Host-feeding patterns of mosquitoes on a global scale and new insights into the vector capacity of *Culex pipiens* s.s./*Cx. torrentium*

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Abstract

Mosquitoes as vectors of several pathogens play a crucial role in public and veterinary health. Their ability to transmit pathogens is summarized as the vector capacity, which is influenced by several intrinsic and extrinsic factors including the mosquito's vector competence as well as their host-feeding patterns. This thesis therefore addresses the vector competence of native and invasive *Culex* and *Aedes* species for European Batai virus (BATV), and host-feeding patterns as a fundamental factor shaping transmission cycles on a global scale and specifically for *Cx. pipiens* s.s. and *Cx. torrentium*.

BATV is a zoonotic arbovirus with veterinary importance and high seroprevalence detected in livestock in Central Europe. While several mosquito species have been observed to be infected, the vectors responsible for pathogen circulation are not known. As a limiting factor for pathogen transmission, the vector competence of two indigenous and three invasive mosquito species (field-collected *Cx. pipiens* biotype *pipiens*, *Cx. torrentium*, *Ae. japonicus japonicus* and laboratory-reared *Ae. albopictus* and *Ae. aegypti*) were investigated at three fluctuating temperature profiles using forced salivation assays. While all tested species could be infected with BATV, *Cx. torrentium* is the only showing transmission of BATV.

Determining the contact rate between the mosquito, the host and the pathogen, host-feeding patterns are a further crucial factor shaping vector capacity and thus transmission cycles. For targeted prevention and control of pathogen transmission, the understanding of host-feeding patterns is essential. In a comprehensive database, the information of 333 published studies on host-feeding patterns from 1942 – 2019 were collated to gain a comprehensive overview on mosquito host-feeding patterns observed globally. Inclusion criteria comprised the sampling of field-caught mosquitoes without bait and serological or molecular biological bloodmeal analysis. The database with more than 600,000 identified bloodmeals of 494 mosquito taxa allows comparison and broader analyses. A majority of the identified mosquitoes belong to the genera *Aedes*, *Anopheles* and *Culex*, with mammalian hosts being

prevalent for the former two, while avian hosts dominated for *Culex* mosquitoes. The examples of the most frequently analysed mosquito species *An. gambiae* and *Cx. quinquefasciatus* demonstrated broad variability of host use between sampling locations, emphasizing the influence of multiple factors on actual host-feeding patterns. An excess of data as from the USA and research gaps as for several regions in Africa could be identified. Classification criteria for a standardized terminology regarding phagia suggested recently were applied on the dataset, classifying two mosquito taxa as anthropophagic, 12 taxa as ornithophagic and 104 as non-human mammalophagic. This literature review aids the understanding of mosquito-host-interactions for a deeper comprehension of global transmission dynamics of mosquito-borne pathogens.

To prevent the loss of information and to facilitate comparison between the studies, a framework was developed for the standardized reporting of vector host-feeding in the future. The suggested data standard includes several criteria regarding the collection and identification methods, as well as the vector and the host species. This was demonstrated with the data of a publication on mosquito host-feeding in Panama.

Furthermore, in a publication on mosquito host-feeding, we focused on the members of the relevant vectors *Cx. pipiens* s.s. and *Cx. torrentium*, as they are rarely differentiated to species and biotype level although exhibiting differing ecologies, possibly including differing host-feeding behaviour. Host-attraction experiments were conducted with *Cx. pipiens* biotype *pipiens* and *Cx. torrentium* regarding bird, mouse and a human lure. Additionally, engorged females were collected in several locations in Germany, Moldova and Iran, genetically identified and the bloodhost identified using barcoding PCR and subsequent Sanger sequencing. In the host-choice experiments, *Cx. pipiens* biotype *pipiens* and *Cx. torrentium* were not significantly attracted to either of the offered hosts bird, mouse or human lure. The field-collections of 992 engorged mosquito specimens expanded the available data on *Cx. pipiens s.s./Cx. torrentium* by two thirds, with *Cx. pipiens* biotype *pipiens* being the predominant taxon.

For all four identified taxa, *Cx. pipiens* biotypes *pipiens* and *molestus*, their hybrids as well as *Cx. torrentium*, great proportions of feeds on avian, human and non-human mammalian hosts were detected. When combined with existing data from 23 published studies, the proportion of avian feeds of *Cx.pipiens* s.s. increased to more than 50%, and up to 39% humans and non-human mammals served as hosts. *Culex torrentium* fed equally on birds and mammals. Notably, the host-feeding patterns exhibited substantial geographical variation.

On the basis of four manuscripts, this thesis investigates two central factors of vector capacity. Vector competence for European BATV was confirmed for *Cx. torrentium*. Knowledge on mosquito host-feeding patterns was summarized and analyzed, and further extended with additional data collections. Particular attention was paid to *Cx. pipiens* s.s. and *Cx. torrentium*, whose members, despite ecological differences and medical importance, due to the high morphological similarity are rarely analysed taxa-specific.

Zusammenfassung

Stechmücken spielen als Überträger einiger Krankheitserreger eine entscheidende Rolle für die öffentliche und veterinärmedizinische Gesundheit. Ihre Fähigkeit Pathogene zu übertragen wird als Vektorkapazität zusammengefasst, die von verschiedenen intrinsischen und extrinsischen Faktoren beeinflusst wird, darunter die Vektorkompetenz der Stechmücken sowie ihre Wirtsnutzungsmuster. Diese Arbeit befasst sich daher mit der Vektorkompetenz einheimischer und invasiver *Culex*- und *Aedes*-Arten für das Europäische Batai-Virus (BATV), sowie mit Wirtsnutzungsmustern als grundlegendem Einflussfaktor auf Übertragungszyklen auf globaler Ebene und speziell für *Cx. pipiens* s.s. und *Cx. torrentium*.

BATV ist ein zoonotisches Arbovirus, das mit hoher Seroprävalenz bei Nutztieren in Mitteleuropa von veterinärmedizinischer Bedeutung ist. Diverse Stechmückenarten wurden bereits infiziert entdeckt, die für die Verbreitung des Virus' verantwortlichen Vektoren sind jedoch nicht bekannt. Als limitierender Faktor für die Pathogenübertragung wurde die Vektorkompetenz von zwei einheimischen und drei invasiven Stechmückenarten (im Feld gesammelte *Cx. Pipiens* Biotyp *pipiens*, *Cx. torrentium* und *Ae. japonicus japonicus*, sowie im Labor gezüchtete *Ae. albopictus* und *Ae. aegypti*) unter drei schwankenden Temperaturprofilen mit Hilfe von Speichelassays untersucht. Während alle getesteten Arten mit BATV infiziert werden konnten, ist *Cx. torrentium* die einzige Art, die BATV-Transmission aufweist.

Ein weiterer entscheidender Faktor, der die Vektorkapazität und damit Übertragungszyklen beeinflusst, sind die Wirtsnutzungsmuster, die die Kontaktfrequenz zwischen Stechmücke, Wirt und Pathogen bestimmen. Für eine gezielte Prävention und Kontrolle der Pathogenübertragung ist das Verständnis der Wirtsnutzungsmuster von entscheidender Bedeutung. In einer umfassenden Datenbank wurden die Informationen von 333 veröffentlichten Studien zu Wirtsnutzungsmustern aus den Jahren 1942 bis 2019 zusammengetragen, um einen umfassenden Überblick über die weltweit beobachteten Wirtsnutzungsmuster von

Stechmücken zu erhalten. Inklusionskriterien waren die Beprobung von im Feld gefangenen Stechmücken ohne Köder und die serologische oder molekularbiologische Analyse der Blutmahlzeit. Die Datenbank mit mehr als 600.000 identifizierten Blutmahlzeiten von 494 Stechmücken-Taxa ermöglicht Vergleiche und umfassendere Analysen. Die Mehrheit der identifizierten Stechmücken gehört zu den Gattungen *Aedes*, *Anopheles* und *Culex*, wobei Säugetierwirte für die beiden erstgenannten überwiegen, während bei *Culex*-Stechmücken Vögel als Wirte dominieren. Die Beispiele der am häufigsten untersuchten Stechmückenarten *An. gambiae* und *Cx. quinquefasciatus* zeigen eine große Variabilität der Wirtsnutzung zwischen den Probenahmeorten, was den Einfluss verschiedener Faktoren auf die tatsächlichen Wirtsnutzungsmuster unterstreicht. Ein Übermaß an Daten wie aus den USA und Forschungslücken wie für mehrere Regionen in Afrika konnten festgestellt werden. Kürzlich vorgeschlagene Klassifizierungskriterien für eine standardisierte Terminologie in Bezug auf Phagie wurden auf den Datensatz angewandt, wodurch zwei Stechmückentaxa als anthropophag, 12 Taxa als ornithophag und 104 als „nicht-human"-mammalophag eingestuft wurden. Diese Literaturarbeit trägt zum Verständnis der Schnittstellen zwischen Stechmücken und Wirten bei und ermöglicht einen besseren Einblick in globale Übertragungsdynamiken von durch Stechmücken übertragenen Pathogenen.

Um den Verlust von Informationen zu vermeiden und den Vergleich zwischen den Studien zu erleichtern, wurde eine Vorlage für die künftige standardisierte Berichterstattung über beobachtete Wirtsnutzungsmuster entwickelt. Die vorgeschlagene Standardvorlage umfasst mehrere Kriterien hinsichtlich der Erfassungs- und Identifizierungsmethoden sowie der Vektor- und Wirtsarten. Dies wurde anhand der Daten einer Veröffentlichung über die Wirtsnutzungsmuster in Panama demonstriert.

Darüber hinaus haben wir uns in einer Publikation über die Wirtsnutzung von Stechmücken auf die bedeutenden Vektoren *Cx. pipiens* s.s. und *Cx. torrentium*

konzentriert, da diese kaum auf Art- und Biotyp-Ebene unterschieden werden, obwohl sie unterschiedliche ökologische Lebensweisen aufweisen, darunter möglicherweise auch ein unterschiedliches Verhalten bei der Wirtsnutzung. Experimente zur Wirtsanziehung wurden mit *Cx. pipiens* Biotyp *pipiens* und *Cx. torrentium* in Bezug auf Vögel, Mäuse und einen menschlichen Lockstoff durchgeführt. Zusätzlich wurden an verschiedenen Orten in Deutschland, Moldawien und dem Iran Stechmücken-Weibchen gesammelt, genetisch bestimmt und der Blutwirt mittels Barcoding-PCR und anschließender Sanger-Sequenzierung identifiziert. Die Experimente zur Wirtswahl zeigten keine signifikante Anziehung von *Cx. pipiens* Biotyp *pipiens* und *Cx. torrentium* zu einem der angebotenen Wirte Vogel, Maus oder menschlichem Lockstoff. Die im Feld gesammelten 992 Stechmücken erweitern die verfügbaren Daten über *Cx. pipiens* s.s./*Cx. torrentium* um zwei Drittel, wobei *Cx. pipiens* Biotyp *pipiens* das häufigste Taxon ist. Bei allen vier identifizierten Taxa, *Cx. pipiens* Biotypen *pipiens* und *molestus*, ihren Hybriden und *Cx. torrentium*, wurden große Anteile von Vögeln, Menschen und nichtmenschlichen Säugetieren als Blutmahlzeitwirte nachgewiesen. Zusammen mit den vorhandenen Daten aus 23 veröffentlichten Studien stieg der Anteil der Vogelwirte von *Cx. pipiens* s.s. auf über 50%, und bei bis zu 39% dienten Menschen und nichtmenschliche Säugetiere als Wirte. *Culex torrentium* ernährte sich gleichermaßen von Vögeln und Säugetieren. Bemerkenswert ist, dass die Wirtsnutzungsmuster erhebliche geografische Unterschiede aufwiesen.

Auf der Grundlage von vier Manuskripten werden in dieser Arbeit zwei zentrale Faktoren der Vektorkompetenz untersucht. Vektorkompetenz für das europäische BATV wurde für *Cx. torrentium* nachgewiesen. Kenntnisse über die Wirtsnutzungsmuster von Stechmücken wurden gesammelt, analysiert und durch zusätzliche Datenerfassungen erweitert. Besonderes Augenmerk wurde auf *Cx. pipiens* s.s. und *Cx. torrentium* gelegt, deren Mitglieder aufgrund großer morphologischer Ähnlichkeit doch trotz ökologischer Unterschiede und medizinischer Bedeutung selten Taxon-spezifisch analysiert werden.

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List of Abbreviations

Abbreviation	**– Full description**
Ae.	- *Aedes*
An.	- *Anopheles*
BATV	- Batai virus
CO_2	- carbon dioxide
COI	- cytochrome c oxidase I
Cq.	- Coquillettidia
Cs.	- *Culiseta*
Cx.	- *Culex*
DNA	- deoxyribonucleic acid
e.g.	- exempli gratia
et al.	- et alia
mobo-virus	- mosquito-borne virus
PCR	- polymerase chain reaction
RNA	- ribonucleic acid
s.l.	- sensu lato
s.s.	- sensu stricto
sp.n.	- species nova
USA	- United States of America
USUV	- Usutu virus
WNV	- West Nile virus

1. Introduction

1.1. Mosquitoes as pathogen vectors

Pathogens like viruses, bacteria and protozoa can be transmitted from host to host in different ways. While some are airborne, waterborne or transmitted through direct host-to-host contact, others are vectored by blood-feeding arthropods like blackflies, biting midges or ticks, and are thus called arthropod-borne pathogens [1]. The most important vectors are mosquitoes, colloquially also called the deadliest animal in the world due to the severe implications for human and animal health through the associated pathogens [2]. These mosquito-borne pathogens comprise protozoa like malaria parasites or viruses such as yellow fever virus or dengue virus [3,4]. The extent of the burden becomes evident from the number of human infections alone: Approximately 390-400 million people become infected annually with dengue virus, leading to 21,000 fatal cases [5–7]. The estimations for yellow fever virus infections lay by 200,000 cases per year with 30,000 fatalities [8]. The recorded numbers of malaria infections reached 247 million cases in 2021 of which an estimate of 619,000 ended fatally [9]. Additionally, mosquito-borne pathogens also pose an economic burden, e.g. costs for mosquito control, and for prevention or treatment of the diseases. Calculations estimated the cumulative economic costs due to pathogens transmitted by mosquitoes of the genus *Aedes* alone of US $ 300 billion [10]. In countries with high malaria prevalence, the annual gross domestic product growth per person grew less by 1.3 % between 1965 and 1990 compared to malaria-free countries [11] and costs for prevention and control of malaria accounted for US $ 4.3 billion in 2016 alone [12].

1.2. West Nile virus, Usutu virus and Batai virus in Europe

While many mosquito-borne pathogens occur in tropical areas, the risk for spread and establishment in temperate regions increases with climate warming, proceeding globalization, land use changes and human demographics [13,14]. The range of exotic vectors expands and specimens introduced by transportation and trade may lead to established populations in previously unimpacted areas. Besides the mosquito species, also pathogens previously confined to tropical regions spread, and single events of infected human travellers or introductions via migratory animals may cause autochthonous transmission, when local conditions are suitable, e.g. climatic conditions and a competent vector population [15]. Several studies have forecasted an increase of human populations under risk by mosquito-borne viruses (mobo-viruses) [16,17]. In Europe, Batai virus (BATV) is a mobo-virus detected already in 1960 [18], while West Nile virus (WNV) and Usutu virus (USUV) have been introduced in the last three decades and established a widespread autochthonous circulation [19].

WNV is a flavivirus of the Japanese encephalitis serocomplex and the disease-causing agent of West Nile fever. Although 80% of infections remain asymptomatic, 20% develop symptoms like fever, headache, nausea, joint pains, body aches or rash. With immunocompromised and elderly people being at higher risk, in 0.67% WNV neuroinvasive disease develops with severe implications such as encephalitis or meningitis, potentially leading to death [20,21]. Furthermore, WNV infection can also have severe impacts on horses' physical health, as up to 20% of infected individuals can develop ataxia, limb weakness or paralysis, fever or failure in proprioception [22]. Next to emotional strain for the owners, this causes financial losses due to medical costs and impairments of use of horses as an economic resource [23,24]. To date, no licenced vaccine for humans exists, while for horses, approved vaccines are available [25,26]. WNV was first discovered in Uganda in 1937 [27]. After single sporadic detections [28], the first larger outbreaks in Europe occurred in 1996 in Romania and

1999 in Russia [29,30]. Since, the virus circulates especially in Italy and south-east Europe, but also in central European countries such as France, Austria and Czech Republic [31,32]. The largest outbreak to date occurred in 2018 with 1,963 autochthonous human cases and 202 fatalities in 12 European countries [33,34]. The same year, WNV was detected in birds and horses for the first time in Germany [35], and five autochthonous human infections followed in 2019 [36]. The transmission cycle of WNV includes bird as amplifying hosts and the mosquito vector in an enzootic cycle [37,38]. Mammals including humans can become infected in spill over events. However, as the viremia remains too low to infect mosquitoes during their blood meal, mammals are dead-end hosts and don't serve as amplifying or reservoir hosts [39]. Additionally, WNV transmission has been reported to occur via blood transfusions, breast-feeding or organ transplantation [40–42].

USUV, which is also a flavivirus of the same serocomplex, shares a similar transmission cycle between birds as the amplifying hosts and mosquitoes like WNV, with mammals as dead-end hosts after spill over events [43]. Transmission from asymptomatic blood donors is not known for USUV [44]. Contrasting to WNV, only few severe cases of USUV infections are known. However, seroprevalence studies imply that USUV may be more widespread in the population than generally recorded [45–48]. USUV infection remains mainly asymptomatic, but reported symptoms comprise headache, fever and rash in mild cases. In very rare cases neurological complications with varying symptoms such as encephalitis, meningoencephalitis, facial paralysis or polyneuritis can develop [49–51]. After multiple introductions to Europe [52], the first known outbreak of USUV among birds in Europe occurred in 1996 in Italy [53]. After a large outbreak five years later in 2001 in Austria with great numbers of dead birds [54], USUV spread throughout Europe, with the virus by 2021 being detected in 17 European countries [55–57]. By 2022, 110 human cases have been reported in Europe, of which 30 individuals developed neurological complications [58]. Clinical symptoms in horses are not known, however, USUV-antibodies have been discovered in horses [19].

The orthobunyavirus BATV of the family *Peribunyaviridae* [59] has been described 1960 for the first time in Europe under the name Čalovo virus in Slovakia [18], with sporadic but consistent detections since [60]. The virus could be detected in several mosquito species, such as *Anopheles maculipennis* s.l., *Cx. pipiens* s.l. and *Ae. vexans* [60]. Detections of the virus in vertebrates are rare; serosurveys, however, show seroprevalences of up to 44.7%, observed in sheep in Germany [61]. Therefore, especially livestock must be exposed to BATV, which is often asymptomatic, but can also cause mild symptoms of febrile illness, and even abortion in ruminants [60,62]. Symptomatic human cases have only been recorded in Asia and Africa [63,64], and presented with mild influenza-like courses with fever, malaise and bronchopneumonia [60]. Serosurveys revealed past infections in humans also in Europe [65,66]. As the genome of BATV and other orthobunyaviruses is segmented, reassortment upon co-infection with other viruses of this genus is possible [60]. Although also transmission by ticks and biting midges has been reported, the main vector of BATV are mosquitoes [62].

1.3. Vector competence and host-feeding patterns shape vector capacity

Transmission cycles of arthropod-borne pathogens are influenced by various factors. The vector capacity is the ability of a population of a vector to become infected and transmit a specific pathogen [67,68]. It is shaped by different variables, that influence the transmission by a specific population at a time and in a specific location, encompassing different environmental, genetic and behavioural factors. These include e.g. the extrinsic incubation period, fecundity, population density, or longevity [68,69]. Due to the many different factors involved, the vectorial capacity varies among mosquito species, vector and host populations, among locations and seasons.

The vector competence is an important part of the vector capacity, focusing on the interaction of the pathogen and the vector. Vector competence describes the pathogen

transmission potential of a vector following exposure to the pathogen [70]. With regard to mobo-viruses, this can be described more precisely as the inert ability of a mosquito species to become infected through an infectious bloodmeal and transmit the pathogen with the next bite, or - with the perspective on the virus - the ability of the virus to infect the mosquito's midgut cells, to proliferate and disseminate throughout the body and to infect the salivary glands, from where it can be transmitted to the next host with the mosquito's saliva [69]. Temperature and pathogen titre are factors influencing vector competence. Also, the vector competence differs for every pathogen strain, vector species and even different vector populations [69]. The value results from the infection rate, meaning the infected specimens per engorged specimens, and the transmission rate, which is the proportion of vector specimens with pathogen positive saliva per infected vector specimens [71,72]. Vector competence is assessed in the lab by experimentally infecting mosquitoes with an infectious bloodmeal, and measuring pathogen loads in body parts and in the saliva after a dissemination period [73–75]. However, although essential, vector competence alone is not sufficient for high vector capacity and thus pathogen transmission, as the vector, the pathogen and a suitable host need to meet in time and space, which is determined by ecological aspects.

A further crucial factor influencing vector capacity and therefore local transmission cycles are host-feeding patterns of the vector, as they determine the contact rates between the mosquito and the hosts [76,77]. Depending on the host-feeding patterns, a pathogen can remain in enzootic or urban human cycles, if the mosquito species is a specialist feeder with clear host preferences. Specific preferences are known for e.g. *Ae. caspius* feeding on non-human mammals [78], or *Ae. aegypti*, which mainly bites humans, thereby being the principle vector for humanopathogenic viruses such as Zika, dengue and chikungunya virus [79,80]. A more catholic mosquito species choosing different host species or host groups opportunistically can promote wider host ranges and can serve as a bridge-vector and cause spill-overs to incidental hosts. Such a generalist is e.g. *Ae. nigripalpus*, which feeds on birds, humans, mammals and

reptiles. Thereby, *Ae. nigripalpus* is both an enzootic and epizootic vector for WNV and St. Louis encephalitis virus in the USA [81–83].

Thus, different mosquito species with different host-feeding patterns can be involved in transmission cycles, e.g. *Cx. torrentium* has been shown to be an enzootic vector among birds for Sindbis virus, while *Ae. cinereus* and *Ae. rossicus* with their more general feeding pattern serve as bridge vectors [84,85]. Similarly, the enzootic cycle of Eastern Equine encephalitis virus among birds is maintained by the ornithophagic *Culiseta melanura*, and transmission to mammals occurs mainly via the generalists *Ae. vexans*, *Coquillettidia perturbans* or *Ae. sollicitans* [86–88]. Another example is dengue virus, which is transmitted among monkeys by forest mosquitoes like *Ae. luteocephalus*, while *Ae. aegypti* and *Ae. albopictus* distributed in urban areas transmit the virus between humans [89].

Possible inherent host preferences of mosquitoes are often studied under laboratory conditions by offering the mosquito different hosts or odours, and analysing the choice of the mosquito [90,91]. This experimental setup can offer important information about the outcome when the mosquito faces multiple hosts and can disclose whether it is e.g. ornithophilic, anthropophilic, mammalophilic, or a generalist feeder. In contrast to philia, which indicates an inherent preference, phagia describes which hosts mosquitoes actually feed on in nature. Actual host-feeding can deviate from inherent preferences in factual ecosystems, as it also depends on extrinsic factors such as host availability, abundance and methods of defence, meaning that even with an experimentally observed preference, a mosquito may still feed on different hosts, if it does not meet the preferred host species [76,92]. Seasonal plasticity has been observed for *Cx. pipiens* in the US, which primarily feed on *Turdus migratorius* as their preferred host, but switch to alternative hosts such as humans when the bird species migrate. This switch in hosts in the end of the season increases WNV infection rates in humans after previous enzootic transmission among American robins [93]. Similarly, also *Cx. tarsalis* has been seen to feed more frequently on mammals in California and Colorado

throughout the year, which could also contribute to epizootics of WNV [93–95]. A change in host-feeding and thus pathogen transmission due to abiotic factors is described for *Cx. nigripalpus*, the primary vector of St. Louis encephalitis virus in Florida. During droughts in spring, the vector dwells in densely vegetated hammocks alongside with birds, and promotes St. Louis encephalitis virus amplification with ornithophagic host-feeding. With the onset of the rainy season, both, the birds and the mosquitoes disperse, and with a shift to mammalian hosts, *Cx. nigripalpus* serves as a bridge vector and causes epizootics of St. Louis encephalitis virus [96–98].

The understanding of actual host-feeding patterns is important to understand possible transmission cycles, to direct efforts in research such as vector competence research, in vector control and in protection. However, also field studies entail difficulties. For example, mosquitoes are collected using traps with cues such as CO_2 or animal bait to attract mosquitoes. These cues are or mimic a potential host and thus the target of host-seeking females, usually without any ingested blood yet [99,100]. Blood fed mosquitoes are rare, and other collection methods provide higher yields, such as the aspiration from resting sites, which are used by mosquitoes to rest after a bloodmeal [101,102]. The positioning of such resting sites, however, requires funded knowledge about the ecology of mosquitoes. Also, collections are always biased towards some mosquito species, as species differ in their ecology, in the cues they are attracted by or in the habitats they inhabit [101,103]. Also, the execution of the collection (e.g. time, rhythm and method), the laboratory analyses (e.g. different primers and assays) and the reporting of the single studies may alter the outcome and the interpretation of results [93,104]. Such differences render the comparison of studies difficult, and an overall perspective on mosquito host-feeding patterns often remains based on subjective impressions instead of combined studies.

1.4. *Culex pipiens* s.l., a widespread disease vector

The *Cx. pipiens* complex is globally relevant with regards of distribution and disease transmission. Members of the complex comprise the taxa *Cx. quinquefasciatus*, *Cx. pallens*, *Cx. pipiens* s.s., *Cx. globocoxitus* and *Cx. australicus* [105]. While the latter two are endemic to Australia and *Cx. pallens* inhabits east Asian regions, *Cx. quinquefasciatus* is distributed throughout all tropical regions [105–107]. *Culex pipiens* s.s. is found in temperate regions all over the world. The species is one of the most common mosquito taxa and most relevant vectors in Europe [108,109]. It comprises the two bioforms *pipiens* and *molestus*, which are morphologically not identifiable, but can only be distinguished genetically. Nonetheless, the bioforms differ in their ecology: *Culex pipiens pipiens* lives aboveground, diapauses in winter, is anautogenous (requirement of a blood meal before the first batch of eggs) and eurygamous (no mating in confined spaces). *Culex pipiens molestus* in contrast occurs in urban underground areas, diapauses, is autogenous (no requirement of a blood meal for the first batch of eggs) and is stenogamous (mates in confined spaces, and is therefore breedable in facilities) [109]. Hybrids of the two bioforms occur in regions where both taxa coexist [109]. Additionally, the sibling species *Cx. torrentium*, which does not belong to the *Cx. pipiens* complex, but can also only be distinguished genetically or by the male genitalia from *Cx. pipiens* s.s., resembles *Cx. pipiens* bioform *pipiens* in its ecology and inhabits Europe [110], possibly even in higher numbers [111]. *Culex torrentium* is a major vector for Sindbis virus [112], with high vector competence also for WNV and USUV [84,113,114]. *Culex pipiens* s.s. is recognized to be a globally significant vector for many pathogens, including WNV, USUV or St. Louis encephalitis virus [115]. In the literature, *Cx. pipiens molestus* is usually described as mammalophilic, *Cx. pipiens pipiens* in contrast is oftentimes characterized as ornithophilic and ornithophagic [116–120]. This often refers to historical studies, in which mosquitoes in the lab were given the choice of two alternatives, e.g. birds and mammals, or cites experts' opinions [121,122]. By the presumption of ornithophagy, *Cx. pipiens pipiens* is often not considered a potential bridge vector for viruses such as WNV, USUV and St. Louis

encephalitis virus, and rather hybrids of the two bioforms with an intermediate host preference are made responsible for epizootics and spill-over events [123–125]. No information is available about the host feeding patterns of *Cx. torrentium*.

1.5. Undefined classification of philia and phagia

Although terms like 'anthrophily' or 'ornithophagy' are widely used, there is no described definition other than 'feeding often on' or 'preferring' a host or host group for both, philia and phagia. For example, the conclusion of ornithophagy of *Cx. pipiens* has been drawn at proportions of e.g. 77.1 % or 91.7 % avian feeds [126–129]. Furthermore, philia and phagia are oftentimes used synonymous, although philia describes the host preference of the vector when having the choice, while phagia refers to the actual host feeding in nature, additionally influenced by host availability and abundance, and can deviate from the vector's preference. This indistinct use of terminology becomes obvious when collected mosquitoes with high proportions of avian bloodmeals in host-feeding studies are referred to as ornithophilic [129–131], or when e.g. 'ornithophilic' becomes explained with 'feeds predominantly on birds' [116].

In a publication on the understanding and interpretation of host-feeding studies, Fikrig & Harrington addressed this gap and proposed a more standardized way to use terminology [132]. The authors suggested a minimum of one third of the bloodmeals being from a certain host or host group in at least three published bloodmeal studies to call a mosquito species -phagic for the respective host or host group. Simultaneously, no more than two studies should constitute the opposite with less than one third of the feeds from the respective host or host group. Regarding philia, at least three choice studies should present a twofold higher likelihood to choose a certain host or host group, while not more than two studies indicate different results, to call a mosquito species -philic for this host or host group. The use of a unified terminology

would facilitate the communication about host-feeding and disclose potential patterns, which is important to understand transmission cycles.

Host-feeding patterns and vector competence are two crucial factors determining transmission risk of mosquito-borne pathogens, and the deeper understanding of which and of the drivers shaping them is necessary, e.g. to take measures for prevention and control of mosquito-borne pathogens.

2. Scope of the thesis

1. Batai virus (BATV) is a mosquito-borne virus prevalent in Europe, Africa and Asia, and widespread seroconversion has been detected in Europe especially in livestock. Although only few acute infections have been recorded, BATV should be monitored to prevent greater economic and health impacts, also due to close relationship to viruses with more severe implications like Cache valley virus and Ngari virus. The mosquito species responsible for BATV transmission are unknown.

To understand, which mosquito species could play a role in the BATV circulation in Europe, we assessed the vector competence of five mosquito taxa. Vector competence is a determining factor in pathogen transmission and is assessed by recording viral particles in the mosquito's saliva upon an infectious bloodmeal. The analysed taxa comprise the two European native and prevalent *Cx. pipiens pipiens* and *Cx. torrentium*, as well as the invasive species *Ae. albopictus*, *Ae. aegypti* and *Ae. japonicus*.

2. A further essential driver in pathogen transmission are host-feeding patterns, as they determine the contact rates between the mosquito, the pathogen and the host. Many studies have been conducted to assess mosquito host-feeding patterns, however, as mosquito host-choice is time- and location-dependent, focusing on single studies can dismiss a more comprehensive picture, and can evoke misleading assumptions.

Therefore, our aim is to collate these studies in a comprehensive database, which allows a capacious and more complete perspective on mosquito host-feeding patterns. This open access database includes mosquito and host information as well as details about the trapping and blood meal analysis. A collation of the data enables a deeper analysis than possible with individual studies, and facilitates the identification of knowledge gaps for future research or prevention and control of mosquito-borne pathogen transmission in a more targeted manner.

3. During the process of collating the published data of mosquito host-feeding studies, we encountered difficulties in the extraction and standardization of the information. Due to differing methods and reporting, a comparison of the single studies is complicated or impossible, thus impeding broader analyses. For other aspects, reporting standards have been developed and presented, such as previously proposed for vector competence studies [70]. To facilitate analyses in the future and to increase the communal gain for the scientific community, we propose a standardized way to report hosts, the collection and the analysis methods of wild caught mosquitoes. This is described by means of an example of published host-feeding patterns from Panama.

4. It is known, that the different members of the *Cx. pipiens* complex differ in their ecology, and thus possibly in their host preference, host-feeding patterns and thus potential roles in pathogen transmission. Few studies addressed the host preference or host-feeding patterns of the bioforms in particular. *Culex pipiens pipiens* is commonly described as ornithophilic, while *Cx. pipiens molestus* is often referred to as mammalophilic. As these members of the *Cx. pipiens* complex are very common across the world and Europe, and known to be competent vectors for viruses such as WNV and Sindbis virus, their host-feeding patterns are a crucial factor in potential pathogen circulation and transmission. Thus, we analyzed the host-feeding patterns of the two bioforms of *Cx. pipiens,* their hybrids and the closely related *Cx. torrentium* found in the literature, and identified blood hosts of self-collected specimens from several locations in Germany, Moldova and Iran over the last 11 years. Additionally, a possible inherent host preference of *Cx. pipiens pipiens* and *Cx. torrentium* was addressed in host-choice experiments.

3. Discussion

3.1. Evaluation of the vector competence for Batai virus of native *Culex* and exotic *Aedes* species in Central Europe

The risk of mosquito-borne diseases has been increasing globally in recent decades, especially in temperate regions [133–135]. Due to expanding globalization and trade, the range of invasive mosquitoes expands, and with migratory birds or returning travellers pathogens get introduced into new areas [136,137]. Furthermore, warming climate makes conditions more favourable for establishing new mosquito populations and for autochthonous circulation of the introduced pathogens [15,137–140]. Recent examples in Europe are given by outbreaks of dengue virus and chikungunya virus in Italy or France [141–145], two viruses originating in tropical regions [146]. The two driving factors of the outbreaks are the spread of the important vector *Ae. albopictus* on one side, of which established populations are already known in many European countries, and rising temperatures on the European continent on the other [147,148].

Batai virus (BATV), an orthobunyavirus of the family *Peribunyaviridae* [59], has also been discovered to circulate in Europe [18,60]. In eastern Germany, antibodies against BATV have been detected in animals such as pigs, horses and a range of bird species, and in high levels in sheep, bovines and goats [61]. While the few infected humans present only mild influenza-like symptoms, in livestock rare severe BATV cases are connected with abortion and congenital defects [60]. Regardless of the little number of observed acute infections in livestock or humans, the virus could have significant economic consequences due to livestock infection and bears the risk of larger veterinary health implications. BATV should be monitored to detect outbreaks early on, to understand transmission and disease dynamics, and to be able to prevent further risks by control measures. Furthermore, the segmentation of the genome of BATV can bear the risk of reassortment. Ngari virus is believed to have originated from the

reassortment upon co-infection of BATV and Bunyamwera virus [149], which was proven to cause haemorrhagic febrile illness in humans in Africa [150,151].

Despite the recorded cases and the potential risks related to BATV, the mosquito species responsible for BATV transmission are not well known. Only *Ae. detritus* has been shown to be vector competent for a European BATV lineage [152], while its current known distribution is patchy and not concordant with occurrences of BATV seroprevalence [153]. *Culex quinquefasciatus* and *Cx. tritaeniorhynchus* transmitted the Asian Chittoor strain under laboratory conditions [154]. Although BATV has repeatedly been isolated from several mosquito species [60,155,156], e.g. *An. maculipennis* s.l., *Cx. pipiens* s.l. and *Ae. vexans* [60], this does not provide any insight into their actual vector competence, meaning the ability to transmit infectious saliva after an infectious bloodmeal.

We therefore analysed the vector competence of selected mosquito taxa for the European BATV lineage by determining body infection rates and presence of the virus in the saliva after feeding them with an infectious bloodmeal. We included field collected *Cx. torrentium* and *Cx. pipiens pipiens*, as they are very common in Germany [157,158], and their vector competence has been already shown for various viruses such as Sindbis virus and WNV [114]. Additionally, vector competence of invasive species was tested, namely field collected *Ae. japonicus* as well as laboratory-reared *Ae. albopictus*, which is highly competent for several viruses, e.g. chikungunya virus and dengue virus [159,160]. Both are established in Germany and other European regions [161,162]. The trials were complemented with laboratory-reared *Ae. aegypti*, a further invasive species globally involved in transmission of many viruses such as Zika, dengue, chikungunya and yellow fever virus [163].

3.1.1. No vector competence of invasive *Aedes* species and *Cx. pipiens pipiens*

All analysed *Aedes* species showed infections with BATV after feeding an infectious bloodmeal at both temperatures 21°C or 24°C and a more tropical temperature of 27°C, but no virus was recorded in the saliva. Thus, *Ae. aegypti*, *Ae. japonicus* and *Ae.*

albopictus therefore seem to be no competent vectors for this European BATV strain. These results confirm the previous findings of a study for *Ae. aegypti* with the Asian lineage of BATV [154]. Only six specimens could be tested for *Aedes japonicus*. This leads to a minimal detection limit of a transmission efficiency of 16% in the case of one infectious specimen. Therefore, although no specimen was infectious in our experiments, we cannot exclude vector competence of *Ae. japonicus* for BATV. Although the here tested *Aedes* species did not transmit European BATV, they might still be competent for other BATV lineages, as a connection has been shown between the effective transmission potential and the specific pairing of both vector and pathogen strain genotypes, again influenced by environmental factors such as temperature [164]. Especially the widespread *Ae. albopictus* could therefore still pose a potential risk for BATV spread upon introduction of other strains. Field collected *Cx. pipiens pipiens* also became infected with BATV at all except the lowest temperature. However, no infectious saliva was collected for this species. This is in line with a study showing no transmission by a *Cx. pipiens* lab colony (hybrids of the two bioforms *Cx. pipiens pipiens* and *Cx. pipiens molestus*) [152].

3.1.2. *Culex torrentium* shows low vector competence for BATV

Field collected *Cx. torrentium* were the only of the tested mosquito species showing infectious saliva, but only at the highest temperature of 27°C with a low transmission efficiency of 3%. This is also the temperature at which the highest BATV RNA copy numbers were detected in the body of the mosquitoes. As the transmission efficiency is very low and occurs only at the highest temperature, *Cx. torrentium* alone cannot explain the high seroprevalences, just as little as *Ae. detritus*, which is also vector competent for BATV but occurs rather in different regions than the detected BATV antibodies in animals [61,152,153,165].

The three *Aedes* species were tested to assess the risk of BATV distribution upon the spread of these invasive mosquito species. The two *Culex* taxa were tested due to their widespread distribution in Europe and known vector competence for other pathogens.

Additionally, *Cx. pipiens* s.l. has already been found to be infected with BATV in Germany [166]. In the future, further mosquito species with detected BATV infection should be examined for their vector competence, e.g. *An. maculipennis* s.l. [60]. As BATV has been discovered repeatedly in these mosquito species, vector competence studies would help to understand if they only become infected or even play a role in BATV transmission.

In this study we analyzed the vector competence of *Cx. pipiens pipiens*, *Cx. torrentium*, *Ae. aegypti*, *Ae. albopictus* and *Ae. japonicus* for a European lineage of BATV. Vector competence, however, is only one of the many factors that shape vector capacity, as it focuses solely on the interaction of the mosquito and the virus. Other important determinants of vector capacity are host availability and abundance, mosquito population density, longevity, and in particular host-feeding patterns [69]. The next presented study focuses specifically on the latter.

3.2. Global database of mosquito host feeding patterns

Mosquito host-feeding patterns shape transmission cycles, as they determine the contact rate between the mosquito as the vector, the pathogen and the host. Understanding transmission cycles is important for the prevention and control of diseases, as it helps to take targeted measures, e.g. in mosquito control or host protection. To analyse mosquito host-feeding patterns, screening of engorged mosquitoes has been conducted across the globe over the last century [167]. To combine the information of single studies and to receive a broader perspective on mosquito host-feeding patterns, we collated the data of 333 publications, which investigated the bloodmeal hosts of field-collected mosquitoes with serological or molecular biological methods. Besides the mosquito and host taxa, we included the time and location of the sampling, as well as mosquito collection and bloodmeal identification methods. In an open access database, 609,243 identified bloodmeals of 494 mosquito taxa and 890 host taxa are documented. Hosts are categorized into the

host groups avian, reptilian, amphibian, fish, annelid, mammalian, non-human mammalian and human. The distinction between the latter two arises from the specific medical importance of anthropophagic mosquito species for human health. The mammalian host group is only recorded for bloodmeal hosts that were not further specified.

3.2.1. Heterogenous distribution of host-feeding studies with critical research gaps

Although studies on host-feeding patterns were conducted on all continents excluding Antarctica, distribution of the studies and of the investigated mosquito specimens was heterogeneous. Most studies were conducted in North America (39.9% of the studies; 27.8% of the bloodmeals), Asia (19.8%; 34.9%) and Africa (19.5%, 22.4%), with clusters in the USA, India and Kenya. Identified global host-feeding patterns could be biased towards local patterns, as 28.9% of the studies and almost 25% of the bloodmeals originate from the USA alone, i.e. host-feeding patterns are influenced by local conditions (such as diversity between mosquito populations) and environmental factors, e.g. host availability [132].

Research gaps were obvious for Africa, as several countries, such as Chad, Ivory Coast, Central African Republic, Gabon or Angola, lack any locally identifiable data, and others, such as Benin or Sudan, are represented only with relatively few collected mosquito specimens. This is crucial, as many mosquito-borne pathogens, e.g. Zika virus, yellow fever virus, chikungunya virus and WNV [168], circulate on the African continent, affecting the local populations and spreading from there to other parts of the globe. Especially here, a detailed understanding of mosquito host-feeding patterns and possible transmission routes in different geographical areas is important to early identify risk areas and to prevent the emergence of new or rare zoonoses. This would reduce both local and worldwide transmission risks, disease burden and economic losses.

Although serious mosquito-borne viruses such as dengue virus, chikungunya virus and Zika virus are present and pose a constant disease burden also in South America [169], only 7.2% (24 of 333) of the studies comprising 5.9% of collected mosquitoes were carried out on this continent. Information on possible local transmission routes therefore is scarce.

3.2.2. Distribution patterns dominated by *Culex*, *Anopheles* and *Aedes*

The most frequently collected and analysed genera are *Culex*, *Anopheles* and *Aedes*, which comprise taxa of global significance as vectors of pathogens like *An. gambiae*, *Ae. aegypti*, *Ae. albopictus* and *Cx. pipiens* [170–173]. Some connections were found between the species in focus with the distribution of associated pathogens. However, despite the fact that the distribution areas of many serious and prevalent pathogens transmitted by mosquitoes of the genus *Aedes* circulate in Asia, e.g. chikungunya and dengue virus [174], only 3.6% of the here collected specimens belong to this genus. Similarly, only 15% of the overall collected *Aedes* specimens were collected in Asia. More than half of the collected *Aedes* originate from North America (53.9%).

Most specimens collected in North America, however, belong to the genus *Culex*, which include important vectors for WNV in this region. Since its introduction into the US in 1999, WNV poses a high burden with more than 50,000 infections and over 2,400 fatalities [175]. *Culex quinquefasciatus*, the primary vector of WNV in the southern US [38,176], alone makes up 25.5% of the here collected specimens. This is also the case for Asia: *Culex* specimens were collected in great numbers, which matches the distribution of prevalent and correlating pathogens such as Japanese encephalitis virus [177].

Anopheles, which encompass the vectors for human malaria transmission [178], is by far the most frequently analysed genus in Africa, Asia and South America as the main circulation areas of malaria [9]. Nevertheless, although *Anopheles* was with 65% the most frequently collected genus in Africa, where malaria incidences are highest [179], most *Anopheles* specimens were collected in Asia, due to higher overall collection numbers.

3.2.3. Host-feeding patterns of key mosquito taxa

Mammals were identified most frequently (78.9%) as a bloodmeal host, including humans and non-human mammals. Bovidae and humans jointly shared with more than 50% a major proportion of identified bloodmeals. This could be a representation of the great proportional difference in biomass of livestock and humans compared to wildlife [180]. It may also result from frequent collections in respective environments, such as farms, villages with livestock, or proximity to human habitation in general. Birds were the hosts in 19.7% of the bloodmeals. Reptiles, amphibia, annelids and fish are host groups detected in less than 1% of all feeds. Especially annelids and fish are only fed on by specialized mosquito species, i.e. only one species each was recorded to bite annelids or fish, respectively.

With 57,966 (10.4%), *Cx. quinquefasciatus* is the mosquito taxon with the most records in our database. The taxon, which inhabits tropical and subtropical regions across the globe [181], was collected especially in the USA, followed by India and Kenya. In single studies, often one host group predominated, suggesting e.g. an anthropophilic feeding behaviour [182,183]. However, the results of the studies and the proportions of the different host groups vary strongly. Thus, the overall host-feeding pattern of the collated studies presents a broader picture with all major host groups represented. 57.7% of the bloodmeals are of avian origin, while also a quarter derives from non-human mammals and 17.3% from humans, and even feeds on amphibia and reptiles are recorded. This variability could be due to differing host availability in the different collection sites, but also due to mosquito population variability between the countries. This emphasizes the need for caution with classifications based on single studies from limited locations with its local conditions. A host census and a calculated forage ratio could help to consider observed host-feeding patterns in relation to actual host availability, and therefore make possible bias recognizable [184,185].

Anopheles gambiae s.l. is the second most collected mosquito taxon with 36,647 specimens (6.6%). The host range remains limited to mammals, with the collated

picture of 23 studies presenting 53.2% of the hosts being humans and 46.7% being non-human or not further identified mammals. The *An. gambiae* complex comprises several taxa, for which different host choice has been described [186,187]. While *An. coluzzii* and *An. gambiae* s.s. are widely regarded as anthropophilic [188], *An. arabiensis* is described to be more of an opportunist feeder and *An. quadriannulatus* as zoophilic [172,186]. However, in many of the studies *An. gambiae* s.l. specimens are not further specified to species level, as morphological identification is not possible [172] or species were only distinguished as different taxa at a later time point. The almost balanced distribution between humans and other mammals may be a result of the aggregation of cryptic anthropophilic, opportunistic or mammalophilic taxa in the species complex.

A mosquito species of global concern is *Ae. albopictus*, which is known to be vector competent for many pathogenic viruses (e.g. dengue virus, chikungunya virus or Zika virus) [189], and expands its range greatly [16]. A great proportion of 37% of the *Ae. albopictus* specimens fed on humans, which is in some literature referred to as anthropophilic [160], being key for human-to-human transmission cycles of viruses like dengue, chikungunya and Zika virus. However, a major proportion of 47.3% fed on non-human mammals, confirming studies describing *Ae. albopictus* as an opportunistic feeder [159]. Feeding on various hosts and host groups could increase the risk of *Ae. albopictus* also being a bridge-vector of further pathogens with enzootic circulation, such as Rift valley fever virus or La Crosse virus, for which vector competence has been demonstrated as well [189].

3.2.4. Application of proposed classification criteria

As the terminology regarding philia and phagia lacks a common definition and is not used consistently, Fikrig and Harrington proposed a standardized classification [132]. Following their suggestion, a mosquito taxon can be called ‘phagic’ for a host or host group, if in three or more studies at least one third of the bloodmeal origins from this host or host group, as long as not more than two studies show the opposite. As such a

classification is based on the number of studies, our database provides a good basis for such classification. Applying these criteria, the two mosquito taxa *Ae. aegypti* and *An. strodei* would be anthropophagic. At the same time, *An. strodei* also fulfils the criteria to be called non-human-mammalophagic. Both, however, results from the evaluation of 23 specimens collected in four independent studies, which calls in question the strength of the statement.

Following the criteria, the term 'non-human-mammalophagic' applies to 104 mosquito taxa of seven genera. Twelve taxa belonging to the genera *Culex*, *Culiseta* and *Coquillettidia* can be called ornithophagic. Here are taxa found that are widely described as ornithophagic in the literature, e.g. *Cs. melanura* and *Cx. modestus*, as well as taxa which have not been described as such, e.g. *Cq. xanthogaster* [190,191]. Amphibiophagic and reptilophagic are only one (*Uranotaenia unguiculata*) and two taxa (*Cx. hortensis* and *Cx. peccator*), respectively. This is in line with the records in literature [192–196], which, however, are limited in numbers. As common traps attract mosquitoes via CO_2, mosquito taxa which use specialized cues such as frog calls are often missed and thus are rarely represented in mosquito host-feeding studies [197,198]. Similarly, also the bloodmeal identification methods could have created a bias towards more commonly tested hosts and host groups, as described in detail below.

The proposed criteria can be a helpful step towards a common terminology for uniform communication about mosquito host-feeding patterns. To improve such standardized classification, we suggest to include the number of specimens in the studies, to counteract possible imbalances arising if studies with few specimens weigh equally as studies with hundreds of specimens. Also, the number of studies opposing the finding should be relative to the total number of studies included, as the number of two opposing studies present a very different proportion of 10 than of 100 included studies.

3.2.5. Impact of bloodmeal identification methods on host diversity and accuracy

The range and accuracy of host identification is strongly influenced by the applied identification method. In the past, serological methods such as precipitin tests were used to identify the bloodmeal host. These methods are based on the reactivity of the ingested blood to pre-prepared antisera, but are as such limited to the range of expected hosts, often including human, cow, pig, chicken and dog. Therefore, less commonly expected host groups such as reptiles and amphibia or even fish have not been tested for as frequently as for birds, humans or other mammals, automatically allowing fewer positive results. Also, these methods show cross-reactivity and often lack specificity [199,200], and most hosts are reported on family or order levels instead of species level, leading to a lower host diversity in the reporting of mosquito host-feeding patterns [201–203]. With the advent of PCR and sequencing techniques, hosts can be detected based on amplified DNA sequences, e.g. often *COI*, *cytochrome b* or 16S, and its comparison with a sequence databank, on the level of species and without any prior knowledge or expectation [202]. As a result, a greater diversity of species can be distinguished, which becomes evident in the larger amount of reported host species per analysed mosquito specimens in comparison to studies applying serological methods. Even though barcoding PCRs aim to eliminate most pre-assumptions, the applied primers are usually designed to amplify vertebrate-sequences. Invertebrates like annelids are still hardly covered, and while only one species in the database has reportedly fed on annelids, discovered by using an annelid-specific primer set, it cannot be excluded that more mosquito species do feed on non-terrestrial or non-vertebrate hosts [204].

Although PCR-based methods largely replaced serological methods, the greatest proportion of available information on mosquito hosts has still been detected serologically, which is due to early large-scale studies, and also contemporary studies especially in regions of Asia, Africa and Oceania still applying serology-based methods, where laboratory capacities may be limited. Studies from Europe and the

Americas instead have largely moved on to PCR-based methods. In future, next generation sequencing could enhance blood host detection for the understanding of transmission cycles, as multiple gene fragments can be detected simultaneously, and thus different hosts in mixed bloodmeals could be identified [205].

3.2.6. Limitations of the study

As this dataset is based on published studies, only information provided by the publications can be extracted and compared. This impacts the outcome, e.g. if only hosts of interest are reported or if collection site description is lacking. The mosquito collection site may have an influence on the host-feeding pattern as it is also driven by host availability. Variability in the reporting of methods and results complicates the standardization of information for comparability and analysis. Aggregation of details such as collections from different locations and at different time points prevents a precise breakdown of the host-feeding patterns and important information might get lost.

Further difficulties arise from mosquito taxonomy, which is often ambiguous, particularly in historic literature [206]. It is underlying constant change, especially since the advent of molecular tools leading to the discovery of new species [207,208], e.g. the distinction of *An. coluzzii* from *An. gambiae* s.s., and *An. amharicus* from *An. quadriannulatus* was described only in 2013 [209]. Another species positioned within the *An. gambiae* complex, *An. fontenillei* sp.n., was described just in 2019 [210].

Additionally, taxa are often not specified down to the lowest possible level, but repeatedly named by their species complex names. By lacking specifying details such as *sensu lato* or *sensu stricto*, it is often not clear, whether the complex or the likewise named species is referred to. This is observed e.g. for members of the *Cx. pipiens* complex and especially the *Cx. pipiens* bioforms and their hybrids, as well as for the frequently collected species of the *An. gambiae* complex. An undifferentiated analysis could conceal actual host-feeding patterns, as even different biotypes of the same species could differ in their host-feeding patterns [109]. This uncertainty extends to the

identification of bloodmeals, as reliance on scientific names of hosts is often hindered by the use of common names. These imprecisions impede deeper analyses of mosquito host-feeding patterns and render many studies not useful for broader perspectives on host-feeding patterns. A standardization would help to reduce some of these challenges and is therefore addressed in the following study.

3.3. A minimum data standard for reporting host-feeding patterns of vectors

To improve systematic data collection and get the most out of the effort of all the single studies, we propose a standardized way for the reporting of host-feeding patterns of vectors, which is inspired by the *minimum data standard for vector competence experiments* proposed by Wu *et al*. [70].

The here presented framework aids the detailed listing of arthropod bloodmeals with the corresponding time and location of collection, method of collection and host identification. More specifically, the naming of the trap and possible lures, as well as used primers and the amplified genes is important for comparability between studies. Although the information about the land use and the surrounding of the trap or collection location can be important, these details were excluded in the suggested standard, to keep it most basic and to minimize the threshold of applying it. The information on the surrounding landscape is difficult to categorize. Similarly, also a host census would provide valuable insights into the correlation of host-feeding and host availability. However, as many studies do not include a host census, we have also dispensed with it for the sake of a minimum data standard.

These included details are important information to investigate and identify possible drivers of and changes in host-feeding patterns. A uniform way of reporting would facilitate the comparison of studies. This way, the research data is more accessible for the community, and new data can be fed straightforward into a communal database. Such a central database allows easier identification of gaps, which could be filled with

further vector collection studies. While this was initiated by the work on mosquito host-feeding patterns, this scheme can also be adapted for the bloodmeal reporting of other vectors, such as ticks, sand flies or biting midges.

3.4. Broad host preference and host-feeding patterns of *Culex pipiens* s.s./*Cx. torrentium*

In many studies on host-feeding patterns of mosquitoes, closely related species or bioforms are often referred to by a taxonomically higher order such as the name of the complex or *sensu lato,* while the host-feeding patterns of the taxa underneath this taxonomic level, e.g. different complex members, could differ [211]. As a result, host-feeding patterns could be masked, hindering the detection of species-specific host-feeding behaviour and species-specific roles in transmission cycles. A prominent example is *Cx pipiens* s.l., a globally distributed mosquito complex, which is often described ornithophilic in the literature [212–214]. Albeit, it is often referred to as the complex without differentiating the comprised species and even more rarely studies discriminated between the *Cx. pipiens* s.s. bioforms *pipiens* and *molestus* or their hybrids *Cx. pipiens pipiens* x *molestus*. Indeed, *Cx. pipiens pipiens* is usually referred to as ornithophilic and ornithophagic, while *Cx. pipiens molestus* is rather called mammalophilic or anthropophilic [116,118,215,216]. However, there are only limited studies that actually identified the collected mosquito specimens genetically to bioform level and thus could substantiate these common assumptions. Additionally, also the sibling species *Cx. torrentium* is often not differentiated from *Cx. pipiens* s.l., as they are morphologically indistinguishable [111]. For *Cx. torrentium*, apart from the work of our research group there are no records on species-specific host-feeding patterns in the literature.

As the members of the *Cx. pipiens* complex are known to be suitable vectors for WNV, Sindbis virus and USUV [217–220], understanding of the species-specific host-feeding patterns is essential for the understanding of their actual role in pathogen circulation.

We therefore addressed the host preferences of *Cx. torrentium* and the two bioforms of *Cx. pipiens* s.s., as well as the differentiated host-feeding patterns of *Cx. pipiens pipiens*, *Cx. pipiens molestus*, their hybrids and *Cx. torrentium* in an experimental setup, in a systematic literature study and by screening of field-collected specimens from Germany, Iran and Moldova.

3.4.1. No significant host preference of *Cx. pipiens pipiens* and *Cx. torrentium*

In our experimental setup, no statistically significant preference for mouse, grey canary or human lure could be observed for *Cx. pipiens pipiens* and *Cx. torrentium*. This is in contrast to experiments, which showed a preference for birds against mammals for *Cx. pipiens pipiens* [123,221]. Nonetheless, the mean preference of both, *Cx. pipiens pipiens* and *Cx. torrentium*, was higher for bird compared to human lure, and higher for mouse compared to bird.

3.4.2. Host-feeding of *Cx. pipiens* s.s. and *Cx. torrentium* in the literature

While the preference for an available host is a good indication for inherent choice in an area with high availability of the different species, transmission routes are determined by the vector-host-contact through actual host-feeding in the field. Host-feeding patterns in nature are influenced by many more factors additional to the mosquitoes' preference, such as host availability and host defence mechanisms. A literature search based on the mosquito bloodmeal database and additional publications since identified 23 publications [116,125,131,215,222–240], in which these species and bioforms are orderly differentiated, with predominance of *Cx. pipiens pipiens* specimens. The available data show an overall high proportion of avian bloodmeals for *Cx. pipiens pipiens*, which is in line with the common assumption of *Cx. pipiens pipiens* being ornithophagic [108]. Looking at the different countries, the host-feeding patterns are heterogeneous regarding the proportion of mammalian feeds, ranging from less than 5% in Portugal, to 62.5% and 64.9% in Iran and the Netherlands, respectively [116,125,131,215,222–231]. This emphasizes the variability between

different locations, which could be driven by differing host availability or genetic mosquito population diversity. However, also *Cx. pipiens molestus* largely fed on birds, which is contrary to many references in literature describing *Cx. pipiens molestus* as mainly mammalophilic or even strongly anthropophilic [215]. Instead, the proportion of mammals (including human and non-human mammals) amounts to only 28.3% [116,125,215,222,226,231–239]. Differences between the locations are visible also for *Cx. pipiens molestus*, with proportions of mammalian bloodmeals as low as 9.1% in the USA and as high as 68% in Argentina [223,224,226,230,234,240]. The hybrids of the two bioforms are understood to represent an intermediate form of *Cx. pipiens pipiens* and *Cx. pipiens molestus* also in host choice, thus feeding more opportunistically and catholic on a range of hosts [116,125]. However, the specimens reported in the literature fed with a high proportion of 67% on birds, also with variation between the countries and including the three main host groups bird, human and non-human mammal [125,215,222,226,227,231,240]. For *Cx. torrentium*, no additional data was available in the literature, although it is referred to as an ornithophilic species, sometimes even described as exclusively ornithophilic, which does not feed on humans [218,241].

3.4.3. Host-feeding of *Cx. pipiens* s.s. and *Cx. torrentium* in Iran, Moldova and Germany

To further investigate the host-feeding patterns of *Cx. pipiens pipiens*, *Cx. pipiens molestus*, their hybrids and *Cx. torrentium* and to consolidate knowledge, we analysed the bloodmeal origin of mosquito specimens collected in Iran, Moldova and Germany from 2011 until 2022. With 992 specimens, our collections increased the overall numbers by two thirds. Compared with the literature [116,125,131,215,222–231], however, our data present a more balanced distribution of hosts. The *Cx. pipiens pipiens* specimens fed about equally on humans and birds (~40% each). With 20.5%, also non-human mammals constitute a significant proportion of the feeds. This distribution is less biased towards birds, but emphasizes the risk of pathogen transmission from birds to non-avian hosts, and the potential role of *Cx. pipiens pipiens* in epizootic transmission

cycles. Also feeds from reptilian and amphibian hosts were detected for the first time for *Cx. pipiens pipiens*. The use of even poikilothermic hosts emphasizes that *Cx. pipiens pipiens* might be less of a specialist, but more opportunistic when it comes to host selection. When combining our data with data from the literature, birds are the most frequently detected host group for *Cx. pipiens pipiens*. However, more than 40% of the specimens opted for human or non-human mammalian hosts. This creates optimal conditions for pathogen transmission across different host groups and an increased risk for spill-over events.

In contrast to the findings from ten studies reported in the literature [116,125,215,222,226,232,233,235–237,239], which identified birds as the primary host group for *Cx. pipiens molestus*, in our collections human and non-human mammalian hosts constitute over 70% of the bloodmeals. As only 14 specimens could be collected, however, the proportion of avian feeds remains high when adding our results to those from previous studies. Human and non-human mammalian feeds were here at 29.6%.

The 18 collected specimens of *Cx. pipiens pipiens* x *molestus* fed in substantial proportions on all three main host groups humans (44.4%), non-human mammals and birds (both 27.8%), which is in line with a broad host spectrum described in literature [115,123,242]. Taken together, the specimens from literature [125,215,222,226,227,231,240] and our collections fed in 61.5% on avian hosts, while also humans and non-human mammals served to a considerable extent as hosts (38.5%).

With 29 specimens, our collections provide first, detailed insight into the host-feeding patterns of *Cx. torrentium*, which so far had not been identified in mosquito host-feeding studies. This species had similar proportions of avian and human hosts (48.3% and 41.4%, respectively). Non-human mammals constitute a smaller fraction with only 10.4%.

The findings present that a substantial proportion of bloodmeals of both, *Cx. pipiens pipiens* and *Cx. pipiens molestus*, were not from birds, but from humans and non-human

mammals, challenging their common categorization as primarily ornithophagic and mammalo- or anthropophagic, respectively [116,118,215,216]. While the terminology is not standardized and these categorizations lack a common definition other than 'feeding often' on a specific host or host group, it is obvious, that *Cx. pipiens pipiens* and *Cx. pipiens molestus* do not represent opposite poles in host-feeding with their hybrids in between. The as ornithophilic described *Cx. pipiens pipiens* displays even the lowest proportion of avian feeds.

The wider host range highlights these species' potential as bridge vectors in the transmission of WNV, USUV and Sindbis virus, aligning with their known vector competence for these pathogens [217,218,243–245]. *Culex torrentium*, although represented in low numbers in this study, but likewise vector competent for these viruses [84,113,218], needs to be considered as potential bridge vector as well.

3.4.4. Identification of mixed bloodmeals

Mixed bloodmeals have been detected very seldom for these mosquito taxa. Previously, only two and one specimens of the *Cx. pipiens* biotypes *pipiens* and *molestus*, respectively, have been reported with blood from more than one host [116,223]. With our collections, we could add 41 mixed bloodmeals for *Cx. pipiens* bioform *pipiens* and one for *Cx. torrentium*. The majority of mixed bloodmeals of *Cx. pipiens pipiens* contained blood of a human and an avian host (85.4%), while also the combinations bird + non-human mammal (7.3%), human + non-human mammal (4.9%) and even bird + amphibia (2.4%) were detected. The *Cx. torrentium* specimen had fed on *Homo sapiens* and *Sus scrofa*.

The information about multiple hosts bitten by the same individual can provide valuable insights regarding transmission risks, however, it requires careful interpretation. Often, as also true for our study, bloodmeals are identified using gel PCR followed by Sanger sequencing [104]. The varying specificity of primers, however, could impact the sensitivity for different host taxa [104]. Furthermore, collections could potentially contain more mixed meals than detected. Different gene

fragments must be amplified and distinct signals observed, to identify more than one host. However, the signals of these different gene fragments could overlap, rendering it challenging to differentiate from signals with low quality. This is especially relevant for hosts from the same host group [201].

4. Conclusion

Mosquitoes as a major vector of pathogens were investigated with a focus on vector competence for European BATV on the one hand, and on host attraction and general host-feeding patterns through a literature-based meta-analysis and an empirical study for *Cx. pipiens* s.s. and *Cx. torrentium* on the other hand.

The number of collected specimens of the *Cx. pipiens* complex analysed in this thesis sums up to two thirds of the amount of those collected and identified to bioform level previously available in the literature. *Culex torrentium* may be relevant in pathogen transmission, as observed host-feeding patterns include the host groups human, avian and non-human mammalian without significant preferences in experimental setups, and as vector competence has been known for Sindbis virus, USUV and WNV, and is now also confirmed for BATV. This makes *Cx. torrentium* a good candidate for epizootic transmission across different host groups. However, information is still scarce due to little numbers of collected specimens and more collections for this species are needed.

The two bioforms *Cx. pipiens pipiens* and *Cx. pipiens molestus* did not differ in their host-feeding patterns as described in the literature, demonstrating that these patterns may be more variable and less pronounced. While 1st generation hybrids *pipiens* x *molestus*, linked to intermediate host-feeding behaviour, could be identified, little is known about backcrossing of the hybrids. This potential genetic mixing should be included in future considerations as possible contributions to the observed similarities in host-feeding patterns by further blurring the ecological distinction between the bioforms. Additionally, Next Generation Sequencing could improve the resolution of host-feeding pattern studies especially regarding the detection of multiple bloodmeals.

The collation of 333 host-feeding studies into a comprehensive database provides a valuable resource for advancing research in this field. While the data come from a wide

geographic range, their distribution is uneven, indicating the need for more research in underrepresented regions like Central Africa or South America. The database primarily offers insights into the feeding behaviour of the genera *Aedes*, *Anopheles* and *Culex*, with humans, non-human mammals and birds being the most frequent host groups. However, the collection methods may introduce biases against mosquito species specialized on other host groups. The host-feeding patterns of mosquito species often varied between different locations and are rarely uniform, suggesting that regional differences in host availability and abundance as well as variability between mosquito populations considerably influence host-feeding patterns. Due to this intricate interplay of factors influencing mosquito host-feeding patterns, the definition of 'phagia' remains complex, rendering it difficult to develop and apply predefined criteria and thresholds. The database is reliant on the given information and detail of each publication included. Ambiguous and changing taxonomy of both, mosquito and host species, impede comparability and aggravate cross-temporal and cross-spatial analyses.

The proposed data standard for reporting host-feeding studies represents a helpful step towards improving the comparability of the diverse datasets, addressing a significant gap in the research on vector host-feeding patterns. This proposed data standard is designed simple for convenient application. For deeper analysis of host-feeding patterns, also a thorough host census of every collection site would be necessary to include.

Finally, both, experimental studies and analyses of field-collected specimens, are crucial, offering complementary insights to better understand natural transmission cycles and infection risks.

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Appendix

Parasites & Vectors

BRIEF REPORT

Open Access

Evaluation of the vector competence for Batai virus of native *Culex* and exotic *Aedes* species in Central Europe

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Abstract

Background Batai virus (BATV) is a zoonotic arbovirus of veterinary importance. A high seroprevalence in cows, sheep and goats and infection in different mosquito species has been observed in Central Europe. Therefore, we studied indigenous as well as exotic species of the genera *Culex* and *Aedes* for BATV vector competence at different fluctuating temperature profiles.

Methods Field caught *Culex pipiens* biotype *pipiens*, *Culex torrentium*, *Aedes albopictus* and *Aedes japonicus japonicus* from Germany and *Aedes aegypti* laboratory colony were infected with BATV strain 53.3 using artificial blood meals. Engorged mosquitoes were kept under four (*Culex* species) or three (*Aedes* species) fluctuating temperature profiles (18 ± 5 °C, 21 ± 5 °C, 24 ± 5 °C, 27 ± 5 °C) at a humidity of 70% and a dark/light rhythm of 12:12 for 14 days. Transmission was measured by testing the saliva obtained by forced salivation assay for viable BATV particles. Infection rates were analysed by testing whole mosquitoes for BATV RNA by quantitative reverse transcription PCR.

Results No transmission was detected for *Ae. aegypti*, *Ae. albopictus* or *Ae. japonicus japonicus*. Infection was observed for *Cx. p. pipiens*, but only in the three conditions with the highest temperatures (21 ± 5 °C, 24 ± 5 °C, 27 ± 5 °C). In *Cx. torrentium* infection was measured at all tested temperatures with higher infection rates compared with *Cx. p. pipiens*. Transmission was only detected for *Cx. torrentium* exclusively at the highest temperature of 27 ± 5 °C.

Conclusions Within the tested mosquito species, only *Cx. torrentium* seems to be able to transmit BATV if the climatic conditions are feasible.

Keywords BATV, *Culex torrentium*, Vector competence, *Aedes albopictus*, *Aedes japonicus japonicus*, *Culex pipiens pipiens*

Background

Batai virus (BATV) [1, 2] belongs to the genus *Orthobunyavirus* within the family *Peribunyaviridae* [3]. Initially detected in *Culex gelidus* trapped in Malaysia in 1955, it has since been identified in southern Slovakia (referred to as Calovo virus, CVOV) [4, 5] as well as in various European countries (for a review, see [6]). Another variant, Chittoor virus (CHITV), has been found in *Anopheles barbirostris* in India [7].

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This zoonotic and especially veterinary important virus is transmitted by mosquitoes and biting midges, with mosquitoes considered as the most important vector group [8]. It affects a variety of vertebrate hosts, including pigs, horses, ruminants and various bird species. BATV has been detected in Africa, Europe and Asia. Human infections are rare and associated with mild flu-like symptoms. Infections of pigs, wild birds and harbour seals have been detected, and in ruminants severe outcomes such as abortions, premature births and genetic defects have been noted [1, 9].

The genomic structure of orthobunyaviruses is tripartite consisting of single-stranded RNA genomes [10]. This tripartite genome organisation leads to the appearance of reassortants, most frequently amongst co-circulating, genetically closely related strains [11].

Reassortments within the genus *Orthobunyavirus* may lead to viruses capable of inducing severe symptoms in humans [12]. Ngari virus, which carries the L- and S-segment of Bunyamwera orthobunyavirus and the M-segment of BATV, is associated with increased viral titres in infected mammalian cells as well as increased pathogenicity compared with the parental viruses [13, 14]. Ngari virus has been responsible for at least two outbreaks of haemorrhagic fever in humans in Central Africa between 1998 and 1999 [15, 16].

Surveillance studies conducted in Germany and Italy have confirmed the presence of antibodies against BATV in cattle, sheep and goats. Overall, these studies have demonstrated a seroprevalence up to 44% [17, 18]. However, in Europe, BATV-associated disease has not yet been reported in ruminants or humans. Notably, a BATV infection has been detected in a German captive harbour seal that exhibited encephalitis symptoms [9].

Furthermore, BATV has been repeatedly detected in Germany *Anopheles maculipennis* s.l., in Germany and twice in Italy [19–21]. Additionally, BATV has been identified in various other taxa, including *Culex pipiens* [22].

Recent laboratory studies with the Asian lineage of BATV showed that the mosquito species *Culex quinquefasciatus* as well as *Culex tritaeniorhynchus* are able to transmit the virus, whereas *Aedes aegypti* could only be infected [23]. British *Cx. pipiens* could also only be infected with BATV, while *Aedes detritus* was a competent vector under laboratory conditions [24].

Taken together, several mosquito species in Central Europe could potentially act as vectors for BATV. We recently showed that especially *Culex torrentium*, one of the three most frequent *Culex* species in Central Europe [*Cx. p. biotype pipiens* (*Cx. p. pipiens*), *Cx. p. biotype molestus* (*Culex p. molestus*) and *Cx. torrentium*] is a potent vector for arboviruses, e.g. West Nile virus (WNV) and Sindbis virus [25, 26]. In addition, the exotic species *Aedes albopictus* has infested more than 20 countries in Europe and is established along the Upper Rhine Valley in Germany and France and is known as a competent vector for chikungunya virus (CHIKV) and dengue virus (DENV) [27–30].

We assessed the vector competence of field-caught *Culex* species *Cx. p. pipiens* and *Cx. torrentium* as well as the invasive species *Ae. albopictus*, *Aedes japonicus japonicus* along with the laboratory colony of *Ae. aegypti* as a reference. Vector competence, in this context, refers the inherent ability of a mosquito to be infected and subsequently transmit the virus [31], confirmed by the presence of infectious viral particles in the mosquito's saliva. Additionally, we investigated the impact of varying temperatures on the risk of BATV transmission by these different mosquito species.

Methods

Culex egg rafts were collected in Hamburg, Neugraben-Fischbek, Germany (longitude 53.467821/latitude 9.831346) in 2018 and 2019. Egg rafts were individually reared at room temperature with a 12:12 light:dark photoperiod. Molecular identification as *Cx. p. pipiens* and *Cx. torrentium* was performed using DNA extraction of a pool of five L1/L2 larvae per egg raft (DNeasy blood & tissue kit, Qiagen, Hilden, Germany) in a multiplex quantitative real-time PCR (HotStarTaq master mix kit, Qiagen, Hilden, Germany) as described [32].

Aedes albopictus were reared from a laboratory colony originally collected from Heidelberg, Germany (F26-29) and *Ae. aegypti* were reared from a historic laboratory colony from the Bayer company (Leverkusen, Germany). *Ae. japonicus* were reared from eggs collected with ovitraps in southwestern Germany (longitude 8.671355/lattitude 49.523888) in summer 2019. All adult mosquitoes were reared at 26 °C, with a relative humidity of 70% and a 12:12 light:dark photoperiod with 30 min twilight.

Females (7–10 days old) were starved for 24 hours (*Aedes*) or 48 hours (*Culex*). The artificial blood feeding was conducted at 24 °C for 2 hours. The blood meal consisted of 50% human blood (expired blood preservation), 30% of an 8% fructose solution, 10% filtrated bovine serum (FBS) and 10% virus stock, and was fed using a cotton stick (*Culex*) or two 50 µl drops (*Aedes*) on the bottom of the vial as previously described [33]. The virus stock contained BATV of the European lineage [strain 53.2, Genbank numbers HQ455790 (S-segment), HQ455791 (M-segment) and HQ457992 (L-segment)] isolated from *An. maculipennis* s.l. collected in Southern Germany [19] at a final concentration of 10^7 plaque forming units per millilitre (PFU/mL). BATV stock was produced and quantified via TCID50 on Vero cells (*Chlorocebus sabaeus*; CVCL_0059, obtained from ATCC, cat.

no. CCL-81), results were converted in PFU/mL and the stock was diluted to reach a final concentration of 10^7 PFU/mL.

Only fully engorged females were used in the following experiments (ten females per vial). An 8% fructose solution was available via soaked cotton pads over the timeframe of the experiment. In general, mosquitoes were incubated for 14 days at 70% humidity and oscillating temperature profiles with mean temperatures of 18, 21, 24 and 27 °C and variations of ± 5 °C within 24 hours to mimic day–night temperature variations as previously described [29]. A diurnal temperature range of approximately 10 °C is commonly observed in the summer months in Germany [34]. The temperature maximum was reached in the middle of the light period, the temperature minimum in the middle of the dark period. Temperature profiles will be referred to by their mean temperature in the following text.

Culex mosquitoes were tested for all four mean temperatures in parallel. *Aedes* mosquitoes were tested at the highest mean temperature and at one lower temperature in parallel (21 °C for *Ae. aegypti*/*Ae. japonicus* and 24 °C for *Ae. albopictus*).

The salivation assay was performed at 14 days post infection (dpi) in alignment with previous studies [28, 29]. In summary, mosquitoes were anaesthetised using CO_2 to facilitate the removal of legs and wings. The proboscis was then placed into a 10 µL tip containing phosphate-buffered saline (PBS) and incubated for 30 min. To test for viable virus particles, each saliva/PBS mix was incubated on Vero cells seeded in a 96-well plate for 7 days. To confirm the presence of BATV RNA, supernatant of Vero cells showing cytopathic effect were prepared for additional RNA testing as recently described by Jansen et al. [29]. RNA was isolated using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany). BATV RNA was detected using the quantitative real-time RT–PCR (qRT–PCR) as previously described [19] using the primers BATAI-Fwd (5′-GCTGGAAGGTTACTGTATTTAATAC-3′) and BATAI-Rev (5′-CAAGGAATCCACTGAGTCTGTG-3′) and the probe BATAI-P (5′-FAM-AACAGTCCAGTTCCAGACGATGGTC-BHQ). A series of a synthetic BATV (1.15×10^3, 1.15×10^4 and 1.15×10^5 copies) standards spanning the qRT–PCR product with an additional 5′ GTA and 3′ ACG overhang (5′-GTAGCTGGAAGGTTACTGTATTTAATACCGTAACAGTCCAGTTCCAGACGATGGTCAGTCACAGACTCAGTGGATTCCTTGACG-3′) was used as a positive control and for quantification of RNA copies within the sample, the threshold for positive PCR results was 100 copies per mosquito.

Every mosquito excluding legs and wings was homogenised using a micro homogeniser (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in 500 µL Dulbecco's modified Eagle medium (DMEM) and RNA was isolated using the 5× MagMax Pathogen RNA/DNA kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) as indicated in the manual. BATV RNA was detected via qRT–PCR as mentioned above. The mean number of RNA copies per mosquito was determined per temperature and species (log10 BATV RNA copies/mosquito).

We determined the feeding rate (FR, the number of engorged mosquitoes per number of mosquitoes that were offered an infectious blood meal) infection rate (IR, number of viral RNA positive mosquitoes per number of engorged mosquitoes), transmission rate (TR, the number of mosquitoes with BATV positive saliva per number of viral RNA positive mosquito bodies), transmission efficiency (TE, the number of mosquitoes with BATV positive saliva/number of engorged mosquitoes) and survival rate (SR, number of surviving mosquitoes on day 14 per number of engorged mosquitoes).

Results

For *Ae. aegypti*, infection rates of 40% at 21 °C and 29% at 27 °C were detected, the mean number of copies ranged between 4.1 and 4.2 log10 RNA copies/mosquito. Transmission could not be detected (Table 1).

Aedes albopictus females were only infected at the two higher temperatures of 27 °C and 24 °C. A rather low infection rate of 3.3% was detected at 24 °C (Table 1). At 27 °C the infection rate was slightly higher with 11.7%. Mean numbers of RNA copies per mosquito ranged between 4.8 and 7.6 log10 RNA copies/mosquito. Transmission could not be detected at either of the investigated temperatures.

For *Ae. japonicus*, infection but no transmission could be shown at the tested temperature of 27 °C (IR of 50%) and 21 °C (IR of 86%) (Table 1). Mean number of RNA copies per mosquito ranged between 5.03 and 5.99 log10 RNA copies/mosquito.

Culex p. pipiens females could be infected with BATV after incubation at 21, 24 and 27 °C, with infection rates between 8.1% and 50% (Table 2).

For *Culex*, no specific temperature effect was detected for the three higher temperatures, while no infection could be detected at the lowest temperature of 18 ± 5 °C. Mean number of RNA copies per mosquito ranged between 5.1 and 6.0 log10 RNA copies/mosquito. Transmission could not be detected for *Cx. p. pipiens*. *Culex torrentium* showed infection at all temperatures, there was no hint towards a temperature dependency concerning the infection. Overall infection rates were higher compared with *Cx. p. pipiens*, with values between 22.6% and 93.3% (Table 2). *C.*

Table 1 Results of vector competence studies with BATV for tested *Aedes* species

Species	FR (%)	Temperature (°C)	*n*	IR (%) [*]	Mean number of RNA copies per mosquito(log10 BATV RNA copies/mosquito) (95% confidence interval)	TR (%) [**]	TE (%) [***]	SR (%)
Ae. aegypti	71	21 ± 5	35	40 [14/35]	4.1 [3.7–4.4]	0 [0/14]	0 [0/35]	85
		27 ± 5	35	29 [10/35]	4.2 [3.9–4.5]	0 [0/10]	0 [0/35]	87
Ae. albopictus	58	24 ± 5	60	3 [2/60]	7.6 [3.5–11.7]	0 [0/2]	0 [0/60]	85
		27 ± 5	60	12 [7/60]	4.8 [4.3–4.8]	0 [0/7]	0 [0/60]	N/A
Ae.japonicus	71	21 ± 5	7	86 [6/7]	5,99 [5.6–6.4]	0 [0/6]	0 [0/7]	N/A
		27 ± 5	6	50 [3/6]	5,03 [5–5.1]	0 [0/6]	0 [0/6]	N/A

Feeding rates (FR), infection rates (IR), mean number of RNA copies per mosquito, transmission rate (TR); transmission efficiency (TE) and survival rates (SR) of *Ae. aegypti*, *Ae. albopictus* and *Ae. japonicus* 14 days post infection (dpi) at different temperatures

FR: number of engorged mosquitoes per number of mosquitoes that were offered an infectious blood meal; IR: number of positive mosquitoes per number of engorged mosquitoes [*]; TR: number of mosquitoes with positive saliva per number of positive mosquitoes [**]; TE: number of mosquitoes with positive saliva/number of engorged mosquitoes [***]; SR: surviving mosquitoes on day 14 per number of engorged mosquitoes; N/A: not analysed (data missing); *n*: number of engorged mosquitoes

Table 2 Results of vector competence studies with BATV tested *Culex* species

Species	FR (%)	Temperature (°C)	*n*	IR (%) [*]	Mean number of RNA copies per mosquito(log10 BATV RNA copies/mosquito) (95% confidence interval)	TR (%) [**]	TE (%) [***]	SR (%)
Cx. pipiens biotype *pipiens*	46	18 ± 5	30	0 [0/30]	/	0 [0/0]	0 [0/30]	84
		21 ± 5	30	50 [15/30]	5.5 [4.6–6.4]	0 [0/15]	0 [0/30]	92
		24 ± 5	33	10 [3/30]	6.0 [5.3–6.6]	0 [0/3]	0 [0/30]	90
		27 ± 5	33	27.3 [9/30]	5.1 [4.8–5.5]	0 [0/9]	0 [0/30]	88
Cx. torrentium	54	18 ± 5	30	93.3 [28/30]	4.6 [4.3–4.9]	0 [0/28]	0 [0/30]	79
		21 ± 5	31	22.6 [7/31]	5.0 [4.4–5.5]	0 [0/31]	0 [0/30]	95
		24 ± 5	33	42.4 [14/33]	5.4 [4.8–5.9]	0 [0/14]	0 [0/30]	100
		27 ± 5	33	54.55 [18/33]	6.0 [4.5–7.1]	5.5 [1/18]	3 [1/33]	100

Feeding rates (FR), infection rates (IR), mean number of RNA copies per mosquito, transmission rate (TR), transmission efficiency (TE) and survival rates (SR) of *Cx. p. pipiens* and *Cx. torrentium* 14 days post infection (dpi) at different temperatures

FR: number of engorged mosquitoes per number of mosquitoes that were offered an infectious blood meal; IR: number of positive mosquitoes per number of engorged mosquitoes [*]; TR: number of mosquitoes with positive saliva per number of positive mosquitoes [**]; TE: number of mosquitoes with positive saliva/number of engorged mosquitoes [***]; SR: surviving mosquitoes on day 14 per number of engorged mosquitoes; N/A: not analysed (data missing); *n*: number of engorged mosquitoes

torrentium was also able to transmit the virus at the highest of the tested temperatures with a low transmission efficiency of 3%. At this temperature, mean number of RNA copies per mosquito reached the highest values of 6.0 log10 RNA copies/mosquito, in comparison with 4.6–5.4 log10 RNA copies/mosquito at the other temperatures (Table 2).

In addition, we measured the survival of *Cx. p. pipiens*, *Cx. torrentium*, *Ae. aegypti* and *Ae. albopictus* (only at 24 °C) at 14 days after infection. Independent of the incubation conditions or the tested mosquito species, survival rates never fell below 79%. For *Cx. torrentium* even survival rates of 100% were detected at the highest temperatures.

Discussion

The presence of BATV antibodies has been studied in Eastern Germany across various livestock species including sheep, goat and cattle [17, 18, 35]. These studies have revealed seroprevalences as high as 44.7%. Antibodies have also been detected in bovine serum samples from the Novarra region in Northern Italy in 2011 [36]. Furthermore, BATV RNA has been detected in aedine and culicine mosquitoes in Germany [19, 22] and in a pool of *An. maculipennis* s.l. mosquitoes in Italy [21]. These findings collectively suggest that the virus is circulating in Central Europe, particularly in regions such as Eastern Germany. Despite the absence of documented BATV infections in humans, a BATV infection has been

detected in Germany in a captured harbour seal showing symptoms of encephalitis [9].

However, no further documented BATV infections have been reported in humans or livestock in Central Europe. Despite this, it remains crucial to continue investigating BATV, as the overall risk of arbovirus transmission is on the rise.

In recent years, the risk of introduction and establishment of arboviral transmission cycles within Central Europe has grown. Notable examples are CHIKV epidemics in Italy and dengue virus (DENV) case clusters in Spain, France and Italy have been described [37, 38]. These outbreaks are attributed to factors such as the expanding distribution of the known CHIKV and DENV vector *Ae. albopictus*, as well as rising temperatures. Furthermore, there is also circulation of endemic viruses such as WNV. It emerged in Germany in 2018, and caused epidemics in Greece and Italy since 2010 [39, 40] with higher temperatures being one of the driving factors [25].

Specifically, BATV could pose a threat, parallels can be drawn from Cache Valley virus (CVV) another member of the Bunyamwera serogroup. In small ruminants, CVV infection may lead to foetal death or severe malformation of the foetus [41]. Cache Valley virus circulates in North, Central and South America and has been isolated from over 40 mosquito species [41]. Although human cases are rare, symptoms can range from mild illness with fever to severe cases of encephalitis. Notably, recent studies in the USA have revealed an increase in CVV infections. They showed that the invasive species *Ae. albopictus* transmits this virus and that *Ae. albopictus* is widespread in the area where CVV cases have been detected [42]. Based on these findings, we conducted tests on *Aedes* species, particularly the invasive ones, to assess their potential impact on the transmission of BATV in Central Europe.

Furthermore, reassortments within the Bunyamwera serogroup occur naturally. The most notable example is a reassortment event between BATV and Bunyamwera virus, resulting in the emergence of Ngari virus. Reassortment has led to an increase in pathogenicity, contributing to two major haemorrhagic fever outbreaks in humans in Africa [15]. Given their close genetic relationship, knowledge about competent vectors for BATV could inform risk assessments related to Ngari virus outbreaks.

As observed before, the laboratory colony of *Ae. aegypti* could be infected with BATV at both of the tested temperatures (27 °C, 21 °C), which were chosen to reflect a tropical and a more moderate temperature. No transmission could be detected, which is in line with previous studies [22]. For the other studied invasive *Aedes* species (*Ae. albopictus* and *Ae. japonicus*) similar results were observed at the two tested temperature, being 27 °C and 24 °C for *Ae. albopictus* and 27 °C and 21 °C for *Ae. japonicus* (infection but no transmission). None of the tested *Aedes* species were competent vectors. However, the sample size of *Ae. japonicus* was smaller than that of the other tested species. With six tested mosquitoes, the minimal detection limit is a TE of 16%, but TEs below this might already be biological relevant, therefore *Ae. japonicus* could still be competent vector here defined as the presence of viable virus particles within the saliva. The number of at least 30 investigated specimens per condition is well established in the field of vector competence studies and allows to determine TEs as low as 3%. Biologically relevant vector competence can be determined (TE > 3%), but the effort of the experiments is still proportionate.

Vector competence studies with *Ae. albopictus* and CVV already revealed that different lineages of CVV have a remarkable effect on transmission [42]. Although no transmission of BATV by *Ae. albopictus* was detected in this study, *Ae. albopictus* still could possibly contribute to the transmission of other strains of BATV if they would be introduced. Therefore, it would be of interest to test *Ae. albopictus* and other *Aedes* species for different BATV strains.

No obvious effect of BATV infection on survival could be seen for any of the tested species. This includes *Cx. torrentium* the only species that tested positive for BATV in the saliva in this study. This is in line with recently published results, where negative effects on survival could only be shown for *Ae. detritus*, but no changes in mortality could be observed for *Ae. aegypti* or *Cx. pipiens*. [24].

As BATV is transmitted by over 40 [41] different species from different genera, we included additional information regarding two specific species: *Cx. p. pipiens* and *Cx. torrentium*. These species are most abundant *Culex* species in Europe and previous research has demonstrated that these two serve as potential bridge vectors [43]. Recently, it has been shown for the Asian lineage of BATV, that *Cx. quinquefasciatus* as well as *Cx. tritaeniorhynchus* are competent vectors [23]. In contrast, it has been shown that a *Cx. pipiens* laboratory colony (hybrids from *Cx. p. pipiens* and *Cx. p. molestus*) was not able to transmit the European variant of BATV [24]. Our results for the field-caught *Cx. p. pipiens* are in line with the results obtained for *Cx. pipiens* [24], which were also not able to transmit BATV. However, at the highest temperature of 27 °C, *Cx. torrentium*, the more prevalent species in Central Europe [44] is able to transmit the virus, but only with a low transmission efficiency of 3%. Our data for *Cx. torrentium* show that highest copy numbers in mosquito bodies are reached at the highest temperature.

It has been described that at 20 °C, the extrinsic incubation period of BATV is at least not longer than

7 days in *Ae. detritus* and moreover this study showed that the transmission rate is higher at 7 days compared with 14 days post infection [24]. Therefore, it would be very important to further analyse whether this is also the case for *Cx. torrentium*. To be an effective vector in nature, vector capacity – rather than just vector competence – plays an important role. Vector capacity encompasses physiological, ecological and environmental factors related to the vector, host and pathogen. Key factors include blood-feeding behaviour, temperature and abundances [31]. However, currently neither *Cx. torrentium* nor *Ae. detritus* seems to be the relevant vector responsible for the high seroprevalence detected in several surveillance studies in Eastern Germany [17, 18]. *Ae. detritus* is a halophilic species predominantly distributed in coastal areas [45] and not in the regions described in the studies [17, 18] and *Cx. torrentium* only transmits BATV at high temperatures with a TE of only 3%.

Conclusion

Within this study, *Cx. torrentium* was found to be a potential vector for BATV at hightemperatures but with a low TE. To unravel the current infection cycle, more mosquito species need to be analysed for their vector competence if technically possible. BATV, for example, has been detected in Germany in a pool of *An. maculipennis* s.l. [19] and in pools of different mosquitoes also containing different *Anopheles* species as well as *Ae. vexans* [21]. Due to their host feeding patterns *Ae. vexans* are important vectors for the transmission from non-human mammals to humans [43]. Combined with the mass appearance of species upon flooding events, they could be an important vector and therefore would be an interesting species to test whether mosquitoes from the field are available. The same is true for *An. maculipennis* s.l.

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Author contributions
JSC, obtained funding. JSC, AH, SJ conceived the study. AH, SJ designed experiments. AH, SJ, MH, MW performed the experiments. NB, KK, RL, HJ sampled the mosquitoes. AH, SJ, RL analysed the data. SJ wrote the first draft of the manuscript. AH, RL, JSC revised the draft. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated by this study and used is presented within this published article.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

Title

Global database of mosquito host feeding patterns

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Abstract

Mosquito host feeding patterns are an important factor in shaping the mosquito's vector capacity. The interaction between vectors and blood hosts determines transmission cycles and risk of pathogen spill-over. Thus, the understanding of host feeding patterns is important to assess the risk for pathogen transmission to humans and animals, identify research priorities or target relevant vectors through control strategies. To investigate mosquito host feeding patterns, we conducted a systematic review collecting the data from 333 publications, covering a timeframe between 1942 and 2019. We included studies, which sampled engorged mosquito females and screened the bloodmeal for hosts using any serological or molecular methods. This standardized database with information on 609,243 blood meals of mosquitoes allow a wide range of in-depth analysis of the host feeding patterns of mosquitoes. Most frequently, taxa belonged to the genera *Anopheles*, *Culex* and *Aedes*. Thereby, nearly one third of all studies were conducted in the USA. Blood meals were predominantly identified with serological methods and a considerable increase in the number of identified host taxa was detected with the introduction of PCR-based analysis methods. Mammalian hosts dominate the dataset for *Anopheles* and *Aedes*, while avian hosts were predominantly identified for *Culex* mosquitoes. A total of 292 of the mosquito taxa (60%) fed on humans, making them potential vectors relevant for public health. In general, the host feeding patterns showed considerable spatial differences on the continental-scale and global scale also for mosquito taxa expected to have a distinct host feeding pattern. Following recently published criteria to classify the host feeding patterns of mosquitoes, only two mosquito taxa can be classified as anthropophagic, 12 taxa as ornithophagic, and 104 as non-human-mammalophagic. This comparative literature study helps to understand the interaction between mosquito and host species to further understand global transmission patterns of mosquito-borne pathogens.

Keywords

Mosquito host-feeding patterns, Meta-Analysis, Blood meals, Mosquitoes

Introduction

Mosquitoes serve as vectors for mosquito-borne pathogens such as the dengue virus, yellow fever virus, and malaria parasites, which can lead to infections in both, humans and animals [2, 3]. Only in 2021, 247 million cases of malaria were recorded with an estimated death toll of 619,000 [4]. Dengue virus accounts for approximately 390-400 million cases of infections and 21,000 deaths per year [5–7], while the estimated yearly numbers for the yellow fever virus lay by 200,000 infections and 30,000 fatal cases [8]. Besides, mosquito-borne pathogens result in economic loss, e.g., prevention and treatment costs for malaria alone reached US $ 4.3 billion in 2016 and a decrease of 1.3% in the yearly gross domestic product growth per person in countries with high malaria prevalence [9]. The cumulative economic costs of *Aedes* and *Aedes*-borne diseases has been estimated in more than US $ 300 billion worldwide [10]. In the course of climate warming a further increase of the population under risk of mosquito-borne pathogens must be expected [11, 12].

The transmission risk of pathogens by a certain mosquito species is determined by different environmental, behavioural and genetic components, which altogether shape the species-specific vector capacity, i.e., the ability of this vector to become infected and transmit a pathogen [13]. This includes e.g., vector competence, longevity, fecundity, pathogen replication (extrinsic incubation period) and population density. Another crucial element of the vector capacity is the host feeding pattern [14, 15]. For example, if a mosquito species predominantly feeds on a specific host species or host group (e.g., birds), the pathogen would be transmitted in a bird-to-bird enzootic cycle. Besides, mosquito species that feed more opportunistically can potentially serve as bridge vectors and can transmit pathogens between different host groups

causing spill-over events. Such a transmission cycle is for example described for the eastern equine encephalitis virus, which is transmitted between birds by the mosquito species *Culiseta melanura* classified as ornithophilic, while transmission from birds to mammals is assumed to be linked to more catholic feeders, i.e., bridge vectors, such as *Aedes vexans*, *Coquillettidia perturbans* and *Ae. sollicitans* [16–18].

Host feeding patterns depend on different intrinsic factors (like host preference, age, infection or nutritional stage) and extrinsic factors (e.g., vector density, host availability or host defensive behaviour) [19, 20]. This can lead to spatio-temporal heterogeneity and plasticity, e.g., seasonally or between different types of land use. While some mosquito species are considered specialists with a strong preference for a certain host species or host groups, e.g., *Ae. caspius* for non-human mammals or *Ae. aegypti* for humans [21, 22], other species are described as broadly feeding generalists, e.g., *Cx. annulirostris*, *Ae. vigilax* and *Ae. notoscriptus* [22].

For example, *Cx. nigripalpus*, which was found to feed on birds and mammals, humans and even reptiles, is considered an important enzootic and epizootic vector of St. Louis encephalitis virus and West Nile virus (WNV) in the United States [23–26]. In contrast, specialists feed on the same host species or host group, as *Ae. aegypti* that predominantly feeds on human hosts being the main vector for dengue, Zika and chikungunya virus [27–32].

The contact probability between vectors and hosts is commonly analysed as host feeding patterns, which is essential to identify research priorities (e.g., vector competence studies), target relevant vectors through control campaigns or protect susceptible hosts more efficiently. There have been many studies on mosquito host feeding patterns all around the world. However, single studies are always only a reference to a specific time and location probably resulting in a biased information of the host feeding patterns. This refers to the wider geographical area, in which mosquitoes are collected, as well as to the local sampling site characterized by different habitats and hosts. Besides, the experimental design of the studies such as the host identification methods could influence the results. To obtain a comprehensive global understanding of

mosquito host feeding patterns, we conducted a systematic bibliographic study analysing the findings of 333 field studies conducted globally between 1942 and 2019 into a single database. The full data, including mosquito taxa, detected host taxa, sampling date, sampling location, method for mosquito collection and host identification are provided open access. This database enables a systematic analysis of the existing knowledge and the identification of potential gaps in our understanding of mosquito host feeding.

Methodology

PRISMA search

On November 18, 2020, the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) was systematically searched for publications on mosquito host feeding patterns using the following strategy: '(Mosquito*[Title] OR Culici*[Title] OR Aedes[Title] OR Culex[Title] OR Anoph*[Title] OR "west nile virus"[Title]) AND (Blood*[Title] OR meal*[Title] OR feed*[Title] OR host*[Title] OR preference*[Title] OR pattern*[Title] OR forage*[Title])'. The publications were independently screened by two researchers (MLW, MJTG) for suitability by title, abstract and full text (Fig. 1), based on the following inclusion criteria. 1) Studies were conducted in the field. 2) If a vertebrate bait was used (e.g., animals or humans as for window traps), the data were only included if the trapped mosquitoes either had no direct contact with the host (e.g. Furvela tent trap [33, 34]) or were immediately collected before biting of the bait was possible. 3) Ingested blood must have been analysed with a serological or molecular biological method. The exclusion criteria were 1) studies that determined the host only by behaviour observation, 2) that were based on laboratory reared mosquitoes, or 3) feeding experiments conducted in the lab. Additional papers mentioned in references or other sources were included if the criteria were matched.

Data collection and standardisation

If two or more studies were entirely or partly not explicitly identifiable as independent studies, only the publication with the higher number of host groups was included in the analysis. Studies without information on the year of collection were excluded for temporal analyses (indicated by column 'time_inclusion') but included for general information on host feeding patterns (indicated by column 'taxa_inclusion'). Studies without spatial information were excluded for spatial analyses (indicated by column 'site_inclusion'). Non-combinable information on precise locations, more exact collection dates or more detailed host breakdown provided by a single study were included separately and used only for the respective analysis, indicated by the columns 'site_inclusion', 'time_inclusion' and 'taxa_inclusion'. Separate decisions were made for each mosquito species if a different detail of information was given for different taxa.

All possible information given on mosquito and detected host taxa, date, country, method of mosquito collection and host identification method were collected and merged into a single database (Supplementary Table S2). Mosquito taxa were standardized using the most updated taxonomy (https://mosquito-taxonomic-inventory.myspecies.info/ and https://wrbu.si.edu/). Taxa currently not listed as valid species or assignable to a valid species were excluded from the analysis: *Anopheles altropos, Anopheles hispaniola*, *Aedes queenslandis*, *Culex culiciformis*, *Anopheles vexans*, *An. n. ovengiensis*, *Culex fuscanus*, *Aedes pseudomediofasciatus*, *Culiseta kanayamensis*, *F. splendens*, *Culex fusco*, *Culex fuscanus* and *Anopheles indiensis*. Although some information was lost by this method, mosquito taxa were standardized on the species level, e.g., Culex pipiens was used for studies reporting *Culex pipiens* sensu lato, *Culex pipiens* biotype pipiens or *Culex pipiens* biotype molestus, and *Anopheles gambiae* includes *Anopheles gambiae* sensu lato, *Anopheles* sensu stricto, *Anopheles gambiae* complex, *Anopheles gambiae* group, *Anopheles gambiae* species A and B, and *Anopheles gambiae* without further specification. However, the reclassification information allows another standardisation for future research.

Scientific host names were standardized referring to NCBI taxonomy and derived from common names if not otherwise provided. Mixed blood meals for mosquito specimens were split in individual rows per host taxon. Thus, in the following all data refers to detected blood meals instead of mosquito specimens. Blood meal hosts were further categorized into the host groups avian, reptilian, amphibian, fish, annelid, human and non-human mammalian. The artificial distinction between the latter was created to analyse the feeding on humans to identify the risk for public health. Also, we used the group mammalian for blood meals without differentiation between human and non-human mammalian. Information on traps and blood meal identification method were reclassified to allow comparisons between the different studies. Corresponding reclassification tables allow another standardisation for future research. If only a sampling period covering several years was indicated the mean of the sampling years was calculated for temporal analysis.

Data analysis

The dataset was used to classify the host feeding patterns of different mosquito taxa as suggested by Fikrig & Harrington [1]. In their publication, the authors propose to use the term 'phagic' for a host taxa or host group, if at least 3 studies confirm this mosquito taxa feeds in >33.3% on this host or host group. Furthermore, we interpreted their hint to be cautious with the terminology if more than 2 studies did not replicate this finding as sharp exclusion criteria to classify the taxa as anthropophagic, ornithophagic, non-human-mammalophagic, reptilophagic or amphibiophagic.

All data analysis and visualization were conducted with the program R [35] using the packages tidyr [36], tidyverse [37], readxl [38], dplyr [39], ggplot2 [40], stringr [41], ggpubr [42], rworldmap [43], rgeos [44], scatterpie [45], plyr [46].

Results

Overview of the literature

The database comprises 333 publications (Supplementary Table S1), of which between 310 and 332 were included in the further presented analyses to avoid overlapping reporting of mosquito blood meals and double counting for the different spatial or temporal analysis (see methods). The analysed studies encompass 609,243 identified blood meals. Each entry in the database represents the number of detected blood meals per study, study year, method of mosquito collection, host identification method, mosquito taxa, host taxa and host group. The studies were performed between 1942 and 2019 in 89 individually distinguishable countries on all continents except for Antarctica (Fig. 2, Fig. 3, Supplementary Fig. S1). By far the most studies were conducted for North America (135 studies, 39.9%; 153,747 blood meals, 27.8%) and especially in the United States of America (111 studies, 28.8%; 130,169 blood meals, 23.5%). Asia is represented with the second most studies (67, 19.8%) and the highest number of reported blood meals over all continents (193,177 blood meals, 34.9%) predominantly conducted in India (29 studies, 7.5%; 89,162 blood meals, 16.1%). A similar number of 66 studies (19.5%) were found for Africa (124,080 blood meals, 22.4%) predominantly focussing on Kenya (22 studies, 5.7%; 40,380 blood meals, 7.3%) (Fig. 3 and 4). Considerably less studies were conducted in South America, Oceania, and Europe with fewer than 30 studies and 38,000 identified blood meals each.

Identification methods for blood-meals

Host detection for mosquito host meals were conducted with a variety of methods with an increasing number of publications per year (Fig. 5A). In the 1940s, this research field of vector ecology started with different serological techniques. Since the end of the 1990s, PCRs are also used in blood meal identification (Fig. 5A). The number of different identified host taxa per study using PCRs increased, although the number of analysed blood meals per study decreased (Fig. 5B, Fig. 5C, Fig. 5D). Studies with several thousand specimens generally used serological

methods, with a median of different identified host taxa of 6.0 for a median of 946.5 identified blood meals. Since the beginning of the 21st century, predominantly PCR-based methods are used, with a median of different identified host taxa of 11.0 for a median of 227.0 identified blood meals (Fig. 5). Thus, studies with PCR-based methods identified almost 7.3 times more host taxa as studies with serological methods (0.059 vs. 0.008 detected host taxa per identified blood meal). Most blood meal hosts were identified using serological methods (477,933; 85.8%). However, we observed differences between the continents (Fig. 2). Studies in North America (76; 55.1%) used PCR-based methods in a larger proportion, just as for Europe (22; 75.9%) and South America (12; 50.0%), where these methods already made up more than half of the identified blood meals per continent. PCR was used relatively less in Asia (21; 31.3%) and Africa (20; 30.3%).

Mosquito genera tested for host feeding patterns

The database comprises 494 mosquito taxa of 25 genera. The five most frequently reported taxa belonging to the genera *Culex* (194; 29.3%), *Anopheles* (159; 24.1%) *Aedes* (148; 22.4%), *Mansonia* (31; 4.7%) and *Psorophora* (22; 3.3%) (Fig. 6A). This correlates with the number of analysed specimens for *Anopheles* (270,133; 48.9%) and *Culex* (206,615; 37.4%) (Fig. 6B). In contrast, although many papers presented results for *Aedes*, considerably less blood meals were identified for this genus (45,965; 8.3%). Less blood meals were identified for the genera *Mansonia* (7,519; 1.4%) and *Psorophora* (5,366; 1.0%) (Fig. 6). For most continents, blood meals from the genus *Anopheles* represented more of the 50% of all blood meals. In contrast, *Culex* dominated for North America (64.0%) or Oceania (52.4%). *Aedes* blood meals were predominantly identified for Europe, North America and Oceania, but also only between 13% and 19% of all blood meals per continent.

Host composition

The database comprises 890 host taxa. For the different host groups, non-human mammalian hosts were most frequently detected (321,123; 57.6%) followed by birds (109,930; 19.7%) and humans (91,874; 16.5%). In addition, for 26,837 (4.8%) blood meals, human and non-human mammalian were reported combined under the term mammalian. The host groups reptilian (4,961), amphibian (1,613), amphibian or reptilian (649), fish (240) and annelid (72) were each detected within less than 1% of all blood meals. A total of 292 mosquito taxa fed on human (59.1% of all mosquito taxa) and a similar amount on birds (295; 59.7%), while non-human mammalian taxa were reported for 412 (83.4 %) mosquito taxa. Blood meals on the host groups reptilian (103; 20.9%) and amphibian (81; 16.4%) were detected less frequently. Only a single mosquito species fed on fish (*Ae. baisasi*) or annelids (*Uranotaenia sapphirina*), respectively. The highest diversity of host taxa (703; 79.0% of all host taxa) were observed for the genus *Culex* with a huge number of bird taxa (455 taxa) (Fig. 7). Considerably less host taxa were observed for the other four most frequent mosquito genera (50-258 taxa), which all were dominated by non-human mammalian taxa (> 50% for all host taxa), while avian taxa were less frequent (< 35% of all host taxa).

The list of the 10 most frequent detect host taxa was dominated by mammalian taxa (Fig. 8). It was headed by the three most-frequent non-human mammalian taxa, i.e. Bovinae (103,757; 19.8%), *Bos taurus* (57,592; 11.0%), Bovidae (43,760; 8.4%), together with humans (91,874; 17.6%), which jointly comprised the majority (56.8%) of all detect blood meals. The blood meals from birds were dominated by Passeriformes (23,831; 21.7%), Galliformes (19,800; 18.0%), Aves (18,869; 17.2%) and *Gallus gallus* (12,905; 11.7%). Blood meals from humans were mostly detected for *Anopheles* (61,194; 66.6%), followed by *Culex* (20,029; 21.8%) and *Aedes* (9,187; 10.0%). A similar pattern was observed for non-human mammalian hosts: *Anopheles* (170,220; 53.0%), *Culex* (101,929; 31.7%) and *Aedes* (32,491; 10.1%). Avian hosts were predominantly reported for the genus *Culex* (78,128; 71.1%), except Galliformes with a high proportion of blood meals detected for *Anopheles darlingi* (Fig. 8, Fig. 9).

Most frequently analysed mosquito taxa

Culex quinquefasciatus is the most frequently analysed mosquito taxon and leads the list with 57,966 (10.4%) mosquito specimens, followed by *Anopheles gambiae* and *Cx. tritaeniorhynchus* with 36,647 (6.6%) and 34,194 (6.1%) blood meals, respectively. Frequently analysed taxa in the genus *Culex* in addition included *Cx. tarsalis* (24,306; 4.4%), *Cx. pipiens* (21,618, 3.9%) and *Cx. nigripalpus* (14,898; 2.7%). For *Anopheles*, the top 10 list further included *An. sacharovi* (29,253; 5.3%), *An. culicifacies* (28,065; 5.0%), *An. darlingi* (23,422; 4.2%) and *An. funestus* (16,630; 3.0%). Taxa of the genus *Aedes* were not under the top 10. 151 taxa (30.6 %) have been analysed only in the single-digit range. Six of the ten most frequently analysed mosquito taxa fed almost exclusively on mammals, with *An. gambiae* and *An. funestus* having more than 50% of the blood meals on humans, and the remaining blood meals on non-human mammals or not further separated mammals (Fig. 9). The majority of blood meals of *Cx. tritaeniorhynchus*, *An. sacharovi* and *An. culicifacies* belonged to non-human mammals (> 90%). *Culex quinquefasciatus*, *Cx. tarsalis, An. darlingi and Cx. nigripalpus* showed a high proportion of birds exceeding 50%. *Culex pipiens* presented one third of the blood meals from each human, avian and non-human mammalian hosts. The host groups amphibia and reptiles were only reported in very small numbers for these ten species (< 0.01%).

The host feeding patterns of the different mosquito taxa showed significant spatial variability (Supplementary Fig. S2). For instance, in the case of *Cx. quinquefasciatus,* the species with the most frequently observed blood meals over all mosquito taxa, there were countries with almost entirely non-human mammalian, avian or human blood meals, while in others a combination of these host groups was observed (Fig. 10). Another example is *An. gambiae*, for which almost exclusively mammalian hosts were detected (Fig. 9). In certain countries, the majority of all blood meals originated from humans (e.g. DR Congo or Benin), while in other countries, the

283 blood meals were predominantly from non-human mammals (e.g. South Africa or Madagascar)
284 (Fig. 11).
285
286 Classification of host-feeding patterns
287 When applying the criteria for the classification of host feeding patterns suggested by Fikrig &
288 Harrington [1], only two mosquito taxa (*An. strodei* and *Ae. aegypti*) can be classified as
289 anthropophagic (0.4% of all mosquito taxa) (Fig. 12). A total of 12 taxa are classified as
290 ornithophagic (2.4%), covering the genera *Culex* (5; 1.0%), *Coquillettidia* (5; 1.0%), and
291 *Culiseta* (2; 0.4%). A total of 104 mosquito taxa (21.1%) can be classified as non-human-
292 mammalophagic, dominated by taxa of the genus *Anopheles* (39; 7.9%) and *Aedes* (36; 7.3%),
293 *Culex* (15; 3.0%), *Psorophora* (5; 1.0%), *Coquillettidia* (4; 0.8%), *Mansonia* (3; 0.6%) and
294 *Culiseta* (2; 0.4%). Besides anthropophagic, *An. strodei* was also classified as non-human-
295 mammalophagic. Only one (*Ur. unguiculata*, 0.2%) and two (*Cx. hortensis*, *Cx. peccator*,
296 0.4%) mosquito taxa can be termed amphibiophagic and reptilophagic, respectively.
297
298 **Discussion**
299 With this database, we unite 333 studies on mosquito host-feeding, covering 494 mosquito taxa
300 and 890 recorded host taxa for at all continents except Antarctica. It is not surprising that the
301 genera *Culex*, *Anopheles* and *Aedes* were studied most frequently, as they are the most
302 widespread and abundant in the world, including important vectors of globally relevant
303 pathogens, as malaria parasites, dengue, Zika or West Nile viruses. *Culex* mosquitoes, in
304 particular, have been extensively studied in the USA, which may be related to the high incidence
305 of West Nile virus [47] predominantly transmitted by mosquitoes of this genus [48, 49]. A
306 concentration of investigations on *Anopheles* mosquitoes for Africa was not observed, although
307 species of the genus are vectors of many mosquito-borne pathogens endemic to this continent
308 and especially malaria parasites [4, 50–52]. The observed hosts were dominated by mammals,

while Bovidae and humans made up for more than 50% of all detected blood meals. This might reflect that the biomass of humans and livestock is many times greater than for wild animals [53].

Studies on mosquito host feeding patterns were conducted nearly all over the world. Nevertheless, we observed larger gaps in Africa with several countries without any individually localizable data (e.g. Algeria, Niger or Chad). This is especially important as the continent poses the origin for worldwide spread and circulation of mosquito-borne pathogens such as malaria parasites or Zika virus [4, 54]. In addition, we observed a disproportionate spatial distribution of reported blood meals. The 111 studies conducted in the USA (28.9% of all studies) made up almost 25% of all reported blood meals. Thus, general host feeding patterns for mosquito taxa might be biased, as these patterns vary across different regions due to local genetic (e.g., intraspecific variation between different mosquito populations) or environmental (e.g., host availability) factors. For example, the globally distributed mosquito species *Cx. quinquefasciatus*, for which blood meals from human, non-human mammalian and birds were recorded. However, again, the host feeding patterns vary strongly between the different countries. While single studies concluded an anthropophilic behaviour of *Cx. quinquefasciatus* [55, 56], other studies [57, 58] and the here presented meta-analysis indicate a much broader host feeding pattern covering human, non-human mammalian and avian taxa. Therefore, it has to be kept in mind that observed host feeding patterns are study and site specific, and often are more variable and less specific than described in the literature. Therefore, at a local level, is important to characterise the host preferences considering host availability through census data together with blood meal analysis, by calculating indices as the Forest Ratio [59, 60].

This is also observed when criteria for a standardized terminology for host feeding patterns are applied for the here presented database. Such standardized terminology can help in the simplified communication about the host feeding patterns of different vector species, as done with the criteria suggested by Fikrig & Harrington [1]. Following these criteria, only the two

335 species *An. strodei* and *Ae. aegypti* are anthropophagic. *Aedes aegypti* is widely accepted as
336 anthropophagic [27, 30, 61]. The host feeding of *An. strodei* instead is only covered in four
337 publications with a total of 23 specimens. At the same time, *An. strodei* also falls under the term
338 non-human mammalophagic, which is with 99 mosquito taxa of seven different genera the most
339 frequent class. Ornithophagy instead was limited to twelve mosquito taxa of the genera
340 *Coquillettidia*, *Culex* and *Culiseta*. Mosquito species classically known to be avian-specific like
341 *Cs. melanura* and *Cx. modestus* are included as well as species with few entries in literature,
342 e.g. *Cq. xanthogaster* [62, 63]. Only one respectively two mosquito species can be termed
343 amphibiophagic (*Uranotaenia unguiculata*) and reptilophagic (*Cx. hortensis* and *Cx. peccator*).
344 This is in accordance with rare literature descriptions, where *Ur. unguiculata* is associated with
345 amphibian hosts [64, 65], and *Cx. hortensis* and *Cx. peccator* are referred to as feeding on
346 ectotherm hosts [66–68]. However, this is probably biased as only few publications and
347 specimens are available for these species and host groups. One reason is that most collection
348 methods rely on CO_2-baited traps, while mosquitoes specialized on amphibia might be rather
349 attracted by other cues, e.g. frog calls [69, 70]. Despite many papers presented results for *Aedes*,
350 considerably fewer blood meals were identified. This can be due, in the case of the daily biting
351 species *Ae. aegypti* and *Ae. albopictus,* to the multiple, interrupted feeding behaviour, that
352 evolved as part of their avoidance behaviour to human host defensive behaviour [71] resulting
353 in more incomplete bloodmeals that are below the detection threshold. In general, the field
354 campaigns for sampling blood-fed females of these species might be more difficult than for
355 others [1].
356 Regarding the criteria for the classification of host feeding patterns of mosquitoes suggested by
357 Fikrig & Harrington [1], we want to draw attention to some critical aspects, which need further
358 discussion and adjustment. First, we propose to take the number of mosquito specimens per
359 study into account, as it seems imbalanced to give studies with very few specimens the same
360 weight as studies with several thousand specimens. Secondly, the maximum number of studies

not fulfilling the minimum criterion of 33.3% of feeds oppose the classification of the phagy should be adjusted relative to the total number of studies per mosquito species, as a fixed threshold of two can represent a very different proportion depending on the number of studies. The method used for the identification of the blood meal host has a considerable impact on the range of identified taxa. The methods applied changed over time, with PCR and sequencing methods largely replaced serology-based procedures. Serological methods can only detect and confirm already expected hosts, i.e. most are based on pre-prepared antisera for an expected range of potential taxa. In many cases, this encompasses few specific host taxa such as cow, pig, human, chicken or dog. Most identified hosts are only reported in subordinate groups, such as families or orders. Additionally, serological methods show cross-reactivity between host taxa and are sometimes unspecific [72, 73]. This way, the resolution and thus the diversity of the hosts is lost in the detection and in the reporting of such. In comparison, sequencing of amplified DNA-sequences (e.g. COI or 16S) of the host blood instead allows the detection of any host without any pre-selection, as long as a corresponding sequence is available in the corresponding sequence databases. This advantage is reflected in a higher number of identified hosts per successful analysed blood meal compared to serological methods. The majority of reported blood meal hosts was analysed serologically, which biases the knowledge on the host feeding patterns for the different mosquito taxa. This is especially true for Asia, Africa and Oceania and might be linked to the laboratory capacities in low- or middle-income countries. In contrast, studies from North America, Europe and South America already used PCR-based methods in a larger proportion, just as in Europe and South America. In the future, meta-barcoding approaches using next Generation Sequencing could be implemented for identification of blood meal hosts [74]. This has the advantage that several fragments of different genes (e.g. COI and 16S) can be detected simultaneously and mixed blood-meals from different hosts can be differentiated [75].

It is in the nature of bibliographic research, that the results depend on the level of detail of methods and results that are presented in previous studies published. For example, some studies only communicated the blood meals for specific hosts of interest, while other detected hosts were not reported at all. In addition, the standardization of the information extracted from the studies posed challenges of various aspects. Mosquito taxonomy itself is often unclear and quickly changing especially referring to historic literature [76], and thus might differ between studies from different publication years. Especially due to the rise of molecular tools for the identification of mosquitoes, new species are discovered regularly, which might have been included under a different species name in the previous studies [77]. Furthermore, repeatedly mosquito taxa, which are also part of a species complex with the same name, are reported without further specifying details like e.g. 'sensu lato', 'sensu stricto', 'complex' or 'group'. It is therefore often unclear to which level the mosquito species has been identified and whether the species complex or a specific species is meant. This is further complicated by the fact that the species complex membership of different taxa also changes over time [78]. This could make a difference for the host feeding patterns, e.g. even the two biotypes of *Cx. pipiens* s.s. could already display differing host feeding patterns [79], which might mix up in the joint analysis of different studies. The standardization of the blood meal host species is based on the scientific name, which poses similar problems as for the mosquitoes. In addition, often only common names were referred to, such as cow or chicken, which do not allow linking to a specific scientific taxon, e.g. duck, deer, wapiti or kangaroo, and have to be assigned to the higher taxonomic orders.

The created database gives the foundation for an open catalogue to which further studies can be added in the future, expanding the knowledge on mosquito host feeding patterns. However, during the process it became evident, that comparable methods and detail on information are needed for comparison of the results and to draw conclusions about the driving factors of

mosquito host feeding patterns, which can help to understand transmission cycles of mosquito-borne pathogens and thus allow research priorities or targeted control strategies.

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Declarations of Competing Interest

None

Data availability

All data are available in the supplementary files.

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Fig. 1: PRISMA flow diagram. Studies included in the dataset are eligible by meeting the criteria but were only included in the analysis when there was no overlap between them.

Fig. 2: Number of publications (A) and detected mosquito blood meals (B) per continent with fill colour indicating the host identification method.

Fig. 3: Map on the number of detected mosquito blood meals per country.

Fig. 4: Top 10 countries with most frequently reported mosquito blood meals with fill colour indicating the mosquito genus.

Fig. 5: Number of publications (A), tested specimens per publication (B), identified host taxa per publication (C) and identified host taxa per analysed blood meal per publication (D) with point colour indicating the host identification method.

Fig. 6: Number of publications (A) and detected mosquito blood meals (B) per mosquito genus with fill colour indicating the continent.

Fig. 7: Number of detected host taxa per mosquito genus with fill colour indicating the host group.

Fig. 8: Top 10 most frequently reported host taxa over all host groups (A), host group human (B), host group avian (C) and host group non-human mammalian (D) with fill colour indicating the mosquito genus.

Fig. 9: Top 10 most frequently analysed mosquito species with fill colour indicating the proportion of the host group.

Fig. 10: Proportion of the host groups for *Culex quinquefasciatus.*

Fig. 11: Proportion of the host groups for *Anopheles gambiae.*

Fig. 12: Number of studies reporting equal or larger 33.3% blood-meals per mosquito taxon per host group plotted against the number of studies reporting less than 33.3% blood-meals per mosquito species for the host groups human (A), avian (B) and non-human mammalian

(C) with point colour indicating the mosquito genus. A jitter was added to the points to support distinguishability of taxa. The red rectangle (+1 to improve visualisation) indicates the area where species fulfil the criteria to classify host-feeding patterns by (Fikrig & Harrington, 2021).

Supplementary Table. S1: References of the 333 articles used for the analyses

Supplementary Table. S2: Structured database of hosts identified in most host feeding studies

Supplementary Fig. S1: Map on the number of publications per country.

Supplementary Fig. S2: Percentage of the host groups human, avian and non-human mammalian per country for the top 10 most frequently analysed mosquito taxa.

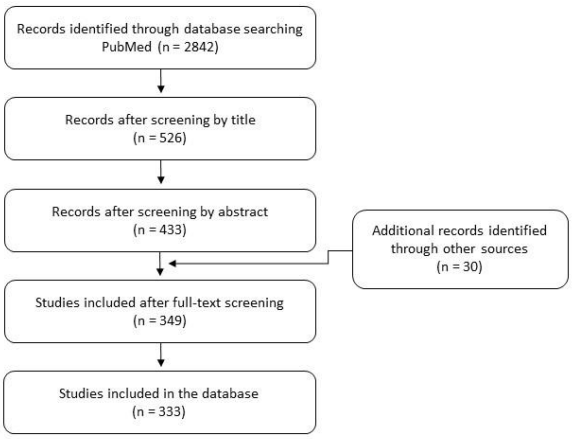


Fig. 1: PRISMA flow diagram. Studies included in the dataset are eligible by meeting the criteria but were only included in the analysis when there was no overlap between them.

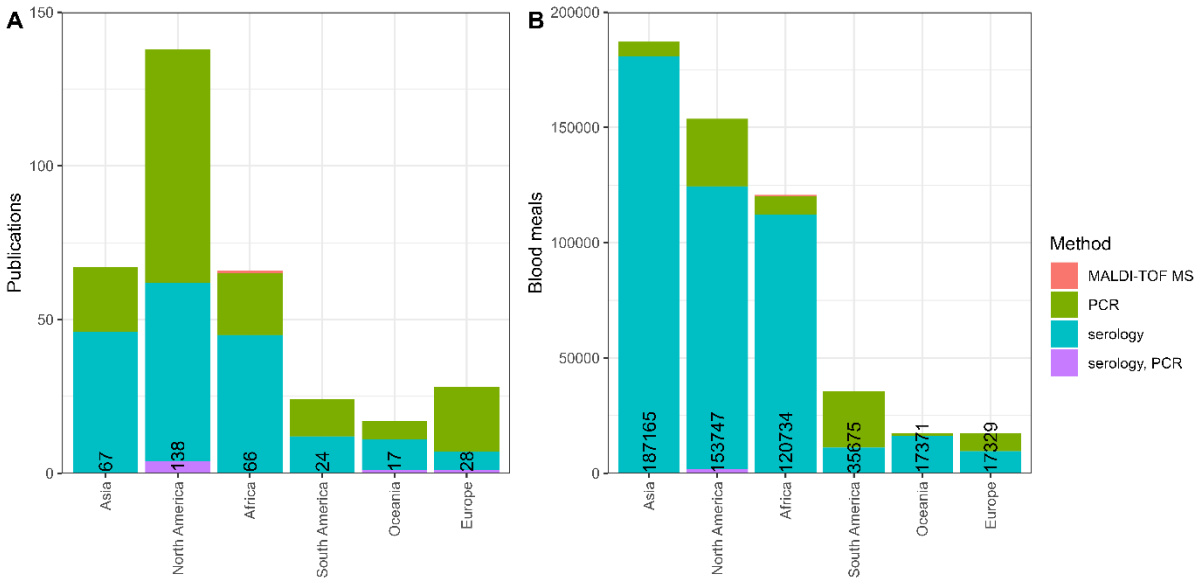


Fig. 2: Number of publications (A) and detected mosquito blood meals (B) per continent with fill colour indicating the host identification method.

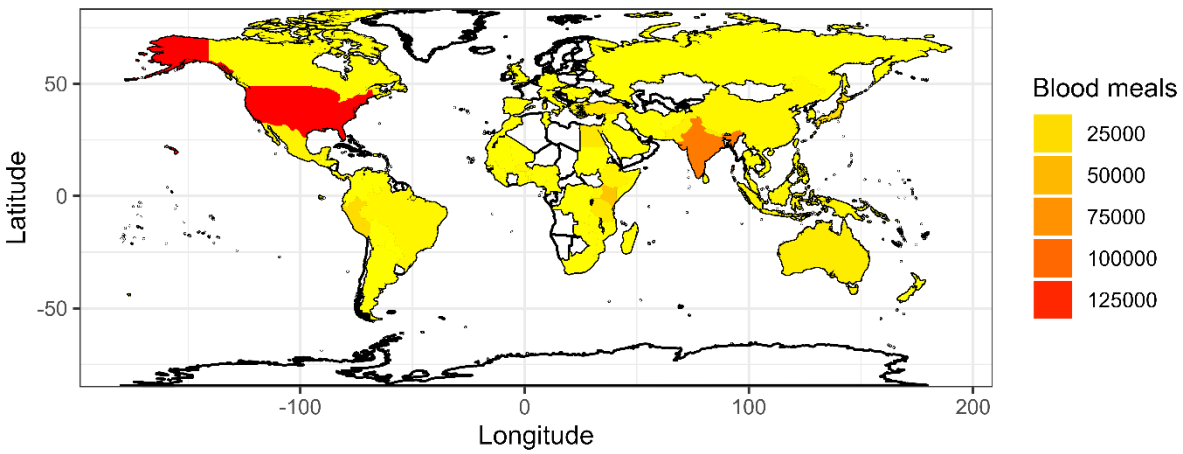


Fig. 3: Map on the number of detected mosquito blood meals per country.

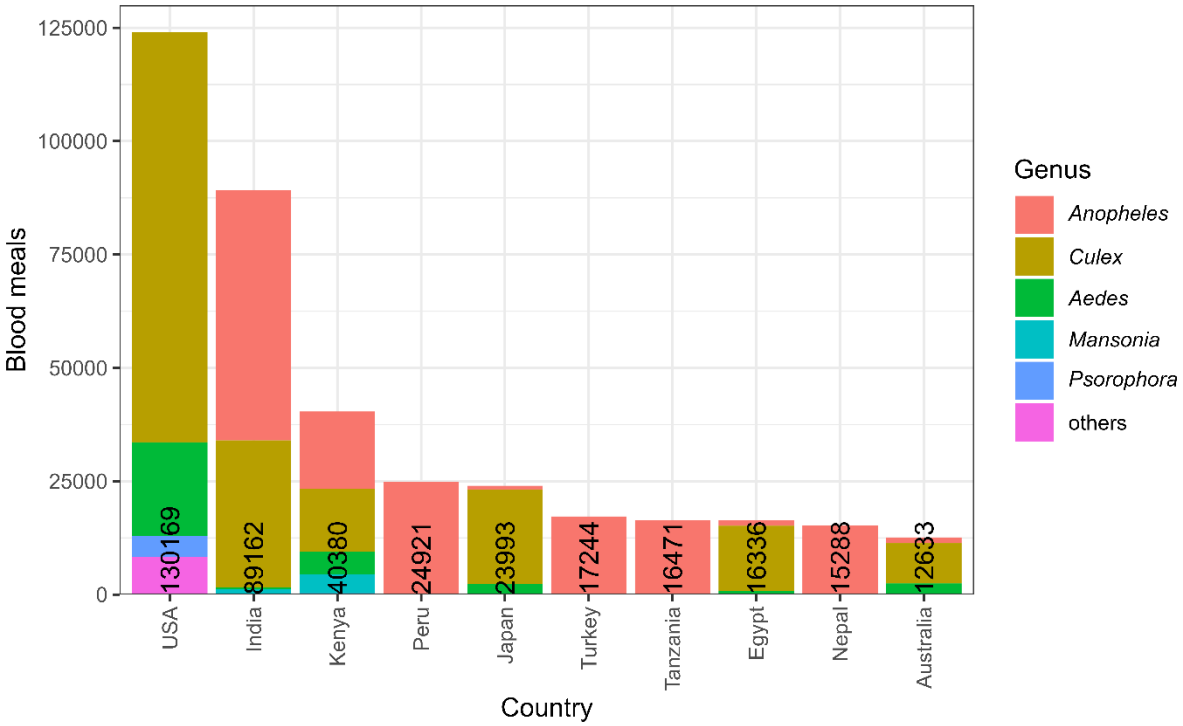


Fig. 4: Top 10 countries with most frequently reported mosquito blood meals with fill colour indicating the mosquito genus.

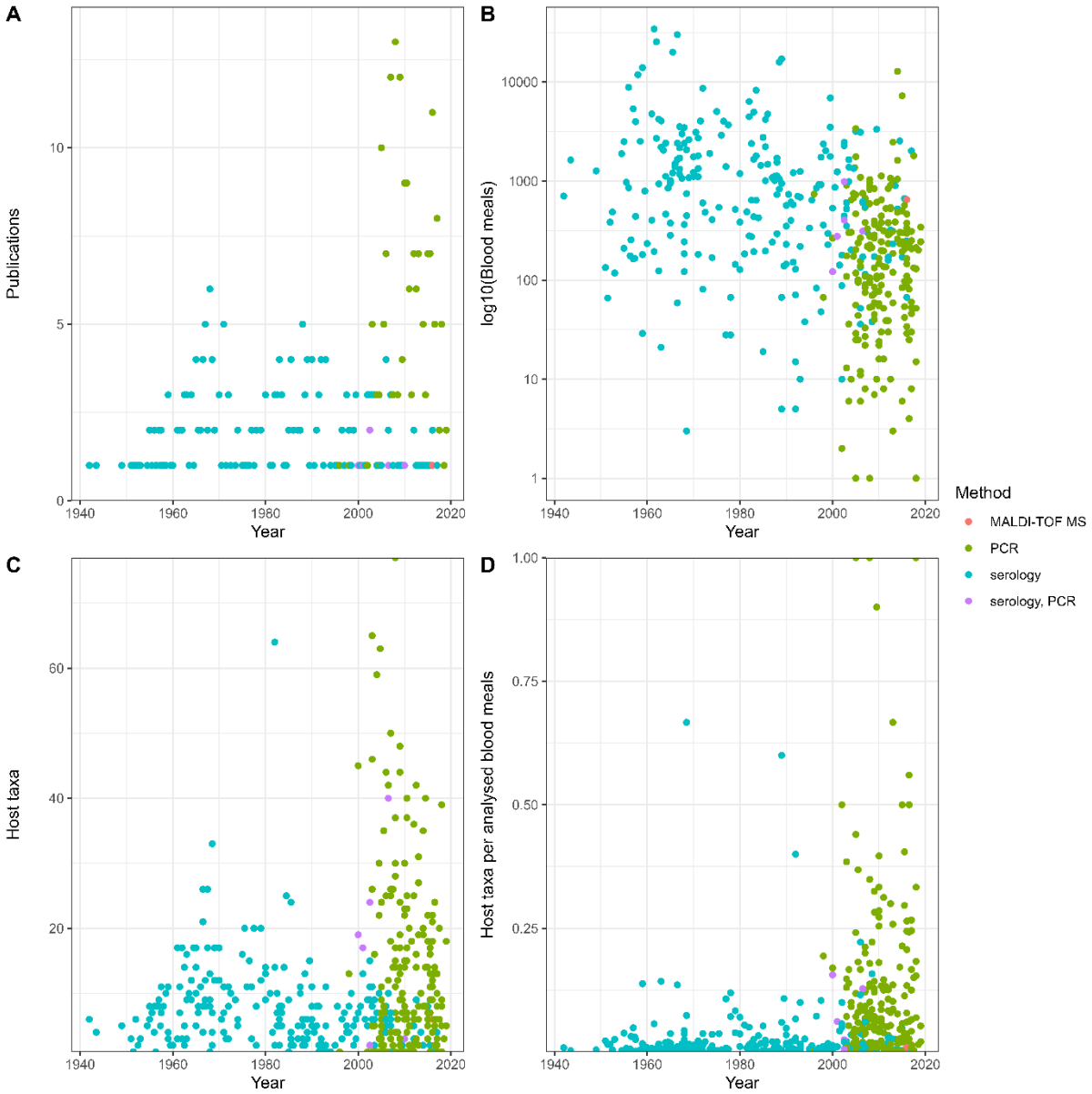


Fig. 5: Number of publications (A), tested specimens per publication (B), identified host taxa per publication (C) and identified host taxa per analysed blood meal per publication (D) with point colour indicating the host identification method.

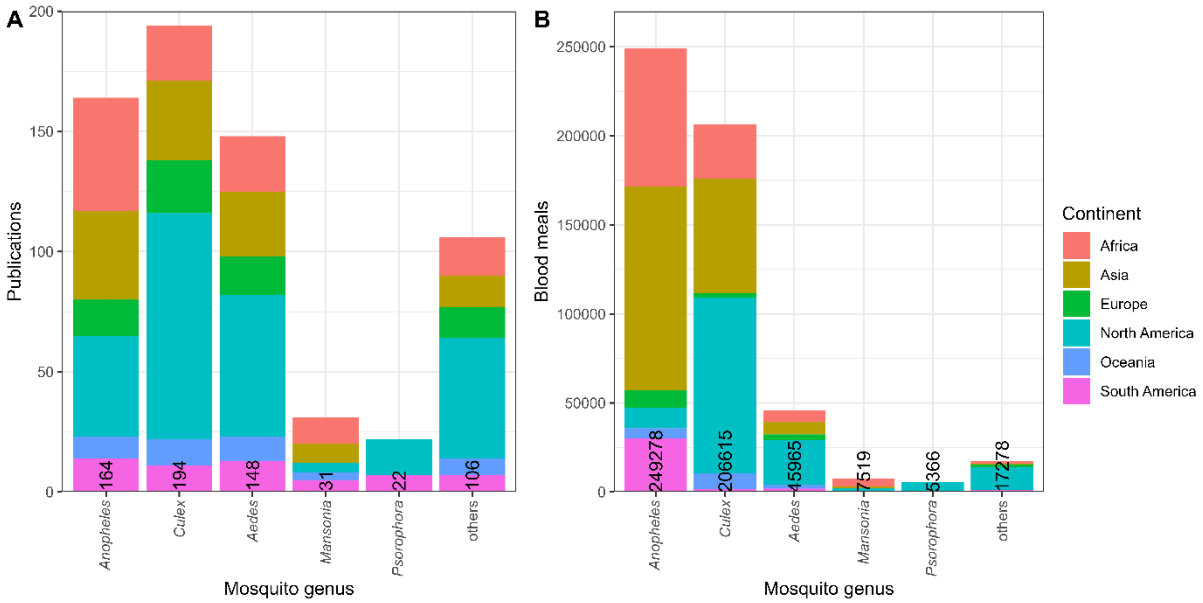


Fig. 6: Number of publications (A) and detected mosquito blood meals (B) per mosquito genus with fill colour indicating the continent.

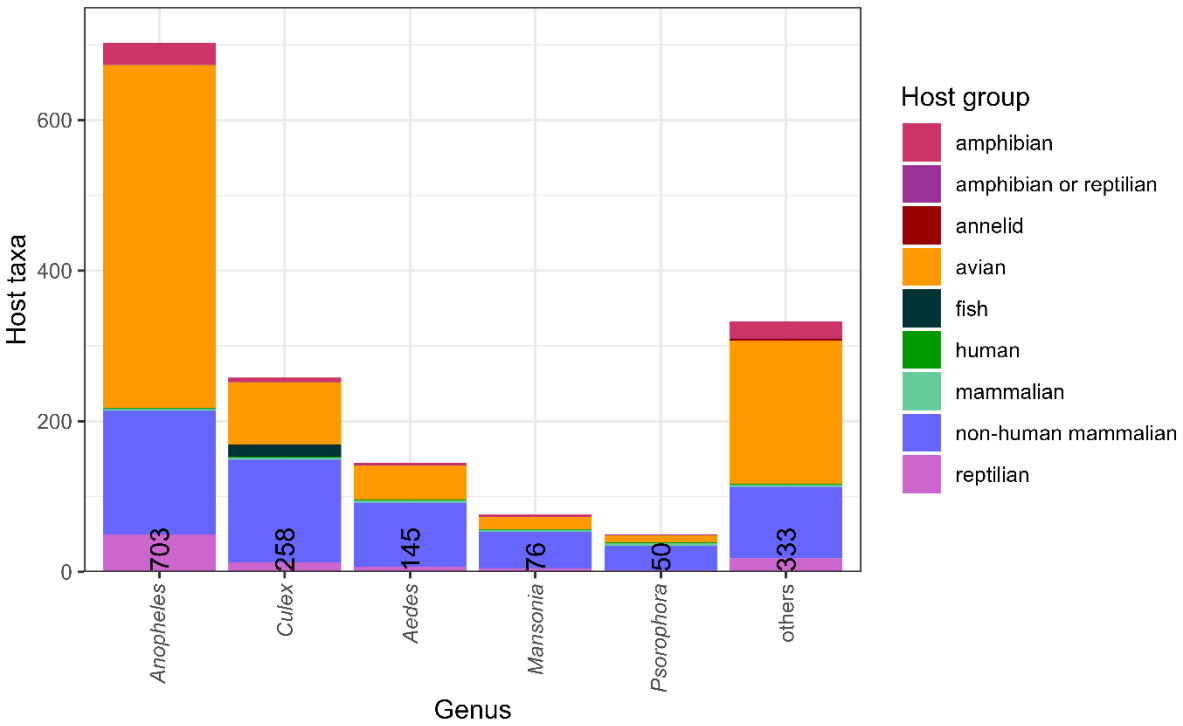


Fig. 7: Number of detected host taxa per mosquito genus with fill colour indicating the host group.

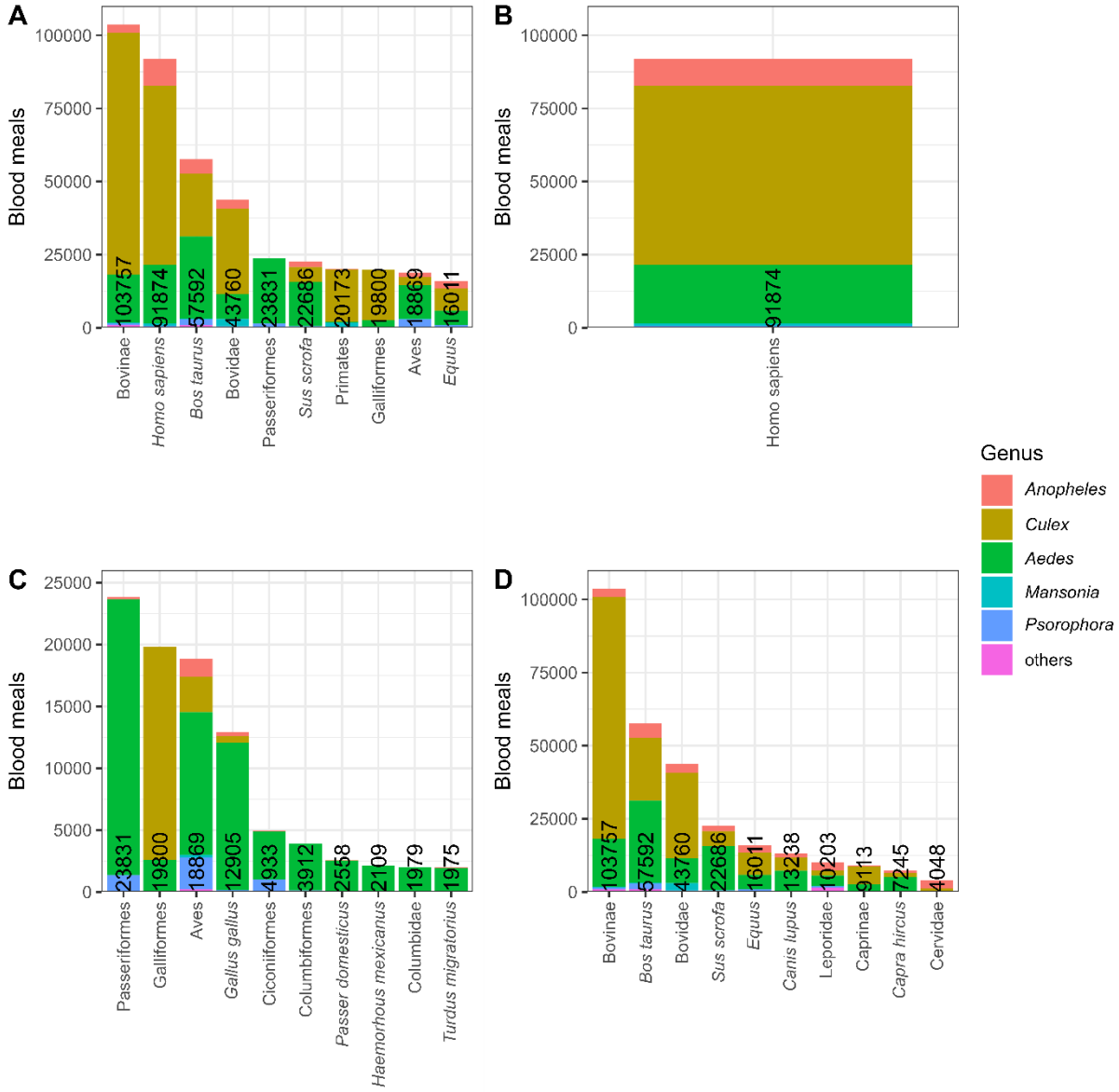


Fig. 8: Top 10 most frequently reported host taxa over all host groups (A), host group human (B), host group avian (C) and host group non-human mammalian (D) with fill colour indicating the mosquito genus.

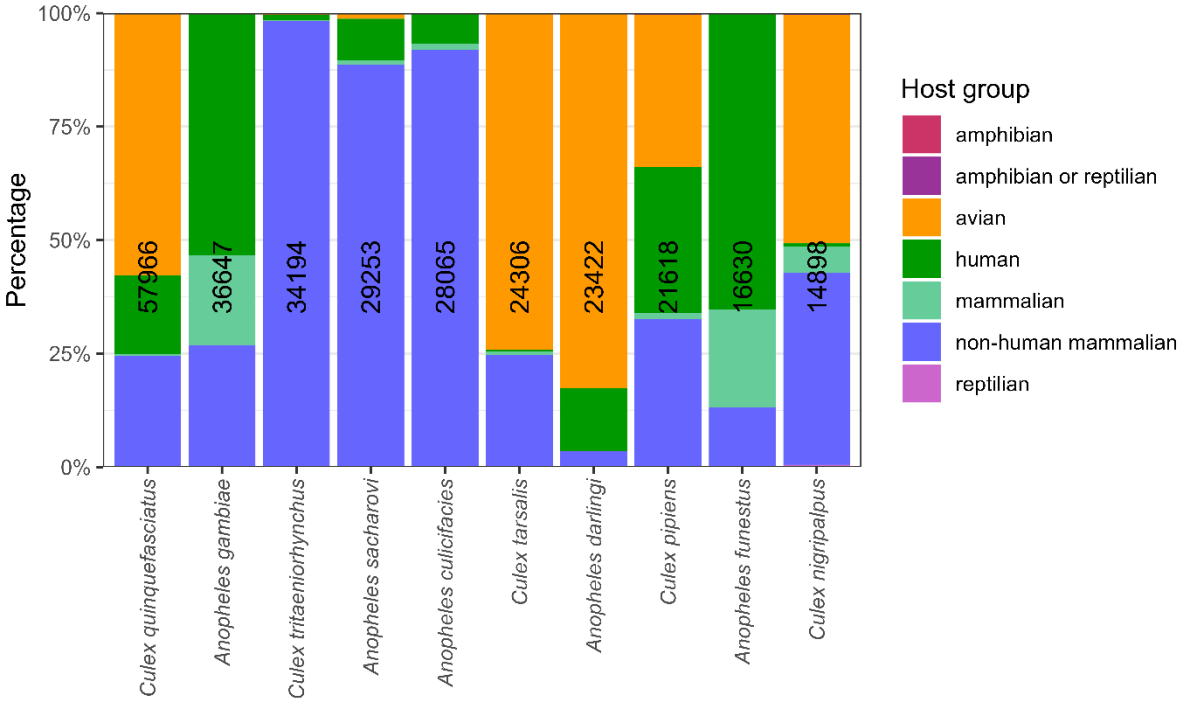


Fig. 9: Top 10 most frequently analysed mosquito species with fill colour indicating the proportion of the host group.

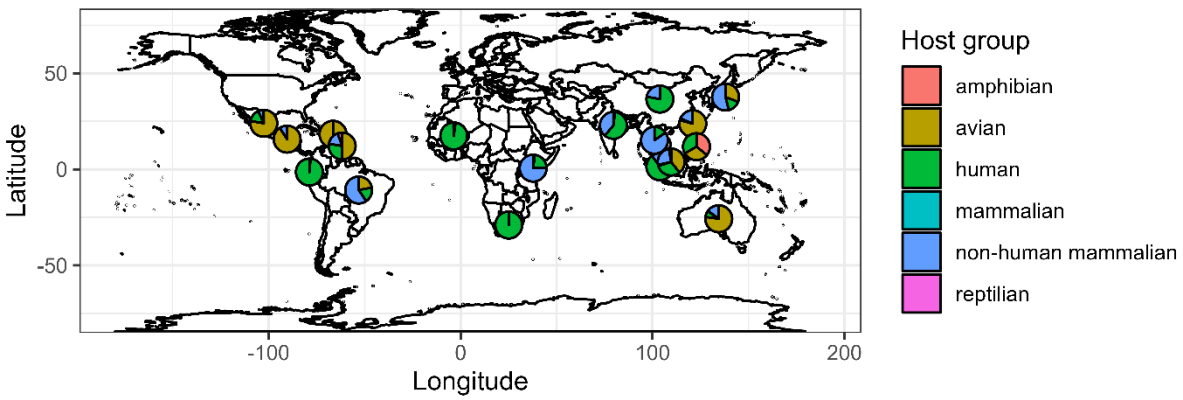


Fig. 10: Proportion of the host groups for *Culex quinquefasciatus.*

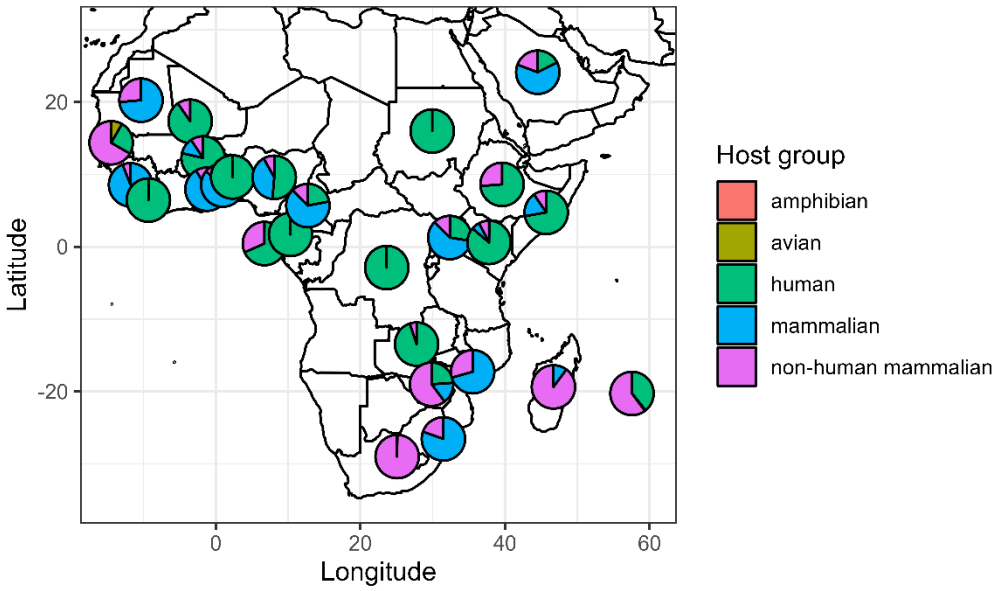


Fig. 11: Proportion of the host groups for *Anopheles gambiae*.

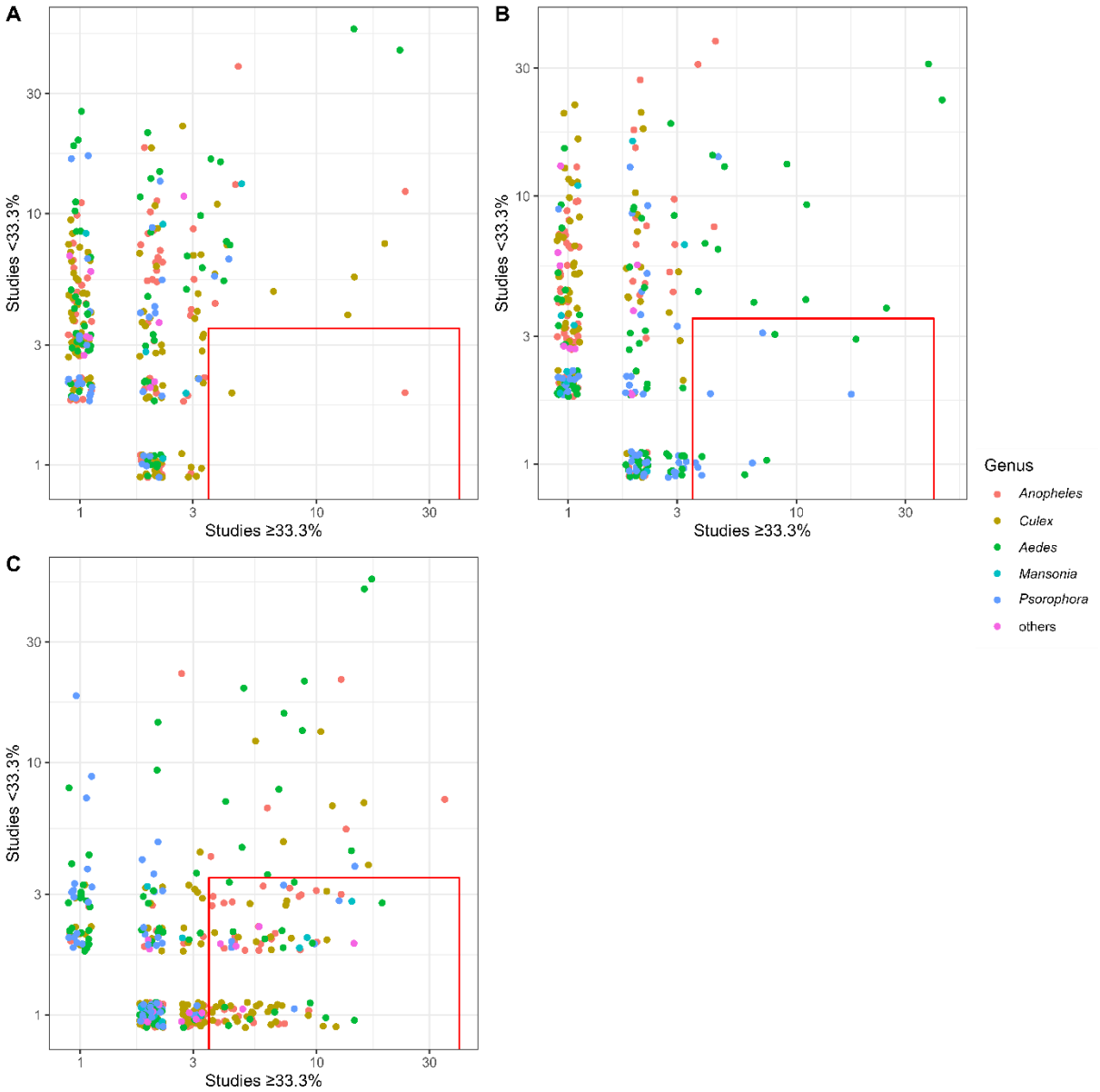
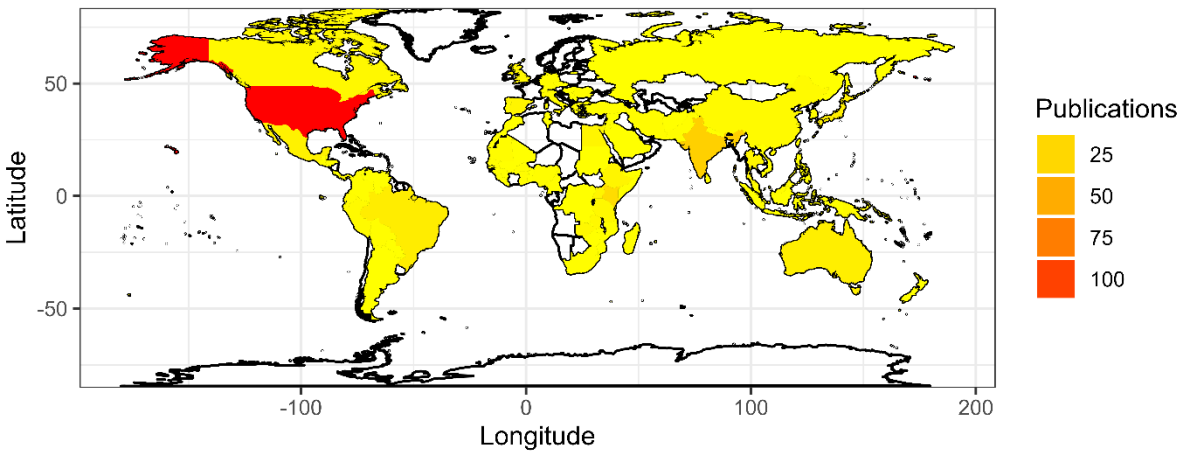
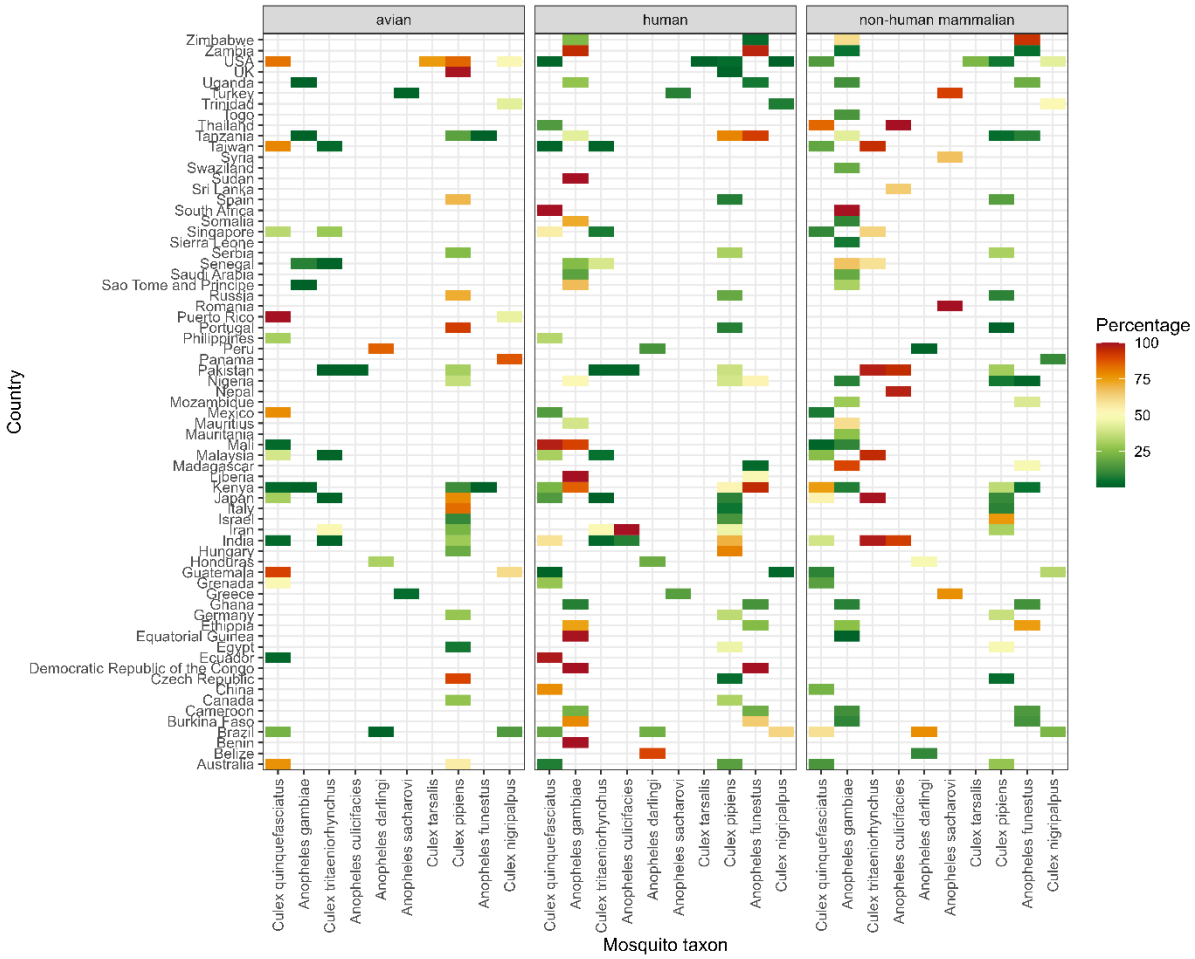


Fig. 12: Number of studies reporting equal or larger 33.3% blood-meals per mosquito taxon per host group plotted against the number of studies reporting less than 33.3% blood-meals per mosquito species for the host groups human (A), avian (B) and non-human mammalian (C) with point colour indicating the mosquito genus. A jitter was added to the points to support distinguishability of taxa. The red rectangle (+1 to improve visualisation) indicates the area where species fulfil the criteria to classify host-feeding patterns by (Fikrig & Harrington, 2021).



Supplementary Fig. S1: Map on the number of publications per country.



Supplementary Fig. S2: Percentage of the host groups human, avian and non-human mammalian per country for the top 10 most frequently analysed mosquito taxa.

A minimum data standard for reporting host-feeding patterns of vectors

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Abstract

Introduction:

Host-feeding patterns provide insights about transmission cycles of vector-borne pathogens. These are investigated by the blood meal identification of engorged vector specimens collected in the field. However, publications on host-feeding patterns often do not report their methods and results in a standardized way. A lot of information is lost due to missing (e.g. GPS coordinates), incomplete (e.g. vector species) or

aggregated information (e.g. sampling site and time point). This prevents systematic analysis in a broad context, e.g. in meta-analysis or comparative studies.

Methods:

We identified information important for the comparability and useability of host-feeding data and created a minimum standard data basis for the reporting of methods and results for studies on the host-feeding patterns of vectors. The usefulness of the proposed variables for standardization are demonstrated with the example of a previously published study on mosquito host-feeding patterns in Panama.

Results:

A proposed table with 18 variables in three sections allows a standardized reporting of details of vector host-feeding studies. These comprise details about the field methods (time, location and method of collection), information about the methods used to identify the vectors and the hosts, and subsequently the outcome data regarding vector species, host species and number of specimens.

Discussion:

With the proposed data standard we aim to facilitate the complete reporting of different host-feeding studies in the future. This will help to compare findings of different host-feeding studies allowing to understand pathogen transmission cycles and to direct further research.

Keywords: host-feeding patterns, mosquito, report, standardization

Introduction

Host-feeding patterns of vector species describe the interaction between vectors and hosts via blood-feeding observed in nature [1]. This can allow an infection of susceptible vectors with pathogens and transmission in another interaction with a

susceptible host. Thus, understanding host-feeding patterns of vectors is essential to understand transmission cycles of associated pathogens.

Transmission cycles of arboviruses are complex, often including different vector and host species. For example, in the sylvatic cycle, dengue virus is assumed to be transmitted between monkeys by different forest mosquito species (e.g. *Aedes luteocephalus*), while the urban *Ae. aegypti aegypti* and *Ae. albopictus* are considered important vectors from humans to humans [2]. In addition, host-feeding patterns can be highly context-dependent, varying in time and space driven by different ecological factors, e.g. the West Nile virus vector *Culex pipiens* in the United States show a seasonal shift from birds to humans driven by the migration dependent host availability of *Turdus migratorius* as preferred bird host [3]. Furthermore, for zoonotic pathogens without human-to-human transmission, vectors are classically divided into enzootic and bridge vectors, e.g. *Cx. torrentium* as an enzootic vector and *Ae. cinereus* as bridge vector for Sindbis virus [4].

The prerequisite to study the complex interaction between vectors and hosts is the collection of blood-engorged specimens, which can be particular challenging for most vector groups. The commonly used trapping systems attract the blood-sucking vectors using carbon dioxide or other visual or olfactory cues that mimic a potential blood host [5, 6]. However, these traps are biased towards unfed host-seeking vectors and only capture a very small proportion of engorged specimens. Therefore, studies on host-feeding patterns often integrate resting site sampling, i.e., the active aspiration of the vectors within microhabitats where they shelter after a blood meal. This sampling method typically yields a higher proportion of blood-engorged specimens compared to the use of baited traps, but it requires a comprehensive understanding of the preferred resting sites of the various vector groups [7, 8]. Thus, studies on host-feeding patters

are mostly focussed on certain vector groups and rely on relatively small number of specimens. These limitations complicate the assessment of underlying spatial-temporal drivers, such as land-use or season, by individual studies. Furthermore, different decisions during the sampling, lab work and reporting of the results can affect the results and their interpretation. For example, the sampling period affect species-specific host-feeding patterns [3] and lab assays for host-screening have a varying sensitivity/specificity for different host species or groups (e.g. vertebrates vs. birds) [9].

There is a strong desire within the overall scientific community to establish data standards for study reporting. Thereby, the benefits of FAIR principles (Findability, Accessibility, Interoperability, and Reusability) are obvious [10]. These recommendations promote the preservation and accessibility of data for future use, facilitate the recovery of unsearchable data, and support open principles for harmonizing data in order to maximize the value of research investments and digital publishing [11]. There are published data standards easily adaptable for the research of vector-borne pathogens, e.g. MIReAD (Minimum Information for Reusable Arthropod abundance Data) [12] can be used to report vector abundance data. A recent study proposed a standard for a more specific vector research, i.e. a standard for vector competence experiments [13].

In a global bibliographic analysis of mosquito host-feeding patterns [14], we observed that necessary metadata is often missing or not reported in a standardized manner. In addition, the results are frequently presented solely in a spatial-temporal aggregated format. This prevents further analysis in a broader context, impeding progress in understanding transmission cycles and the measures derived from such insights. Consequently, advancements in the control and research of vector-borne pathogens are hindered, e.g. as highlighted in a study on the mosquito phylogeny and host-feeding

patterns [15]. Therefore, inspired by the recent proposal for a minimum data standard for vector competence experiments by Wu *et al*. [13], we here propose a minimum data standard for the reporting of the field and laboratory methods and the results of studies on the host-feeding patterns of vectors. We illustrate the usefulness of our standardised data basis by extracting information of a previous publication on mosquito host-feeding patterns from Panama [16].

Methods

In this paper, we propose minimum data standard for the reporting of host-feeding pattern studies, covering field methods, lab methods and the results (table 1). The high number of potential parameters possible to include makes it challenging to develop general, flexible standard for the collection of host-feeding data. We aimed to develop a standardized way to publish accompanying metadata in studies reporting host-feeding patterns, which allows analysis through new users, e.g. GPS-coordinates instead of land-use descriptions, permitting to use satellite data to analyse land-use, or raw data instead of indices (e.g. human blood index).

During a systematic literature study on mosquito host-feeding patterns, we observed that several parameters pose a challenge for the unification and merging of the data. Most importantly, the terminology of mosquito species was not uniform across the publications. For instance, the designations *Cx. pipiens* sensu lato and *Cx. pipiens* sensu stricto are precise, whereas *Cx. pipiens* leaves open whether it refers to *Cx. pipiens* s.s. or an unidentified member of the species complex, which includes *Cx. pipiens* with two forms, pipiens and molestus, *Cx. pallens*, *Cx. quinquefasciatus*, *Cx. australicus* and *Cx. globocoxitus* [17, 18]. The distinction of subspecies can entail clear differences in host-

feeding patterns, which are made unrecognizable by indistinct naming. The same applies to the host names. In addition, the hosts are often identified solely by their common names, which are complex to standardize and often have to be classified to a higher taxonomic level, e.g. “Bovinae”, “Bovine”, “Cow”, “Buffalo”, “Ox”, “Ruminantia” all summarized under “Bovinae”. Finally, metadata like sampling site coordinates were often missing completely, and the time of collection was frequently only given as a period of years, so that conclusions about spatial-temporal changes in host-feeding patterns were not possible. Furthermore, some publications offered many of the information in a mix of separate tables, which clustered the details differently and thus could not be joined together into one table. Thus, for comparative analysis it is important to keep the information as segregated as practical possible, e.g. do not merge information for blood-engorged specimens trapped at different locations or time points.

Results

With the example of a study on mosquito host-feeding in Panama by Navia-Gine *et al*. [16], we demonstrate the use of our data standard to report field methods, host-screening methods and outcome data (table 2). Navia-Gine *et al.* [16] present mosquito species and identified blood meal hosts in structured tables. All additional information regarding the mosquito sampling and lab work are provided in the text.

Furthermore, table 2 presents an extract of all the relevant information combined by us in one table with the suggested structure outlined in the methods section (see supplementary Table 1). This version provides the most relevant metadata and allows to directly analyse their correlation with the vector and host information. Different points in time, locations, mosquito collection and blood host identification methods can

be clearly linked to the respective identified blood meals without confusion and remain useable for the scientific community.

Discussion

Studies on the host-feeding patterns of vectors can have a significant impact on our understanding of transmission cycles of vector-borne pathogens. Thereby, the knowledge on the potentially relevant vector species can have direct practical implications. For example, it can affect the decision on the species-specific control measurements to reduce the risk of transmission, e.g. targeted control of the vectors' breeding sites depending on the identified relevant species [19]. Furthermore, it can influence the selection of priority species for research, e.g. a better understanding of host-feeding patterns can help to identify potential bridge vectors, which should be given special consideration in vector competence studies [20].

At least since the beginning of the 20th century, systematic evaluations on the host-feeding patterns of vectors have been conducted [21, 22]. These studies were published across various locations and at different points in time all over the world. However, due to incomplete or highly aggregated reporting of methods and results, the reusability and comparability of the data from this publication is often difficult or impossible. In a systematic bibliographic work merging 333 studies on mosquito host-feeding patterns, especially the unspecific reporting of mosquito and host taxa was found to hamper a systematic aggregation of the results, e.g. it remains unclear to which level the mosquito and host species has been identified and whether the species complex or a specific species is meant [14]. Another problem is that data are often presented in an aggregated form, whereby information from different sampling sites or time points are combined. This results in a loss of spatial-temporal information, which prevents a deeper

understanding of the ecological drivers, which might affect host-feeding patterns, e.g. land use or seasonal changes.

The here proposed standard for reporting of methods and results for studies on vector host-feeding patterns would make the data much more directly useable in a wider context, and even allow it to be fed directly into a global database for broader analyses [14]. This would facilitate comparisons between vector species, sampling sites or time points on a global scale, which is essential for a better understanding of pathogen transmission cycles and to identify knowledge gaps. The here presented standardised tables can be easily adapted for different vector groups, allowing to systematically analyse the interactions between arthropod vectors and hosts.

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Availability of data and materials

All data are available in the manuscript or open databases.

Authors' contributions

Conceptualization: R.L..; data collection: M.L.W.; literature synthesis and first drafting: M.L.W., F.G.S., R.L.; writing and editing: all authors.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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Table 1 A minimum standard for field methods, mosquito identification, host-screening methods, and outcome data

	Variable	Description
field methods	**X coordinate**	X coordinate of sampling site
	Y coordinate	Y coordinate of sampling site
	start year	start of sampling [year]
	start month	start of sampling [month]
	start day	start of sampling [day]
	end year	end of sampling [year]
	end month	end of sampling [month]
	end day	end of sampling [day]
	trapping method	sampling method (e.g. aspiration) and used trap
	lure	lure of the trap (e.g. CO_2, octenol)
mosquito identification and host-screening methods	**mosquito identification method**	method for identification of blood meal origin (e.g., precipitin test, ELISA, PCR+species-specific gel bands, PCR + sequencing)
	blood host identification method	amplified and sequenced gene (e.g., *Cytochrome b*, 16S)
	gene for sequencing	method for the identification of the mosquito species, e.g. morphology, PCR+species-specific gel bands, PCR + sequencing
outcome data	**mosquito species**	full scientific name (species) (most recent taxonomy, e.g. based on NCBI taxonomy current name or even using the NCBI:txid e.g. 1424507)
	mosquito subspecies	epithet for the species' subspecies mosquito subspecies
	additional mosquito species information	epithet of mosquito biotype (e.g., "biotype pipiens", for *Culex pipiens* biotype pipiens)
	host	full scientific name (species) (most recent taxonomy, e.g. based on NCBI taxonomy current name or even using the NCBI:txid e.g. 7159)
	specimens	number of specimens

Table 2 Presentation of the field methods, mosquito identification, host-screening methods, and outcome data in a publication on mosquito host-feeding patterns from Panama [16] with example of extracted data (see Supplementary Table 1 for the complete dataset)

	Variable	From the paper	Extracted column 1	Extracted column 2	…
field methods	**X coordinate**	"Our principle collections occurred in Aruza Abajo (8° 21.67′ N, 77° 56.44′ W)"	8° 21.67' N	8° 21.67' N	…
	Y coordinate		77° 56.44' W	77° 56.44' W	…
	start year	"The first collection round started on 18 June 2010 during the outbreak period, whilst the second round started on 23 October 2010 during the post-outbreak phase."	2010	2010	…
	start month		June	June	…
	start day		18	18	…
	end year		2010	2010	…
	end month		June	June	…
	end day		23	23	…
	trapping method	"Mosquitoes were collected using standard Centers for Disease Control and Prevention miniature light traps (John W. Hock Co., Gainesville, FL)"	CDC miniature light traps	CDC miniature light traps	…
	lure	"Light traps were baited with one kilogram of solid carbon dioxide (e.g. dry ice, CO2)"	solid carbon dioxide	solid carbon dioxide	…
mosquito identification and host-screening methods	**mosquito identification method**	"Mosquitoes were identified to species level using a dissecting microscope, a chill table and morphological keys"	morphology	morphology	…
	blood host identification method	"PCR products were cycle sequenced using BigDye Terminator v3.1 kit (Applied Biosystems, Foster City, CA) followed by Sephadex P-50 purification and sequencing using a 3130x1 Genetic Sequencer"	PCR + sequencing	PCR + sequencing	…

	gene for sequencing	"Published vertebrate primers targeting cytochrome C oxidase I (COI), 16S ribosomal DNA (16S), and mammalian cytochrome-*b* (cyt-*b*) were used for this study, herein COI, 16S, and cyt-*b* primers, respectively"	vertebrate cytochrome C oxidase I, 16S ribosomal DNA, mammalian cytochrome-b	vertebrate cytochrome C oxidase I, 16S ribosomal DNA, mammalian cytochrome-b	…
outcome data	**mosquito species**	Table 3 and Table 4	*Coquillettidia venezuelensis*	*Coquillettidia venezuelensis*	…
	mosquito subspecies		NA	NA	…
	additional mosquito species information		NA	NA	…
	host		*Sus scrofa*	*Equus caballus*	…
	specimens		110	41	…

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Supplementary Table 1 Extracted field methods, mosquito identification, host-screening methods, and outcome data in a publication on mosquito host-feeding patterns from Panama [16]

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Host attraction and host feeding patterns indicate generalist feeding of *Culex pipiens* s.s. and *Cx. torrentium*

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Abstract

Background Mosquito host feeding patterns are an important factor of the species-specific vector capacity determining pathogen transmission routes. *Culex pipiens* s.s./*Cx. torrentium* are competent vectors of several arboviruses, such as West Nile virus and Usutu virus. However, studies on host feeding patterns rarely differentiate the morphologically indistinguishable females.

Methods We analyzed the host feeding attraction of *Cx. pipiens* and *Cx. torrentium* in host-choice studies for bird, mouse, and a human lure. In addition, we summarized published and unpublished data on host feeding patterns of field-collected specimens from Germany, Iran, and Moldova from 2012 to 2022, genetically identified as *Cx. pipiens* biotype *pipiens*, *Cx. pipiens* biotype *molestus*, *Cx. pipiens* hybrid biotype *pipiens* × *molestus*, and *Cx. torrentium*, and finally put the data in context with similar data found in a systematic literature search.

Results In the host-choice experiments, we did not find a significant attraction to bird, mouse, and human lure for *Cx. pipiens pipiens* and *Cx. torrentium*. Hosts of 992 field-collected specimens were identified for Germany, Iran, and Moldova, with the majority determined as *Cx. pipiens pipiens*, increasing the data available from studies known from the literature by two-thirds. All four *Culex pipiens* s.s./*Cx. torrentium* taxa had fed with significant proportions on birds, humans, and nonhuman mammals. Merged with the data from the literature from 23 different studies showing a high prevalence of blood meals from birds, more than 50% of the blood meals of *Cx. pipiens* s.s. were identified as birds, while up to 39% were human and nonhuman mammalian hosts. *Culex torrentium* fed half on birds and half on mammals. However, there were considerable geographical differences in the host feeding patterns.

Conclusions In the light of these results, the clear characterization of the *Cx. pipiens* s.s./*Cx. torrentium* taxa as ornithophilic/-phagic or mammalophilic/-phagic needs to be reconsidered. Given their broad host ranges, all four *Culex* taxa could potentially serve as enzootic and bridge vectors.

Keywords Mosquito, Host attraction, Host feeding patterns, *Culex pipiens* biotype *pipiens*, *Culex pipiens* biotype *molestus*, *Culex pipiens* hybrid biotype *pipiens* × *molestus*, *Culex torrentium*

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Background

Host feeding patterns describe an important component of vector capacity, i.e., the probability of a vector–host contact [1]. This interaction is essential to understanding pathogen transmission cycles, e.g., to identify potential vector species [2]. Host feeding patterns of mosquitoes are characterized by intrinsic (genetic) and extrinsic (environmental) factors [3–5]. Intrinsic factors are considered the main drivers of host preference for mosquito species with a narrow range of host species, e.g., high preference of *Culex territans* or *Uranotaenia unguiculata* for amphibians [6, 7], while extrinsic factors are expected to be relevant for species with a broad range of host species, e.g., host availability for *Cx. pipiens* [8].

It is proposed that specialists evolve when there is a fitness gain achieved by consuming one optimal host compared with feeding on a range of suboptimal hosts [9]. In contrast, generalists are expected to occur in environments with a low probability of host encounter, and the advantage of waiting for an optimal host is weighed against the risk of death prior to blood feeding and reproduction [1]. To understand the transmission cycle of mosquito-borne pathogens, it is important to accurately describe species-specific differences in host feeding patterns, as it enables the classification of mosquito species as enzootic vectors or bridge vectors of a given pathogen, e.g., *Cx. torrentium* is considered the enzootic vector (bird–mosquito–bird) of Sindbis virus, while *Aedes cinereus* the bridge vector (bird–mosquito–human) [10].

Misconceptions about mosquito host feeding patterns are deeply rooted in the literature. One prominent example is *Cx. pipiens* s.s./*Cx. torrentium*, including the taxa *Cx. pipiens* biotype *pipiens* (*Cx. pipiens pipiens*), *Cx. pipiens* biotype *molestus* (*Cx. pipiens molestus*), the hybrid between both biotypes *Cx. pipiens* biotype *pipiens* × *molestus* (*Cx. pipiens pipiens* × *molestus*), and *Cx. torrentium*. The females cannot be identified by classic morphology [11], but the taxa differ considerably in their ecology [12–15]. In the literature, *Cx. pipiens pipiens* and *Cx. torrentium* are commonly described as ornithophilic/-phagic [13, 16–18], while there is no unified definition for this terminology other than feeding "often" or preferring to feed on the respective host group compared with other host groups without a defined threshold [19]. In contrast, *Cx. pipiens molestus* is predominantly considered mammalophilic/-phagic [20]. The hybrid between both biotypes with an intermediate host feeding pattern is considered to function as bridge vectors for zoonotic diseases in Northern America [21]. In contrast, recent studies from Europe and Asia show opportunistic host feeding patterns for *Cx. pipiens* s.s./*Cx. torrentium* with a considerable proportion of mammals, including humans. There might be no taxa-specific association with one host group and the taxa have to be considered both potential enzootic and bridge vectors [22–25].

Culex pipiens s.s./*Cx. torrentium* are potential vectors of different mosquito-borne pathogens with a high relevance for veterinary and public health. This also applies to Germany, Moldova, and Iran, which are examined in more detail in the present study. *Culex pipiens* s.s./*Cx. torrentium* is widespread in each of the three countries [22, 23], and field-collected specimens are regularly found to be positive for arboviruses as well as their vector competence was confirmed in the laboratory, for example, Usutu virus or West Nile virus [26–32]. This is also reflected in the published information on the host feeding patterns for the countries, which showed that *Cx. pipiens* s.s./*Cx. torrentium* have to be considered potential bridge vectors feeding on birds and mammals, including humans [22, 23]. Nevertheless, although there are several other studies analyzing the host feeding patterns of *Cx. pipiens* s.l. with more than 20,000 identified blood meals all over the world, many studies did not differentiate between the members of the species complex [33].

Therefore, the aim of this study was to provide comprehensive insight into the host feeding patterns of *Cx. pipiens pipiens*, *Cx. pipiens molestus*, *Cx. pipiens pipiens* × *molestus*, and *Cx. torrentium* by (1) analyzing the host attraction of *Cx. pipiens pipiens* and *Cx. torrentium* in a host-choice experiment, (2) summarizing the published and unpublished host feeding patterns for specimens collected in field studies over the last decade analyzed with the same laboratory protocols, allowing for a comparability of the results between Germany, Moldova, and Iran, and (3) finally comparing our results on the host feeding patterns of these taxa with those previously described in the globally available literature.

Methods

Experiment on the host attraction of *Cx. pipiens pipiens* and *Cx. torrentium*

Culex pipiens s.s./*Cx. torrentium* were reared from egg rafts collected in Weinheim, Germany (49.54° N, 8.66° E) between May and August 2020 using gravid-trap bins baited with a yeast hay infusion. About 1–5 egg rafts were placed in larval rearing trays (22 cm × 15 cm × 7 cm) containing 1 L of tap water. Larvae were fed daily with a small amount of crushed flake fish food (TetraMin Flakes, Tetra GmbH, Melle, Germany). Larval rearing was conducted at 22–26 °C and 40–60% relative humidity. Emerging adults were maintained in 32.5 cm × 32.5 cm × 32.5 cm screened cages under the same temperature and relative humidity conditions and were daily provided with 10% sucrose solution ad libitum. Females used in the host

selection trials emerged 4 days prior and deprived of sucrose solution 12 h prior to testing.

The trials were conducted with two animals: one grey canary (*Serinus canaria* form *domestica*) and one house mouse (*Mus musculus*). In addition, as an attractant that mimics human skin scents, a packet of BG-Sweetscent (Biogents, Regensburg, Germany) was used with 25 ml CO_2/min, which is similar to the amount of CO_2 emitted by the mouse. The CO_2 emission of the canary (9.22 ml CO_2/min (SD 1.09) and the mouse (24.82 ml CO_2/min (SD 1.64) was previously measured with a CO_2 monitor (AIRCO2NTROL 5000, TFA Dostmann, Wertheim-Reicholzheim, Germany). For this purpose, the individual animals were placed in a box (32 × 25 × 37 cm) and the CO_2 content was measured before adding the animal and after 10 min. The experiment was repeated three times. A 1.5 m × 1.5 m mesh enclosure was placed inside the laboratory and two lard can traps (25 × 25 × 80 cm) were hung side-by-side separated by one meter [34] (Fig. 1). The lard can traps were constructed from a large tube (⌀ 25 cm) covered at both ends with removable sampling devices with mesh funnels that allowed mosquitoes to enter but prevented them from escaping the tube. A cage with the attractant was placed inside the tube. Trials were performed with the following combinations inserted within the lard can traps: bird–bird, bird–lure, bird–mouse, lure–lure, mouse–lure, and mouse–mouse. The animal or attractant was randomly assigned to one of the lard can traps. Each trial was repeated five times.

Culex pipiens s.s./*Cx. torrentium* females entered the trap through one of two removable funnels on either end of the trap. The funnels contained a mosquito-proof mesh that prevented direct contact between the animals and mosquitoes. The trials were conducted from 6 pm to 8 am with an average of 122 females (between 43 and 212 females) for each trial, depending on the availability of 4-day-old females. Mosquitoes in the lard can traps and the remaining mosquitoes in the mesh enclosure were removed with a manual mouth sucking aspirator, stored separately in tubes at −20 °C. All specimens were identified as *Cx. pipiens pipiens*, *Cx. pipiens molestus*, *Cx. pipiens pipiens* × *molestus*, or *Cx. torrentium* using a molecular DNA typing assay [12].

Host attraction was analyzed using individual binomial generalized linear models (GLM) per combination of hosts and mosquito species. The proportions of host-seeking female mosquitoes per lard can trap (from now on "attraction") was used as response variable ($N = 10$ per GLM) and animal/attractant as two-factorial explanatory variable, e.g., "bird" and "mouse." Mosquitoes that did not enter one of the lard can traps were not considered as host-seeking and were excluded from the statistical analysis.

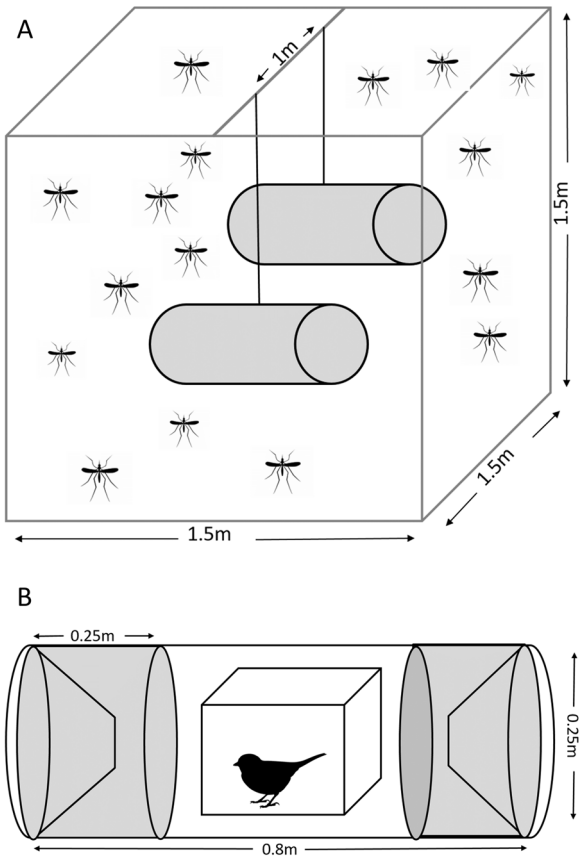

Fig. 1 **A** Mesh enclosure with two lard can traps each equipped with an animal or attractant, mosquito pictogram taken from © clipart-library, **B** lard can traps included in the mesh enclosure (Fig. 1); bird pictogram taken from © clipart-library

Analysis of the host feeding patterns of *Cx. pipiens* s.s./*Cx. torrentium* collected in Germany, Moldova, and Iran

Our field data on the host feeding patterns of *Cx. pipiens* s.s./*Cx. torrentium* combine previously collected data by us during field studies conducted in Germany [22] and Iran [23] and new, unpublished data collected in different sampling campaigns between 2012 and 2022 in Germany and Moldova. All specimens from the already published studies, as well as the newly collected specimens, were analyzed with the same laboratory workflow [22, 23]. This allows for a better comparability between the results from the three countries, for example, polymerase chain reaction (PCR) primers have been shown to have different specificity [35], potentially influencing the sensitivity for different host taxa between different studies. Sampling sites in all of the three countries covered different dominant land-use categories from urban over rural to natural in each of the countries [22, 23], although an analysis of the differences in host feeding patterns between different land-use categories were not in focus of this study, as it was shown to have no statistically significant impact

in our previous studies in Germany [22] and Iran [23]. Mosquitoes were collected with pop-up garden bags as artificial resting sites using a hand-held aspirator [36] or within a nationwide mosquito and pathogen surveillance program using CO_2-baited Heavy Duty Encephalitis Vector Survey traps (BioQuip Products, Rancho Dominguez, California, USA), Centers for Disease Control miniature light trap (BioQuip Products, Rancho Dominguez, California, USA), and Biogents Sentinel or BG-Pro traps (Biogents, Regensburg, Germany). The collected mosquitoes were left in the trap bags and stored at −20 °C prior to analysis. Each specimen was morphologically identified under permanent cooling [37].

Whole blood-engorged, morphologically identified *Cx. pipiens* s.s./*Cx. torrentium* specimens were placed individually into 2 ml tubes and about 20 pieces of 2.0 mm zirconia beads (BioSpec Products, Bartlesville, USA) as well as 1 ml of cell culture medium (high-glucose Dulbecco's modified Eagle's medium; Sigma-Aldrich, St. Louis, MO, USA) were added. The homogenization was performed with a TissueLyser or TissueLyser II (Qiagen, Hilden, Germany) for 2 min at 50 oscillations/s. After clarifying by centrifugation for 1 min at 8000 rpm and 4 °C, the suspension was transferred to a new safe-lock tube. DNA was extracted from 200 µl of the homogenate using the KingFisher™ Flex Magnetic Particle Processor with the MagMAX™ Pathogen ribonucleic acid/DNA Kit (both Thermo Fisher Scientific, Waltham, MA USA).

Two primer sets targeting the cytochrome *b* or *16S* rRNA gens were used [38, 39] following the previously published protocol [22, 23]. All amplicons were further processed with Sanger sequencing (LGC Genomics, Berlin, Germany), sequences pre-processed with Geneious® 7.1.9 [40], and finally compared with GenBank sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Host species were determined using a 95% threshold for percentage identity. Using the same template, all morphologically identified *Cx. pipiens* s.s./*Cx. torrentium* specimens were identified as *Cx. pipiens pipiens*, *Cx. pipiens molestus*, *Cx. pipiens pipiens* × *molestus*, or *Cx. torrentium* using a molecular DNA typing assay [12].

Differences in the proportion for the avian, human, and nonhuman mammalian host feeding groups were evaluated among the three countries by the test of equal or given proportions (*prop.test*) in R (Version: 4.2.2) [41].

Global literature review on the host feeding patterns of *Cx. pipiens* s.s./*Cx. torrentium*

Data on host feeding patterns were extracted for *Cx. pipiens pipiens*, *Cx. pipiens molestus*, *Cx. pipiens pipiens* × *molestus*, or *Cx. torrentium* from publications identified in a systematic search on 17 June 2024 using the PubMed database with the following strategy: '(Mosquito*[Title] OR Culici*[Title] OR Aedes[Title] OR Culex[Title] OR Anoph*[Title] OR "west nile virus"[Title]) AND (Blood*[Title] OR meal*[Title] OR feed*[Title] OR host*[Title] OR preference*[Title] OR pattern*[Title] OR forage*[Title])'. The methods were described in detail by Wehmeyer et al. [33]. In short, two researchers independently screened the publications for suitability on the basis of following inclusion criteria: (1) the study was conducted in the field, (2) studies using vertebrate baits were included only if mosquitoes had no direct contact with the host or were collected before biting, and (3) ingested blood was analyzed using serological or molecular methods. Studies that were only based on behavior observation, laboratory-reared mosquitoes, or laboratory-based feeding experiments were excluded. For this publication, studies were included where *Cx. pipiens* s.s./*Cx. torrentium* were identified as *Cx. pipiens pipiens*, *Cx. pipiens molestus*, *Cx. pipiens pipiens* × *molestus*, or *Cx. torrentium* using a molecular DNA typing assay. All possible information given on mosquito, detected host taxa, and country were collected and merged into a single database. Blood meal hosts were further categorized into the host groups avian, amphibian or reptilian, reptilian, amphibian, mammalian, human, and nonhuman mammalian.

Data analysis

All computational analysis was performed in R (Version: 4.2.2) using the R-Studio IDE (Version: 2022.12.0) [41]. Additionally, functions from the following packages were used for data preparation and visualization: dplyr [42], ggplot2 [43], tidyverse [44], readxl [45], stringr [46], plyr [47], and magrittr [48].

Results

Experiment on the host attraction of *Cx. pipiens pipiens* and *Cx. torrentium*

A total of 268 *Cx. pipiens pipiens* and 350 *Cx. torrentium* females were used in the experimental trials comparing the proportional attraction for bird versus lure, bird versus mouse, and mouse versus lure. Both species showed a higher mean attraction for birds compared with lure with a mean of 60.3% [95% confidence interval (95% CI) 30.9–89.8%] against 39.7% (95% CI 10.2–69.1%) for *Cx. pipiens pipiens* and 58.9% (95% CI 38.4–99.4%) against 38.9% (95% CI 7.1–70.8%) for *Cx. torrentium*. For the trial bird against mouse it was the other way around with a higher mean attraction for mouse against bird with a mean of 53.3% (95% CI 0.7–100.0%) against 77.3% (95% CI 49.1–100.0%) for *Cx. pipiens pipiens* and 41.7% (95% CI 14.2–69.1%) against 58.3% (95% CI 30.9–85.8%) for *Cx. torrentium*. No clear pattern regarding the mean

values was observed for the trial lure versus mouse. The 95% confidence intervals of mean attraction for the different trials were highly overlapping (Fig. 2) and neither species showed any statistically significant difference for a host or attractant (binomial GLMs, $P > 0.05$). In addition, no statistical pattern was observed for the same host/attractant in both lard can traps (Additional file 1: Fig. S1).

Analysis of the host feeding patterns of *Cx. pipiens* s.s./*Cx. torrentium* collected in Germany, Moldova, and Iran

The host species were identified for a total of 931 blood-fed *Cx. pipiens pipiens*, 29 *Cx. torrentium*, 18 *Cx. pipiens pipiens* × *molestus*, and 14 *Cx. pipiens molestus* collected in Iran, Moldova, and Germany (Fig. 3). For *Cx. pipiens pipiens*, blood meals from human (371, 39.8%) and avian hosts (363, 39.0%) were detected in the highest numbers, followed by non-mammalian hosts detected with 191 blood meals (20.5%) and 4 amphibian blood meals (0.4%). Blood meals of *Cx. torrentium* were dominated by birds (14, 48.3%) and humans (12, 41.4%), while only 3 blood meals (10.3%) were observed from nonhuman mammalian taxa. *Culex pipiens pipiens* × *molestus* fed on humans (8, 44.4%) and showed equal proportions of avian and non-human mammalian blood meals (5, 27.8%). Finally, for *Cx. pipiens molestus*, blood meals from human (5, 35.7%) and non-human mammals (5, 35.7%) were equally frequently detected, shortly followed by avian hosts (4, 28.6%).

As demonstrated above, a high prevalence of humans is evident for all four studied *Culex* taxa (> 35%, Fig. 4). Focusing exclusively on *Cx. pipiens pipiens* with a sufficient sample size, further frequent host taxa were *Bos taurus* (122 blood meals, 13.1% of all blood meals for this taxon), *Columba palumbus* (68, 7.3%), *Anas* spp. (62, 6.7%), *Turdus merula* (54, 5.8%), and *Gallus gallus* (44, 4.7%). The other blood meals (210, 22.6%) were distributed over many less frequent hosts dominated by different bird species and domestic animals (e.g., *Canis lupus*, *Felis catus*). Comparing the host feeding patterns for the three countries in comparison with the remaining two, a significant lower proportion of nonhuman mammals was observed for Germany (Germany versus Iran: $\chi^2 = 33.1$, $df = 1$, $P < 0.001$; Germany versus Moldova: $\chi^2 = 6.3$, $df = 1$, $P < 0.012$; Iran versus Moldova: $\chi^2 = 0.27$, $df = 1$, $P = 0.6$), while we found lower proportions of humans in Moldova (Germany versus Iran: $\chi^2 = 2.7$, $df = 1$, $P = 0.09$; Germany versus Moldova: $\chi^2 = 13.2$, $df = 1$, $P < 0.001$; Iran versus Moldova: $\chi^2 = 18.8$, $df = 1$, $P < 0.001$) and lower proportions of birds in Iran (Germany versus Iran: $\chi^2 = 42.1$, $df = 1$, $P < 0.001$; Germany versus Moldova: $\chi^2 = 2.8$, $df = 1$, $P < 0.09$; Iran versus Moldova: $\chi^2 = 29.7$, $df = 1$, $P < 0.001$).

For 41 *Cx. pipiens pipiens* specimens (4.4%), two different hosts were detected: 35 mixed blood meals with

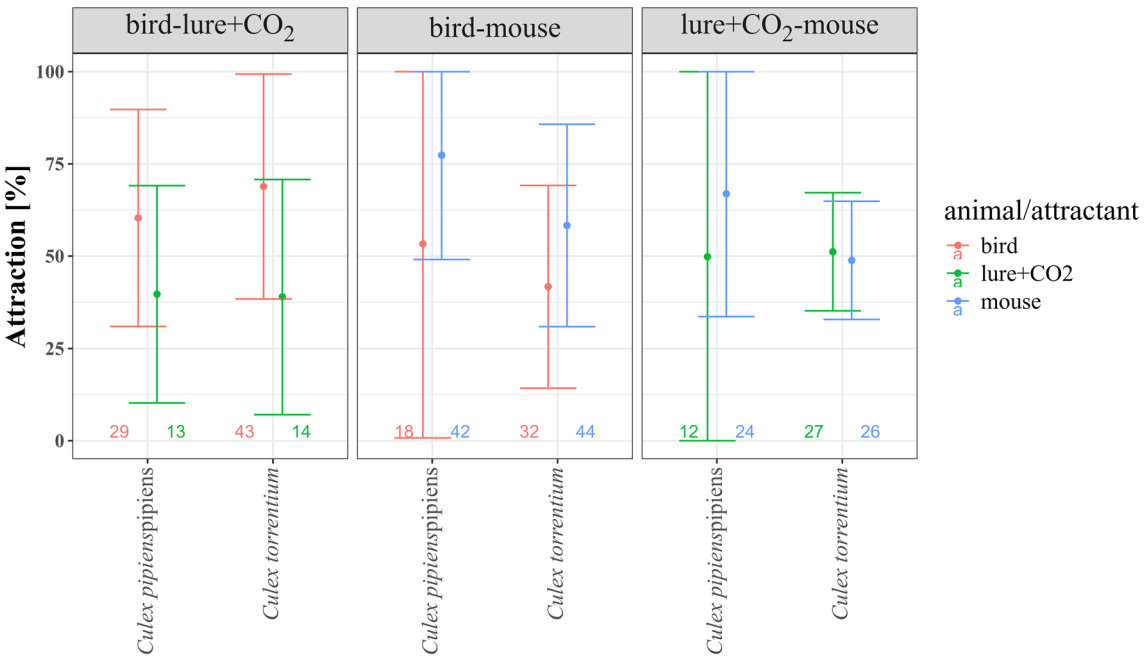

Fig. 2 Mean attraction with 95% confidence interval for host/attractant for *Culex pipiens pipiens* and *Culex torrentium*. Numbers on the bottom indicate the total number of specimens collected in the specific lard can trap over five replicates

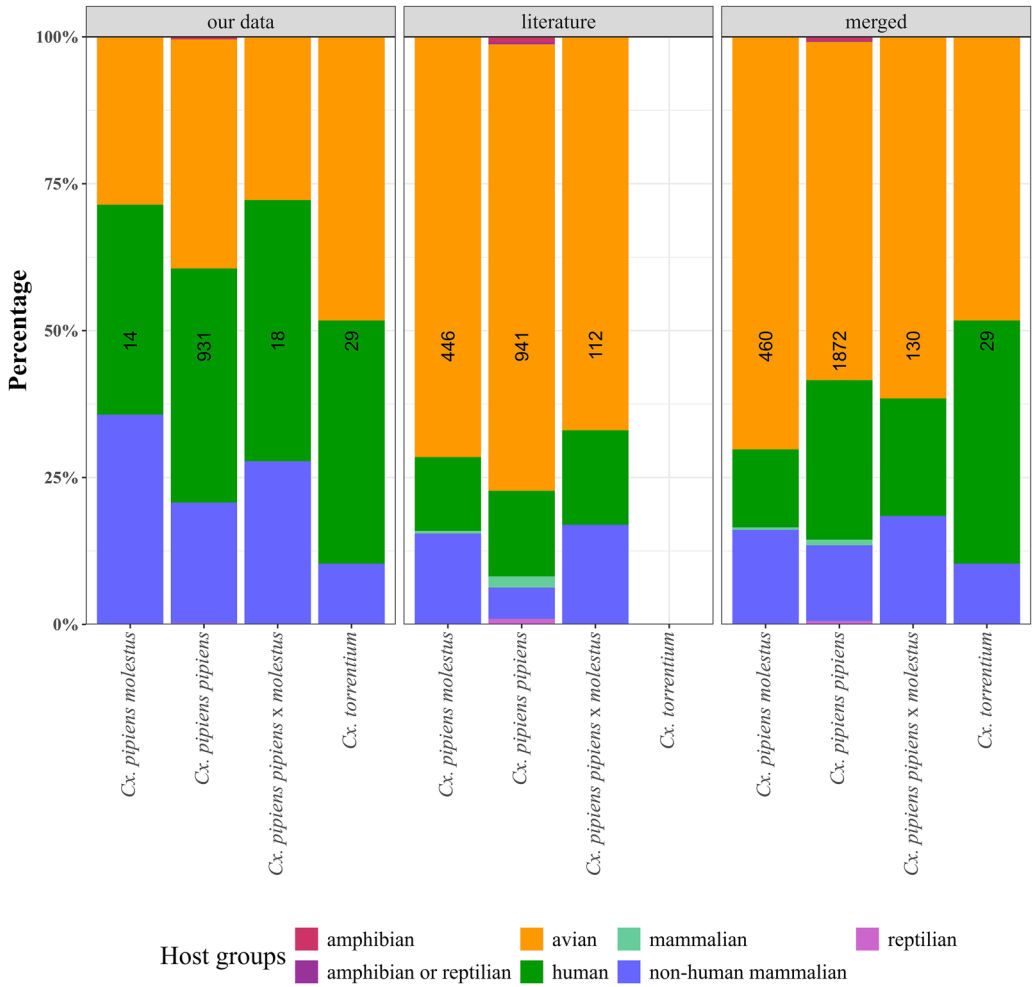

Fig. 3 Proportion of host groups detected for *Cx. pipiens molestus*, *Cx. pipiens pipiens*, *Cx. pipiens pipiens* × *molestus*, and *Cx. torrentium*. Data collected in our studies (left), data from literature (middle), and both datasets merged (right). Numbers in the bar indicate the number of blood meals per taxon and dataset. The host group "mammalian" is used if studies do not identify the mammalian species

human and avian blood, 3 with avian and nonhuman mammalian blood, 2 specimens fed on a human and a nonhuman mammal, and 1 specimen contained blood of a bird and an amphibian. One *Cx. torrentium* specimen (3.4%) contained blood from *Homo sapiens* and *Sus scrofa*.

Global literature review on the host feeding patterns of *Cx. pipiens* s.s./*Cx. torrentium*

We found a total of 23 publications on host feeding patterns that used molecular assays to differentiate *Cx. pipiens pipiens*, *Cx. pipiens molestus*, *Cx. pipiens pipiens* × *molestus*, and *Cx. torrentium* (5 × USA [49–53]; 4 × Japan [54–57] [50–52, 54–56]; 3 × Spain [25, 58, 59]; 2 × each for Australia, Portugal, and UK [17, 60–64]; and 1 × each for Argentina, Iran, the Netherlands, Romania, and Russia [18, 65–68]). When this dataset was merged with our dataset, 1872 identified blood meals were available for *Cx. pipiens pipiens*, 460 for *Cx. pipiens molestus*, and 130 for *Cx. pipiens pipiens* × *molestus* (Fig. 3). No additional data from the literature were available for *Cx. torrentium*. Compared with the new data presented in this study for Germany, Iran, and Moldova with blood meals from birds < 50%, the three *Cx. pipiens* taxa in the merged dataset had more than 50% blood meals from birds, while human and mammalian species each had less than 30%.

Results from the different countries were heterogeneous. Studies from Romania, the USA, and Portugal showed that *Cx. pipiens pipiens* predominantly fed on birds, with up to 95.5% (Fig. 5). In contrast, higher proportions of mammalian taxa were observed for the newly collected data from Moldova and Germany (42.7% and 35.5%, respectively), and even reached 64.9% and 75.8% in the Netherlands and Iran, respectively. Similarly, low

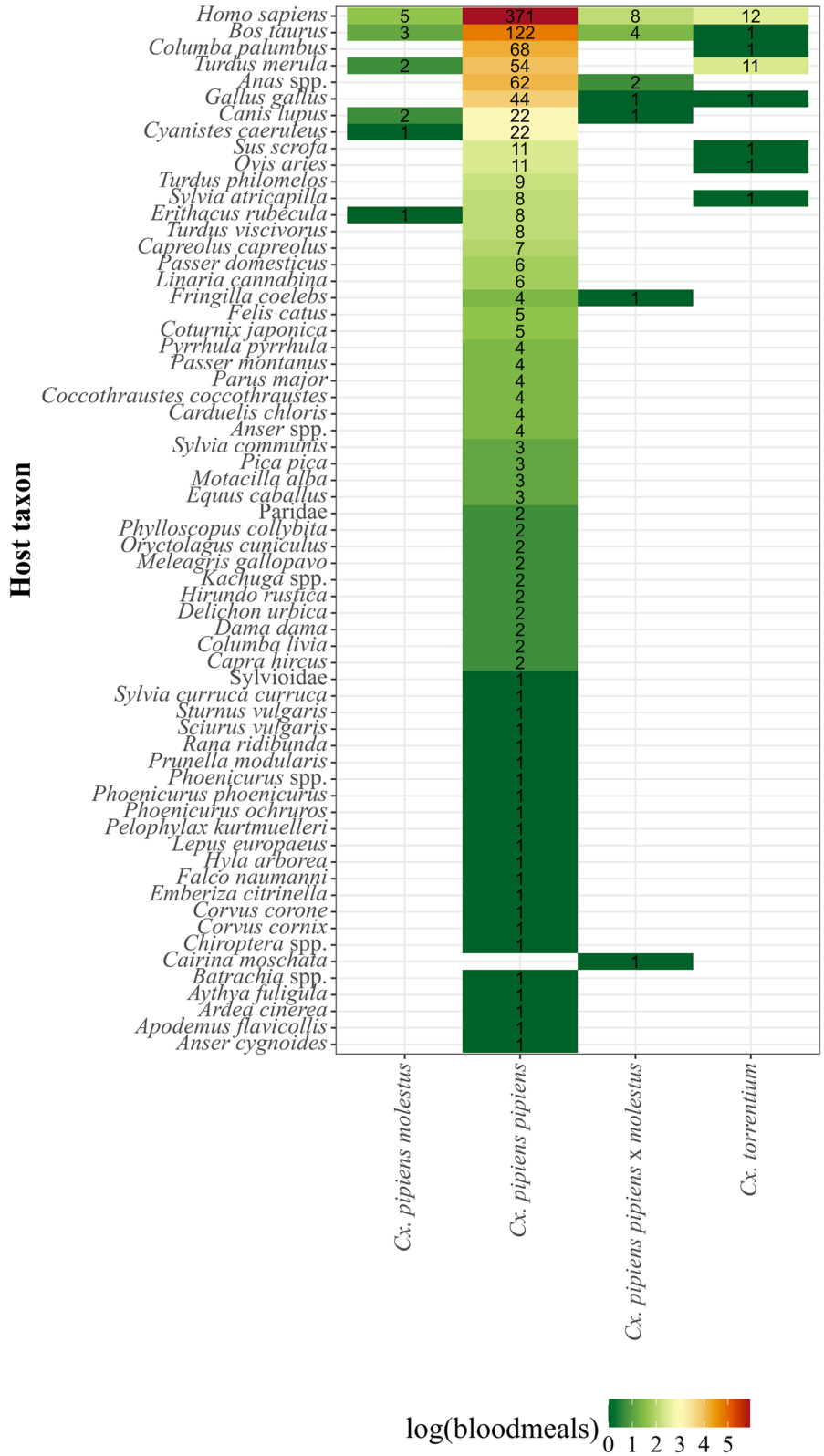

Fig. 4 Number of blood meals per host taxon detected in our studies for *Cx. pipiens molestus*, *Cx. pipiens pipiens*, *Cx. pipiens pipiens* × *molestus*, and *Cx. torrentium*

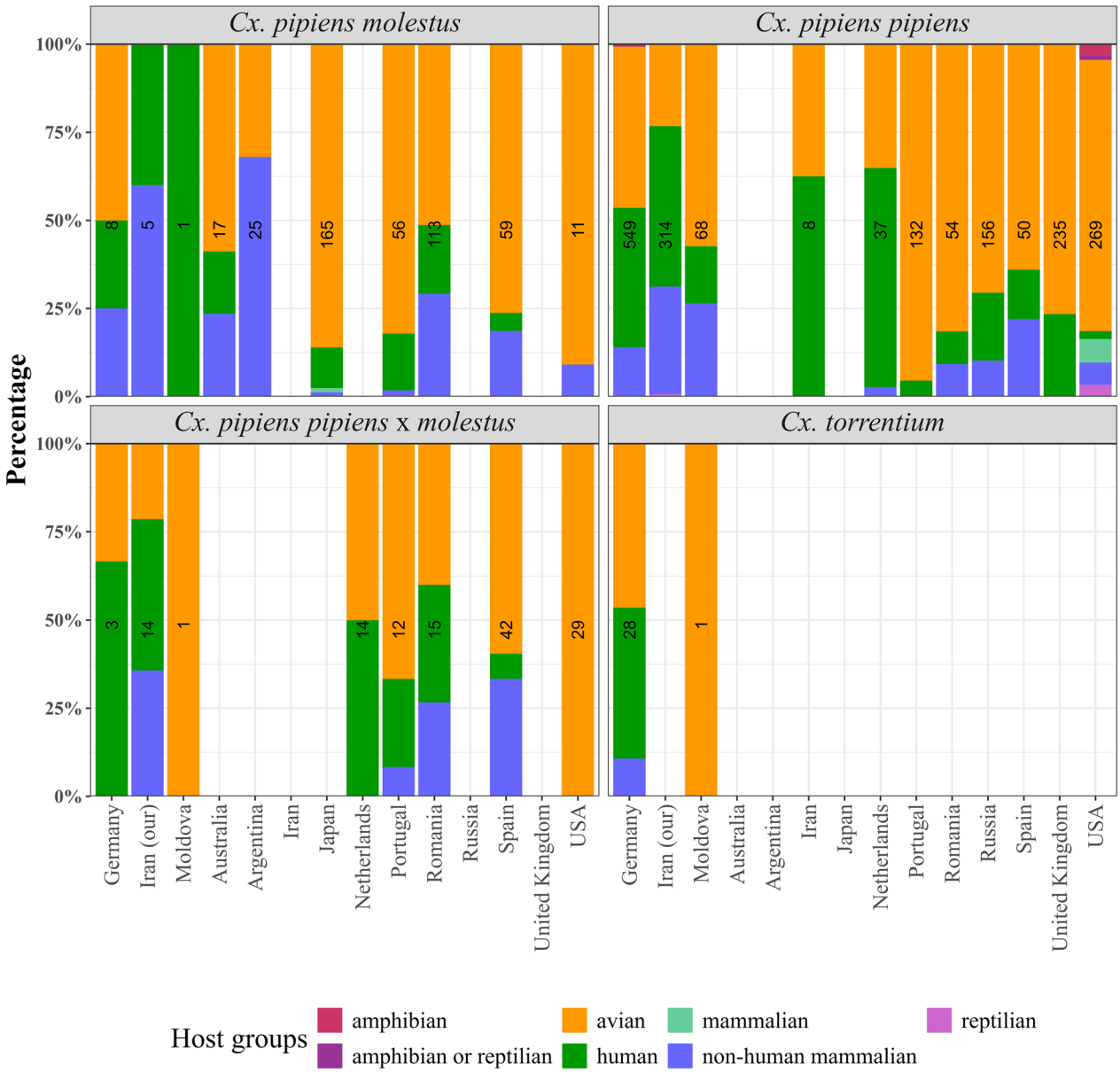

Fig. 5 Proportion of host groups for *Cx. pipiens molestus*, *Cx. pipiens pipiens*, *Cx. pipiens pipiens* × *molestus*, and *Cx. torrentium* per country. Data combined blood meals collected by us (Germany, Iran, Moldova) and data from the literature. Numbers in the bar indicate the number of blood meals per taxon and country. The host group "mammalian" is used if studies do not identify the mammalian species

proportions of mammalian hosts were observed for *Cx. pipiens molestus* in the USA, Spain, Japan, and Portugal (< 25%); around half of the feeds in Germany, Australia, and Romania; and a high proportion of 68% in Argentina. The few specimens from Iran and Moldova did not contain any avian blood. For *Cx. pipiens pipiens* × *molestus*, a dominance of mammals was found for Germany, Iran, the Netherlands, and Romania (> 50%); less than 50% for Portugal and Spain; and only blood meals from birds in the USA.

Discussion

Due to their wide distribution, abundance, and vector competence for WNV, USUV, or SINV, *Culex pipiens pipiens*, *Cx. pipiens molestus*, and *Cx. torrentium* are potentially important vectors of arboviruses in Europe [26–30]. The transmission cycles promoted by these vectors are shaped by their host-feeding patterns, i.e., maintaining enzootic cycles within one host group (e.g., birds) or leading to a spill-over from one host group to another.

We did not observe a significant attraction for mouse, grey canary, or human lure for *Cx. pipiens pipiens* and *Cx. torrentium*. In similar experiments conducted in the USA, *Cx. pipiens pipiens* showed a significant attraction for birds against mammals [69, 70]. For the USA it is especially discussed that hybridization between *Cx. pipiens pipiens* and *Cx. pipiens molestus* is the driver of host attraction with intermediate host acceptance for the hybrid taxon [70]. However, we did not find any differences in the host attraction between *Cx. pipiens pipiens* and *Cx. torrentium* either, which do not hybridize.

Host feeding patterns can differ from host choice experiments under laboratory conditions, that is, they are expected to depend on the availability and abundance of the hosts [8]. Many studies have been conducted worldwide to identify the blood hosts of more than 20,000 *Cx. pipiens* specimens [33], but only a few have differentiated the bioforms of *Cx. pipiens* s.s., and none included *Cx. torrentium*. Nevertheless, in the literature, *Cx. pipiens pipiens* is regularly referred to as ornithophilic/-phagic, whereas *Cx. pipiens molestus* is described as mammalophilic/-phagic or anthropophilic/-phagic [16–18, 71]. Unfortunately this terminology is not based on a standardized classification and is generally used without a clear definition [19].

Studies from the literature differentiating *Cx. pipiens* s.s./*Cx. torrentium* were collated here and showed that *Cx. pipiens pipiens* fed predominantly on avian hosts. Much less data were available for *Cx. pipiens molestus* and *Cx. pipiens pipiens* × *molestus*, but showed a similar pattern with a high proportion of birds. No data were available for *Cx. torrentium*. Nevertheless, there were considerable differences between the countries, with some combinations of countries and taxa reaching more than 62% mammalian hosts, for example, *Cx. pipiens pipiens* collected in the Netherlands [67] and *Cx. pipiens molestus* collected in Argentina. Additionally, for the field-collected specimens analyzed in our laboratory, a broad host use was observed with up to 50% mammalian hosts. The reasons for these differences can be manifold. First, only very few studies differentiated the *Cx. pipiens* s.s./*Cx. torrentium*. Worldwide, more than 20,000 undifferentiated *Cx. pipiens* specimens were analyzed and revealed a broad host feeding pattern with one-third of the blood meals from each human, avian, and nonhuman mammalian host [33]. Our studies on the host feeding patterns in Germany, Iran, and Moldova increased the total number of available taxa-specific information on the host feeding patterns of *Cx. pipiens* s.s./*Cx. torrentium* by two-thirds. Another factor might be the species identification of the different *Cx. pipiens* s.s./*Cx. torrentium* taxa, that is, *Cx. pipiens* s.s. host attraction is considered to be the result of genetic introgressive hybridization between *Cx. pipiens pipiens* and *Cx. pipiens molestus* populations [25]. In addition, host availability is often assumed to drive the host feeding patterns observed in the field [8], but this information is mostly not collected in the field. Our data from Germany, Iran, and Moldova analyzed with the same laboratory workflow showed statistically significant differences for the proportions of the different host groups, e.g., lower proportion of nonhuman mammals for Germany or lower proportion of birds for Iran. However, the underlying drivers of these differences remain unclear and need further evaluation in further work. Our previous studies in Germany and Iran showed that land-use as most obvious driver might not explain these differences in host feeding patterns [22, 23].

The birds mainly detected in blood meals of *Cx. pipiens pipiens* belonged especially to the species *Gallus gallus Columba palumbus, Hirundo rustica,*, and *Turdus merula.* The latter was also present in the feeds of *Cx. pipiens molestus Cx. pipiens pipiens* × *molestus* and dominated the feeds of *Cx. torrentium*. Of these bird species, especially the blackbird *Turdus merula* in particular is known to be part of the transmission cycle of WNV and USUV in Europe, as it was found to die in large numbers during USUV outbreaks [72–74]. At the same time, we observed considerable proportions of human hosts for each *Culex* taxon, highlighting their potential role as enzootic and bridge vectors.

In the field-collected *Culex* specimens analyzed in our laboratory, mixed blood meals were detected in 41 *Cx. pipiens pipiens* and one *Cx. torrentium* specimen. Up to now, only a few mixed blood meals have been described in the literature, for example, for *Cx. pipiens pipiens* or *Cx. pipiens molestus* [17, 49]. The detection of mixed blood meals is interesting information, as it is evidence of the transmission potential transmission risk between two host species. However, the frequency of mixed blood meals must be interpreted with caution. Generally, gel PCRs with subsequent Sanger sequencing were used to identify the blood meal hosts. Different primers have been shown to have different specificity [35], potentially influencing the sensitivity for different host taxa. The presence of gene fragments of two or more hosts could lead to overlapping signals after sequencing, which are difficult to distinguish from low-quality signals, for example, requiring advanced techniques using next-generation sequencing [75]. Thus, actual amounts of specimens with ingested blood of more than one host could be higher than observed.

Conclusions

Cx. pipiens pipiens, *Cx. pipiens molestus*, and *Cx. torrentium* were found to feed with a significant proportion on each avian, human and nonhuman mammalian

host. Thus, the classification of *Cx. pipiens pipiens* and *Cx. pipiens molestus* as strictly ornithophilic/-phagic and anthropo- or mammalophilic, respectively, should be reconsidered. The broad host range of these taxa combined with a high vector competence suggests a high relevance as both enzootic and bridge vectors in the transmission cycles of various mosquito-borne pathogens, for example, WNV, USUV, and SINV [26–30]. At the same time, we observed significant differences between data collected from different countries. Future studies especially should focus on the underlying intrinsic and extrinsic factors, e.g., the influence of population genetics, host availability, or general environmental conditions on the host feeding patterns.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-024-06439-7.

Additional file 1: Figure S1. Mean attraction with 95% confidence interval for host/attractant for *Culex pipiens pipiens* and *Culex torrentium*. Numbers on the bottom indicate the total number of specimens collected in the specific lard can trap over five replicates.

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Author contributions
Conceptualization: H.J., E.K., F.G.S., and R.L.; data collection: M.L.W., L.J., H.J., T.S., L.R., S.M.M.A., M.N., K.K., U.L., F.G.S., and R.L.; data analysis: M.L.W., L.J., H.J., F.G.S., and R.L.; first drafting: M.L.W., L.J., H.J., and R.L.; and writing and editing: all authors.

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Availability of data and materials
All data are available in the manuscript and in the supplementary files.

Declarations

Ethics approval and consent to participate
The competent authority (Regierungspräsidium Karlsruhe) classified the experimental trials not as animal experiments, as the used attractant animals were not exposed to pain, damage, or suffering.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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