stethophyma*	*lineatum*	2n=23(22+X)	Shaw, 1971
Acrididae	Oedipodinae	Parapleurini	*Stethophyma*	*magister*	2n=23(22+X)	Bugrov, 1988
Acrididae	Oedipodinae	Psidiniini	*Derotmema*	*saussureanum*	2n=23(22+X)	Rentz and Weissman, 1981

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Psinidiini	*Psinidia*	*amplicornis*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Sphingonotini	*Eusphingonotus*	*japonicus*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Sphingonotini	*Heliopteryx*	*humeralis*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Helioscirtus*	*moseri*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Sphingonotini	*Hyalorrhipis*	*clausi*	2n=23(22+X)	Vysotskaya, 1993
Acrididae	Oedipodinae	Sphingonotini	*Microtes*	*nicola*	2n=23(22+X)	Weissman, 1984
Acrididae	Oedipodinae	Sphingonotini	*Microtes*	*nicola*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Sphingonotini	*Microtes*	*occidentalis*	2n=23(22+X)	Weissman, 1984
Acrididae	Oedipodinae	Sphingonotini	*Microtes*	*occidentalis*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Sphingonotini	*Pseudoceles*	*persa*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingoderus*	*carinatus*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingoderus*	*carinatus*	2n=23(22+X)	Vysotskaya, 1993
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*azurescens*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*azurescens*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*bey-bienkoi*	2n=23(22+X)	Vysotskaya, 1993
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*caerulans*	2n=23(22+X)	Gosálvez et al., 1985
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*caerulans*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*caerulans*	2n=23(22+X)	Santos et al., 1983
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*caerulans*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*caerulans*	2n=23(22+X)	Viseras et al., 1991
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*caerulans*	2n=23(22+X)	Gosálvez et al., 1986
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*coerulipes*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*eurasius*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*guanchus*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*haitensis*	2n=23(22+X)	Perez-Gelabert, 1988
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*indus*	2n=23(22+X)	Singh, 2006

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*maculatus*	2n=23(22+X)	Bugrov, 1988
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*nebulosus persa*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*obscuratus*	2n=23(22+X)	Bugrov, 1996
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*pilosus*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*rubescens*	2n=23(22+X)	This study
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*savignyi*	2n=23(22+X)	Vysotskaya, 1993
Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus*	*sp.*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Oedipodinae	Trilophidiini	*Trilophidia*	*annulata*	2n=23(22+X)	Singh, 2006
Acrididae	Oedipodinae	Trilophidiini	*Trilophidia*	*annulata japonica*	2n=23(22+X)	Inoue, 1985
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*carlianus*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*coconino*	2n=21(20+X)	Evans, 1954
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*coconino*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*coconino*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*crotalum*	2n=21(20+X)	Evans, 1954
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*crotalum*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*crotalum*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*inaculatus*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*lobatus*	2n=23(22+X)	Carothers, 1917
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*rabula*	2n=21(20+X)	Evans, 1954
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*rabula*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*rabula*	2n=21(20+X)	White, 1951a
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*rabula altior*	2n=21(20+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*shastanus*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*splendidus*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*stenometopus*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*undulatus*	2n=21(20+X)	Evans, 1954

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*undulatus*	2n=21(20+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*undulatus*	2n=21(20+X)	White, 1951a
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*undulatus*	2n=21(20+X)	White, 1973
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*undulatus*	2n=23(22+X), 2n=21(20+X)	Evans 1954; White 1949
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*undulatus thalassinus*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Circotettix*	*undulatus thalassinus*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Conozoa*	*sulcifrons sulcifrons*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Conozoa*	*wallula*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Trimerotropini	*Dissosteira*	*pictipennis*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Spharagemon*	*campestris*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Trimerotropini	*Spharagemon*	*clementina*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Spharagemon*	*cristatum*	2n=23(22+X)	DBW unpublished
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*acta*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*agrestis*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*agrestis hewitti*	2n=23(22+X)	Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*albescens*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*arenacea*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*bernardi*	2n=23(22+X)	Rentz and Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*bifasciata*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*bilobata*	2n=23(22+X)	John, 1973
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*bilobata*	2n=23(22+X)	Walters, 1968
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*bilobata*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*bilobata*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*californica*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*californica*	2n=23(22+X)	Rentz and Weissman, 1981

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*campestris*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*cincta*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*citrina*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*citrina*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*coeruleipennis*	2n=23(22+X)	King, 1923
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*cristata*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*cyaneipennis*	2n=21(20+X)	King, 1923
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*cyaneipennis*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*cyaneipennis*	2n=21(20+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*cyaneipennis*	2n=21(20+X)	White, 1973
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*cyaneipennis*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*cyaneipennis*	2n=23(22+X)	White, 1951a
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*diversellus*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*diversellus*	2n=23(22+X)	White, 1951a
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*diversellus*	2n=23(22+X)	White, 1973
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*fallax*	2n=23(22+X)	Carothers, 1917
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*fontana*	2n=23(22+X)	Coleman, 1948
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*fontana*	2n=23(22+X)	Coleman, 1948
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*fontana*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*fontana*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*fratercula*	2n=23(22+X)	Shaw et al., 1998
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*fratercula*	2n=23(22+X)	Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*fratercula x cyaneipennis*	2n=22	Shaw et al., 1998
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*gracilis*	2n=23(22+X)	White, 1973
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*gracilis gracilis*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*gracilis gracilis*	2n=23(22+X)	Weissman and Rentz, 1980

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*gracilis sordida*	2n=23(22+X)	Coleman, 1948
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*helferi*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*huroniana*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*inconspicua*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*inconspicua*	2n=23(22+X)	White, 1973
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*infantilis*	2n=23(22+X)	Rentz and Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*inyo*	2n=23(22+X)	Rentz and Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*koebelei*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*koebelei*	2n=23(22+X)	Weissman and Rentz, 1978
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*laticincta*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*latifasciata*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*latifasciata*	2n=23(22+X)	White, 1951a
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*lauta*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*leucophaea*	2n=23(22+X)	Rentz and Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*magnifica*	2n=23(22+X)	David B. Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*maritima*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*maritima*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*melanoptera*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*munda*	2n=23(22+X)	Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*occidentalis*	2n=21(20+X)	John and Weissman, 1977
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*occidentalis*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*occidentalis*	2n=21(20+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*occidentaloides*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*ochraceipennis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*ochraceipennis*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*ochraceipennis*	2n=23(22+X)	Weissman and Rentz, 1980

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pacifica*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pacifica*	2n=23(22+X)	Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Coleman, 1948
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Confalonieri, 1995
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Confalonieri, 1988
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Confalonieri, 1992
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	de Vaio et al., 1979
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Goni et al., 1985
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Mesa, 1971
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pallidipennis pallidipennis*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pistrinaria*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pogonata*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*praeclara*	2n=23(22+X)	Coleman, 1948
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pseudofasciata*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pseudofasciata*	2n=23(22+X)	Weissman, 1976
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*pseudofasciata*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*rebellis*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*santabarbara*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*saxatilis*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*schaefferi*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*sp.*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*sparsa*	2n=21(20+X)	Weissman and Rentz, 1980

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*sparsa*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*sparsa*	2n=23(22+X)	White, 1951b
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*sparsa*	2n=23(22+X)	White, 1951a
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*sparsa*	2n=23(22+X)	White, 1951c
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*sparsa*	2n=23(22+X), 2n=21(20+X)	White, 1951c
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*strenua*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa*	2n=23(22+X)	Carothers, 1917
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa*	2n=23(22+X)	Coleman, 1948
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa*	2n=23(22+X)	John et al., 1983
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa*	2n=23(22+X)	Wenrich, 1917
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa*	2n=23(22+X)	White, 1949
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa*	2n=23(22+X)	White, 1951a
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa*	2n=23(22+X)	White, 1973
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*suffusa x cyaneipennis*	2n=22	John et al., 1983
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*texana*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*thalassica*	2n=23(22+X)	John and Weissman, 1977
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*thalassica*	2n=23(22+X)	King, 1923
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*thalassica*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*thalassica*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*thalassica x occidentalis*	2n=22	John and Weissman, 1977
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*titusi*	2n=23(22+X)	Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*tolteca modesta*	2n=23(22+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*topanga*	2n=23(22+X)	Rentz and Weissman, 1981
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*verruculatus*	2n=21(20+X)	Helwig, 1955

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*verruculatus*	2n=21(20+X)	Weissman and Rentz, 1980
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*whitei*	2n=23(22+X)	Rentz and Weissman, 1984
Acrididae	Oedipodinae	Trimerotropini	*Trimerotropis*	*verruculatus*	2n=21(20+X)	Helwig, 1933
Acrididae	Oedipodinae	Tropidolophini	*Tropidolophus*	*formosus*	2n=23(22+X)	DBW unpublished
Acrididae	Ommatolampidinae	Abracrini	*Abracis*	*dilecta*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Abracis*	*flavolineata*	2n=23(22+X)	Milani and Cabral-de-Mello, 2014
Acrididae	Ommatolampidinae	Abracrini	*Abracis*	*sp.*	2n=21(20+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Abracrini	*Abracis*	*sp. B*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Abracris*	*flavolineata*	2n=23(22+X)	Bueno et al., 2013
Acrididae	Ommatolampidinae	Abracrini	*Abracris*	*flavolineata*	2n=23(22+X)	Menezes-de-Carvalho et al., 2015
Acrididae	Ommatolampidinae	Abracrini	*Abracris*	*flavolineata*	2n=23(22+X)	Palacios-Gimenez et al., 2014
Acrididae	Ommatolampidinae	Abracrini	*Eujivarus*	*fusiformis*	2n=21(20+X)	Rocha et al., 2011
Acrididae	Ommatolampidinae	Abracrini	*Eujivarus*	*fusiformis*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Eujivarus*	*sp.*	2n=21(20+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Abracrini	*Eujivarus*	*sp. A*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Eujivarus*	*sp. B*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Eujivarus*	*sp. C*	2n=21(20+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Ommatolampidinae	Abracrini	*Eujivarus*	*vittatus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Eusitalces*	*sp. A*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Eusitalces*	*vulneratus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Jodacris*	*chapadensis*	2n=19(18+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Jodacris*	*ferrugineus ferrugineus*	2n=19(18+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Jodacris*	*furcillata*	2n=19(18+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Jodacris*	*sp.*	2n=19(18+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Abracrini	*Omalotettix*	*obliquum*	2n=21(20+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Omalotettix*	*sp.*	2n=21(20+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Abracrini	*Orthoscapheus*	*rufipes*	2n=23(22+X)	de F. Rocha et al., 2011
Acrididae	Ommatolampidinae	Abracrini	*Osmilia*	*flavolineata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Psiloscirtus*	*bolivianus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Psiloscirtus*	*olivaceus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Psiloscirtus*	*sp. A*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Roppacris*	*griseipes*	2n=23(22+X)	Mesa and Fontanetti, 1983
Acrididae	Ommatolampidinae	Abracrini	*Sitalces*	*dorsalis*	2n=23(22+X)	Sylvester and Blackmon, 2019

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Ommatolampidinae	Abracrini	*Sitalces*	*infuscatus*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Ommatolampidinae	Abracrini	*Sitalces*	*sp.*	2n=19(18+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Abracrini	*Sitalces*	*volxemi*	2n=19(18+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Abracrini	*Xiphiola*	*borellii*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	n.a.	*Lagidacris*	*hebes*	2n=17(16+X)	Mesa and Fontanetti, 1983
Acrididae	Ommatolampidinae	n.a.	*Lagidacris*	*sp.*	2n=17(16+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Ommatolampidini	*Eulampiacris*	*leucoptera*	2n=23(22+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Ommatolampidini	*Eulampiacris*	*leucoptera*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Ommatolampidini	*Lamiacris*	*microguttata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Ommatolampidini	*Lamiacris*	*nigroguttata*	2n=23(22+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Ommatolampidini	*Machaeropeles*	*rostratum*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Ommatolampidini	*Muriciacris*	*triflavovittata*	2n=23(22+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Ommatolampidini	*Ommatolampis*	*perspicillata*	2n=23(22+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Ommatolampidini	*Ommatolampis*	*perspicillata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Ommatolampidini	*Peruana*	*palpata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Ommatolampidini	*Peruana*	*palpata*	2n=23(22+X)	da Costa et al., 2014

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Ommatolampidinae	Ommatolampidini	*Sitalces*	*dorsalis*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Ommatolampidini	*Sitalces*	*infuscatus*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Pycnosarcini	*Pycnosarcus*	*atavus*	2n=17(16+X)	Mesa et al., 1982
Acrididae	Ommatolampidinae	Pycnosarcini	*Pycnosarcus*	*sp.*	2n=17(16+X)	da Costa et al., 2014
Acrididae	Ommatolampidinae	Syntomacrini	*Syntomacrella*	*guyanensis*	2n=23(22+X)	Mesa and Fontanetti, 1983
Acrididae	Oxyinae	n.a.	*Caryanda*	*paravicinia*	2n=23(22+X)	Singh, 2006
Acrididae	Oxyinae	Oxyini	*Bermius*	*brachycerus*	2n=23(22+X)	King and John, 1980
Acrididae	Oxyinae	Oxyini	*Gesonula*	*punctifrons*	2n=23(22+X)	Momma, 1943
Acrididae	Oxyinae	Oxyini	*Gesonula*	*punctifrons*	2n=23(22+X)	Singh, 2006
Acrididae	Oxyinae	Oxyini	*Oxya*	*chinensis*	2n=23(22+X)	Bugrov, 1988
Acrididae	Oxyinae	Oxyini	*Oxya*	*fuscovittata*	2n=23(22+X)	Singh, 2006
Acrididae	Oxyinae	Oxyini	*Oxya*	*fuscovittata*	2n=23(22+X)	Bugrov, 1988
Acrididae	Oxyinae	Oxyini	*Oxya*	*hyla*	2n=23(22+X)	Singh, 2006
Acrididae	Oxyinae	Oxyini	*Oxya*	*intricata*	2n=23(22+X)	Momma, 1943
Acrididae	Oxyinae	Oxyini	*Oxya*	*japonica japonica*	2n=23(22+X)	Inoue, 1985
Acrididae	Oxyinae	Oxyini	*Oxya*	*jezoensis*	2n=23(22+X)	Momma, 1943
Acrididae	Oxyinae	Oxyini	*Oxya*	*nitidula*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Oxyinae	Oxyini	*Oxya*	*universalis*	2n=23(22+X)	Momma, 1943
Acrididae	Oxyinae	Oxyini	*Oxya*	*velox*	2n=23(22+X)	Chadha and Mehta, 2011
Acrididae	Oxyinae	Oxyini	*Oxya*	*yezoensis*	2n=23(22+X)	Inoue, 1985
Acrididae	Oxyinae	Oxyini	*Pseudoxya*	*diminuta*	2n=23(22+X)	Phimphan and Sangpakdee, 2018
Acrididae	Oxyinae	Oxyini	*Tolgadia*	*infirma*	2n=21(20+X)	John and Freeman, 1976
Acrididae	Pauliniinae	n.a.	*n.a.*	*n.a.*	2n=23(22+X)	White, 1973

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Acrididae	Pauliniinae	n.a.	*Paulinia*	*acuminata*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Pezzotettiginae	n.a.	*Pezotettix*	*giornae*	2n=23(22+X)	Santos et al., 1983
Acrididae	Proctolabinae	Coscineutini	*Coscineuta*	*virens*	2n=21(20+X)	Helwig, 1942
Acrididae	Proctolabinae	Proctolabini	*Eucephalacris*	*borellii*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Proctolabinae	Proctolabini	*Eucephalacris*	*borellii*	2n=23(22+X)	Sylvester and Blackmon, 2019
Acrididae	Rhytidochrotinae	n.a.	*Paropaon*	*laevifrons*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Rhytidochrotinae	n.a.	*Paropaon*	*pilosus tingomariae*	2n=23(22+X)	Mesa et al., 1982
Acrididae	Romaleinae	Romaleini	*Zoniopoda*	*tarsata*	2n=23(22+X)	Vilardi, 1986a
Acrididae	Spathosterninae	Spathosternini	*Spathosternum*	*prasiniferum*	2n=23(22+X)	Aswathanarayana, 2006
Acrididae	Spathosterninae	Spathosternini	*Spathosternum*	*prasiniferum*	2n=23(22+X)	Bhunya and Manna, 1968
Acrididae	Spathosterninae	Spathosternini	*Spathosternum*	*prasiniferum*	2n=23(22+X)	Bhunya and Manna, 1968
Acrididae	Spathosterninae	Spathosternini	*Spathosternum*	*prasiniferum*	2n=23(22+X)	Singh, 2006
Acrididae	Spathosterninae	Spathosternini	*Spathosternum*	*pygmaeum*	2n=23(22+X)	Seino and Dongmo, 2013
Acrididae	Tetriginae	Tetrigini	*Paratettix*	*sp*	2n=19(18+X)	Berhanu et al., 2012
Acrididae	Tetriginae	Tetrigini	*Paratettix*	*uvarovi*	2n=13(12+X)	Bugrov, 1996
Acrididae	Tropidopolinae	Tristriini	*Tristria*	*pulvinata*	2n=21(20+X)	Manna and Mazumder, 1965
Acrididae	Tropidopolinae	Tristriini	*Tristria*	*pulvinata*	2n=21(20+X)	Manna and Mazumder, 1967
Acrididae	Tropidopolinae	Tristriini	*Tristria*	*pulvinata*	2n=21(20+X)	Singh, 2006
Acrididae	Tropidopolinae	Tropidopolini	*Tropidopola*	*turanica*	2n=23(22+X)	Bugrov, 1988
Acrididae	Melanoplinae	Podismini	*Podisma*	*aberrans*	2n=23(22+X)	Bugrov et al., 1994
Acrididae	Melanoplinae	Podismini	*Podisma*	*carpentana ignatii*	2n=23(22+X)	Bella et al., 1991
Catantopidae	Melanoplinae	Podismini	*Appalachia*	*arcana*	2n=23(22+X)	Fontana and Vickery, 1976
Dericorythidae	Conophyminae	Conophymatini	*Bienkoa*	*fedtshenkoi*	2n=23(22+X)	Bugrov, 1988
Dericorythidae	Conophyminae	Conophymatini	*Conophyma*	*alaense haidarkenicum*	2n=23(22+X)	Sergeev and Bugrov, 1988

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Dericorythidae	Conophyminae	Conophymatini	*Conophyma*	*bolyrevi ambiguum*	2n=23(22+X)	Sergeev and Bugrov, 1988
Dericorythidae	Conophyminae	Conophymatini	*Conophyma*	*przewalski*	2n=23(22+X)	Bugrov, 1988
Dericorythidae	Conophyminae	Conophymatini	*Conophyma*	*semenovi*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Dericorythidae	Conophyminae	Conophymatini	*Conophyma*	*sokolovi*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Dericorythidae	Conophyminae	Conophymatini	*Conophyma*	*turkestanicum*	2n=23(22+X)	Sergeev and Bugrov, 1988
Dericorythidae	Conophyminae	Conophymatini	*Tarbinskia*	*kittaryi*	2n=23(22+X)	Bugrov, 1988
Dericorythidae	Dericorythinae	n.a.	*Dericorys*	*albidula*	2n=23(22+X)	Bugrov and Vysotskaya, 1981
Dericorythidae	Dericorythinae	n.a.	*Dericorys*	*annulata*	2n=23(22+X)	Bugrov, 1988
Dericorythidae	Dericorythinae	n.a.	*Dericorys*	*tibialis*	2n=23(22+X)	Bugrov, 1988
Episactidae	Teicophryinae	n.a.	*Teicophrys*	*californiae*	2n=17(16+X)	DBW unpublished
Eumastacidae	Biroellinae	n.a.	*n.a.*	*n.a.*	2n=17(16+X)	White, 1973
Eumastacidae	Chininae	n.a.	*n.a.*	*n.a.*	2n=14	White, 1973
Eumastacidae	Chorotypinae	n.a.	*n.a.*	*n.a.*	2n=21(20+X), 19	White, 1973
Eumastacidae	Eruciinae	n.a.	*n.a.*	*n.a.*	2n=21(20+X)	White, 1973
Eumastacidae	Eumastacinae	n.a.	*n.a.*	*n.a.*	2n=21(20+X)	White, 1973
Eumastacidae	Gomphomastacinae	n.a.	*Gomphomastax*	*clavata*	2n=19(18+X)	Vysotskaya, 1993
Eumastacidae	Gomphomastacinae	n.a.	*Gomphomastax*	*gussakovskii*	2n=19(18+X)	Bugrov, 1988
Eumastacidae	Gomphomastacinae	n.a.	*Gomphomastax*	*juniperi*	2n=21(20+X)	Bugrov, 1988
Eumastacidae	Gomphomastacinae	n.a.	*n.a.*	*n.a.*	2n=19(18+X)	White, 1973

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Eumastacidae	Gomphomastacinae	n.a.	*Phytomastax*	*artemisiana*	2n=19(18+X)	Bugrov, 1988
Eumastacidae	Gomphomastacinae	n.a.	*Phytomastax*	*opaca*	2n=19(18+X)	White, 1968
Eumastacidae	Gomphomastacinae	n.a.	*Clinomastax*	*ninae*	2n=19(18+X)	Bugrov, 1996
Eumastacidae	Mastacideinae	n.a.	*n.a.*	*n.a.*	2n=21(20+X)	White, 1973
Eumastacidae	Miraculinae	n.a.	*n.a.*	*n.a.*	2n=25(24+X)	White, 1973
Eumastacidae	Morabinae	n.a.	*n.a.*	*n.a.*	2n=17(16+X), 21, 19, 18, 16, 15, 13	White, 1973
Eumastacidae	Morseinae	Morseini	*Eumorsea*	*truncaticeps*	2n=23(22+X)	DBW unpublished
Eumastacidae	Morseinae	n.a.	*n.a.*	*n.a.*	2n=23(22+X)	White, 1973
Eumastacidae	Morseinae	Psychomastacini	*Psychomastax*	*robusta*	2n=23(22+X)	DBW unpublished
Eumastacidae	Paramastacinae	n.a.	*n.a.*	*n.a.*	2n=19(18+X)	White, 1973
Eumastacidae	Parepisactinae	n.a.	*n.a.*	*n.a.*	2n=19(18+X)	White, 1973
Eumastacidae	Pseudoschmidtiinae	n.a.	*n.a.*	*n.a.*	2n=21(20+X), 19	White, 1973
Eumastacidae	Teicophryinae	n.a.	*n.a.*	*n.a.*	2n=17(16+X)	White, 1973
Eumastacidae	Thericleinae	n.a.	*n.a.*	*n.a.*	2n=19(18+X), 17, 16	White, 1973
Lentulidae	Lentulinae	n.a.	*Basutacris*	*minuta*	2n=23(22+X)	White, 1967
Lentulidae	Lentulinae	n.a.	*Eremidium*	*denticerus*	2n=23(22+X)	White, 1967
Lentulidae	Lentulinae	n.a.	*Karruia*	*paradoxa*	2n=23(22+X)	White, 1967
Lentulidae	Lentulinae	n.a.	*Lentula*	*callani*	2n=23(22+X)	White, 1967
Lentulidae	Lentulinae	n.a.	*Mecostibus*	*nyassae*	2n=23(22+X)	White, 1967
Lentulidae	Lentulinae	n.a.	*Paralentula*	*marcida*	2n=23(22+X)	White, 1967
Lentulidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=23(22+X), 21, 20, 19	White, 1973
Lentulidae	Shelforditinae	n.a.	*Karruacris*	*browni*	2n=19/20(18/19+X)	White, 1967

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Lentulidae	Shelforditinae	n.a.	*Shelfordites*	*nanus*	2n=21(20+X)	White et al., 1967
Morabidae	Morabinae	Keyacridini	*Vandiemenella*	*viatica*	2n=17(16+X)	Mrongovius, 1979
Morabidae	Morabinae	Morabini	*Moraba*	*sp.*	2n=17(16+X)	White et al., 1967
Morabidae	Morabinae	Morabini	*Moraba*	*viatica*	2n=17(16+X)	White et al., 1964
Morabidae	Morabinae	Morabini	*Moraba*	*viatica*	2n=17(16+X)	White et al., 1967
Morabidae	Morabinae	Warramungini	*Warramaba*	*virgo*	2n=15(14+X)	White and Webb, 1968
Ommexechidae	Aucacridinae	Aucacridini	*Aucacris*	*bullocki*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Aucacridinae	Aucacridini	*Conometopus*	*sulcaticollis*	2n=25(24+X)	Mesa et al., 1982
Ommexechidae	Aucacridinae	Aucacridini	*Cumainocloidus*	*cordillerae*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Aucacridinae	Aucacridini	*Neuquenina*	*fictor*	2n=22(20+XY)	Mesa et al., 1982
Ommexechidae	Illapeliinae	n.a.	*Illapelia*	*penai*	2n=23(22+X)	Sylvester and Blackmon, 2019
Ommexechidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=23(22+X), 25	White, 1973
Ommexechidae	Ommexechinae	Ommexechini	*Calcitrena*	*maculosa*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Clarazella*	*bimaculata*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Clarazella*	*patagona*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Descampsacris*	*serrulata*	2n=23(22+X)	Mesa and Fontanetti, 1983
Ommexechidae	Ommexechinae	Ommexechini	*Descampsacris*	*serrulata*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Graea*	*horrida*	2n=23(22+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Ommexechidae	Ommexechinae	Ommexechini	*Ommexecha*	*germari*	2n=21(20+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Ommexecha*	*macroptera*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Ommexecha*	*virens*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Ommexecha*	*virens*	2n=23(22+X)	Souza et al., 2015
Ommexechidae	Ommexechinae	Ommexechini	*Pachyossa*	*signata*	2n=22(20+XY)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Spathalium*	*audouini*	2n=22(20+XY)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Tetrixocephalus*	*chilensis*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Tetrixocephalus*	*micropterus*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Tetrixocephalus*	*sergioi*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Tetrixocephalus*	*sp.n.*	2n=23(22+X)	Mesa et al., 1982
Ommexechidae	Ommexechinae	Ommexechini	*Tetrixocephalus*	*willemsei*	2n=22(20+XY)	Mesa et al., 1982
Pamphagidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=19(18+X)	White, 1973
Pamphagidae	Pamphaginae	Nocarodeini	*Nocaracris*	*cyanipes*	2n=18(XY)	Bugrov et al., 1991
Pamphagidae	Pamphaginae	Nocarodeini	*Nocaracris*	*rubripes*	2n=18(XY)	Bugrov et al., 1991
Pamphagidae	Pamphaginae	Pamphagini	*Eumigus*	*cucullatus*	2n=19(18+X)	Santos et al., 1983
Pamphagidae	Pamphaginae	Pamphagini	*Ocnerodes*	*brunneri*	2n=19(18+X)	Santos et al., 1983
Pamphagidae	Thrinchinae	Haplotropidini	*Haplotropis*	*brunneriana*	2n=19(18+X)	Bugrov, 1996
Pamphagidae	Thrinchinae	Thrinchini	*Melanotmethis*	*fuscipennis*	2n=19(18+X)	Bugrov, 1996
Pamphagidae	Thrinchinae	Thrinchini	*Pezotmethis*	*ferghanensis*	2n=19(18+X)	Bugrov, 1996

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Pamphagidae	Thrinchinae	Thrinchini	*Prionotropis*	*flexuosa*	2n=19(18+X)	Santos et al., 1983
Pamphagidae	Thrinchinae	Thrinchini	*Strumiger*	*desertorum*	2n=19(18+X)	Bugrov, 1996
Pamphagidae	Thrinchinae	Thrinchini	*Thrinchus*	*arenosus*	2n=19(18+X)	Bugrov, 1996
Pamphagidea	Thrinchinae	Thrinchini	*Asiotmethis*	*heptapotamicus*	2n=18(XY)	Bugrov, 1986
Pamphagidea	Thrinchinae	Thrinchini	*Asiotmethis*	*muricatus*	2n=19(18+X)	Bugrov, 1996
Pamphagidea	Thrinchinae	Thrinchini	*Asiotmethis*	*zacharjini*	2n=18(XY)	Bugrov, 1996
Pamphagidea	Thrinchinae	Thrinchini	*Atrichotmethis*	*semenovi*	2n=18(XY)	Bugrov, 1986
Pamphagodidae	n.a.	n.a.	*Charilaus*	*carinatus*	2n=23(22+X)	White, 1967
Pamphagodidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=23(22+X)	White, 1973
Pneumoridae	n.a.	n.a.	*n.a.*	*n.a.*	2n=23(22+X)	White, 1973
Proscopiidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=17(16+X), 19	White, 1973
Pyrgomorphidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=19(18+X), 18, 17, 15, 11	White, 1973
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*australis*	2n=19(18+X)	Nankivell, 1976
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*australis*	2n=19(18+X)	White, 1973
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*bedeli*	2n=19(18+X)	Sannomiya, 1964
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*bedeli*	2n=19(18+X)	Sannomiya, 1973
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*bedeli*	2n=19(18+X)	Sannomiya and Kayano, 1968
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*crenaticeps*	2n=19(18+X)	White, 1957
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*crenulata*	2n=19(18+X)	Singh, 2006

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*lata*	2n=19(18+X)	Inoue ,1985
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*lata*	2n=19(18+X)	Seino et al., 2013
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*similis*	2n=19(18+X)	King and John, 1980
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*similis*	2n=19(18+X)	Nankivell, 1976
Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha*	*similis*	2n=19(18+X)	Peters, 1984
Pyrgomorphidae	Pyrgomorphinae	Chrotogonini	*Chrotogonus*	*incertus*	2n=19(18+X)	Srivastava, 1954
Pyrgomorphidae	Pyrgomorphinae	Chrotogonini	*Chrotogonus*	*oxypterus*	2n=19(18+X)	Gururaj and Rajasekarasetty, 1967
Pyrgomorphidae	Pyrgomorphinae	Chrotogonini	*Chrotogonus*	*trachypterus*	2n=19(18+X)	Sharma et al., 1965
Pyrgomorphidae	Pyrgomorphinae	Chrotogonini	*Chrotogonus*	*trachypterus*	2n=19(18+X)	Sandhu and Chadha, 2012
Pyrgomorphidae	Pyrgomorphinae	Chrotogonini	*Chrotogonus*	*trachypterus*	2n=19(18+X)	Singh, 2006
Pyrgomorphidae	Pyrgomorphinae	Chrotogonini	*Chrotogonus*	*turanicus*	2n=19(18+X)	Bugrov, 1988
Pyrgomorphidae	Pyrgomorphinae	Dictyophorini	*Dictyophorus*	*griseus*	2n=19(18+X)	Seino et al., 2013
Pyrgomorphidae	Pyrgomorphinae	Monistriini	*Monistria*	*concinna*	2n=19(18+X)	King and John, 1980
Pyrgomorphidae	Pyrgomorphinae	Omurini	*Algete*	*brunneri*	2n=19(18+X)	Mesa and Fontanetti, 1983
Pyrgomorphidae	Pyrgomorphinae	Omurini	*Omura*	*congrua*	2n=19(18+X)	Mesa et al., 1982
Pyrgomorphidae	Pyrgomorphinae	Phymateini	*Phymateus*	*leprosus*	2n=19(18+X)	Motara, 1985

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Pyrgomorphidae	Pyrgomorphinae	Phymateini	*Zonocerus*	*variegatus*	2n=19(18+X)	Seino et al., 2013
Pyrgomorphidae	Pyrgomorphinae	Poekilocerini	*Poecilocera (=Poikilocerus)*	*pictus*	2n=19(18+X)	Koduru et al., 1985
Pyrgomorphidae	Pyrgomorphinae	Poekilocerini	*Poecilocera (=Poikilocerus)*	*pictus*	2n=19(18+X)	Rahiman and Rajasekarasetty, 1967
Pyrgomorphidae	Pyrgomorphinae	Poekilocerini	*Poekilocerus*	*pictus*	2n=19(18+X)	Goswami et al., 1982
Pyrgomorphidae	Pyrgomorphinae	Pyrgomorphini	*Pyrgomorpha*	*ambigua*	2n=19(18+X)	Momma, 1943
Pyrgomorphidae	Pyrgomorphinae	Pyrgomorphini	*Pyrgomorpha*	*conica*	2n=19(18+X)	Santos et al., 1983
Pyrgomorphidae	Pyrgomorphinae	Pyrgomorphini	*Pyrgomorpha*	*conica bispinosa*	2n=19(18+X)	Bugrov, 1988
Pyrgomorphidae	Pyrgomorphinae	Pyrgomorphini	*Pyrgomorpha*	*kraussi*	2n=19(18+X)	Lewis and John, 1959
Pyrgomorphidae	Pyrgomorphinae	Tagastini	*Tagasta*	*indica*	2n=19(18+X)	Singh, 2006
Pyrgomorphidae	Pyrgomorphinae	Taphronotini	*Taphronota*	*thaelephora*	2n=19(18+X)	Seino et al., 2013
Pyrgomorphidae	Pyrgomorphinae	Taphronotini	*Taphronota*	*thaelephora*	2n=19(18+X)	Seino et al., 2007
Romaleidae	Romaleinae	Chariacrini	*Chariacris*	*miniacea*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Elaeochlorini	*Agriacris*	*basalis*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Elaeochlorini	*Elaeochlora*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Elaeochlorini	*Staleochlora*	*pulchella brachyptera*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Elaeochlorini	*Staleochlora*	*trilineata*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Elaeochlorini	*Staleochlora*	*viridicata*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Phaeopariini	*Abila*	*bolivari*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Phaeoparinii	*Phaeoparia*	*lineaalba*	2n=23(22+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Romaleidae	Romaleinae	Phaeoparinii	*Phaeoparia*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Alcamenes*	*clarazianus*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Prionolopha*	*serrata*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Procolpia*	*minor*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Securigera*	*acutangula*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*attenuatus*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*discoideus*	2n=23(22+X)	Loreto et al., 2008
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*discoideus*	2n=23(22+X)	Machado et al., 2014
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*discoideus*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*discoideus angulatus*	2n=23(22+X)	Machado et al., 2014
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*gracilis*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*insignis*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*laevipes*	2n=22(20+XY)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*modestus*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*sp. 2*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Procolpini	*Xyleus*	*sp. 3*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Antandrus*	*viridis*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Chromacris*	*miles*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Chromacris*	*peruviana*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Chromacris*	*speciosa*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Coryacris*	*angustipennis*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Diponthus*	*clarazianus*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Diponthus*	*communis*	2n=22(20+XY)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Diponthus*	*dispar*	2n=21(20+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Diponthus*	*electus*	2n=21(20+X)	Mesa et al., 1982

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Romaleidae	Romaleinae	Romaleini	*Diponthus*	*maculiferus*	2n=21(20+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Diponthus*	*prope communis*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Diponthus*	*sp.*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Dracotettix*	*monstrosus*	2n=23(22+X)	Rentz and Weissman, 1981
Romaleidae	Romaleinae	Romaleini	*Dracotettix*	*newboldi*	2n=23(22+X)	DBW unpublished
Romaleidae	Romaleinae	Romaleini	*Dracotettix*	*plutonius*	2n=23(22+X)	DBW unpublished
Romaleidae	Romaleinae	Romaleini	*Litoscirtus*	*insularis*	2n=23(22+X)	Lightfoot and Weissman, 1991
Romaleidae	Romaleinae	Romaleini	*Litoscirtus*	*platynotus*	2n=23(22+X)	Lightfoot and Weissman, 1991
Romaleidae	Romaleinae	Romaleini	*Radacridium*	*mariajoseae*	2n=23(22+X)	Rocha et al., 1997
Romaleidae	Romaleinae	Romaleini	*Radacridium*	*nordestinum*	2n=23(22+X)	Rocha et al., 1997
Romaleidae	Romaleinae	Romaleini	*Spaniacris*	*deserticola*	2n=23(22+X)	DBW unpublished
Romaleidae	Romaleinae	Romaleini	*Tytthotyle*	*maculata*	2n=23(22+X)	DBW unpblished
Romaleidae	Romaleinae	Romaleini	*Xestotrachelus*	*robustus*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Zoniopoda*	*hempeli*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Zoniopoda*	*iheringi*	2n=21(20+X)	Sylvester and Blackmon, 2019
Romaleidae	Romaleinae	Romaleini	*Zoniopoda*	*juncorum*	2n=23(22+X)	Sylvester and Blackmon, 2019
Romaleidae	Romaleinae	Romaleini	*Zoniopoda*	*omnicolor*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Zoniopoda*	*similis*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Zoniopoda*	*sp. 1*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Zoniopoda*	*sp. 2*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Romaleini	*Zoniopoda*	*tarsata*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Tropidacrini	*Eutropidacris*	*collaris*	2n=23(22+X)	Mesa et al., 1982
Romaleidae	Romaleinae	Tropidacrini	*Tropidacris*	*collaris*	2n=23(22+X)	de França Rocha et al., 2015
Romaleidae	Romaleinae	Tropidacrini	*Tropidacris*	*cristata grandis*	2n=23(22+X)	de França Rocha et al., 2015
Tetrigidae	Batrachideinae	Batrachideini	*Tettigidea*	*lateralis*	2n=13(12+X)	Fontana and Vickery, 1973
Tetrigidae	Batrachideinae	Batrachideini	*Tettigidea*	*lateralis*	2n=13(12+X)	Fontana and Vickery, 1975

family	subfamily	tribe	genus	species	Chromosome number (male)	Reference
Tetrigidae	Batrachideinae	Batrachideini	*Tettigidea*	*parvipennis*	2n=13(12+X)	Robertson, 1917
Tetrigidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=13(12+X)	White, 1973
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*ceperoi*	2n=13(12+X)	Henderson, 1961
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*japonica*	2n=13(12+X)	Yung, 1965
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*subulata*	2n=13(12+X)	Henderson, 1961
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*subulata*	2n=13(12+X)	Bugrov, 1996
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*tartara*	2n=13(12+X)	Bugrov, 1996
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*tenuicornis*	2n=13(12+X)	Bugrov, 1996
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*tenuicornis*	2n=13(12+X)	Maryańska-Nadachowska and Warchałowska-Śliwa, 1991
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*tenuicornis*	2n=13(12+X)	Warchałowska-Śliwa et al., 2005
Tetrigidae	Tetriginae	Tetrigini	*Tetrix*	*undulata*	2n=13(12+X)	Henderson, 1961
Tridactylidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=13(12+X)	White, 1973
Trigonopterygidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=23(22+X)	White, 1973
Tristiridae	Atacamacridinae	n.a.	*Atacamacris*	*diminuta*	2n=20(18+XY)	Mesa et al., 1982
Tristiridae	Illapeliinae	n.a.	*Illapelia*	*penai*	2n=23(22+X)	Mesa et al., 1982
Tristiridae	Tristirinae	Elasmoderini	*Philippiacris*	*rabiosus*	2n=23(22+X)	Mesa et al., 1982
Tristiridae	Tristirinae	Tristirini	*Bufonacris*	*sp.*	2n=19(18+X)	Mesa et al., 1982
Tristiridae	Tristirinae	Tristirini	*Moluchacris*	*cinerascens*	2n=21(20+X)	Mesa et al., 1982
Tristiridae	Tristirinae	Tristirini	*Peplacris*	*recutita*	2n=21(20+X)	Mesa et al., 1982
Tristiridae	Tristirinae	Tropidosthetini	*Elysiacris*	*angusticollis*	2n=21(20+X)	Mesa et al., 1982
Tristiridae	Tristirinae	Tropidosthetini	*Tropidostethus*	*bicarinatus*	2n=21(20+X)	Mesa et al., 1982
Xyronotidae	n.a.	n.a.	*n.a.*	*n.a.*	2n=23(22+X)	White, 1973

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SI CHAPTER 5.4

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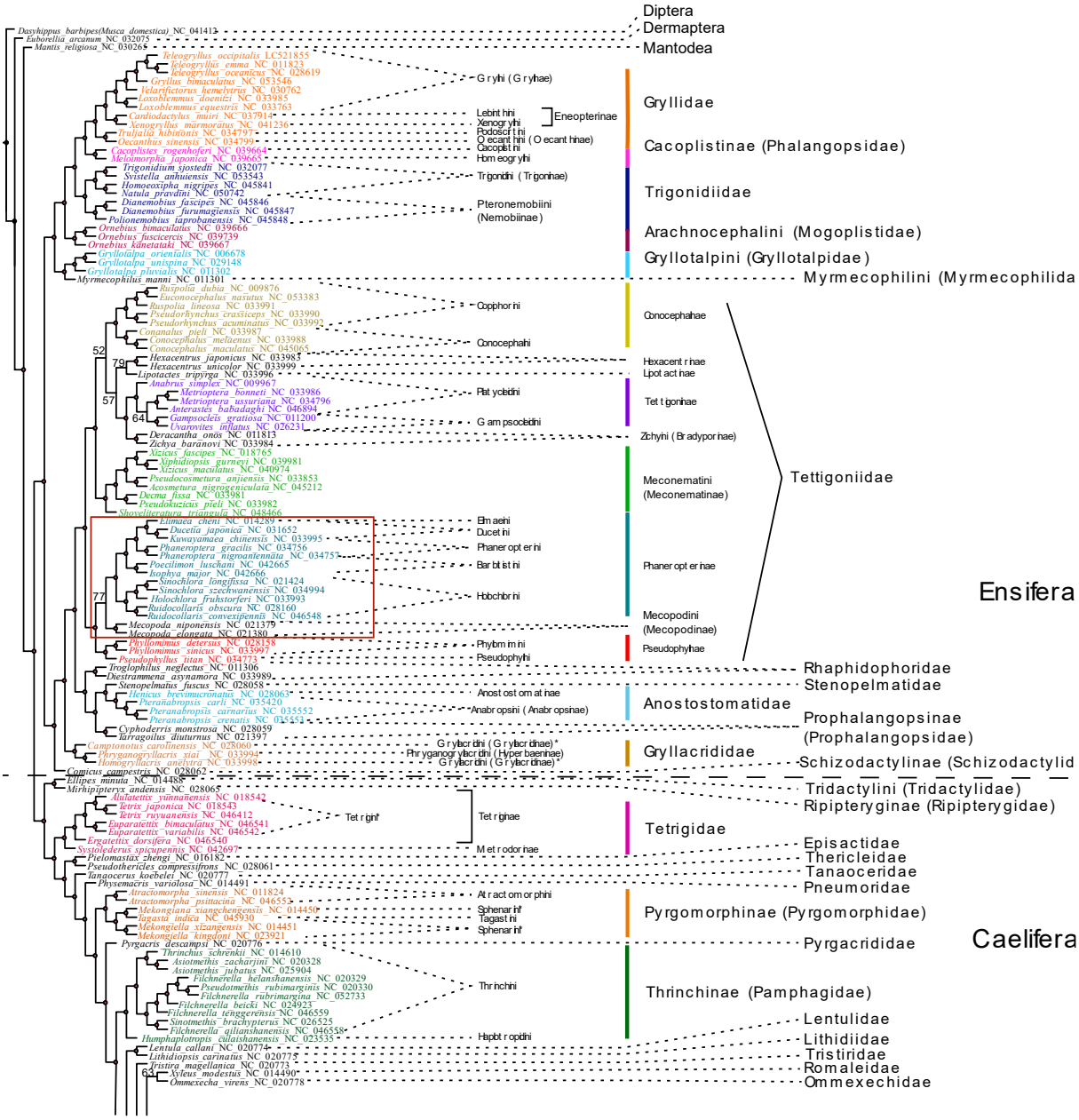
DOES (GENOME) SIZE MATTER?

Evolution of mitogenomic gene order in Orthoptera

Sarah Maria Gaugel, Oliver Hawlitschek, Lara-Sophie Dey & Martin Husemann

Figure S1. ML phylogenetic tree based on mitochondrial sequences inferred with IQTree. Numbers near the nodes indicate bootstrap values (BS). Red dots on nodes indicate a BS value > 90. Black dots near the root indicate unknown BS values. The taxon labels are highlighted in different colors assigned to major groups based on the currently accepted taxonomy (Obtained from Orthoptera Species File; Cigliano et al., 2022) which are shown on the right. The '*' sign is added to all clades which are considered either paraphyletic or polyphyletic by the 'r'sent results. No assigned tribe/subfamily implies that the taxon in question is currently not classified into any of the groups within a family. Tribes in blue letters: classified into Oedipodinae, tribes in red letters: classified into Gomphocerinae. Rectangles with red lines highlight some inconsistencies with BI topology. Editing was performed with Inkscape and Figtree software.


Diptera
Dermaptera
Mantodea
Gryllidae
Cacoplistinae (Phalangopsidae)
Trigonidiidae
Arachnocephalini (Mogoplistidae)
Gryllotalpini (Gryllotalpidae)
Myrmecophilini (Myrmecophilida
Tettigoniidae
Ensifera
Rhaphidophoridae
Stenopelmatidae
Anostostomatidae
Prophalangopsinae (Prophalangopsidae)
Schizodactylinae (Schizodactylid
Tridactylini (Tridactylidae)
Ripipteryginae (Ripipterygidae)
Tetrigidae
Episactidae
Thericleidae
Tanaoceridae
Pneumoridae
Pyrgomorphinae (Pyrgomorphidae)
Pyrgacrididae
Caelifera
Thrinchinae (Pamphagidae)
Lentulidae
Lithidiidae
Tristiridae
Romaleidae
Ommexechidae

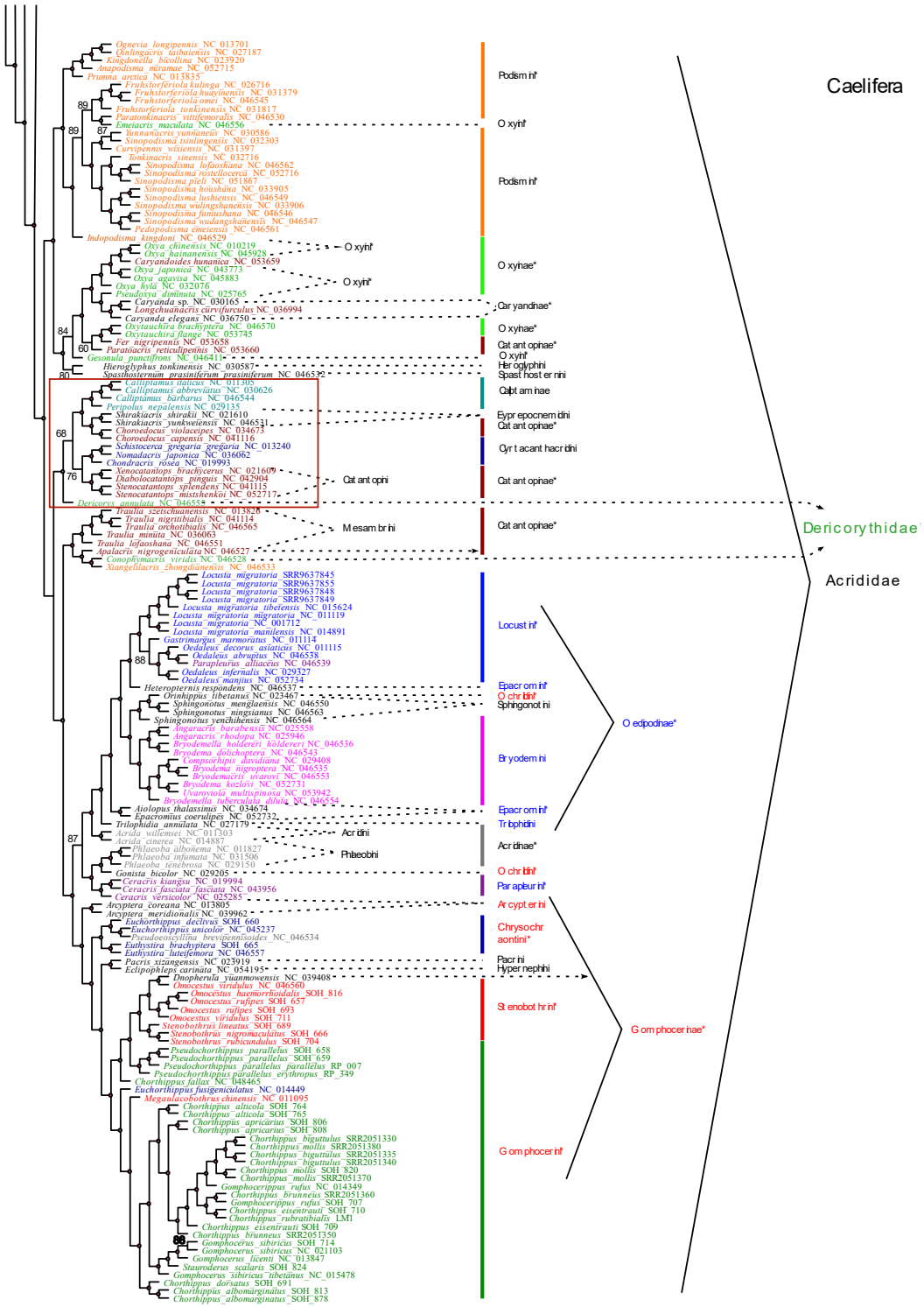
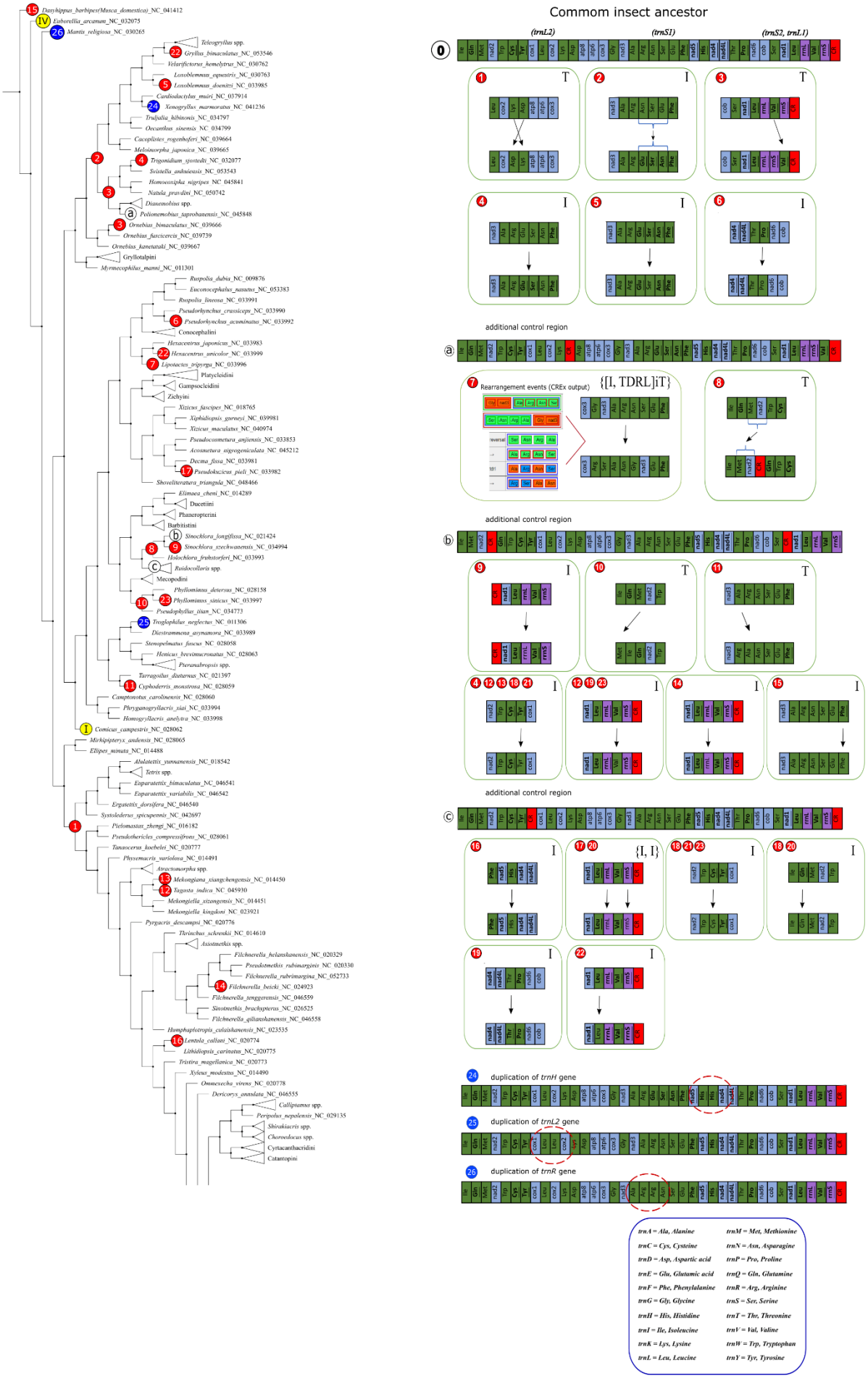
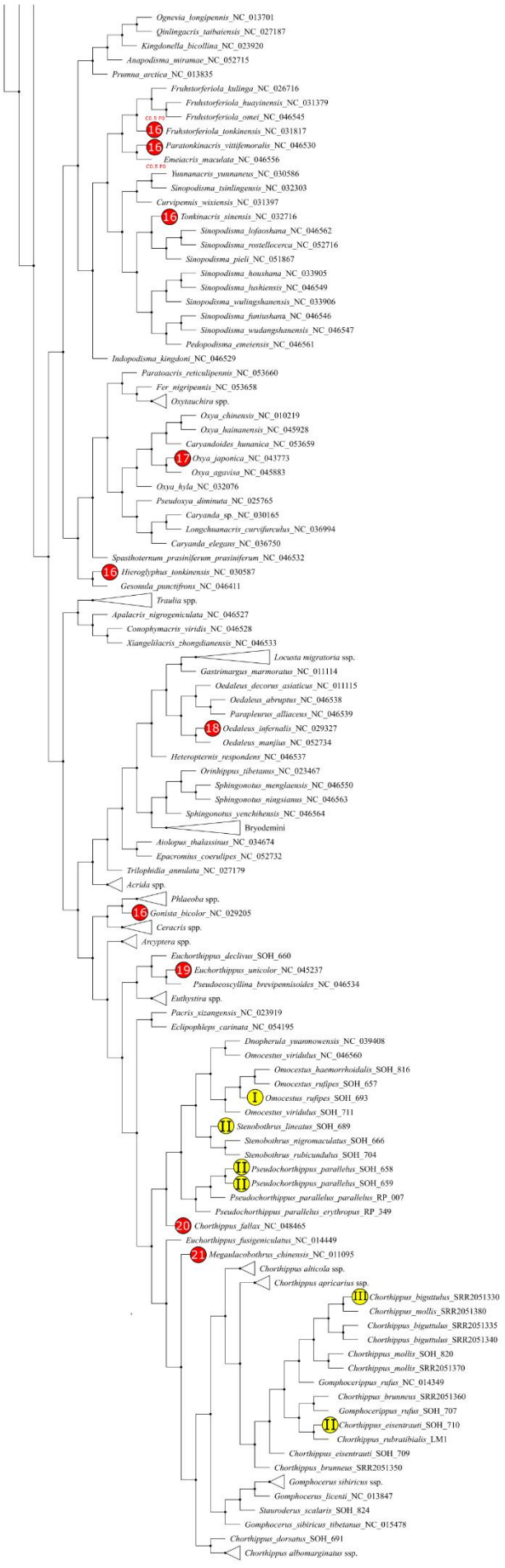

Caelifera
Dericorythidae
Acrididae
Podism inf
O xyinf
Podism inf
O xyinae*
Car yandinae*
O xyinae*
Cat ant opinae*
O xyinf
Her oglyphini
Spast host er nini
Capt am nae
Eypr epocnem idini
Cat ant opinae*
Cyr t acant hacr idini
Cat ant opinae*
Cat ant opinae*
Locust inf
Epacr om inf
O chr idinf
Sphingonot ni
Br yodem ini
Epacr om inf
Tr typhlini
Acr idinae*
O chr idinf
Par apleur inf
Ar cypt er ini
Chrysochr aontini*
Pacr ini
Hyper nephini
St enobot hr inf
G om phocer inf
O edipodinae*
G om phocer nae*

Figure S2. Complete phylogenetic reconstruction (Bayesian Inference) of Orthoptera from mitogenome data (left). MTR events predicted by TreeREx mapped on the tree (right). Numerals refer to rearrangement events of taxa; red circles: inversions, transpositions, inverse transpositions or TDRLs; blue circles: duplication; yellow circles: deletions or missing information; see Table S3 for those excluded taxa. Letters in white circles: novel control region position. Collapsed branches are indicated by triangles. Dots on nodes indicate a confidence level of 1 (= consistent). Underlined genes are encoded on the light strand. An output scheme inferred with CrEx is shown for visualization of multiple MTR events of *Lipotactes tripyrga*. Monophyletic genera occur as "Genus spp." monophyletic species as "ssp." Note that the MTR scenarios of *Euborellia arcanum* are not shown despite its multiple modifications, since it was excluded from the TreeREx analysis. Gene names are according to IUPAC IUB (Cornishbowden, 1984). The legend on the right translates amino acid coding gene names.


Commom insect ancestor
(trnL2)
(trnS1)
(trnS2, trnL1)
additional control region
Rearrangement events (CREx output)
{[I, TDRL]iT}
additional control region
additional control region
duplication of trnH gene
duplication of trnL2 gene
duplication of trnR gene
trnA = Ala, Alanine
trnC = Cys, Cysteine
trnD = Asp, Aspartic acid
trnE = Glu, Glutamic acid
trnF = Phe, Phenylalanine
trnG = Gly, Glycine
trnH = His, Histidine
trnI = Ile, Isoleucine
trnK = Lys, Lysine
trnL = Leu, Leucine
trnM = Met, Methionine
trnN = Asn, Asparagine
trnP = Pro, Proline
trnQ = Gln, Glutamine
trnR = Arg, Arginine
trnS = Ser, Serine
trnT = Thr, Threonine
trnV = Val, Valine
trnW = Trp, Tryptophan
trnY = Tyr, Tyrosine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Qinlingacris_taibaiensis_NC_027187
Kingdonella_bicollina_NC_023920
Anapodisma_miramae_NC_052715
Prumna_arctica_NC_013835
Fruhstorferiola_kulinga_NC_026716
Fruhstorferiola_huayinensis_NC_031379
Fruhstorferiola_omei_NC_046545
16 Fruhstorferiola_tonkinensis_NC_031817
16 Paratonkinacris_vittifemoralis_NC_046530
Emeiacris_maculata_NC_046556
Yunnanacris_yunnanens_NC_030586
Sinopodisma_tsinlingensis_NC_032303
Curvipennis_wixiensis_NC_031397
16 Tonkinacris_sinensis_NC_032716
Sinopodisma_lofaoshana_NC_046562
Sinopodisma_rostellocerca_NC_052716
Sinopodisma_pieli_NC_051867
Sinopodisma_houshana_NC_033905
Sinopodisma_tushiensis_NC_046549
Sinopodisma_wulingshanensis_NC_033906
Sinopodisma_funiushana_NC_046546
Sinopodisma_wudangshanensis_NC_046547
Pedopodisma_emeiensis_NC_046561
Indopodisma_kingdoni_NC_046529
Paratoacris_reticulipennis_NC_053660
Fer_nigripennis_NC_053658
Oxytauchira spp.
Oxya_chinensis_NC_010219
Oxya_hainanensis_NC_045928
Caryandoides_hunanica_NC_053659
17 Oxya_japonica_NC_043773
Oxya_agavisa_NC_045883
Oxya_hyla_NC_032076
Pseudoxya_diminuta_NC_025765
Caryanda_sp._NC_030165
Longchuanacris_curvifurculus_NC_036994
Caryanda_elegans_NC_036750
Spasthoternum_prasiniferum_prasiniferum_NC_046532
16 Hieroglyphus_tonkinensis_NC_030587
Gesonula_punctifrons_NC_046411
Traulia spp.
Apalacris_nigrogeniculata_NC_046527
Conophymacris_viridis_NC_046528
Xiangelilacris_zhongdianensis_NC_046533
Locusta migratoria ssp.
Gastrimargus_marmoratus_NC_011114
Oedaleus_decorus_asiaticus_NC_011115
Oedaleus_abruptus_NC_046538
Parapleurus_alliaceus_NC_046539
18 Oedaleus_infernalis_NC_029327
Oedaleus_manjius_NC_052734
Heteropternis_respondens_NC_046537
Orinhippus_tibetanus_NC_023467
Sphingonotus_menglaensis_NC_046550
Sphingonotus_ningsianus_NC_046563
Sphingonotus_yenchihensis_NC_046564
Bryodemini
Aiolopus_thalassinus_NC_034674
Epacromius_coerulipes_NC_052732
Trilophidia_annulata_NC_027179
Acrida spp.
Phlaeoba spp.
16 Gonista_bicolor_NC_029205
Ceracris spp.
Arcyptera spp.
Euchorthippus_declivus_SOH_660
19 Euchorthippus_unicolor_NC_045237
Pseudoecoscyllina_brevipennisoides_NC_046534
Euthystira spp.
Pacris_xizangensis_NC_023919
Eclipophleps_carinata_NC_054195
Dnopherula_yuanmowensis_NC_039408
Omocestus_viridulus_NC_046560
Omocestus_haemorrhoidalis_SOH_816
Omocestus_rufipes_SOH_657
I Omocestus_rufipes_SOH_693
Omocestus_viridulus_SOH_711
II Stenobothrus_lineatus_SOH_689
Stenobothrus_nigromaculatus_SOH_666
Stenobothrus_rubicundulus_SOH_704
II Pseudochorthippus_parallelus_SOH_658
II Pseudochorthippus_parallelus_SOH_659
Pseudochorthippus_parallelus_parallelus_RP_007
Pseudochorthippus_parallelus_erythropus_RP_349
20 Chorthippus_fallax_NC_048465
Euchorthippus_fusigeniculatus_NC_014449
21 Megaulacobothrus_chinensis_NC_011095
Chorthippus alticola ssp.
Chorthippus apricarius ssp.
III Chorthippus_biguttulus_SRR2051330
Chorthippus_mollis_SRR2051380
Chorthippus_biguttulus_SRR2051335
Chorthippus_biguttulus_SRR2051340
Chorthippus_mollis_SOH_820
Chorthippus_mollis_SRR2051370
Gomphocerippus_rufus_NC_014349
Chorthippus_brunneus_SRR2051360
Gomphocerippus_rufus_SOH_707
II Chorthippus_eisentrauti_SOH_710
Chorthippus_rubratibialis_LM1
Chorthippus_eisentrauti_SOH_709
Chorthippus_brunneus_SRR2051350
Gomphocerus sibiricus ssp.
Gomphocerus_licenti_NC_013847
Stauroderus_scalaris_SOH_824
Gomphocerus_sibiricus_tibetanus_NC_015478
Chorthippus_dorsatus_SOH_691
Chorthippus albomarginatus ssp.

Table S1. List of taxa used in the study for phylogenetic inference and gene order analyses. Additional taxonomic information according to the Orthoptera species file (Cigliano et al., 2022) and genome length is provided. "GB" = GenBank, "OH" = O. Hawlitschek, "Prepr." = preprint

No	Suborder	Family	Subfamily	Tribe	Species & subspecies	Mt genome length (bp)	Accession ID	Source	Reference
1	Caelifera	Acrididae	Acridinae	Acridini	*Acrida cinerea*	15599	NC_014887	GB	(Liu and Huang, 2010)
2	Caelifera	Acrididae	Acridinae	Acridini	*Acrida willemsei*	15601	NC_011303	GB	(Fenn et al., 2008)
3	Caelifera	Acrididae	Acridinae	Phlaeobini	*Phlaeoba albonema*	15657	NC_011827	GB	(Shi et al., 2008)
4	Caelifera	Acrididae	Acridinae	Phlaeobini	*Phlaeoba infumata*	16276	NC_031506	GB	(Liu and Qiu, 2016b)
5	Caelifera	Acrididae	Acridinae	Phlaeobini	*Phlaeoba tenebrosa*	15648	NC_029150	GB	(Song et al., 2016b)
6	Caelifera	Acrididae	Acridinae		*Pseudoeoscyllina brevipennisoides*	15933	NC_046534	GB	(Chang et al., 2020)
7	Caelifera	Acrididae	Calliptaminae		*Calliptamus abbreviatus*	16123	NC_030626	GB	(Han et al., 2016a)
8	Caelifera	Acrididae	Calliptaminae		*Calliptamus barbarus*	15639	NC_046544	GB	(Chang et al., 2020)
9	Caelifera	Acrididae	Calliptaminae		*Calliptamus italicus*	15675	NC_011305	GB	(Fenn et al., 2008)
10	Caelifera	Acrididae	Calliptaminae		*Peripolus nepalensis*	15858	NC_029135	GB	(Zhi et al., 2016c)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
11	Caelifera	Acrididae	Caryandinae		*Caryanda elegans*	15450	NC_036750	GB	(Yuan et al., 2019)
12	Caelifera	Acrididae	Caryandinae		*Caryanda sp.*	15445	NC_030165	GB	(Hu et al., 2017)
13	Caelifera	Acrididae	Catantopinae	Catantopini	*Diabolocatantops pinguis*	15569	NC_042904	GB	(Chen et al., 2019)
14	Caelifera	Acrididae	Catantopinae	Catantopini	*Stenocatantops mistshenkoi*	15930	NC_052717	GB	(Chen et al., 2020)
15	Caelifera	Acrididae	Catantopinae	Catantopini	*Stenocatantops splendens*	16293	NC_041115	GB	(Li et al., 2019c)
16	Caelifera	Acrididae	Catantopinae	Catantopini	*Xenocatantops brachycerus*	15605	NC_021609	GB	(Yang et al., 2016a)
17	Caelifera	Acrididae	Catantopinae	Mesambriini	*Traulia lofaoshana*	15567	NC_046551	GB	(Chang et al., 2020)
18	Caelifera	Acrididae	Catantopinae	Mesambriini	*Traulia minuta*	15459	NC_036063	GB	(Qiu et al., 2021)
19	Caelifera	Acrididae	Catantopinae	Mesambriini	*Traulia nigritibialis*	15574	NC_041114	GB	(Li et al., 2019c)
20	Caelifera	Acrididae	Catantopinae	Mesambriini	*Traulia orchotibialis*	15445	NC_046565	GB	(Chang et al., 2020)
21	Caelifera	Acrididae	Catantopinae	Mesambriini	*Traulia szetschuanensis*	15768	NC_013826	GB	(Huang and Zhang, 2010b; unpublished)
22	Caelifera	Acrididae	Catantopinae		*Apalacris nigrogeniculata*	15654	NC_046527	GB	(Chang et al., 2020)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
23	Caelifera	Acrididae	Catantopinae		*Caryandoides hunanica*	16321	NC_053659	GB	(Zeng and Huang, 2021; unpublished)
24	Caelifera	Acrididae	Catantopinae		*Choroedocus capensis*	15762	NC_041116	GB	(Li et al., 2019c)
25	Caelifera	Acrididae	Catantopinae		*Choroedocus violaceipes*	18255	NC_034673	GB	(Guan and Xu, 2017b; unpublished)
26	Caelifera	Acrididae	Catantopinae		*Fer nigripennis*	15555	NC_053658	GB	(Zeng and Huang, 2021; unpublished)
27	Caelifera	Acrididae	Catantopinae		*Longchuanacris curvifurculus*	16328	NC_036994	GB	(Hu et al., 2018)
28	Caelifera	Acrididae	Catantopinae		*Paratoacris reticulipennis*	16250	NC_053660	GB	(Zeng and Huang, 2021; unpublished)
29	Caelifera	Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Chondracris rosea*	15646	NC_019993	GB	(Jiang and Qiang, 2013; unpublished)
30	Caelifera	Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Nomadacris japonica*	15636	NC_036062	GB	(Qiu et al., 2017; unpublished)
31	Caelifera	Acrididae	Cyrtacanthacridinae	Cyrtacanthacridini	*Schistocerca gregaria gregaria*	15625	NC_013240	GB	(Erler et al., 2010)
32	Caelifera	Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Shirakiacris shirakii*	15649	NC_021610	GB	(Liu and Huang, 2013; unpublished)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
33	Caelifera	Acrididae	Eyprepocnemidinae	Eyprepocnemidini	*Shirakiacris yunkweiensis*	15596	NC_046531	GB	(Chang et al., 2020)
34	Caelifera	Acrididae	Gomphocerinae	Arcypterini	*Arcyptera coreana*	15783	NC_013805	GB	(Liu and Huang, 2007)
35	Caelifera	Acrididae	Gomphocerinae	Arcypterini	*Arcyptera meridionalis*	16225	NC_039962	GB	(Han et al., 2018; unpublished)
36	Caelifera	Acrididae	Gomphocerinae	Chrysochraontini	*Euchorthippus declivus*	15703	SOH_660	OH	(Hawlitschek et al., 2022)
37	Caelifera	Acrididae	Gomphocerinae	Chrysochraontini	*Euchorthippus fusigeniculatus*	15772	NC_014449	GB	(Zhao et al., 2010)
38	Caelifera	Acrididae	Gomphocerinae	Chrysochraontini	*Euchorthippus unicolor*	15679	NC_045237	GB	(Qiu et al., 2019)
39	Caelifera	Acrididae	Gomphocerinae	Chrysochraontini	*Euthystira brachyptera*	15621	SOH_665	OH	(Hawlitschek et al., 2022)
40	Caelifera	Acrididae	Gomphocerinae	Chrysochraontini	*Euthystira luteifemora*	15661	NC_046557	GB	(Chang et al., 2020)
41	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus albomarginatus*	15612	SOH_813	OH	(Hawlitschek et al., 2022)
42	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus albomarginatus*	15617	SOH_878	OH	(Hawlitschek et al., 2022)
43	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus alticola*	15613	SOH_764	OH	(Hawlitschek et al., 2022)
44	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus alticola*	15618	SOH_765	OH	(Hawlitschek et al., 2022)
45	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus apricarius*	15622	SOH_806	OH	(Hawlitschek et al., 2022)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
46	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus apricarius*	15614	SOH_808	OH	(Hawlitschek et al., 2022)
47	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus biguttulus*	15618	SRR2051330	GB	(Berdan et al., 2015)
48	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus biguttulus*	15618	SRR2051335	GB	(Berdan et al., 2015)
49	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus biguttulus*	15618	SRR2051340	GB	(Berdan et al., 2015)
50	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus brunneus*	15617	SRR2051350	GB	(Berdan et al., 2015)
51	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus brunneus*	15615	SRR2051360	GB	(Berdan et al., 2015)
52	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus dorsatus*	15625	SOH_691	OH	(Hawlitschek et al., 2022)
53	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus eisentrauti*	15621	SOH_709	OH	(Hawlitschek et al., 2022)
54	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus eisentrauti*	15624	SOH_710	OH	(Hawlitschek et al., 2022)
55	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus fallax*	16152	NC_048465	GB	(Deng et al., 2019)
56	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus mollis*	15607	SOH_820	OH	(Hawlitschek et al., 2022)
57	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus mollis*	15617	SRR2051370	GB	(Berdan et al., 2015)
58	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus mollis*	15748	SRR2051380	GB	(Berdan et al., 2015)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
59	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Chorthippus rubratibialis*	15618	LM_1	OH	(Nolen et al., 2020)
60	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerippus rufus*	15598	NC_014349	GB	(Sun et al., 2010)
61	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerippus rufus*	15617	SOH_707	OH	(Hawlitschek et al., 2022)
62	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus licenti*	15597	NC_013847	GB	(Gao et al., 2009)
63	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus sibiricus*	15590	NC_021103	GB	(Zhang et al., 2013b)
64	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus sibiricus*	15614	SOH_714	OH	(Hawlitschek et al., 2022)
65	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Gomphocerus sibiricus tibetanus*	15571	NC_015478	GB	(Yin et al., 2012)
66	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Pseudochorthippus parallelus*	15623	SOH_658	OH	(Hawlitschek et al., 2022)
67	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Pseudochorthippus parallelus*	15607	SOH_659	OH	(Hawlitschek et al., 2022)
68	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Pseudochorthippus parallelus erythropus*	15616	RP_349	OH	(Hagberg et al., 2021; prepr.)
69	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Pseudochorthippus parallelus parallelus*	15623	RP_007	OH	(Hagberg et al., 2021; prepr.)
70	Caelifera	Acrididae	Gomphocerinae	Gomphocerini	*Stauroderus scalaris*	15613	SOH_824	OH	(Hawlitschek et al., 2022)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
71	Caelifera	Acrididae	Gomphocerinae	Hypernephiini	*Eclipophleps carinata*	15617	NC_054195	GB	(Qian et al., 2021; unpublished)
72	Caelifera	Acrididae	Gomphocerinae	Ochrilidiini	*Gonista bicolor*	15618	NC_029205	GB	(Zhang et al., 2016a)
73	Caelifera	Acrididae	Gomphocerinae	Orinhippini	*Orinhippus tibetanus*	15611	NC_023467	GB	(Song et al., 2016c)
74	Caelifera	Acrididae	Gomphocerinae	Pacrini	*Pacris xizangensis*	15622	NC_023919	GB	(Zhang et al., 2016b)
75	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Megaulacobothrus chinensis*	15599	NC_011095	GB	(Yan and Yuan, 2008)
76	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Omocestus haemorrhoidalis*	15620	SOH_816	OH	(Hawlitschek et al., 2022)
77	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Omocestus rufipes*	15627	SOH_657	OH	(Hawlitschek et al., 2022)
78	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Omocestus rufipes*	15622	SOH_693	OH	(Hawlitschek et al., 2022)
79	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Omocestus viridulus*	15902	NC_046560	GB	(Chang et al., 2020)
80	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Omocestus viridulus*	15609	SOH_711	OH	(Hawlitschek et al., 2022)
81	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus lineatus*	15556	SOH_689	OH	(Hawlitschek et al., 2022)
82	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus nigromaculatus*	15622	SOH_666	OH	(Hawlitschek et al., 2022)
83	Caelifera	Acrididae	Gomphocerinae	Stenobothrini	*Stenobothrus rubicundulus*	15616	SOH_704	OH	(Hawlitschek et al., 2022)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
84	Caelifera	Acrididae	Gomphocerinae		*Dnopherula yuanmowensis*	16018	NC_039408	GB	(Li et al., 2019b)
85	Caelifera	Acrididae	Hemiacridinae	Hieroglyphini	*Hieroglyphus tonkinensis*	15558	NC_030587	GB	(Chang and Huang, 2016)
86	Caelifera	Acrididae	Melanoplinae	Podismini	*Anapodisma miramae*	15622	NC_052715	GB	(Chen et al., 2020)
87	Caelifera	Acrididae	Melanoplinae	Podismini	*Curvipennis wixiensis*	15642	NC_031397	GB	(Chen and Xu, 2017)
88	Caelifera	Acrididae	Melanoplinae	Podismini	*Fruhstorferiola huayinensis*	15528	NC_031379	GB	(Liu and Qiu, 2016a)
89	Caelifera	Acrididae	Melanoplinae	Podismini	*Fruhstorferiola kulinga*	15655	NC_026716	GB	(Yang et al., 2016d)
90	Caelifera	Acrididae	Melanoplinae	Podismini	*Fruhstorferiola omei*	15894	NC_046545	GB	(Chang et al., 2020)
91	Caelifera	Acrididae	Melanoplinae	Podismini	*Fruhstorferiola tonkinensis*	15483	NC_031817	GB	(Zhang and Lin, 2016)
92	Caelifera	Acrididae	Melanoplinae	Podismini	*Indopodisma kingdoni*	15625	NC_046529	GB	(Chang et al., 2020)
93	Caelifera	Acrididae	Melanoplinae	Podismini	*Kingdonella bicollina*	15630	NC_023920	GB	(Zhi et al., 2016b)
94	Caelifera	Acrididae	Melanoplinae	Podismini	*Ognevia longipennis*	15621	NC_013701	GB	(Huang and Zhang, 2010a; unpublished)
95	Caelifera	Acrididae	Melanoplinae	Podismini	*Paratonkinacris vittifemoralis*	15655	NC_046530	GB	(Chang et al., 2020)

No	Suborder	Family	Subfamily	Tribe	Species & subspecies	Mt genome length (bp)	Accession ID	Source	Reference
96	Caelifera	Acrididae	Melanoplinae	Podismini	*Pedopodisma emeiensis*	15867	NC_046561	GB	(Chang et al., 2020)
97	Caelifera	Acrididae	Melanoplinae	Podismini	*Prumna arctica*	15628	NC_013835	GB	(Sun et al., 2010)
98	Caelifera	Acrididae	Melanoplinae	Podismini	*Qinlingacris taibaiensis*	15774	NC_027187	GB	(Guan and Xu, 2015a; unpublished)
99	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma funiushana*	15834	NC_046546	GB	(Chang et al., 2020)
100	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma houshana*	16122	NC_033905	GB	(Zhongying et al., 2020)
101	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma lofaoshana*	16261	NC_046562	GB	(Chang et al., 2020)
102	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma lushiensis*	15948	NC_046549	GB	(Chang et al., 2020)
103	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma pieli*	15189	NC_051867	GB	(Liu et al., 2017)
104	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma rostellocerca*	15573	NC_052716	GB	(Chen et al., 2020)
105	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma tsinlingensis*	16044	NC_032303	GB	(Guan and Xu, 2017a; unpublished)
106	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma wudangshanensis*	16437	NC_046547	GB	(Chang et al., 2020)
107	Caelifera	Acrididae	Melanoplinae	Podismini	*Sinopodisma wulingshanensis*	16077	NC_033906	GB	(Zhongying et al., 2020)

No	Suborder	Family	Subfamily	Tribe	Species & subspecies	Mt genome length (bp)	Accession ID	Source	Reference
108	Caelifera	Acrididae	Melanoplinae	Podismini	*Tonkinacris sinensis*	15818	NC_032716	GB	(Zhang et al., 2017b)
109	Caelifera	Acrididae	Melanoplinae	Podismini	*Xiangelilacris zhongdianensis*	15629	NC_046533	GB	(Chang et al., 2020)
110	Caelifera	Acrididae	Melanoplinae	Podismini	*Yunnanacris yunnaneus*	15625	NC_030586	GB	(Hu et al., 2016)
111	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Angaracris barabensis*	15930	NC_025558	GB	(Han et al., 2016b)
112	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Angaracris rhodopa*	15930	NC_025946	GB	(Han et al., 2016c)
113	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Bryodema dolichoptera*	15578	NC_046543	GB	(Chang et al., 2020)
114	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Bryodema kozlovi*	15592	NC_052731	GB	(Chang and Huang, 2021; unpublished)
115	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Bryodema nigroptera*	15929	NC_046535	GB	(Chang et al., 2020)
116	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Bryodemacris uvarovi*	15927	NC_046553	GB	(Chang et al., 2020)
117	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Bryodemella holdereri holdereri*	16265	NC_046536	GB	(Chang et al., 2020)
118	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Bryodemella tuberculata diluta*	15463	NC_046554	GB	(Chang et al., 2020)
119	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Compsorhipis davidiana*	16085	NC_029408	GB	(Zhou and Huang, 2016; unpublished)

No	Suborder	Family	Subfamily	Tribe	Species & subspecies	Mt genome length (bp)	Accession ID	Source	Reference
120	Caelifera	Acrididae	Oedipodinae	Bryodemini	*Uvaroviola multispinosa*	15781	NC_053942	GB	(Yu et al., 2019)
121	Caelifera	Acrididae	Oedipodinae	Epacromiini	*Aiolopus thalassinus*	16832	NC_034674	GB	(Zhang et al., 2017a)
122	Caelifera	Acrididae	Oedipodinae	Epacromiini	*Epacromius coerulipes*	15661	NC_052732	GB	(Chang and Huang, 2021; unpublished)
123	Caelifera	Acrididae	Oedipodinae	Epacromiini	*Heteropternis respondens*	16251	NC_046537	GB	(Chang et al., 2020)
124	Caelifera	Acrididae	Oedipodinae	Locustini	*Gastrimargus marmoratus*	15924	NC_011114	GB	(Ma et al., 2009)
125	Caelifera	Acrididae	Oedipodinae	Locustini	*Locusta migratoria*	15722	NC_001712	GB	(Flook et al., 1995)
126	Caelifera	Acrididae	Oedipodinae	Locustini	*Locusta migratoria*	15748	SRR9637845	GB	(Wang et al., 2014)
127	Caelifera	Acrididae	Oedipodinae	Locustini	*Locusta migratoria*	15747	SRR9637848	GB	(Wang et al., 2014)
128	Caelifera	Acrididae	Oedipodinae	Locustini	*Locusta migratoria*	15748	SRR9637849	GB	(Wang et al., 2014)
129	Caelifera	Acrididae	Oedipodinae	Locustini	*Locusta migratoria*	15501	SRR9637855	GB	(Wang et al., 2014)
130	Caelifera	Acrididae	Oedipodinae	Locustini	*Locusta migratoria manilensis*	15895	NC_014891	GB	(Huang and Liu, 2016; unpublished)
131	Caelifera	Acrididae	Oedipodinae	Locustini	*Locusta migratoria migratoria*	16053	NC_011119	GB	(Xiao et al., 2012b)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
132	Caelifera	Acrididae	Oedipodinae	Locustini	*Locusta migratoria tibetensis*	15568	NC_015624	GB	(Zhang et al., 2011b; unpublished)
133	Caelifera	Acrididae	Oedipodinae	Locustini	*Oedaleus abruptus*	16252	NC_046538	GB	(Chang et al., 2020)
134	Caelifera	Acrididae	Oedipodinae	Locustini	*Oedaleus decorus asiaticus*	16259	NC_011115	GB	(Ma et al., 2009)
135	Caelifera	Acrididae	Oedipodinae	Locustini	*Oedaleus infernalis*	15898	NC_029327	GB	(Guo et al., 2017)
136	Caelifera	Acrididae	Oedipodinae	Locustini	*Oedaleus manjius*	14999	NC_052734	GB	(Chang and Huang, 2021; unpublished)
137	Caelifera	Acrididae	Oedipodinae	Parapleurini	*Ceracris fasciata fasciata*	15905	NC_043956	GB	(Gao et al., 2018)
138	Caelifera	Acrididae	Oedipodinae	Parapleurini	*Ceracris kiangsu*	15665	NC_019994	GB	(Jiang et al., 2013; unpublished)
139	Caelifera	Acrididae	Oedipodinae	Parapleurini	*Ceracris versicolor*	15616	NC_025285	GB	(Xu et al., 2016)
140	Caelifera	Acrididae	Oedipodinae	Parapleurini	*Parapleurus alliaceus*	15326	NC_046539	GB	(Chang et al., 2020)
141	Caelifera	Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus menglaensis*	15644	NC_046550	GB	(Chang et al., 2020)
142	Caelifera	Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus ningsianus*	16261	NC_046563	GB	(Chang et al., 2020)
143	Caelifera	Acrididae	Oedipodinae	Sphingonotini	*Sphingonotus yenchihensis*	15642	NC_046564	GB	(Chang et al., 2020)

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144	Caelifera	Acrididae	Oedipodinae	Trilophidiini	*Trilophidia annulata*	15775	NC_027179	GB	(Guan and Xu, 2015b; unpublished)
145	Caelifera	Acrididae	Oxyinae	Oxyini	*Emeiacris maculata*	15608	NC_046556	GB	(Chang et al., 2020)
146	Caelifera	Acrididae	Oxyinae	Oxyini	*Gesonula punctifrons*	15218	NC_046411	GB	(Chang et al., 2020)
147	Caelifera	Acrididae	Oxyinae	Oxyini	*Oxya agavisa*	15443	NC_045883	GB	(Li et al., 2020)
148	Caelifera	Acrididae	Oxyinae	Oxyini	*Oxya chinensis*	15443	NC_010219	GB	(Zhang and Huang, 2008)
149	Caelifera	Acrididae	Oxyinae	Oxyini	*Oxya hainanensis*	15531	NC_045928	GB	(Li et al., 2020)
150	Caelifera	Acrididae	Oxyinae	Oxyini	*Oxya hyla*	15627	NC_032076	GB	(Song et al., 2016a)
151	Caelifera	Acrididae	Oxyinae	Oxyini	*Oxya japonica*	15427	NC_043773	GB	(Li et al., 2020)
152	Caelifera	Acrididae	Oxyinae	Oxyini	*Pseudoxya diminuta*	15541	NC_025765	GB	(Tang et al., 2014)
153	Caelifera	Acrididae	Oxyinae		*Oxytauchira brachyptera*	15883	NC_046570	GB	(Chang et al., 2020)
154	Caelifera	Acrididae	Oxyinae		*Oxytauchira flange*	15620	NC_053745	GB	(Zeng and Huang, 2021; unpublished)
155	Caelifera	Acrididae	Spasthosterninae	Spasthosternini	*Spasthoternum prasiniferum prasiniferum*	15507	NC_046532	GB	(Chang et al., 2020)

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156	Caelifera	Dericorythidae	Conoyphyminae		*Conophymacris viridis*	15627	NC_046528	GB	(Chang et al., 2020)
157	Caelifera	Dericorythidae	Dericorythinae		*Dericorys annulata*	15570	NC_046555	GB	(Chang et al., 2020)
158	Caelifera	Episactidae	Episactinae		*Pielomastax zhengi*	15602	NC_016182	GB	(Yang and Huang, 2011)
159	Caelifera	Lentulidae	Lentulinae		*Lentula callani*	15944	NC_020774	GB	(Leavitt et al., 2013)
160	Caelifera	Lithidiidae			*Lithidiopsis carinatus*	15652	NC_020775	GB	(Leavitt et al., 2013)
161	Caelifera	Ommexechidae	Ommexechinae	Ommexechini	*Ommexecha virens*	15536	NC_020778	GB	(Leavitt et al., 2013)
162	Caelifera	Pamphagidae	Thrinchinae	Haplotropidini	*Humphaplotropis culaishanensis*	15659	NC_023535	GB	(Li et al., 2016)
163	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Asiotmethis jubatus*	15669	NC_025904	GB	(Li et al., 2015)
164	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Asiotmethis zacharjini*	15660	NC_020328	GB	(Zhang et al., 2013a)
165	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Filchnerella beicki*	15658	NC_024923	GB	(Li et al., 2014)
166	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Filchnerella helanshanensis*	15657	NC_020329	GB	(Zhang et al., 2013a)
167	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Filchnerella qilianshanensis*	15659	NC_046558	GB	(Chang et al., 2020)
168	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Filchnerella rubrimargina*	15590	NC_052733	GB	(Chang and Huang, 2021; unpublished)

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169	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Filchnerella tenggerensis*	15635	NC_046559	GB	(Chang et al., 2020)
170	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Pseudotmethis rubimarginis*	15661	NC_020330	GB	(Zhang et al., 2013a)
171	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Sinotmethis brachypterus*	15662	NC_026525	GB	(Shi et al., 2016)
172	Caelifera	Pamphagidae	Thrinchinae	Thrinchini	*Thrinchus schrenkii*	15672	NC_014610	GB	(Zhang et al., 2011a)
173	Caelifera	Pneumoridae			*Physemacris variolosa*	17004	NC_014491	GB	(Sheffield et al., 2010)
174	Caelifera	Pyrgacrididae			*Pyrgacris descampsi*	15618	NC_020776	GB	(Leavitt et al., 2013)
175	Caelifera	Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha psittacina*	15929	NC_046552	GB	(Chang et al., 2020)
176	Caelifera	Pyrgomorphidae	Pyrgomorphinae	Atractomorphini	*Atractomorpha sinensis*	15558	NC_011824	GB	(Ding et al., 2007)
177	Caelifera	Pyrgomorphidae	Pyrgomorphinae	Sphenariini	*Mekongiana xiangchengensis*	15567	NC_014450	GB	(Zhao et al., 2010)
178	Caelifera	Pyrgomorphidae	Pyrgomorphinae	Sphenariini	*Mekongiella kingdoni*	15932	NC_023921	GB	(Zhi et al., 2016a)
179	Caelifera	Pyrgomorphidae	Pyrgomorphinae	Sphenariini	*Mekongiella xizangensis*	15885	NC_014451	GB	(Zhao et al., 2010)
180	Caelifera	Pyrgomorphidae	Pyrgomorphinae	Tagastini	*Tagasta indica*	15432	NC_045930	GB	(Qiu et al., 2020)
181	Caelifera	Ripipterygidae	Ripipteryginae		*Mirhipipteryx andensis*	15307	NC_028065	GB	(Song et al., 2015)

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182	Caelifera	Romaleidae	Romaleinae	Procolpini	*Xyleus modestus*	15723	NC_014490	GB	(Sheffield et al., 2010)
183	Caelifera	Tanaoceridae	Tanaocerinae		*Tanaocerus koebelei*	15515	NC_020777	GB	(Leavitt et al., 2013)
184	Caelifera	Tetrigidae	Metrodorinae		*Systolederus spicupennis*	15604	NC_042697	GB	(Zhang, 2019; unpublished)
185	Caelifera	Tetrigidae	Tetriginae	Tetrigini	*Euparatettix bimaculatus*	15194	NC_046541	GB	(Chang et al., 2020)
186	Caelifera	Tetrigidae	Tetriginae	Tetrigini	*Euparatettix variabilis*	15924	NC_046542	GB	(Chang et al., 2020)
187	Caelifera	Tetrigidae	Tetriginae	Tetrigini	*Tetrix japonica*	15128	NC_018543	GB	(Xiao et al., 2012a)
188	Caelifera	Tetrigidae	Tetriginae	Tetrigini	*Tetrix ruyuanensis*	15584	NC_046412	GB	(Chang et al., 2020)
189	Caelifera	Tetrigidae	Tetriginae		*Alulatettix yunnanensis*	15104	NC_018542	GB	(Xiao et al., 2012a)
190	Caelifera	Tetrigidae	Tetriginae		*Ergatettix dorsifera*	15221	NC_046540	GB	(Chang et al., 2020)
191	Caelifera	Thericleidae	Thericleinae	Pseudothericleini	*Pseudothericles compressifrons*	15081	NC_028061	GB	(Song et al., 2015)
192	Caelifera	Tridactylidae	Tridactylinae	Tridactylini	*Ellipes minuta*	15451	NC_014488	GB	(Sheffield et al., 2010)
193	Caelifera	Tristiridae	Tristirinae	Tristirini	*Tristira magellanica*	16494	NC_020773	GB	(Leavitt et al., 2013)
194	Dermaptera	Anisolabididae	Anisolabidinae		*Euborellia arcanum*	16087	NC_032075	GB	(Song et al., 2016a)

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195	Dictyoptera	Mantidae	Mantinae		*Mantis religiosa*	15534	NC_030265	GB	(Ye et al., 2016)
196	Diptera	Muscidae			*Musca domestica*	15568	NC_041412	GB	(Han et al., 2019a; unpublished)
197	Ensifera	Anostostomatidae	Anabropsinae	Anabropsini	*Pteranabropsis carli*	16119	NC_035420	GB	(Song, 2017; unpublished)
198	Ensifera	Anostostomatidae	Anabropsinae	Anabropsini	*Pteranabropsis carnarius*	16099	NC_035552	GB	(Song, 2017; unpublished)
199	Ensifera	Anostostomatidae	Anabropsinae	Anabropsini	*Pteranabropsis crenatis*	15638	NC_035553	GB	(Song, 2017; unpublished)
200	Ensifera	Anostostomatidae	Anostostomatinae		*Henicus brevimucronatus*	15140	NC_028063	GB	(Song et al., 2015)
201	Ensifera	Gryllacrididae	Gryllacridinae	Gryllacridini	*Camptonotus carolinensis*	15211	NC_028060	GB	(Song et al., 2015)
202	Ensifera	Gryllacrididae	Gryllacridinae	Gryllacridini	*Homogryllacris anelytra*	15694	NC_033998	GB	(Zhou et al., 2017b)
203	Ensifera	Gryllacrididae	Hyperbaeninae	Phryganogryllacridini	*Phryganogryllacris xiai*	15949	NC_033994	GB	(Zhou et al., 2017b)
204	Ensifera	Gryllidae	Eneopterinae	Lebinthini	*Cardiodactylus muiri*	15629	NC_037914	GB	(Dong et al., 2017)
205	Ensifera	Gryllidae	Eneopterinae	Xenogryllini	*Xenogryllus marmoratus*	16111	NC_041236	GB	(Ma et al., 2019b)
206	Ensifera	Gryllidae	Gryllinae	Gryllini	*Gryllus bimaculatus*	15669	NC_053546	GB	(Park et al., 2021)
207	Ensifera	Gryllidae	Gryllinae	Gryllini	*Loxoblemmus doenitzi*	15820	NC_033985	GB	(Zhou et al., 2017b)

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208	Ensifera	Gryllidae	Gryllinae	Gryllini	*Loxoblemmus equestris*	16227	NC_030763	GB	(Yang et al., 2016b)
209	Ensifera	Gryllidae	Gryllinae	Gryllini	*Teleogryllus emma*	15660	NC_011823	GB	(Ye et al., 2008)
210	Ensifera	Gryllidae	Gryllinae	Gryllini	*Teleogryllus occipitalis*	15501	LC521855	GB	(Kataoka et al., 2020)
211	Ensifera	Gryllidae	Gryllinae	Gryllini	*Teleogryllus oceanicus*	15660	NC_028619	GB	(Zhou et al., 2017a)
212	Ensifera	Gryllidae	Gryllinae	Gryllini	*Velarifictorus hemelytrus*	16314	NC_030762	GB	(Yang et al., 2016b)
213	Ensifera	Gryllidae	Oecanthinae	Oecanthini	*Oecanthus sinensis*	15598	NC_034799	GB	(Li et al., 2019c)
214	Ensifera	Gryllidae	Podoscirtinae	Podoscirtini	*Truljalia hibinonis*	18051	NC_034797	GB	(Li et al., 2019c)
215	Ensifera	Gryllotalpidae	Gryllotalpinae	Gryllotalpini	*Gryllotalpa orientalis*	15521	NC_006678	GB	(Kim et al., 2005)
216	Ensifera	Gryllotalpidae	Gryllotalpinae	Gryllotalpini	*Gryllotalpa pluvialis*	15525	NC_011302	GB	(Fenn et al., 2008)
217	Ensifera	Gryllotalpidae	Gryllotalpinae	Gryllotalpini	*Gryllotalpa unispina*	15513	NC_029148	GB	(Zhang et al., 2016c)
218	Ensifera	Mogoplistidae	Mogoplistinae	Arachnocphalini	*Ornebius bimaculatus*	16589	NC_039666	GB	(Ma and Li, 2018)
219	Ensifera	Mogoplistidae	Mogoplistinae	Arachnocphalini	*Ornebius fuscicercis*	15776	NC_039739	GB	(Ma and Li, 2018)
220	Ensifera	Mogoplistidae	Mogoplistinae	Arachnocphalini	*Ornebius kanetataki*	16368	NC_039667	GB	(Ma and Li, 2018)

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221	Ensifera	Myrmecophilidae	Myrmecophilinae	Myrmecophilini	*Myrmecophilus manni*	15323	NC_011301	GB	(Fenn et al., 2008)
222	Ensifera	Phalangopsidae	Cacoplistinae	Cacoplistini	*Cacoplistes rogenhoferi*	15880	NC_039664	GB	(Ma and Li, 2018)
223	Ensifera	Phalangopsidae	Cacoplistinae	Homeogryllini	*Meloimorpha japonica*	16136	NC_039665	GB	(Ma and Li, 2018)
224	Ensifera	Prophalangopsidae	Prophalangopsinae		*Cyphoderris monstrosa*	16590	NC_028059	GB	(Song et al., 2015)
225	Ensifera	Prophalangopsidae	Prophalangopsinae		*Tarragoilus diuturnus*	16144	NC_021397	GB	(Zhou et al., 2014)
226	Ensifera	Rhaphidophoridae	Aemodogryllinae	Aemodogryllini	*Diestrammena asynamora*	16110	NC_033989	GB	(Zhou et al., 2017b)
227	Ensifera	Rhaphidophoridae	Troglophilinae		*Troglophilus neglectus*	15810	NC_011306	GB	(Fenn et al., 2008)
228	Ensifera	Schizodactylidae	Schizodactylinae		*Comicus campestris*	15691	NC_028062	GB	(Song et al., 2015)
229	Ensifera	Stenopelmatidae	Stenopelmatinae	Stenopelmatini	*Stenopelmatus fuscus*	15767	NC_028058	GB	(Song et al., 2015)
230	Ensifera	Tettigoniidae	Bradyporinae	Zichyini	*Deracantha onos*	15650	NC_011813	GB	(Zhou et al., 2009)
231	Ensifera	Tettigoniidae	Bradyporinae	Zichyini	*Zichya baranovi*	16256	NC_033984	GB	(Zhou et al., 2017b)
232	Ensifera	Tettigoniidae	Conocephalinae	Conocephalini	*Conanalus pieli*	15309	NC_033987	GB	(Zhou et al., 2017b)
233	Ensifera	Tettigoniidae	Conocephalinae	Conocephalini	*Conocephalus maculatus*	16271	NC_045065	GB	(Tang et al., 2014)

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234	Ensifera	Tettigoniidae	Conocephalinae	Conocephalini	*Conocephalus melaenus*	15865	NC_033988	GB	(Zhou et al., 2017b)
235	Ensifera	Tettigoniidae	Conocephalinae	Copiphorini	*Euconocephalus nasutus*	16494	NC_053383	GB	(Gao et al., 2019)
236	Ensifera	Tettigoniidae	Conocephalinae	Copiphorini	*Pseudorhynchus acuminatus*	15876	NC_033992	GB	(Zhou et al., 2017b)
237	Ensifera	Tettigoniidae	Conocephalinae	Copiphorini	*Pseudorhynchus crassiceps*	16056	NC_033990	GB	(Zhou et al., 2017b)
238	Ensifera	Tettigoniidae	Conocephalinae	Copiphorini	*Ruspolia dubia*	14971	NC_009876	GB	(Zhou et al., 2007)
239	Ensifera	Tettigoniidae	Conocephalinae	Copiphorini	*Ruspolia lineosa*	15899	NC_033991	GB	(Zhou et al., 2017b)
240	Ensifera	Tettigoniidae	Hexacentrinae		*Hexacentrus japonicus*	15396	NC_033983	GB	(Zhou et al., 2017b)
241	Ensifera	Tettigoniidae	Hexacentrinae		*Hexacentrus unicolor*	15753	NC_033999	GB	(Zhou et al., 2017b)
242	Ensifera	Tettigoniidae	Lipotactinae		*Lipotactes tripyrga*	15706	NC_033996	GB	(Zhou et al., 2017b)
243	Ensifera	Tettigoniidae	Meconematinae	Meconematini	*Acosmetura nigrogeniculata*	15629	NC_045212	GB	(Han et al., 2019b)
244	Ensifera	Tettigoniidae	Meconematinae	Meconematini	*Decma fissa*	16120	NC_033981	GB	(Zhou et al., 2017b)
245	Ensifera	Tettigoniidae	Meconematinae	Meconematini	*Pseudocosmetura anjiensis*	15872	NC_033853	GB	(Zhou et al., 2017b)
246	Ensifera	Tettigoniidae	Meconematinae	Meconematini	*Pseudokuzicus pieli*	15645	NC_033982	GB	(Zhou et al., 2017b)

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247	Ensifera	Tettigoniidae	Meconematinae	Meconematini	*Shoveliteratura triangula*	15817	NC_048466	GB	(Mao et al., 2020)
248	Ensifera	Tettigoniidae	Meconematinae	Meconematini	*Xiphidiopsis gurneyi*	16358	NC_039981	GB	(Mao et al., 2018)
249	Ensifera	Tettigoniidae	Meconematinae	Meconematini	*Xizicus fascipes*	16166	NC_018765	GB	(Yang et al., 2012)
250	Ensifera	Tettigoniidae	Meconematinae	Meconematini	*Xizicus maculatus*	15701	NC_040974	GB	(Shaoli et al., 2018)
251	Ensifera	Tettigoniidae	Mecopodinae	Mecopodini	*Mecopoda elongata*	15284	NC_021380	GB	(Zhou et al., 2013)
252	Ensifera	Tettigoniidae	Mecopodinae	Mecopodini	*Mecopoda niponensis*	15364	NC_021379	GB	(Zhou et al., 2013)
253	Ensifera	Tettigoniidae	Phaneroptinae	Barbitistini	*Isophya major*	15262	NC_042666	GB	(Öztürk and Çıplak, 2019)
254	Ensifera	Tettigoniidae	Phaneroptinae	Barbitistini	*Poecilimon luschani*	15724	NC_042665	GB	(Öztürk and Çıplak, 2019)
255	Ensifera	Tettigoniidae	Phaneroptinae	Ducetiini	*Ducetia japonica*	15638	NC_031652	GB	(Guan et al., 2016)
256	Ensifera	Tettigoniidae	Phaneroptinae	Ducetiini	*Kuwayamaea chinensis*	15692	NC_033995	GB	(Zhou et al., 2017b)
257	Ensifera	Tettigoniidae	Phaneroptinae	Elimaeini	*Elimaea cheni*	15831	NC_014289	GB	(Zhou et al., 2010)
258	Ensifera	Tettigoniidae	Phaneroptinae	Holochlorini	*Holochlora fruhstorferi*	15875	NC_033993	GB	(Zhou et al., 2017b)
259	Ensifera	Tettigoniidae	Phaneroptinae	Holochlorini	*Ruidocollaris convexipennis*	15869	NC_046548	GB	(Chang et al., 2020)

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260	Ensifera	Tettigoniidae	Phaneroptinae	Holochlorini	*Ruidocollaris obscura*	16424	NC_028160	GB	(Yang et al., 2016c)
261	Ensifera	Tettigoniidae	Phaneroptinae	Holochlorini	*Sinochlora longifissa*	18133	NC_021424	GB	(Liu et al., 2013)
262	Ensifera	Tettigoniidae	Phaneroptinae	Holochlorini	*Sinochlora szechwanensis*	15932	NC_034994	GB	(Liu, 2016)
263	Ensifera	Tettigoniidae	Phaneroptinae	Phaneropterini	*Phaneroptera gracilis*	16227	NC_034756	GB	(Wang et al., 2017; unpublished)
264	Ensifera	Tettigoniidae	Phaneroptinae	Phaneropterini	*Phaneroptera nigroantennata*	15858	NC_034757	GB	(Wang et al., 2017; unpublished)
265	Ensifera	Tettigoniidae	Pseudophyllinae	Phyllomimini	*Phyllomimus detersus*	16007	NC_028158	GB	(Yang et al., 2016c)
266	Ensifera	Tettigoniidae	Pseudophyllinae	Phyllomimini	*Phyllomimus sinicus*	15752	NC_033997	GB	(Zhou et al., 2017b)
267	Ensifera	Tettigoniidae	Pseudophyllinae	Pseudophyllini	*Pseudophyllus titan*	15120	NC_034773	GB	(Li et al., 2019a)
268	Ensifera	Tettigoniidae	Tettigoniinae	Gampsocleidini	*Gampsocleis gratiosa*	15929	NC_011200	GB	(Zhou et al., 2008)
269	Ensifera	Tettigoniidae	Tettigoniinae	Gampsocleidini	*Uvarovites inflatus*	15956	NC_026231	GB	(Wang et al., 2016)
270	Ensifera	Tettigoniidae	Tettigoniinae	Platycleidini	*Anabrus simplex*	15766	NC_009967	GB	(Fenn et al., 2007)
271	Ensifera	Tettigoniidae	Tettigoniinae	Platycleidini	*Anterastes babadaghi*	16143	NC_046894	GB	(Karşi and Ciplak, 2019)

No	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Species & subspecies**	**Mt genome length (bp)**	**Accession ID**	**Source**	**Reference**
272	Ensifera	Tettigoniidae	Tettigoniinae	Platycleidini	*Metrioptera bonneti*	15852	NC_033986	GB	(Zhou et al., 2017b)
273	Ensifera	Tettigoniidae	Tettigoniinae	Platycleidini	*Metrioptera ussuriana*	16142	NC_034796	GB	(Li et al., 2019c)
274	Ensifera	Trigonidiidae	Nemobiinae	Pteronemobiini	*Dianemobius fascipes*	15350	NC_045846	GB	(Ma et al., 2019a)
275	Ensifera	Trigonidiidae	Nemobiinae	Pteronemobiini	*Dianemobius furumagiensis*	16641	NC_045847	GB	(Ma et al., 2019a)
276	Ensifera	Trigonidiidae	Nemobiinae	Pteronemobiini	*Polionemobius taprobanensis*	15552	NC_045848	GB	(Ma et al., 2019a)
277	Ensifera	Trigonidiidae	Trigonidiinae	Trigonidiini	*Homoeoxipha nigripes*	15363	NC_045841	GB	(Ma et al., 2019a)
278	Ensifera	Trigonidiidae	Trigonidiinae	Trigonidiini	*Natula pravdini*	15625	NC_050742	GB	(Ma et al., 2019a)
279	Ensifera	Trigonidiidae	Trigonidiinae	Trigonidiini	*Svistella anhuiensis*	16075	NC_053543	GB	(Ma et al., 2019a)
280	Ensifera	Trigonidiidae	Trigonidiinae	Trigonidiini	*Trigonidium sjostedti*	15627	NC_032077	GB	(Song et al., 2016a)

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Table S2. Abbreviation list

Abbreviation	Explanation
AICc	Corrected Akaike information criterion
ATP	Adenosine 5'-triphosphate
atp 6 + 8	ATP synthase membrane subunit 6 and 8
AT-rich region	Adenine and thymine-rich region
BI	Bayesian inference
CI	Consistency index
cob	Cytochrome B gene
cox 1-3	Cytochrome C oxidase subunit 1-3
CR	Control region
CREx	Common interval rearrangement explorer
D-Loop	Displacement loop
DNA	Desoxyribonucleic acid

Abbreviation	Explanation
e.g.	“exempli gratia”: for example
ESS	Effective sample size
et al.	“et. alia": and others
GTR	Generalized time reversible model
GTR + G	Generalized time reversible model with Gamma distributed rate variation among sites
GTR + I + G	Generalized time reversible model with invariant sites and gamma distributed rate variation among sites
ID	Identification
i.e.	“Id est”: that is
IUPAC	International Union of Pure and Applied Chemistry
kb	Kilobases
MAFFT	Multiple Alignment using Fast Fourier Transform

Abbreviation	Explanation
MCMC	Markov chain Monte Carlo
ML	Maximum likelihood
Mt DNA	Mitochondrial DNA
Mt genome, mitogenome	Mitochondrial genome
MTR	Mitochondrial genome rearrangement
Mya.	Million years ago
NADH	Nicotinamide adenine dinucleotide + hydrogen
nad 1-6	NADH dehydrogenase subunit 1-6
nad4L	NADH dehydrogenase subunit 4L
Nst	Number of substitution types
ori	Origin of replication
PAM	Point accepted mutation
PCG	Protein coding gene

Abbreviation	Explanation
pp	Posterior probability
RaxML	Randomized axelerated maximum likelihood
Rcluster	Relaxed cluster algorithm
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
rrnL	Large rRNA subunit (16S subunit)
rrnS	Small rRNA subunit (12S subunit)
spp.	Several species
TDRL	Tandem duplication/random loss
TreeREx	Tree Rearrangement Explorer
tRNA	Transfer ribonucleic acid
trnA, Ala	Alanine tRNA coding unit
trnC, Cys	Cysteine tRNA coding unit

Abbreviation	Explanation
trnD, Asp	Aspartic acid tRNA coding unit
trnE, Glu	Glutamic acid tRNA coding unit
trnF, Phe	Phenylalanine tRNA coding unit
trnG, Gly	Glycine tRNA coding unit
trnH, His	Histidine tRNA coding unit
trnI, Ile	Isoleucine tRNA coding unit
trnK, Lys	Lysine tRNA coding unit
trnL, Leu	Leucine tRNA coding unit
trnM, Met	Methionine tRNA coding unit
trnN, Asn	Asparagine tRNA coding unit
trnP, Pro	Proline tRNA coding unit
trnQ, Gln	Glutamine tRNA coding unit
trnR, Arg	Arginine tRNA coding unit
trnS, Ser	Serine tRNA coding unit
trnT, Thr	Threonine tRNA coding unit
trnV, Val	Valine tRNA coding unit

Abbreviation	Explanation
trnW, Trp	Tryptophan tRNA coding unit
trnY, Tyr	Tyrosine tRNA coding unit

Table S3. List of taxa with mitogenomes containing gene duplications, deletions or *missing gene information, e.g. due to incomplete assembling. These taxa were not used in the TreeREx analysis

TAXON ID	TAXON NAME	REARRANGEMENT
SRR2051330	*Chorthippus biguttulus*	Deletion of gene *trnN**
SOH_710	***CHORTHIPPUS EISENTRAUTI***	**DELETION OF GENE *TRNQ****
NC_028062	*Comicus campestris*	Deletion of gene *trnI**
NC_032075	***EUBORELLIA ARCANUM***	**DELETION OF GENE *TRNY****
SOH_693	*Omocestus rufipes*	Deletion of gene *trnI**
SOH_658	***PSEUDOCHORTHIPPUS PARALLELUS***	**DELETION OF GENE *TRNQ****
SOH_659	*Pseudochorthippus parallelus*	Deletion of gene *trnQ**
SOH_689	***STENOBOTHRUS LINEATUS***	**DELETION OF GENE *TRNQ****
NC_011306	*Troglophilus neglectus*	Duplication of gene *trnL2*
NC_041236	***XENOGRYLLUS MARMORATUS***	**DUPLICATION OF GENE *TRNH***
NC_030265	*Mantis religiosa*	Duplication of *trnR*

Appendix 1: Gene order file (as .txt file) uploaded to https://github.com/laradey/mitogenome_gene_order.

Appendix 2: Original TreeREx output (Bayesian inference tree as .svg file) with consistency indices (CI) indicated in green and yellow boxes. "I": inversion, "T": transposition, "iT": inverse transposition, TDRL: tandem-duplication/random loss uploaded to https://github.com/laradey/mitogenome_gene_order.

SI CHAPTER 6

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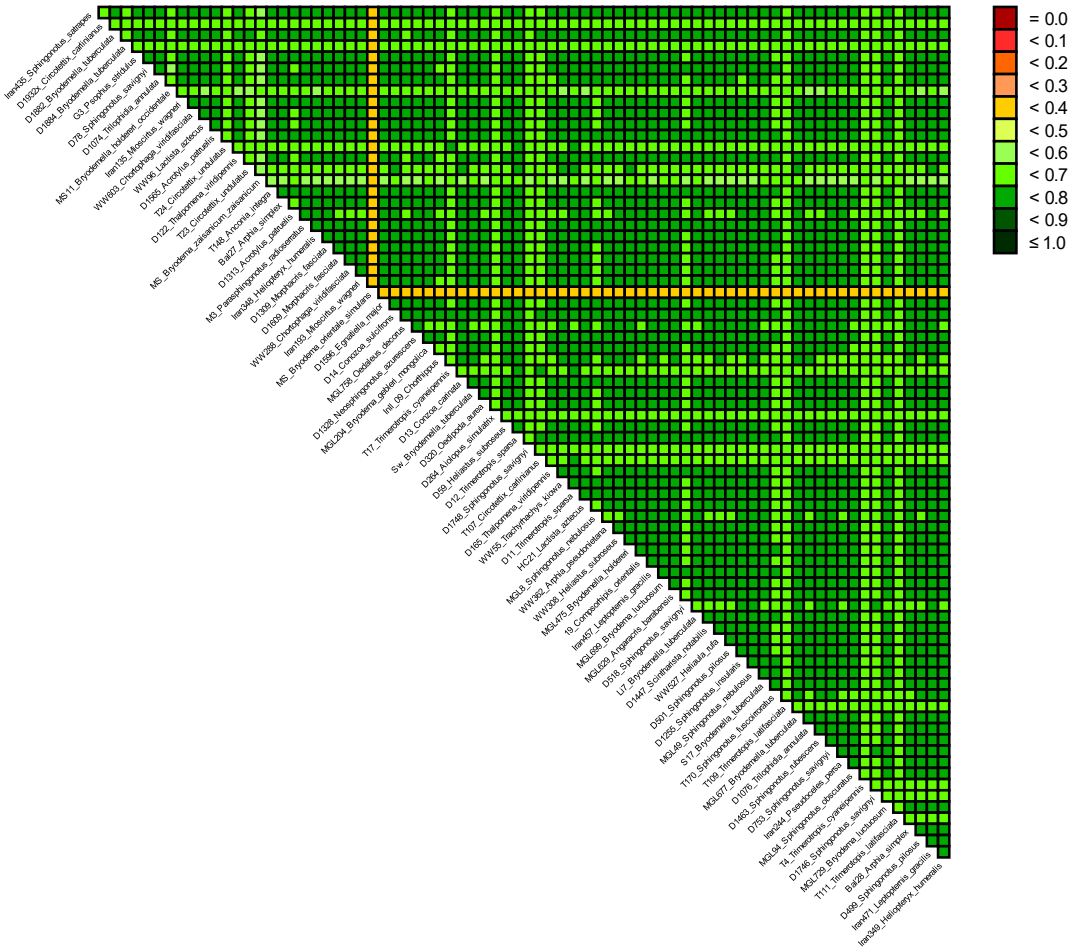
SAME SAME BUT DIFFFERENT

Genomic data, morphometrics and ecological modelling confirm large scale convergence of wing morphology in band winged grasshoppers (Orthoptera: Oedipodinae)

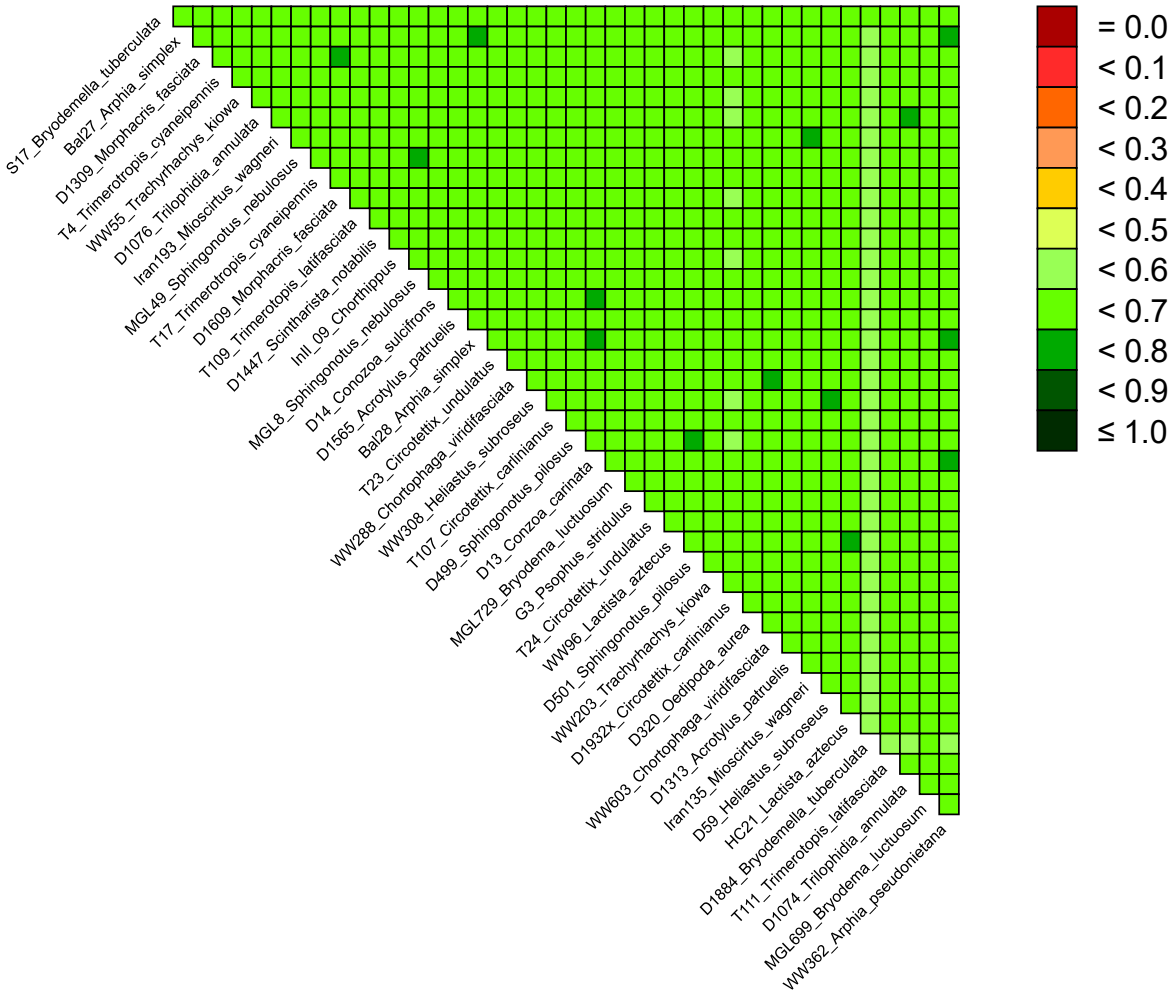
Lara-Sophie Dey, Axel Hochkirch, Hojun Song, Marianna Simoes, Karen Meusemann, Oliver Hawlitschek & Martin Husemann

SI 1: Symtest supermatrix for the full and reduced genomic datasets.

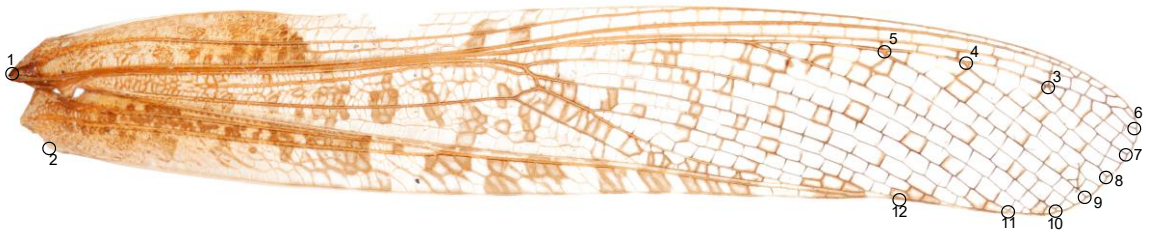
Full dataset



Reduced dataset


S17_Bryodemella_tuberculata
Bal27_Arphia_simplex
D1309_Morphacris_fasciata
T4_Trimerotropis_cyaneipennis
WW55_Trachyrhachys_kiowa
D1076_Trilophidia_annulata
Iran193_Mioscirtus_wagneri
MGL49_Sphingonotus_nebulosus
T17_Trimerotropis_cyaneipennis
D1609_Morphacris_fasciata
T109_Trimerotopis_latifasciata
D1447_Scintharista_notabilis
Inll_09_Chorthippus
MGL8_Sphingonotus_nebulosus
D14_Conozoa_sulciffons
D1565_Acrotylus_patruelis
Bal28_Arphia_simplex
T23_Circotettix_undulatus
WW288_Chortophaga_viridifasciata
WW308_Heliastus_subroseus
T107_Circotettix_carlinianus
D499_Sphingonotus_pilosus
D13_Conzoa_carinata
MGL729_Bryodema_luctuosum
G3_Psophus_stridulus
T24_Circotettix_undulatus
WW96_Lactista_aztecus
D501_Sphingonotus_pilosus
WW203_Trachyrhachys_kiowa
D1932x_Circotettix_carlinianus
D320_Oedipoda_aurea
WW603_Chortophaga_viridifasciata
D1313_Acrotylus_patruelis
Iran135_Mioscirtus_wagneri
D59_Heliastus_subroseus
HC21_Lactista_aztecus
D1884_Bryodemella_tuberculata
T111_Trimerotopis_latifasciata
D1074_Trilophidia_annulata
MGL699_Bryodema_luctuosum
WW362_Arphia_pseudonietana
= 0.0
< 0.1
< 0.2
< 0.3
< 0.4
< 0.5
< 0.6
< 0.7
< 0.8
< 0.9
≤ 1.0

SI 2: Landmarks acquired from Elytra.



SI 3: Tps file used for morphometrical analyses

Available on GitHub
https://github.com/laradey/Convergence_Oedipodinae/all_no_na.tps

SI 4: Species occurrences downloaded from GBif, Species occurrences based on literature data, references and data points.

Chorthippus alticola Ramme, 1921 - DOI 10.15468/dl.42py3m – outgroup - 27

Heliastus sumichrasti (Saussure, 1861) - DOI10.15468/dl.ehc497 - 144

Conozoa sulcifrons (Scudder, 1876) - DOI10.15468/dl.m2vhbe - 366

Morphacris fasciata (Thunberg, 1815) - DOI10.15468/dl.h4vswz - 179Circotettix carlinianus (Thomas, 1870) - DOI10.15468/dl.vem69s - 94

Lactista azteca (Saussure, 1861) - DOI10.15468/dl.mbx3tp - 287

Arphia pseudonietana (Thomas, 1870) - DOI10.15468/dl.ygyhnk - 885

Psophus stridulus (Linnaeus, 1758) - DOI10.15468/dl.fxx546 - 3864

Arphia simplex Scudder, 1875 - DOI10.15468/dl.y25qtg - 328

Scintharista notabilis (Walker, 1870) - DOI10.15468/dl.aadyvc - 36

Trimerotropis sparsa (Thomas, 1875) - DOI10.15468/dl.xntv3h - 67

Trimerotropis cyaneipennis Bruner, 1889 - DOI10.15468/dl.cvet9n - 204

Trilophidia annulata (Thunberg, 1815) - DOI10.15468/dl.mdnwcx - 674

Chortophaga viridifasciata (De Geer, 1773) - DOI10.15468/dl.ttzp8x - 5185

Trimerotropis latifasciata Scudder, 1880 - DOI10.15468/dl.73dztf - 180

Circotettix undulatus (Thomas, 1872) - DOI10.15468/dl.g82aer - 80

Trachyrhachys kiowa (Thomas, 1872) - DOI10.15468/dl.f9urxg - 908

Mioscirtus wagneri (Eversmann, 1859) - DOI10.15468/dl.s4wz26 – 61

Bryodema luctuosum – DOI10.15468/dl.7ntmbf; Tishechkin, 2010; Bey-Bienko, 1929; Zhi et al. 2013; Benediktov, 2016; Zhang et al. 2006. - 27

Acrotylus patruelis – Wehrt et al. unpublished - 243

Mioscirtus wagneri – Cordero et al. 2007; Uvarov, 1934; Mistshenko, 1937; Hollier, 2012; Descamps, 1970; Ortego et al. 2009 . - 61

Oedipoda aurea – Abusarhan et al. 2017; Sevgili et al. 2011, 2012; Naskrecki and Ünal, 1995; Uvarov, 1934; Hodjat et al. 2018; Willemse, Kleukers, & Odé, 2018; Collection Material from Naturalis Leiden; DOI10.15468/dl.2uc2y8 - 39

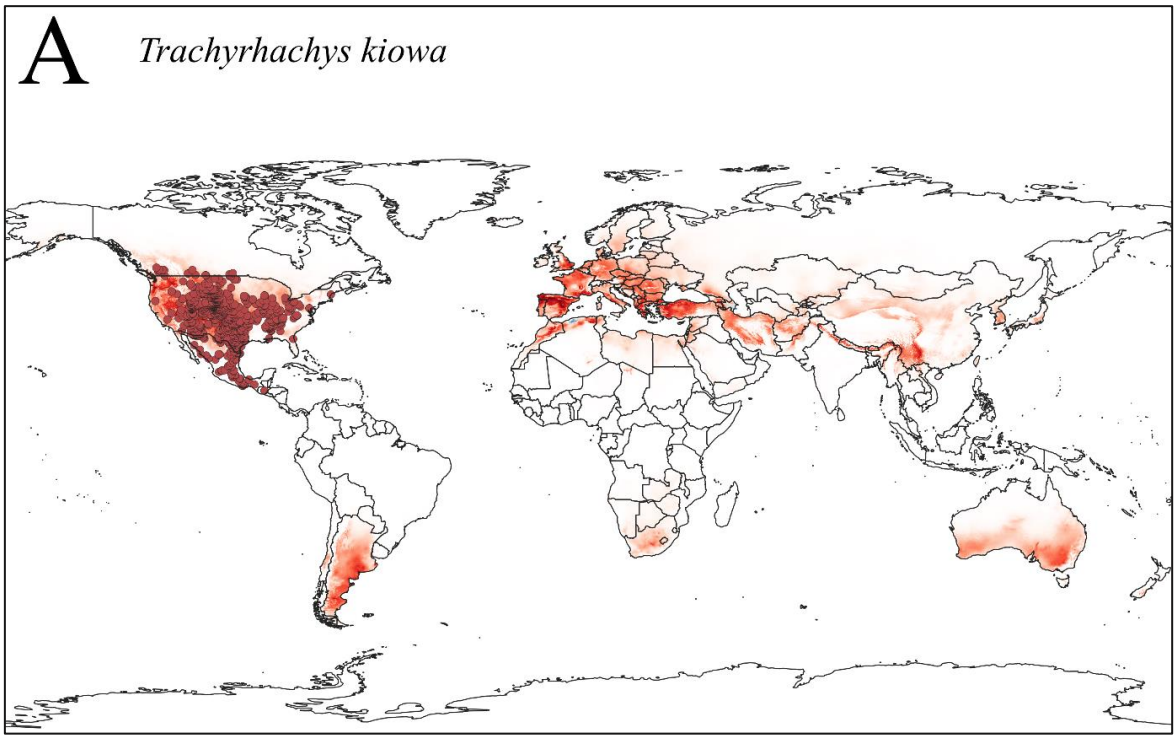
Sphingonotus pilosus – Sevgili et al. 2012; Ünal, 1999; Dey et al. 2018; Hodjat et al. 2018; Garai et al. 2010; Ghahari et al. 2009. - 32

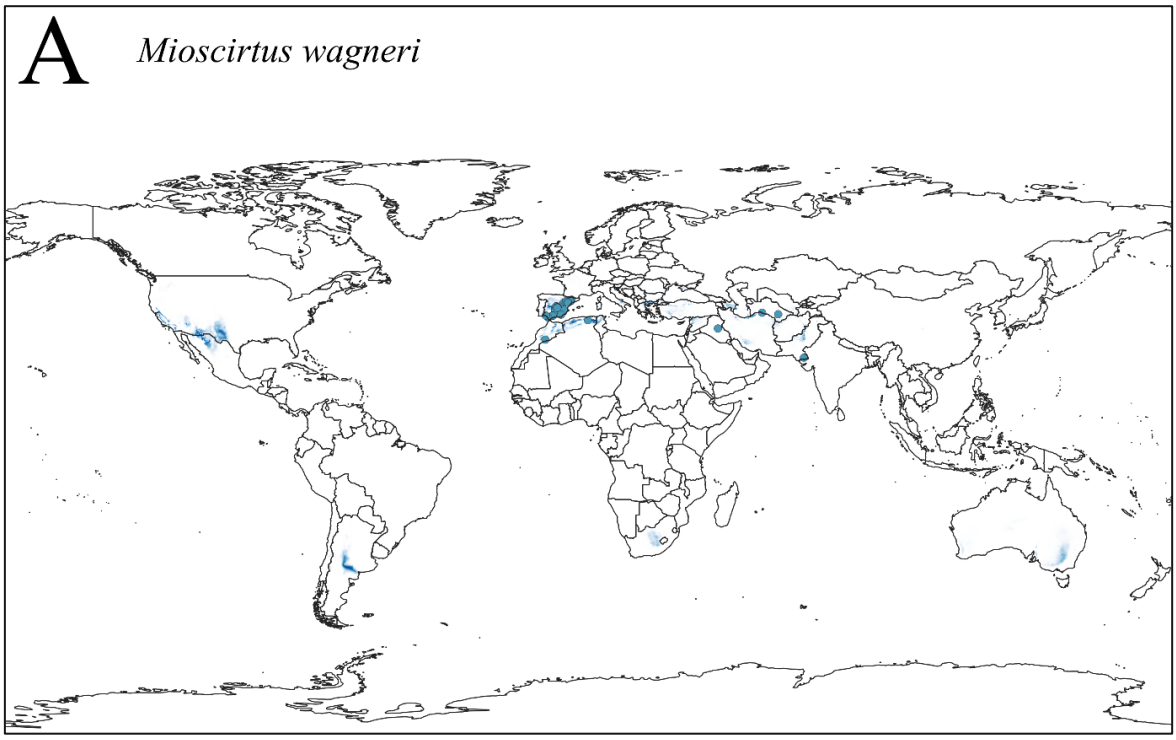
Sphingonotus nebulosus - Sevgili et al. 2012; Ünal, 1999; Dey et al. 2018; Hodjat et al. 2018; Ghahari et al. 2009. - 13

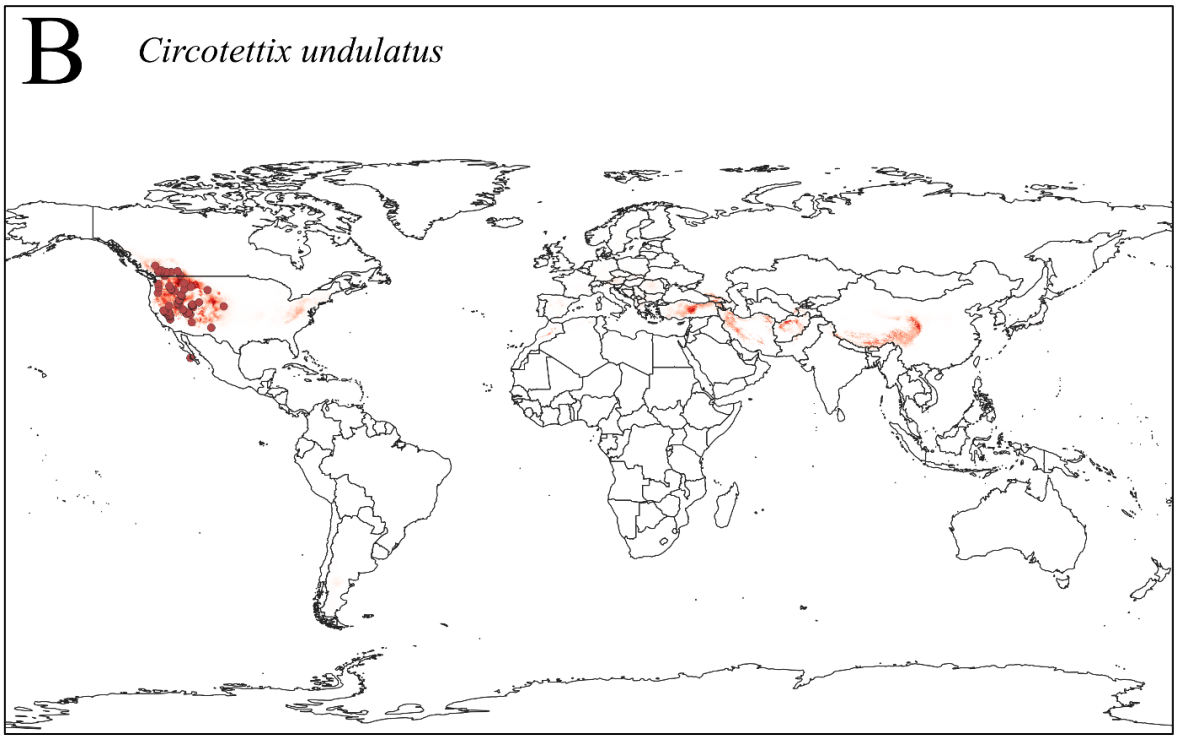
Bryodemella tuberculata – Dey et al. 2021 – 380

All occurrence data are available on GitHub.
https://github.com/laradey/Convergence_Oedipodinae

Occurrences plotted on suitability maps.



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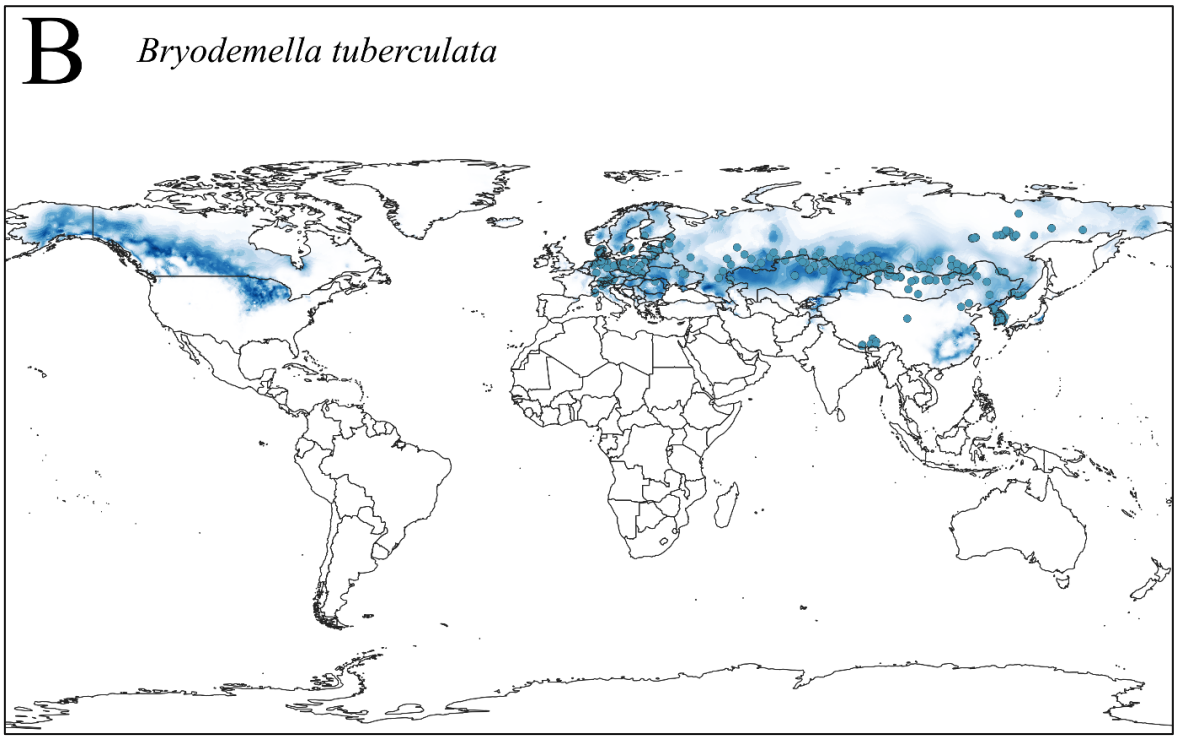
Circotettix undulatus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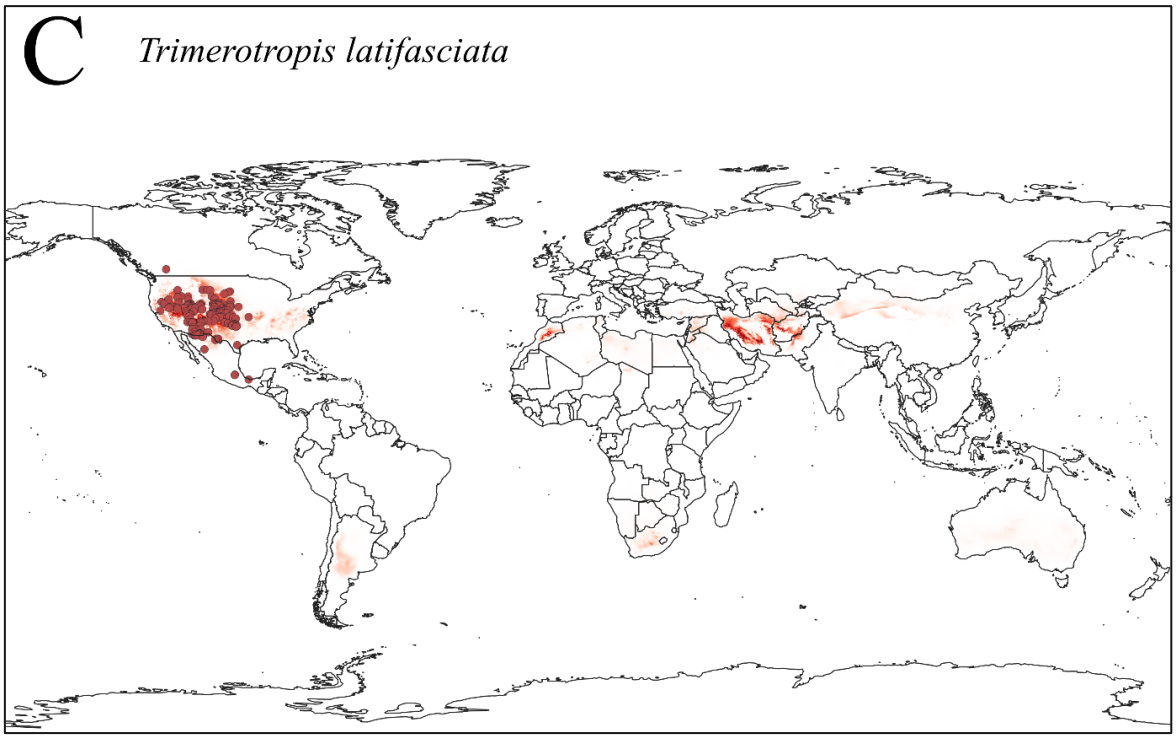
Bryodemella tuberculata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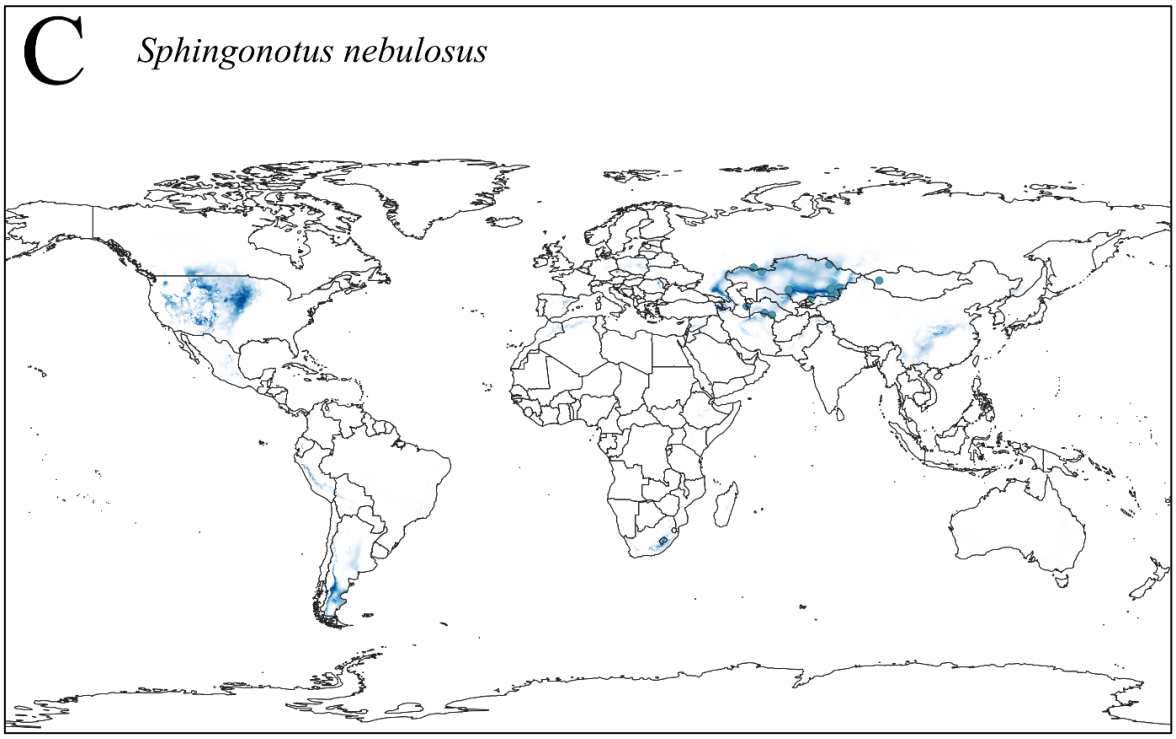
Trimerotropis latifasciata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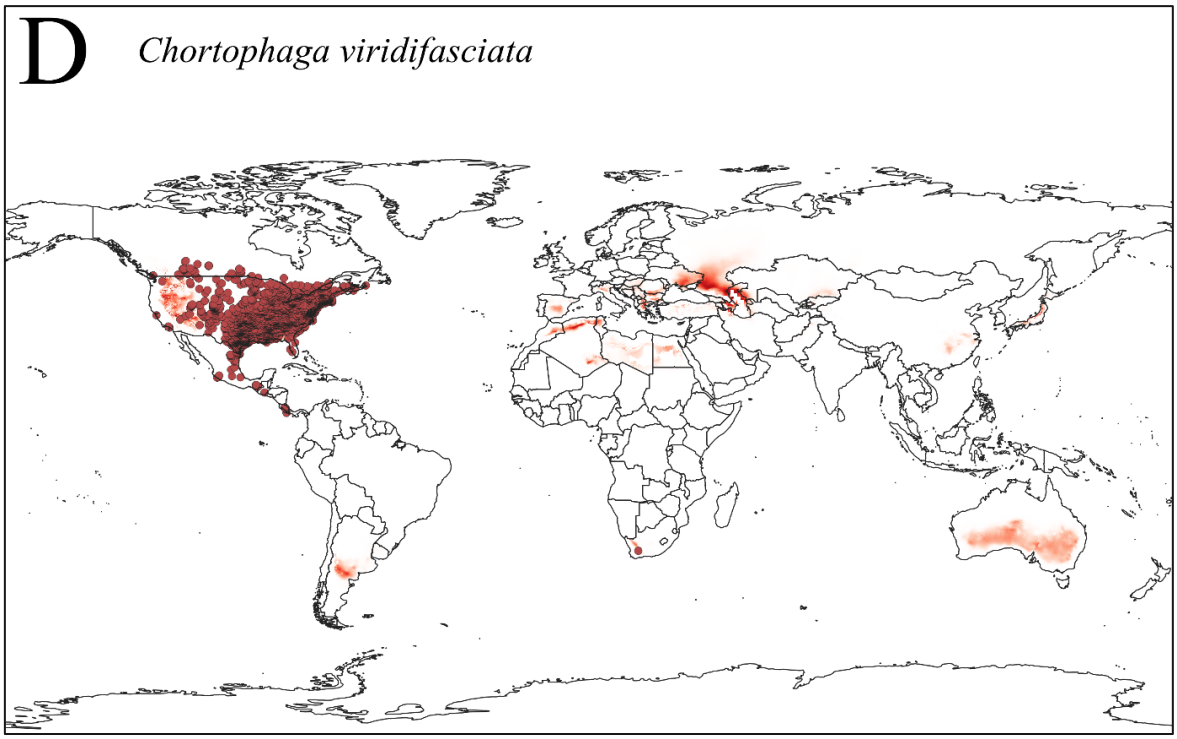
Sphingonotus nebulosus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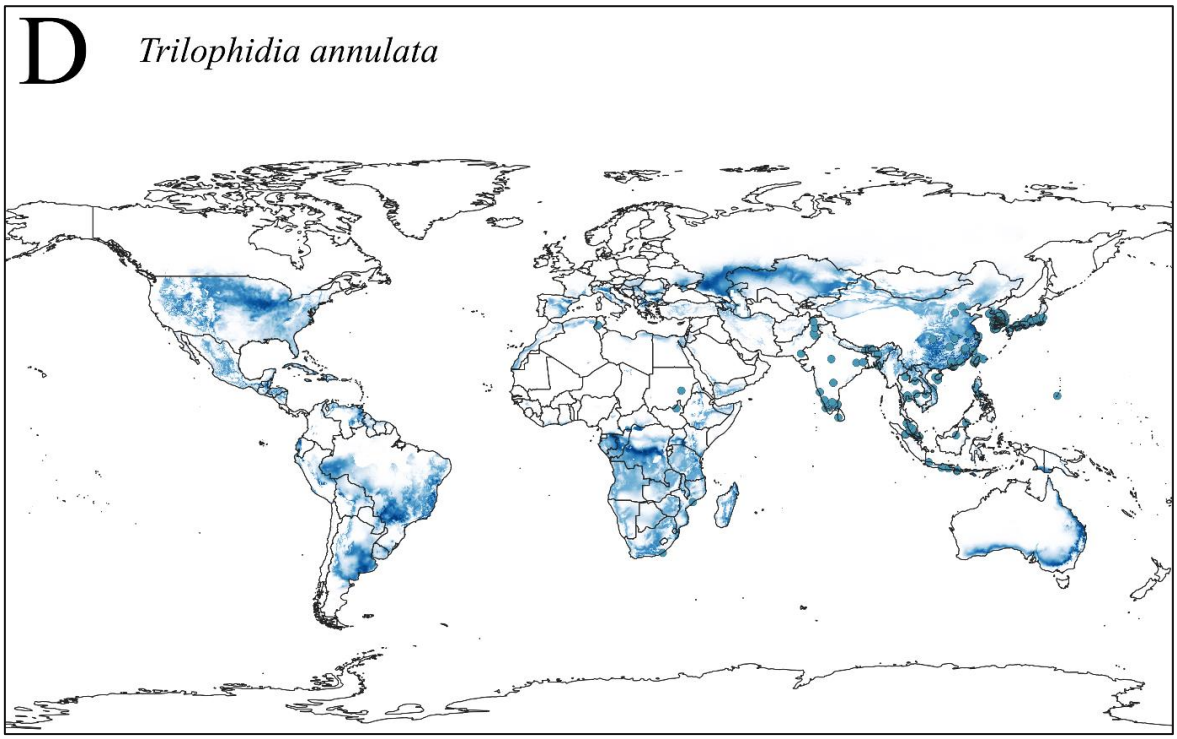
Chortophaga viridifasciata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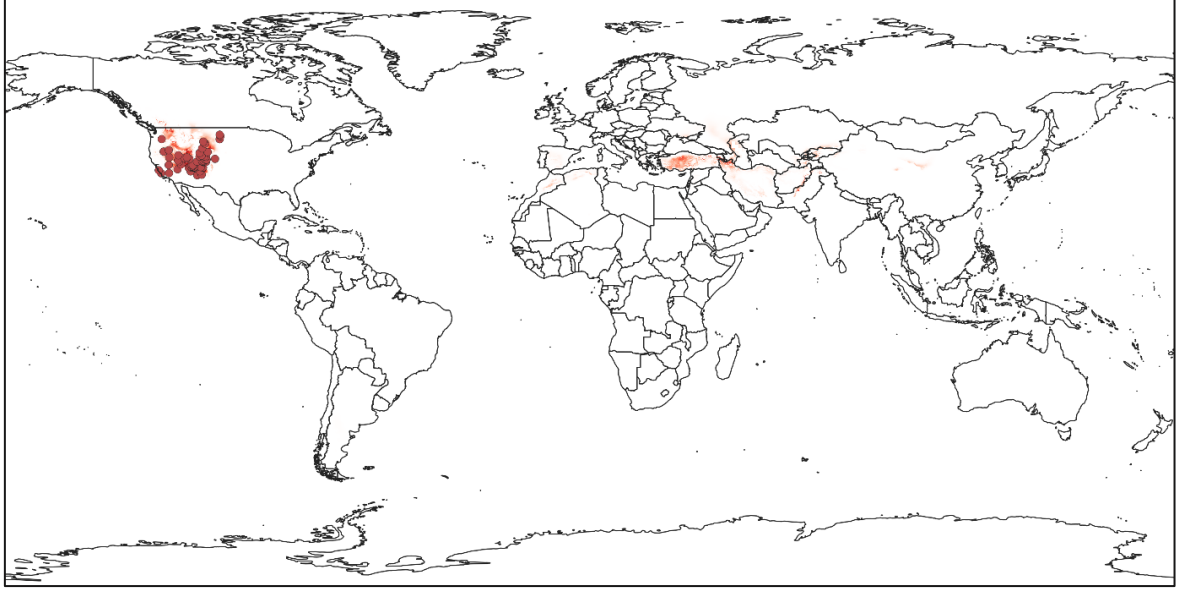
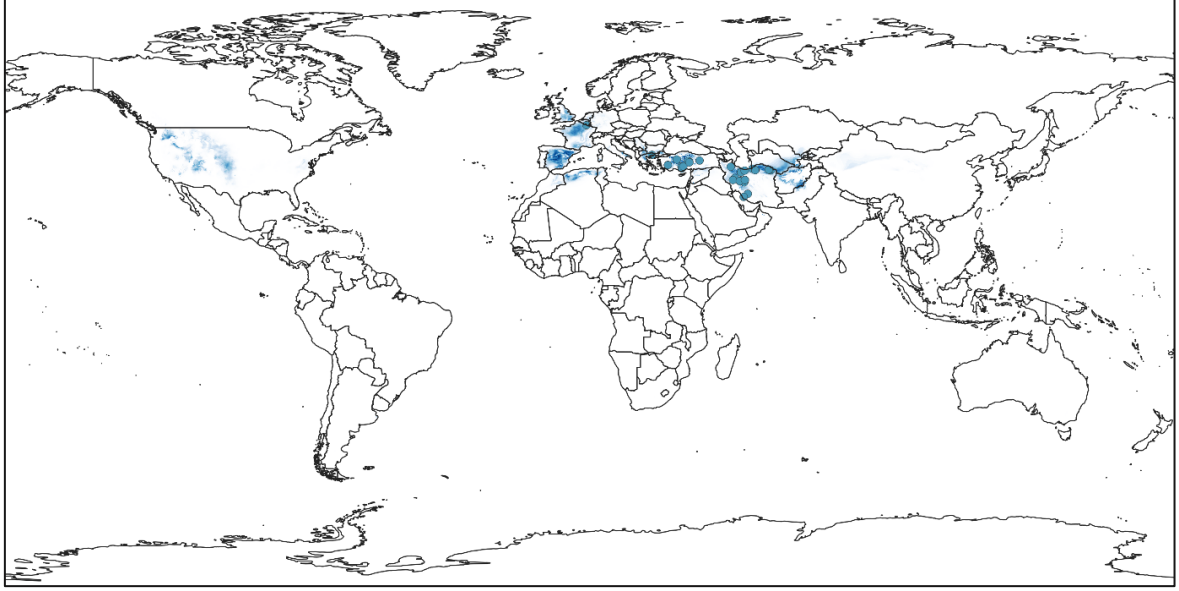
Trilophidia annulata

F *Trimerotropis sparsa*



F *Sphingonotus pilosus*



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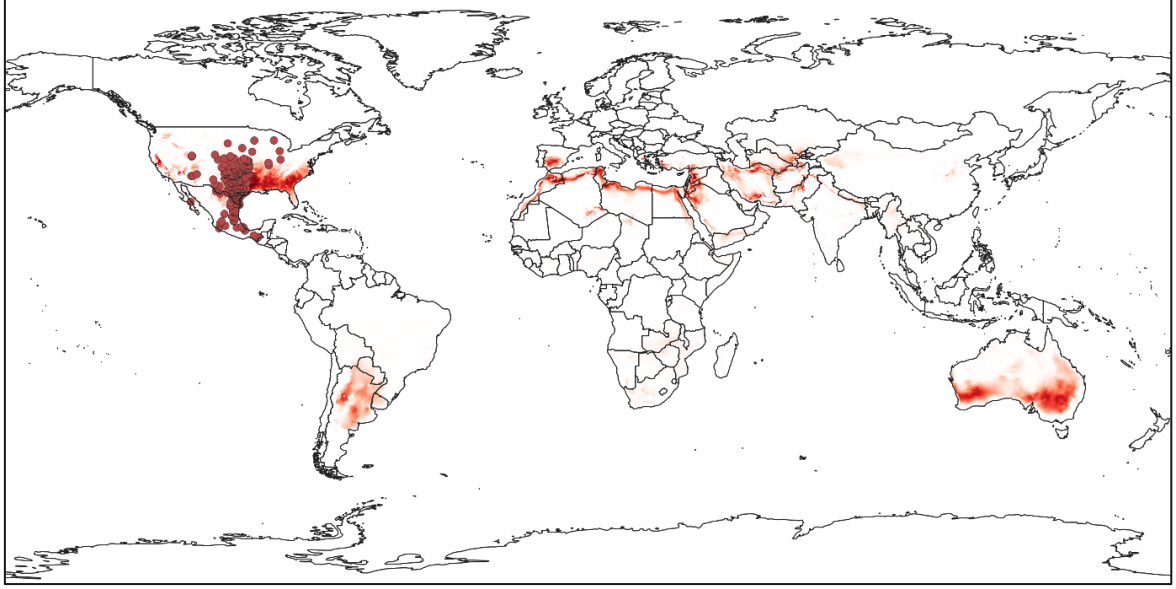
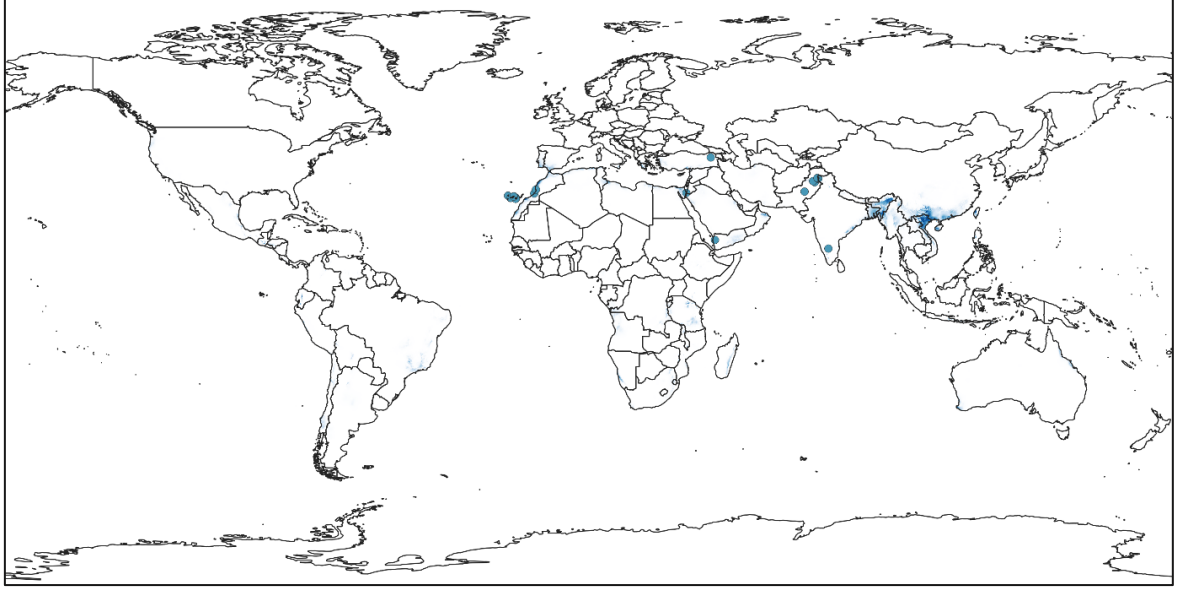
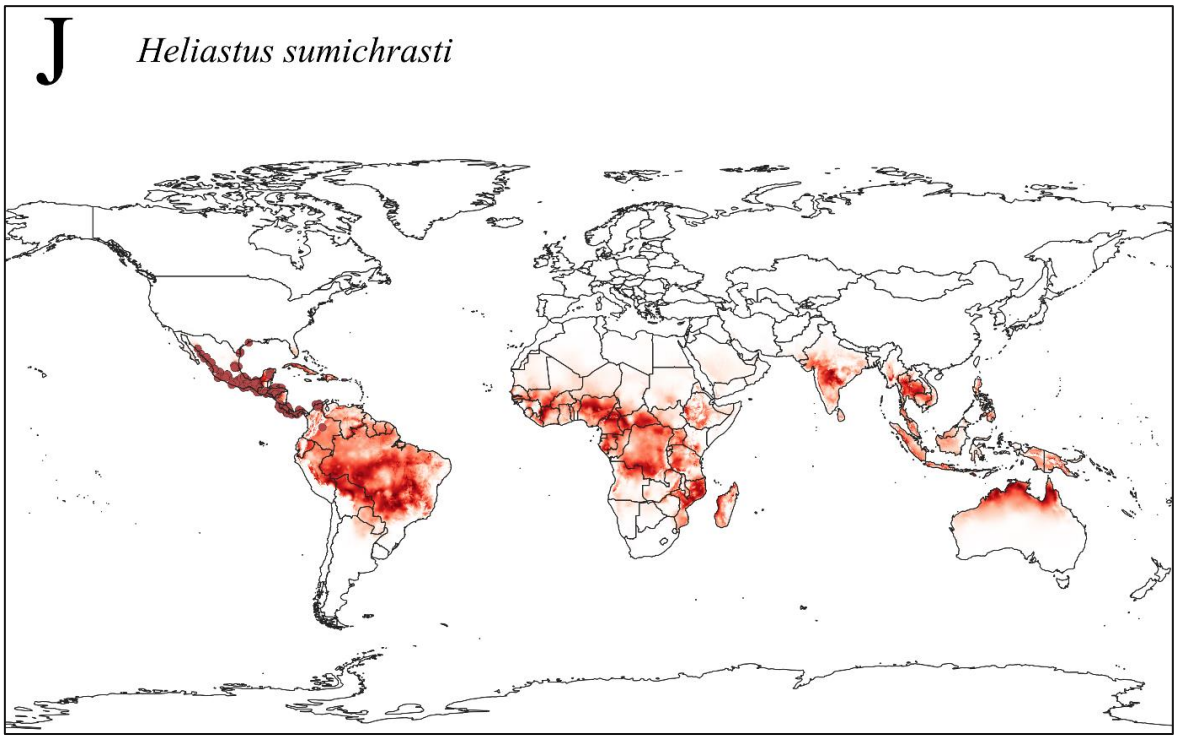


G *Scintharista notabilis*

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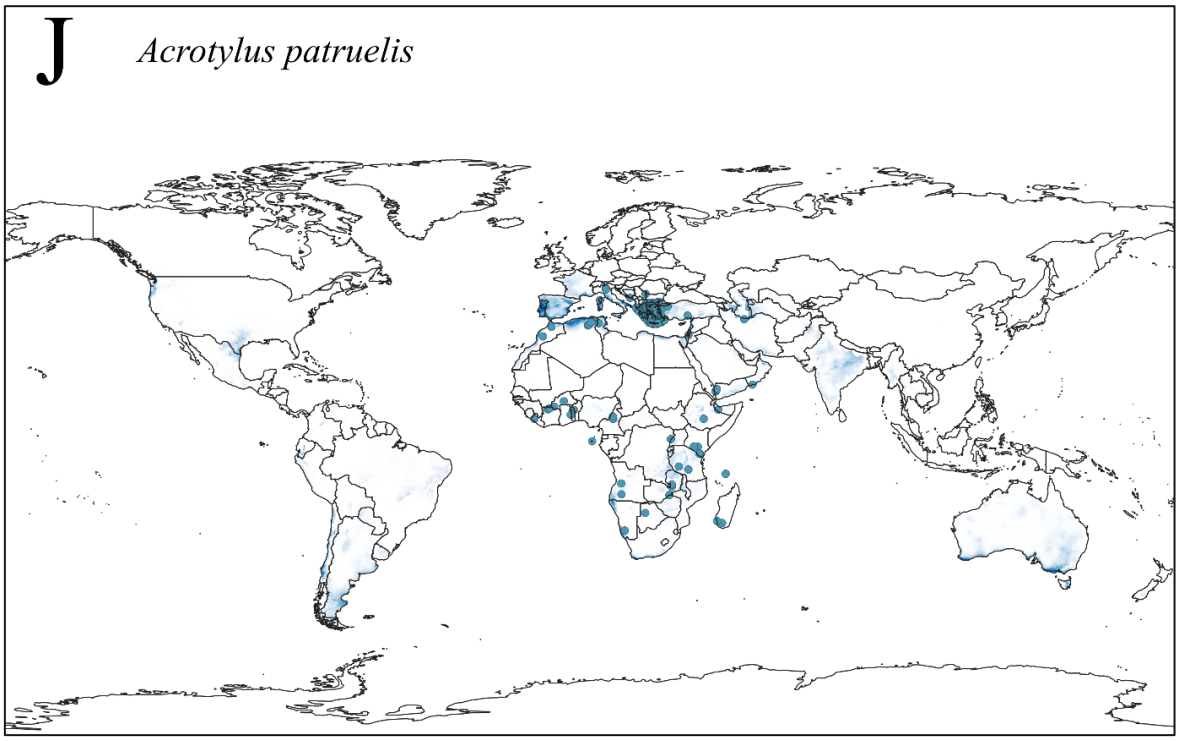
Heliastus sumichrasti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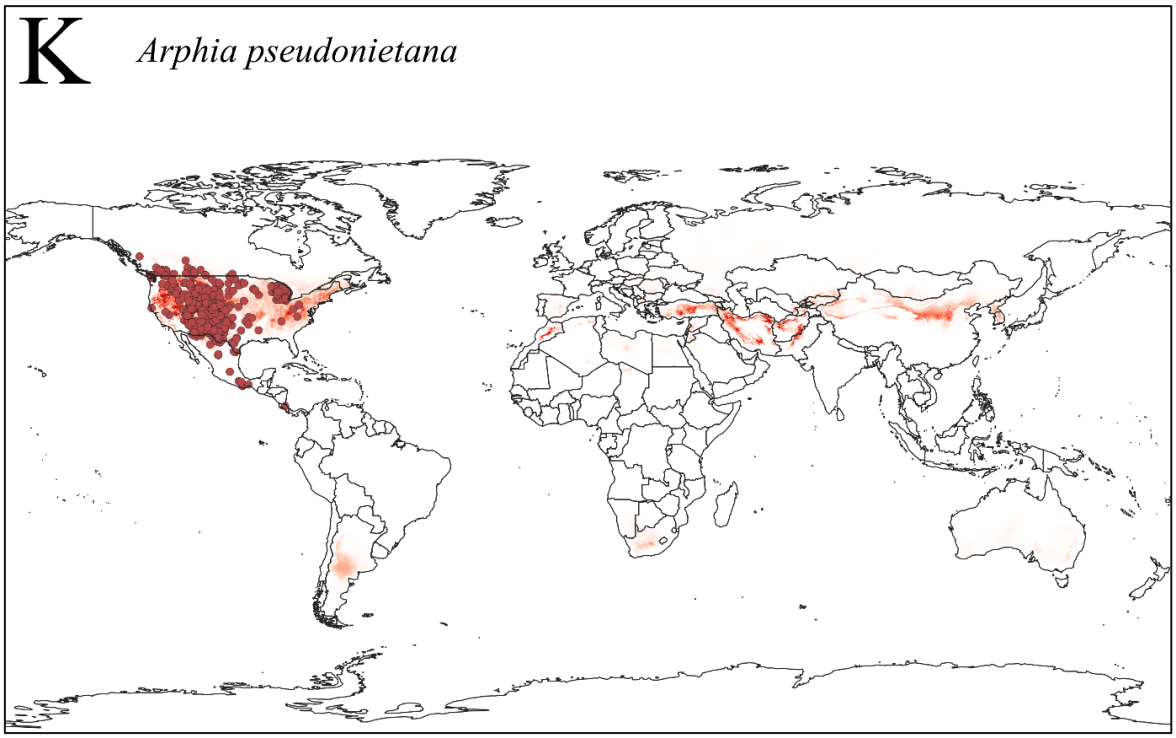
Acrotylus patruelis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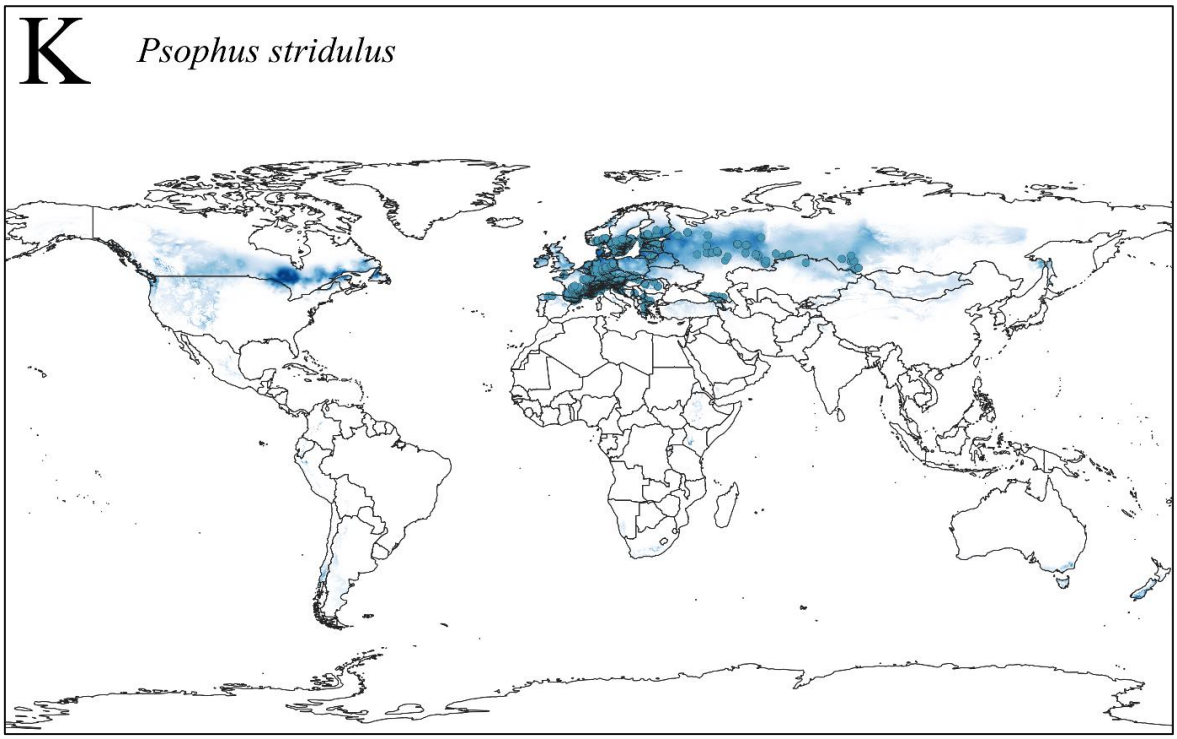
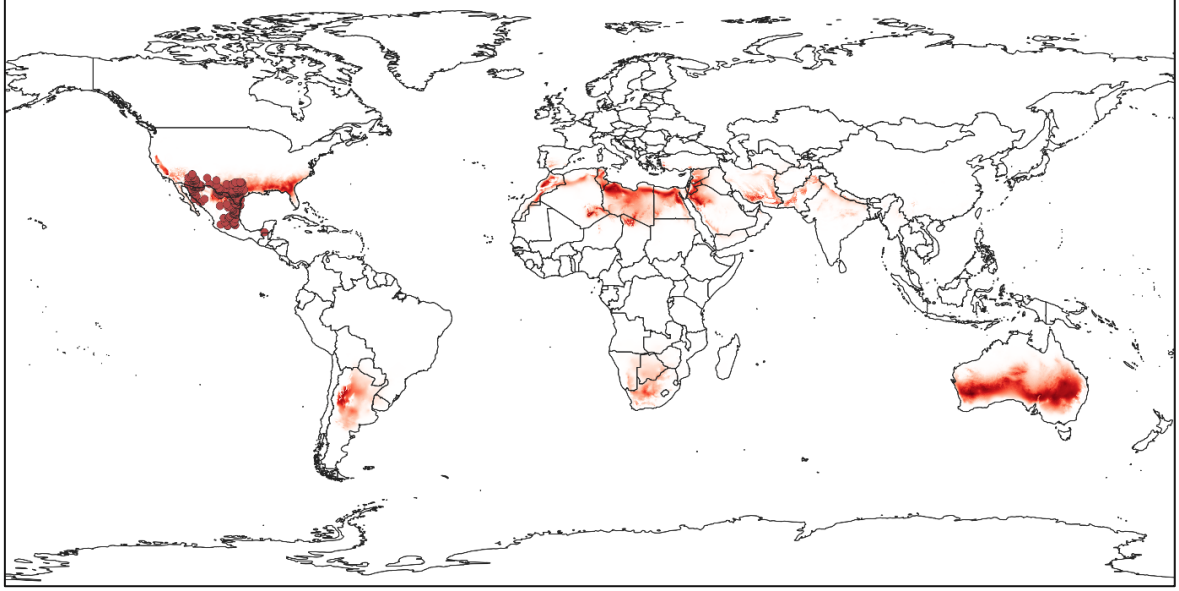
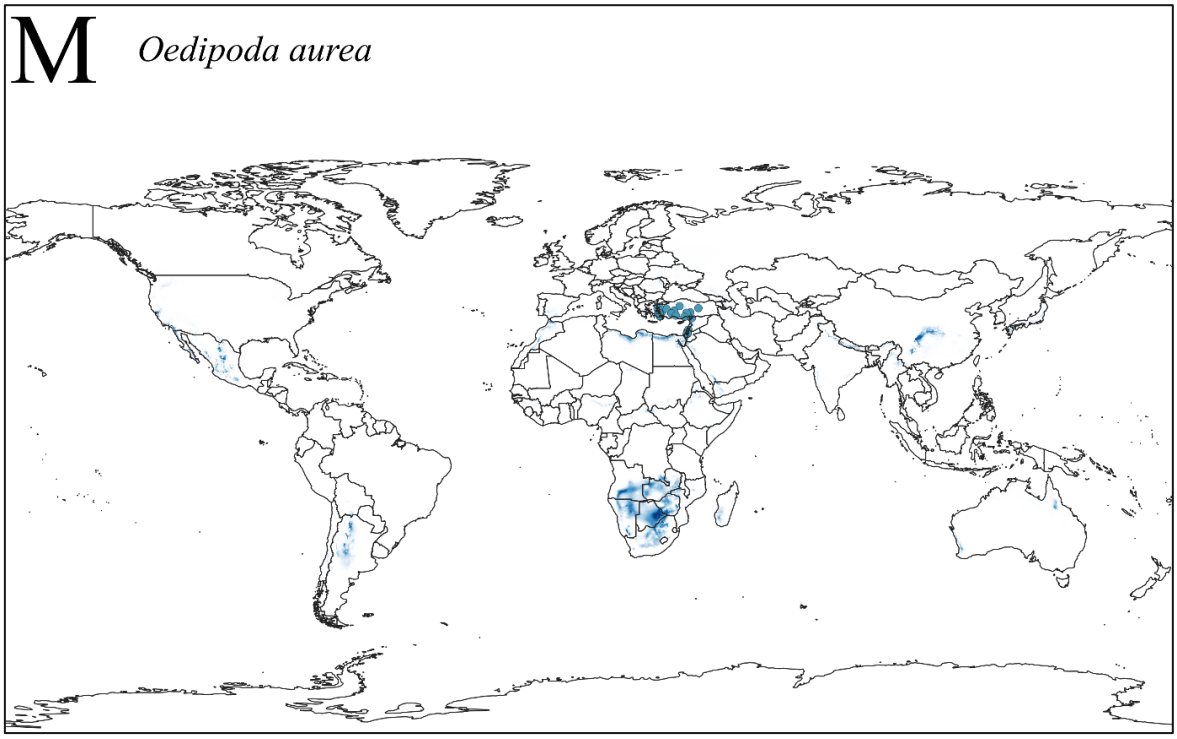
Arphia pseudonietana

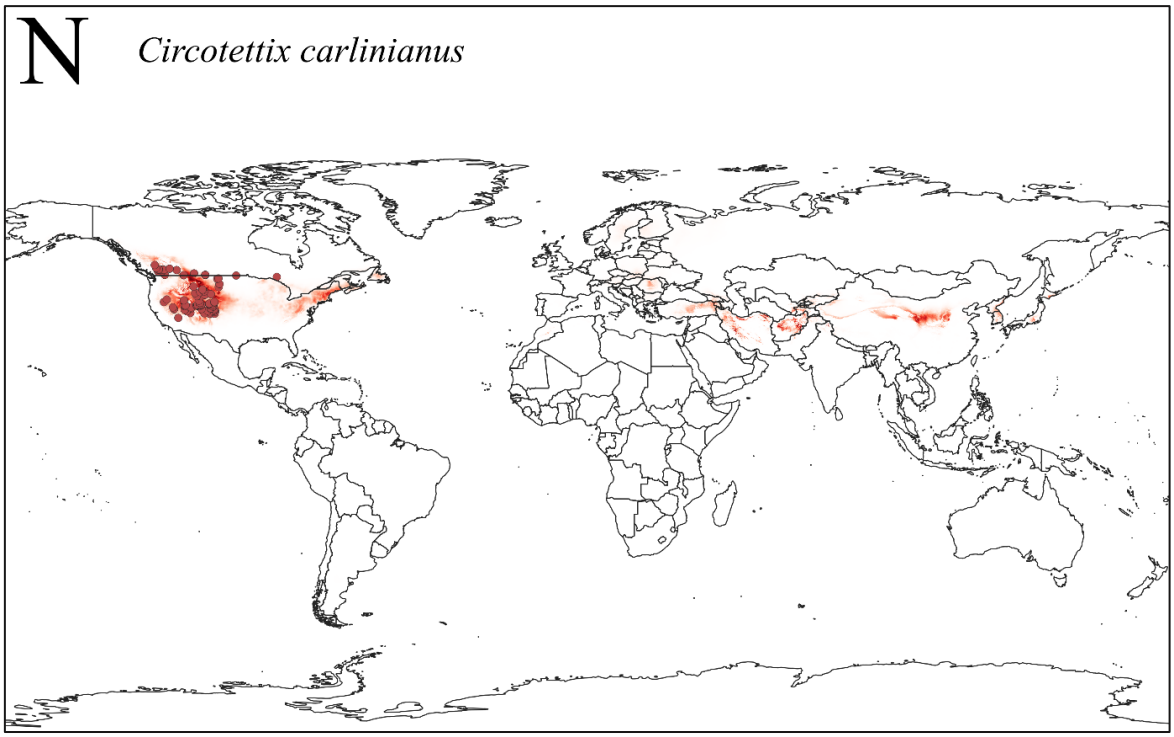

K
Psophus stridulus

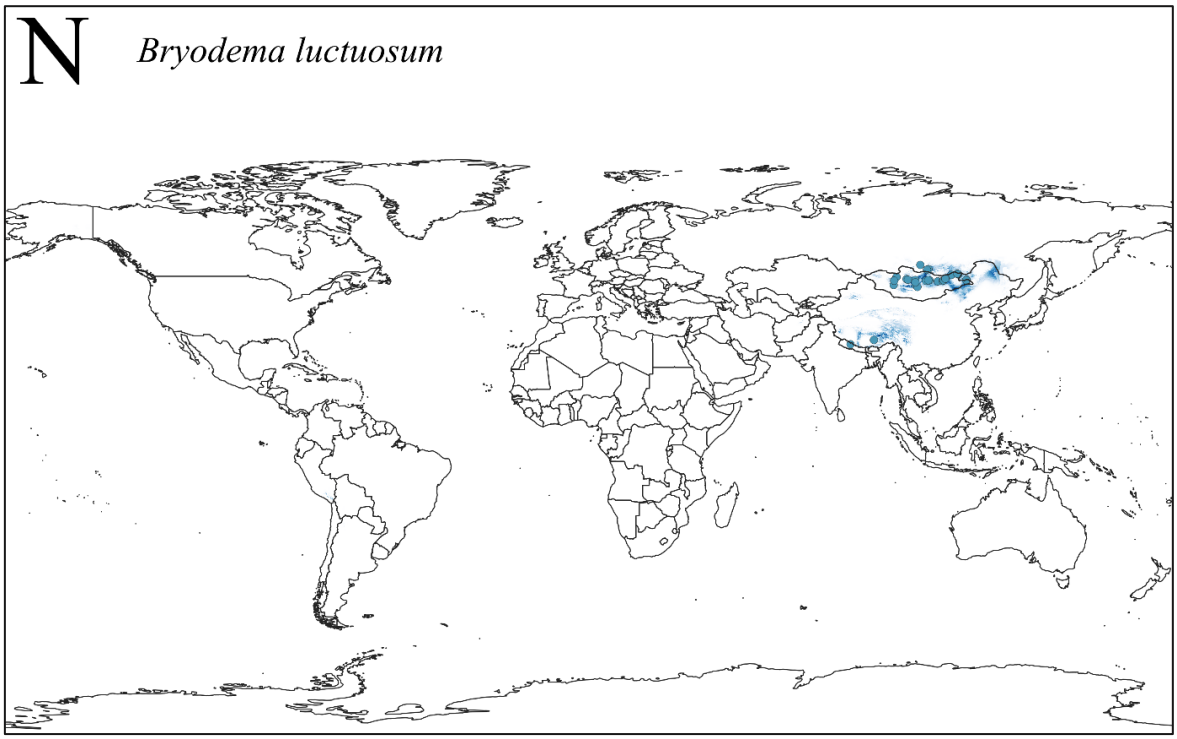
M *Lactista azteca*

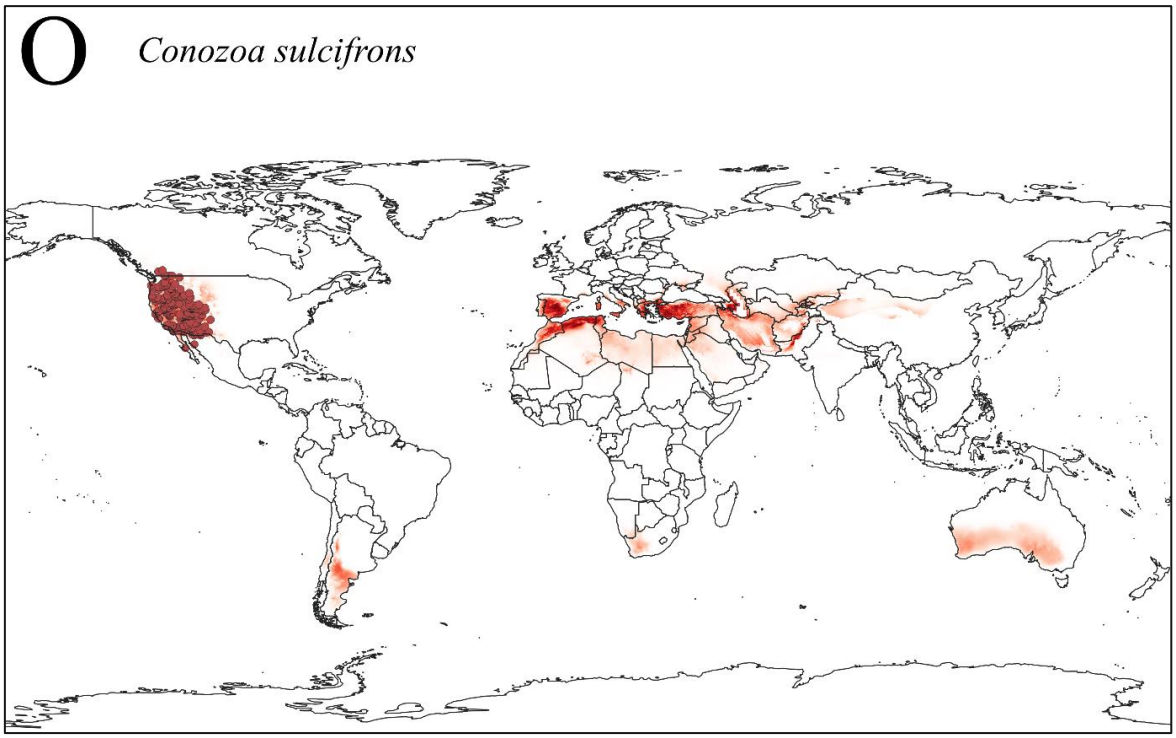


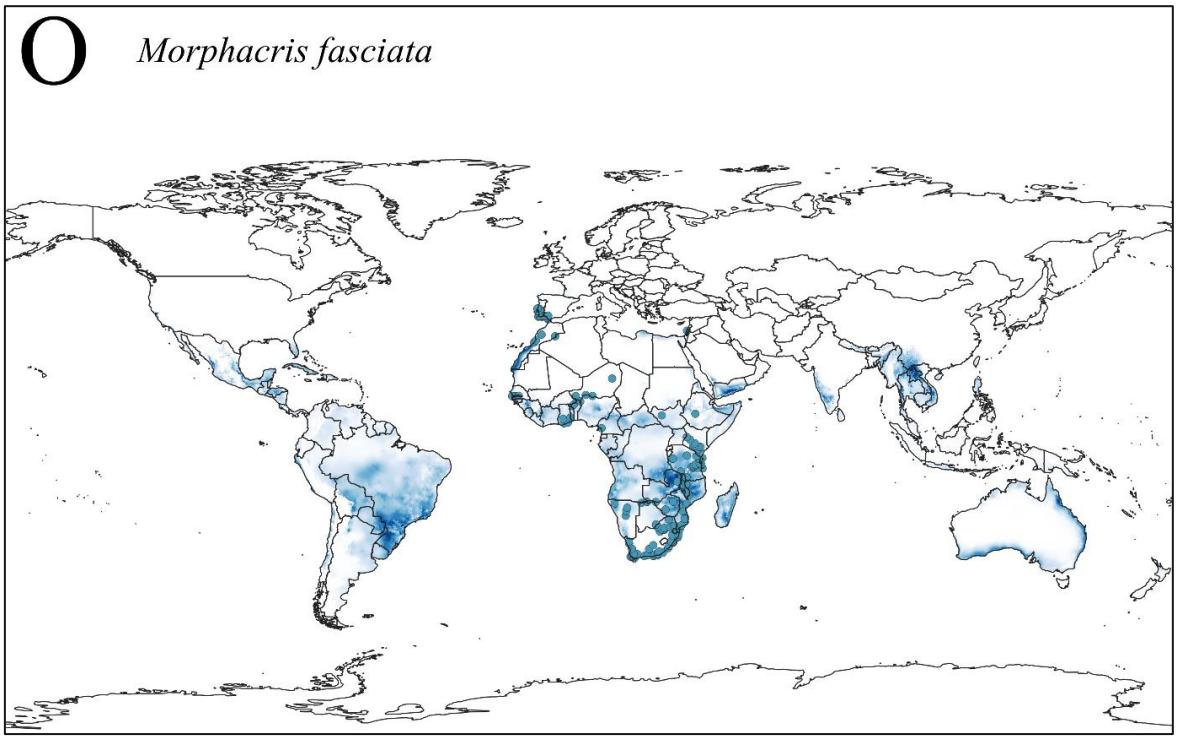
M *Oedipoda aurea*




N
Circotettix carlinianus

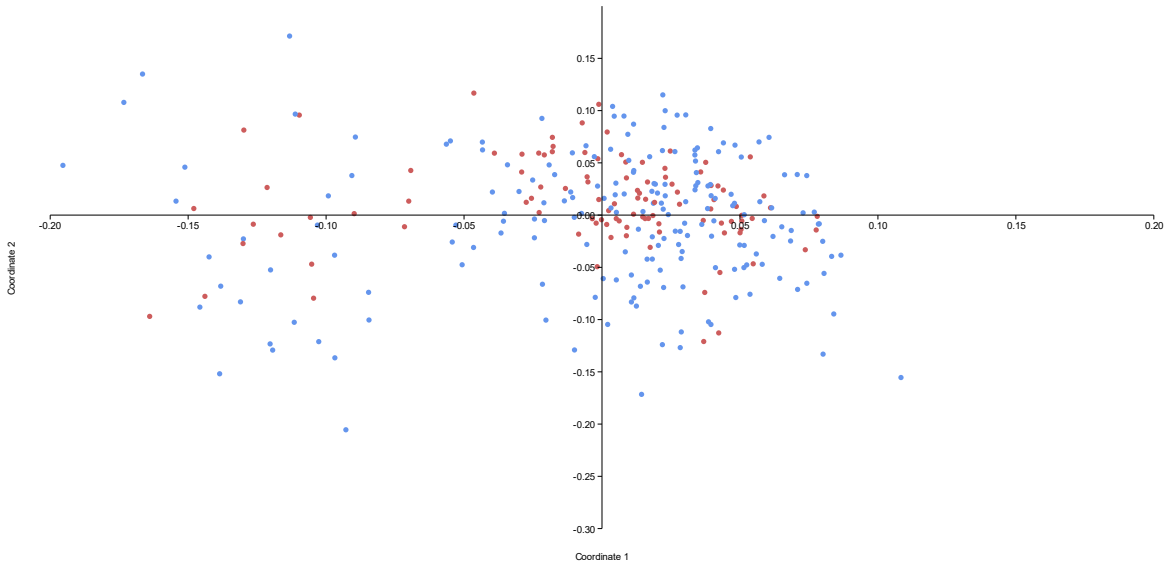

N
Bryodema luctuosum


Conozoa sulcifrons

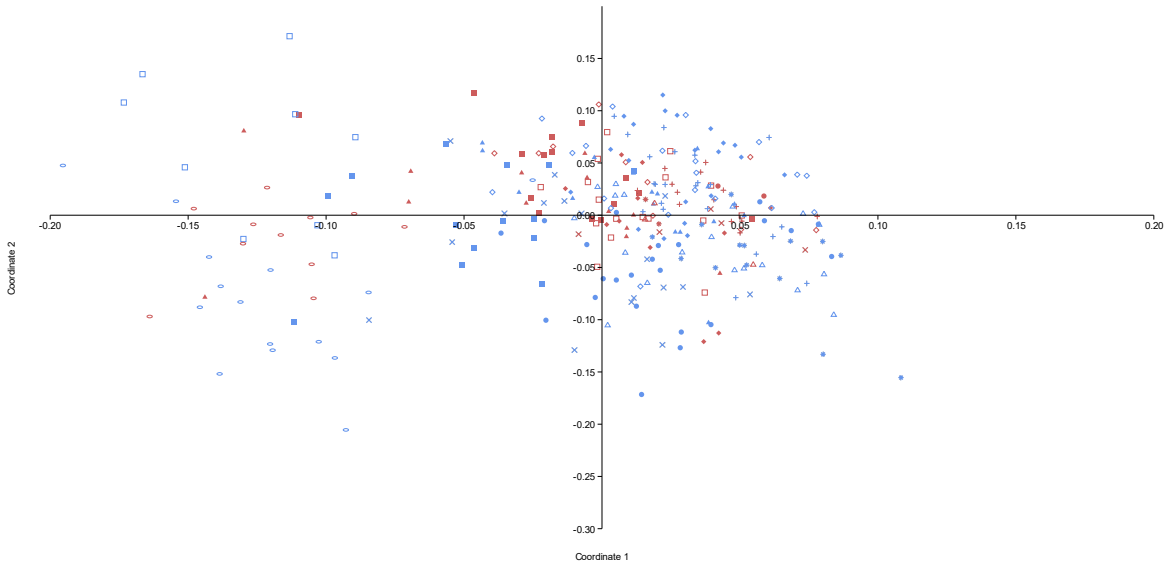

Morphacris fasciata

SI 5: Past 3 PCoA analyses for the morphometric dataset

Realms

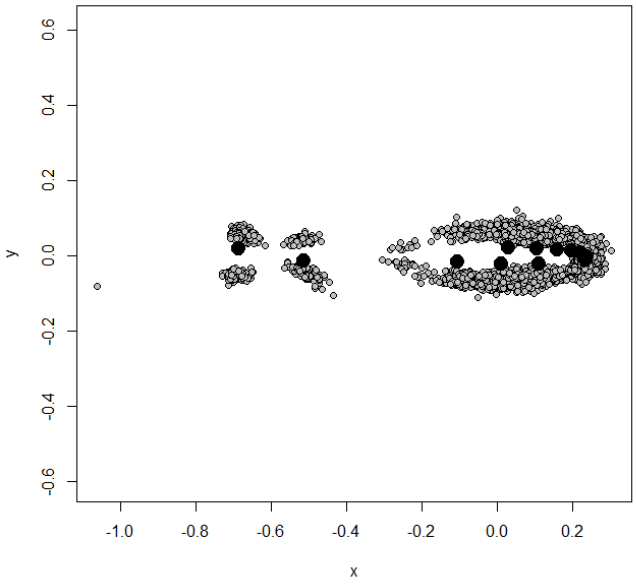


species

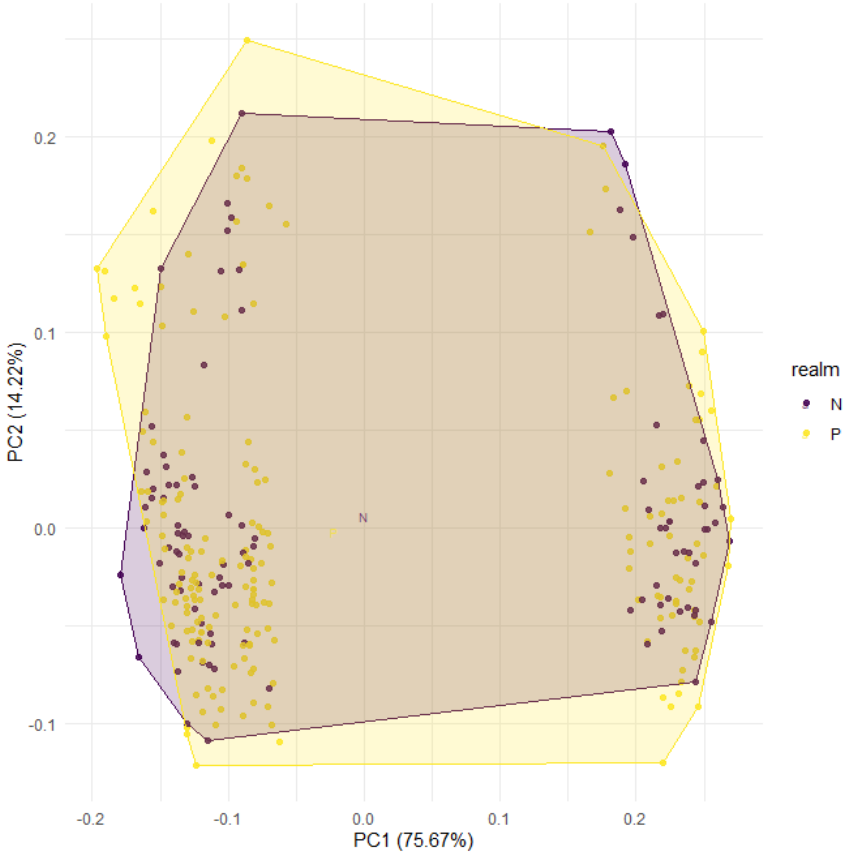


SI 6: Resulting PCA analyses and outlier analysis of R "geomorph" package.

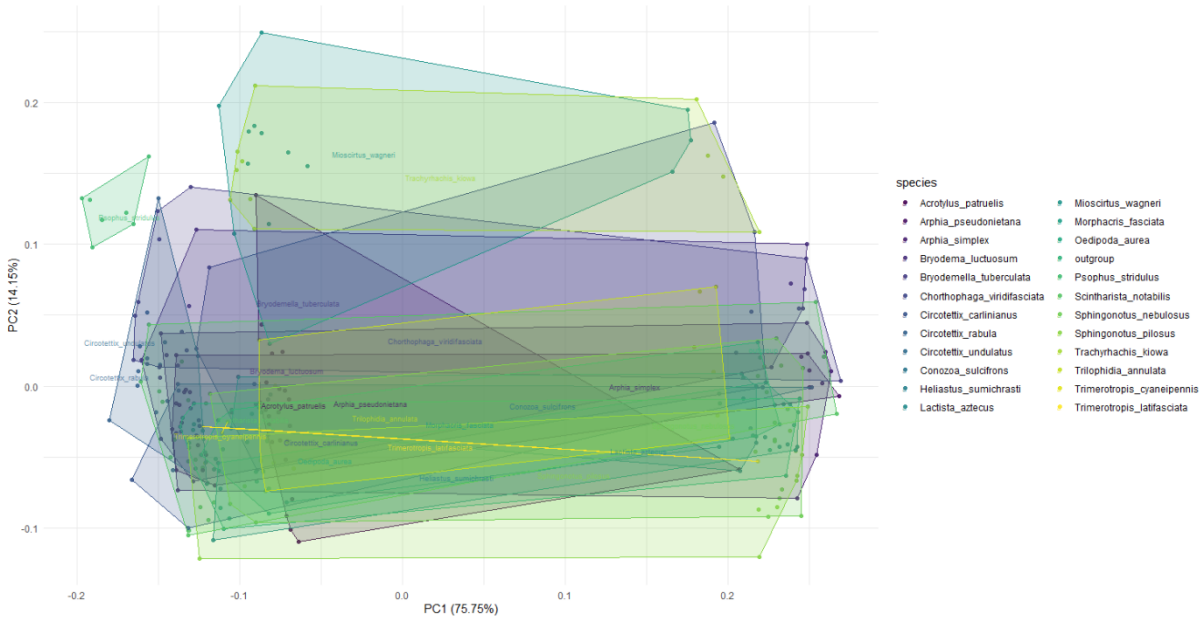
Outlier analysis



PCA realm (N – New World, P – Old World)

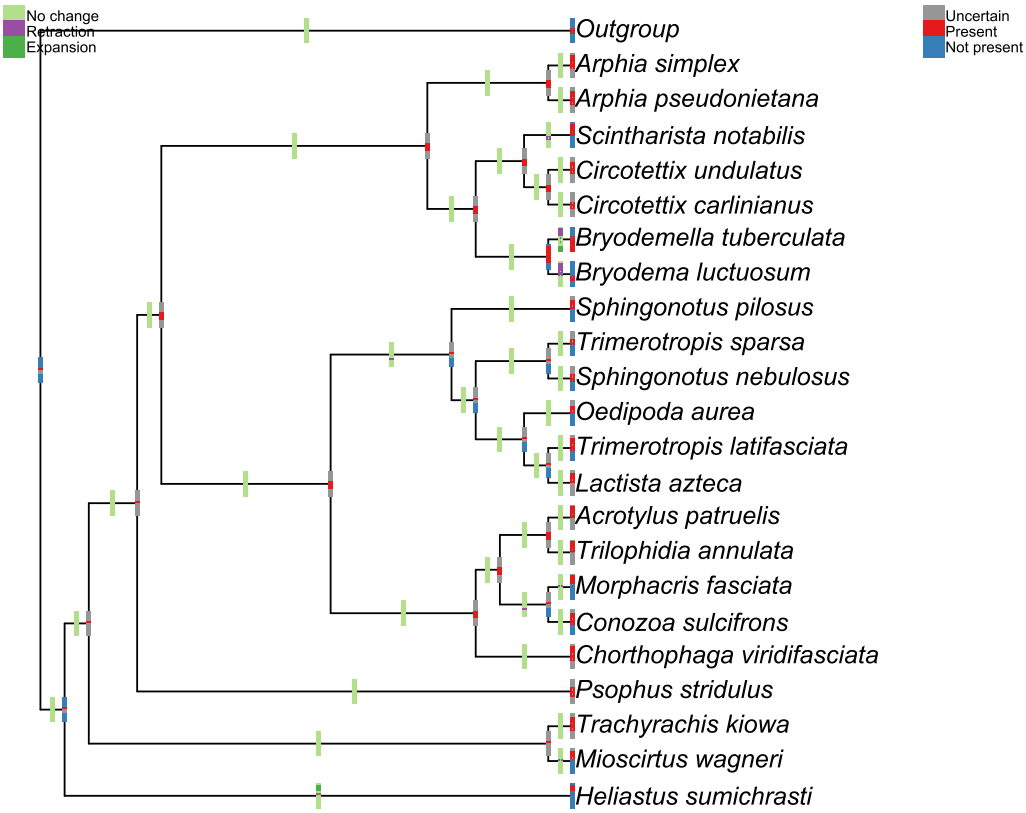


PCA species

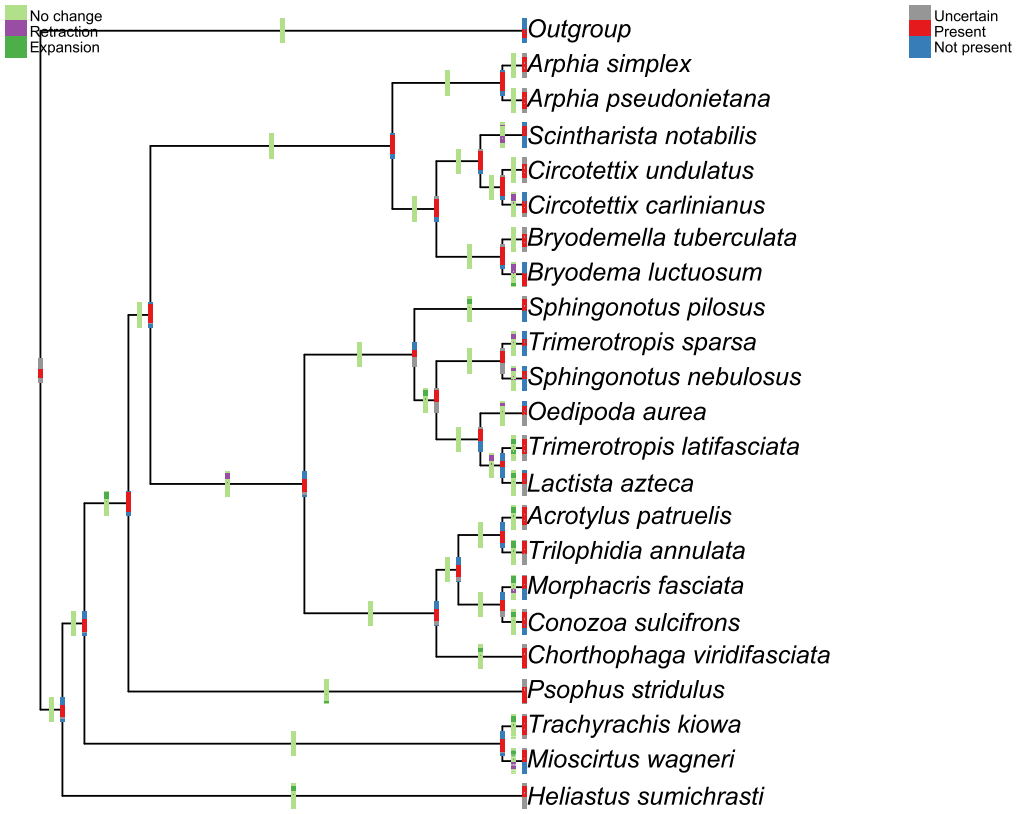

PC2 (14.15%)
PC1 (75.75%)
0.2
0.1
0.0
-0.1
-0.2
-0.1
0.0
0.1
0.2
species
Acrotylus_patruelis
Arphia_pseudonietana
Arphia_simplex
Bryodema_luctuosum
Bryodemella_tuberculata
Chorthophaga_viridifasciata
Circotettix_carlinianus
Circotettix_rabula
Circotettix_undulatus
Conozoa_sulcifrons
Heliastus_sumichrasti
Lactista_aztecus
Mioscirtus_wagneri
Morphacris_fasciata
Oedipoda_aurea
outgroup
Psophus_stridulus
Scintharista_notabilis
Sphingonotus_nebulosus
Sphingonotus_pilosus
Trachyrhachis_kiowa
Trilophidia_annulata
Trimerotropis_cyaneipennis
Trimerotropis_latifasciata

SI 7: Nichevol reconstructions of ancestral suitable areas for all 15 bioclimatic layers for the morphological and phylogentic trees.

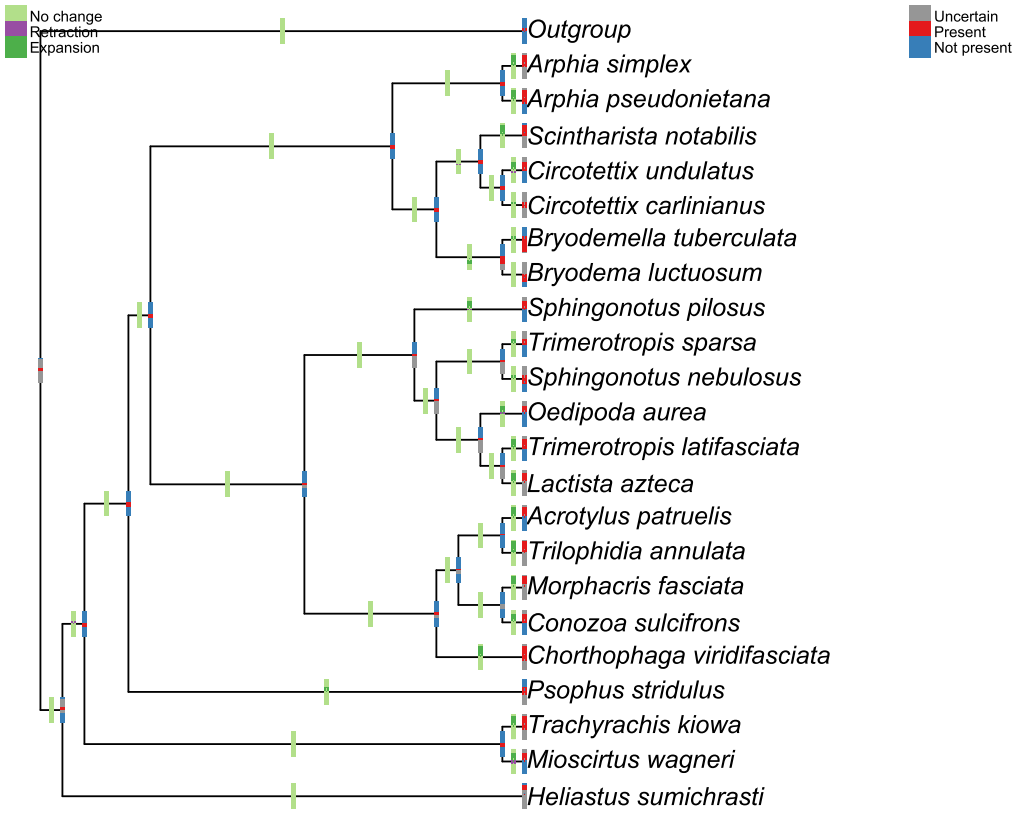
Morphological dataset – Bio1 - Annual Mean Temperature



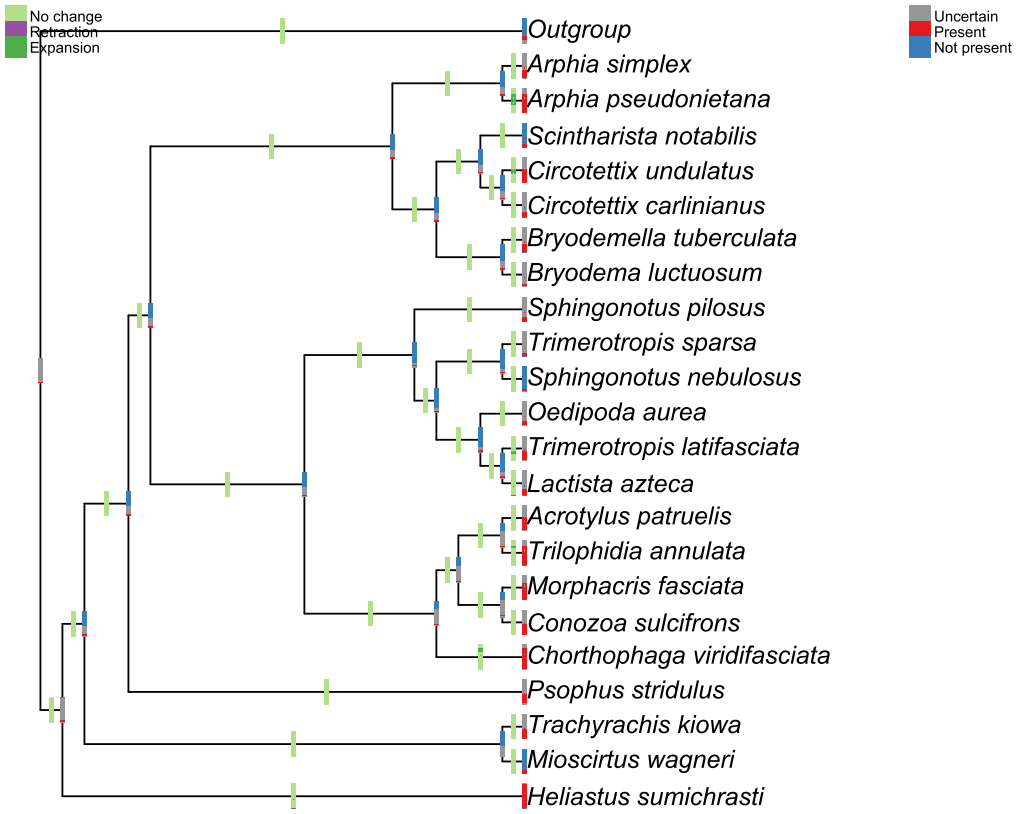
Morphological dataset – Bio2 - Mean Diurnal Range (Mean of monthly (max temp - min temp))



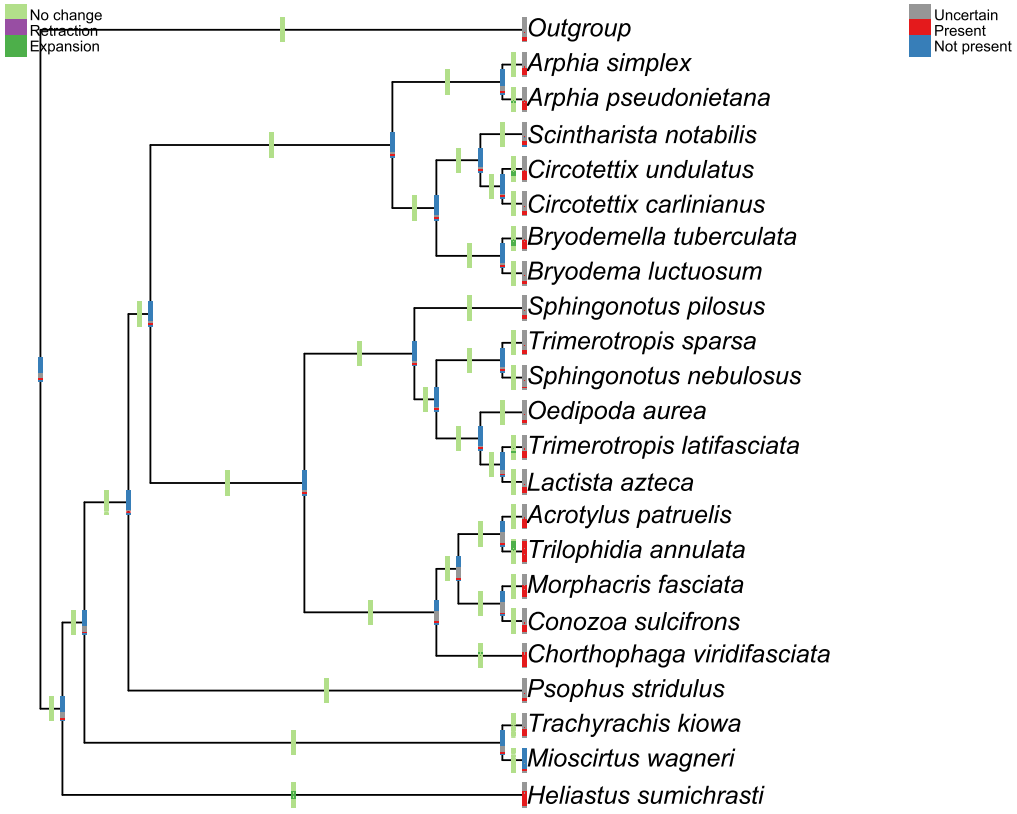
Morphological dataset – Bio3 - Isothermality (BIO2/BIO7) (×100)



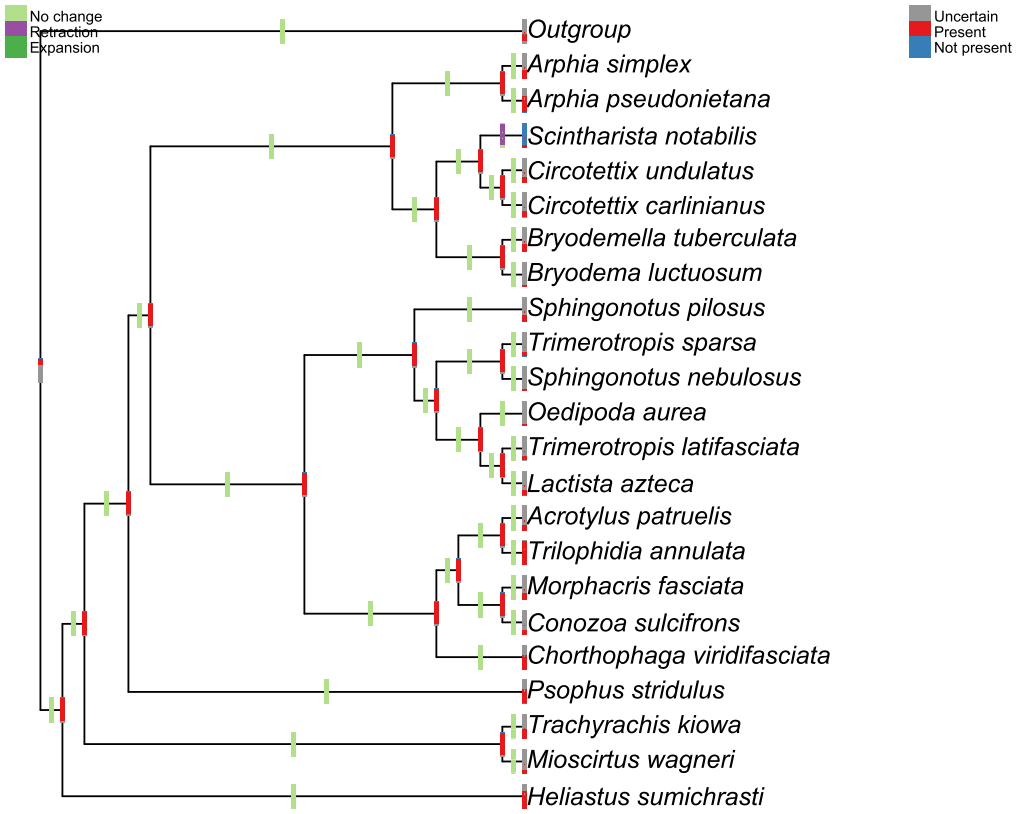
Morphological dataset – Bio4 - Temperature Seasonality (standard deviation ×100)



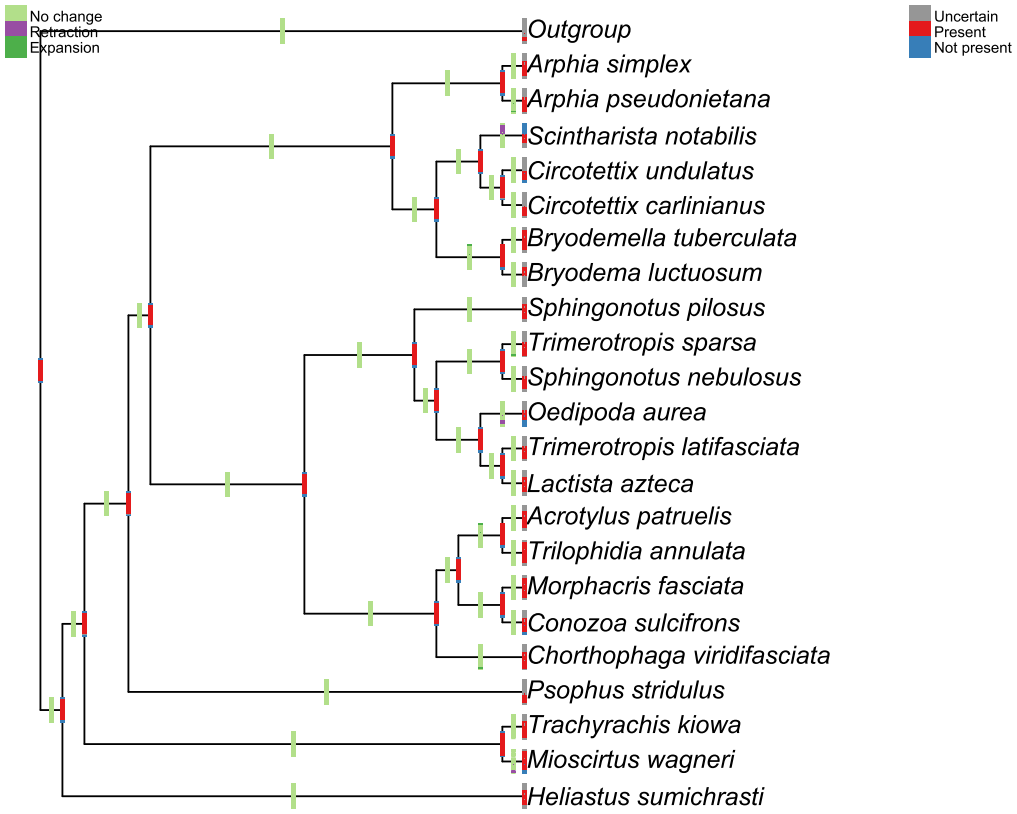
Morphological dataset – Bio5 - Max Temperature of Warmest Month



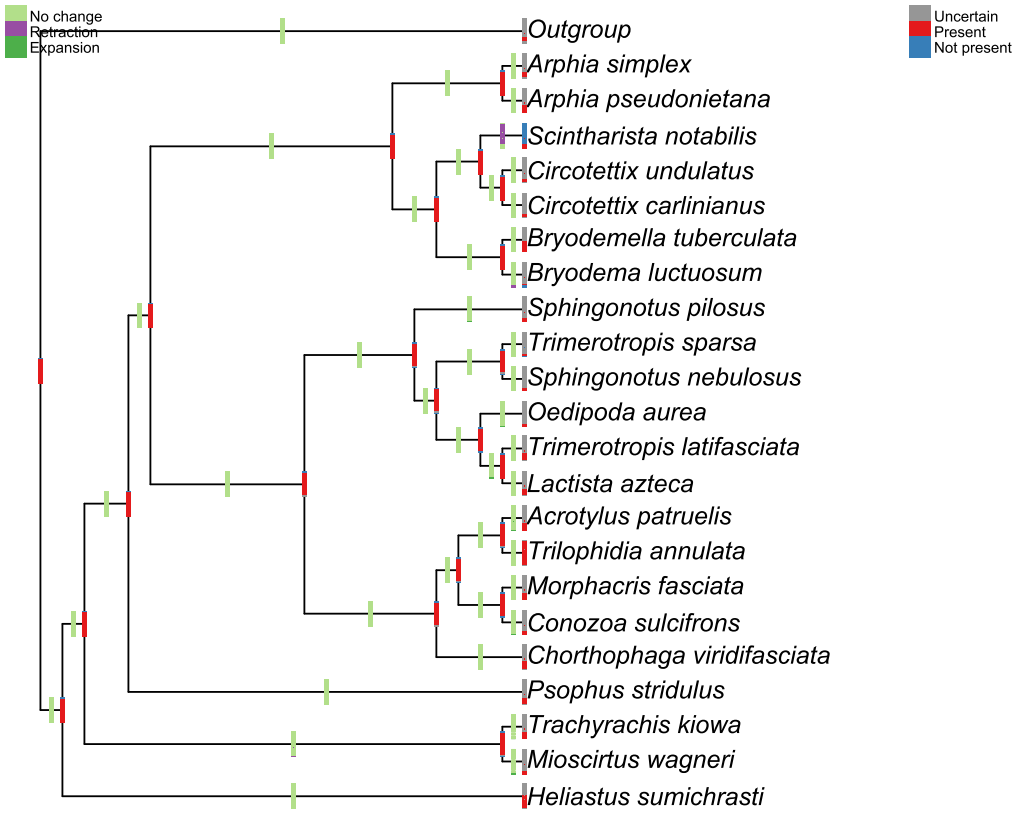
Morphological dataset – Bio6 - Min Temperature of Coldest Month



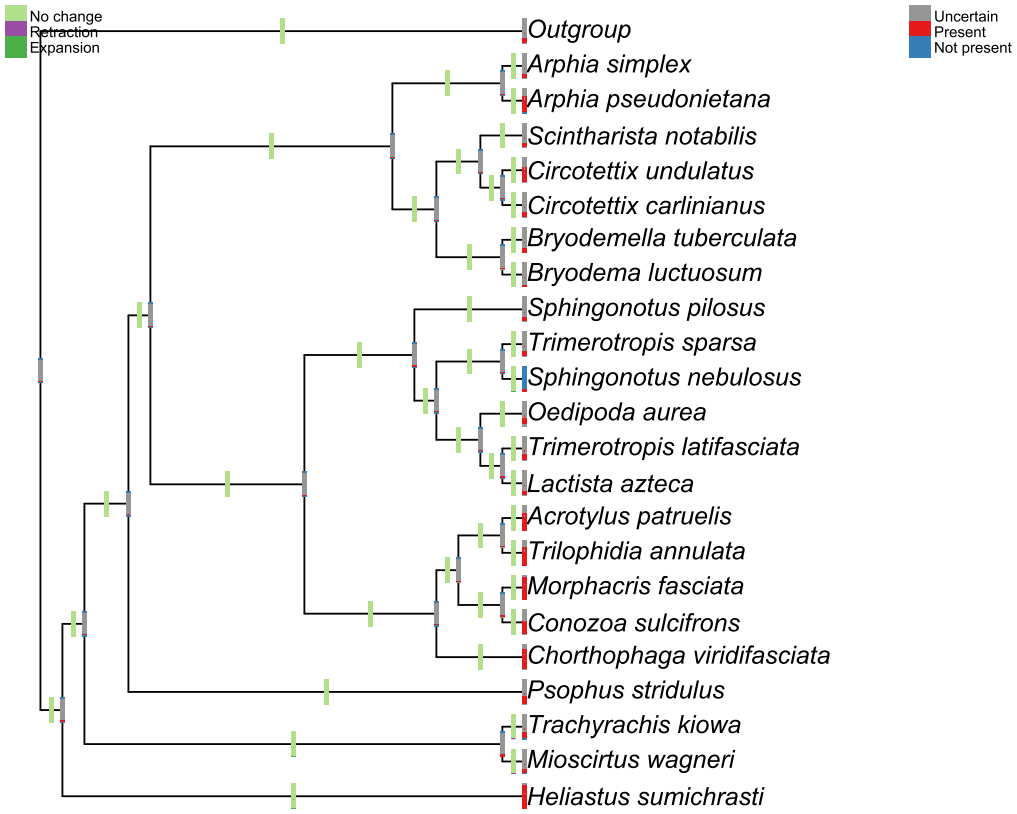
Morphological dataset – Bio7 - Temperature Annual Range (BIO5-BIO6)



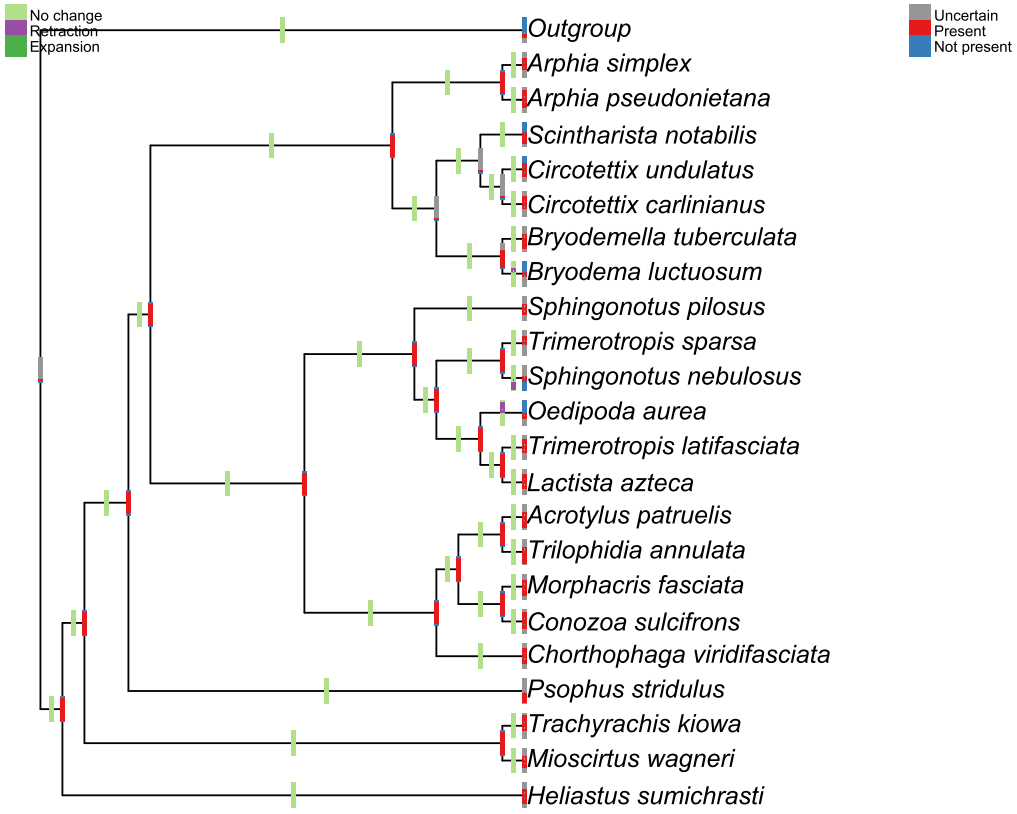
Morphological dataset – Bio10 - Mean Temperature of Warmest Quarter



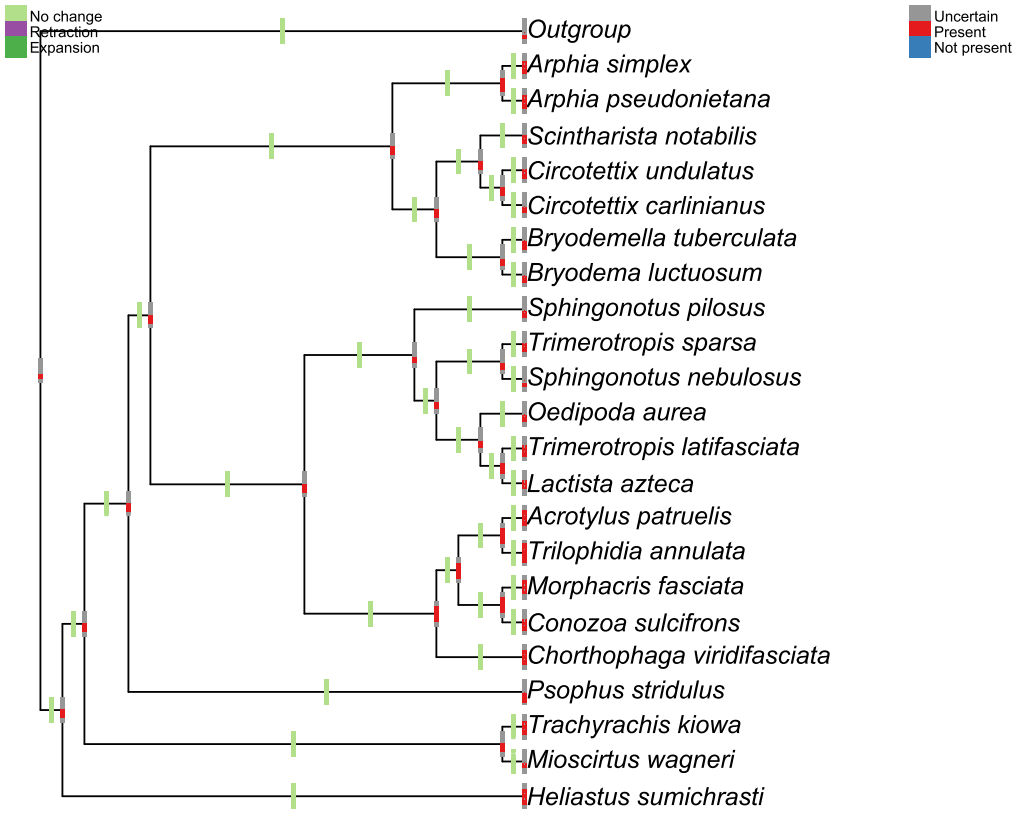
Morphological dataset – Bio11 - Mean Temperature of Coldest Quarter



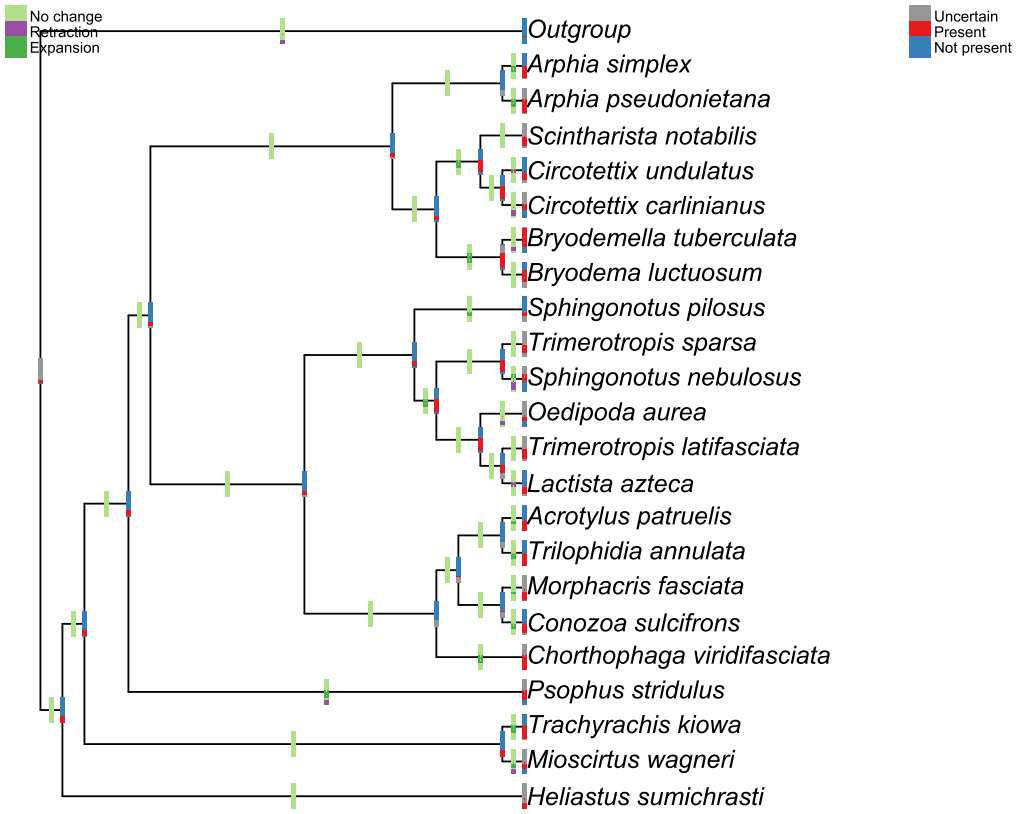
Morphological dataset – Bio12 - Annual Precipitation



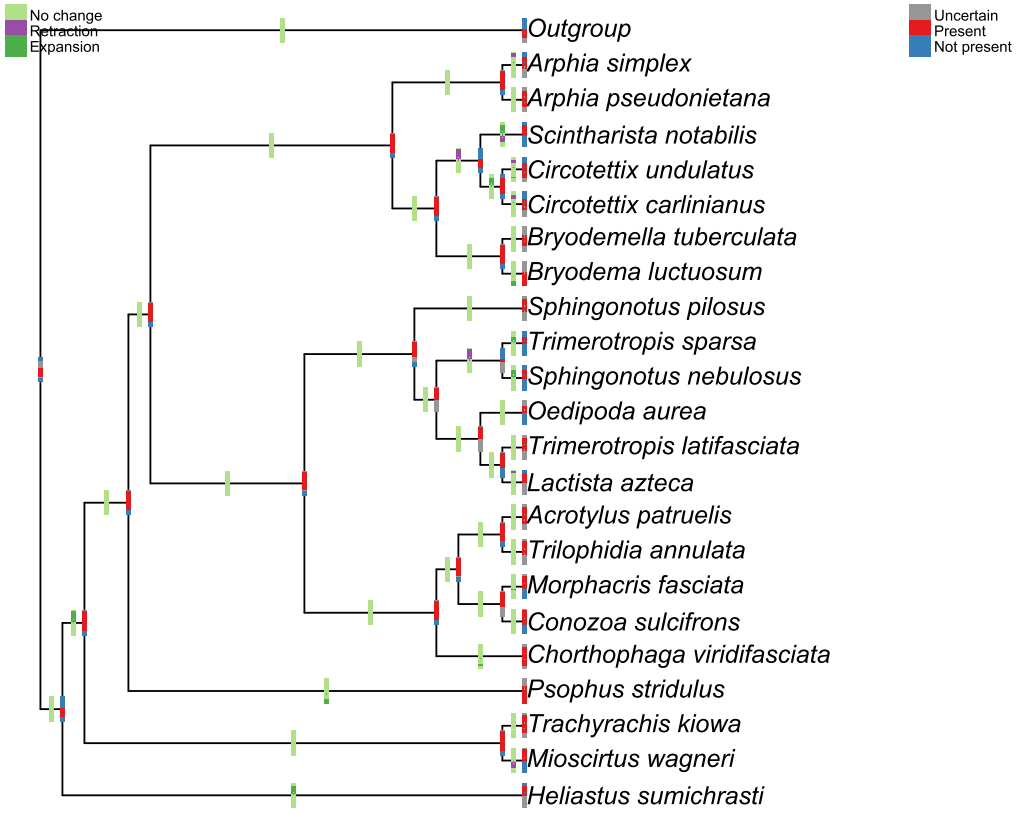
Morphological dataset – Bio13 - Precipitation of Wettest Month



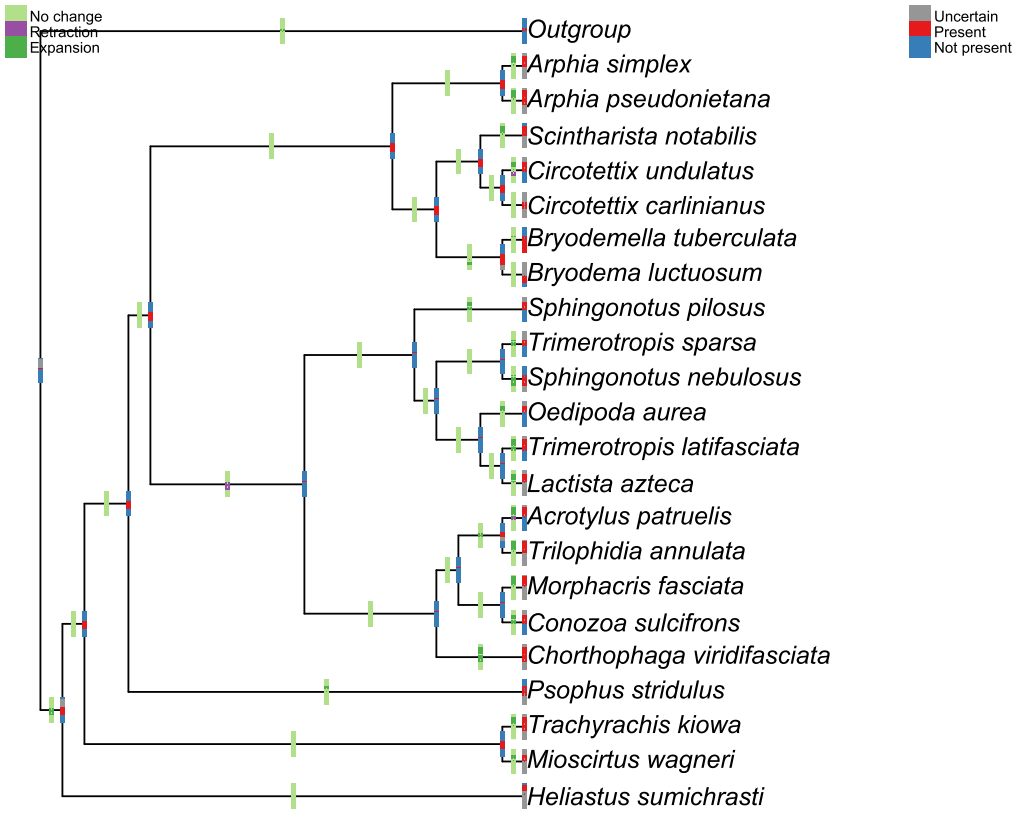
Morphological dataset – Bio14 - Precipitation of Driest Month



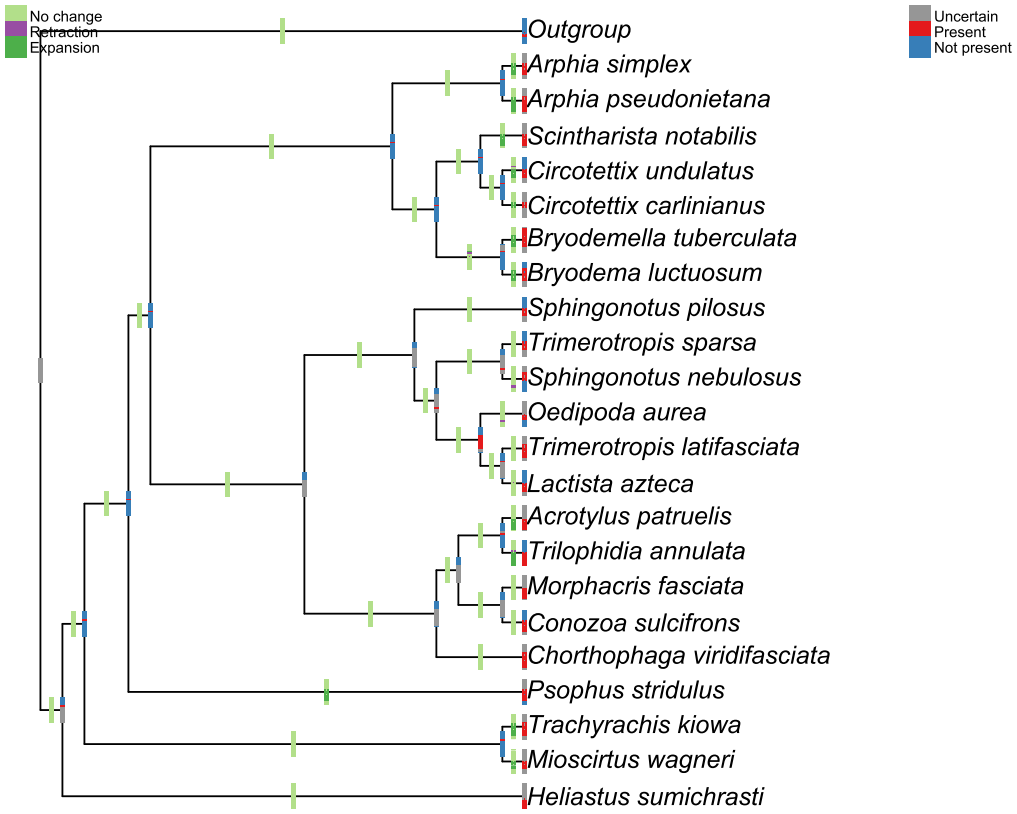
Morphological dataset – Bio15 - Precipitation Seasonality (Coefficient of Variation)



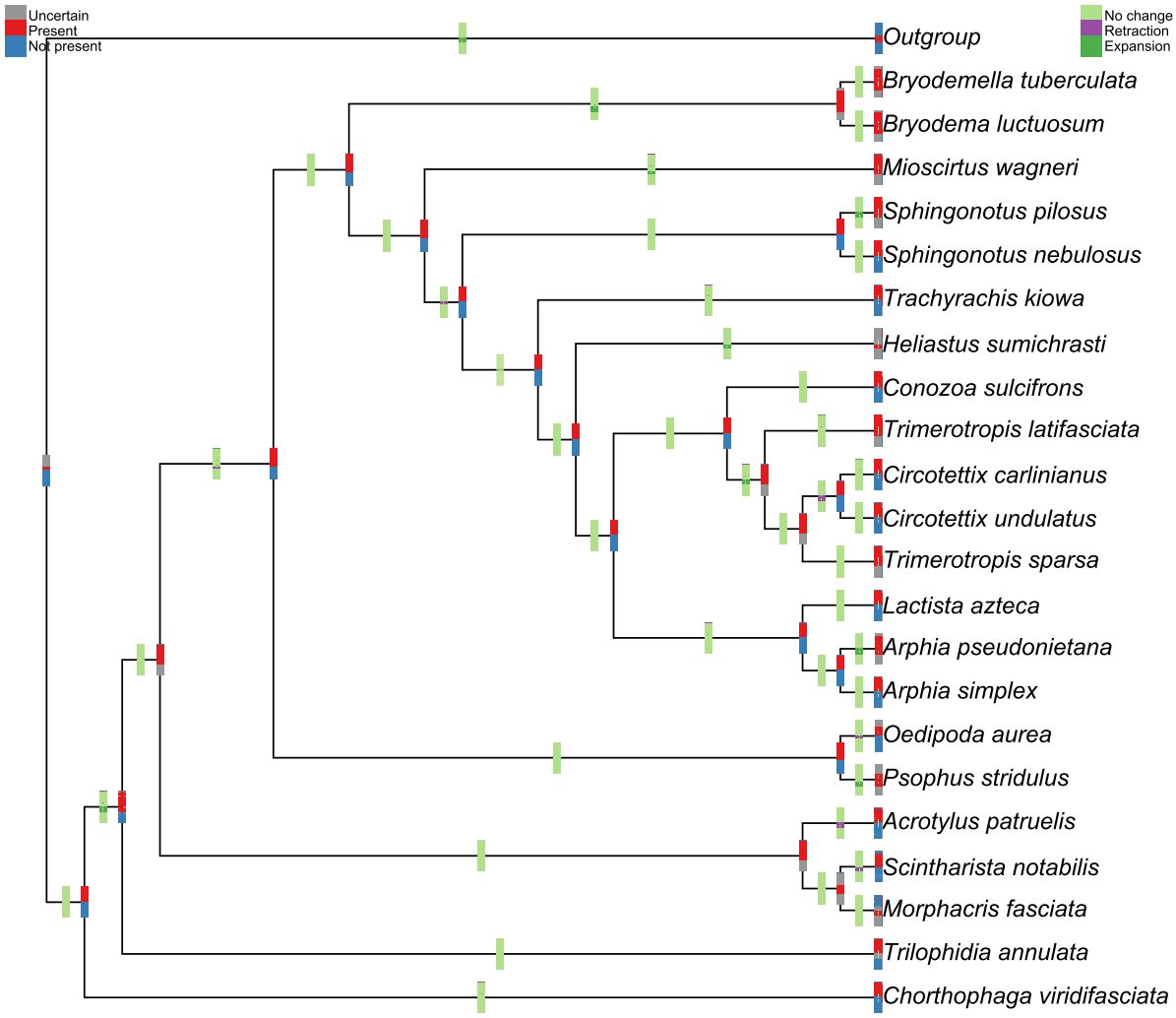
Morphological dataset – Bio16 - Precipitation of Wettest Quarter



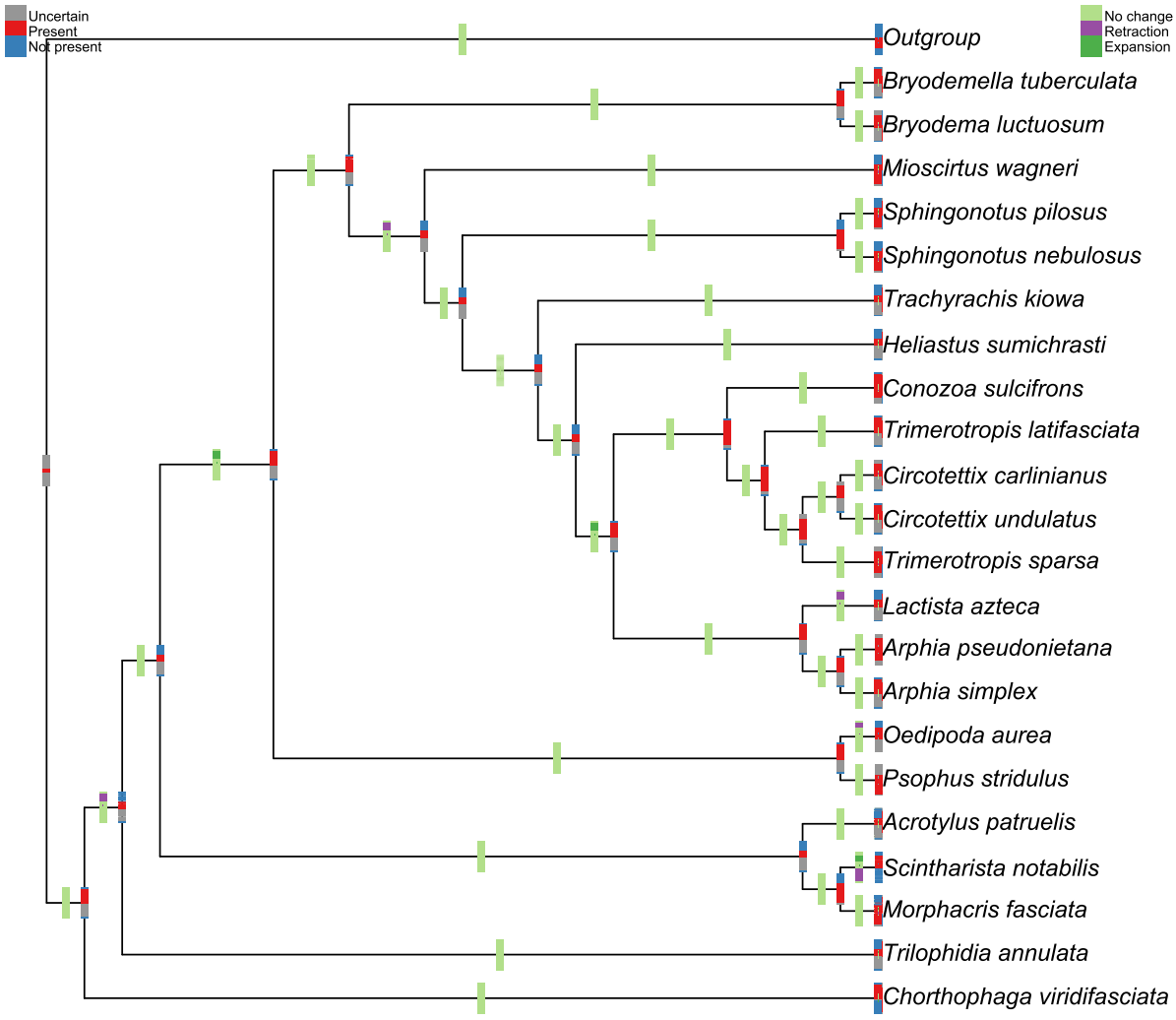
Morphological dataset – Bio17 - Precipitation of Driest Quarter



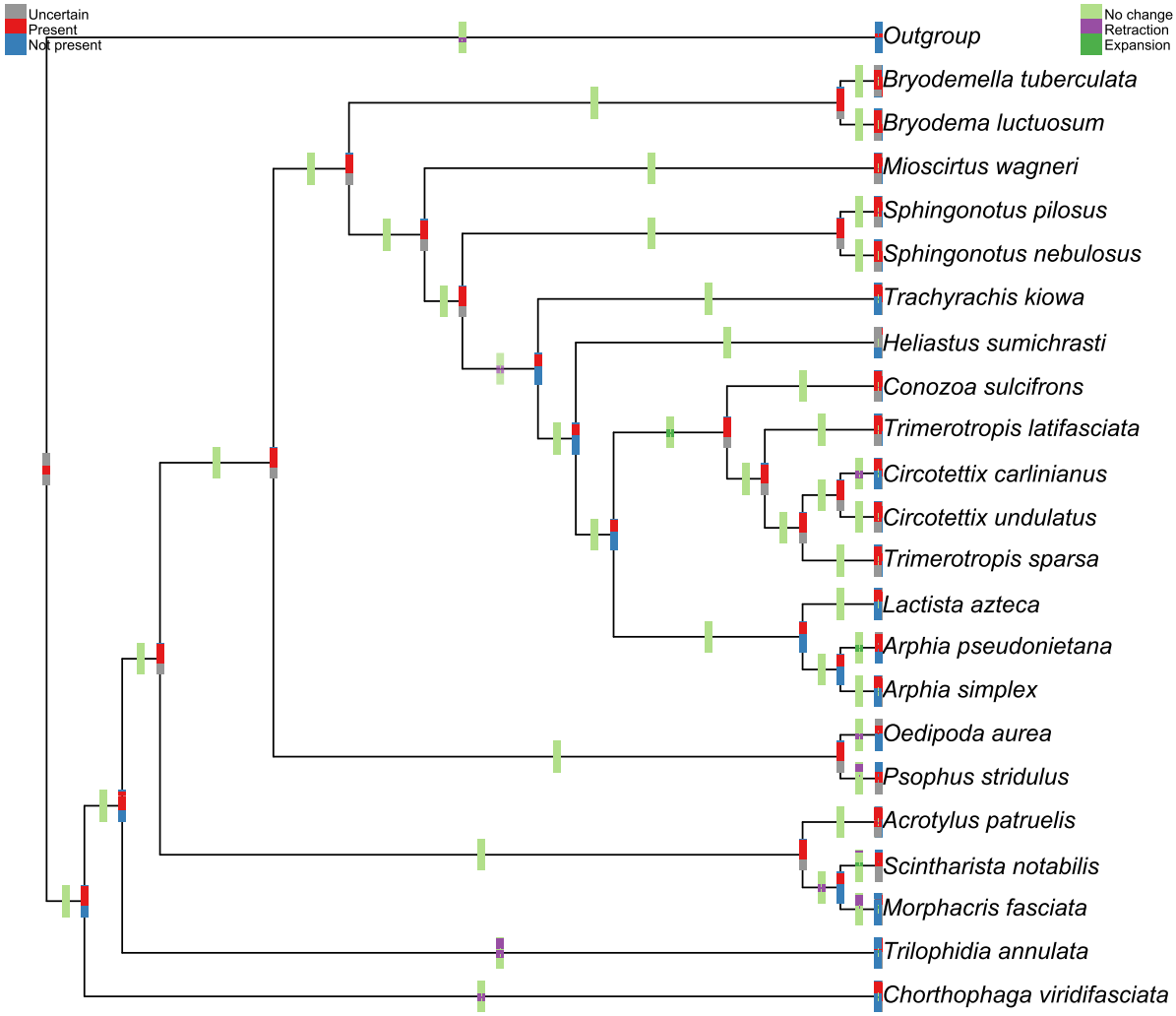
Phylogenetic dataset – Bio1 - Annual Mean Temperature



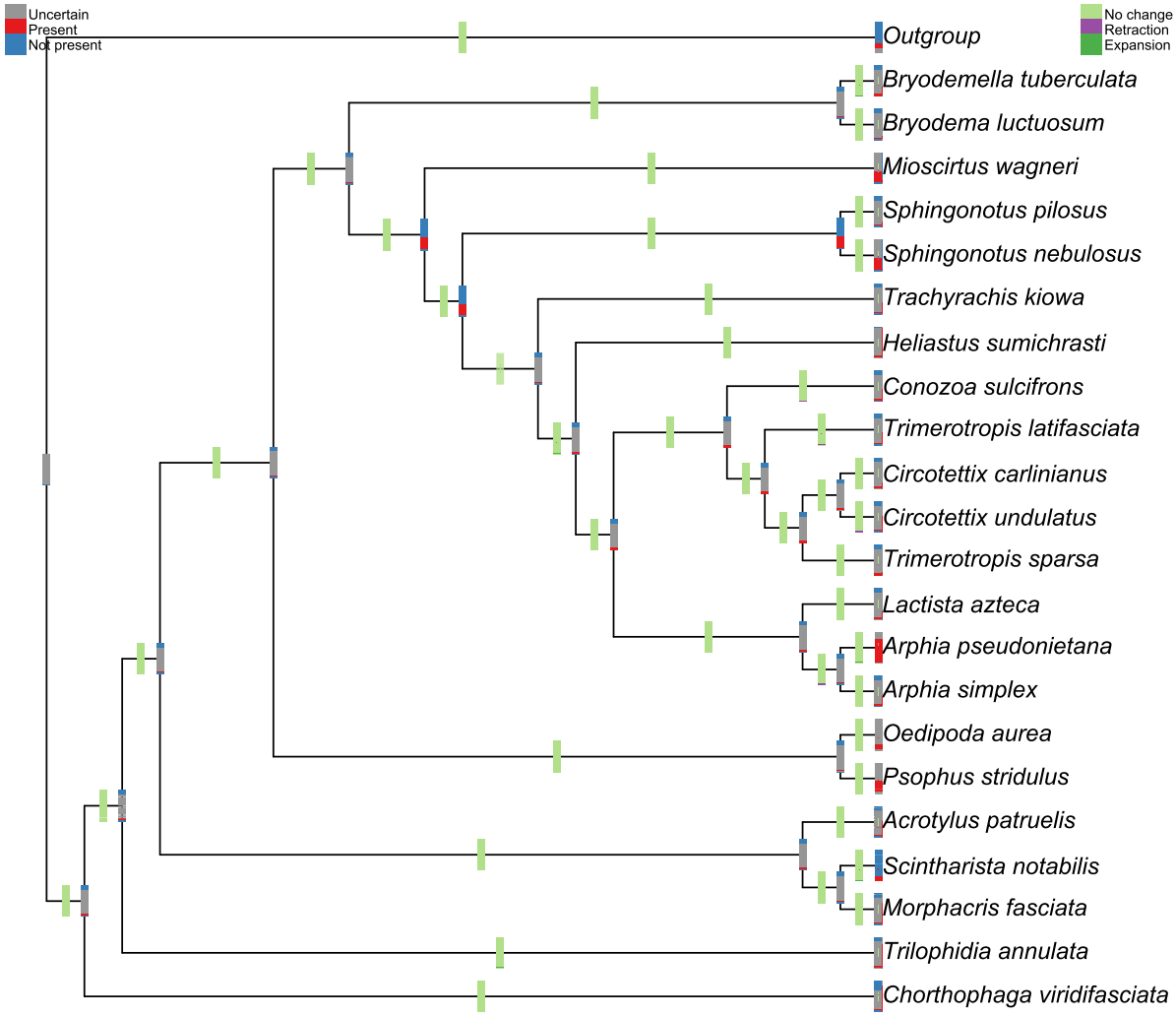
Phylogenetic dataset – Bio2 - Mean Diurnal Range (Mean of monthly (max temp - min temp))



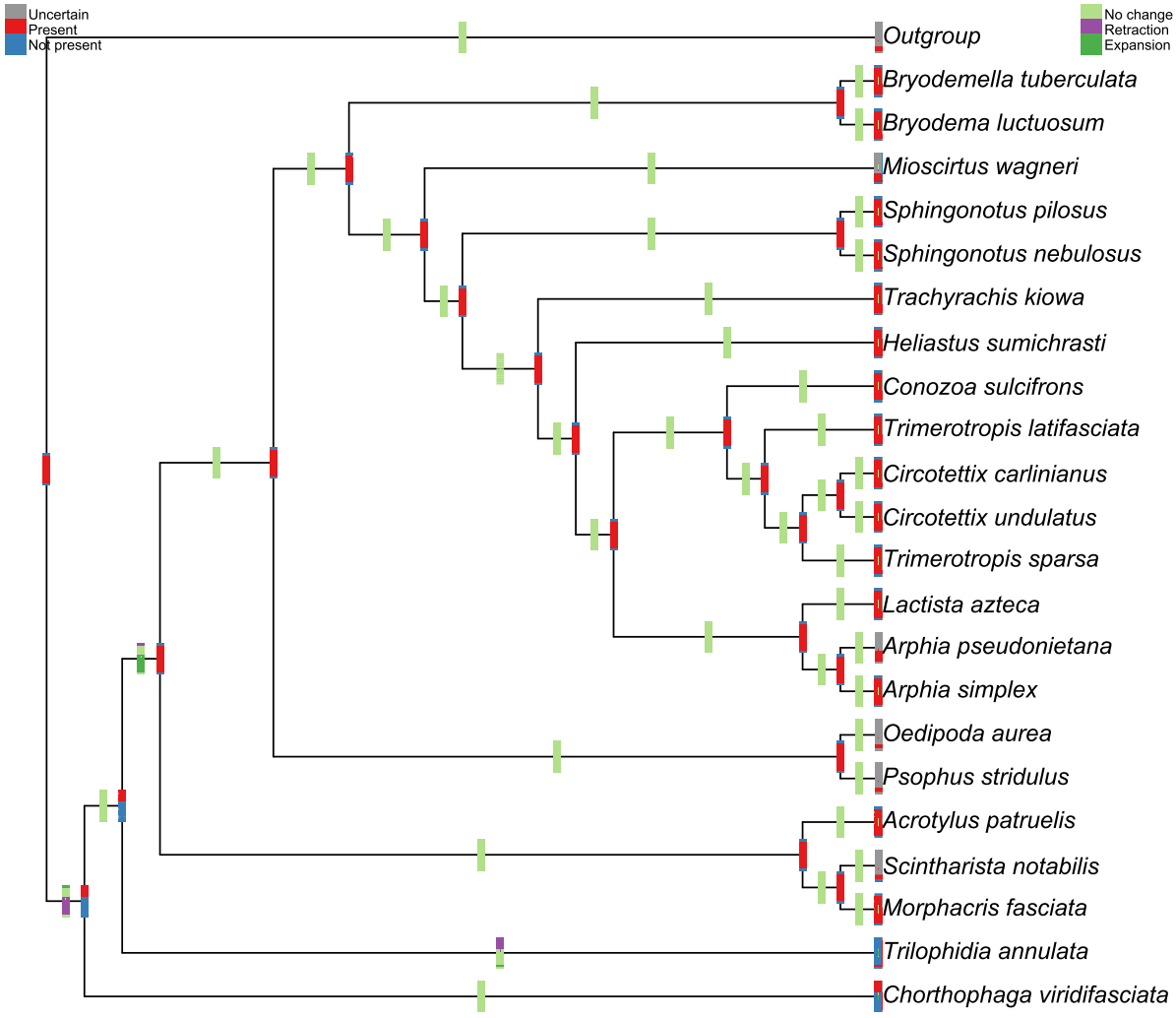
Phylogenetic dataset – Bio3 - Isothermality (BIO2/BIO7) (×100)



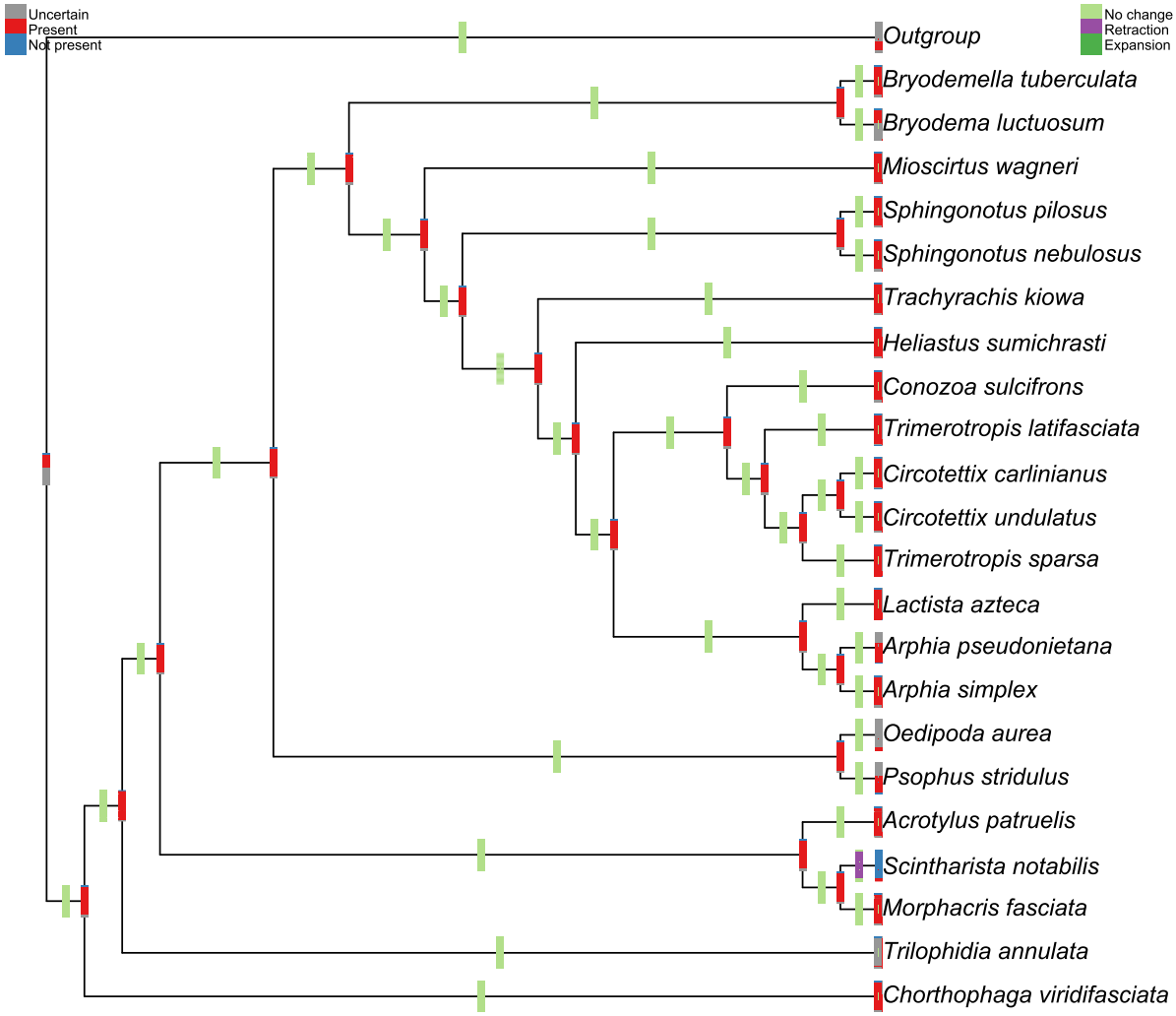
Phylogenetic dataset – Bio4 - Temperature Seasonality (standard deviation ×100)



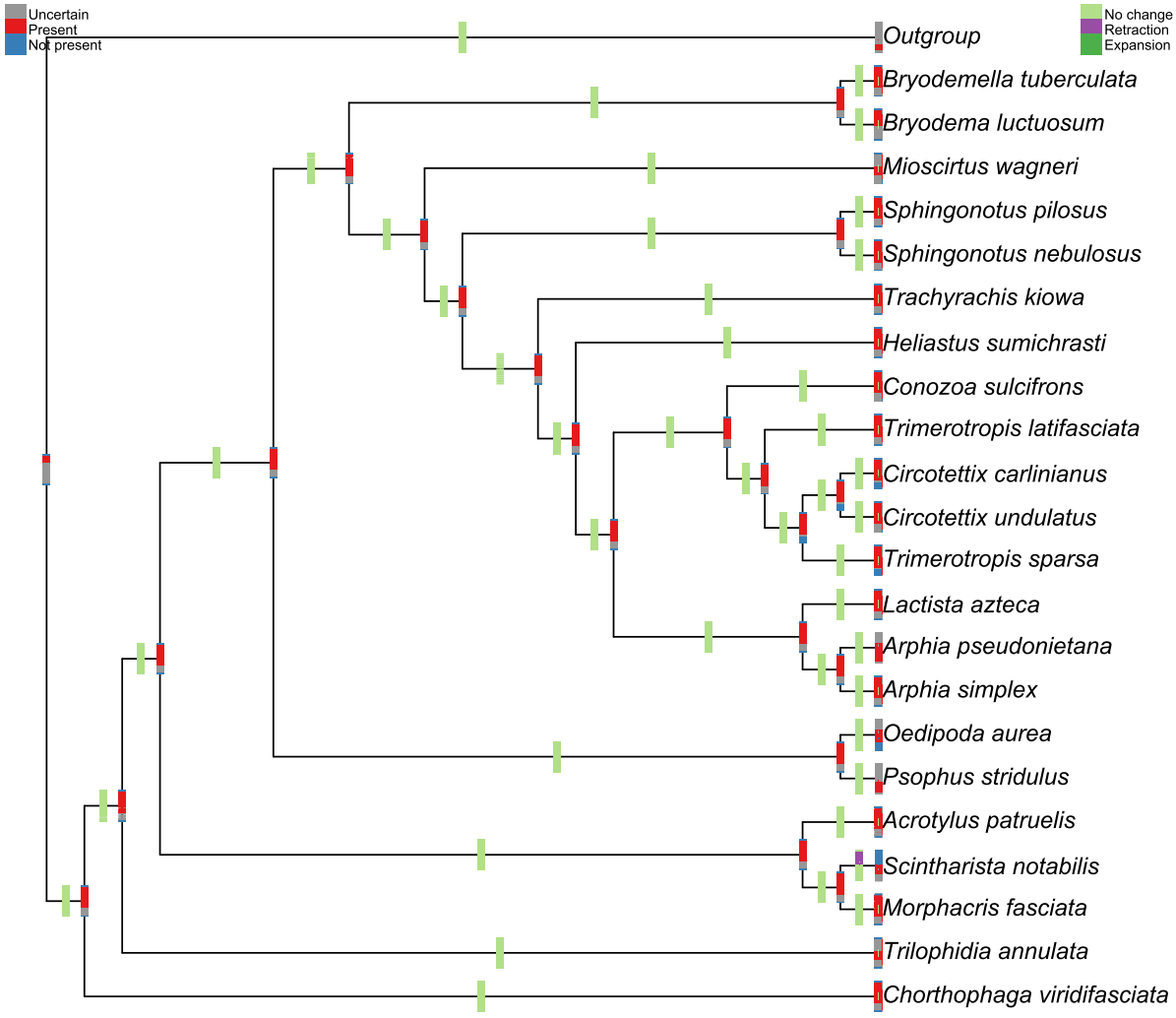
Phylogenetic dataset – Bio5 - Max Temperature of Warmest Month



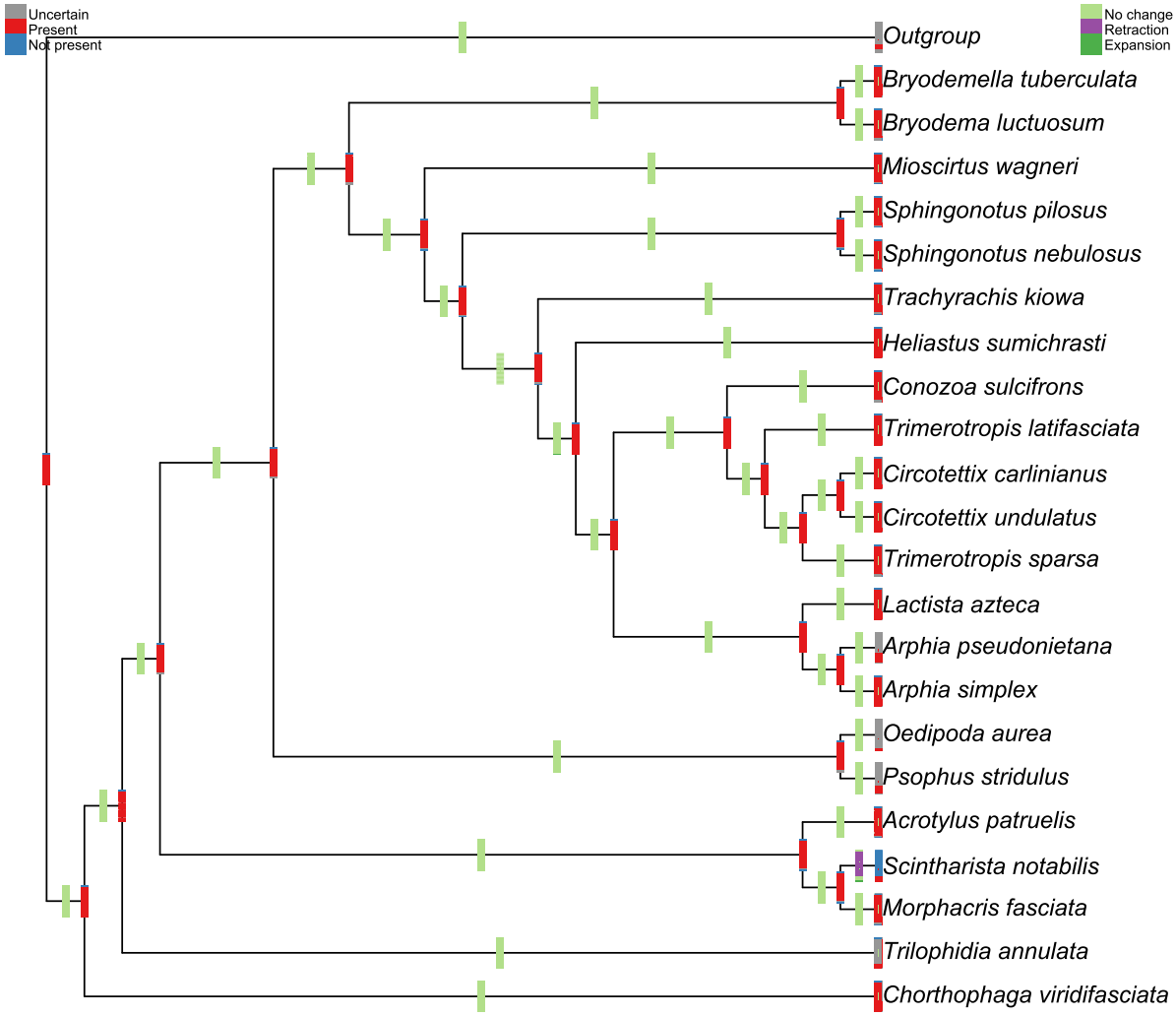
Phylogenetic dataset – Bio6 - Min Temperature of Coldest Month



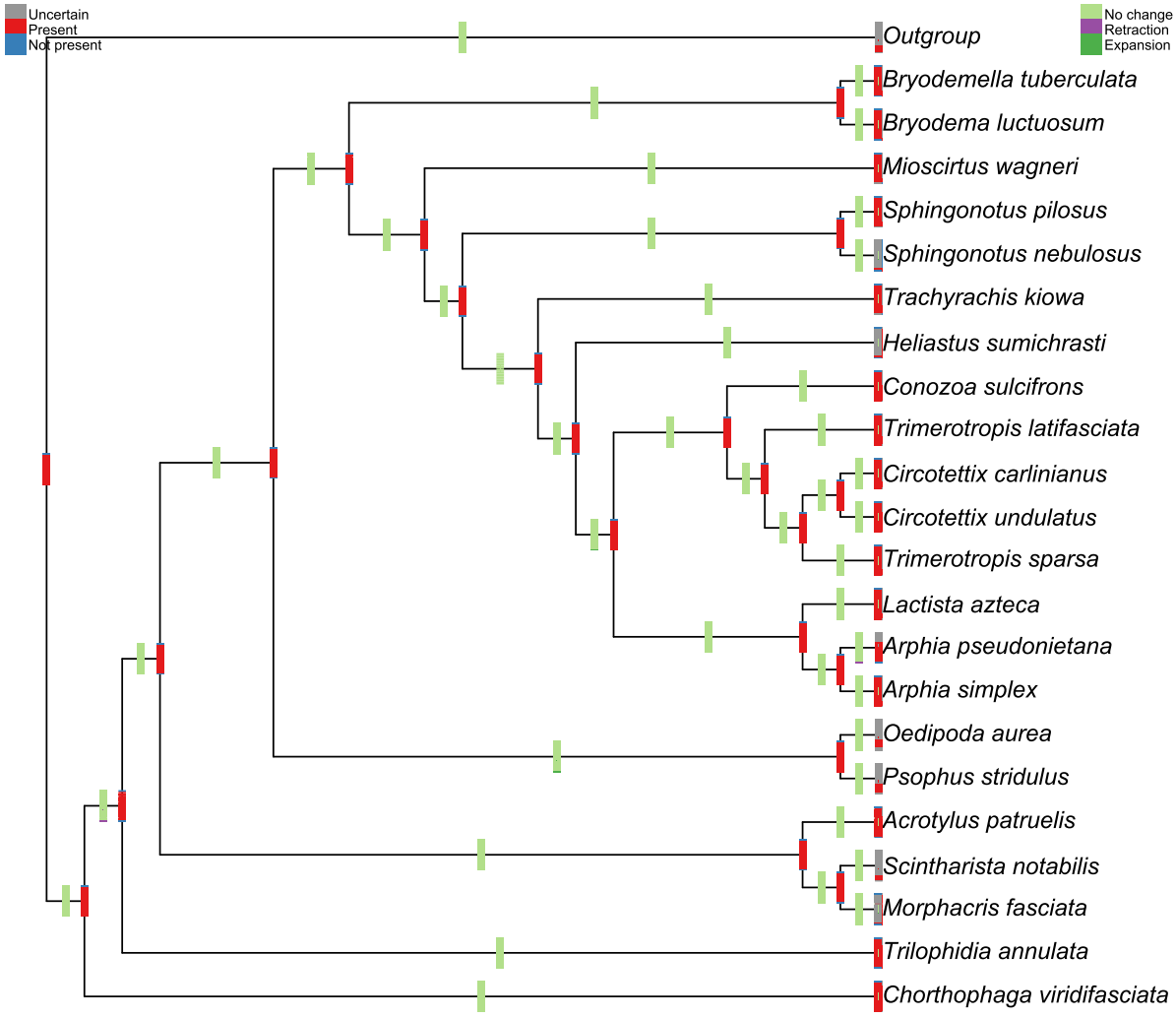
Phylogenetic dataset – Bio7 - Temperature Annual Range (BIO5-BIO6)



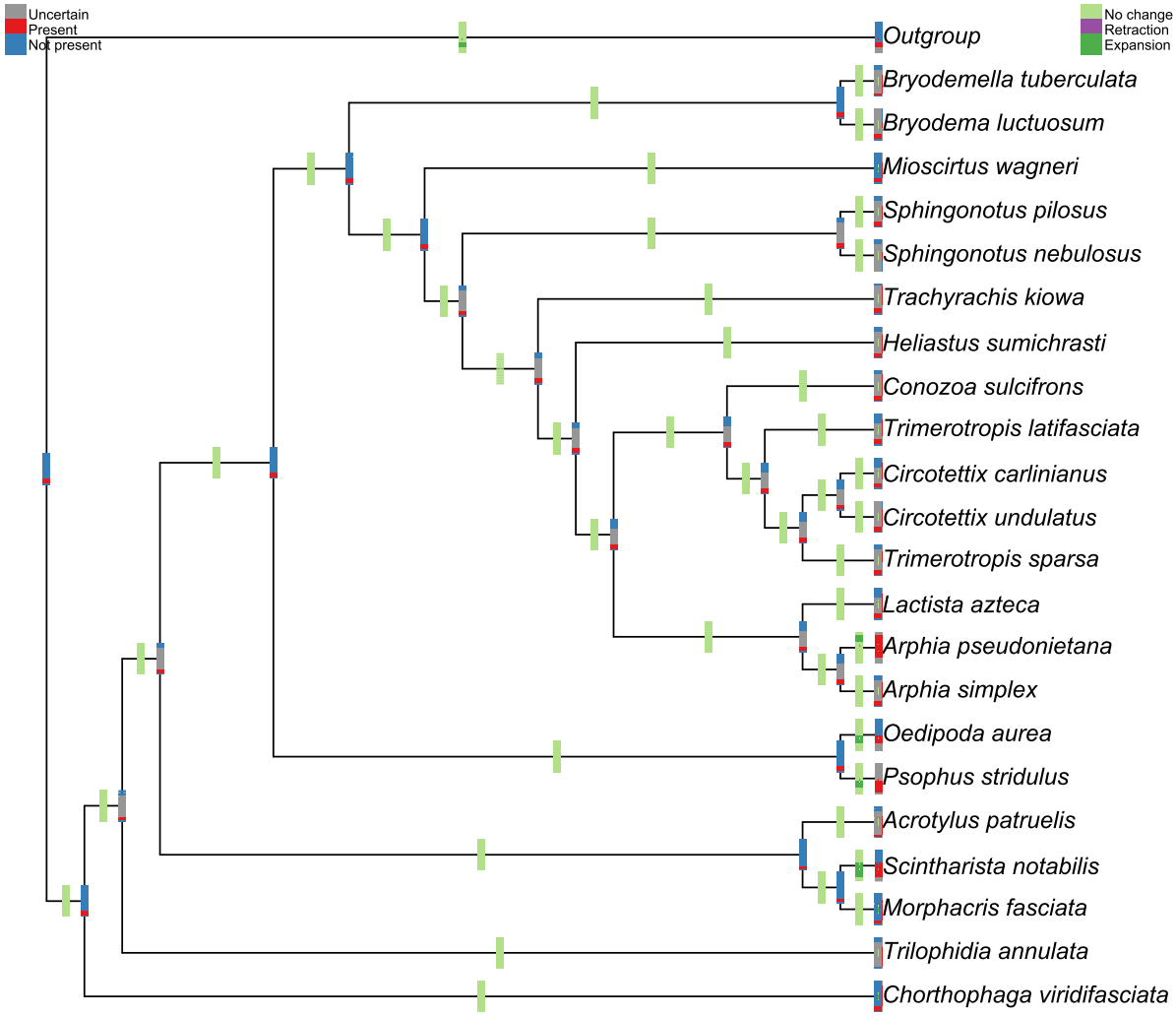
Phylogenetic dataset – Bio10 - Mean Temperature of Warmest Quarter



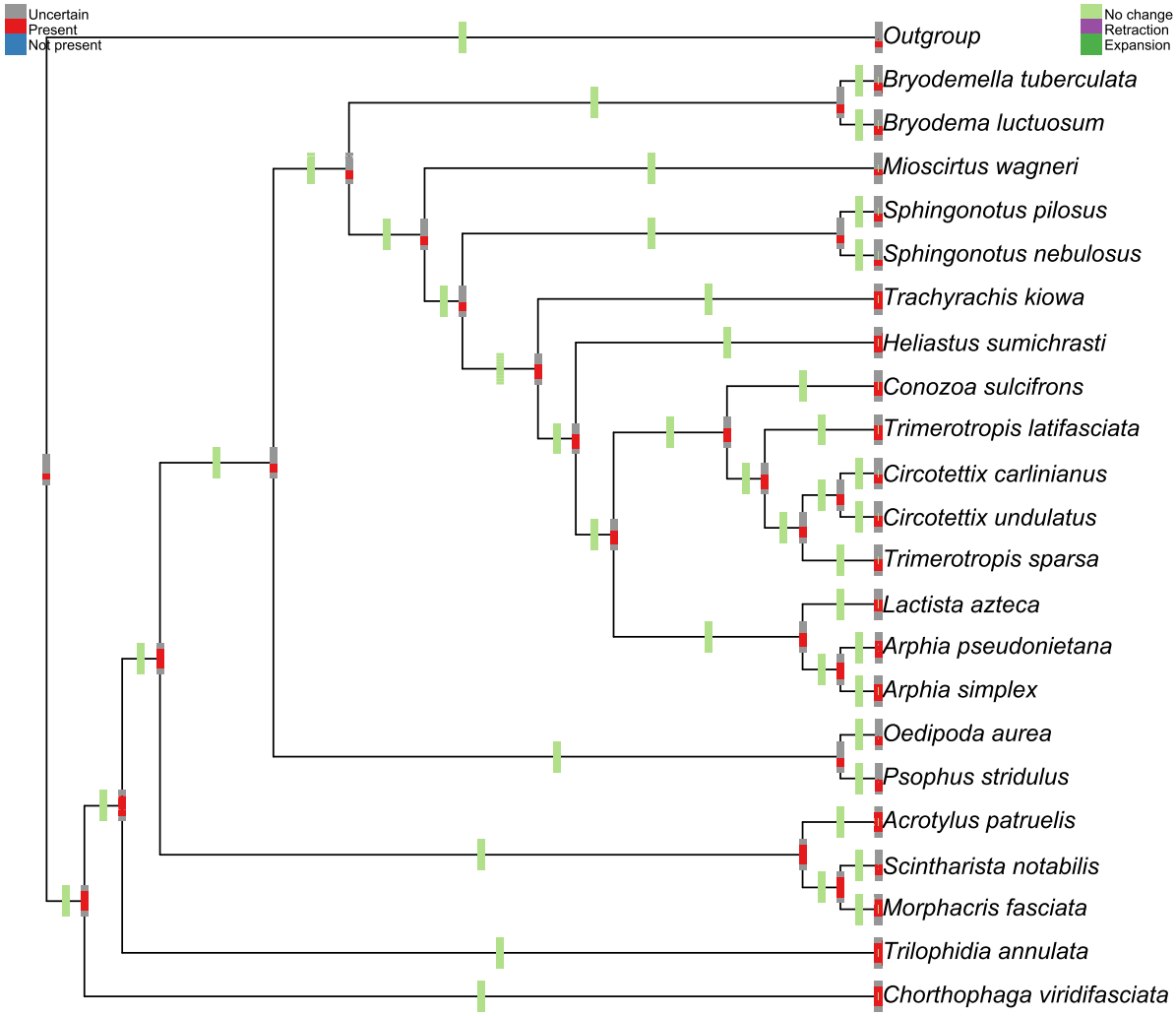
Phylogenetic dataset – Bio11 - Mean Temperature of Coldest Quarter



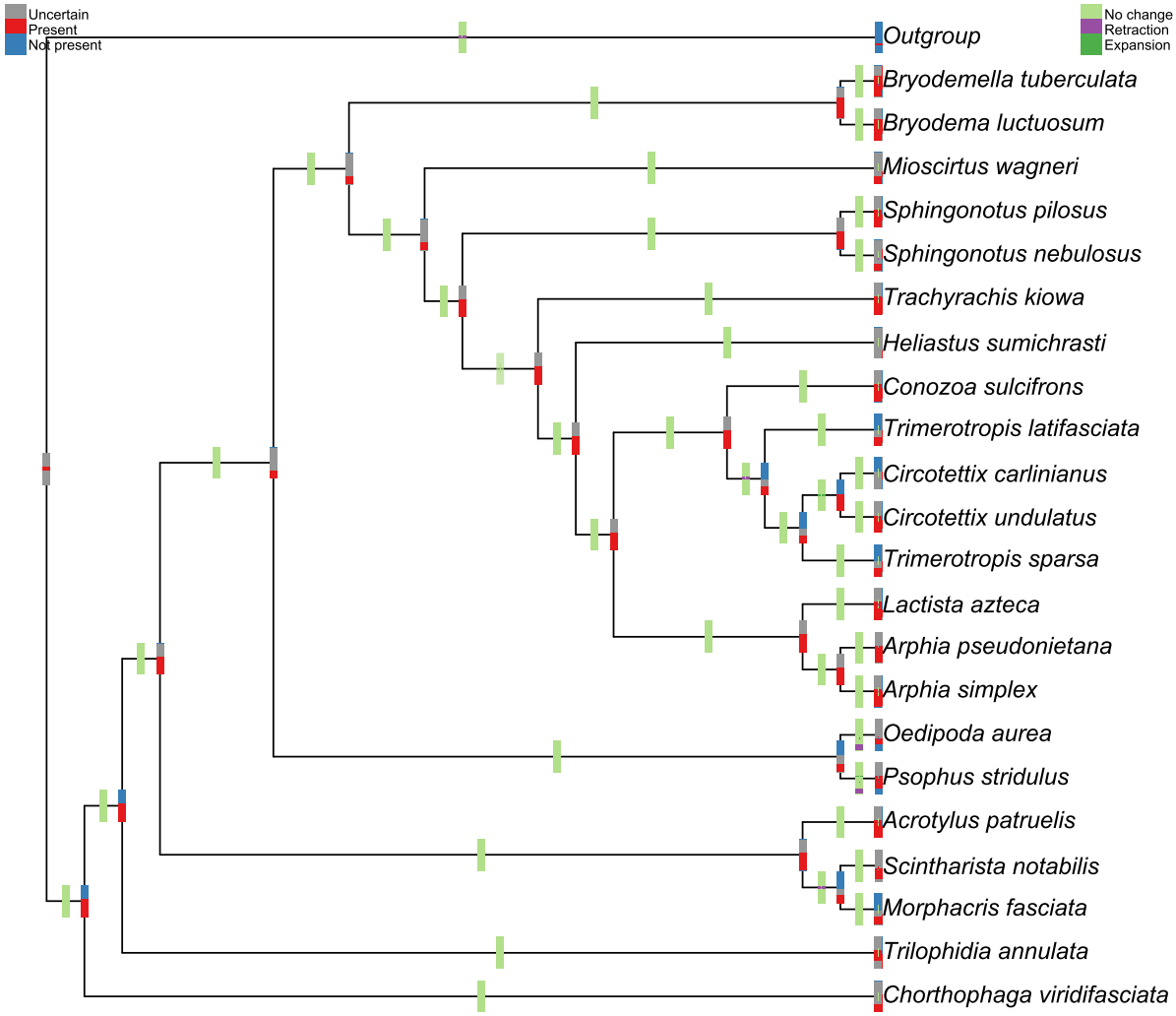
Phylogenetic dataset – Bio12 - Annual Precipitation



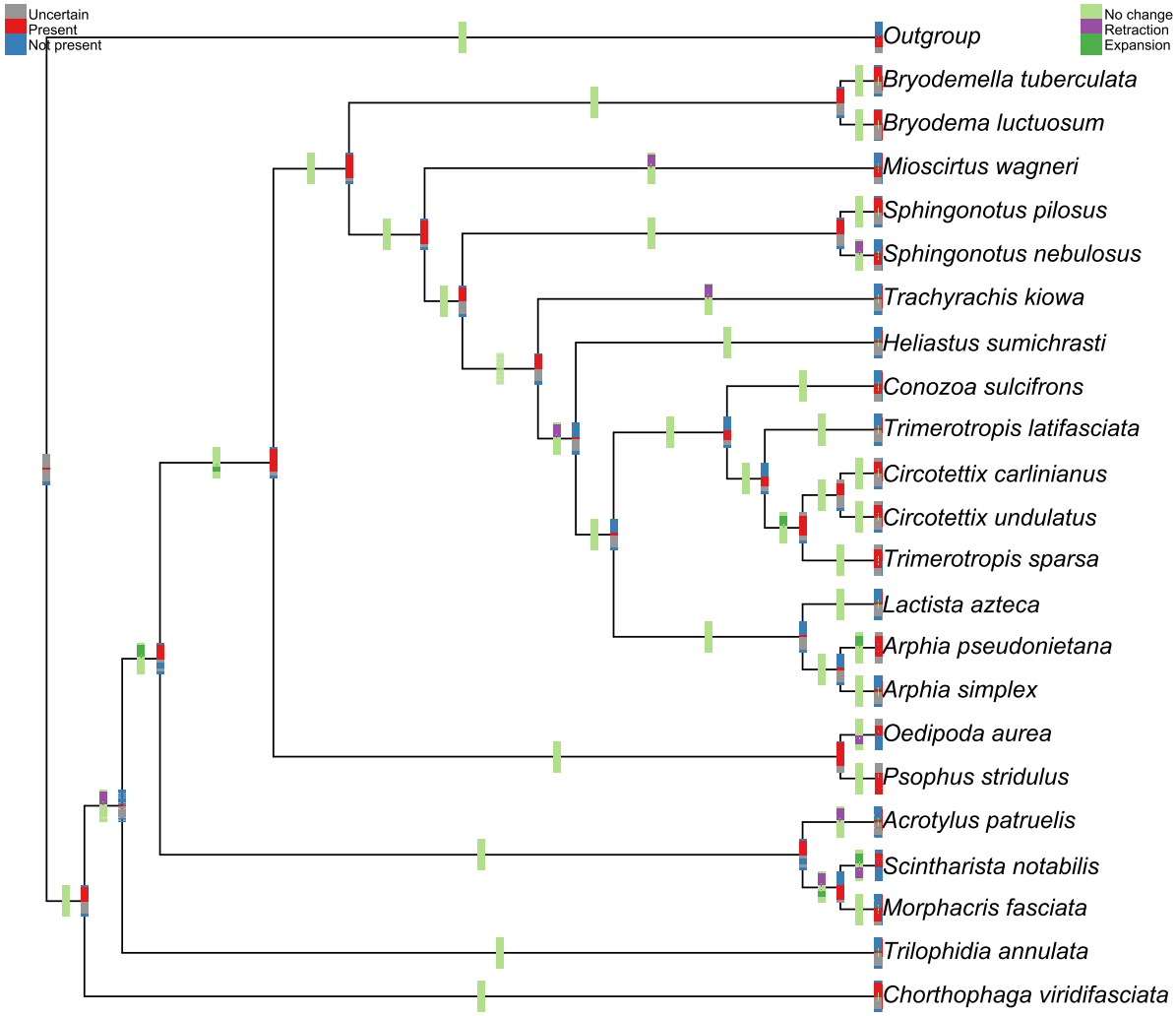
Phylogenetic dataset – Bio13 - Precipitation of Wettest Month



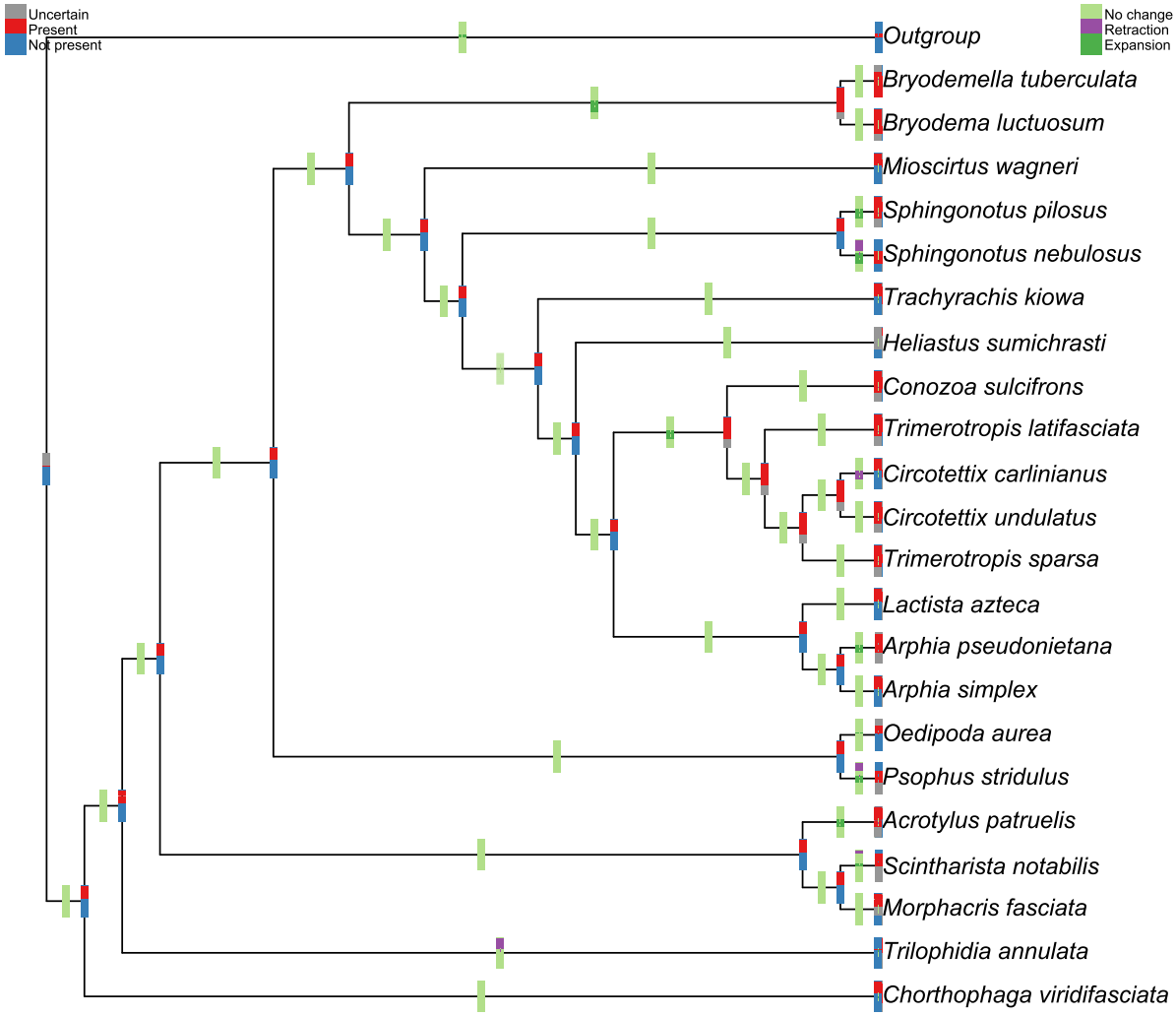
Phylogenetic dataset – Bio14 - Precipitation of Driest Month



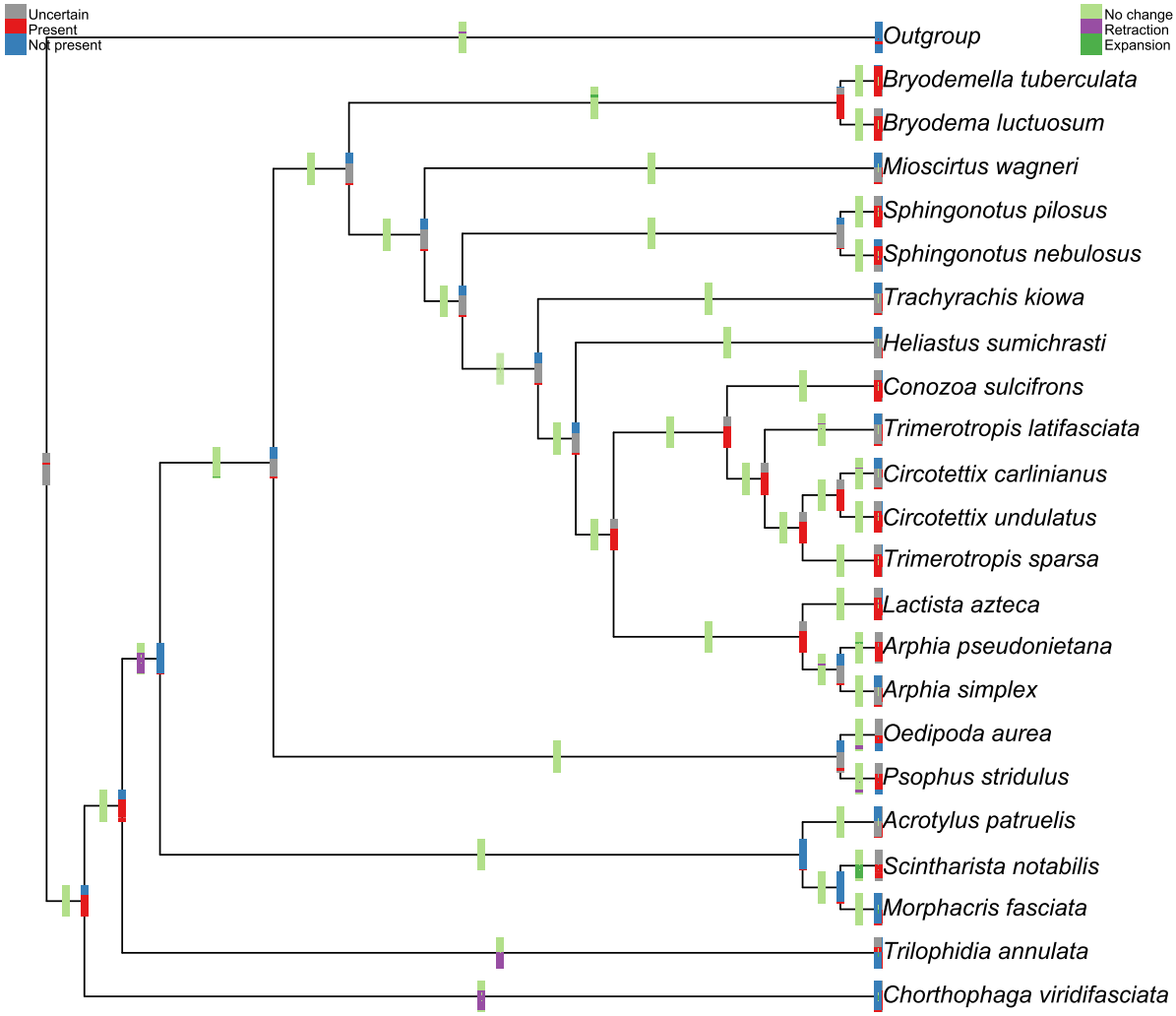
Phylogenetic dataset – Bio15 - Precipitation of Driest Month



Phylogenetic dataset – Bio16 - Precipitation of Wettest Quarter



Phylogenetic dataset – Bio17 - Precipitation of Driest Quarter



ERKLÄRUNG DER GLEICHHEIT DER EXEMPLARE

Hiermit versichere ich, Lara-Sophie Dey, dass das vorliegende Exemplar der Dissertation "Phylogenetic and phylogenomic analyses and distribution modelling of a challenging taxon – the band-winged grasshoppers" dem beim Studienamt zur Archivierung eingereichten Exemplar entspricht.

Hamburg, 02.05.2023

Ort/Datum

Unterschrift