# Trophic specialization of paludomid gastropods from 'ancient' Lake Tanganyika reflected by radular tooth morphologies and material properties

Dissertation zur Erlangung des Doktorgrades an der Fakultät für Mathematik, Informatik und Naturwissenschaften Fachbereich Biologie der Universität Hamburg

> vorgelegt von Wencke Krings Hamburg, 2020

Vorsitzender der Prüfungskommission: Prof. Dr. Jörg Ganzhorn Erst- und Zweitgutachter: Prof. Dr. Susanne Dobler, Prof. Dr. Stanislav Gorb Datum der Disputation: 06.11.2020

## Table of contents

Table of contents
Chapter 1. Introduction
Molluscan diversity and evolution7
Ecological niches and trophic specialization7
The radula as a Molluscan key innovation and mechanical interface
Hypotheses integrating tooth structure, mechanical properties, ecology, and evolution: model system Paludomidae from Lake Tanganyika
An approach to identify the driving mechanisms in gastropod evolution resulting in species diversity based on the radula
Objectives and hypotheses:
References 20
Chapter 2. Functional morphology of paludomid radular teeth
<ol> <li>1 Filling the lake: tracing convergent trophic specialization in the evolution of paludomid gastropods in Lake Tanganyika</li> </ol>
Abstract
Introduction
Materials and Methods
Results
Discussion and Conclusion 40
References
Supplementary Material
2. 2 Tightening it up: Diversity of the Chitin Anchorage of Radular-Teeth in paludomid Freshwater- Gastropods
Abstract
Introduction
Material and Methods
Results
Discussion
References
Supplementary Material

Chapter 3. Functional morphology and material properties of <i>Spekia zonata</i> – a case study	3
3. 1 Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods	3
Abstract 8	۲ ۲
Introduction	5
Matarials & Mathada	ر د
	0 2
Results	5
Discussion	5
References	0
3. 2 Finite element analysis of individual taenioglossan radular teeth (Mollusca)	5
Abstract	5
Introduction	7
Materials and Methods11	3
Results	0
Discussion	8
References	5
Supplements	3
Chapter 4. Trophic specialization	7
4. 1 Trophic specialisation reflected by radular tooth material properties in an 'ancient' Lake Tanganyikar	n 7
gastropod species nock	/ -
Abstract	/
Background	9
Results	4
Discussion	8
Methods	4
Declarations	7
Literature	8
Supplementary	6
Chapter 5. Summary and Discussion	1
Morphology, mechanical properties, and ecology19	1
The role of morphology (Objectives 1, 2, 4, 5; chapter 2.1, 2.2, 3.2)	1
The role of mechanical properties (functional gradients) in combination with morphology (Objectives 3, 4, 5; chapter 3.1, 3.2, 4.1)	8
The role of trophic specialization - from paludomid case study to Molluscan evolution?	5

Hypotheses on paludomid evolution (Objective 6; chapter 2.1, 2.2, 4.1)	205
Outlook	210
Evolutionary history and radular tooth functionality of Lake Tanganyikan paludomids	210
Understanding radular function	211
References	212
Chapter 6. List of Publications	223
Beiträge/Contributions	225
Eidesstattliche Versicherung/Declaration on oath	231
Danksagung	233

## Chapter 1. Introduction

#### Molluscan diversity and evolution

The Molluscan phylum, exhibiting an ancient evolutionary history with the oldest known common ancestor roaming the seafloor 550 Mya (e.g. Parkhaev 2008, 2017; Haszprunar & Wanninger 2012; Wanninger & Wollesen 2015), is the second speciose animal group (e.g. Chapman 2009). In particular the class Gastropoda, containing about 80,000 recent species (e.g. Bouchet et al. 2005), can be considered successful since they inhabit extraordinary distinct environments: from marine and freshwater habitats to deserts, from hydrothermal vents in the deep sea to mountaintops, from rocky areas to rainforests and urban regions. The colonialization of these ecosystems, not only by gastropods but various molluscan taxa, is enabled by their diverse body plans and amour systems, their complex nervous systems (e.g. Haszprunar & Wanninger 2012; Wollesen et al. 2017; Wanninger & Wollesen 2019), but probably also by the evolution of a key innovation for mechanical food gathering and processing, the radula. This complex and highly diverse organ enables the feeding on a wide range of sources with different mechanical properties throughout the phylum; this can be considered as one engine for species diversification and evolution possibly leading to the establishment of distinct ecological niches.

#### Ecological niches and trophic specialization

Hypotheses relating species diversity with the formation of ecological niches have seen various approaches, many of them based on vertebrates in a close phylogenetic context exhibiting a great morphological diversity (e.g. species flocks as result of adaptive radiations). Famous examples include the Darwin finches (e.g. Lack 1947; Grant 1986; Grant & Weiner 1999; Grant & Grant 2008, 2014; Farrington et al. 2014), or the cichlid fishes in the East African lakes (e.g. Barlow 2000; Seehausen 2006; Malinsky & Salzburger 2016; Rocco et al. 2019). But there are however also spectacular examples of invertebrate species flocks exhibiting a great diversity, especially among

7

molluscs (see also Glaubrecht 2009, 2011) as the lacustrine and riverine freshwater gastropods on Sulawesi (Rintelen et al. 2004, 2007, 2010; Rintelen & Glaubrecht 2005, Glaubrecht & Rintelen 2008; Hilgers et al. 2020), Madagascar (Köhler & Glaubrecht 2010), in the Thai rivers (Glaubrecht & Köhler 2004; Köhler et al. 2010), or 'ancient' Lake Tanganyika (Wilson et al. 2004; Glaubrecht & Strong 2007; Glaubrecht 2008), that could potentially be the result of an adaptive radiation.

In the search for drivers of evolution involving adaptations it is crucial to identify those, as in the context of trophic specialization morphological structures associated with feeding need to be examined. Bills or skulls in birds (e.g. on Darwin's finches: Herrel et al. 2005a, 2005b; Soons et al. 2015; Tokita et al. 2015; on oviraptorosaurian dinosaurs: Ma et al. 2020), skull bones in cichlid fishes (e.g. Muschick et al. 2012; Wanek & Sturmbauer 2015; Pérez-Miranda et al. 2020), or teeth in mammals (e.g. review on mammal teeth as interface Ungar 2015; analysis of food sources based on tooth interface e.g. Calandra et al. 2016; Winkler et al. 2019; Schulz-Kornas et al. 2020) can exhibit such functional adaptations and indicate trophic specialization as they serve as an interface between the organism and its ingesta (all substances that are taken in as food, minerals, particles covering the food, etc.). For Molluscan taxa, with the exception of all Bivalvia and some Gastropoda species characterized by the lack of this organ, the radula serves as such an interface and can be targeted in research approaches relating diversity with ecological niches and trophic specialisations.

#### The radula as a Molluscan key innovation and mechanical interface

In the 19th and early 20th century the radula was designated as a Molluscan autapomorphy. In the course of this recognition Troschel (1856–1863) introduced it as the most important character complex for systematics at any level resulting in Thiele (1931–1935) reorganizing the originally shell-based Molluscan classifications in accordance with radular characters. The Molluscan feeding organ, viz. the buccal mass, does not only comprise the radula but also odontophoral cartilages, subradular cartilages, various muscles, and in some taxa also the jaw. The radula itself consists of a non-extensible chitinous membrane (e.g. Guralnick & Smith 1999) with rows of embedded teeth (Fig. 1A). During resting it is tightly enrolled and stored in the oral cavity (Fig. 1B), but during feeding

action muscles of the buccal mass pull the radula across the odontophoral cartilages unfolding it. A system of diverse muscle groups moves it during feeding bringing the teeth, serving as actual interfaces, in a position which allows the acting on the ingesta (Fig. 1C). Traditionally the radula was compared to a flat ribbon or a 'belt-and-pulley' model (Ankel 1938; Märkel 1964; Solem 1974; Hickman 1980), but this view had fortunately been replaced (e.g. Morris & Hickman 1981). The precise radular motion is quite understudied, but previous research on the buccal mass muscles (e.g. Graham 1973; Fretter & Graham 1976; Crampton 1977; Morse 1984; Simone 2005, 2011; Evans et al. 2009; Golding et al. 2009; Balog et al. 2012) and feeding tracks (e.g. Ankel 1936, 1938; Eigenbrodt 1941; Hickman & Morris 1985; Janssen & Triebskorn 1987; Mackenstedt & Märkel 2001) already indicate that motion patterns are highly diverse in Molluscan taxa; complex and well controlled protractive motion of the membrane gives rise to sweeps, including the collecting of various particles and cutting and grinding action as potential ingesta may be too large to ingest in one or is fixed to the substrate. Thus, teeth can have distinct functions like cutting, tearing, and scratching but also gathering or raking when interacting with the ingesta. In this context it must be highlighted that due to bending and stretching of the membrane teeth presumably do not act as individual and independent structures, but rely on each other during feeding, transmitting and distributing forces and stresses from one tooth to another (e.g. Solem 1972; Morris & Hickman 1981;

Fig. 1. A. SEM image of the radula from Lavigeria grandis in natural orientation; distinct ontogenetic regions are highlighted: the building zone in the radular sack (RS) with immature teeth (yellow box), the working zone where teeth are actually used for acting on the ingesta (red box), and the digestive zone with worn teeth (blue box). B. Schematic illustration depicting the position of the radula during resting and C. during feeding (the arrow indicates the movement pattern observed in these taxa). BM = buccal mass, FP = food particle, RM = radular membrane, RS = radular sack, RT = radular tooth/teeth, SC = subradular cartilage. Scale bar =  $1000 \,\mu m$ .



Hickman 1984; Padilla 2004; Herrera et al. 2015; Montroni et al. 2019). The few studies approaching the complex interaction of teeth with the ingesta (Ankel 1938; Eigenbrodt 1941; Wägele 1983; Hawkins et al. 1989) are unfortunately on few taxa.

However, the acting on the ingesta leads to tooth wear (e.g. Runham & Thornton 1967; Mackenstedt & Märkel 1987; Franz 1990; Padilla et al. 1996; Shaw et al. 2002, 2010), but through a continuous growth of the radular ribbon in the radular sack tooth rows are constantly rebuilt. After passing the working zone they break loose in the degenerative zone (Fig. 1) (see e.g. Runham 1962; Mackenstedt & Märkel 1987). The recognition of high amounts of Fe in teeth of Polyplacophora and *Patella* facilitated the hypothesis that this leads to a higher wear resistance and the studies identifying tooth's mechanical properties (hardness, elasticity) relate this with these chemical constituents (e.g. van der Wal et al. 2000; Wealthall et al. 2005; Shaw et al. 2009, 2010; Weaver at al. 2010; Saunders et al. 2011; Han et al. 2011; Lu & Barber 2012; Wang et al. 2014; Barber et al. 2015). But most gastropod species – except *Patella* – are thought to have lost the ability of incorporating such elements (e.g. Simone 2011), even though the chemical properties of the vast majority of gastropod teeth are still understudied.

While the precise interaction between ingesta and tooth, the tooth's function, its chemical compositions, and mechanical properties still lacks a profound examination and understanding for most taxa, the radular tooth morphologies are quite well studied, especially after SEM imaging became more and commonplace in Malacology. Not the precise tooth morphology, but the quantities and arrangements of certain tooth types per row (central, lateral, marginal teeth; see Fig. 2) led to the categorization of radulae into about 6–8 basic types (e.g. docoglossan, rhipidoglossan, neritinomorph, taenioglossan, stenoglossan; Fig. 2) in order to gain the upper hand over the Molluscan diversity (e.g. Gray 1853; Hyman 1967; Steneck & Watling 1982; Haszprunar & Götting 2007). These basic types do not always and consistently reflect phylogeny between the major lineages due to convergences (Haszprunar et al. 2011), but overall the radula and its teeth are still a valuable character in taxonomy and systematics. However, the rather finer tooth morphology seems to be influenced by feeding ecology reflecting the various transitions from zoovorous to

10



Fig. 2. Images of radulae in feeding orientation representing some basic radular types (note the different quantities of teeth) collated with the shells of the species: A. Docoglossan, found in Polyplacophora taxa, here from *Lepidochitona cinerea*; B. Neritinomorph, found in Gastropoda, here from *Vittina natalensis*; C. Rhipidoglossan, found in Gastropoda, here from *Rochia conus*; D. Taenioglossan, found in Gastropoda, here from *Marisa cornuarietis*; E. '*Helix'*-form, found in Gastropoda, here from *Anentome helena* (image of this shell from garnelio.de). CT = central tooth, DT = dominant tooth, LT = lateral tooth, MT = marginal tooth. Scale bars of radulae: A, E = 100 µm, B, D = 200 µm, C = 400 µm, F = 40 µm; scale bars of shells: A = 5 mm, B = 30 mm, C = 60 mm, D = 15 mm, E = 2 mm, F = 3 mm.

herbivorous feedings traits (e.g. Solem 1974; Walsby 1975; Steneck & Watling 1982; Burch & Jeorg 1984; Blinn et al. 1989; Hawkins et al. 1989; Duda et al. 2001; Ukmar-Godec et al. 2015). In this context hypotheses on the evolution of feeding and competitor avoidance strategies based on the analysis of radular teeth have been put forward (e.g. Steneck & Watling 1982; Black et al. 1988; Blinn et al. 1989).

Additional to the food source, also the substrate that the food is attached to possibly influences tooth structure, not only morphology, but also their mechanical properties (e.g. hardness) and chemical composition (e.g. Breure & Gittenberger 1981; van der Wal et al. 2000). Unfortunately, the significance of substrate and ingesta in most Molluscan taxa is still poorly understood because current models are descriptive reports on differences in tooth shape and mechanical properties were measured in only few taxa resulting in a patchwork of information.

Overall, radular function is quite complex (see also Padilla 2004) and in order to approach the radular diversity and build hypotheses about tooth function (e.g. gathering, scratching) and to relate this with possible trophic adaptations (to the food source or the feeding substrate), we here established the following research program.

### <u>Hypotheses integrating tooth structure, mechanical properties, ecology, and evolution: model</u> <u>system Paludomidae from Lake Tanganyika</u>

All these past approaches unfortunately lack: (i) testable hypotheses; (ii) quantifiable characters; (iii) a synthetic model of ingesta driven evolution in gastropods; (iv) an evolutionary and biological context of the interpretations. The overflowing diversity of radulae (e.g. distinct quantities and arrangement of teeth, radular motion patterns involving different musculature and hence providing diverse interaction with surfaces and ingesta) complicates comparisons and especially the detection of possible trophic specializations reflected by radular structure. Therefore, a model system is here targeted that (a) has in a close phylogenetic context (a species flock), but (b) shows an interspecific diversity in radular tooth morphologies, (c) shows only little phenotypic plasticity, and (d) has a long history of biological research providing ecological and phylogenetic data. Due to their detailed

knowledge about anatomy and their life history (e.g. Leloup 1953; Brown 1994; Strong & Glaubrecht 2002, 2003, 2007, 2008, 2010; West et al. 2003; Glaubrecht 2008, 2010) and close phylogenetic relationship (Wilson et al. 2004; Glaubrecht & Strong 2007; Glaubrecht 2008) as well as their highly distinct radular tooth morphologies (see references above) the paludomid gastropods from Lake Tanganyika represent an ideal model system for the focus of this work.

Lake Tanganyika as the second deepest lake in the world is well known as natural lab in the search for drivers of evolution since it harbours unique assemblage of highly endemic species flocks not only of cichlid fish but also of paludomid gastropods. These gastropods, representing one of the most spectacular examples of closely related Molluscan taxa (about 50-70 species) with an extraordinary interspecific diversity in shell (Fig. 3) and radular tooth morphology (Fig. 4), have been of high research interest throughout the last centuries due to the question of their origin and evolution. Their marine-like appearance (termed 'thalassoid' by Bourguignat 1885 and 'halolimnic' by Moore 1898) resulted in the hypothesis that Lake Tanganyika was once connected to the ocean and triggered many expeditions and a long-lasting controversy about the origin and evolution of the lake and its fauna. However, this eventually led to the refutation of Moore's (1897, 1898, 1903) controversial hypothesis of the lake being once directly connected to the ocean and of a marine origin of its faunal elements (see e.g. in Hudleston 1904; Cunnington 1920; Yonge 1938; Brooks 1950; Hubendick 1952; Boss 1978; Coulter 1991) and recent geological data show that the lake has actually formed by rifting of the East African subcontinent 4–11 Mya (Lezzar et al. 2002; Spiegel et al. 2007; Bauer et al. 2010; Roller et al. 2010). For a long time the idea that this largest and deepest of the African lakes has supplied its gastropod fauna with a stable inland environment and offered unique opportunities for within-lake diversification ('ancient endemic radiation' see Cunnington 1920; Brooks 1950) resulting in a truly 'adaptive' radiation cf. Mayr (1963) and Mayr & Ashlock (1991) was common (e.g. Boss 1978; Johnston & Cohen 1987; Brown & Mandahl-Barth 1987; Berthold 1990; Coulter 1991; Michel et al. 1992; Michel 1994, 2000, 2004; West & Cohen 1996; Martens 1997; West & Michel 2000; Van Damme & Pickford 2003). However, strong evidence for an ancient origin of disparity and diversity in this flock has been presented, indicating the existence of major gastropod lineages before the formation of the lake itself or its proto-lakes. With a molecular clock approach in support (Wilson et al. 2004), this alternative hypothesis of the former existence of several originally riverine paludomid lineages later inhabiting the lake and bringing possible adaptations to former riverine environments with them was suggested by Glaubrecht & Strong (2007) and Glaubrecht (2008). However, in order to allocate hypotheses about paludomid evolution and radiation - especially in the context of adaptive radiation - the identification of actual adaptations, here in the radular teeth, is crucial.

Fig. 3. Shell habitus of some species belonging to the paludomid species flock from Lake Tanganyika and surrounding rivers systems; coloured boxes indicate the taxonomic groups: green box: Hauttecoeuriini, yellow box: Tiphobiini, blue box: Spekiini and Reymondia, turquoise box: Cleopatra (inhabits African river systems), red box: Lavigeria, orange box: Potadomoides (inhabits river systems adjacent to Lake Tanganyika). A. Stanleya neritinoides ZMB 102624, B. Tanganyicia rufofilosa ZMB 102621, C. Martelia tanganyicensis ZMB 220134, D. Syrnolopsis lacustris ZMB 220046, E. Anceya giraudi ZMB 220000, F. Chytra kirkii SMF 290543, G. Limnotrochus thomsoni SMF 290542, H. Paramelania crassigranulata SMF 290528, I. Paramelania iridescens SMF 290538, J. Bathanalia howesi DBL (without number), K. Paramelania imperialis SMF 290534, L. Paramelania damoni SMF 290531, M. Tiphobia horei SMF 290550, N. Leloupiella minima ZMB 220076, O. Bridouxia rotundata ZMB 220063, P. Bridouxia grandidieriana BMNH-1889.6.23.57-61, Q. Bridouxia giraudi MNHN-IM-2000-32552, R. Bridouxia praeclara DBL (without number), S. Reymondia pyramidalis ZMB 220220, T. Bridouxia ponsonbyi ZMB 220137, U. Spekia zonata ZMB 220158, V. Reymondia horei SMF 290494, W. Cleopatra bulimoides ZMB 31148, X. Lavigeria crassilabris ZMB 38254, Y. Lavigeria spinulosa ZMB 38254, Z. Lavigeria nassa ZMB 220172, AA. Lavigeria locardiana ZMB 220165, BB. Lavigeria grandis SMF 292827, CC. Potadomoides hirta MRAC H7H32/H7H33, DD. Potadomoides bequaerti MRAC H7355. BMNH = British Museum of Natural History, DBL = Danish Bilharziasis Laboratory, MNHN = Muséum National d'Histoire Naturelle, MRAC = Musée royal de l'Afrique Centrale, SMF = Senckenberg Museum Frankfurt, ZMB = Museum für Naturkunde Berlin. Scale bars: A-B, F-M, U-DD = 20 mm, C-E, N-T = 5 mm.





Fig. 4. Schematic illustrations of taenioglossan radular teeth from representative paludomid species from Lake Tanganyika and surrounding rivers systems (*Cleopatra*) indicating the diverse tooth morphologies present in this flock.

# An approach to identify the driving mechanisms in gastropod evolution resulting in species <u>diversity based on the radula</u>

The goal of this project (Fig. 5) is the integration of tooth structure and function, phylogeny, and ecology (substrate preference), and presenting it in an evolutionary context. The here developed functional models of the radula allow the testing of general hypotheses relating parameters in structure and species diversity. With this project and by defining mechanical and morphological properties in radular teeth in connection with niche properties we approach the question whether the found diversity, i.e. here disparity of the radula, in this gastropod species flock can possibly be explained with trophic specialization allowing niche formation and thus resulting e.g. in avoidance or reduction of competition. We here do not address the food (biofilm, algae) but focus on feeding substrate, which had been documented and described for Lake Tanganyikan paludomids by many collectors. Adaptations of radular teeth to feeding substrate has never been examined in such a comprehensiveness before.



Fig. 5. Schematic illustrations of the here presented research project involving radular tooth structure and function, phylogenetic approaches, and ecology (substrate preference; pictures of feeding substrate taken in Lake Tanganyika, from top to bottom: *Limnotrochus thomsoni* alive on algae covering soft substrate; *Tiphobia horei* alive on soft substrate; habitat of *Spekia zonata*, rocky shores; pictures are courtesy of Heinz Büscher who collected paludomid specimens for this project in Lake Tanganyika).

#### Objectives and hypotheses:

The project seeks to answer the following questions for the model system paludomid gastropods:

*Objective 1: To which extent does the morphology of teeth relate to the preferred feeding substrate (chapter 2.1)?* 

Hypothesis 1: Broader and thicker teeth can be found in solid-substrate feeders, thinner and more slender teeth can be found in soft-substrate feeders.

*Objective 2: Does the attachment of teeth with the membrane relate to the preferred feeding substrate (chapter 2.2)?* 

Hypothesis 2: A more intense attachment can be found in solid-substrate feeders, soft-substrate feeders show a lighter attachment.

*Objective 3: To which extent do the mechanical properties (here hardness and elasticity) relate to preferred feeding substrate (chapter 4.1)?* 

Hypothesis 3: Teeth of species that feed on solid substrate have harder and stiffer teeth than species feeding on soft substrate.

*Objective 4: How do morphology and material properties complement each other (chapter 3.2)? Hypothesis 4: Material properties and morphology have a high influence on the tooth's functionality.* 

*Objective 5: Can we identify distinct functions of teeth (e.g. scratching, gathering of food particles) in examined radulae and how does this relate with ecology (chapter 3.1, 3.2, 4.1)?* 

Hypothesis 5: Within each radula tooth types (central, lateral, marginal I and marginal II) have different morphologies and mechanical properties (e.g. functional gradients). This indicates monoor multifunctional feeding organs.

Objective 6: Is the high quantity of paludomid gastropod species the result of niche partitioning in respect to feeding substrate (chapter 2.1 and 4.1)?

Hypothesis 6: By analysing morphology and mechanical properties and relating this with the gastropods feeding substrate possible adaptations can be determined indicating niche partitioning and competitor avoidance.

#### <u>References</u>

- Ankel, W.E., 1936. Die Fraßspuren von *Helcion* und *Littorina* und die Funktion der Radula. Verh. Dtsch. Zool. Ges. Freib. 38, 174.
- Ankel, W.E., 1938. Erwerb und Aufnahme der Nahrung bei den Gastropoden. Verh. Dtsch. Zool. Ges., Zoologischer Anzeiger 11, 223–295.
- Balog, G., Voronezhskaya, E.E., Hiripi, L., Elekes, K., 2012. Organization of the serotonergic innervation of the feeding (Buccal) musculature during the maturation of the pond snail *Lymnaea stagnalis*: a morphological and biochemical study. J. Comp. Neurology 520, 315–329.
- Barber, A.H., Lu, D., Pugno, N.M., 2015. Extreme strength observed in limpet teeth. J. Royal Soc. Interface 12, 20141326.
- Barlow, G.W., 2000. The Cichlid Fishes: Nature's Grand Experiment in Evolution. Perseus Publishing, Cambridge.
- Bauer, F.U., Glasmacher, U.A., Ring, U., Schumann, A., Nagudi, B., 2010. Thermal and exhumation history of the central Rwenzori Mountains, Western rift of the east African rift system, Uganda. Int. J. Earth Sci. 99(7), 1575–1597.
- Berthold, T., 1990. Intralacustrine speciation and the evolution of shell sculpture in gastropods of ancient lakes application of Günther's niche concept. Abhandlungen des Naturwissenschaftlichen Vereins Hamburg (NF) 31/32, 85–118.
- Black, R., Lymbery, A., Hill, A., 1988. Form and Function: size of radular teeth and inorganic content of faeces in a guild of grazing molluscs at Rottnest Island, Western Australia. J. Exp. Mar. Biol. Ecol. 121, 23–35.
- Blinn, W., Truitt, R.E., Pickart, A., 1989. Feeding ecology and radular morphology of the freshwater limpet *Ferrissia fragilis*. J. N. Am. Benthol. Soc. 237–242.
- Boss, K.J., 1978. On the evolution of gastropods in ancient lakes. In: Pulmonates Systematics, Evolution and Ecology, Vol. 2a (Eds. V. Fretter and J. Peake). Academic Press, London.
- Bouchet, P., Rocroi, J.-P. (Ed.); Frýda, J., Hausdorf, B., Ponder, W., Valdes, A., Warén, A., 2005. Classification and Nomenclator of Gastropod Families. Malacologia 47(1-2). ConchBooks, Hackenheim.
- Bourguignat, M.J.R., 1885. Notice prodromique sur les mollusques terrestres et fluviatiles. Savy, Paris.
- Breure, A.S.H., Gittenberger, E., 1981. The rock-scraping radula, a striking case of convergence (Mollusca). Neth. J. Zool. 32, 307–312.
- Brooks, J.L., 1950. Speciation in ancient lakes. Q. Rev. Biol. 25, 30–60, 131–176.
- Brown, D., 1994. Freshwater Snails of Africa and their Medical Importance. Taylor and Francis, London.
- Brown, D.S., Mandahl-Barth, G., 1987. Living molluscs of Lake Tanganyika: a revised and annotated

list. J. Conchol. 32, 305–327.

Burch, J.B., Jeong, K.H., 1984. The Radula Teeth of selected Planorbidae. Malacological Review 17(1–2), 67–84.

- Calandra, I., Labonne, G., Schulz-Kornas, E., Kaiser, T.M., Montuire, S., 2016. Tooth wear as a means to quantify intra-specific variations in diet and chewing mechanics. Sci. Rep. 6, 34037.
- Chapman, A.D., 2009, Numbers of living species in Australia and the world, 2nd ed. Australian Biodiversity Information Services, Toowoomba, Australia.
- Coulter, G.W., 1991. Lake Tanganyika and its life. Oxford University Press, Oxford.
- Crampton, D., 1977. Functional anatomy of the buccal apparatus of Onchidoris bilamellata (Mollusca: Opisthobranchia). Trans. Zool. Soc. Lond. 34, 45–86.
- Cunnington, W.A., 1920. The fauna of the African Lakes: a study in comparative limnology with special reference to Tanganyika. Proc. Zool. Soc. Lond. 1920, 507–622.
- Duda, T.F., Kohn, A.J., Palumbi, S.R. 2001. Origins of diverse feeding ecologies within *Conus*, a genus of venomous marine gastropods. Biol. J. Linn. Soc. 73(4), 391–409.
- Eigenbrodt, H., 1941. Untersuchungen über die Funktion der Radula einiger Schnecken. Z. Morphol. Oekol. Tiere 37, 735–791.
- Evans, C.C.E., Dickinson, A.J.G., Croll, R.P., 2009. Major muscle systems in the larval Caenogastropod, *Ilyanassa obsoleta*, display different patterns of development. J. Morphol. 270, 1219–1231.
- Farrington, H.L., Lawson, L.P., Clark, C.M., Petren, K., 2014. The Evolutionary History of Darwin's Finches: Speciation, Geneflow, and Introgression in a Fragmented Landscape. Evolution 68(10), 2932–2944.
- Franz, C.J., 1990. Feeding patterns of *Fissurella* species on Isla de Margarita, Venezuela: use of radulae and food passage rates. J. Mollus. Stud. 56, 25–35.
- Fretter, V., Graham, A., 1976. A functional anatomy of Invertebrates. Academic Press, London, UK.
- Glaubrecht, M., 2008. Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology and systematization of a paludomid species flock in an ancient lake. Zoosystematics Evol. 84, 71–122.
- Glaubrecht, M., 2009. On "Darwinian Mysteries" or molluscs as models in evolutionary biology: From local speciation to global radiation. Am. Malacol. Bull. 27, 3–23.
- Glaubrecht, M., 2010. The enigmatic *Cleopatra broecki* Putzeys, 1899 of the Congo River system in Africa retransfer from Potadomoides Leloup, 1953 (Caenogastropoda, Cerithioidea, Paludomidae). Zoosystematics Evol. 86(2), 283–293.
- Glaubrecht, M., 2011. Toward solving Darwin's "mystery": speciation and radiation in freshwater gastropods. Am. Malacol. Bull. 29(1/2), 187–216.
- Glaubrecht, M., Köhler, F., 2004. Radiating in a river: systematics, molecular genetics and

morphological differentiation of viviparous freshwater gastropods endemic to the Kaek River, central Thailand. Biol. J. Linn. Soc. 82(3), 275–311.

- Glaubrecht, M., Rintelen, T.v., 2008. The species flocks of lacustrine gastropods: *Tylomelania* on Sulawesi as models in speciation and adaptive radiation. Proceedings of the "Speciation in Ancient Lake IV" Symposium, Berlin. Hydrobiologia 615, 181–199.
- Glaubrecht, M., Strong, E.E., 2007. Ancestry to an endemic radiation in Lake Tanganyika? Evolution of the viviparous gastropod *Potadomoides* Leloup, 1953 in the Congo River system (Cerithioidea, Paludomidae). Biol. J. Linn. Soc. 92(2), 367–401.
- Golding, R.E., Ponder, W.F., Byrne, M., 2009. Three-dimensional reconstruction of the odontophoral cartilages of Caenogastropoda (Mollusca: Gastropoda) using micro-CT: Morphology and phylogenetic significance. J. Morphol. 270, 558–587.
- Graham, A., 1973. The anatomical basis of function in the buccal mass of prosobranch and amphineuran molluscs. J. Zool. Lond. 169, 317–348.
- Grant, P.R., 1986. Ecology and Evolution of Darwin's Finches. Princeton University Press, Princeton.
- Grant, P.R., Grant, B.R., 2008. How and Why Species Multiply. The Radiation of Darwin's Finches. Princeton University Press, Princeton.
- Grant, P.R., Grant, B.R., 2014. 40 Years of Evolution: Darwin's Finches on Daphne Major Island. Princeton University Press, Princeton.
- Grant, P.R., Weiner, J., 1999. Ecology and Evolution of Darwin's Finches. Princeton University Press, Princeton.
- Gray, J.E., 1853. On the division of ctenobranchous gasteropodous Mollusca into larger groups and families. Ann. Mag. Nat. Hist. 11(2), 124–133.
- Guralnick, R., Smith, K., 1999. Historical and biomechanical analysis of integration and dissociation in molluscan feeding, with special emphasis on the true limpets (Patellogastropoda: Gastropoda). J. Morphol. 241, 175–195.
- Han, Y., Liu, C., Zhou, D., Li, F., Wang, Y., Han, X., 2011. Magnetic and structural properties of magnetite in radular teeth of chiton *Acanthochiton rubrolinestus*. Bioelectromagnetics 32, 226– 233.
- Haszprunar, G., Götting, K.J., 2007. Mollusca, Weichtiere. In: Spezielle Zoologie. Teil 1. Einzeller und wirbellose Tiere (Eds. W. Westheide and G. Rieger). Springer, Berlin.
- Haszprunar, G., Speimann, E., Hawe, A., Heß, M., 2011. Interactive 3D anatomy and affinities of the Hyalogyrinidae, basal Heterobranchia (Gastropoda) with a rhipidoglossate radula. ODE 11(3), 201–236.
- Haszprunar, G., Wanninger, A., 2012. Molluscs. Curr. Biol. 22, 510–514.
- Hawkins, S.J., Watson, D.C., Hill, A.S., Harding, S.P., Kyriakides, M.A., Hutchinson, S., Norton, T.A., 1989. A comparison of feeding mechanisms in microphagus, herbivorous, intertidal, Prosobranchs in relation to resource partitioning. J. Moll. Stud. 55, 151–165.

- Hawkins, S.J., Watson, D.C., Hill, A.S., Harding, S.P., Kyriakides, M.A., Hutchinson, S., Norton, T.A., 1989. A comparison of feeding mechanisms in microphagous, herbivorous, intertidal, prosobranchs in relation to resource partitioning. J. Molluscan Stud. 55(2), 151–165.
- Herrel, A., Podos, J., Huber, S.K., Hendry, A.P., 2005a. Bite performance and morphology in a population of Darwin's finches: implications for the evolution of beak shape. Funct. Ecol. 19(1), 43–48.
- Herrel, A., Podos, J., Huber, S.K., Hendry, A.P., 2005b. Evolution of bite force in Darwin's finches: a key role for head width. J. Evol. Biol. 18(3), 669–675.
- Herrera, S.A., Grunenfelder, L., Escobar, E., Wang, Q., Salinas, C., Yaraghi, N., Geiger, J., Wuhrer, R., Zavattieri, P., Kisailus, D., 2015. Stylus support structure and function of radular teeth in *Cryptochiton stelleri*. 20th International Conference on Composite Materials Copenhagen, 19-24th July 2015.
- Hickman, C.S., 1980. Gastropod radulae and the assessment of form in evolutionary paleontology. Paleobiology 6, 276–294.
- Hickman, C.S., 1984. Implications of radular tooth-row functional-integration for archaeogastropod systematics. Malacologia 25, 143–160.
- Hickman, C.S., Morris, T.E., 1985. Gastropod feeding tracks as a source of data in analysis of the functional-morphology of radulae. Veliger 27, 357–365.
- Hilgers, L., Hartmann, S., Pfaender, J., Lentge-Maaß, N., Rintelen, T.v., Hofreiter, M., 2020. Radula diversification promotes ecomorph divergence in an adaptive radiation of freshwater snails. bioRxiv.01.17.910034.
- Hubendick, B., 1952. On the evolution of the so-called thalassoid molluscs of Lake Tanganyika. Arkiv för Zoologi 3, 319–323.
- Hudleston, W.H., 1904. On the origin of the marine (halolimnic) fauna of Lake Tanganyika. Geol. Mag. Suppl. 337–382.
- Hyman, L.H., 1967. Mollusca I. Aplacophora, Polyplacophora, Monoplacophora. Gastropoda, the Coelomate Bilateria. The Invertebrates 6. McGraw-Hill Book Company, New York.
- Janssen, H.H., Triebskorn, R., 1987. Comparative morphology of the radulae in *Pomatia elegans* and in *Littorina littorea* (Gastropoda: Taenioglossa). Zool. Anz. 219(1/2), 73–82.
- Johnston, M.R., Cohen, A.S., 1987. Morphological divergence in endemic gastropods from Lake Tanganyika: implications for models of species flock formation. Palaios 2, 413–425.
- Köhler, F., Glaubrecht, M., 2010. Uncovering an overlooked radiation: morphological and mitochondrial DNA differentiation in endemic freshwater snails on Madagascar (Caenogastropoda: Pachychilidae) and their biogeography. Biol. J. Linn. Soc. 99(4), 867–894.
- Köhler, F., Panha, S., Glaubrecht, M., 2010. Speciation and Radiation in a River: Assessing the Morphological and Genetic Differentiation in a Species Flock of Viviparous Gastropods (Cerithioidea: Pachychilidae). In: Evolution in Action. Case Studies in Adaptive Radiation, Speciation and the Origin of Biodiversity (Ed. M. Glaubrecht). Springer, Heidelberg.

Lack, D., 1947. Darwin's Finches. Cambridge University Press, Cambridge.

- Leloup, E., 1953. Exploration hydrobiologique du Lac Tanganika (1946–1947). Bruxelles.
- Lezzar, K.E., Tiercelin, J.J., Le Turdu, C., Cohen, A.S., Reynolds, D.J., Le Gall, B., Scholz, C.A., 2002. Control of normal fault interaction on the distribution of major Neogene sedimentary depocenters, Lake Tanganyika, East African rift. AAPG Bull. 86, 1027–1059.
- Lu, D., Barber, A.H., 2012. Optimized nanoscale composite behaviour in limpet teeth. J. Royal Soc. Interface 9, 1318–1324.
- Ma, W., Brusatte, S.L., Lü, J., Sakamoto, M., 2020. The skull evolution of oviraptorosaurian dinosaurs: the role of niche partitioning in diversification. J. Evol. Biol. 33(2), 178–188.
- Mackenstedt, U., Märkel, K., 1987. Experimental and comparative morphology of radula renewal in pulmonates (Mollusca, Gastropoda). Zoomorphology 107, 209–239.
- Mackenstedt, U., Märkel, K., 2001. Radular structure and function. In: The biology of terrestrial molluscs (Ed. G.M. Barker). CABI Publishing, Oxon.
- Malinsky, M., Salzburger, W., 2016. Environmental context for understanding the iconic adaptive radiation of cichlid fishes in Lake Malawi. PNAS 113(42), 11654–11656.
- Märkel, K., 1964. Modell-Untersuchungen zur Klärung der Arbeitsweise der Gastropodenradula. Verh. Dtsch. Zool. Ges. 18, 232–243.
- Martens, K., 1997. Speciation in ancient lakes. Trends Ecol. Evol. 12(5), 177–182.
- Mayr, E., 1963. Animal, species, and evolution. Harvard University Press, Cambridge.
- Mayr, E., Ashlock, P.D., 1991. Principles of systematic zoology. McGraw-Hill, New York.
- Michel, E., 1994. Why snails radiate: a review of gastropod evolution in long-lived lakes, both Recent and fossil. In: Speciation in ancient lakes (Eds. K. Martens, B. Goddeeris, G. Coulter). Advances in Limnology, Stuttgart.
- Michel, E., 2000. Phylogeny of a gastropod species flock: exploring speciation in Lake Tanganyika in a molecular framework. In: Biology of Ancient Lakes. Advances in Ecological Research. (Ed. A. Rossiter). Academic Press, London.
- Michel, E., 2004. *Vinundu*, a new genus of Gastropod (Cerithioidea: 'Thiaridae) with two species from Lake Tanganyika, east Africa, and its molecular phylogenetic relationships. J. Molluscan Stud. 70, 1–19.
- Michel, E., Cohen, A.S., West, K., Johnston, M.R., Kat, P.W., 1992. Large African lakes as natural laboratories for evolution: examples from the endemic gastropod fauna of Lake Tanganyika. Mitteilungen der Internationalen Vereinigung für Limnologie 23, 85–99.
- Montroni, D., Zhang, X., Leonard, J., Kaya, M., Amemiya, C., Falini, G., Rolandi, M., 2019. Structural characterization of the buccal mass of *Ariolimax californicus* (Gastropoda; Stylommatophora). PLoS One 14(8), e0212249.
- Moore, J.E.S., 1897. The fresh-water fauna of Lake Tanganyika. Nature 56, 198–200.

- Moore, J.E.S., 1898. Descriptions of the genera *Bathanalia* and *Bythoceras*, from Lake Tanganyika. Proc. Malacol. Soc. Lond. 3, 92–93.
- Moore, J.E.S., 1903, The Tanganyika problem. Burst and Blackett, London.
- Morris, T.E., Hickman, C.S., 1981. A method for artificially protruding gastropod radulae and a new model of radula function. Veliger 24, 85–89.
- Morse, M.P., 1984. Functional Adaptations of the Digestive System of the Carnivorous Mollusc *Pleurobranchaea californica* MacFarland. 1966. J. Morphol. 180, 253–269.
- Muschick, M., Indermaur, A., Salzburger, W., 2012. Convergent evolution within an adaptive radiation of cichlid fishes. Curr. Biol. 22, 2362–2368.
- Padilla, D.K., 2004. Form and function of radular teeth of herbivorous molluscs: Focus on the future. Am. Malacolog. Bull. 18, 163–168.
- Padilla, D.K., Dittman, D.E., Franz, J., Sladek, R., 1996. Radular production rates in two species of *Lacuna* Turton (Gastropoda: Littorinidae). J. Mollus. Stud. 62, 275–280.
- Parkhaev, P.Y., 2008. The early Cambrian radiation of Mollusca. In: Phylogeny and Evolution of the Mollusca (Eds. W.F. Ponder and D.R. Lindberg). University of California Press, Berkeley.
- Parkhaev, P.Y., 2017. Origin and the early evolution of the phylum Mollusca. Paleontol. J. 51, 663–686.
- Pérez-Miranda, F., Mejía, O., González-Díaz, A.A., Martínez-Méndez, N., Soto-Galera, E., Zúñiga, G., Říčan, O., 2020. The role of head shape and trophic variation in the diversification of the genus *Herichthys* in sympatry and allopatry. J. Fish Biol. 96(6), 1370–1378.
- Rintelen, T.v., Bouchet, P., Glaubrecht, M., 2007. Ancient lakes as hotspots of diversity: a morphological review of an endemic species flock of *Tylomelania* (Gastropoda: Cerithioidea: Pachychilidae) in the Malili lake system on Sulawesi, Indonesia. Hydrobiologia 592(1), 11–94.
- Rintelen, T.v., Glaubrecht, M., 2005. Anatomy of adaptive radiation: a unique reproductive strategy in the endemic freshwater gastropod *Tylomelania* (Cerithiodea: Pachychilidae) on Sulawesi, Indonesia and its biogeographical implications. Biol. J. Linn. Soc. 85(4), 513–542.
- Rintelen, T.v., Rintelen, K.v., Glaubrecht, M., 2010. The species flocks of the viviparous freshwater gastropod *Tylomelania* (Mollusca: Cerithioidea: Pachychilidae) in the ancient lakes of Sulawesi, Indonesia: The role of geography, trophic morphology and color as driving forces in adaptive radiation. In: Evolution in Action. Case Studies in Adaptive Radiation, Speciation and the Origin of Biodiversity (Ed. M. Glaubrecht). Springer, Heidelberg.
- Rintelen, T.v., Wilson, A.B., Meyer, A., Glaubrecht, M., 2004. Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. Proc.
  R. Soc. Lond. 271(1557), 2541–2549.
- Roller, S., Hornung, J., Hinderer, M., Ssemmanda, I., 2010. Middle Miocene to Pleistocene sedimentary record of rift evolution in the southern Albert rift (Uganda). Int. J. Earth Sci. 99, 1643–1661.

- Ronco, F., Büscher, H.H., Indermaur, A., Salzburger, W., 2019. The taxonomic diversity of the cichlid fish fauna of ancient Lake Tanganyika, East Africa. J. Great Lakes Res., doi:10.1016/j.jglr.2019.05.009.
- Runham, N.W., 1962. Rate of replacement of the molluscan radula. Nature 194, 992–993.
- Runham, N.W., Thornton, P.R., 1967. Mechanical wear of the gastropod radula: a scanning electron microscope study. J. Zool. 153, 445–452.
- Saunders, M., Kong, C., Shaw, J.A., Clode, P.L., 2011. Matrix-mediated biomineralization in marine mollusks: A combined transmission electron microscopy and focused ion beam approach. Microsc. Microanal. 17, 220–225.
- Schulz-Kornas, et al., 2020. Everything matters: molar microwear texture in goats (*Capra aegagrus hircus*) fed diets of different abrasiveness. Palaeogeogr. Palaeoclimatol. Palaeoecol. 552, 109783.
- Seehausen, O., 2006. African cichlid fish: A model system in adaptive radiation research. Proc. R. Soc. Lond. B 273(1597), 1987–1998.
- Shaw, J.A., Brooker, L.R., Macey, D.J., 2002. Radula tooth turnover in the chiton, *Acanthopleura hirtosa* (Blainville, 1825) (Mollusca: Polyplacophora). Molluscan Res. 22, 93–99.
- Shaw, J.A., Macey, D.J., Brooker, L.R., Clode, P.L., 2010. Tooth Use and Wear in Three Iron-Biomineralizing Mollusc Species. Biol. Bull 218, 132–144.
- Shaw, J.A., Macey, D.J., Brooker, L.R., Stockdale, E.J., Saunders, M., Clode, P.L., 2009. The chiton stylus canal: An element delivery pathway for tooth cusp biomineralization. J. Morphol. 270, 588–600.
- Simone, L., 2005. Comparative morphological study of representatives of the three families of Stromboidea and the Xenophoroidea (Mollusca, Caenogastropoda), with an assessment of their phylogeny. Arquivos de Zoologia 37(2), 141–267.
- Simone, L., 2011. Phylogeny of the Caenogastropoda (Mollusca), based on comparative morphology. Arquivos de Zoologia 42(4), 161–323.
- Solem, A., 1972. Malacological applications of scanning electron microscopy II. Radular structure and functioning. Veliger 14, 327–336.
- Solem, A., 1974. The shell makers: introducing mollusks. Jon Wiley & Sons, New York.
- Soons, J., Genbrugge, A., Podos, J., Adriaens, D., Aerts, P., Dirckx, J., Herrel, A., 2015. Is Beak Morphology in Darwin's Finches Tuned to Loading Demands? PLoS One 10(6).
- Spiegel, C., Kohn, B.P., Belton, D.X., Gleadow, A.J.W., 2007. Morphotectonic evolution of the Central Kenya rift flanks: implications for late Cenozoic environmental change in East Africa. Geology 35, 427–430.
- Steneck, R.S., Watling, L., 1982. Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. Marine Biology 68, 299–319.
- Strong, E.E., Glaubrecht, M., 2002. Evidence for convergent evolution of brooding in an unique gastropod from Lake Tanganyika: anatomy and affinity of *Tanganyicia rufofilosa*

(Caenogastropoda, Cerithioidea, Paludomidae). Zool. Scr. 31(2), 167–184.

- Strong, E.E., Glaubrecht, M., 2003. Anatomy and systematic affinity of *Stanleya neritinoides* (Smith, 1880), an enigmatic member of the thalassoid gastropod species flock from Lake Tanganyika, East Africa (Cerithioidea: Paludomidae). Acta Zool. 84(4), 249–265.
- Strong, E.E., Glaubrecht, M., 2007. The morphology and independent origin of ovoviviparity in *Tiphobia* and *Lavigeria* (Caenogastropoda: Cerithioidea: Paludomidae) from Lake Tanganyika. Org. Divers. Evol. 7(2), 81–105.
- Strong, E.E., Glaubrecht, M., 2008. Anatomy and systematics of the minute Syrnolopsine gastropods from Lake Tanganyika (Caenogastropoda, Cerithioidea, Paludomidae). Acta Zool. 89(4), 289–310.
- Strong, E.E., Glaubrecht, M., 2010. Anatomy of the Tiphobiini from Lake Tanganyika (Cerithioidea, Paludomidae). Malacologia 52(1), 115–153.
- Thiele, J., 1931–1935. Handbuch der systematischen Weichtierkunde. Gustav Fischer, Jena.
- Tokita, M., Yano, W., James, H.F., Abzhanov, A., 2016. Cranial shape evolution in adaptive radiations of birds: comparative morphometrics of Darwin's finches and Hawaiian honeycreepers. Philos. Trans. R. Soc. B. 372(1713), 20150481.
- Troschel, F.H., 1856–1863. Das Gebiss der Schnecken zur Begründung einer natürlichen Classification. Berlin, Germany.
- Ukmar-Godec, T., Kapun, G., Zaslansky, P., Faivre, D., 2015. The giant keyhole limpet radular teeth: A naturally-grown harvest machine. J. Struct. Biol. 192(3), 392–402.
- Ungar, P.S., 2015. Mammalian dental function and wear: A review. Biosurf. Biotribol. 1, 25–41.
- Van Damme, D., Pickford, M., 2003. The late Cenozoic Thiaridae (Mollusca, Gastropoda, Cerithioidea) of the Albertine Rift Valley (Uganda-Congo) and their bearing on the origin and evolution of the Tanganyikan thalassoid malacofauna. Hydrobiologia 498, 1–83.
- van der Wal, P., Giesen, H., Videler, J., 2000. Radular teeth as models for the improvement of industrial cutting devices. Mater. Sci. Eng. C 7, 129–142.
- Wägele, H., 1983. Rasterelektronenmikroskopische Untersuchungen an Radulae einiger Nordseeschnecken (Gastropoda: Prosobranchia) mit Anmerkungen zur Funktionsmorphologie. Drosera 83(1), 68–78.
- Walsby, J.R., 1975. Feeding and the Radula in the Marine Pulmonate Limpet, *Trimusculus reticulatus*. Veliger 18, 139–145.
- Wanek, K.A., Sturmbauer, C., 2015. Form, function and phylogeny: comparative morphometrics of Lake Tanganyika's cichlid tribe Tropheini. Zool Scr. 44(4), 362–373.
- Wang, C., Li, Q.Y., Wang, S.N., Qu, S.X., Wang, X.X., 2014. Microstructure and self-sharpening of the magnetite cap in chiton tooth. Mater. Sci. Eng. C 37, 1–8.
- Wanninger, A., Wollesen, T., 2015. Mollusca. In: Evolutionary Developmental Biology of Invertebrates. Volume 2: Lophotrochozoa (Spiralia) (Ed. A. Wanninger). Springer, Wien.

Wanninger, A., Wollesen, T., 2019. The evolution of molluscs. Biol. Rev. 94, 102–115.

- Wealthall, R.J., Brooker, L.R., Macey, D.J., Griffin, B.J., 2005. Fine structure of the mineralized teeth of the chiton *Acanthopleura echinata* (Mollusca: Polyplacophora). J. Morphol. 265, 165–175.
- Weaver, J.C., Wang, Q., Miserez, A., Tantuccio, A., Stromberg, R., Bozhilov, K.N., Maxwell, P., Nay, R., Heier, S.T., DiMasi, E., 2010. Analysis of an ultra hard magnetic biomineral in chiton radular teeth. Mater. Today 13, 42–52.
- West, K., Cohen, A., 1996. Shell microstructure of gastropods from Lake Tanganyika, Africa: adaptation, convergent evolution, and escalation. Evolution 50(2), 672–681.
- West, K., Michel, E., 2000. The dynamics of endemic diversification; molecular phylogeny suggests an explosive origin of the thiarid gastropods of Lake Tanganyika. Adv. Ecol. Res. 31, 331–354.
- West, K., Michel, E., Todd, J., Brown, D., Clabaugh, J., 2003. The Gastropods of Lake Tanganyika: Diagnostic key, classification and notes on the fauna. Special publications: Societas Internationalis Limnologiae - Int. Assoc. of Theoretical and Applied Limnology.
- Wilson, A.B., Glaubrecht, M., Meyer, A., 2004. Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika. Proc. Royal Soc. Lond. B 271(1538), 529–536.
- Winkler, D.E., Schulz-Kornas, E., Kaiser, T.M., Tütken, T., 2019. Dental microwear texture reflects dietary tendencies in extant Lepidosauria despite their limited use of oral food processing. P. Roy. Soc. B 286(1903), 20190544.
- Wollesen, T., Scherholz, M., Rodriguez-Monje, S.V., Redl, E., Todt, C., Wanninger, A., 2017. Brain regionalization genes are coopted into shell field patterning in Mollusca. Sci. Rep. 7, 5486.

Yonge, C.M., 1938. The prosobranchs of Lake Tanganyika. Nature 142, 464–466.

### Chapter 2. Functional morphology of paludomid radular teeth

# 2. 1 Filling the lake: tracing convergent trophic specialization in the evolution of paludomid gastropods in Lake Tanganyika

Wencke Krings<sup>1\*</sup>, Marco T. Neiber<sup>1</sup>, Matthias Glaubrecht<sup>1</sup>

<sup>1</sup> Center of Natural History (CeNak), Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

\*corresponding author: wencke.krings@uni-hamburg.de

#### <u>Abstract</u>

Lake Tanganyika harbours high proportions of endemic and morphologically distinct taxa, not only in cichlids but also paludomid gastropods. The latter form a flock of special interest due to longlasting debates about its origin and the processes that might have contributed to its evolution. Moreover, those gastropods are remarkable because of their distinct shell shapes and heteromorphic radular tooth morphologies. In order to shed light on the evolutionary history, we present a well-resolved phylogenetic hypothesis for the Paludomidae from Lake Tanganyika and adjacent river systems. It connects evidence from a molecular clock approach suggesting that species diversity evolved long before the formation of the Lake Tanganyika basin. Through independent migration events several lineages of riverine paludomids colonized the lake. Additionally the radular tooth morphologies were evaluated and the most characteristic ones were related with the gastropods' specific feeding substrate. Subsequently, an ancestral state reconstruction for these morphologies and substrates was conducted, allowing insights into the role of ecological speciation. Our results strongly indicate that several riverine lineages brought preadaptations to the former riverine environments into the lake. Taxa evolved convergently radular teeth adapted to foraging algae from solid or mixed substrates, even secondary adaptations to soft substrates were recorded.

Keywords: Tanganyika; adaptive radiation; Paludomidae; ancestral state reconstruction; radula

#### Introduction

Lake Tanganyika as the second deepest lake in the world belongs to the African Great Lakes. Next to Lake Malawi and Lake Victoria it is a major reservoir of freshwater diversity in Africa, harbouring high proportions of endemic and morphologically distinct taxa, e.g. not only cichlids (for Lake Tanganyika: at least 241 species, 99.17 % of them are endemic, [1]) but also bivalves and gastropods [2]. These lakes, with their surrounding rivers, have been of high interest because they provide natural laboratories for investigating the drivers of speciation under specific ecological conditions. Here, one 'engine' is ecological speciation by adaptive divergence [3–7], which is most likely a key feature particularly in sympatry [8–14]. Evidence for such a process can be parallel evolution, i.e. the development of the same morphologies in closely related but independent lineages under identical extrinsic conditions [8, 15-23]. In adaptive radiations convergent or parallel evolution of phenotypes is a widespread phenomenon. In most scenarios these lineages originated in geographic isolation from each other (e.g. similar ecotypes of anoles lizards on different Caribbean islands [24-25], stickleback fish in post-glacial lakes [15, 18], spider ecomorphs on Hawaiian Islands [26–27], and cichlid fishes in the East African Lakes Malawi and Tanganyika [28-29]) before colonializing the same habitat. But there are examples of parallel evolution in sympatry as well; recent theoretical models [30] predict that convergence should be common within all species-rich assemblages. Specifically, these models predict this process for "species-saturated" communities where the number of species exceeds the number of available niches [31]. [32] described multiple instances of convergence in body and trophic morphology in cichlid fish from Lake Tanganyika and proposed that the parallel phenotypes resulted from the combination of coexistence in the same habitat and the limited number of available niches. Distantly related, yet ecomorphologically similar species show convergent morphologies that can be associated with adaptations to specific, limited habitats and resources and parallel evolution therefore offers an explanation for the greatly elevated species numbers in cichlid species [32].

In order to develop plausible scenarios or hypotheses explaining the evolution of certain lineages, the identification of adaptations is crucial, especially in the context of ecological speciation, explicitly trophic specialization. Morphological structures like bills in birds, vomer bones in cichlid fishes or teeth in mammals that serve as an interface between the organism and its ingesta (e.g. food or the substrate the food is attached to) can provide insights into evolution of these taxa. The radula is the mechanical organ of ingesta processing in gastropods and acts as such as an interface. Its teeth can show very distinct morphologies and are considered adaptive to food or feeding substrate, showing various kinds of ecological specializations and are hence closely associated with feeding strategies, competitor avoidance and thus trophic specialization [33–42].

The paludomid gastropods from Lake Tanganyika and its surrounding rivers form a species flock [see 43 for a definition of the term] and are of high interest not only because of their distinct shell morphologies, but also in the context of trophic specialization since they show an extraordinarily high interspecific diversity in their radular tooth morphologies; this flock had thus been the focus of many studies [e.g. 44–54]. The evolutionary history of these gastropods had been under considerable debate: the idea that this largest and deepest of the African lakes has supplied its gastropod diversity with a stable inland environment and offered unique opportunities for within-lake diversification ['ancient endemic radiation' see 55–56] resulting in a truly 'adaptive' radiation [cf. 57–58] had been present for a long time [59–67] and still is [68]. But strong evidence for an ancient origin of disparity and diversity, long before the formation of the lake itself (the oldest formation estimates are 9–12 Mya) or its proto-lakes [69–71], followed by independent colonization events of different paludomid lineages into the Lake Tanganyikan basin had been presented [44, 72]. This is supported by phylogenetic trees and molecular clock approaches [68], unfortunately interpreted differently.

Here we present a comprehensive phylogeny of the Tanganyikan Paludomidae and their sister taxa from the surrounding river systems including a molecular clock approach. Since molecular data is not consistently and completely available for all taxa, we combine molecular and morphological data and established a systematization [see 73 for further details of this term]. Instead of a single adaptive radiation within the lake we alternatively anticipate the former existence of several lineages of originally riverine snails, much older than Lake Tanganyika, colonializing the lake at different times. This adds to recent studies on the origin of Lake Tanganyikan cichlid tribes emphasizing the differentiation of Lake Tanganyikan precursor cichlid lineages in ancient rivers and precursor lakes followed by an amalgamation in the extant Lake Tanganyika [e.g. 74–75].

For reconstructing the evolutionary history of the African Paludomidae and the processes that could have contributed to their diversity we evaluated the different radular tooth morphologies and correlated those with their specific feeding substrates (soft, mixed, or solid), since radular teeth as highly functionate interfaces do not only interact with the food but also with the substrate the food is attached to. Our results strongly indicate that several riverine paludomid species brought preadaptations [*sensu strictu* 76; refers to a change in function with little change in structure or morphology] in their radula to the former river environments with them and evolved convergently teeth adapted to different substrates, possibly within Lake Tanganyika.

#### Materials and Methods

#### Phylogeny

Specimens were identified based on shell morphology in comparison with type material, following [44]. Available DNA sequences of African paludomids including the riverine species were taken from previous studies [59, 69], Melanoides tuberculata (Müller, 1774) [77] was used as outgroup. Total genomic DNA from species, which have not been sequenced before, was taken from museum specimens stored in 70% ethanol and was extracted using a modified version of the protocol for mucopolysaccharide-rich tissues [78] as detailed in [79] (see Supplementary Tab. 1). Partial sequences of the mitochondrial 16S rRNA (16S) gene and the cytochrome c oxidase subunit I (COI) gene were targeted. Sequences were amplified by PCR using the primer pairs LCO1490 plus HCO2198 [80] for COI as well as 16S F mod plus 16S R AS1 [81] and 16S F Thia2 plus 16SR\_FA\_AWmod [81] and cycling conditions for mitochondrial genes as detailed in [79]. Sequencing was carried out at Macrogen Europe Laboratory (Amsterdam, The Netherlands). The sequences were aligned with MAFFT 7 [82] using the Q-INS-I algorithm, the  $1PAM/\kappa = 2$  option for the scoring matrix for nucleotide sequences and otherwise default settings. Appropriate data partitions and models of nucleotide evolution were selected using PartitionFinder 2 [83]. Maximum likelihood analysis with Garli 2.0 [84], Bayesian inference with MrBayes 3.2.6 [85] and maximum parsimony analysis with Paup\* version 4b10 [86] were used to reconstruct phylogenetic relationship. Identified tribes were named in accordance with [87].

#### Molecular clock

We dated the divergence times for the African Paludomidae included in this study using the Bayesian algorithm implemented in BEAST 2 [88] based on the concatenated mitochondrial data set assuming an uncorrelated relaxed log-normal molecular clock as the test implemented in MEGA 7 [89] rejected a strict molecular clock at  $\alpha$  = 0.05. The same partitioning scheme and nucleotide substitution models as in the MRBAYES analysis described above was used. As tree prior the Yule speciation model was chosen. Calibration was based on previous results obtained from the study of

[68]. A normal prior was applied to the calibration node (i.e. African Paludomidae) assuming a mean age of 47.5 Ma +/- 2.5 Ma [see also 68] corresponding to the Early Eocene. The BEAST 2 analysis was run for 100,000,000 generations with a sampling frequency set to 10,000. TRACER 1.7 [90] was used to assess convergence of runs and to check whether effective sample sizes for all estimated parameters were above 200. A maximum clade credibility tree with median node heights was calculated with TREEANNOTATOR v1.8.2 from the BEAST 2 package discarding 10% of generations of each run as burn-in.

#### Systematization

No molecular information could be obtained for *Potadomoides* Leloup, 1953 [46] because of the age of the samples, storage conditions and the lack of fresh material since attempt failed to collect it at it type locality, the Malagarasi River. By comparing morphological characters and identifying synapomorphies, it is however possible to place species in the resulting phylogeny leading to a systematization [see 73 for further details of this term]. Thus, since *Potadomoides* and *Lavigeria* show similar morphological features [see also 44, 72], this riverine taxon was placed tentatively as sister group to the lacustrine *Lavigeria*.

#### Radular characters, feeding substrates, ancestral state reconstruction

For each species radulae (see Supplementary Tab. 1) were prepared following the protocol of [91] and visualized with SEM. Radular morphologies were characterized according to [44, 54] or information about the character states were taken from [44, 72, 92–98]. Character states that can be found in all paludomids were excluded and only character states that are most likely linked with substrate were analysed, overall six (see Supplementary and Fig. 1). Our characters focus mostly on the central teeth because they differ extremely in their morphology between soft substrate and solid substrate feeders (Fig. 1). Studied species are substrate-specific, information on the feeding substrate (algae from rocks = solid substrate; algae from sand = soft substrate; algae from plant surfaces and soft substrate = mixed substrate; algae from rocks and sand = mixed substrate) was

taken from the literature [44, 46, 49–50, 59] or is based on data from the collectors in the field (Heinz Büscher, Matthias Glaubrecht) and was included in the ancestral state reconstruction. Ancestral states and feeding substrates were reconstructed and mapped on the molecular phylogeny using the maximum likelihood algorithm implemented in Mesquite 3.10 [99].



Fig. 1. Radular character states of the central, lateral, inner and outer marginal tooth with SEM images of representative taxa. Character states are matched with certain colours also used in Fig. 2.

#### <u>Results</u>

#### Phylogeny

The phylogenetic analyses support four major clades of African paludomid gastropods (Fig. 2). Group 1: Spekiini Ancey, 1906 [100], *Reymondia* Bourguignat, 1885 [101] and the mostly riverine Cleopatrini Pilsbry & Bequaert, 1927 [102]; Group 2: Hauttecoeuriini Bourguignat, 1885 [101], including the Syrnolopsini Bourguignat, 1890 [103]; Group 3: Tiphobiini Bourguignat, 1886 [104]; Group 4: Nassopsini Kesteven, 1903 [105], including *Vinundu* Michel, 2004 [93] and *Lavigeria* Bourguignat, 1888 [106].

#### Molecular clock

Our molecular clock analysis (Fig. 2) estimates the first differentiation to 47 Mya (between Group 4 and the lineage leading to Group 1–3) according to our calibration point. Group 3 has its origin 39.5 Mya and Group 1 and 2 were estimated to have had their last common ancestor 31.9 Mya. Within Group 1 the Cleopatrini were estimated to have originated about 24.7 Mya. However, differentiation patterns within certain groups are also much younger: the origin of the Nassopsini species ranges from 0.2 to 8 Mya depending on the species, *Bridouxia* Bourguignat, 1885 [101] species differentiated 5.8 Mya and *Syrnolopsis* species 0.4 Mya.

#### Ancestral state reconstruction, feeding substrates, radular characters

The reconstructed ancestral feeding substrate is soft (Fig. 2), changes to solid substrate can be detected within Group 1 (in *Reymondia* and the Spekiini) after the differentiation between this lineage and the Cleopatrini; within the Hauttecoeuriini (in *Stanleya* Bourguignat, 1885 [101] and *Tanganyicia* Crosse, 1881 [107]) after the differentiation between this lineage and the lineage leading to *Martelia* Dautzenberg, 1907 [108], *Anceya* Bourguignat, 1885 [101], and *Syrnolopsis* Smith, 1880 [109]; within the Tiphobiini (in *Bathanalia* Moore, 1898a [110]) after the differentiation between the differentiation between the differentiation between Bathanalia and *Chytra* Moore, 1898a [110]; within the Nassopsini after the differentiation
between them and the lineage leading to Group 1–3. The estimates from the molecular clock analysis date the change in feeding substrate for the Nassopsini to 24.2 Mya, for Group 1 to 22.4 Mya, within the Hauttecoeuriini to 20.1 Mya, within the Tiphobiini to 11.8 Mya. Within the Spekiini, for *Bridouxia grandidieriana* (Bourguignat, 1885 [101]), we can detect a secondary shift from solid to mixed (soft substrate and algae attached to plant surfaces) feeding substrate dated to 5.8 Mya. Within the Tiphobiini we found a shift from soft to mixed (soft and solid) feeding substrate for *Limnotrochus thomsoni* 19.5 Mya and *Paramelania damoni* 10.6 Mya.

The reconstructed ancestral states (Fig. 2) for the different tooth types are: a long, slender central tooth (CT) with numerous denticles of equal size, a lateral tooth (LT) with one main and few smaller denticles, an inner marginal tooth (IMT) with many denticles (up to 10) confined to the distal tip, an outer marginal tooth (OMT) with numerous (more than 10) denticles.

After the differentiation between the lineage leading to Potadomoides and the Nassopsini and the lineage leading to Group 1–3 (47.4 Mya), we can detect the evolution of a spatulate or prominent central denticle on the CT and a reduction in the amount of denticles for the IMT and OMT in the Group constituted of Potadomoides and the Nassopsini. After the differentiation of the Hauttecoeuriini and Group 1 (31.9 Mya) we can see a shift from a long and slender CT to a squarish CT in Group 1, still found in the recent Cleopatrini. Additionally, Cleopatra Troschel, 1856 [111] evolved IMT with numerous, small denticles, parallel to Leloupiella Neiber & Glaubrecht, 2019 [87]. In the Spekiini and Reymondia a broad CT appeared showing all kinds of reductions in the amount of denticles (Reymondia: one prominent denticle, Bridouxia grandidieriana: few long denticles of equal size, B. ponsonbyi (Smith, 1889 [112]) and Spekia Bourguignat, 1879 [113]: no middle denticles, but small denticles flanking the sides; Leloupiella: no denticles). In Leloupiella we can, besides of the change in the morphology in the IMT, detect also a change in the LT: an increased amount of denticles resulting in numerous denticles of almost equal size. Within the Hauttecoeuriini Martelia evolved a broad CT and an IMT with denticles extending along the edges of the stylus, Stanleya a LT with numerous denticles of almost equal size, Tanganyicia displays an IMT not with numerous but many denticles, hence the amount of denticles is here decreased.

Within the Tiphobiini the lineage leading to *Bathanalia* and *Chytra* evolved a squarish CT, whereas *Limnotrochus* Smith, 1880 [109] and *Paramelania iridescens* (Moore, 1898b [114]) convergently evolved a broad CT. In the LT the ancestral state is retained, only in *P. damoni* (Smith, 1881 [115]) the LTs show an increased amount of denticles. In the IMTs we can detect a different location of the denticles, they are not confined to the distal tip, but extend along the edges of the stylus (convergent in *Bathanalia, Tiphobia* Smith, 1880 [109], and *P. damoni*) or along the outer edge (convergent in *Chytra* and *Limnotrochus*). *Limnotrochus* and *P. iridescence* display an increased amount of denticles in the OMT can be observed. Within the Nassopsini most ancestral character states are retained, only *Vinundu* displays a squarish CT as well as a reduction of denticles on the OMT. *Lavigeria grandis* (Smith, 1881 [115]) and *L. livingstoniana* (Bourguignat, 1885 [101]) show an increased amount of denticles on the OMTs.

When correlating the feeding substrate with the tooth morphologies we can see, that feeding on solid substrate goes along with three morphologies of the CT (Fig. 2) (a) a long and slender CT with a spatulate or prominent and central denticle (in Nassopsini and *Reymondia*), (b) a wide and broad CT with few or no denticles along the outer edges (in *Bridouxia, Spekia,* and *Leloupiella*) characterizing the sister clade of the Cleopatrini, and (c) a CT with broad and long denticles of the same size (*Stanleya, Bathanalia,* and *Tanganyicia*). Sometimes species foraging on solid substrate exhibit a LT bearing a prominent denticle (Nassopsini, *Reymondia, Bridouxia praeclara, B. ponsonbyi, B. rotundata, Spekia, Tanganyicia, and Bathanalia*).

Fig. 2. Systematization of the paludomid species of Lake Tanganyika and the adjacent river systems. Results from the molecular clock analyses, estimated in Mya, are given at the nodes. Blue background indicates the existence of Lake Tanganyika or a proto-lake and hence the time span when a colonialization of the lake from the surrounding river systems could have taken place. Feeding substrate (grey: solid, brown: soft, green: plant surfaces, white: no information), character states (coloured boxes resemble character states from Fig. 1), and reconstructed character states on the branches are depicted. Bayesian posterior probability values (left) as well as maximum likelihood (middle) and maximum parsimony (right) bootstrap values are given at the nodes (above the line).



#### Discussion and Conclusion

The geological age of the Lake Tanganyika basin is subject of an ongoing debate. The oldest cited maximum age is based on sedimentation rates and sets the geological origin of the proto-Lake, formed through rifting of the East African subcontinent in an area of junction of several rivers to 9–12 Mya [69–71]. But this are maximum ages and more recent studies date the pre-rift formation to 4–11 Mya and the earliest onset of a true rifting activity to 5.5 Mya [117–120].

Comparing the age estimates for the Lake Tanganyikan paludomid tribes obtained from the molecular clock approach with the estimated ages for the formation of the lake basin we propose that the lineages of the four groups evolved before the formation of Lake Tanganyika or a protolake, respectively. The tribes have their origin probably in the surrounding river systems. Our results go hand in hand with recent studies on the divergence ages of the most recent common ancestor of several cichlid tribes in Lake Tanganyika [74] providing substantial evidence for an origin of Lake Tanganyikan cichlids that predates the formation of the lake. Depending on the group results estimate the origin of the Lake Tanganyikan cichlid tribes between 11.89 and 25.34 Mya, accordingly the occurrence of an independent diversification of Lake Tanganyikan cichlid precursor lineages in different drainages and precursor lakes is followed by an amalgamation in the extant Lake Tanganyikan basin. Lake Tanganyika has direct faunal exchange with the surrounding water bodies, e.g. river systems, because of changes in hydrographic connectivity [2; this has also been reported for Tanganyikan paludomid taxa found in the adjacent river systems 121]; hence several Tanganyikan cichlid clades contain derived taxa that only occur in the Malagarasi River or the Congo River [122–123]. Similar patterns can be observed for the paludomid gastropods, not only due to the results of the phylogenetic analysis with Cleopatra clustering within the Tanganyikan paludomid species flock, but also in combination with the molecular dating approach and the systematization that includes Potadomoides. [59, 68] hypothesize that paludomid gastropods colonized the lake once followed by *Cleopatra*, having its origin within the lake, and distributing with about 20 species [49] across Africa and Madagascar. Alternatively, we already proposed in [44, 72] that several lineages of extinct Paludomidae migrated into the lake from surrounding river systems at different times. Most lineages have an ancient origin, older than the lake, only few lineages are younger. The

origin of the Nassopsini species ranges from 8 to 0.2 Mya depending on the taxon. *Bridouxia* differentiated 5.8 Mya and *Syrnolopsis* 0.4 Mya, hence after the formation of the lake [e.g. 69–71]. These tribes are probably the only lineages that have diversified within the lake. *Potadomoides* and *Cleopatra* (and possibly *Pseudocleopatra* with about 4 species [49] which is probably closely related to *Cleopatra* [124]) are presumably the only remaining riverine lineages.

#### Result of trophic specialization?

The reconstructed ancestral states of the feeding substrate and teeth suggest an ancestral riverine paludomid species adapted to feeding on soft substrate. This species displayed long and slender teeth with numerous denticles of equal size, all tooth types were similar in their morphology. This radula could be used for raking algae from a soft sediment and can be described as 'monofunctional'; it can be seen as preadaptation [*sensu strictu* 76] to the former riverine soft substrate.

When correlating the tooth morphologies with the feeding substrate, it is important to highlight, that the only available information on the feeding substrate of *Potadomoides* is based on [46] describing the locality, the Malagarasi delta, of *P. pelseneeri* Leloup, 1953 [46] as muddy substrate. Unfortunately, *Potadomoides* has not been found again in the last decades, hence we do not have more information about its specific feeding substrate. But the Malagarasi River as well as the habitat of the other *Potadomoides* taxa, the Congo River drainage, is not only characterized by swampy areas with soft substrate but contains also rapids with rocks. From its radular tooth characters very similar to *Lavigeria* species, we rather conclude that *Potadomoides* also feeds on algae from solid substrates. If this is indeed the case the ancestor of the Nassopsini species and *Potadomoides* would also have been a solid-substrate feeder, bringing this preadaptation in its tooth morphologies into Lake Tanganyika. We thus conclude that within the rivers, probably in rapids, two lineages evolved radular teeth adapted to feeding algae from solid substrates, viz. ancestor of (i) Spekiini and *Reymondia* as well as (ii) Nassopsini and *Potadomoides*. This has been facilitated by evolving more distinct tooth types, a 'multifunctional' radula showing either (a) a long and slender CT with a spatulate or prominent central denticle (ancestor of 1. Nassopsini and *Potadomoides*, 2. *Reymondia*)

or (b) a wide and broad CT with few or no denticles along the outer edges (ancestor of Spekiini). The here proposed function of the CTs is scratching across the substrate loosening food [see also 125–126]. During this action, the muscular force is deemed to be transferred by the tooth onto the substrate, this results in tensile and compressive stresses, which in turn result in an elastic deformation in contact. Since these teeth are rather short and broad with few denticles deformations are reduced. *Limnotrochus thomsoni* and *Paramelania damoni* are exceptional since their tooth morphology resembles soft substrate feeders even though they are also capable to forage on solid feeding substrate at least for some time. This shift to mixed substrate probably evolved in the riverine environment as well.

Some riverine, soft substrate feeding species, presumably adapted after the colonialization of Lake Tanganyika convergently to solid substrate (ancestor of 1. *Bathanalia*, 2. *Stanleya*, and *Tanganyicia*). This could have taken place in the course of an exclusively intralacustrine process and involved the evolution of a CT with broad and long denticles of the same size.

The other lacustrine species are still adapted to foraging on soft substrates carrying their ancestral characters, long and slender teeth with numerous denticles of equal size (*Martelia, Anceya, Syrnolopsis, Chytra, Tiphobia,* and most *Paramelania* Smith, 1881 [115] species) for gathering algae from a sandy or muddy substrate. We propose that *Bridouxia grandidieriana,* as the only Spekiini species foraging on soft substrate and on plant surfaces (mixed substrate), became secondly adapted within Lake Tanganyika by evolving a CT with small denticles allowing the structure to serve as a scraper with tines [work on *Bridouxia* as a candidate of an in situ adaptive radiation is accepted for publication in Plos One].

We propose that one main engine in the evolution of paludomids was trophic specialization on different feeding substrates (soft, mixed, and solid ones), allowing the coexistence of several taxa in the same environment by the formation of different niches. Additionally, we found evidence for parallel evolution: morphological adaptations in the CT for loosening algae from solid substrates evolved at least five times within this flock. Interestingly, the morphological adaptations of the CT resulted in different general tooth morphologies, involving a spatulate or prominent central

denticle, broad and long denticles of the same size, or a wide and broad CT. Hence, tooth phenotypes of species sharing a similar ecological niche are still distinct but seem to follow a general pattern. As proposed by [32] for cichlid fish from Lake Tanganyika, stating that the found parallel phenotypes result from the combination of coexistence in the same habitat and the limited number of available niches, we can detect a similar pattern for the lacustrine paludomids.

Our study resulted not only in an essentially well-resolved phylogenetic hypothesis for African paludomids, it also connects evidence from a molecular clock approach supporting the hypothesis of independent colonialization events into Lake Tanganyika from adjacent river systems. The correlation of the distinct radular tooth morphologies with the feeding substrates in connection with ancestral state reconstructions allow insights into the role of ecological speciation within the evolutionary history of these gastropod lineages.

# <u>Acknowledgements</u>

The authors acknowledge Renate Walter (Department of Biology, University of Hamburg) for her support with the SEM imaging and Benedikt Wiggering (CeNak, University of Hamburg) for his input and fruitful discussions, and Heinz Büscher for collecting specimens in the field and documenting the feeding substrate. The authors thank the anonymous reviewers for their constructive suggestions.

# Authors' contributions

MG initiated and designed the study, WK and MN compiled and analysed the data. WK drew the figures and wrote drafts of the manuscript together with the other two authors who contributed equally to the final version of the paper.

#### <u>References</u>

- 1. Ronco F, Büscher HH, Indermaur A, Salzburger W. 2019 The taxonomic diversity of the cichlid fish fauna of ancient Lake Tanganyika, East Africa. *J. Great Lakes Res.* (doi:10.1016/j.jglr.2019.05.009)
- 2. Salzburger W, Van Bocxlaer B, Cohen AS. 2014 Ecology and Evolution of the African Great Lakes and Their Faunas. *Annu. Rev. Ecol. Evol. Syst.* **45**(1), 519–545. (doi:10.1146/annurev-ecolsys-120213-091804)
- 3. Nosil P. 2012 *Ecological Speciation*. Oxford: Oxford University Press.
- 4. Mayr E. 1942 *Systematics and the origin of species from the viewpoint of a zoologist*. New York: Columbia University Press.
- 5. Mayr E. 1947 Ecological factors in speciation. *Evolution* **1**(4), 263–288. (doi:10.1111/j.1558-5646.1947.tb02723.x)
- 6. Schluter D. 2001 Ecology and the origin of species. *Trends. Ecol. Evol.* **16**, 372–380. (doi:10.1016/S0169-5347(01)02198-X)
- 7. Rundle HD, Nosil P. 2005 Ecological speciation. *Ecol. Lett.* **8**, 336–352. (doi:10.1111/j.1461-0248.2004.00715.x)
- 8. Glaubrecht M. 2011 Toward solving Darwin's "mystery": speciation and radiation in freshwater gastropods. *Am. Malacol. Bull.* **29**, 187–216. (doi:10.4003/006.029.0211)
- 9. Friesen ML, Saxer G, Travisano M, Doebeli M. 2004 Experimental evidence for sympatric ecological diversification due to frequency-dependent competition in *Escherichia coli*. *Evolution* **58**, 245–260. (doi:10.1111/j.0014-3820.2004.tb01642.x)
- 10. Jiggins CD. 2008 Ecological Speciation in Mimetic Butterflies, *BioScience* **58**(6), 541–548. (doi:10.1641/B580610)
- Schwarz D, Robertson H, Feder J, Varala K, Hudson M, Ragland G et al. 2009 Sympatric ecological speciation meets pyrosequencing: sampling the transcriptome of the apple maggot *Rhagoletis pomonella*. *BMC Genom.* **10**, 633. (doi:10.1186/1471-2164-10-633)
- Hadid Y, Tzur S, Pavlícek T, Šumbera R, Šklíba J, Lövy M et al. 2013 Possible incipient sympatric ecological speciation in blind mole rats (*Spalax*). *Proc. Natl. Acad. Sci. U.S.A.* **110**, 2587–2592. (doi:10.1073/pnas.1222588110)
- Lemoine M, Barluenga M, Lucek K, Mwaiko S, Haesler M, Chapman LJ et al. 2019 Recent sympatric speciation involving habitat-associated nuptial colour polymorphism in a crater lake cichlid *Hydrobiologia* 832, 297. (doi:10.1007/s10750-018-3746-1)
- Fulgione D, Buglione MB, Rippa D, Trapanese M, Petrelli S, Monti DM et al. 2019 Selection for background matching drives sympatric speciation in Wall Gecko. *Sci. Rep.* 9, 1288 (2019). (doi:10.1038/s41598-018-37587-3)
- 15. Schluter D, Nagel LM. 1995 Parallel speciation by natural selection. Am. Nat. 146, 292–301.
- Pigeon D, Chouinard A, Bernatchez L. 1997 Multiple modes of speciation involved in the parallel evolution sympatric morphotypes of Lake Whitefish (*Coregonus clupeaformis*, Salmonidae). *Evolution* 421, 196–205. (doi:10.1111/j.1558-5646.1997.tb02401.x)
- 17. McPeek MA, Wellborn GA. 1998 Genetic variation and reproductive isolation among phenotypically divergent amphipod populations. *Limnol. Oceanogr.* **43**, 1169. (doi:10.4319/lo.1998.43.6.1162)
- 18. Rundle HD, Nagel L, Boughman JW, Schluter D. 2000 Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287**, 306–308. (doi:10.1126/science.287.5451.306)
- 19. Taylor EB, McPhail JD. 2000 Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus. Proc. R. Soc. Lond. B.* **267**(1460), 2375–2384. (doi:10.1098/rspb.2000.1294)
- Mundy NI, Badcock NS, Hart T, Scribner K, Janssen K, Nadeau NJ. 2004 Conserved genetic basis of a quantitative plumage trait involved in mate choice. *Science* **303**, 1870–1873. (doi:10.1126/science.1093834)
- 21. Foster SA, Baker JA. 2004 Evolution in parallel: New insights form a classic system. *Trends. Ecol. Evol.* **19**, 456–459. (doi:10.1016/j.tree.2004.07.004)
- 22. Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G Jr., Dickson M, Grimwood JJ et al. 2005 Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science* **307**, 1928–1933.

(doi:10.1126/science.1107239)

- 23. Schluter D. 2009 Evidence for Ecological Speciation and Its Alternative. *Science* **323**(5915), 737–741. (doi:10.1126/science.1160006)
- Losos JB, Jackman TR, Larson A, Queiroz K, Rodriguez-Schettino L. 1998 Contingency and determinism in replicated adaptive radiations of island lizards. *Science* 279, 2115–2118. (doi:10.1126/science.279.5359.2115)
- 25. Losos JB, Leal M, Glor RE, De Queiroz K, Hertz PE, Rodriguez-Schettino, L et al. 2003 Niche lability in the evolution of a Caribbean lizard community. *Nature* **424**, 542–545. (doi:10.1038/nature01814)
- 26. Blackledge TA, Gillespie RG. 2004 Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 16228–16233. (doi:10.1073/pnas.0407395101)
- 27. Gillespie R. 2004 Community assembly through adaptive radiation in Hawaiian spiders. *Science* **303**, 356–359. (doi:10.1126/science.1091875)
- Kocher TD, Conroy JA, McKaye KR, Stauffer JR. 1993 Similar morphologies of cichlid fish in Lakes Tanganyika and Malawiare due to convergence. *Mol. Phylogenet. Evol.* 2, 158–165. (doi:10.1006/mpev.1993.1016)
- 29. Salzburger W. 2009 The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Mol. Ecol.* **18**, 169–185. (doi:10.1111/j.1365-294X.2008.03981.x)
- 30. Scheffer M, van Nes EH. 2006 Self-organized similarity, the evolutionary emergence of groups of similar species. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 6230–6235. (doi:10.1073/pnas.0508024103)
- 31. terHorst CP, Miller TE, Powell E. 2010 When can competition for resources lead to ecological equivalence? *Evol. Ecol. Res.* **12**, 843–854.
- 32. Muschick M, Indermaur A, Salzburger W. 2012 Convergent evolution within an adaptive radiation of cichlid fishes. *Curr. Biol.* **22**, 2362–2368. (doi:10.1016/j.cub.2012.10.048)
- 33. Breure ASH, Gittenberger E. 1981 The rock-scraping radula, a striking case of convergence (Mollusca). *Neth. J. Zool.* **32**, 307–312. (doi:10.1163/002829681X00347)
- 34. Steneck RS, Watling L. 1982 Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. *Mar. Biol.* **68**(3), 299–319. (doi:10.1007/BF00409596)
- 35. Kesler DH, Jokinen EH, Munns WR Jr. 1986 Trophic preferences and feeding morphology of two pulmonate snail species from a small New England pond, USA. *Can. J. Zool.* **64**, 2570–2575. (doi:10.1139/z86-377)
- Black R, Lymbery A, Hill A. 1988 Form and Function: size of radular teeth and inorganic content of faeces in a guild of grazing molluscs at Rottnest Island, Western Australia. J. Exp. Mar. Biol. Ecol. 121, 23–35. (doi:10.1016/0022-0981(88)90021-4)
- 37. Blinn W, Truitt RE, Pickart A. 1989 Feeding ecology and radular morphology of the freshwater limpet *Ferrissia fragilis. J. N. Aa. Benthol. Soc.* **8**(3), 237–242. (doi:10.2307/1467327)
- 38. Iken K. 1999 Feeding ecology of the Antarctic herbivorous gastropod *Laevilacunaria antarctica* Martens. *J. Exp. Mar. Biol. Ecol.* **236**, 133–148. (doi:10.1016/S0022-0981(98)00199-3)
- 39. Jensen KR. 1980 A review of sacoglossan diets, with comparative notes on radular and buccal anatomy. *Malacol. Rev.* **13**, 55–77.
- 40. Jensen KR. 1981 Observations on feeding methods in some Florida ascoglossans. *J. Molluscan Stud.* **47**, 190–199. (doi:10.1093/oxfordjournals.mollus.a065567)
- 41. Jensen KR. 1983 Factor affecting feeding selectivity in herbivorous Ascoglossa (Mollusca: Opisthobranchia). *J. Exp. Mar. Biol. Ecol.* **66**, 135–148. (doi:10.1016/0022-0981(83)90035-7)
- 42. Trowbridge CD. 1991 Diet specialization limits herbivorous sea slug's capacity to switch among food species. *Ecology* **72**(5), 1880–1888. (doi:10.2307/1940985)
- 43. Greenwood PH. 1974 The cichlid fishes of Lake Victoria, east Africa: The biology and evolution of a species flock. *Bull. Br. Mus. Nat. Hist. Zool.* **6**, 1–134.
- 44. Glaubrecht M. 2008 Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: Morphology and systematization of a paludomid species flock in an ancient lake. *Zoosystematics Evol.* 84, 71–122. (doi:10.1002/zoos.200700016)
- 45. Pilsbry HA, Bequaert J. 1927 The aquatic mollusks of the Belgian Congo. With a geographical and ecological account of Congo malacology. *Bull. Am. Mus. Nat. Hist.* **53**, 69–602.
- 46. Leloup E. 1953 *Exploration hydrobiologique du Lac Tanganika (1946 1947)*. Bruxelles.

- 47. Brown DS, Mandahl-Barth G. 1987 Living molluscs of Lake Tanganyika: a revised and annotated list. *J. Conchol.* **32**, 305–327.
- 48. Coulter GW. 1991 Lake Tanganyika and its life. Oxford: Oxford University Press.
- 49. Brown D. 1994 Freshwater Snails of Africa and their Medical Importance. London: Taylor and Francis.
- 50. West K, Michel E, Todd J, Brown D, Clabaugh J. 2003 *The Gastropods of Lake Tanganyika: Diagnostic key, classification and notes on the fauna.* Special publications: Societas Internationalis Limnologiae Int. Assoc. of Theoretical and Applied Limnology.
- 51. Glaubrecht M. 1999 Systematics and evolution of viviparity in tropical freshwater gastropods (Cerithioidea: Thiaridae s.l.). Courier Forschungs-Institut Senckenberg **203**, 91–96.
- 52. Glaubrecht M. 2006 Independent evolution of reproductive modes in viviparous freshwater Cerithioidea (Gastropoda, Sorbeoconcha) a brief review. *Basteria* **69**(3), 28–32.
- Lydeard C, Holznagel WE, Glaubrecht M, Ponder WF. 2002 Molecular phylogeny of a circum-global, diverse gastropod superfamily (Cerithioidea: Mollusca: Caenogastropoda): pushing the deepest phylogenetic limits of mitochondrial LSU rDNA sequences. *Mol. Phylogenetics Evol.* 22(3), 399–406. (doi:10.1006/mpev.2001.1072)
- 54. Strong EE, Colgan DJ, Healy JM, Lydeard C, Ponder WF, Glaubrecht M. 2011 Phylogeny of the gastropod superfamily Cerithioidea using morphology and molecules. *Zool. J. Linn. Soc.* **162**, 43–89. (doi:10.1111/j.1096-3642.2010.00670.x)
- 55. Cunnington WA. 1920 The fauna of the African Lakes: a study in comparative limnology with special reference to Tanganyika. *Zool. J. Linn. Soc.* **90**(4), 507–622. (doi:10.1111/j.1096-3642.1920.tb03243.x)
- 56. Brooks JL. 1950 Speciation in ancient lakes. *Quart. Rev. Biol.* **25**, 30–60, 131–176. (doi:10.1016/S0169-5347(97)01039-2)
- 57. Mayr E. 1963 Animal, species, and evolution. Cambridge, Mass.: Harvard University Press.
- 58. Mayr E, Ashlock PD. 1991 Principles of systematic zoology. New York: McGraw-Hill.
- 59. West K, Michel E. 2000 The dynamics of endemic diversification; molecular phylogeny suggests an explosive origin of the thiarid gastropods of Lake Tanganyika. *Adv. Ecol. Res.* **31**, 331–354. (doi:10.1016/S0065-2504(00)31018-2)
- 60. Boss KJ. 1978 On the evolution of gastropods in ancient lakes. In *Pulmonates Systematics, Evolution and Ecology*, vol. 2a (eds V Fretter, J Peake), pp. 385–428. London: Academic Press.
- 61. Johnston MR, Cohen AS. 1987 Morphological divergence in endemic gastropods from Lake Tanganyika: implications for models of species flock formation. *Palaios* **2**, 413–425. (doi:10.2307/3514613)
- 62. Coulter GW. 1991 Lake Tanganyika and its life. Oxford: Oxford University Press.
- Michel E, Cohen AS, West K, Johnston MR, Kat PW. 1992 Large African lakes as natural laboratories for evolution: examples from the endemic gastropod fauna of Lake Tanganyika. *Mitt. Internat. Verein. Limnol.* 23, 85–99. (doi:10.1080/05384680.1992.11904012)
- 64. Michel E. 1994 Why snails radiate: a review of gastropod evolution in long-lived lakes, both recent and fossil. In *Speciation in ancient lakes. Advances in Limnology* (eds K Martens, B Goddeeris, GW Coulter), pp. 285–317. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung.
- 65. Michel E. 2000 Phylogeny of a gastropod species flock: exploring speciation in Lake Tanganyika in a molecular framework. *Adv. Ecol. Res.* **31**, 275–302. (doi:10.1016/S0065-2504(00)31016-9)
- 66. West K, Cohen A. 1996 Shell microstructure of gastropods from Lake Tanganyika, Africa: adaptation, convergent evolution, and escalation. *Evolution* **50**(2), 672–681. (doi:10.1111/j.1558-5646.1996.tb03877.x)
- 67. Martens K. 1997 Speciation in ancient lakes. *Trends. Ecol. Evol.* **12**(5), 177–182. (doi:10.1016/S0169-5347(97)01039-2)
- Wilson AB, Glaubrecht M, Meyer A. 2004 Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika. *Proc. R. Soc. Lond. B.* 271(1538), 529–536. (doi:10.1098/rspb.2003.2624)
- 69. Tiercelin JJ, Mondeguer A. 1991 The geology of the Tanganyika trough. In *Lake Tanganyika and Its Life* (ed GW Coulter), pp. 7–48. New York: Oxford University Press.
- Cohen AS, Soreghan MJ, Scholz CA. 1993 Estimating the age of formation of lakes: an example from Lake Tanganyika, East African Rift system. *Geology* 21, 511–514. (doi:10.1130/0091-7613(1993)021<0511:ETAOFO>2.3.CO;2)

- 71. Cohen AS, Talbot MR, Awramik SM, Dettman DL, Abell P. 1997 Lake level and paleoenvironmental history of Lake Tanganyika, African, as inferred from late Holocene and modern stromatolithes. *Geol. Soc. Am. Bull.* **109**(4), 444–460. (doi:10.1130/0016-7606(1997)109<0444:LLAPHO>2.3.CO;2)
- 72. Glaubrecht M, Strong EE. 2007 Ancestry to an endemic radiation in Lake Tanganyika? Evolution of the viviparous gastropod *Potadomoides* Leloup, 1953 in the Congo River system (Cerithioidea, Paludomidae). *Biol. J. Linn. Soc.* **92**, 367–401. (doi:10.1111/j.1095-8312.2007.00867.x)
- 73. Ax P. 1984 Das Phylogenetische System: Systematisierung der lebenden Natur aufgrund ihrer Phylogenese. Stuttgart: Gustav Fischer Verlag.
- Schedel FDB, Musilova Z, Schliewenet UK. 2019 East African cichlid lineages (Teleostei: Cichlidae) might be older than their ancient host lakes: new divergence estimates for the east African cichlid radiation. *BMC Evol. Biol.* 2019, 19–94. (doi:10.1186/s12862-019-1417-0)
- 75. Weiss JD, Cotterill FPD, Schliewen UK. 2015 Lake Tanganyika—A 'Melting Pot' of Ancient and Young Cichlid Lineages (Teleostei: Cichlidae)? *PLoS ONE* **10**(7), e0132615. (doi:10.1371/journal.pone.0132615)
- 76. Osche G. 1962 Das Praeadaptationsphänomen und seine Bedeutung für die Evolution. *Zool. Anz.* **169**, 14–49.
- 77. Müller OF. 1774 *Vermium terrestrium et fluviatilium, seu animalium infusorium, Helminthicorum, et testaceorum, non marinorum, succincta historia. Vol 2: I-XXXVI*, pp. 1—214. Havniae et Lipsiae, apud Heineck et Faber, ex officina Molleriana.
- 78. Soklov EP. 2000 An improved method for DNA isolation from mucopolysaccharide-rich molluscan tissues. *J. Molluscan Stud.* **66**, 573–575. (doi:10.1093/mollus/66.4.573)
- 79. Neiber MT, Walther F, Hausdorf B. 2018 Phylogeny and reclassification of the Caucasigenini radiation from the Caucasus region (Gastropoda, Hygromiidae). *Zool. Scr.* **47**, 54–62. (doi:10.1111/zsc.12259)
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994 DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3(5), 294– 299.
- Gimnich F. 2015 Molecular approaches to the assessment of biodiversity in limnic gastropods (Cerithioidea, Thiaridae) with perspectives on a Gondwanian origin. Doctoral dissertation, Humboldt-Universität zu Berlin. (doi:10.18452/17264)
- 82. Katoh K, Standley DM. 2013 MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **30**(4), 772–780. (doi:10.1093/molbev/mst010)
- Lanfear R, Calcott B, Ho SYW, Guindon S. 2012 Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* 29(6), 1695–1701. (doi:10.1093/molbev/mss020)
- 84. Zwickl DJ. 2006 Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Doctoral dissertation, University of Texas at Austin.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S et al. 2012 MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61(3), 539–542. (doi:10.1093/sysbio/sys029)
- 86. Swofford DL. 2002 PAUP\*. Phylogenetic Analysis Using Parsimony \*and other methods. Version 4.0b10. Sunderland, MA: Sinauer Associates. (doi:10.1111/j.0014-3820.2002.tb00191.x)
- 87. Neiber MT, Glaubrecht M. 2019 Annotated nomenclator of extant and fossil taxa of the Paludomidae (Caenogastropoda, Cerithioidea). *ZooKeys* **850**, 1–132. (doi:10.3897/zookeys.850.34238)
- 88. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D et al. 2014 BEAST 2: A software platform for Bayesian evolutionary analysis. *PLOS Comput. Biol.* **10**, e1003537. (doi:10.1371/journal.pcbi.1003537)
- 89. Kumar S, Stecher G, Tamura K. 2016 MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**(7), 1870–1874. (doi:10.1093/molbev/msw054)
- 90. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018 Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* **67**(5), 901–904. (doi:10.1093/sysbio/syy032)
- 91. Holznagel WE. 1998 A nondestructive method for cleaning gastropod radulae from frozen, alcohol-fixed, or dried material. *Am. Malacol. Bull.* **14**, 181–183.
- 92. Strong EE, Glaubrecht M. 2010 Anatomy of the Tiphobiini from Lake Tanganyika (Cerithioidea, Paludomidae). *Malacologia* **52**(1), 115–153. (doi:10.4002/040.052.0108)

- 93. Michel E. 2004 *Vinundu*, a new genus of gastropod (Cerithioidea: 'Thiaridae') with two species from Lake Tanganyika, East Africa, and its molecular phylogenetic relationships. *J. Molluscan Stud.* **70**(1), 1–19. (doi:10.1093/mollus/70.1.1)
- 94. Strong EE, Glaubrecht M. 2002 Evidence for convergent evolution of brooding in an unique gastropod from Lake Tanganyika: anatomy and affinity of *Tanganyicia rufofilosa* (Caenogastropoda, Cerithioidea, Paludomidae). *Zool. Scr.* **31**(2), 167–184. (doi:10.1046/j.1463-6409.2002.00072.x)
- 95. Strong EE, Glaubrecht M. 2003 Anatomy and systematic affinity of *Stanleya neritinoides* (Smith, 1880), an enigmatic member of the thalassoid gastropod species flock from Lake Tanganyika, East Africa (Cerithioidea: Paludomidae). *Acta Zool.* **84**(4), 249–265. (doi:10.1046/j.1463-6395.2003.00145.x)
- 96. Strong EE, Glaubrecht M. 2007 The morphology and independent origin of ovoviviparity in *Tiphobia* and *Lavigeria* (Caenogastropoda: Cerithioidea: Paludomidae) from Lake Tanganyika. *Org. Divers. Evol.* **7**(2), 81–105. (doi:10.1016/j.ode.2006.02.003)
- 97. Strong EE, Glaubrecht M. 2008 Anatomy and systematics of the minute Syrnolopsine gastropods from Lake Tanganyika (Caenogastropoda, Cerithioidea, Paludomidae). *Acta Zool.* **89**(4), 289–310. (doi:10.1111/j.1463-6395.2007.00318.x)
- Glaubrecht, M. 2010 The enigmatic *Cleopatra broecki* Putzeys, 1899 of the Congo River system in Africa retransfer from *Potadomoides* Leloup, 1953 (Caenogastropoda, Cerithioidea, Paludomidae). *Zoosystematics Evol.* 86(2), 283–293. (doi:10.1002/zoos.201000011)
- 99. Maddison WP, Maddison DR. 2018 Mesquite: a modular system for evolutionary analysis. Version 3.10. http://www.mesquiteproject.org.
- 100. Ancey C-F. 1906 Reflexions sur la fauna malacologique du Lac Tanganika et cataloque des mollusques de ce lac. *Bull. Biol. Fr. Bel.* **5**(9), 229–270.
- 101. Bourguignat MJR. 1885 Notice prodromique sur les mollusques terrestres et fluviatiles. Paris: Savy.
- 102.Pilsbry HA, Bequaert J. 1927 The aquatic mollusks of the Begian Congo. With a geographical and ecological account of Congo malacology. *Bull. Am. Mus. Nat. Hist.* **53**, 69–602.
- 103.Bourguignat MJR. 1890 Histoire Malacologique du Lac Tanganika (Afrique Equatoriale). *Ann. Sci. Nat., Zool. Biol. Anim.* **10**, 1–267.
- 104. Bourguignat MJR. 1886 Nouveautés Malacologiques: Unionidæ et Iridinidæ du Lac Tanganika. Paris: Savy.
- 105.Kesteven L. 1903 Notes on Prosobranchiata. No. ii. Proc. Linn. Soc. N.S.W. 27(4), 620–636.
- 106.Bourguignat MJR. 1888 Iconographie malacologiques des animaux mollusques fluviatiles du Lac Tanganika. Crété: Corbeil.
- 107. Crosse H. 1881 Faune malacologique du Lac Tanganyika. *Journal de Conchyliologie* **29**, 105–272.
- 108. Dautzenberg P. 1907 Description de coquilles nouvelles de diverses provenances et de quelques cas térattologiques. *Journal de Conchyliologie* **55**, 327–341.
- 109.Smith EA. 1880 Diagnoses of new shells from Lake Tanganyika and East Africa. *Ann. Mag. Nat. Hist.* **6**, 425–430.
- 110. Moore JES. 1898a Descriptions of the genera *Bathanalia* and *Bythoceras*, from Lake Tanganyika. *Proc. Malacol. Soc. Lond.* **3**, 92–93.
- 111. Troschel, F. H. (1856-1863). Das Gebiss der Schnecken zur Begründung einer natürlichen Classification. Berlin, Germany.
- 112.Smith EA. 1889 Diagnosis of new shells from Lake Tanganyika. Ann. Mag. Nat. Hist. 6, 173–175.
- 113.Bourguignat MJR. 1879 Description de diverses espèces terrestres et fluviatiles et de différents genres de molluscques de l'Egypte, de l'Abyssinies, de Zanzibar, du Senegal et du centre de l'Afrique. Paris.
- 114. Moore JES. 1898b The molluscs of the Great African Lakes. II. The anatomy of the *Typhobias*, with a description of the new genus (*Bathanalia*). *Quarterly Journal of Microscopic Sciences (new series)* **41**, 181–204.
- 115.Smith EA. 1881 Descriptions of two new species of shells from Lake Tanganyika. *Proc. R. Soc. Lond.* **49**(3), 558–561. (doi:10.1111/j.1096-3642.1881.tb01312.x)
- 116.Giraud V. 1885 Description du nouveau genre Bourguignatia du Tanganika. Bulletins de la Société Malacologique de France 2, 193–194.
- 117.Lezzar KE, Tiercelin JJ, Le Turdu C, Cohen AS, Reynolds DJ, Le Gall B, Scholz CA. 2002 Control of normal fault interaction on the distribution of major Neogene sedimentary depocenters, Lake Tanganyika, East African

rift. AAPG Bull. 86, 1027-1059. (doi:10.1306/61EEDC1A-173E-11D7-8645000102C1865D)

- 118.Spiegel C, Kohn BP, Belton DX, Gleadow AJW. 2007 Morphotectonic evolution of the Central Kenya rift flanks: implications for late Cenozoic environmental change in East Africa. *Geology* **35**, 427–430. (doi:10.1130/G23108A.1)
- 119. Bauer FU, Glasmacher UA, Ring U, Schumann A, Nagudi B. 2010 Thermal and exhumation history of the central Rwenzori Mountains, Western rift of the east African rift system, Uganda. *Int. J. Earth Sci.* **99**(7), 1575–1597.
- 120.Roller S, Hornung J, Hinderer M, Ssemmanda I. 2010 Middle Miocene to Pleistocene sedimentary record of rift evolution in the southern Albert rift (Uganda). *Int. J. Earth Sci.* **99**, 1643–1661.
- 121. Wembo Ndeo O, Clewing C, Stelbrink B, Albrecht C. 2020 Lake Tanganyika endemic gastropods also occur in the Lukuga River. *J Great Lakes Res*. (doi:10.1016/j.jglr.2019.10.007)
- 122.Salzburger W, Mack T, Verheyen E, Meyer A. 2005 Out of Tanganyika: genesis, explosive speciation, keyinnovations and phylogeography of the haplochromine cichlid fishes. BMC Evol. Biol. **5**, 17. (doi:10.1186/1471-2148-5-17)
- 123.Sturmbauer C, Salzburger W, Duftner N, Schelly R, Koblmüller S. 2010 Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei: Perciformes) derived from mitochondrial and nuclear DNA data. *Mol. Phylogenet. Evol.* **57**(1), 266–284. (doi:10.1016/j.ympev.2010.06.018)
- 124. Neiber, MT, Kahl, SM, Wiggering, B, Glaubrecht, M. 2019 Adding the West-African riverine component: Revision of the Recent freshwater snails belonging to *Pseudocleopatra* Thiele, 1928 (Caenogastropoda, Cerithioidea, Paludomidae). *Zootaxa* **4674**(3), 301–328.
- 125. Steneck RS, Waitling L. 1982 Feeding capabilities and limitation of herbivorous Molluscs: a functional group approach. *Mar. Biol.* **68**(3), 299–319. (doi:10.1007/BF00409596)
- 126.Krings W, Kovalev A, Glaubrecht M, Gorb SN. 2019 Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods. *Zoology* **137**, 125713. (doi:10.1016/j.zool.2019.125713)

## Supplementary Material

Morphological characters and states:

- 1. Central tooth (CT), overall shape: 0 higher than wide, 1 squarish, 2 wider than high.
- CT dentition on cutting edge: 0 numerous of equal size, 1 one main middle denticle flanked by one or more smaller denticles, 2 - few long of equal size, 3 - no denticle, 4 - no central denticles but denticles flanking the sides.
- 3. Lateral tooth (LT) dentition: 0 with main denticle and few smaller denticles, 1 with numerous denticles of more or less equal size.
- Inner marginal tooth (IMT) cusp: 0 denticles confined to distal tip, 1 denticles extending from tip along both edges, 2 - denticles along inner edge only, 3 - denticles along outer edge only.
- 5. IMT cusp: 0 with only few denticles (1–4), 1 with many denticles (up to 10), 2 with numerous denticles (more than 10).
- Outer marginal tooth (OMT) cusp: 0 only few denticles (1–4), 1 with many (up to 10), 2 with numerous (more than 10).

Supplementary Tab. 1: GenBank accession numbers of COI and 16S sequences used for the phylogenetic analyses (including museum number, locality and reference); museum number, locality and references for the morphological radular character analysis.

Species	Molecula	r data, GenBa	ink accessior	Radular analysis				
	Museu m number	Locality	СОІ	165	Referenc e of molecula r data	Museu m number	Locality	Reference of morphologic al data
Anceya giraudi	ZMB 220.000 b	Zambia, Kasenga Point	AY45652 9	AY45652 9	[68]	ZMB 220.132 ; ZMB 220.070	Tanzania, Kigoma; Zambia, Kasenga Point	[44], this study
Bathanalia howesii	-	-	AY21314 1	-	[93]	-	-	[92]
Bridouxia ponsonbyi	ZMB 220.137	Tanzania, Kigoma	-	Will be uploaded	this study	ZMB 220.137	Tanzania, Kigoma	[44], this study
Bridouxia grandidieria na Chytra kirkii	ZMB 220.009 x	Zambia, Mpulungu Field Station Zambia,	AY45653 3 AY21314	AY45658 7 -	[68]	ZMB 220.138 ; ZMB 220.009 ; ZMB 220.084 ; ZMB 220.138	Tanzania, Kigoma; Zambia, Mpulungu Field Station; Zambia, Kumbula Island; Tanzania, Kigoma Tanzania,	[44], this study [92], this
Cleonatra	ZMB	Wonzye Point	2 Will be	Will be	this	no. 63; IRSNB no. 68; ZMB 220.155	Malagarasi delta; Democratic Republic of Congo, Cape Tembwe; Russago Medinet el	study
bulimoides	103.720	-	uploaded	uploaded	study	31148– 1	fayum	[90]
Cleopatra johnstoni	ZMB 220.102 a	Zambia <i>,</i> Lake Mweru	AY45653 6	AY45659 0	[68]	ZMB 220.102 a	Zambia, Lake Mweru	this study
Lavigeria arenarum	ZMB 220.073 a	Zambia, Kumbula Island	AY45653 7	AY45659 1	[68]	ZMB 220.073	Zambia, Kumbula Island	this study
Lavigeria crassilabris	ZMB 220.115	Tanzania, Kigoma	-	Will be uploaded	this study	ZMB 220.115	Tanzania, Kigoma	this study

Lavigeria arandis	ZMB	Zambia, Kasenga	AY45653	AY45659	[68]	ZMB	Zambia, Kasenga	this study
granais	a	Point	0	2		a	Point	
Lavigeria limnaea	ZMB 220.097 a	Tanzania, Kigoma	AY45655 0	AY45660 4	[68]	ZMB 220.020	Zambia, Kasenga Point	this study
Lavigeria littoralis	ZMB 220.048 x	Zambia, Kasakalaw e	AY45654 5	AY45659 9	[68]	ZMB 220.015	Zambia, Mpulungu Field Station	this study
Lavigeria livingstonian a	ZMB 107.097	Tanzania, Utinta	Will be uploaded	Will be uploaded	this study	ZMB 220.116	Tanzania, Kigoma	this study
Lavigeria locardiana	ZMB 220.055 a	Zambia, Kasenga Point	AY45654 6	AY45660 0	[68]	ZMB 220.071 a	Zambia, Kasenga Point	this study
Lavigeria nassa	ZMB 220.019 a	Zambia, Kasenga Point	AY45654 8	AY45660 2	[68]	ZMB 220.127 ; ZMB 220.040 ; ZMB 220.125	Burundi, Bujumbura; Tanzania, Kigoma; Tanzania, Kigoma	[44], this study
Lavigeria smithi	ZMB 220.021 c	Zambia, Kasakalaw e	AY45655 3	AY45660 7	[68]	ZMB 220.021	Zambia, Kasakalawe	this study
Lavigeria spinulosa	ZMB 220.014 a	Zambia, Kumbula Island	AY45655 5	AY45660 9	[68]	ZMB 220.014	Zambia, Kumbula Island	this study
Limnotrochu s thomsoni	ZMB 220.038 a	Zambia, Kumbula Island	AY45655 8	AY45661 2	[68]	ZMB 220.038 ; ZMB 220.031	Zambia, Kumbula Island; Tanzania, Karema	[92], this study
Martelia tanganyicens is	ZMB 220.006 x	Zambia, Kumbula Island	AY45657 8	AY45663 2	[68]	ZMB 220.133 ; ZMB 220.067	Tanzania, Kigoma; Zambia, Kumbula Island	[44], this study
Melanoides tuberculata	ZMB 220.060	Malawi	AY45656 2	AY45661 6	[68]	-	-	-
Paludomus siamensis	ZMB 200.231	-	AY45656 0	AY45661 4	[68]	ZMB 200.234	Thailand, Kanchanabu ri	this study
Paramelania imperialis	ZMB 220.111	Zambia, Kasenga Point	AY45656 5	AY45661 9	[68]	ZMB 220.111	Zambia, Kasenga Point	this study
Paramelania damoni	-	-	AY21315 2	-	[59]	ZMB 220.052	Burundi, Bujumbura	this study

Paramelania	ZMB	Zambia,	AY45656	AY45662	[68]	ZMB	Tanzania,	[92], this
iridescens	220.099	Chipata	6	0		220.037	Kigoma; Zambia	study
	d	Day				, 2101B 220.099	Chipata bay	
Potodomoid	-	-	-	-	-	DBL w/o	Democratic	[72]
es bequaerti						no.	Republic of	
						(possibl	Congo,	
						y ex MRAC	Lualaba	
						341.980		
						)		
Potodomoid	-	-	-	-	-	DBL w/o	-	[72]
es hirta						no.		
Potodomoid	-	-	-	-	-	paratyp	Democratic	[72]
es schoutedeni						e, MRAC	Republic of	
schouteden						814	Kibombo	
Potodomoid	-	-	-	-	-	paratyp	Tanzania,	[72]
es pelseneeri						e, IRSNB	Malagarasi	
Deurseendig	7140	Zanahia			[(0)]	no. 145	Tonnonio	
keymonala horei	21VIB 220.013	Zampia, Kasenga	A145656 8	A145662	[68]	21VIB 220 147	Tanzania, Kigoma	[44], this study
norer	a	Point	0	2		; ZMB	Zambia,	Study
						220.012	Kasenga	
							Point	
Reymondia	ZMB	Tanzania,	-	Will be	this	ZMB	Tanzania,	[44], this
Reymondia pyramidalis	ZMB 220.148	Tanzania, Kigoma	-	Will be uploaded	this study	ZMB 220.148	Tanzania, Kigoma	[44], this study
Reymondia pyramidalis Spekia	ZMB 220.148 ZMB 220.026	Tanzania, Kigoma Zambia, Kumbula	- AY45656	Will be uploaded AY45662	this study [68]	ZMB 220.148 ZMB	Tanzania, Kigoma Burundi,	[44], this study [44], this
Reymondia pyramidalis Spekia zonata	ZMB 220.148 ZMB 220.026 a	Tanzania, Kigoma Zambia, Kumbula Island	- AY45656 9	Will be uploaded AY45662 3	this study [68]	ZMB 220.148 ZMB 220.144 : ZMB	Tanzania, Kigoma Burundi, Bujumbura; Zambia.	[44], this study [44], this study
Reymondia pyramidalis Spekia zonata	ZMB 220.148 ZMB 220.026 a	Tanzania, Kigoma Zambia, Kumbula Island	- AY45656 9	Will be uploaded AY45662 3	this study [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula	[44], this study [44], this study
Reymondia pyramidalis Spekia zonata	ZMB 220.148 ZMB 220.026 a	Tanzania, Kigoma Zambia, Kumbula Island	- AY45656 9	Will be uploaded AY45662 3	this study [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island	[44], this study [44], this study
Reymondia pyramidalis Spekia zonata Stanleya	ZMB 220.148 ZMB 220.026 a	Tanzania, Kigoma Zambia, Kumbula Island	- AY45656 9 AY21314	Will be uploaded AY45662 3	this study [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic	[44], this study [44], this study [95]
Reymondia pyramidalis Spekia zonata Stanleya neritinoides	ZMB 220.148 ZMB 220.026 a	Tanzania, Kigoma Zambia, Kumbula Island -	- AY45656 9 AY21314 6	Will be uploaded AY45662 3	this study [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Conro	[44], this study [44], this study [95]
Reymondia pyramidalis Spekia zonata Stanleya neritinoides	ZMB 220.148 ZMB 220.026 a	Tanzania, Kigoma Zambia, Kumbula Island -	- AY45656 9 AY21314 6	Will be uploaded AY45662 3	this study [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba	[44], this study [44], this study [95]
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella	ZMB 220.148 ZMB 220.026 a	Tanzania, Kigoma Zambia, Kumbula Island - -	- AY45656 9 AY21314 6 AY45657	Will be uploaded AY45662 3 -	this study [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania,	[44], this study [44], this study [95] [44], this
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima	ZMB 220.148 ZMB 220.026 a -	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula	- AY45656 9 AY21314 6 AY45657 2	Will be uploaded AY45662 3 - - AY45662 6	this study [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma;	[44], this study [44], this study [95] [44], this study
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima	ZMB 220.148 ZMB 220.026 a - - - ZMB 220.008 x	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island	- AY45656 9 AY21314 6 AY45657 2	Will be uploaded AY45662 3 - - AY45662 6	this study [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia,	[44], this study [44], this study [95] [44], this study
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima	ZMB 220.148 ZMB 220.026 a - - ZMB 220.008 x	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island	- AY45656 9 AY21314 6 AY45657 2	Will be uploaded AY45662 3 - AY45662 6	this study [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB 220.076	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia, Kumbula Island	[44], this study [44], this study [95] [44], this study
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima	ZMB 220.148 ZMB 220.026 a - - - ZMB 220.008 x ZMB	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island Zambia,	- AY45656 9 AY21314 6 AY45657 2 AY45657	Will be uploaded AY45662 3 - AY45662 6 AY45662	this study [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB 220.135 ; ZMB 220.076	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia, Kumbula Island Tanzania,	[44], this study [44], this study [95] [44], this study [44], this
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima Syrnolopsis lacustris	ZMB 220.148 ZMB 220.026 a - - ZMB 220.008 x ZMB 220.008 X	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island Zambia, Mpulungu	- AY45656 9 AY21314 6 AY45657 2 AY45657 4	Will be uploaded AY45662 3 - - AY45662 6 AY45662 8	this study [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB 220.135 ; ZMB 220.076 ZMB 220.130	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia, Kumbula Island Tanzania, Kigoma;	[44], this study [44], this study [95] [44], this study [44], this study
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima Syrnolopsis lacustris	ZMB 220.148 ZMB 220.026 a - - - ZMB 220.008 x ZMB 220.046 a	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island Zambia, Mpulungu Field	- AY45656 9 AY21314 6 AY45657 2 AY45657 4	Will be uploaded AY45662 3 - - AY45662 6 AY45662 8	this study [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB 220.135 ; ZMB 220.130 ; ZMB	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia, Kumbula Island Tanzania, Kigoma; Tanzania,	[44], this study [44], this study [95] [44], this study [44], this study
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima Syrnolopsis lacustris	ZMB 220.148 ZMB 220.026 a - - ZMB 220.008 x ZMB 220.008 x	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island Zambia, Mpulungu Field Station	- AY45656 9 AY21314 6 AY45657 2 AY45657 4	Will be uploaded AY45662 3 - - AY45662 6 AY45662 8	this study [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB 220.135 ; ZMB 220.130 ; ZMB 220.131	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia, Kumbula Island Tanzania, Kigoma; Tanzania, Kigoma; Tanzania, Kigoma; Zambia	[44], this study [44], this study [95] [44], this study [44], this study
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima Syrnolopsis lacustris	ZMB 220.148 ZMB 220.026 a - - ZMB 220.008 x ZMB 220.008 a	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island Zambia, Mpulungu Field Station	- AY45656 9 AY21314 6 AY45657 2 AY45657 4	Will be uploaded AY45662 3 - - AY45662 6 AY45662 8	this study [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB 220.135 ; ZMB 220.130 ; ZMB 220.131 ; ZMB 220.131 ; ZMB	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia, Kigoma; Tanzania, Kigoma; Tanzania, Kigoma; Zambia, Kigoma; Zambia, Kasakalawe	[44], this study [44], this study [95] [44], this study [44], this study
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima Syrnolopsis lacustris	ZMB 220.148 ZMB 220.026 a - - ZMB 220.026 x ZMB 220.026 x ZMB 220.046 a ZMB	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island Zambia, Mpulungu Field Station Zambia,	- AY45656 9 AY21314 6 AY45657 2 AY45657 4 AY45657	Will be uploaded AY45662 3 - - AY45662 6 AY45662 8 AY45663	this study [68] [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB 220.135 ; ZMB 220.130 ; ZMB 220.131 ; ZMB 220.131 ; ZMB 220.078 ZMB	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia, Kumbula Island Tanzania, Kigoma; Tanzania, Kigoma; Zambia, Kasakalawe Tanzania,	[44], this study [44], this study [95] [44], this study [44], this study this study
Reymondia pyramidalis Spekia zonata Stanleya neritinoides Leloupiella minima Syrnolopsis lacustris Syrnolopsis minuta	ZMB 220.148 ZMB 220.026 a - - ZMB 220.008 x ZMB 220.008 a ZMB 220.046 a	Tanzania, Kigoma Zambia, Kumbula Island - Zambia, Kumbula Island Zambia, Mpulungu Field Station Zambia, Kasenga	- AY45656 9 AY21314 6 AY45657 2 AY45657 4 AY45657 6	Will be uploaded AY45662 3 - AY45662 6 AY45662 8 AY45662 8	this study [68] [68] [68] [68]	ZMB 220.148 ZMB 220.144 ; ZMB 220.026 MNHN ZMB 220.135 ; ZMB 220.135 ; ZMB 220.130 ; ZMB 220.131 ; ZMB 220.131 ; ZMB 220.078 ZMB 220.131	Tanzania, Kigoma Burundi, Bujumbura; Zambia, Kumbula Island Democratic Republic of Congo, Pemba Tanzania, Kigoma; Zambia, Kumbula Island Tanzania, Kigoma; Tanzania, Kigoma; Zambia, Kasakalawe Tanzania, Kigoma	[44], this study [44], this study [95] [44], this study [44], this study this study

Tanganyicia rufofilosa	ZMB 220.039 a	?	AY45658 0	AY45663 4	[68]	ZMB 102.622	Tanzania, Kigoma	[94], this study
Tiphobia horei	ZMB 220.095	Zambia, Chipata Bay	AY45658 2	AY45663 6	[68]	ZMB 220.095 ; MCZ 30.1576	Zambia, Chipata Bay; Tanzania, Magambo	[96]
Vinundu guillemei	-	Kachese	AY21317 3	-	[59]	-	Tanzania, Kipili	[93]
Vinundu westae	-	Burundi, Gitaza	AY21315 4	-	[59]	-	Burundi, Bujumbura	[93]

# *2. 2 Tightening it up: Diversity of the Chitin Anchorage of Radular-Teeth in paludomid Freshwater-Gastropods*

Wencke Krings<sup>1\*</sup>, Jan-Ole Brütt<sup>1</sup>, Stanislav Gorb<sup>2</sup>, Matthias Glaubrecht<sup>1</sup>

<sup>1</sup> Center of Natural History (CeNak), Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

<sup>2</sup> Functional Morphology and Biomechanics, Zoological Institute of the Christian-Albrechts-Universität zu Kiel, Am Botanischen Garten 9, 24118 Kiel, Germany

\*corresponding author: wencke.krings@uni-hamburg.de

#### <u>Abstract</u>

The radula is the anatomical structure used for feeding in most Molluscan species and is one important autapomorphy. Previous studies were focused on the radular teeth and their functional morphology, but their anchorage in the radular membrane and its function have never been examined. Here we analyse the micro-structure of connecting chitinous fibres and its implication on the radular function in 13 closely related paludomid species (Gastropoda) from the so called 'ancient lake' Tanganyika. This species flock shows a stunning interspecific diversity in their radular tooth morphology and tooth embedding in the membrane as well. The species studied here feed organics from different substrates (soft, mixed and solid) and are substrate-specific. Here the morphological composition of the membrane is described in detail for the first time, it consists of parallel fibres that extend also within the teeth. Besides, we show that morphologies of the anchorage correlated with the specific preferred feeding substrate can be identified as functional adaptation.

Keywords: radular teeth, feeding, functional morphology, histology, embedding

#### Introduction

### Biodiversity of gastropods and their feeding organ

Mollusca are a species-rich animal group, second only to insects (e.g., Chapman, 2009). Their biodiversity, in particular within Gastropoda, goes along with the colonialization of extraordinary diverse environments and with the establishment of different ecological niches: gastropods feed on a range of different food sources with different mechanical properties. This is probably possible because they carry an evolutionary key innovation for mechanical food collecting and processing, the radula.

The great natural historians of the 19th and early 20th century designated the radula as an organ that most Mollusca share (e.g. Troschel, 1856–1863) and later defined it as autapomorphy of this group. The first extensive study of this organ was done by Troschel (1856–1863), he introduced the feeding organ as the most important character complex for systematics at any level. Following this paradigm gastropods were classified by the 'form' of the radula and its teeth, replacing the more traditional view based on shells only. Thiele (1931–1935) revised the Mollusca based on this, resulting in the reorganization of the system of gastropods at all taxonomic levels. Today the systematic value of the radular morphology is generally accepted, however, its importance depends on the group. Some closely related species show extremely diverse radular morphologies (e.g. the Paludomidae from Lake Tanganyika; see Glaubrecht, 2008) and even specimens of the same species can produce differently shaped teeth, when fed with different food (Padilla 1998, 2001). As in other animal groups processing ingesta mechanically the morphology of the radula can also be understood as an interface between the organisms and its environment, holding phylogenetic as well as ecological information.

The feeding organ, the buccal mass, does not only comprise the radula but also the odontophore cartilages, the oral tube, and numerous muscles. The radula is composed of a chitinous radular membrane (Guralnick and Smith, 1999) holding the embedded transverse and longitudinal rows of chitinous, sometimes mineralized teeth. To date the vast majority of studies on radular tooth mineralization were conducted on the molluscan class Polyplacophora and on Patellogastropoda -

a basal clade of Gastropoda. They gained interest because their tooth cups are strongly wear resistant due to significant amounts of iron-based biominerals and silica incorporated in their chitinous teeth. Since the amounts of these minerals are different the teeth have different mechanical properties which make this feeding organ interesting for materials science (e.g., van der Wal et al., 2000; Brooker et al., 2003; Lee et al., 2003; Wealthall et al., 2005; Shaw et al., 2009, 2010; Weaver at al., 2010; Saunders et al., 2011; Han et al., 2011; Brooker and Shaw, 2012; Lu and Barber, 2012; Nemoto et al., 2012; Wang et al., 2014; Barber et al., 2015) – but most gastropods, except Patellogastropoda, seem to lack this (Simone, 2011). Additionally, silicon and other elements like calcium and magnesium have been detected (e.g., Sollas, 1907; Jones et al. 1935; Lowenstam 1962; Liddiard et al., 2004; Hua and Li, 2007). However, physical properties of the vast majority of radular teeth, as well as its implications on their function, are still understudied.

Radular teeth can show extremely different morphologies between taxa, but all possess a universal bauplan: base, stylus, and cutting plate, the latter can contain cusps (e.g., Runham & Thornton, 1967; Brooker & Macey, 2001). New radular teeth are formed continuously in the formation zone (Fig. 3 a-1) before they enter the working zone (Fig. 3 a-2); only the anterior rows of teeth are used for feeding (e.g., Runham, 1963; Runham & Isarankura, 1966; Isarankura & Runham, 1968; Mackenstedt & Märkel, 1987; Franz, 1990; Padilla et al., 1996; Shaw et al., 2008). While feeding, the muscles of the buccal mass protract and retract the radular membrane transmitting the movement to the radular cartilage (Simone, 2011). Several gastropod species are predatory while others gastropods feed on endolithic and epilithic algae that are rasped from the substrate (e.g., Jensen, 1980, 1981, 1983, 1993; Trowbridge, 1991). Since the species studied here are herbivorous, we focus on this feeding behaviour. While feeding on e.g. algae, the teeth come in contact with the food on the surface of the substrate, and by scratching the surface snails harvest particles of a variety of sizes. Herbivorous feeding can be the grazing on soft substrate collecting algae, but may also include cutting and grinding action as potential ingesta may be too large to ingest at once or be fixed to the substrate. Hypotheses on the evolution of feeding strategies as well as strategies of competitor avoidance, like trophic specialization, have been put forward by e.g., Kesler et al. (1986) or Black et al. (1988). Studies on gastropods foraging selectively on algae in response to the algae position, mode of attachment, toughness and cell size have led also to the notion of competition

avoidance (Steneck & Watling, 1982; Blinn et al., 1989; Ilken, 1999). Besides the food, also the substrate to which the food is deposited on or attached to could influence specializations of the functional morphology of the radula, since different forces while foraging must be exerted. It had already been postulated that e.g. feeding on solid substrate is enabled by teeth with an upright standing, hard, mineralized cutting plate (van der Wal et al., 2000) and that those morphs evolved convergently several times (e.g., Breure & Gittenberger, 1981).

Radular teeth and their function have been well studied, but the anchorage of those within the membrane and its functional morphology have not been examined before. It had been shown, that the radular membrane consists, like the teeth, of chitin (e.g., Peters, 1972). During ontogeny, the teeth can be mineralized and tanned in contrast to the membrane: in the formation zone the membrane as well as the teeth are just composed of chitin, but in the course of the ontogenesis different elements are embedded in the teeth (e.g., Mischor & Märkel, 1984 for *Pomacea bridgesi*, Ampullarioidea, Caenogastropoda; references for mineralization in Polyplacophora and Patellogastropoda please see above). This chitinous membrane consists of fibrillar structures that were detected for taxa of the Caenogastropoda and Euthyneura (Wiesel & Peters, 1978; Mischor & Märkel, 1984; Mackenstedt & Märkel, 1987), but no inferences were done from these observations.

Here, the composition of the membrane is described in detail for the first time. The influence of the ingesta (feeding substrates) on the functional morphology of gastropod feeding organs can be tested in closely related species foraging on different substrates (solid, mixed, and soft ones). The paludomid species flock from Lake Tanganyika is a good model system since species are substrate-specific and show a great interspecific diversity of radular tooth morphologies (e.g., West et al., 2003; Glaubrecht, 2008). The number of teeth per row is identical (taenioglossan radulae), but distinct morphologies can be found in the central, lateral and marginal teeth and their anchorage in the membrane. This can be explained by different ecological niches, adaptations to feeding on organics from different feeding substrates.

58



Figure 1. Shells of examined species. Background colours indicate the phylogeny (see also Fig. 9). Black scale bar: from Lake Tanganyika. Blue scale bar: from adjacent river systems. Forms right to the letters a-m indicate preferred feeding substrate (square: soft substrate feeders specialised on sand [yellow], mud [brown] or both soft [yellow box with brown edges]; soft with occasional feeding on solid substrate [yellow box with black edges] or plant surface [yellow box with green edges]; red triangles: solid substrate feeders). a-b Tiphobiini: a *Paramelania damoni* SMF 290531-2, b *Limnotrochus thomsoni* SMF 290542-1. c-i Spekiini: c *Spekia zonata* ZMB 220.007-2, d *Leloupiella minima* ZMB 220.008-3, e *Bridouxia ponsonbyi* ZMB 220.137-1, f *B. praeclara* DBL 19-4, g *B. rotundata* ZMB 220.063-3, h *B. grandidieriana* BMNH 1889.6.23.57-61, i *Reymondia horei* ZMB 220.007-1. j Cleopatrini: *Cleopatra johnstoni* ZMB 220.102. k-m Nassopsini: k *Lavigeria grandis* ZMB 220.018-1, l *L. nassa* ZMB 220.172-1, m *L. paucicostata* ZMH 119368/999. Scale bars: a, b, i, k-m = 20 mm; c = 10 mm; d-h, j = 5 mm.

#### Material and Methods

46 adult specimens of 12 representative species from Lake Tanganyika and one from the surrounding river systems with clear substrate-specificity (9 solid substrate-, 1 soft substrate-feeder, 3 mixed substrate feeders; Tab. 1, Fig. 1 and Fig. 2), preserved in 70% ethanol and inventoried at the Zoologisches Museum Hamburg (ZMH) and the Museum für (ZMB), Naturkunde Berlin were examined. Identification was done by an assignment based on morphospecies concept (in the this case conchological) in comparison with type material. Information on feeding substrate (Fig. 1) were taken from literature (Bourguignat, 1885, 1888; Moore, 1903; Germain, 1908; Pilsbry & Bequaert, 1927; Leloup, 1953; Brown, 1980; Coulter, 1991; West et al., 2003; Glaubrecht, 2008) or from comments of the collectors; phylogeny (Fig. 1 and Fig. 9) was reconstructed according to Wilson et al (2004) and Krings et al. (unpublished data, here chapter 2.1).

Figure 2. Sample sites in Lake Tanganyika (for taxa at the certain locality see Tab. 1). 1: Tanzania, 07°07'00''S, 30°29'52''E, 2: Zambia, 08°47'50''S, 31°01'02''E, 3: Zambia, 08°45'547''S, 31°05'825''E, 4: Tanzania, Kigoma, 5: Zambia, 08°42'887''S, 31°08'476''E, 6: Zambia, 08°45'258''S, 31°05'116''E, 7: Zambia, 08°42'887''S, 31°08'476''E, 8: Zambia, 08°46'50''S, 31°05'05''E, 9: Zambia, 09°20'866''S, 28°43'886''E, 10: Zambia, 08°29'23''S, 30°28'46''E, 11: Burundi, Bujumbura, 12: Zambia, 08°36'26''S, 30°49'52''E, 13: Zambia, 08°37'23''S, 31°12'01''E.



### Sectioning

The inner structure of the membrane was studied using histological sectioning of material embedded in epoxy resin, light (LM) and scanning electron microscopy (SEM) imaging. Additionally artificially damaged membranes were studied in SEM. For the histology three radulae of Lavigeria nassa (ZMH 119369/999-2, -5, -7), two of *L. grandis* (ZMB 220.089-2, ZMH 150051/999-1) and two of Spekia zonata (ZMH without number-1, -2) initially collected and preserved in 70% ethanol were rehydrated in a decreasing ethanol series and some (ZMH 119369/999-2, -5, ZMB 220.089-2, ZMH without number-1) decalcified with ascorbic acid (2% solution), then dehydrated in an ascending ethanol series and embedded into paraffin. Radulae were cut in 8 µm thick sections with the microtome (Thermo Scientific<sup>™</sup> HM 340E Electronic Rotary Microtome, Thermo Fisher Scientific, Waltham, Massachusetts) and placed on microscope slides. After deparaffinization with Roti-Histol (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) one radula (ZMH119369/999-2) was stained following the protocol of Masson (Masson-trichrome-staining, see Romeis, 2010), one (ZMB 220.089-2) with a hemalum-eosin-staining (see Romeis, 2010), and selected slides of five radulae (ZMH without number-1, -2, ZMH 150051/999-1, ZMH 119369/999-5, -7) with tolouidin blue staining (see Romeis, 2010). Stained sections were fixated with Roti-Histokitt (mounting fluid; Carl Roth GmbH + Co. KG, Karlsruhe, Germany), whereas some unstained slices were not embedded for further SEM investigations. Images of the slices with staining or without staining were taken at magnifications of x100 with the Keyence VHX-5000 digital microscope (KEYENCE, Neu-Isenburg, Germany) and with the Leica DM 2500 LED digital microscope equipped with the Leica DMX 4500 microscope camera (Leica Microsystems GmbH, Wetzlar, Germany) in order to visualize the fibres connecting teeth and membrane.

Radulae of 13 specimens (Tab. 1) were extracted, cleaned, dried and embedded in epoxy resin (RECKLI®EPOXIWST, RECKLI GmbH, Herne, Germany). For the embedding procedure the radulae were attached with double-sided adhesive tape to a glass slide, surrounded by a small metallic ring filled with epoxy, and left for polymerization at room temperature. The tape was important to accurately arrange the radula. After embedding and resin polymerization the glass slide and the tape was removed and radulae were polished with gradual diamond pastes (Buehler MetaDi Ultra

Paste 6 µm 3 µm, 1 µm) and finally smoothened with a polishing machine (Buehler MataServ 250 with Struers OP-U, 0.04 µm suspension with 250 rpm) for a plain surface displaying the longitudinal section of the teeth and membrane. Sections were photographed with an imaging system from Dun, Inc. (Lake Monticello, Virginia, USA) equipped with a Canon EOS 5D Mark III and automated composite stacking. Images were processed with Capture One Pro software (Phase One, Kopenhagen, Denmark) and Zerene Stacker software (Richland, Washington State, USA) with PMax algorithm.

## Scanning Electron Microscopy (SEM)

For the visualization of the chitinous fibres 33 specimens were dissected and their radulae freed from the buccal mass. Two radulae of *Spekia zonata* (ZMH-withoutnumber-3, -4) were extracted according to the protocol of Holznagel (1998), here proteins were digested with proteinase K. After cleaning in an ultrasonic bath and mounting, upside down and plain, on a standard electron microscope stub using double-sided adhesive carbon tape they were coated with carbon and then visualized with the SEM Zeiss LEO 1525 (One Zeiss Drive, Thornwood, NY). Tooth anchorages of 384 central and 714 lateral teeth were traced in Adobe Illustrator (Fig. 7) and by calibration with the metering bar, the area of attachment for the central and marginal teeth was calculated; the area of the marginals was not calculated because it was not plain. In order to compare the anchorage area between species with strong differences in their body and radula size, the percentage of the tooth anchorage was calculated (radular area vs. area of tooth anchorage; Fig. 7). Mean values and standard deviations were calculated with JMP® Pro, Version 13 (SAS Institute Inc., Cary, NC, 1989-2007): a Tukey-Kramer test (p-value < 0.001) was carried out and revealed homogeneous groups (Fig. 9). Fissures in the membrane allowed us imaging the fibrillose membrane layers (Fig. 5 a, b).



Figure 3. a Radula of *Spekia zonata* ZMB 220.077-2 embedded in epoxy and polished, 1: formation zone, 2: working zone. b Radular teeth and membrane of *Reymondia horei* ZMB 220.147-10 embedded in epoxy and polished. c Radula of *Spekia zonata* ZMB 220.077-3 embedded in epoxy and polished, arrows point at the processus that stabilize the central teeth while feeding. Scale bars: a = 0.5 mm, b, c = 100  $\mu$ m. Rm = Radular membrane, Tb = Tooth basis, Tc = Tooth cutting plate, Tp = Tooth processus.



Figure 4. a-b Masson-trichrome-stained longitudinal sections of the radula of *Lavigeria nassa* (ZMH119369/999-2) depicting the chitinous fibres' connection (arrows) between the tooth and membrane. c Connection (arrows) between radular teeth and membrane of *L. nassa* (ZMH 119369/999-7) (section without staining). d *L. grandis* ZMB 220.018-1 embedded in epoxy and polished. e Diagram explaining the hypothesis on the functionality of the anchorage morphology: when force (grey arrow) is exerted while feeding, the teeth are pushed backwards until reaching the next tooth row, the chitinous fibres connecting the tooth basis provide stabilization of the teeth. Scale bars: a-b = 20  $\mu$ m, c = 100  $\mu$ m. Rm = Radular membrane, Tb = Tooth basis, Tc = Tooth cutting plate.



Figure 5. SEM-images of the chitinous tooth anchorages. a-c Arrows with small arrowhead pointing at the fibres and arrows with large arrowhead pointing at fibre bundles. d-e Fibres running parallel to the length of the radular membrane (d is magnification of c). f-g Fibres connecting membrane with central and lateral teeth (f is magnification of e). a, f, g *Bridouxia ponsonbyi* ZMB 220.137-11, b *Spekia zonata* ZMB 220.042-1, c *B. grandidieriana* ZMH 119367/999-3, d-e *Lavigeria nassa* ZMH 119369/999-3. Scale bars: a, c, g = 2  $\mu$ m, b = 4  $\mu$ m, d = 20  $\mu$ m, e-f = 10  $\mu$ m. Rm = Radular membrane, Tb = Tooth basis, Tc = Tooth cutting plate, TL = Thick layer, tL = thin layer.



Figure 6. SEM images of the two fibre anchorage types. a-b *Reymondia horei* ZMB 220.147-3, central and lateral teeth are stabilized by fibres (fibre of the Type 1) running parallel to the length of the membrane (b is magnification of a). d-e *Lavigeria grandis* ZMB 220.089-1, central teeth are stabilized by fibres (fibre of the Type 2) at angles of 30-45° (e is magnification of d). c, f Schematic drawings of the connection of tooth with the membrane: c Fibre of the Type 1 with fibres running parallel to the membrane and anchoring the tooth at an angle of 90°. f Fibre of the Type 2 with fibres anchoring the tooth at angles of 30-45°. Scale bars: a, d = 100  $\mu$ m, b = 20  $\mu$ m, e = 10  $\mu$ m. Mf = Membrane fibres, Tb = Tooth basis.



Figure 7. Anchorage area calculation: the anchorage area of the teeth (dark black lines) is measured and compared with the area of the radula (black lines), SEM images of *Reymondia horei* ZMB 220.007-1. Scale bar = 100  $\mu$ m. Rm = Radular membrane, Tb = Tooth basis.

# <u>Results</u>

# Basic pattern of the anchorage system

The membrane and the tooth itself are composed of bundles consisting of almost parallel fibres running continuously from the membrane into the tooth cutting plate connecting the tooth with the membrane directly (found for the sectioned species *Spekia zonata, Lavigeria grandis* and *L. nassa*; see Fig. 4 a-c, e). These fibres have a diameter of ~0.1  $\mu$ m (Fig. 5 a-c) and are assembled together into bundles with diameter of ~0.5  $\mu$ m (Fig. 5 a-c). Besides of connecting the tooth basis with the membrane these fibres also connect the teeth directly (Fig. 4 c- e). The outermost parts of the membrane as well as from the tooth itself consist of a thicker and thin layer surrounding the bundles (Fig. 5 a-c). Overall the membrane has a thickness of 8-9  $\mu$ m in most species, only in *Lavigeria* and *Spekia* the membrane was comparatively thicker (15-16  $\mu$ m; Fig. 3 b-c). No differences regarding the bundles, layers or fibres were detected in specimens treated with or without proteinase K.

# Fibre orientation

The SEM images show that fibres anchorage the teeth at two different angles, depending on the tooth types (Fig. 6 a-c and d-f):

Type 1: Fibres running parallel to the length of the radula connecting the tooth basis with themselves and with the membrane in an angle of 90° (Fig. 6 a-c). This anchorage can be found in the lateral teeth of all examined species but only in the central teeth of the examined Spekiini and *Cleopatra johnstoni* (Fig. 9).

Type 2: Fibres fan out and connect teeth with the membrane at an angle of 30-45° (Fig. 6 d-f). This pattern can be found in the central tooth anchorages of all examined Nassopsini and Tiphobiini (Fig. 9).



Figure 8. SEM images of the central and lateral tooth anchorages (SEM image looking at the bottom of the radular membrane) of the examined Paludomidae from Lake Tanganyika; a *Paramelania damoni*, b *Limnotrochus thomsoni*, c *Spekia zonata*, d *Bridouxia ponsonbyi*, e *B. praeclara*, f *B. rotundata*, g *B. grandidieriana*, h *Reymondia horei*, i *Leloupiella minima*, j *Cleopatra johnstoni*, k *Lavigeria grandis*, I *L. nassa*. Scale bars: a-b, d-g, i = 40 µm; c = 200 µm, h, j = 20 µm; k-l = 100 µm.



Figure 9. Left side: phylogenetic tree (from Wilson et al., 2004; Krings et al., unpublished data, here chapter 2.1]) of the examined Paludomidae from Lake Tanganyika (black edging) and the adjacent river systems (blues edging), background colours indicate different phylogenetic groups (see also Fig. 1). Solid substrate feeders: black triangle; soft substrate feeders: brown (mud) or yellow (sand) squares; mixed substrate feeders: yellow square (sand) with black edges (rock) or yellow square (sand) with green edges (plant surface). The different anchorage Fibre Types (see also Fig. 6) are added to the tree. Right side: calculated anchorage area in percent for the examined species (for N see Tab. 1): blue boxplots for central teeth and red for lateral teeth. Tukey-Kramer test revealed homogenous groups (blue letters: central teeth, red letters: lateral teeth).

# Area and shape of anchorage

The SEM images taken from the bottom side of the radula depict the anchorage area of the different tooth types so it is possible to compare between species (Fig. 8). The bases of all teeth are completely anchored by fibres into the membrane. All central teeth have a smaller anchorage area (depending on the species 7–30%) than the lateral teeth (depending on the species 20–38%; see also Fig. 9). Between the phylogenetic groups we found differences in the anchorage area and its shape:

1. All examined Nassopsini and Tiphobiini taxa show a general central anchorage pattern that has a triangulate shape (Fig. 8), that is, compared to the anchorage area of the lateral teeth (27–44%), significantly smaller (3–10%; Fig. 9) since in both taxa central teeth are reduced. Central tooth anchorages of the Tiphobiini are more reduced (5–10%) than those of the Nassopsini (10–14%) and show an even, slim, slightly bended and very long anchorage of lateral teeth (Fig. 8).

2. All examined Spekiini and Cleopatrini have (with the exception of *Reymondia horei* with an anchorage similar to those of the Nassopsini) also a similar anchorage pattern. The central tooth anchorage area is slender and slightly curved: it bulges to the lateral sides interacting with the lateral teeth. Lateral tooth anchorages are broad and short showing distinct waves, similar to those of the Nassopsini taxa (Fig. 9). *Bridouxia grandidieriana* differs in the anchorage area from other species of the genus *Bridouxia*: central tooth area is significantly smaller (22%) and lateral teeth anchorage is comparable to that of the Nassopsini and *Limnotrochus* (Fig. 9).

#### <u>Discussion</u>

#### Chitinous fibres anchor and stabilize the teeth

Chitinous fibres in the radular membrane were already described for Pomacea bridgesi, Lymnaea stagnalis, Cepaea nemoralis and Biomphalaria glabrata (Wiesel & Peters, 1978, Mischor & Märkel, 1984, Mackenstedt & Märkel, 1987) and the fibres within the tooth for Flabellina verrucosa (Mikhlina et al., 2018) but without considering their functional morphology. By applying histological sectioning, we were able to show that fibres oriented in the same direction connect the membrane directly with the tooth cutting plate (Fig. 4 a-c, e) and serve as anchors as well as prevent fractures while feeding. While foraging the forces exerted by the radula on the substrate are transmitted from the cutting plate by these fibres directly to the membrane (Fig. 4 e) and hence the stresses are distributed over a larger area. Additionally, since fibres connect the teeth directly with each other, we propose that when the radula is in touch with the ground and the teeth are bent backwards, they are supported by structures of the next tooth rows (Fig. 4 d-e). In this manner the fibres contribute to a self-stabilization of the neighbouring teeth (Fig 4 e). In most Spekiini species and in *Cleopatra* we could also see that the anchorages of the central teeth interact with those of the lateral teeth (Fig. 8) so forces can be transferred while feeding not only to the neighbouring teeth in the same row but also to the teeth of neighbouring rows (for example from the central row to the lateral one).

#### Chitinous anchorages show reflect the phylogeny as well as adaptations to feeding substrates

Most central teeth studied here have a smaller tooth basis (Fig. 9), which means that the studied characters of the anchorage correlate with the tooth morphology and are not an independent character for phylogenetic analysis. Most species that feed on solid substrate have broader central teeth and hence larger central tooth anchorage areas (except for *Lavigeria*) than species feeding also on soft substrates (*Paramelania, Limnotrochus, Bridouxia grandidieriana*) (Fig. 8, 9). The broader tooth anchorages prevent them from breaking loose while feeding on solid substrates. *B. grandidieriana* is the only known species within *Bridouxia* feeding also on soft substrates
(Glaubrecht, 2008), so here the morphology of the anchorage hints at the trophic specialization of this species.

The orientation of the fibres anchoring the teeth correlates with tooth basis size rather than feeding substrate, because we detected both types on animals specialised on all feeding substrates. Larger teeth are stabilized with fibres of the Type 1 (Spekiini and *Cleopatra*) and smaller teeth with fibres of the Type 2 preventing the small teeth to slip to the sides while feeding (Tiphobiini and Nassopsini). Species with a broad central tooth have relatively large anchorage area (Fig. 8, 9) and presumably do not need lateral stabilizations.

The basic morphology seems to be phylogenetically relevant but can also provide an ecological signal since the anchorage area and fibre type corresponds to the size of the teeth. Most species that feed on solid substrates have relatively large anchorage area (Spekiini species) connecting the lateral and central teeth with the membrane. Only representatives of *Lavigeria* (solid substrate feeder) show different pattern: central teeth here are small and hence have a smaller attachment area but teeth are stabilized by the fibres of the Type 2 (Fig. 8, 9). Thus, we can conclude that by examining the anchorage angle in connection with the attachment area one can differentiate between soft and solid substrate feeders. The morphology of the fibres indicates different functional loads and hence ecological specialisation.

#### <u>Conclusion</u>

Here, the morphological composition of the membrane is described in detail for the first time, it consists of parallel fibres that extend also within the teeth. The chitinous fibres anchor and stabilize the teeth. Besides we show that morphologies and the attachment area as well as the fibre orientation correlate not only with the size of the tooth basis but also with the specific preferred feeding substrate and can hence be identified as functional adaptation.

#### <u>Acknowledgements</u>

The authors thank Peter Stutz (Mineralogisch-Petrographisches Institut, University of Hamburg, Germany) for preparing the samples embedded in epoxy, Renate Walter (Department of Biology, University of Hamburg) for her support with the SEM imaging. Heinz Büscher from Basel helped to great extend collecting specimens at Lake Tanganyika. The authors thank the anonymous reviewers for their constructive suggestions.

# Authors' contributions

Matthias Glaubrecht and Wencke Krings conceived this study. Jan-Ole Brütt and Wencke Krings prepared the samples including histology, analysed the data, and worked on the manuscript. Wencke Krings analysed the data, drew the figures, and wrote the manuscript. Stanislav N. Gorb contributed to the overall question in the context of functional morphology and worked on the manuscript. Matthias Glaubrecht as malacologist is an expert in the Tanganyikan gastropod fauna and collected most specimens. All authors contributed to the manuscript and approved the final version of the manuscript.

#### <u>References</u>

Barber, A.H., Lu, D. & Pugno, N.M., 2015, Extreme strength observed in limpet teeth. *Journal of the Royal Society. Interface*, 12: 105.

Black, R., Lymbery & A., Hill, A., 1988, Form and Function: size of radular teeth and inorganic content of faeces in a guild of grazing molluscs at Rottnest Island, Western Australia. *Journal of Experimental Marine Biology and Ecology*, 121: 23–35.

Blinn, W., Truitt, R. E. & Pickart, A., 1989, Feeding ecology and radular morphology of the freshwater limpet *Ferrissia fragilis*. *Journal of the North American Benthological Society*, 8(3): 237–242.

Bourguignat, M. J. R., 1885, Notice prodromique sur les mollusques terrestres et fluviatiles. Savy, Paris. 110 pp.

Bourguignat, M. J. R., 1888, Iconographie malacologiques des animaux mollusques fluviatiles du Lac Tanganika. Savy, Paris. 82 pp.

Breure, A. S. H. & Gittenberger, E., 1981, The rock-scraping radula, a striking case of convergence (Mollusca). *Netherlands Journal of Zoology*, 32: 307–312.

Brooker, L. R. & Macey, D. J., 2001, Biomineralization in chiton teeth and its usefulness as a taxonomic character in the genus *Acanthopleura* Guilding, 1829 (Mollusca: Polyplacophora). *American Malacological Bulletin*, 16: 203–215.

Brooker, L., Lee, A., Macey, D., Van Bronswijk, W. & Webb, J., 2003, Multiple-front iron-mineralisation in chiton teeth (*Acanthopleura echinata*: Mollusca: Polyplacophora). *Marine Biology*, 142: 447-454.

Brooker, L.R. & Shaw, J.A., 2012, The chiton radula: a unique model for biomineralization studies. *Intech Open Access Publisher*.

Brown, D., 1994, *Freshwater snails of Africa and their medical importance*, 2<sup>nd</sup> ed. Taylor and Francis. 609 pp.

Chapman, A. D., 2009, *Numbers of living species in Australia and the world*, 2<sup>nd</sup> ed. Australian Biodiversity Information Services, Toowoomba, Australia, 84 pp.

Coulter, G. W., 1991, Lake Tanganyika and its life. Oxford University Press, Oxford, 354 pp.

Franz, C. J., 1990, Feeding patterns of *Fissurella* species on Isla de Margarita, Venezuela: use of radulae and food passage rates. *Journal of Molluscan Studies*, 56: 25–35.

Germain, L., 1908, Mollusques du Lac Tanganyika et de ses environs. Extrait des resultats secientifiques des voyages en Afrique d'Edouard Foa. *Bulletin du Museum d'Histoire Naturelle*, 14: 1–612.

Glaubrecht, M., 2008, Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology and systematization of a paludomid species flock in an ancient lake. *Zoosystematics and Evolution*, 84, 71–122.

Guralnick, R. & Smith, K., 1999, Historical and biomechanical analysis of integration and dissociation in molluscan feeding, with special emphasis on the true limpets (Patellogastropoda: Gastropoda). *Journal of Morphology*, 241: 175–195.

Han, Y., Liu, C., Zhou, D., Li, F., Wang, Y. & Han, X., 2011, Magnetic and structural properties of magnetite in radular teeth of chiton *Acanthochiton rubrolinestus*. *Bioelectromagnetics*, 32: 226-233.

Holznagel, W. E., 1998, A nondestructive method for cleaning gastropod radulae from frozen, alcohol-fixed, or dried material. *American Malacological Bulletin*, 14: 181–183.

Hua, T.-E., & Li, C.-W. (2007). Silica biomineralization in the radula of a limpet *Notoacmea schrenckii* (Gastropoda: Acmaeidae). *Zoological Studies*, 46: 379-388.

Ilken, K., 1999, Feeding ecology of the Antarctic herbivorous gastropod *Laevilacunaria antarctica* Martens. *Journal of Experimental Marine Biology and Ecology*, 236: 133–148.

Isarankura, K. & Runham, N. W., 1968, Studies on the replacement of the gastropod radula. *Malacologia*, 7: 71–91.

Jensen, K. R., 1980, A review of sacoglossan diets, with comparative notes on radular and buccal anatomy. *Malacological Review*, 13: 55–77.

Jensen, K. R., 1981, Observations on feeding methods in some Florida ascoglossans. *Journal of Molluscan Studies*, 47: 190–199.

Jensen, K. R., 1983, Factor affecting feeding selectivity in herbivorous *Ascoglossa* (Mollusca: Opisthobranchia). *Journal of Experimental Marine Biology and Ecology*, 66: 135–148.

Jensen, K. R., 1993, Morphological adaptations and plasticity of radular teeth of the *Sacoglossa* (= *Ascoglossa*) (Mollusca: Opisthobranchia) in relation to their food plants. *Biological Journal of the Linnean Society*, 48: 135–155.

Jones, E.I., McCance, R.A., & Shackleton, L.R.B., 1935, The role of iron and silica in the structure of the radular teeth of certain marine molluscs. *Journal of Experimental Biology*, 12: 59-64.

Kesler, D. H., Jokinen, E. H. & Munns Jr, W. R., 1986, Trophic preferences and feeding morphology of two pulmonate snail species from a small New England pond, USA. *Canadian Journal of Zoology*, 64: 2570–2575.

Lee, A.P., Brooker, L.R., Macey, D.J., Webb, J. & van Bronswijk, W., 2003, A new biomineral identified in the cores of teeth from the chiton *Plaxiphora albida*. *Journal of Biological Inorganic Chemistry*, 8: 256-262.

Leloup, E., 1953, *Exploration hydrobiologique du Lac Tanganika (1946-1947). Résultats scientifiques. Vol. 3, Gastéropodes.* Bruxelles, Institut Royal des Sciences Naturelles de Belgique, 273 pp.

Liddiard, K.J., Hockridge, J.G., Macey, D.J., Webb, J., & van Bronswijk, W., 2004, Mineralisation in the teeth of the limpets *Patelloida alticostata* and *Scutellastra laticostata* (Mollusca: Patellogastropoda). *Molluscan Research*, 24: 21-31.

Lowenstam, H.A., 1962, Goethite in radular teeth of recent marine gastropods. *Science*, 137: 279-280.

Lu, D. & Barber, A.H., 2012, Optimized nanoscale composite behaviour in limpet teeth. *Journal of the Royal Society*. *Interface*, 9: 1318-1324.

Mackenstedt, U. & Märkel, K., 1987, Experimental and comparative morphology of radula renewal in pulmonates (Mollusca, Gastropoda). *Zoomorphology*, 107: 209–239.

Mikhlina, A., Tzetlin, A., & Vortsepneva, E., 2018, Renewal mechanisms of buccal armature in *Flabellina verrucosa* (Nudibranchia: Aeolidida: Flabellinidae). *Zoomorphology*, 137(1): 31–50.

Mischor, B. & Märkel, K., 1984, Histology and regeneration of the radula of *Pomacea bridgesi* (Gastropoda, Prosobranchia). *Zoomorphology*, 104: 42–66.

Moore, J. E. S., 1903, *The Tanganyika problem*. Burst and Blackett, London, 371 pp.

Nemoto, M., Wang, Q., Li, D., Pan, S., Matsunaga, T. & Kisailus, D., 2012, Proteomic analysis from the mineralized radular teeth of the giant Pacific chiton, *Cryptochiton stelleri* (Mollusca). *Proteomics*, 12: 2890-2894.

Padilla, D. K., Dittman, D. E., Franz, J. & Sladek, R., 1996, Radular production rates in two species of *Lacuna* Turton (Gastropoda: Littorinidae). *Journal of Molluscan Studies*, 62: 275–280.

Padilla, D.K., 1998, Inducible phenotypic plasticity of the radula in *Lacuna* (Gastropoda: Littorinidae). *Veliger*, 41: 201-204.

Padilla, D.K., 2001, Food and environmental cues trigger an inducible offense. *Evolutionary Ecology Research*, 3: 15-25.

Peters, W., 1972, Occurrence of chitin in molluscs. *Comparative Biochemistry and Physiology B*, 41: 541–550.

Pilsbry, H. A. & Bequaert, J., 1927, The aquatic mollusks of the Begian Congo. With a geographical and ecological account of Congo malacology. *Bulletin of the American Museum of Natural History*, 53: 69–602.

Romeis, B., 2010, *Mikroskopische Technik*, 18<sup>th</sup> ed. Leibnitz-Verlag, München, 551 pp.

Runham, N. W. & Isarankura, K., 1966, Studies on radula replacement. *Malacologia*, 5: 73.

Runham, N. W. & Thornton, P. R., 1967, Mechanical wear of the gastropod radula: a scanning electron microscope study. *Journal of Zoology*, 153: 445–452.

Runham, N. W., 1963, A study of the replacement mechanism of the pulmonate radula. *Journal of Cell Science*, 3: 271–277.

Saunders, M., Kong, C., Shaw, J.A. & Clode, P.L., 2011, Matrix-mediated biomineralization in marine mollusks: A combined transmission electron microscopy and focused ion beam approach. *Microscopy and Microanalysis*, 17: 220-225.

Shaw, J. A., Macey, D. J. & Brooker, L. R., 2008, Radula synthesis by three species of iron mineralizing molluscs: production rate and elemental demand. *Journal of the Marine Biological Association of the United Kingdom*, 88: 597–601.

Shaw, J.A., Macey, D.J., Brooker, L.R. & Clode, P.L., 2010, Tooth Use and Wear in Three Iron-Biomineralizing Mollusc Species. *Biological Bulletin*, 218: 132-144.

Shaw, J.A., Macey, D.J., Brooker, L.R., Stockdale, E.J., Saunders, M. & Clode, P.L., 2009, The chiton stylus canal: An element delivery pathway for tooth cusp biomineralization. *Journal of Morphology*, 270: 588-600.

Simone, L., 2011, Phylogeny of the Caenogastropoda (Mollusca), based on comparative morphology. *Arquivos de Zoologia*, 42(4): 161-323.

Smith, E. A., 1880, Diagnoses of new shells from Lake Tanganyika and East Africa. *Annals and Magazine of Natural History*, 6: 425–430.

Smith, E. A., 1881, Descriptions of two new species of shells from Lake Tanganyika. *Proceedings of the Royal Society of London*, 49(3): 558–561.

Smith, E. A., 1889, Diagnosis of new shells from Lake Tanganyika. *Annals and Magazine of Natural History*, 6: 173–175.

Smith, E. A., 1893, On a Collection of Land and Freshwater Shells transmitted by Mr. H. H. Johnston, C. B., from British Central Africa. *Proceedings of the General Meetings for Scientific Business of the Zoological Society of London*, 1893: 632–641.

Smith, E. A., 1904, Some remarks on the Mollusca of Lake Tanganyika. *Proceedings of the Malacological Society London*, 6(2): 77–104.

Smith, E. A., 1908, Description of new species of freshwater shells form Central Africa. *Proceedings of the Malacological Society of London*, 8: 398–401.

Sollas, I.B.J., 1907, The molluscan radula: Its chemical composition, and some points in its development. *Journal of Cell Science*, s2-51, 115-136.

Steneck, R. S., Watling, L., 1982, Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. *Marine Biology*, 68: 299319.

Thiele, J., 1931-1935, Handbuch der systematischen Weichtierkunde. Gustav Fischer, Jena, Germany, 1154 pp.

Troschel, F. H., 1856-1863, *Das Gebiss der Schnecken zur Begründung einer natürlichen Classification*. Berlin, Germany, 252 pp.

Trowbridge, C. D., 1991, Diet specialization limits herbivorous sea slug's capacity to switch among food species. *Ecology*, 72(5): 1880–1888.

van der Wal, P., Giesen, H. & Videler, J., 2000, Radular teeth as models for the improvement of industrial cutting devices. *Material Science Engineering C*, 7: 129–142.

Wang, C., Li, Q.Y., Wang, S.N., Qu, S.X. & Wang, X.X., 2014, Microstructure and self-sharpening of the magnetite cap in chiton tooth. *Materials Science and Engineering: C*, 37: 1-8.

Wealthall, R.J., Brooker, L.R., Macey, D.J. & Griffin, B.J., 2005, Fine structure of the mineralized teeth of the chiton *Acanthopleura echinata* (Mollusca: Polyplacophora). *Journal of Morphology*, 265: 165-175.

Weaver, J.C., Wang, Q., Miserez, A., Tantuccio, A., Stromberg, R., Bozhilov, K.N., Maxwell, P., Nay, R., Heier, S.T. & DiMasi, E., 2010, Analysis of an ultra hard magnetic biomineral in chiton radular teeth. *Materials Today*, 13: 42-52.

West, K., Michel, E., Todd, J., Brown, D. & Clabaugh, J., 2003, *The Gastropods of Lake Tanganyika: Diagnostic key, classification and notes on the fauna*. Special publications: Societas Internationalis Limnologiae – Int. Assoc. of Theoretical and Applied Limnology, 130 pp.

Wiesel, R. & Peters, W., 1978, Licht- und elektronenmikroskopische Untersuchungen am Radulakomplex und zur Radulabildung von *Biomphalaria glabrata* Say (*= Australorbis* gl.) (Gastropoda, Basommatophora). *Zoomorphologie*, 89: 73–92.

Wilson, A. B., Glaubrecht, M. & Meyer, A., 2004, Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika. *Proceedings of the Royal Society B*, 271(1538): 529–536.

Woodward, S.P., 1859, On some new freshwater shells from Central Africa. *Proceedings of the Zoological Society of London*, 27: 348–351.

# Supplementary Material

Table 1. Examined species, specimens with locality, collection number and preparation technique, N of measured anchorage areas.

Таха	Species	Collection number	Locality	/		On map (Fig. 2)	Date of collecti on	Prepar ation	N of measured anchorage areas by SEM
Tiphobiini	Paramelania damoni (Smith, 1881)	ZMB 107.096-7	Tanza nia	07°07 '25''S	30°31 '08''E	1	25.10.2 004	SEM	Central: 17 Lateral: 64
	<i>Limnotrochus thomsoni</i> Smith, 1880	ZMH 119371/99 9-1	Zamb ia	08°47 '50''S	31°01 '02''E	2	20.09.2 016	Ероху	
		ZMH 119371/99 9-2						SEM	Central: 12 Lateral: 73
		ZMH 119371/99 9-3						SEM	Central: 48 Lateral: 95
Spekiini	<i>Spekia zonata</i> (Woodward, 1859)	ZMB 220.077-2 ZMB	Zamb ia	08°45 '547'' S	31°05 '825'' E	3	12.02.2 000	Ероху	
		220.077-3		•	-			Ероху	
		ZMB 220.142-2 ZMB 220.143-3	Tanza nia	Kigo ma		4	24.02.1 995	SEM SEM	Central: 43 Lateral: 36
		ZMB 220.042-1	Zamb ia	08°42 '887'' S	31°08 '476'' E	5	19.11.1 999	SEM	
		ZMH without number-1 ZMH without number-2	Zamb ia	08°37 '23''S	31°12 '01''E	13	09.201 8	Section s	
		ZMH without number-3 ZMH without number-4						Protein ase K Protein ase K	
	Bridouxia ponsonbyi (Smith, 1889)	ZMB 220.137-9 ZMB 220.137- 10	Tanza nia	Kigo ma		4	28.02.1 995 28.02.1 995	Epoxy SEM	

		ZMB 220.137- 11					24.02.1 995	SEM	
		ZMB 220.137- 13	Zamb ia	08°42 '887'' S	31°08 '476'' E	5	19.11.1 999	SEM	Central: 11 Lateral: 36
	Bridouxia praeclara Bourguignat, 1885	ZMB 220.010-3 ZMB 220.010-4	Zamb ia	08°45 '258'' S	31°05 '116'' E	6	15.11.1 999	Epoxy SEM	
		ZMB 220.010-5						SEM	Central: 25 Lateral: 40
	Bridouxia rotundata	ZMB 220.063-3	Tanza nia	Kigo ma		4	02.199 5	Ероху	
	(Smith, 1904)	ZMB 220.063-4						SEM	
		220.063-5 ZMB						SEM	Central: 29
	Bridouxia grandidieriana	ZMH 119367/99	Zamb ia	08°43 '25''S	31°09 '00''E	7	30.08.2 016	Ероху	Lateral: 18
	(Bourguignat, 1885)	9-1 ZMH 119367/99 9-2						SEM	Central:11 Lateral: 25
		ZMH 119367/99 9-3						SEM	
		ZMB 220.009-3	Zamb ia	08°46 '50''S	31°05 '05''E	8	16.11.1 999	SEM	
	Reymondia horei (Smith, 1880)	ZMB 220.007-1 ZMB	Zamb ia	08°45 '258'' S	31°05 '116'' E	6	31.10.1 999	SEM SEM	Central: 67 Lateral: 71 Central: 23
		ZZ0.007-3 ZMB 220.147-2	Tanza nia	Kigo ma		4	26.02.1 995	SEM	Central: 34 Central: 15 Lateral: 57
		ZMB 220.147-3						SEM	
		ZMB 220.147- 10						Ероху	
	Leloupiella minima (Smith, 1908)	ZMB 220.008-1 ZMB	Zamb ia	08°45 '258'' S	31°05 '116'' F	6	23.11.1 999	Ероху	
	(511101, 1908)	2101B 220.008-2 ZMB		3	E			SEM	Central: 6
	<i>a</i> , .	220.008-3		00000	200.00		40.45.5	-	Lateral: 16
Cleopatriní	<i>Cleopatra</i> <i>johnstoni</i> Smith, 1893	21VIB 220.102b- 1	Zamb ia	09°20 '866'' S	28°43 .886'' E	9	19.12.2 000	Ероху	

		-					1	1	i .
		ZMB 220.102b- 2						SEM	
		ZMB 220.102b- 3						SEM	Central: 30 Lateral: 39
Nassopsini	Lavigeria grandis (Smith, 1881)	ZMB 220.018-1	Zamb ia	08°42 '887'' S	31°08 '476'' E	7	08.11.1 999	Ероху	
		ZMB 220.089-1 ZMB	Zamb ia	08°47 '480'' S	31°04 '494'' E	8	05.12.2 000	SEM Section	
		220.089-2 ZMH 150051/99 9-1	Zamb ia	08°37 '23''S	31°12 '01''E	13	16.12.2 017	s Section s	
	Lavigeria nassa (Woodward, 1859)	ZMH 119369/99 9-1 ZMH 119369/99	Zamb ia	08°29 '23''S	30°28 '46''E	10	09.09.2 016	Epoxy Section s	
		9-2 ZMH 119369/99 9-3						SEM	Central: 20 Lateral: 35
		ZMH 119369/99 9-5 ZMH 119369/99 9-7						Section s SEM Section s	
		ZMB 220.127-1	Buru ndi	Buju mbur a		11	03.198 8	SEM	
	Lavigeria paucicostata (Bourguignat,	ZMH 119368/99 9-1	Zamb ia	08°36 '26''S	30°49 '52''E	12	03.09.2 016	Ероху	
	1888)	ZMH 119368/99 9-2						SEM	
		ZMH 119368/99 9-3						SEM	Central: 11 Lateral: 46
		ZMH 119368/99 9-4						SEM	Central: 16 Lateral: 29

# Chapter 3. Functional morphology and material properties of *Spekia zonata* – a case study

# *3. 1 Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods*

Wencke Krings<sup>1</sup>\*, Alexander Kovalev<sup>2</sup>, Matthias Glaubrecht<sup>1</sup>, Stanislav N. Gorb<sup>2</sup>

<sup>1</sup> Center of Natural History (CeNak), Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

<sup>2</sup> Functional Morphology and Biomechanics, Zoological Institute of the Christian-Albrechts-Universität zu Kiel, Am Botanischen Garten 9, 24118 Kiel, Germany

\*corresponding author: wencke.krings@uni-hamburg.de

#### <u>Abstract</u>

One important autapomorphy of molluscs is the radula, which is the anatomical structure used for feeding in most species of Mollusca. As this phylum represents the second species-richest animal group inhabiting very diverse environments, it is not surprising that the morphology of the radula and its teeth is also very diverse between taxa. However, the taenioglossan radulae are remarkable because its tooth types (central, lateral, and marginal teeth) are highly distinct in their morphology within the same radula. There are several hypotheses attempting to explain this tooth diversity by their functional specialisations. Here for the first time, the functional morphology of taenioglossan radular teeth from one single species was analysed and their material properties (hardness and elasticity modulus) were characterised by nanoindentation. Spekia zonata Bourguignat, 1879 belongs to the Paludomidae inhabiting and feeding on solid substrates in Lake Tanganyika. All tooth types show gradual and significant differences in their stiffness and hardness: from the basis, as the softest and most flexible area, to the stylus and the cusps as the stiffest and hardest areas. The flexibility of the stylus allows the teeth to act as one single feeding organ: the central and lateral teeth can stabilize each other during feeding by the rear teeth providing support to the next row. Tooth types also differ significantly in their stiffness and hardness: the central teeth consist of the hardest and stiffest material, followed by the lateral teeth and finally marginal teeth. This can be explained by different functional loads of teeth: central and lateral ones are used for scratching over the substrate while the marginals serve mainly as brooms collecting food particles.

Keywords: Functional morphology, nanoindentation, mechanical properties, Gastropoda, Mollusca

#### Introduction

The radula is the anatomical structure used for feeding in most Mollusca species and one important autapomorphy of this animal group. With 80,000 recent species in the class Gastropoda alone (Bouchet et al., 2005) Mollusca is the second species-rich animal group with a diversity only topped by Insecta (e.g. Chapman, 2009). This diversity goes along with the colonialization of diverse ecosystems and with the establishment of different ecological niches, including various food sources. This became possible partially due to a key innovation for mechanical food processing, the radula.

The great natural historians of the 19th and early 20th century equipped with rather simple light microscopes were the first to understand this type of feeding organ as being what we today call an autapomorphy of the Mollusca. The first extensive study of the radula was done by Troschel (1856–1863), who introduced it as the most important character complex for mollusc systematics at various levels. Following this paradigm gastropods were eventually classified by the 'form' of the radula and its teeth, replacing the more traditional view based on shells only. Thiele (1931–1935) first revised the Mollusca based on radular characters. This in turn resulted in the reorganization of the system of gastropods at all taxonomic levels. Today the systematic value of the radular morphology remains valuable; however, it is not applicable at every hierarchical level partly due to ecological adaptations. Nevertheless, the morphology of the radula is understood as a link between the organism and its environment holding phylogenetic as well as ecological information.

The gastropods' feeding organ, the buccal mass, is faced ventrally in the oral cavity and includes not only the radula, but also the odontophoral cartilages, numerous muscles, and in some taxa the jaw. The odontophoral cartilages are surrounded by the chitinous radular membrane (Guralnick and Smith, 1999), in which transverse and longitudinal rows of sometimes mineralized teeth are embedded (fig. 1b). While feeding, the non-extensible chitinous membrane is stretched while being pulled over the odontophoral cartilage and moved by numerous muscles so that teeth can scratch on the substrate loosening food items.

According to different amounts and arrangements of teeth, seven basic types of radular morphologies have been recognized (e.g. Gray, 1853; Hyman, 1967; Steneck and Watling, 1982). In addition, radulae can show different tooth morphologies between taxa, but with a similar universal structure. They consist of a basis connecting the tooth to the radular membrane, a stylus, and a cusp consisting of denticles (e.g. Runham and Thornton, 1967; Brooker and Macey, 2001). Tooth morphologies can be very diverse also within single radula. For example, in the case of the taenioglossan radula, there are four distinct teeth: within each row one central tooth, flanked to the sides by one lateral tooth and two marginal teeth (fig. 1c-g). In addition to the variety of tooth morphologies there is diversity in material properties of radular teeth. Within the last two decades, material properties of rhipidoglossan and docoglossan radular teeth had been studied extensively in Patellogastropoda and Polyplacophora; it has been shown that different elements, sometimes a substantial amount of iron, is incorporated in the chitinous teeth material making the teeth more wear resistant (e.g. Runham, 1961; Towe et al., 1963; Runham et al., 1969; van der Wal, 1989; Evans et al., 1991, 1992; Lee et al., 2003; van der Wal et al., 2000; Brooker et al., 2003; Shaw et al., 2010; Weaver et al., 2010; Brooker and Shaw, 2012; Lu and Barber, 2012; Ukmar-Godec et al., 2015). Since the amount and the composition of embedded elements differ between the previously studied mollusc species, the teeth have different mechanical properties (e.g. Weaver et al., 2010; Lu and Barber, 2012; Grunenfelder et al., 2014; Barber et al., 2015; Ukmar-Godec et al., 2017). However, radular teeth are not everlasting, and through the continuous growth of the radular ribbon from posterior to anterior new rows of teeth constantly enter the wearing zone while at the same time the teeth in the last row break loose (e.g. Runham, 1962, 1963; Runham and Isarankura, 1966; Isarankura and Runham, 1968; Mackenstedt and Märkel, 1987; Lowenstam and Weiner, 1989; Franz, 1990; Padilla et al., 1996; Shaw et al., 2008). Only the outermost few rows of teeth are actively used (e.g. Runham, 1963; Runham and Isarankura, 1966; Isarankura and Runham, 1968; Mackenstedt and Märkel, 1987, Franz, 1990; Padilla et al., 1996; Shaw et al., 2008).

The main function of the radula is scratching across a substrate loosening ingesta (food, minerals, etc.). Usually, these movements include cutting and grinding actions as potential dimensions of particles may be too large to take in at once or food can be potentially fixed to the substrate. So far only the mechanical properties of docoglossan radular teeth from Patellogastropoda and

Polyplacophora have been studied (Weaver et al., 2010; Lu and Barber, 2012; Grunenfelder et al., 2014; Barber et al., 2015; Ukmar-Godec et al., 2017). However, tooth types within one radula with strong differences in their morphology such as those of a taenioglossan radula might have different functions. In this study we hypothesize that the central and lateral teeth are responsible for loosening ingesta from the substrate whereas the two marginals harvest the loosened particles. In order to test this, we identified material properties of the taenioglossan radular teeth from freshwater Cerithioidea. Our species *Spekia zonata* Bourguignat, 1879 (fig. 1a) belongs to the Paludomidae inhabiting solid substrates in the so-called 'ancient' Lake Tanganyika (e.g. Wilson et al., 2004; Glaubrecht, 2008) and was chosen because its radula shows morphological differences between the central, lateral and marginal teeth. Lake Tanganyika is the second deepest lake in the world and well known for its unique assemblage of highly endemic animal species. The paludomid gastropods from Lake Tanganyika are of particular interest, because they represent the most spectacular example among molluscs of closely related taxa (about 50–70 species) with an extraordinary diversity especially in their shell and taenioglossan radular morphology (e.g. Leloup, 1953; Brown and Mandahl-Barth, 1987; Coulter, 1991; Brown, 1994; West et al., 2003).

Material properties (hardness and elasticity modulus) were measured by nanoindentation. Measurements of both the deformation and recovery of the material provided information about elasticity of the material (e.g. Ebenstein and Pruitt, 2006). This technique has been previously used to identify local mechanical properties of various biological materials (e.g. Arzt et al., 2002; Enders et al., 2004; Barbakadze et al., 2006; Klein and Gorb, 2012; Schulz-Kornas et al., 2017). Knowing the mechanical properties of the teeth we can deduce potential functions of different teeth (scratching or gathering). Within each tooth we also aimed at detecting gradients of material properties in order to understand functional significance of different regions on the tooth (bending, twisting, or resistance to obstacle).

#### Materials & Methods

Adult specimens were inventoried at the Museum für Naturkunde Berlin (ZMB) or the Zoological Museum Hamburg (ZMH) and are stored in ethanol; they were collected by one of us (MG) at Kigoma (Tanzania) in 1995, by Anthony Wilson at Kumbula Island (Zambia) in 2000, and by Heinz Büscher at Kalambo Falls Lodge (Zambia) in 2018. By testing samples that had been stored in ethanol for different periods of time we can identify to which extend alcohol changes material properties through time. For scanning electron microscopy (SEM), the radula of S. zonata (ZMB 220.144-1) was extracted, digested with proteinase K according to the protocol of Holznagel (1998), cleaned for a few seconds in an ultrasonic bath, mounted on an aluminium stub, coated with carbon and visualized with the SEM Zeiss LEO 1525 (One Zeiss Drive, Thornwood, NY). Seven radulae of S. zonata (ZMB 220.077-1, ZMB 220.077-2, ZMB 220.143-1, ZMB 220.143-2, ZMB 220.143-3, ZMB 220.143-4, ZMH 150008/999-1) were extracted, cleaned, dried and embedded in epoxy resin (RECKLI®EPOXIWST, elasticity modulus of the epoxy is 1 GPa). For the embedding procedure the radulae were tapped with double-sided adhesive tape to a glass object slide (the radulae laid on its side - at the bottom marginal, then lateral, central, lateral, and on the top marginal teeth). The tape was important to accurately arrange the radula. Then samples were surrounded by a small metallic ring that was filled with epoxy, which was polymerized at room temperature. We choose this specific epoxy because it did not infiltrate the teeth. After embedding the object slide and the tape was removed and radulae were first polished with gradual diamond pastes (Buehler MetaDi Ultra Paste 6 μm 3 μm, 1 μm) and finally smoothened with a polishing machine (Buehler MataServ 250 with Struers OP-U, 0.04 µm suspension with 250 rpm) for a plain surface displaying the longitudinal section of the teeth for nanoindentation (fig. 1h-k). Since the radula was arranged to its side only one tooth type was superficial at the plain surface. After testing the outer marginals samples were again smoothened until the inner marginals were on display. These steps were repeated until all teeth were measured.

Nanoindentation was performed using a Nanoindenter SA2 (MTS Nano Instrument, Oak Ridge, TN, USA; CSM) equipped with Berkovich indenter tip. With this technique hardness and the effective elastic modulus can be determined from force-distance curves. Application of the continuous

stiffness measurement technique (Oliver and Pharr, 1992) allows to measure the properties continuously during indentation as a function of depth. The Nanoindenter SA2 system is equipped with a dynamic contact module (DCM) head allowing to test materials with low contact stiffness as soft biological tissues. All tests were performed under normal room conditions (relative humidity 28–30%, temperature 22–24°C; during the single tests the relative humidity and temperature remained almost constant). The indents were made at two sites (stylus and cusp) along the longitudinally-polished central and lateral teeth and at three sites (basis, stylus, and cusp) along inner marginal and outer marginal teeth (fig. 2a). This approach provided us with information on differences in mechanical properties from the cusp to the stylus of each tooth. After each loading cycle the specimen was fully unloaded and the indenter moved to the next preselected position. Each indentation curve was manually controlled for correct surface finding after the tests were completed. In each specimen 10–20 tooth rows of the wear zone were analysed, resulting in more than 660 measured areas of indentation. Elasticity moduli (E) and hardness (H) of materials were determined at penetration depths of 480–520 nm with about 30 values per indentation. We decided to focus on the material properties at this depth, because due to the surface roughness both E and H strongly fluctuated at low indentation depths (fig. 2b). Also, the measured structures are quite small (fig. 1h-k) and thereby we excluded the side effects of the surrounding epoxy. All statistical analyses were performed with JMP<sup>®</sup> Pro, Version 13 (SAS Institute Inc., Cary, NC, 1989-2007). Mean values and standard deviations were calculated, Shapiro-Wilk-W-test for testing of normality and one-way ANOVA followed by a Student-t-test for detecting homogenous groups were carried out in order to compare mechanical properties of different areas of the central, lateral, inner and outer marginal teeth within each specimen (i), between single specimens (ii), between the different sample locations (iii), and between different preservation times of specimens (iv).



Figure 1. (a) Shells of Spekia zonata (ZMB 220.077-1); (b) schematic drawing of the radula when feeding; (c-g) taenioglossan radula of S. zonata (ZMB 220.144-1); (c) mature worn teeth in the wear zone, black arrows = area of nanoindentation for central, lateral and marginal teeth; (d) central and lateral teeth; (e) immature and unworn mature central, lateral, inner marginal and outer marginal teeth; (f) central and lateral teeth viewed from the side; (g) inner and outer marginal teeth; (h) radula (ZMB220.143-2) embedded in epoxy resin and polished for nanoindentation (longitudinal section along the radula) with (j-k) magnification of some tested areas (j: with nanoindentation mark; k: crosses indicate points of indentation). Scale bars: a = 4 mm; c, e, g =100  $\mu$ m; d, f, i, j =30  $\mu$ m; h =250  $\mu$ m; k = 60  $\mu$ m. CT = central tooth, FP = food particle, IMT = inner marginal tooth, IRT = immature radular teeth, LT =lateral tooth, MRT = mature radular teeth, O = odontophore, OMT = outer marginal tooth, RM = radular muscles, RT = radular teeth.

Figure 2. (a) Areas of nanoindentation, scale bar = 30  $\mu$ m; (b) results of nanoindentation measurements for stylus of lateral teeth, elastic modulus versus displacement into tooth material. The mean values within the indentation depth of 480–520 nm were used for further comparison between measurements; (c) summary of Young's moduli for different regions with significant differences between them (shown by different letters). CTC = central tooth cusp, CTS = central tooth stylus, IMTB = inner marginal tooth basis, IMTC = inner marginal tooth cups, IMTS = inner marginal tooth basis, OMTC = outer marginal tooth cup, OMTS = outer marginal tooth stylus.



## <u>Results</u>

The radula of *Spekia zonata* has an extraordinary length (about 6 mm in an animal with 12.5–12.8 mm shell height) and consists of about 105 tooth rows. This species has a remarkable diversity between its distinct teeth.

The central tooth is very broad (mean value: ~ 170  $\mu$ m) and three times wider than long (fig. 1d). It is shaped like a wing and typically lacks a central cusp. The anterior rim is concave, forming a broad scraping edge without median cusps. This median portion of the cutting edge is usually evenly rounded with a widely triangular outline and sometimes of more irregular shape with rudimentary cusps. The margins of the cutting edge are inclined toward frontal marginal cusps on the basal platform and carry 4–6 successively smaller pointed cusps. The basal platform is solid and of crescent shape with a widely bulging posterior central portion accompanied by a pair of rounded small posterior cusps. The tooth formula (amount of cusps for this tooth type) is 4–6/0/4–6.

The lateral teeth have a posterior lateral extension that occupies one half of the width of the tooth (fig. 1c). The basal platform of the tooth is thickened (mean value: ~ 130  $\mu$ m) and not continuous with the lateral extensions. Its cutting edge carries a broad spatulate central cusp and a variable number of accompanying cusps, the tooth formula is 2–3/1/2–5 (fig. 1d).

The inner and outer marginal teeth are long, slender (mean value: ~ 209  $\mu$ m; 5 times longer than wide) and their flattened stalks have grooves at the lateral sides. Both marginal teeth are similar in shape and length but differ in their cutting edges. These are paw-like and bear eight broader cusps on the inner marginal, while the outer marginal is more slender with 11 finger-like cusps (fig. 1g).

The Young's (elastic) modulus is a measure of the stiffness of a solid material and describes the relationship between mechanical stress and indentation depth whereas the hardness is a measure of the resistance to local plastic deformation. Statistical analysis revealed normal distribution and significant differences (p<0.0001, ANOVA; F-ratio: 9; df: 8902.705). Student-t-test unveiled homogeneous groups (represented by letters in fig. 2c) showing that (i) areas (cusps, basis, stylus) differ between the tooth types (central, lateral, inner and outer marginal teeth), (ii) the measured areas (base, stylus and cusps) differ within each tooth type revealing a gradient of elasticity modulus

(fig. 2c) and hardness (hardness: Central Tooth Cusps [CTC] =  $0.41 \pm 0.02$  GPa; Central Tooth Stylus [CTS] =  $0.32 \pm 0.03$  GPa; Lateral Tooth Cusps [LTC] =  $0.28 \pm 0.02$  GPa; Lateral Tooth Stylus [LTS] =  $0.24 \pm 0.03$  GPa; Inner Marginal Tooth Cusps [IMTC] =  $0.23 \pm 0.03$  GPa; Inner Marginal Tooth Stylus [IMTS] =  $0.21 \pm 0.02$  GPa; Inner Marginal Tooth Basis [IMTB] =  $0.13 \pm 0.02$  GPa; Outer Marginal Tooth Cusps [OMTC] =  $0.22 \pm 0.02$  GPa; Outer Marginal Tooth Stylus [OMTS] =  $0.18 \pm 0.02$  GPa; Outer Marginal Tooth Basis [OMTC] =  $0.22 \pm 0.02$  GPa; Outer Marginal Tooth Stylus [OMTS] =  $0.18 \pm 0.02$  GPa; Outer Marginal Tooth Basis [OMTB] =  $0.14 \pm 0.02$  GPa). The stiffness and hardness decrease significantly in each taenioglossan tooth type from the cusp over the stylus to the base. The central tooth cusps consist of the stiffest material (elastic modulus:  $8.09 \pm 0.65$  GPa; hardness:  $0.41 \pm 0.02$  GPa; N = 110 fully mineralized teeth), followed by the lateral tooth cups (elastic modulus:  $5.78 \pm 0.42$  GPa; hardness:  $0.28 \pm 0.02$  GPa; hardness:  $0.23 \pm 0.03$  GPa; N = 57 fully mineralized teeth) and the outer marginal tooth cusps (elastic modulus:  $4.60 \pm 0.47$  GPa; hardness:  $0.22 \pm 0.02$  GPa; N = 60 fully mineralized teeth). No significant differences in elasticity moduli and hardness were found in the same measured areas between different individuals. No significant differences were detected between the samples from different years and different collection sites.

#### <u>Discussion</u>

# Function of taenioglossan teeth

Here we present the first comparative study on the mechanical properties of the taenioglossan radular teeth based on a large sample size. All teeth show gradual and significant differences in their stiffness and hardness: with the basis as the softest and most flexible area over to the cusps as the stiffest and hardest areas. We hypothesize that this is likely an adaptation to the reduction of the probability of fractures by reducing contact stress concentrations when teeth contact obstacles during active feeding process.

The distinct taenioglossan teeth differ significantly in their stiffness and hardness: the central tooth consists of the hardest and stiffest material, followed by the lateral teeth and both marginal teeth. This can be explained by different functional loads of teeth: central and lateral teeth are used for scratching over the substrate and removing food items attached to the surface. During this action the muscular force must be transferred by the tooth to the substrate. This results in tensile and compressive stresses which in turn result in an elastic deformation in contact. Since these teeth are rather short and broad deformations are reduced. Central and lateral teeth are stiff and hard, which might be an adaptation to transmit the high forces needed to rasp off ingesta. Furthermore, the flexibility of the stylus allows them to act as one single feeding organ: the central and lateral teeth are stabilized during feeding by the support from the central and lateral teeth of the next tooth row (this was also reported in Hickman, 1984; Padilla, 2003).

The marginals of a taenioglossan radula are thought to be functionally different, to serve as brooms (e.g. Steneck and Watling, 1982; Mackenstedt and Märkel, 2001). The mechanical properties reported here and the morphology of the marginal teeth strongly indicate that their function is the harvesting of food items or particles which had been loosened from the substrate by the grinding action of the central and lateral teeth. We suppose that when the radula is pulled and stretched across the odontophore during the feeding process, marginal teeth are folded to the side so that they do not touch the surface during scraping of the substrate by the central and lateral teeth (fig. 3b). When the radula relaxes (fig. 3a) these teeth fold back gathering the loosened food. During

folding and unfolding, they have to cover the longest distance along the substrate surface with a high possibility to hit an obstacle. The lower stiffness and hardness of the stylus and the basis of these teeth allow them to reduce stress concentration at their cusps in case of hitting the substrate asperities (even to the higher extent than it is the case for central and lateral teeth). The cusps can be catapulted back in place after passing the obstacle without fractures or ruptures. The combination of stiff cusps and softer styli and bases results in the ability to process even the food on solid substrates properly at the minimal risk of damage. Similar mechanism was recently demonstrated for the copepod gnathobases (Michels et al., 2012), for the wear-reducing mechanism of the snake skin (Klein and Gorb, 2012) or for human teeth (Wang and Weiner, 1998; Fong et al., 2000, Zheng et al., 2003). The inner and outer marginals of the studied taenioglossan radula also stabilize each other, with usually one inner and one outer marginal interlocking (fig. 1g) and thus functioning as one single unit. This latter mechanism had also been previously reported in the teeth of other animals such as catfish (Geerinckx et al., 2007). Thus, the whole taenioglossan radula can be viewed as highly adapted mechanical organ of ingesta processing with different mechanical functions of its parts.

# *Comparison with mechanical properties of radulae of other molluscs*

Our values of the elasticity modulus of the central and lateral tooth cusps (mean value  $\pm$  standard deviation; CTC = 8.09  $\pm$  0.65 GPa; LTC = 5.78  $\pm$  0.42 GPa) in the paludomid *Spekia zonata* are very low in comparison with the elasticity measured in other radular teeth, ranging from 16 GPa over 90-125 GPa up to 52-140 GPa (Weaver et al., 2010; Lu and Barber, 2012; Grunenfelder et al., 2014; Barber et al., 2015; Ukmar-Godec et al., 2017). The hardness, previously measured in different mollusc species, is also much higher, ranging from 9-12 GPa (Weaver et al., 2010; Grunenfelder et al., 2014; Ukmar-Godec et al., 2017) in comparison to those measured here (0.11-0.43 GPa). However, all previous studies focused only on chitons and limpets. Chitons belong to the Mollusca, but form an own class (Polyplacophora) and are not closely related to Gastropoda (Kocot et al., 2011); limpets belong to the gastropods, but as a sister group (Patellogastropoda) to most other gastropods, they are not closely related with our studied species (Aktipis et al., 2008). We do not

know to which extend biomineralization of radular teeth and, depending on this, their hardness and elasticity, is phylogenetically fixed or adaptive, because only few studies focus on this (Lowenstam and Weiner, 1985, 1989; Brooker and Macey, 2001; Brooker et al., 2006; Brooker and Shaw, 2012). Interesting in this context would be to further study material properties of species that are closely related to that studied here.



Figure 3. Hypothetical position of the radular teeth while resting (*a*); and while scratching over the substrate surface (*b*). Marginal teeth are flipped while feeding and due to the collapse of the radula, the marginal teeth swing back in the resting position collecting the loosened food items. CT = central tooth, IMT = inner marginal tooth, LT = lateral tooth, O = odontophore, OMT = outer marginal tooth, RM = radular membrane.

Additionally, representatives of Polyplacophora and Patellogastropoda are known for feeding on hard calcified algae (Weaver et al., 2010; Lu and Barber, 2012; Grunenfelder et al., 2014; Barber et al., 2015; Ukmar-Godec et al., 2017) and in contrast, *S. zonata* feed on soft algae scratched off a stone. In order to determine their feeding preferences in greater detail, it would be necessary to test the feeding substrates and intaken biofilms directly *in situ* in Africa.

The differences in hardness could also be explained by different amounts of calcium in the water bodies (e.g. Besson et al., 2014), but it is not yet clarified to which extent calcium carbonate is taken up from the surrounding water or from the diet (e.g. Furuhashi et al., 2009). It had been shown that marine species have lower calcium ion concentrations in their extrapallial fluids than those in freshwater (Wilbur and Saleuddin, 1983; Coimbra et al., 1988). Since the radula consists of calcified chitin *S. zonata*, inhabiting Lake Tanganyika with a relatively low calcium-concentration (Branchu and Bergonzini, 2004), might have less access to harder elements than the marine species examined in previous studies. Calcium is also an important element of the snail's shell, but despite of the different ion concentrations in marine and freshwater habitats no evidence for average thicker or thinner shells had been found.

Furthermore, we do not know the turnover rate of paludomid radula teeth. It has been previously reported that chitons replace about 0.32-0.36 tooth rows/day (e.g. Shaw et al., 2002; Sigwart and Carey, 2014). Thus, we anticipate that softer teeth might have stronger wear rate, but could be replaced faster. Previous studies, however, reported on snails with a different (i.e. docoglossan) radular type. Those teeth are rather uniform in their morphology and are mainly used for scratching (e.g. Steneck and Watling, 1982; Mackenstedt and Märkel, 2001) in comparison to the distinct tooth type of a taenioglossan radula as in *Spekia* having different functionality of teeth in each row.

#### **Conclusion**

The hardness and effective elasticity modulus of taenioglossan radular teeth were measured by nanoindentation in our model species *Spekia zonata*. (*a*) Every tooth shows gradual and significant differences in stiffness and hardness: from the basis (the softest and most flexible area) to the stylus (intermediate) and the cusps (the stiffest and hardest areas). The flexibility of the stylus allows the teeth to act as one single feeding organ: the central and lateral teeth can stabilize each other during feeding by the rear teeth providing support. (*b*) The four different tooth types differ significantly in their stiffness and hardness: the central teeth. This can be explained by different functional loads of teeth: central and lateral ones are used for scratching over the substrate while the marginals serve mainly as brooms collecting food particles.

#### <u>Acknowledgements</u>

We would like to thank: Peter Stutz from the Mineralogical-Petrographic Institute of the University of Hamburg for the great support in the sample preparation. Heinz Büscher from Basel helped to great extend collecting specimens at Lake Tanganyika. Renate Walter from the Zoological Institute of the University of Hamburg visualized the radulae at the SEM. Thomas M. Kaiser from the CeNak discussed results and earlier versions of the manuscript. We thank the anonymous reviewers for their helpful comments.

#### Authors' contribution

Wencke Krings wrote the manuscript, drew the figures, performed nanoindentation and analysed the data. Alexander Kovalev is an expert in nanoindentation; he also discussed the data. Matthias Glaubrecht initialized the project and its design, discussed and planed; his expertise in malacology contributed greatly to the connection to molluscan biology. Stanislav N. Gorb designed the experiments and discussed the data, the manuscript, the figures; his expertise was critical for understanding the results and the functional morphology. All authors contributed to the manuscript.

#### <u>References</u>

Aktipis, S.W., Giribet, G., Lindberg, D.R., Ponder, W.F., 2008. Gastropoda: An overview and analysis, In: Ponder, W.F., Linderberg, D.R. (Eds.), Phylogeny and Evolution of the Mollusca. University of California Press, London, pp. 201–238.

Arzt, E., Enders, S., Gorb, S., 2002. Towards a micromechanical understanding of biological surface devices. Z. Metalkd. 93, 345–351. https://doi:10.3139/146.020345.

Barbakadze, N., Enders, S., Gorb, S., Arzt, E., 2006. Local mechanical properties of the head articulation cuticle in the beetle *Pachnoda marginata* (Coleoptera, Scarabaeidae). J. Exp. Biol. 209, 722–730. https://doi:10.1242/jeb.02065.

Barber, A.H., Lu, D., Pugno, N.M., 2015. Extreme strength observed in limpet teeth. J. Royal Soc. Interface 12, 20141326. https://doi:10.1098/rsif.2014.1326.

Besson, P., Degboe, J., Berge, B., Chavagnac, V., Fabre, S., Berger, G., 2014. Calcium, Na, K and Mg concentrations in seawater by inductively coupled plasma-atomic emission spectrometry: Applications to IAPSO seawater reference material, hydrothermal fluids and synthetic seawater solutions. Geostand. Geoanal. Res. 38(3), 355–362. https://doi:10.1111/j.1751-908X.2013.00269.x.

Bouchet P., Rocroi J.-P. (Ed.); Frýda J., Hausdorf B., Ponder W., Valdes A. and Warén A., 2005. Classification and Nomenclator of Gastropod Families. Malacologia 47(1-2). ConchBooks: Hackenheim, Germany.

Bourguignat, M.J.R., 1879. Description de diverses espèces terrestres et fluviatiles et de différents genres de molluscques de l'Egypte, de l'Abyssinies, de Zanzibar, du Senegal et du centre de l'Afrique. Paris.

Branchu, Ph., Bergonzini, L., 2004. Chloride concentrations in Lake Tanganyika: an indicator of the hydrological budget? Hydrol. Earth Syst. Sci. 8, 256-265. https://doi:10.5194/hess-8-256-2004.

Brooker, L., Lee, A., Macey, D., Van Bronswijk, W., Webb, J., 2003. Multiple-front iron-mineralisation in chiton teeth (*Acanthopleura echinata*: Mollusca: Polyplacophora). Marine Biology 142, 447–454. https://doi:10.1007/s00227-002-0957-8.

Brooker, L.R., Lee, A.P., Macey, D.J., Webb, J., van Bronswijk, W., 2006. In situ studies of biomineral deposition in the radula teeth of chitons of the suborder Chitonina. Venus 65(1-2), 71–80.

Brooker, L.R., Macey, D.J., 2001. Biomineralization in chiton teeth and its usefulness as a taxonomic character in the genus *Acanthopleura* Guilding, 1829 (Mollusca: Polyplacophora). Am. Malacol. Bull. 16, 203–215.

Brooker, L.R., Shaw, J.A., 2012. The chiton radula: a unique model for biomineralization studies. Intech Open Access Publisher. https://doi:10.5772/31766.

Brown, D., 1994. Freshwater Snails of Africa and their Medical Importance. Taylor and Francis.

Brown, D.S., Mandahl-Barth, G., 1987. Living molluscs of Lake Tanganyika: a revised and annotated list. J. Conchol. 32, 305–327.

Chapman, A. D., 2009. Numbers of living species in Australia and the world. 2nd edition. Toowoomba, Australia: Australian Biodiversity Information Services.

Coimbra, J., Machado, J., Fernandes, P.L., Ferreira, H.G., Ferreira, K.G., 1988. Electrophysiology of the mantle of *Anodonta cygnea*. J. Exp. Biol. 140, 65.

Coulter, G.W., 1991. Lake Tanganyika and its Life. Oxford University Press, Oxford.

Ebenstein, D.M., Pruitt, L.A., 2006. Nanoindentation of biological materials. Nano Today 1, 26-33. https://doi:10.1016/S1748-0132(06)70077-9. Enders, S., Barbakadze, N., Gorb, S. N., Arzt, E., 2004. Exploring biological surfaces by nanoindentation. J. Mater. Res. 19, 880–887. https://doi:10.1557/jmr.2004.19.3.880.

Evans, L.A., Macey, D.J., Webb, J., 1991. Distribution and composition of matrix protein in the radula teeth of the chiton *Acanthopleura hirtosa*. Mar. Biol. 109, 281–286. https://doi:10.1007/BF01319396.

Evans, L.A., Macey, D.J., Webb, J., 1992. Calcium biomineralization in the radular teeth of the chiton, *Acanthopleura hirtosa*. Calcif. Tissue Int. 51, 78–82. https://doi:10.1007/BF00296222.

Fong, H., Sarikaya, M., White, S.N., Snead, M.L., 2000. Nano-mechanical properties profiles across dentin-enamel junction of human incisor teeth. Mat. Sci. Eng. C- Bio. S. 7, 119–128. https://doi:10.1016/S0928-4931(99)00133-2.

Franz, C.J., 1990. Feeding patterns of *Fissurella* species on Isla de Margarita, Venezuela: use of radulae and food passage rates. J. Molluscan Stud. 56, 25–35. https://doi:10.1093/mollus/56.1.25.

Furuhashi, T., Schwarzinger, C., Miksik, I., Smrz, M., Beran, A., 2009. Molluscan shell evolution with review of shell calcification hypothesis. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 154, 351–371. https://doi:10.1016/j.cbpb.2009.07.011.

Geerinckx T., De Poorter, J., Adriaens, D., 2007. Morphology and development of teeth and epidermal brushes in loricariid catfishes. J. Morphology 268, 805–814.

Glaubrecht, M., 2008. Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology and systematization of a paludomid species flock in an ancient lake. Zoosystematics Evol. 84, 71–122. https://doi:10.1002/zoos.200700016.

Gray, J.E., 1853. On the division of ctenobranchous gasteropodous Mollusca into larger groups and families. Ann. Mag. Nat. Hist. 11(2), 124–133. https://doi:10.1111/j.1469-7998.1853.tb07174.x.

Grunenfelder, L.K., de Obaldia, E.E., Wang, Q., Li, D., Weden, B., Salinas, C., Wuhrer, R., Zavattieri, P., Kisailus, D., 2014. Biomineralization: Stress and damage mitigation from oriented nanostructures within the radular teeth of *Cryptochiton stelleri*. Adv. Funct. Mater. 24/39: 6085. https://doi:10.1002/adfm.201401091.

Guralnick, R., Smith, K., 1999. Historical and biomechanical analysis of integration and dissociation in molluscan feeding, with special emphasis on the true limpets (Patellogastropoda: Gastropoda). J. Morphol. 241, 175–195. https://doi:10.1002/(SICI)1097-4687(199908)241:2<175::AID-JMOR7>3.0.CO;2-0.

Hickman, C.S., 1984. Implications of radular tooth-row functional integration for archaeogastropod systematics. Malacologia 25, 143–160.

Holznagel, W., 1998. A nondestructive method for cleaning gastropod radulae from frozen, alcohol-fixed, or dried material. Am. Malacol. Bull. 14(2), 181–183.

Hyman, L.H., 1967. Mollusca I. Aplacophora, Polyplacophora, Monoplacophora. Gastropoda, the Coelomate Bilateria. The Invertebrates 6, McGraw-Hill Book Company, New York.

Isarankura, K., Runham, N.W., 1968. Studies on the replacement of the gastropod radula. Malacologia 7, 71–91.

Klein, M.-C.G., Gorb, S.N., 2012. Epidermis architecture and material properties of the skin of four snake species. J. Roy. Soc. Interface 9, 3140–3155. https://doi:10.1098/rsif.2012.0479.

Kocot, K.M., Cannon, J.T., Todt, C., Citarella, M.R., Kohn, A.B., Meyer, A., Santos, S.R., Schander, C., Moroz, L.L., Lieb, B., Halanych, K.M., 2011. Phylogenomics reveals deep molluscan relationships. Nature 477, 452–456. https://doi:10.1038/nature10382.

Lee, A.P., Brooker, L.R., Macey, D.J., Webb, J. van Bronswijk, W., 2003. A new biomineral identified in the cores of teeth from the chiton *Plaxiphora albida*. J. Biol. Inorg. Chem. 8, 256–262. https://doi:10.1007/s00775-002-0410-y.

Leloup, E., 1953. Exploration Hydrobiologique du Lac Tanganika (1946-1947). Bruxelles.

Lowenstam, H.A., Weiner, S., 1985. Transformation of amorphous calcium phosphate to crystalline dahllite in the radula teeth of chitons. Science 227, 51–52.

Lowenstam, H.A., Weiner, S., 1989. Mollusca, In: Lowenstam, H.A., Weiner, S. (Eds.), On Biomineralization. Oxford University Press, Oxford, pp. 88–305.

Lu, D., Barber, A.H., 2012. Optimized nanoscale composite behaviour in limpet teeth. J. Royal Soc. Interface 9, 1318–1324. https://doi:10.1098/rsif.2011.0688.

Mackenstedt, U., Märkel, K., 1987. Experimental and comparative morphology of radula renewal in pulmonates (Mollusca, Gastropoda). Zoomorphology 107, 209–239. https://doi:10.1007/BF00312262.

Mackenstedt, U., Märkel, K., 2001. Radular structure and function, In: Barker, G.M. (Eds.), The Biology of Terrestrial Molluscs. CABI Publishing, Oxon, pp. 213–236.

Michels, J., Vogt, J., Gorb, S.N., 2012. Tools for crushing diatoms - opal teeth in copepods feature a rubber-like bearing composed of resilin. Sci. Rep. 2, 1–5. https://doi:10.1038/srep00465.

Oliver, W.C., Pharr, G.M., 1992. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J. Mater. Res. 7, 1564-1583. https://doi:10.1557/JMR.1992.1564.

Padilla, D.K., 2003. Form and function of radular teeth of herbivorous molluscs: Focus on the future. Am. Malacol. Bull. 18(1), 163–168.

Padilla, D.K., Dittman, D.E., Franz, J., Sladek, R., 1996. Radular production rates in two species of Lacuna Turton (Gastropoda: Littorinidae). J. Molluscan Stud. 62, 275–280. https://doi:10.1093/mollus/62.3.275.

Runham, N.W., 1961. The histochemistry of the radula of Patella vulgata. J. Cell Sci. 3, 371–380.

Runham, N.W., 1962. Rate of replacement of the molluscan radula. Nature 194, 992–993. https://doi:10.1038/194992b0.

Runham, N.W., 1963. A study of the replacement mechanism of the pulmonate radula. J. Cell Sci. 3, 271–277.

Runham, N.W., Isarankura, K., 1966. Studies on radula replacement. Malacologia 5, 73.

Runham, N.W., Thornton, P.R., 1967. Mechanical wear of the gastropod radula: a scanning electron microscope study. J. Zool. 153, 445–452. https://doi:10.1111/j.1469-7998.1967.tb04976.x.

Runham, N.W., Thornton, P.R., Shaw, D.A., Wayte, R.C., 1969. The mineralization and hardness of the radular teeth of the limpet *Patella vulgata* L. Z. Zellforsch. Mikrosk. Anat. 99, 608–626. https://doi:10.1007/BF00340948.

Schulz-Kornas, E., Braune, C., Winkler, D.E., Kaiser, T.M., 2017. Does silica concentration and phytolith ultrastructure relate to phytolith hardness? Biosurf. Biotribol. 3, 135–143. https://doi.org/10.1016/j.bsbt.2017.12.004.

Shaw, J.A., Brooker, L.R., Macey, D.J., 2002. Radula tooth turnover in the chiton, *Acanthopleura hirtosa* (Blainville, 1825) (Mollusca: Polyplacophora). Molluscan Res. 22, 93–99. https://doi:10.1071/MR02005.

Shaw, J.A., Macey, D.J., Brooker, L.R., 2008. Radula synthesis by three species of iron mineralizing molluscs: production rate and elemental demand. J. Mar. Biol. Assoc. U.K. 88, 597–601. https://doi:10.1017/S0025315408000969.

Shaw, J.A., Macey, D.J., Brooker, L.R., Clode, P.L., 2010. Tooth use and wear in three iron-biomineralizing mollusc species. Biol. Bull. 218, 132–144. https://doi:10.1086/BBLv218n2p132.

Sigwart, J.D., Carey, N., 2014. Grazing under experimental hypercapnia and elevated temperature does not affect the radula of a chiton (Mollusca, Polyplacophora, Lepidopleurida). Mar. Environ. Res. 102, 73–78. https://doi:10.1016/j.marenvres.2014.05.004.

Steneck, R.S., Watling, L., 1982. Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. Mar. Biol. 68, 299–319. https://doi:10.1007/BF00409596.

Thiele, J., 1931–1935. Handbuch der systematischen Weichtierkunde. Gustav Fischer, Jena.

Towe, K.M., Lowenstam, H.A., Nesson, M.H., 1963. Invertebrate ferritin: occurrence in Mollusca. Science 142, 63–64. https://doi:10.1126/science.142.3588.63.

Troschel, F.H., 1856-1863. Das Gebiss der Schnecken zur Begründung einer natürlichen Classification. 1, 1–252. Berlin.

Ukmar-Godec, T., Bertinetti, L., Dunlop, J.W.C., Godec, A., Grabiger, M.A., Masic, A., Nguyen, H., Zlotnikov, I., Zaslansky, P., Faivre, D., 2017. Materials nanoarchitecturing via cation-mediated protein assembly: Making limpet teeth without mineral. Adv. Mater. 29, 1701171. https://doi: 10.1002/adma.201701171.

Ukmar-Godec, T., Kapun, G., Zaslansky, P., Faivre, D., 2015. The giant keyhole limpet radular teeth: A naturally-grown harvest machine. J. Struct. Biol. 192(3), 392–402. https://doi: 10.1016/j.jsb.2015.09.021.

van der Wal, P., 1989. Structural and material design of mature mineralized radula teeth of *Patella vulgata* (Gastropoda). J. Ultrastruct. Mol. Struct. Res. 102, 147–161. https://doi:10.1016/0889-1605(89)90052-9.

van der Wal, P., Giesen, H., Videler, J., 2000. Radular teeth as models for the improvement of industrial cutting devices. Mater. Sci. Eng. C 7, 129–142. https://doi:10.1016/S0928-4931(99)00129-0.

Wang, R. Z., Weiner, S., 1998. Strain-structure relations in human teeth using Moire fringes. J. Biomech. 31(2), 135–141. https://doi:10.1016/S0021-9290(97)00131-0.

Weaver, J.C., Wang, Q., Miserez, A., Tantuccio, A., Stromberg, R., Bozhilov, K.N., Maxwell, P., Nay, R., Heier, S.T., Di Masi, E., 2010. Analysis of an ultra hard magnetic biomineral in chiton radular teeth. Mater. Today 13, 42–52. https://doi:10.1016/S1369-7021(10)70016-X.

West, K., Michel, E., Todd, J., Brown, D., Clabaugh, J., 2003. The Gastropods of Lake Tanganyika: Diagnostic key, classification and notes on the fauna. Special publications: Societas Internationalis Limnologiae – Int. Assoc. of Theoretical and Applied Limnology.

Wilbur, K.M., Saleuddin, A.S.M., 1983. Shell formation, In: Saleuddin, A.S.M., Wilbur, K.M. (Eds.), The Mollusca. Academic Press, New York, pp. 1-235.

Wilson, A.B., Glaubrecht, M., Meyer, A., 2004. Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika. Proc. Royal Soc. Lond. B 271(1538), 529–536. https://doi:10.1098/rspb.2003.2624.

Zheng, J., Zhou, Z.R., Zhang, J., Li, H., Yu, H.Y., 2003. On the friction and wear behaviour of human tooth enamel and dentin. Wear 255, 967–974. https://doi:10.1016/S0043-1648(03)00079-6.

# *3. 2 Finite element analysis of individual taenioglossan radular teeth (Mollusca)*

Wencke Krings<sup>1\*,</sup> Jordi Marcé-Nogué<sup>234</sup>, Hasan Karabacak<sup>1</sup>, Matthias Glaubrecht<sup>1</sup>, Stanislav N. Gorb<sup>5</sup>

<sup>1</sup> Center of Natural History (CeNak), Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

<sup>2</sup> Department of Pathology and Anatomical Sciences, Jacobs School of Medicine and Biomedical Sciences, University of Buffalo, State University of New York, NY, USA

<sup>3</sup> Department of Mechanical Engineering, Universitat Rovira i Virgili, Tarragona, Spain

<sup>4</sup> Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Barcelona, Spain

<sup>5</sup> Functional Morphology and Biomechanics, Zoological Institute of the Christian-Albrechts-Universität zu Kiel, Am Botanischen Garten 9, 24118 Kiel, Germany

\*corresponding author: wencke.krings@uni-hamburg.de; +49 40 428388126, Center of Natural History (CeNak), Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

## <u>Abstract</u>

Molluscs are a highly successful group of invertebrates characterised by a specialised feeding organ called the radula. The diversity of this structure is associated with distinct feeding strategies and ecological niches. However, the precise function of the radula (each tooth type and their arrangement) remains poorly understood. Here for the first time, we use a quantitative approach, Finite-Element-Analysis (FEA), to test hypotheses regarding the function of particular taenioglossan tooth types. Taenioglossan radulae are of special interest, because they are comprised of multiple teeth that are regionally distinct in their morphology. For this study we choose the freshwater gastropod species *Spekia zonata*, endemic to Lake Tanganyika, inhabiting and feeding on algae attached to rocks. As a member of the African paludomid species flock, the enigmatic origin and evolutionary relationships of this species has received much attention. Its chitinous radula comprises several tooth types with distinctly different shapes. We characterise the tooth's position, material properties and attachment to the radular membrane and use this data to evaluate 18 possible FEA scenarios differing in above parameters. Our estimations of stress and strain indicate different functional loads for different teeth. We posit that the central and lateral teeth are best

suitable for scratching substrate loosening ingesta, whereas the marginals are best suited for gathering food particles. Our successful approach and workflow are readily applicable to other mollusc species.

Keywords: Functional morphology, FEA, radula, mechanical properties, Gastropoda

# Introduction

# 1.1 Success of molluscs

Mollusca is the second most taxonomically diverse animal group: estimates comprise 70,000–76,000 [1], up to 130,000 [2], or 200,000 [3–5] extant species. Within Mollusca, even though there are many problems with recording the malacofauna and species numbers [6], Gastropoda is the most diverse constituent clade with 80,000 [7] described recent species. The Molluscan diversity, dating back to more than 550 million years [8–9], is enabled by the colonialization of nearly all aquatic and terrestrial ecosystems leading to the establishment of different ecological niches [10]. This became possible due to the immense diversity in their body plans and shells, their complex nervous systems [10–12], and partially due to a key innovation for mechanical food processing termed radula resembling an important autapomorphy, a distinct feature that is unique to Mollusca.

# 1.2 Previous work on radular basic structure

The gastropods' feeding organ, the buccal mass, does not only include the radula, but also odontophoral cartilages, muscles, and in some taxa the jaw. The cartilages are covered by the chitinous radular membrane [13], embedding rows of sometimes mineralized teeth [Krings et al., accepted for publication in Malacologica, here chapter 2.2]. During foraging the membrane is pulled over the odontophoral cartilage by radular muscles, leading to the interaction of teeth with the ingesta (Fig. 1b). This can lead to wear and potentially structural failure, but continuously the radula is formed posteriorly (building zone, radular sack) and in the course of an individual ontogeny become mature before entering the working zone whereas anteriorly the teeth in the outermost row break loose (wearing zone) [14–22].

# 1.3 Previous work on radular diversity and material properties

The notification that radulae differ in the amounts and arrangements of teeth led to the definition of about 5–7 basic radular types [23–25] which do not consistently reflect phylogeny due to convergences [26]. The tooth morphologies can be distinct between the radular 'morphotypes' but

also within each radula (e.g. taenioglossan radula with three morphologically distinct tooth types per row: one central tooth, two lateral teeth and two marginal teeth (Figs. 1c-h, 2). Additionally, radulae can be taxon-specific regarding their tooth morphologies, even in closely related species (e.g. the Paludomid species flock in Lake Tanganyika, [27]). This recognition led to Troschel [28] introducing this character complex as most important for mollusc systematics, resulting in Thiele [29] revising the Mollusca based on this. Nowadays radular tooth morphologies are still understood as of systematic value, but not at every level due to ecological adaptations. Additionally, material properties seem to be diverse in radular teeth. Especially the studies on Patellogastropoda and Polyplacophora show that elements, e.g. Fe, can be incorporated in the chitin matrix probably leading to a higher wear resistance [e.g. 30–43]. The different properties of the previously studied Patellogastropoda and Polyplacophora species [e.g. 40, 42, 44–46].

## 1.4 Previous work on radular function

The morphology, position and chemical composition are widely considered adaptive to ingesta or substrate – linking the organism as interface with its environment. Hypotheses relating the radula with the evolution of feeding strategies and trophic specialization have been put forward [47, 48]. There are famous examples of gastropod species that are active predators [e.g. *Conus*]; but many gastropod feed on endolithic and epilithic algae that are rasped from the substrate [e.g. Sacoglossa, 49–53]. The notification that gastropods selectively forage on algae in response to the position, mode of attachment, toughness and cells size have led to the notion of competition avoidance [25, 54, 55]. Additionally, the substrate that the food lies on or is attached to could influence the mechanical composition of radular teeth [see also Krings et al., under review in BMC Evolutionary Biology, here chapter 4.1]. The assumptions that feeding from rocky substrate is enabled by teeth with an upright standing, hard cusp [37, 56] and that those morphs evolved convergently several times [e.g. 57] have been postulated. However, the evolutionary significance of substrate and food is still poorly understood, because current models are descriptive reports on 'differences' in tooth shape and hypotheses derived from these observations [58, 59].
#### 1.5 FEA as potential method

To understand the function of morphological structures, biomechanical models and quantifiable characters are necessary. Previous research [60] has highlighted the difficulties of producing models for understanding the functionality of radular teeth, especially since many factors control the morphology (categories of factors as defined by Hickman [60]: phylogenetic, programmatic, constructional, ecological, maturational, degenerative) and further work emphasized the need to understand the radular function [61–64]. Padilla [65] gave a comprehensive summary on the past studies and suggested to apply approaches with high research potential for the future contributing to a deeper understanding of the ecology and evolution through the light of the functional morphology of radular teeth. While experimenting with grazing molluscs, she already developed biomechanical techniques to measure forces that are required to remove algae [66, 67] which was modified by Krings et al. [68] measuring the in vivo forces exerted by the radula. In this context, especially Padilla's [65] the insistence and emphasis on establishing further 'methods for testing and demonstrating function', the necessity to 'integration of structure and function' and to include the 3-dimensional morphology are highly important. She recommended including the shape, the ingesta-tooth interface, the material properties and the teeth's interaction into future considerations on radular evolution.

Finite-element-analysis (FEA), a software-based virtual method, allows to model and test 3dimensional bodies with defined material properties under the action of outer forces with detailed visualization of deformation and distribution of stresses and strain within the structure. In this context, FEA had been employed in studies of various biological structures including qualitative (stress/strain distribution plots) and quantitative approaches (examining stress/strain at homologous points and comparing the strength of the whole models by computing means). Both, data at homogenous points [69–76] as well as averages are considered valuable in functionalmorphological, ecomorphological, and macroevolutionary analyses [77–85] involving standard statistical methods [86–88]. FEA was also applied in studying food processing structures as beaks of Darwin's finches providing engineering evidence for trophic specialization [89] and is a useful approach to provide a comparative perspective on radular teeth mechanics. In Malacological objects FEA had already been used for understanding the functionality of *Patella*, Polyplacophora [37] and *Euhadra* [90] radular teeth. Van der Wal et al. [37] designed a FEA study including considerations on the material gradients and mechanical properties of teeth. However, their study lacks the exact 3D morphology, which at that time could not be included in FEA due to lacking computing capacity. Fortunately, we are capable of this today due to the progress in data processing technology. Additionally, in more recent FEA studies on radular teeth [90] material properties, especially gradients, are lacking. However, since they are of high functional importance [e.g. 91–98] they should be included in FEA [see also 99].

To lay a keystone for further studies that appeal the overflowing diversity of radular teeth and to connect radular diversity with functionality and hence possible adaptations to the ingesta, we propose here the first biomechanical radular tooth model that includes the exact 3D morphology, the position, the embedment and the mechanical properties (material gradients) of different tooth types. We conducted overall 18 different FEA scenarios with the taenioglossan radula of the gastropod Spekia zonata [96] (Fig. 1a). This species belongs to the African Paludomidae foraging algae attached to rocky, solid substrates in Lake Tanganyika and was chosen as model because a) its radula, even though taenioglossan radulae usually have morphologically distinct centrals, laterals and marginals, shows very distinct and hence unusual tooth types (Fig. 1c-h) and b) as representative of a flock this species is interesting in the search for drivers in a potential adaptive radiation, especially since the origin and evolution of these Paludomid gastropods have been discussions for decades [e.g. 27, 101–109]. They represent with about 50–70 species a very spectacular species flock among the molluscs, because even though they are closely related, they show an extraordinary interspecific diversity not only in their shell but also their taenioglossan radular tooth morphology [e.g. 27, 103, 110–112]. In the future we hope to address this paludomid tooth diversity by analysing the functionality of more paludomid radular teeth by FEA to discuss the evolution and potential trophic specialization as it has already been done for Darwin's finches [89]. Here we combined the exact 3D morphology with the material properties in connection with the position of the tooth and its embedment in the membrane and conducted 18 FEA scenarios. By altering conditions in the model, we were able to consider the role of a) the tooth morphology, b) the tooth's position on the radular membrane, c) the mode of embedment in the radular membrane, d) the material gradients of the tooth types (Tab. 1). Comparing the results of the stress and strain in the tooth structures between the scenarios allowed us to put forward hypothesises about the functionality of the taenioglossan radular tooth types and the influence of the different conditions on the mechanical behaviour of teeth and their functional constrains. Additionally we would like to highlight the importance of mechanical properties in biological structures. We hope this basic research contributes to the overall topics of functional gradients or to the design of artificial soft graspers as it has been addressed in [113].

Figure 1. (a) Shells of Spekia zonata (ZMB 220.077-1); (b) schematic drawing of the radula when feeding; (c-h) taenioglossan radula of S. zonata (c-e, g: ZMB 220.144-1, f, h: ZMH 150008/999-4); (c) mature worn teeth in the wearing zone; (d) CT and LT from the side; (e) immature and unworn mature CT, LT, IMT and OMT, the teeth surrounded with black lines do not show signs of wear, and hence these cusps were then modelled in Maya 3D software; (f) MT and LT manually teared out to obtain detailed information on the 3D structure; (g) IMT and OMT; (h) worn CT manually teared out to obtain detailed information on the 3D structure. Scale bars: a = 4 mm; c, e, g =100  $\mu$ m; d, h = 30  $\mu$ m; f = 40  $\mu$ m. CT = central tooth, FP = food particle, IMT = inner marginal tooth, IRT = immature radular teeth, LT =lateral tooth, MRT = mature radular teeth, MT = marginal tooth, O = odontophore, OMT = outer marginal tooth, RM = radular muscles, RT = radular teeth.



## Materials and Methods

### 2.1 Specimens

Adult specimens of *Spekia zonata* are inventoried at the Museum für Naturkunde Berlin (ZMB) or the Zoological Museum Hamburg (ZMH) and stored in ethanol. They were collected from stones at the shores of Lake Tanganyika in Burundi (ZMB 220.144) by F. Riedel and at Kalambo Falls Lodge (Zambia) in 2017 (ZMH 150008/999) by Heinz Büscher.

# 2.2 Morphological analysis and visualization

To obtain a model suitable for FEA the teeth were formed manually. Radular teeth of S. zonata are rather small with ~ 130–200  $\mu$ m length and are of low contrast and thus could not be visualized applying  $\mu$ -CT technique (employing standard desktop  $\mu$ -CTs, e.g. the SkyScan 1172 HR micro-CT [Bruker microCT, Kontich, Belgium]), as it has been applied in previous studies on gastropod anatomy [e.g. 114, 115] or radular tooth morphology [43, 44]. To create a 3D model of the distinct taenioglossan radular teeth, radulae of two specimens (ZMB 220.144-1, ZMH 150008/999-4) were extracted, digested with proteinase K following the protocol of [116], cleaned with an ultrasonic bath and mounted on a scanning electron microscopy (SEM) aluminium sample holder. We only dissected two gastropods, because previous research on Paludomidae showed [e.g. 27] that tooth morphologies are rather constant within the same species. To obtain images from all sides of an individual tooth the radula was manually destroyed, teeth were extracted, twisted and mounted (Fig. 1f, h). Then teeth were coated with carbon and visualized employing the SEM Zeiss LEO 1525 (One Zeiss Drive, Thornwood, NY). Using the 3D software Maya 2019 (Autodesk, Inc., San Rafael, USA), the teeth were then formed by hand (Fig. 2) always comparing the model with the SEM images taken from different sides. In the same manner the position and embedment of the teeth within the membrane were reconstructed (Fig. 3a). The one side of the generated model was then cut and mirrored to generate symmetry. Surface irregularities from model generation were repaired using Geomagic Wrap 2017 (3D Systems, Inc., Moerfelden-Walldorf, Germany) and models were converted to CAD file format necessary for using ANSYS FEA Package.



Figure 2. 3D Modell, before creating symmetry, generated in accordance with SEM images (*a*) from the top view; (*b*) from the side; (*c*) from the bottom. Visualization in Meshlab 2016. CT = central tooth, IMT = inner marginal tooth, LT =lateral tooth, OMT = outer marginal tooth, TB = tooth basis, TC = tooth cusp, TS = tooth stylus.

## 2.3 Material properties

Material properties (Young's modulus) were taken from [56] (Fig. 3b). In that study, radulae of *S. zonata* were embedded in epoxy, polished and hardness and elasticity was measured by nanoindentation. A diamond tip was pressed onto the material under a known load resulting in quantitative variables in the unit of measurement GPa (harder material has a higher GPa). The materials deformation and reformation allowed us to infer the elasticity of the material [see also 117]. Three indentations were made of each marginal tooth (MT), on the basis, stylus and cusp (Fig. 2), and two indentations where made on the lateral tooth (LT) and central tooth (CT), on the stylus and cusp, since those are shorter. The stiffest material (N = 110 fully mineralized teeth) was found in the CT cusps, followed by the LT cups (N = 112 fully mineralized teeth) and finally MT cusps (N = 60 fully mineralized teeth). The mean value of the measured Young's modulus (CT cups:  $8.09 \pm 0.65$  GPa; CT stylus:  $6.67 \pm 0.54$  GPa; LT cups:  $5.78 \pm 0.42$  GPa; LT stylus:  $4.95 \pm 0.49$  GPa; MT cusps:  $4.60 \pm 0.47$  GPa; MT stylus:  $3.29 \pm 0.50$  GPa; MT basis:  $2.43 \pm 0.30$  GPa) was assigned to different tooth areas (Fig. 3b) computing the *heterogeneous models*. It was assigned to the points of the models where it was analysed and by employing the thermal diffusion method values were smoothly diffused through the teeth [118].

For the *homogeneous models*, we applied a unique Young's modulus to the whole tooth and the membrane. For the soft embedment we used a E=0.0225 GPa, for the medium-hard embedment we used a E=0.225 GPa, and for the hard embedment we used E=2.25 GPa. This last value corresponds to the softest measured area in the teeth (Young's modulus of the outer marginal tooth basis). Due to the low thickness of the membrane and due to the rapid mechanical changes while drying, we were not able to measure the hardness and elasticity of the membrane by nanoindentation. Therefore, we altered the mechanical properties of this structure in our model. E=2.25 GPa is the hardest and stiffest embedding condition, because preliminary unpublished results suggested that applying of a higher Young's modulus results in a plateau in stress and strain.



Figure 3. Conditions for the FEA: (*a*) left side: cut 3D model used for FEA, right side: attachment area with the radular membrane; (*b*) areas with different material properties used for FEA scenarios with heterogeneous materials; area of OMT: dark blue E=2.23 GPa, marine blue E=3.29 GPa, blue-green E=4.60 GPa; area of LT: dark green E=4.95 GPa, light green E=5.78 GPa; area of CT: yellow E=6.67 GPa, red E=8.09 GPa; (*c*-*d*, *e*-*f*, *h*-*i*) contact area (red) between tooth cusps and food and modelled direction of force acting on the teeth (red arrow); (*e*-*g*) first hypothetical direction of force for the MTs, from anterior to posterior resulting in (*g*) higher stress; (*h*-*j*) second hypothetical direction of force for the MTs, from lateral to medial resulting in (*j*) lower stress.

# 2.4 Area of embedment in the radular membrane

Information about the connection between underlying radular membrane and the tooth itself was taken from [Krings et al., accepted for publication in Malacologica, here chapter 2.2]: the membrane and the tooth itself is composed of chitinous bundles [119] consisting of almost parallel fibres running continuously from the membrane into the tooth cusps, connecting the tooth with the membrane directly. The attachment area (the area connecting tooth with membrane) was identified in [Krings et al., accepted for publication in Malacologica, here chapter 2.2] by mounting the radula upside down on a stub and visualizing the attachment by scanning electron microscopy (SEM). This area was transferred into the 3D model (blue area in Fig. 3a) and we applied a lateral elastic stiffness creating a partial restraint of the movement. We adopted the following values for the elastic foundation stiffness: K<sub>Hard</sub>=7500 N/mm<sup>3</sup>, K<sub>Medium-Hard</sub>=7500 N/mm<sup>3</sup> and K<sub>Soft</sub>=750 N/mm<sup>3</sup>. These values are obtained assuming a thickness of the membrane of d=0.03 mm, when dividing the Young's modulus for the hard, medium-hard and soft cases (E/d).

# 2.5 Force applied

A force of 1 N was applied to the cusps of different teeth (red areas in Fig. 3c-f, h-j) along the anterior-posterior axis in the anterior direction (Fig. 3e-g) or along the anterior-medial axis (Fig. 3h-j; see also [37]). For the MT we have chosen to apply the force along the anterior-medial axis (Fig. 3h-j) since stress and strain values are smaller (Fig. 3j) in comparison to applying the force in the anterior-posterior axis (Fig. 3e-g). Since the objective of this work was comparing the different

scenarios under the action of the same loading conditions, we were not interested in the applying real values of force, but rather to provide a comparison between models under some arbitrary force.

### 2.6 FEA model

A structural static analysis was performed employing the finite element package ANSYS 17.1 (Ansys, Canonsburg, USA) in a Dell Precision Workstation T7820 with 64 GB RAM. To evaluate the biomechanical behaviour of the radula when feeding 18 different scenarios were designed depending on the tooth type analysed (marginal, central, and lateral tooth), the stiffness of the embedment (soft, medium-hard, and hard) and the distribution of the material properties (homogeneous or heterogeneous/gradient). See Tab. 1 for the list of all the cases. The different feedings scenarios were meshed using the ANSYS mesh module with an adaptive mesh of hexahedral elements [120] resulting in about 100,000 elements per model.

# 2.7 Average values and quasi-ideal mesh

In this work we computed the average values of von Mises stress and strain. For non-uniform meshes comprising elements of different sizes, we need to consider this non-uniformity in computing these average values. Therefore, we used the mesh-weighted arithmetic mean (MWAM) and the mesh-weighted median (MWM) as proposed by [121]. Alternatively, we computed the von Mises stress and strain in 11 homologous points for all the cases (Fig. 8). Statistical analyses resulting in medians and standard deviations depicted as boxplots were performed with JMP<sup>®</sup> Pro, Version 13 (SAS Institute Inc., Cary, NC, 1989–2007).

Tab. 1: Summary of the 18 conducted scenarios with the different conditions of the model: embedment hard, medium-hard, soft (including the Young's modulus E of the membrane and the specific weight K); material properties of the teeth either homogenous (including the Young's modulus E of the teeth) or heterogeneous (with the measured material gradients).

Tooth type	Scenario number	Embedding membrane	E membrane (GPa)	K membrane (N/mm3)	Material properties of teeth	E teeth (GPa)	
	1	hard	2.25	75000	homogeneous	2.25	
	2		2.25	75000	heterogeneous	gradient	
central	3	medium-hard	0.225	7500	homogeneous	0.225	
tooth	4		0.225	7500	heterogeneous	gradient	
	5	soft	0.0225	750	homogeneous	0.0225	
	6		0.0225	750	heterogeneous	gradient	
lateral tooth	7	hard	2.25	75000	homogeneous	2.25	
	8		2.25	75000	heterogeneous	gradient	
	9	medium-hard	0.225	7500	homogeneous	0.225	
	10		0.225	7500	heterogeneous	gradient	
	11	soft	0.0225	750	homogeneous	0.0225	
	12		0.0225	750	heterogeneous	gradient	
outer marginal tooth	13	hard	2.25	75000	homogeneous	2.25	
	14		2.25	75000	heterogeneous	gradient	
	15	medium-hard	0.225	7500	homogeneous	0.225	
	16		0.225	7500	heterogeneous	gradient	
	17	soft	0.0225	750	homogeneous	0.0225	
	18		0.0225	750	heterogeneous	gradient	

## <u>Results</u>

Von Mises stress and von Mises strain, their distribution and mean values, were obtained for each scenario. Von Mises stress is an equivalent stress that summarize the nine stress values of the stress tensor in one unique equivalent value, so it makes the comparison between models easier. Despite von Mises stress is a criterion used to analyse stress distribution in the FEA model, similar equations can be used in strain, so we can compute also a unique and equivalent value of strain for each model. Figs. 4 and 5 display the distribution of von Mises stress and strain in each scenario and Figs. 6 and 7 depict the Mesh-Weighted Arithmetic Mean (MWAM) and Mesh-Weighted Median (MWM).

## 3.1 Von Mises stresses

# 3.1.1 Effect of model conditions on stress distribution in different tooth types

In all scenarios (for values see Tab. 2) the marginal tooth (MT) always exhibits higher stress values, whereas the central (CT) and lateral teeth (LT) show lower stress values. In each tooth type the highest stress values were obtained for models with material gradients. The models with gradients showed highest stress values for soft, followed by medium-hard and hard embedding. The highest stress values were obtained for the MT with soft embedding and material gradient, followed by MT with a medium-hard embedding and material gradient, and finally MT with hard embedding and material gradient. Both LT and CT showed also the highest stress for soft, whereas medium-hard and hard embedding showed lower stress values. Every tooth type modelled with homogenous material showed a) lower stress values, and b) the same stress values for all embedding modes (Tab. 2).

# 3.1.2 Homologous points

Von Mises stress in the defined points (Fig. 8; Supplements Tab. 1) on the marginal tooth was always higher at the tooth basis (12.13–15.19 MPa) than at the stylus (0.29–7.13 MPa) or at the cusp (0.51 MPa). As for the MWAM and MWM, the highest forces at the basis were found in the models with gradients (12.13–15.19 MPa) for all types of embedment. For the other defined points in the MT,

stress values are slightly different at different model conditions. In the lateral and central teeth, the highest stress values were calculated for scenarios with material gradients and soft embedment, but the range of force values for all points was much smaller (0.04–0.64 MPa). As for the MWAM and MWM, each point of the models calculated without material gradients always showed lower stress and these values were independent from the embedding mode (MT: P1: 12.13–12.16 MPa; P2: 7.14 MPa; P3: 1.22 MPa; P4: 0.29 MPa; P5: 0.51 MPa; LT: P1: 0.03 MPa; P2: 0.34 MPa; P3: 0.44 MPa; CT: P1: 0.17 MPa; P2: 0.05 MPa; P3: 0.14 MPa).

#### 3.2 Von Mises strain

# 3.2.1 Effect of model conditions on strain distribution in different tooth types

The marginal tooth exhibits the highest von Mises strain values in comparison to the lateral and central one (Tab. 2; Figs. 4–7). However, the values for different model conditions are opposite in comparison with the von Mises stress: the highest strain was obtained for models without material gradients. The models with gradients show, as for the stress, the highest strain values for the soft embedding: both medium-hard and hard embedding models had lower strain values. The highest stress values were calculated for the marginal tooth with soft embedding and material gradients, followed by the MT with a medium-hard embedding with material gradients and the MT with hard embedding with material gradients and the MT with hard the soft, then medium-hard and finally hard embedding (Tab. 2).

# 3.2.2 Homologous points

Von Mises strain in the defined points (Fig. 8; Supplements Tab. 1) of the marginal tooth basis was always higher (0.0050–0.5389 MPa) than that in the stylus (0.0001–0.3171 MPa) or in the cusp (0.0001–0.0225 MPa). As for the MWAM and MWM, in all points of the MT the highest strain was found for the model scenarios without gradients (0.0001–0.5389 MPa), with soft, then mediumhard, and finally hard embedment. The same holds true for both central and lateral teeth.



Figure 4. Results of the FEA (stress and strain, both in MPA) for (*a-f*) the CT and (*g-l*) LT (front and back view) with soft (*a-b*, *g-h*), medium-hard (*c-d*, *i-j*) and hard (*e-f*, *k-l*) embedding membrane. Images represent scenarios with and without material gradients, since for CT and LT there is not much difference in stress and strain values between homogenous and heterogeneous material properties (for the values of MWAM and MWM see Figs. 6, 7 and Tab. 2). The scaling for Figs. 4 and 5 is identical for comparison between tooth types.



Figure 5. Results of the FEA (stress and strain, both in MPA) for the OMT (front and back view) with soft (*a-d*), medium-hard (*e-h*) and hard (*i-l*) embedding membrane. *a, c, e, g, i, k:* scenarios without material gradient, blue circle (soft): tooth and membrane E=0.0225 GPa, green circle (medium-hard): tooth and membrane E=0.225 GPa, red circle (hard): tooth and membrane E=2.25 GPa; *b, d, f, h, j, l* with measured material gradients (for the values of MWAM and MWM see Figs. 6, 7 and Tab. 2). The scaling for Figs. 4 and 5 is identical for comparison between tooth types.



Figure 6. Results of the FEA (values of MWAM and MWM, both in MPa) of stress and strain (lin. and log.), for the CT, LT, and OMT with different conditions of the model: embedment hard, medium-hard, soft and with or without material gradients. Blue = strain MWAM; red = strain MWM; green = stress MWAM; purple = stress MWM. For the values see Tab. 2.



Figure 7. Results of FEA, range of stress and strain values (lin. and log., both in MPa), for the CT, LT, and OMT with different conditions: embedment hard, medium-hard, soft and with or without material gradients. Blue = strain MWAM; red = strain MWM; green = stress MWAM; purple = stress MWM. For the values see Tab. 2.



Figure 8. Results of the FEA of stress and strain (*b* lin. and log., both in MPa) for certain defined areas (*a* 1-11) on the CT, LT, and OMT with different conditions of the model: embedment hard, mediumhard, soft and with or without material gradients; on the right side of the figure legend with the colour code for the different areas. For the values see Supplements Tab. 1.

Tooth type	Scenario	Embedding membrane	Material properties of teeth	Stress MWAM, MPa	Stress MWM, MPa	Strain MWAM, MPa	Strain MWM, MPa	
central tooth	1	hard	homogeneous	0.1888929	0.164465000	86.2	75.1	
	2		heterogeneous	0.2086405	0.181253171	28.4	24.5	
	3	medium-hard	homogeneous	0.1896065	0.166365587	853.3	749.3	
	4		heterogeneous	0.2384223	0.205177677	32.7	27.6	
	5	soft	homogeneous	0.1895905	0.166362911	8532.4	7493.7	
	6		heterogeneous	0.2446671	0.209700000	33.5	28.1	
lateral tooth	7	hard	homogeneous	0.2065989	0.161000000	93.8	73.0	
	8		heterogeneous	0.2189697	0.174349367	42.6	34.4	
	9	medium-hard	homogeneous	0.2074396	0.162210000	931.1	727.2	
	10		heterogeneous	0.2686988	0.229189415	52.2	44.6	
	11	soft	homogeneous	0.2065989	0.161000000	9382.4	7295.7	
	12		heterogeneous	0.2898695	0.245265000	57.0	47.9	
	13	hard	homogeneous	1.5554244	0.420405942	736.5	194.2	
outer marginal tooth	14		heterogeneous	1.5607737	0.423819854	595.7	102.5	
	15	medium-hard	homogeneous	1.5554244	0.420405942	7364.6	1942.3	
	16	1	heterogeneous	1.7632298	0.424554246	699.7	102.5	
	17	soft	homogeneous	1.5554244	0.420405942	73645.5	19422.6	
	18	]	heterogeneous	1.9434961	0.424714985	790.9	102.5	

Tab. 2: Values of the stress and strain, in Mpa, for the different FEA scenarios.

#### <u>Discussion</u>

As already proposed by Padilla [65] biomechanical modelling approaches that include the 3dimensional shape of the tooth, the interaction between the teeth, the material composition, and the interface between tooth and ingesta are crucially important to access the functional significance of morphological structures. Here we provide the first FEA model of taenioglossan radular teeth including these properties. The visual representation of the stress distribution for the FEA models is valuable for comparisons and hypotheses on the biomechanical behaviour.

#### 4.1 Tooth morphology and the position on the radular membrane

The results of the FEA models for the distinct radular tooth types (Figs. 4–7) can be explained by their morphologies: short, broad morphologies will not deform as much as taller, thinner ones. The marginal tooth always experiences higher stress and strain than the central and lateral tooth since the latter ones are rather short and broad (CT width mean value:  $\sim$  170  $\mu$ m, LT width mean value:  $\sim$ 130 µm) important for transferring force effectively to the substrate [see also 52, 56, 65]. CTs and LTs display additionally a thick cutting edge at the interface between the tooth and ingesta. Jensen [52] highlighted different effects of the tooth shape on the ingesta and Padilla [65] pointed out the importance of this contact area since its size determines local pressure (the amount of force per unit area) applied to ingesta. Pointy teeth exhibit a stronger pressure on their tooth cusps which makes them more effective at piercing and tearing whereas blunt teeth, as the CT and LT of S. zonata, are presumably more effective for loosening material from substrate surfaces (in this specific case from solid surfaces, see also [52, 56, 65-68]). In S. zonata thick rounded bulges are present at the basis of the LT and the CT. The bulges contribute to the reinforcement of the tooth structure and hence support the force transmission to the radular membrane. The MT in contrast consists of rather slender and thin stylus (MT length mean value:  $\sim 209 \,\mu$ m) with small bulges at the edges reinforcing the structure before terminating in a cusp containing small denticles. In contrast to the hypothesis stating that long teeth are more effective in removing algae tissue [122] these long MT are more affected in our model by stress and strain due to their thinness. In turn this results in higher deformation when in contact with the substrate, but also in an enlarged risk of breaking. This had already been hypothesized in [56, 65, 123] and is now supported by the results of this paper. It is important to note that the modelled highest values of stress and strain were always observed in the thinnest parts of teeth: in the MT at the stylus and denticles, in the LT at the denticles and basis, in the CT at the cutting edge and basis (Figs. 4–5).

Reconstructing the 3D-model gave us insight into the precise position of the teeth to each other. Their arrangement on the radular membrane results in the interaction and interlocking between them. This effect in turn aids in the force transmission from the single tooth to the neighbouring teeth as had been previously postulated by [60] and [65]. The CTs from adjacent rows support each other by the interaction of their bulky bases with the rounded bulges of their styli. The rounded and broad bases of the LT fit perfectly together; hence adjacent tooth rows can stabilize each other while interacting with the substrate surface. The MTs support each other as well: the two marginal teeth – the inner and the outer – can interlock tightly. Here the outer, larger MT embraces the inner, smaller MT; they can hence function as one single unit [62]. The performance of single teeth is of high interest as well [37, 90], but to link morphology and function it is utterly necessary to consider the radula with all its teeth as a complex unit with mechanically interacting, non-independent structures [56, 62, 63, 65].

#### 4.2 Material gradients

Our experiments on models with material gradients (heterogeneous tooth material properties obtained experimentally) resulted in higher values of stress in the teeth (Figs. 5–7) with both central and lateral teeth being less effected than the marginal ones. However, the incorporation of the Young's modulus into our models has stronger effect on the values of the strain than on the values of stress: the strain was much higher in homogenous teeth than in heterogeneous ones showing that homogenous teeth deform more. Van der Wal et al. [37] highlighted the importance of material gradients in radular teeth. They found for *Patella* and representatives of Polyplacophora that the 'leading part' of the tooth (the area of interaction between the tooth and substrate) is harder and

stiffer than the 'trailing part'. It seems to be important for its functionality, because hard materials with softer underlying layers might be less prone to abrasion [44, 94, 124 and 125 on snake skin]. Teeth of *S. zonata* are morphologically distinct from the teeth of *Patella* and Polyplacophora, but we also revealed material gradient in the species studied: the cutting edge of the tooth cusps is the hardest and stiffest area, teeth become softer and more flexible over the stylus to the basis [see also 56, 123]. The harder and stiffer material properties in the cusps, especially in the LTs and CTs, are needed for transferring force to the ingesta (in the case of *S. zonata*, teeth acting on algae attached to a rocky surface). This could either lead to natural wear at the cusps documented for gastropods and Polyplacophora [18, 20, 21, 39, 126, 127], but might also result in a risk of fracture when teeth are exposed to higher stresses. The latter has not been documented naturally but was simulated in breaking stress experiments on taenioglossan teeth (unpublished data). However, since teeth continuously enter the wearing zone they can be replaced in both scenarios.

Deformation in these structures on the other hand would be very problematic since teeth must maintain their shape while acting on ingesta. Inclusion of real material properties in our model resulted in less deformation under the load.

Each tooth region, especially pronounced in the MT, is affected differently by stress and strain: the basis and the stylus are affected more than the tooth cusps - with the exception of the small denticles that show higher values of stress and strain due to their direct interaction with the substrate. This is a direct results of specific material properties: the relatively stiff and hard cusp is not affected by deformation (strain), but the flexible and soft basis and the stylus are. This pattern is not observed for the CTs and LTs showing a quite uniform distribution of both stress and strain resulting in the reduction of structural failure while scratching across the substrate surface. This system is analogous to other biological systems, such as mouthparts in Arthropods showing sclerotized and sometimes strongly mineralised cutting edges in their mouthparts enabling the crushing of food with a resistance to wear and the avoidance of structural failure [94, 96]. The function of the flexible MT basis and stylus is in providing shock absorption against mechanical impacts [123]. This behaviour, the combination of different material properties in a complex network, appears to be functionally analogous to resilin-dominated areas in Arthropods (e.g. wings

or mouthparts [94, 96, 128–132], reptile skins [124, 125]), or squid beaks resulting from the regionalization of cross-linking and the degree of hydration [133–136]; the combination of a stiff and hard surface with a flexibility of underlying layers allows the structure to be less prone to failure. While foraging the MT can hence flip and rotate due to its ability to deform (this is not possible for the LTs and CTs). This leads to the conclusion, that teeth have different functions: the CTs and LTs loosen food from the substrate whereas the MTs gather the algae from the substrate like brooms.

# 4.3 Mode of embedment

The central and lateral teeth have quite large attachment areas connecting the tooth basis with the membrane whereas the marginal teeth display a relatively small area (Fig. 3a). In the CT and LT forces can easily be transmitted from the tooth tip to the underlying membrane resulting in less stress and strain in the tooth structure itself, whereas the MTs have limited options transferring stress to the surrounding membrane. However, the small attachment area of the MT together with the thin and slender stylus and the material properties allows the tooth to have a stronger range of deflection.

The hardest embedding condition (membrane E=2.25 GPa) results in the lowest stress and strain mean values, but the mean stress values of the scenarios differs only little in comparison to the mean strain values. We can hence conclude that the mode of embedment has more influence on the deformation than on the stress. Stress and strain are reduced when material properties at the tooth basis and the membrane are as similar as possible. To function properly teeth have to be embedded in a membrane made of rather stiff material. The stiffness of the radular membrane itself is ensured by numerous muscles pulling it across the odontophoral cartilage while feeding.

# 4.4 An optimum set of model conditions and teeth functionality

Hard embedment in combination with homogenous material properties result in the smallest stress values whereas hard embedment in combination with heterogeneous material properties exhibit the lowest strain values. However, since stress values slightly differ between scenarios in contrast

to strain values, we hypothesize that in radular teeth the resistance to deformation is of high importance. Besides, reduction of stress is important to avoid fracture, but snails replace the radular teeth often. Thus, the hypothetical best model condition for the teeth would be hard embedment and the presence of the material gradient.

By combining results of all FEAs we can reveal differences in functional specialisations: it seems that central and lateral teeth can transmit stresses from the cusp across the basis to the membrane. This is ensured by morphology, the large attachment area, and the interlocking system between neighbouring teeth (CT and LT support each other while feeding, which may result in more uniform stress distribution). The hardest and stiffest material is detected in the tooth cusps enabling them to loosen algae from a solid substrate with small deformation. Marginal teeth must have a different function than CTs and LTs: FEA unravels that MTs are much more affected by stress and strain which could result in a higher risk of tooth fracture when used in the same manner.

It has been postulated that in the evolution of the taenioglossan radula (viz. the reduction in the quantity of teeth to only seven teeth/row) especially the reduction of the quantity of marginal teeth is important [25]. This is supposed to be closely related with the reduction of musculature and the shift from a sweeping to a rasping or scraping mode of feeding [137, 138], hence resulting in a more forceful way of feeding from the substrate [25]. It had been hypothesized previously, that the CTs are only used for gathering food [25, 137, 139], but our results depict that the CTs in concert with the LTs rather loosen ingesta from the substrate surface. The MTs have a different function: Steneck & Watling [25] already highlighted the possibility of marginal teeth to gather food from a greater surface area by 'inward raking' as the teeth converge to the central axis of the radula during retraction; our results comply with this hypothesis. We found that the MTs are less affected from stress and strain when the force is applied along the anterior-medial axis (Fig. 3h-j), rather supporting this 'inward raking' hypothesis. The small tooth basis as the most flexible and soft part in connection with its small attachment area with the membrane allows the flipping and rotation while retraction of the radula. During this action the comparable elongated structure of this tooth leads to a higher risk of hitting large obstacles during retraction since teeth have to cover a longer distance. The flexibility of the basis and the stylus makes the structures less prone to failure and

fraction but does not allow the direct transfer of forces from the radula to the ingesta. Therefore, we postulate that the MTs rather gather food after the LTs and CTs has loosened it from the surface (see also [56]).

We here established a workflow for building substantial hypotheses on radular tooth functions. In the future we hope to address the tooth diversity of the Lake Tanganyikan Paludomidae by analysing the functionality of more teeth by FEA. Understanding how form and material properties influences the functionality allows us the allocation of adaptations and subsequently the development of possible scenarios on the evolution, including potential trophic specializations.

# <u>Conclusions</u>

Here for the first time, the functionality of taenioglossan radular teeth was analysed employing Finite-Element-Analysis (FEA) resulting in values of stress and strain. We characterized the radular complex with respect to the 3D morphology, the position of each tooth, material properties, and nature of tooth attachment. To understand the relationship between tooth morphology, tooth material properties, and tooth attachment we compared 18 different FEA scenarios (representing variation in properties and fixations of the model). Our results of stress and strain mean values and distributions clearly depict different functions of teeth. We conclude that the central and lateral teeth are best structured for scratching over the substrate loosening ingesta, whereas the marginals are broom-like and best structured for collecting food particles. Detailed biomechanical analyses of radula from other species are required to provide an integrated view of macroevolution in Mollusca as a whole.

#### <u>Acknowledgements</u>

We would like to acknowledge: Peter Stutz from the Mineralogical-Petrographic Institute of the University of Hamburg, Germany, and Dr. Alexander Kovalev from the Zoological Institute of the Christian-Albrechts-Universität zu Kiel, Germany, for the great support on the nanoindentation. Heinz Büscher from Basel, Switzerland, helped to great extent by collecting specimens at Lake Tanganyika. Renate Walter from the Zoological Institute of the University of Hamburg, Germany, visualized the radulae at the SEM. Thomas M. Kaiser from the CeNak Hamburg, Germany, discussed results and provided valuable suggestions to earlier versions of the manuscript. Jan-Ole Brütt from the CeNak Hamburg, Germany, provided us insight into the attachment area of the radular teeth. Lena Schwinger from the CeNak Hamburg, Germany, supported 3D printing of models. JMN acknowledges Dr. Juan Liu and the Laboratory for the Evolution and Anatomy of Fish (LEAF) of the University at Buffalo, USA, and the CERCA Programme (Generalitat de Catalunya, Spain). We are grateful for the reviews that helped to a great extent improving this paper.

#### Authors' contributions

WK wrote the manuscript, drew the figures, generated data for the model conditions and analysed the FEA data. JMN is an expert in FEA and conducted all analyses, discussed the data and wrote the manuscript. HK provided the 3D model for this analysis in the context of his bachelor thesis and discussed results. MG helped to connect the biomechanical results to molluscan biology. SG initiated, designed and planned this study, discussed the data, the manuscript, the figures; his expertise was critical for understanding the results and the functional morphology. All authors contributed to the final version of the manuscript.

#### <u>References</u>

[1] G. Rosenberg, A New Critical Estimate of Named Species-Level Diversity of the Recent Mollusca, Amer. Malac. Bull. 32(2) (2014) 308–322. https://doi:10.4003/006.032.0204.

[2] A.D. Chapman, Numbers of living species in Australia and the world, second ed., Toowoomba, Australia: Australian Biodiversity Information Services, 2009.

[3] S.M. Wells, Molluscs and the conservation of biodiversity, in: A.C. van Bruggen, S.M. Wells, T.C.M. Kemperman (Eds.), Biodiversity and conservation of the Mollusca, Backhuys, Oegstgeest-Leiden, Netherlands, 1995.

[4] B. Groombridge, M. Jenkins, World Atlas of Biodiversity: Earth's Living Resources in the 21st Century, University of California Press, Berkeley, California, 2002.

[5] F.W. Ponder, D.R. Lindberg, Phylogeny and Evolution of the Mollusca, University of California Press, Berkeley, 2008.

[6] M. Glaubrecht, On "Darwinian Mysteries" or molluscs as models in evolutionary biology: From local speciation to global radiation, Amer. Malac. Bull. 27 (2009) 3–23. https://doi:10.4003/006.027.0202.

[7] P. Bouchet, J.-P. Rocroi (Eds.), J. Frýda, B. Hausdorf, W. Ponder, A. Valdes, A. Warén, Classification and Nomenclator of Gastropod Families, Malacologia 47(1–2), ConchBooks, Hackenheim, Germany, 2005.

[8] M.A. Fedonkin, A. Simonetta, A.Y. Ivantsov, New data on *Kimberella*, the Vendian mollusc-like organism (White Sea region, Russia): palaeoecological and evolutionary implications, Geological Society, London, Special Publications 286(1) (2007) 157–179. https://doi:10.1144/SP286.12.

[9] P.Y. Parkhaev, Origin and the early evolution of the phylum Mollusca. Paleontol. J. 51 (2017) 663–686.

[10] A. Wanninger, T. Wollesen, The evolution of molluscs, Biol. Rev. 94 (2019) 102–115. https://doi:10.1111/brv.12439.

[11] G. Haszprunar, A. Wanninger, Molluscs, Curr. Biol. 22 (2012) 510–514. https://doi:10.1016/j.cub.2012.05.039.

[12] T. Wollesen, M. Scherholz, S.V. Rodriguez-Monje, E. Redl, C. Todt, A. Wanninger, Brain regionalization genes are coopted into shell field patterning in Mollusca, Sci. Rep. 7 (2017) 5486. https://doi:10.1038/s41598-017-05605-5.

[13] R. Guralnick, K. Smith, Historical and biomechanical analysis of integration and dissociation in molluscan feeding, with special emphasis on the true limpets (Patellogastropoda: Gastropoda), J. Morphol. 241 (1999) 175–195. https://doi:10.1002/(SICI)1097-4687(199908)241:2<175::AID-JMOR7>3.0.CO;2-0.

[14] N.W. Runham, Rate of replacement of the molluscan radula, Nature 194 (1962) 992–993. https://doi:10.1038/194992b0.

[15] N.W. Runham, A study of the replacement mechanism of the pulmonate radula, J. Cell Sci. 3 (1963) 271–277.

[16] N.W. Runham, K. Isarankura, Studies on radula replacement, Malacologia 5 (1966) 73.

[17] K. Isarankura, N.W. Runham, Studies on the replacement of the gastropod radula, Malacologia 7 (1968) 71–91.

[18] U. Mackenstedt, K. Märkel, Experimental and comparative morphology of radula renewal in pulmonates (Mollusca, Gastropoda), Zoomorphology 107 (1987) 209–239. https://doi:10.1007/BF00312262.

[19] H.A. Lowenstam, S. Weiner, Mollusca, in: H.A. Lowenstam, S. Weiner (Eds.), On Biomineralization, Oxford University Press, Oxford, 1989, 88–305.

[20] C.J. Franz, Feeding patterns of *Fissurella* species on Isla de Margarita, Venezuela: use of radulae and food passage rates, J. Molluscan Stud. 56 (1990) 25–35. https://doi:10.1093/mollus/56.1.25.

[21] D.K. Padilla, D.E. Dittman, J. Franz, R. Sladek, Radular production rates in two species of *Lacuna* Turton (Gastropoda: Littorinidae), J. Molluscan Stud. 62 (1996) 275–280. https://doi:10.1093/mollus/62.3.275.

[22] J.A. Shaw, D.J. Macey, L.R. Brooker, Radula synthesis by three species of iron mineralizing molluscs: production

rate and elemental demand, J. Mar. Biol. Assoc. U.K. 88 (2008) 597–601. https://doi:10.1017/S0025315408000969.

[23] J.E. Gray, On the division of ctenobranchous gasteropodous Mollusca into larger groups and families, Ann. Mag. Nat. Hist. 11(2) (1853) 124–133. https://doi:10.1111/j.1469-7998.1853.tb07174.x.

[24] L.H. Hyman, Mollusca I. Aplacophora, Polyplacophora, Monoplacophora. Gastropoda, the Coelomate Bilateria. The Invertebrates 6, McGraw-Hill Book Company, New York, 1967.

[25] R.S. Steneck, L. Watling, Feeding capabilities and limitation of herbivorous molluscs: a functional group approach, Mar. Biol. 68 (1982) 299–319. https://doi:10.1007/BF00409596.

[26] G. Haszprunar, E. Speimann, A. Hawe, M. Heß, Interactive 3D anatomy and affinities of the Hyalogyrinidae, basal Heterobranchia (Gastropoda) with a rhipidoglossate radula, ODE 11(3) (2011). https://doi:201–236. 10.1007/s13127-011-0048-0.

[27] M. Glaubrecht, Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology and systematization of a paludomid species flock in an ancient lake, Zoosystematics Evol. 84 (2008) 71–122. https://doi:10.1002/zoos.200700016.

[28] F.H. Troschel, Das Gebiss der Schnecken zur Begründung einer natürlichen Classification. 1, Berlin, 1856-1863.

[29] J. Thiele, Handbuch der systematischen Weichtierkunde, Gustav Fischer, Jena, 1931–1935.

[30] N.W. Runham, The histochemistry of the radula of *Patella vulgate*, J. Cell Sci. 3 (1961) 371–380.

[31] K.M. Towe, H.A. Lowenstam, M.H. Nesson, Invertebrate ferritin: occurrence in Mollusca, Science 142 (1963) 63–64. https://doi:10.1126/science.142.3588.63.

[32] N.W. Runham, P.R. Thornton, D.A. Shaw, R.C. Wayte, The mineralization and hardness of the radular teeth of the limpet *Patella vulgata* L., Z. Zellforsch. Mikrosk. Anat. 99 (1969) 608–626. https://doi:10.1007/BF00340948.

[33] P. van der Wal, Structural and material design of mature mineralized radula teeth of *Patella vulgata* (Gastropoda), J. Ultrastruct. Mol. Struct. Res. 102 (1989) 147–161. https://doi:10.1016/0889-1605(89)90052-9.

[34] L.A. Evans, D.J. Macey, J. Webb, Distribution and composition of matrix protein in the radula teeth of the chiton *Acanthopleura hirtosa*, Mar. Biol. 109 (1991) 281–286. https://doi:10.1007/BF01319396.

[35] L.A. Evans, D.J. Macey, J. Webb, Calcium biomineralization in the radular teeth of the chiton, *Acanthopleura hirtosa*, Calcif. Tissue Int. 51 (1992) 78–82. https://doi:10.1007/BF00296222.

[36] A.P. Lee, L.R. Brooker, D.J. Macey, J. Webb, W. van Bronswijk, A new biomineral identified in the cores of teeth from the chiton *Plaxiphora albida*, J. Biol. Inorg. Chem. 8(3) (2003) 256–262. https://doi:10.1007/s00775-002-0410-y.

[37] P. van der Wal, H. Giesen, J. Videler, Radular teeth as models for the improvement of industrial cutting devices, Mater. Sci. Eng. C 7 (2000) 129–142. https://doi:10.1016/S0928-4931(99)00129-0.

[38] L. Brooker, A. Lee, D. Macey, W. Van Bronswijk, J. Webb, Multiple-front iron-mineralisation in chiton teeth (*Acanthopleura echinata*: Mollusca: Polyplacophora), Mar. Biol. 142 (2003) 447–454. https://doi:10.1007/s00227-002-0957-8.

[39] J.A. Shaw, D.J. Macey, L.R. Brooker, P.L. Clode, Tooth use and wear in three iron-biomineralizing mollusc species, Biol. Bull. 218 (2010) 132–144. https://doi:10.1086/BBLv218n2p132.

[40] J.C. Weaver, Q. Wang, A. Miserez, A. Tantuccio, R. Stromberg, K.N. Bozhilov, P. Maxwell, R. Nay, S.T. Heier, E. Di Masi, Analysis of an ultra hard magnetic biomineral in chiton radular teeth, Mater. Today 13 (2010) 42–52. https://doi:10.1016/S1369-7021(10)70016-X.

[41] L.R. Brooker, J.A. Shaw, The chiton radula: a unique model for biomineralization studies, Intech Open Access Publisher, 2012. https://doi:10.5772/31766.

[42] D. Lu, A.H. Barber, Optimized nanoscale composite behaviour in limpet teeth, J. Royal Soc. Interface 9 (2012) 1318–1324. https://doi:10.1098/rsif.2011.0688.

[43] T. Ukmar-Godec, G. Kapun, P. Zaslansky, D. Faivre, The giant keyhole limpet radular teeth: A naturally-grown harvest machine, J. Struct. Biol. 192(3) (2015) 392–402. https://doi:10.1016/j.jsb.2015.09.021.

[44] L.K. Grunenfelder, E.E. de Obaldia, Q. Wang, D. Li, B. Weden, C. Salinas, R. Wuhrer, P. Zavattieri, D. Kisailus, Biomineralization: Stress and damage mitigation from oriented nanostructures within the radular teeth of *Cryptochiton stelleri*, Adv. Funct. Mater. 24/39: 6085 (2014). https://doi:10.1002/adfm.201401091.

[45] A.H. Barber, D. Lu, N.M. Pugno, Extreme strength observed in limpet teeth, J. Royal Soc. Interface 12 (2015) 20141326. https://doi:10.1098/rsif.2014.1326.

[46] T. Ukmar-Godec, L. Bertinetti, J.W.C. Dunlop, A. Godec, M.A. Grabiger, A. Masic, H. Nguyen, I. Zlotnikov, P. Zaslansky, D. Faivre, Materials nanoarchitecturing via cation-mediated protein assembly: Making limpet teeth without mineral, Adv. Mater. 29 (2017) 1701171. https://doi: 10.1002/adma.201701171.

[47] D.H. Kesler, E.H. Jokinen, W.R. Munns Jr., Trophic preferences and feeding morphology of two pulmonate snail species from a small New England pond, USA. Can. J. Zool. 64(11) (1986) 2570–2575. https://doi:10.1139/z86-377.

[48] R. Black, A. Lymbery, A. Hill, Form and Function: size of radular teeth and inorganic content of faeces in a guild of grazing molluscs at Rottnest Island, Western Australia, J. Exp. Mar. Biol. Ecol. 121 (1988) 23–35. https://doi:10.1016/0022-0981(88)90021-4.

[49] K.R. Jensen, A review of sacoglossan diets, with comparative notes on radular and buccal anatomy, Malacological Review 13 (1980) 55–77.

[50] K.R. Jensen, Observations on feeding methods in some Florida ascoglossans, J. Molluscan Stud. 47(2) (1981) 190–199. https://doi:10.1093/oxfordjournals.mollus.a065567.

[51] K.R. Jensen, Factor affecting feeding selectivity in herbivorous Ascoglossa (Mollusca: Opisthobranchia), J. Exp. Mar. Biol. Ecol. 66(2) (1983), 135–148. https://doi:10.1016/0022-0981(83)90035-7.

[52] K.R. Jensen, Morphological adaptations and plasticity of radular teeth of the Sacoglossa (= Ascoglossa) (Mollusca: Opisthobranchia) in relation to their food plants, Biol. J. Linn. Soc. 48(2) (1993) 135–155. https://doi:10.1006/bijl.1993.1011.

[53] C.D. Trowbridge, Diet specialization limits herbivorous sea slug's capacity to switch among food species, Ecology 72(5) (1991) 1880–1888. https://doi:10.2307/1940985.

[54] W. Blinn, R.E. Truitt, A. Pickart, Feeding ecology and radular morphology of the freshwater limpet *Ferrissia fragilis*, J. N. Am. Benthol. Soc. 8(3) (1989) 237–242. https://doi: 10.2307/1467327.

[55] K. Iken, Feeding ecology of the Antarctic herbivorous gastropod *Laevilacunaria antarctica* Martens, J. Exp. Mar. Biol. Ecol. 236(1) (1999) 133–148. https://doi:10.1016/S0022-0981(98)00199-3.

[56] W. Krings, A. Kovalev, M. Glaubrecht, S.N. Gorb, Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods, Zoology 137 (2019a) 125713. https://doi:10.1016/j.zool.2019.125713.

[57] A.S.H. Breure, E. Gittenberger, The rock-scraping radula, a striking case of convergence (Mollusca), Neth. J. Zool. 32(3) (1981) 307–312. https://doi:10.1163/002829681X00347.

[58] M. Nishi, A.J. Kohn, Radular teeth of Indo-Pacific molluscivorous species of *Conus*: a comparative analysis, J. Molluscan Stud 65(4) (1999) 483–497. https://doi:10.1093/mollus/65.4.483.

[59] T.F. Duda, A.J. Kohn, S.R. Palumbi, Origins of diverse feeding ecologies within *Conus*, a genus of venomous marine gastropods, Biol. J. Linn. Soc. 73(4) (2001) 391–409. https://doi:10.1006/bijl.2001.0544.

[60] C.S. Hickman, Gastropod radulae and the assessment of form in evolutionary paleontology, Paleobiology 6 (1980) 276–294.

[61] C.S. Hickman, Ecological and phylogenetic implications of the unusual radula of *Laevinesta atlantica* (Mollusca, Gastropoda), Veliger 25 (1983) 323–325.

[62] C.S. Hickman, Implications of radular tooth-row functional-integration for archaeogastropod systematics, Malacologia 25 (1984) 143–160.

[63] T.E. Morris, C.S. Hickman, A method for artificially protruding gastropod radulae and a new model of radula function, Veliger 24 (1981) 85–89.

[64] C.S. Hickman, T.E. Morris, Gastropod feeding tracks as a source of data in analysis of the functional-morphology of radulae, Veliger 27 (1985) 357–365.

[65] D.K. Padilla, Form and function of radular teeth of herbivorous molluscs: Focus on the future, Am. Malacolog. Bull. 18 (2004) 163–168.

[66] D.K. Padilla, Structural resistance of algae to herbivores. A biomechanical approach, Mar. Biol. 90 (1985) 103–109.

[67] D.K. Padilla, Algal structural defenses: form and calcification in resistance to tropical limpets, Ecology 70 (1989) 835–842.

[68] W. Krings, T. Faust, A. Kovalev, M.T. Neiber, M. Glaubrecht, S.N. Gorb, In slow motion: radula motion pattern and forces exerted to the substrate in the land snail *Cornu aspersum* (Mollusca, Gastropoda) during feeding, R. Soc. Open Sci. 6(7) (2019b) 2054–5703. https://doi:10.1098/rsos.190222.

[69] J. Fortuny, J. Marcé-Nogué, S. de Esteban-Trivigno, L. Gil, Á. Galobart, Temnospondyli bite club: ecomorphological patterns of the most diverse group of early tetrapods, J Evol Biol. 24(9) (2011) 2040–54. https://doi:10.1111/j.1420-9101.2011.02338.x.

[70] J. Fortuny, J. Marcé-Nogué, J.-S. Steyer, S. de Esteban-Trivigno, E. Mujal, L. Gil, Comparative 3D analyses and palaeoecology of giant early amphibians (Temnospondyli: Stereospondyli), Sci Rep. 6 (2016) 30387. https://doi:10.1038/srep30387.

[71] M.R.G. Attard, W.C.H. Parr, L.A.B. Wilson, M. Archer, S.J. Hand, T.L. Rogers, S. Wroe, Virtual reconstruction and prey size preference in the mid cenozoic thylacinid, *Nimbacinus dicksoni* (Thylacinidae, Marsupialia). PLoS One 9(4) (2014) e93088. https://doi.org/10.1371/journal.pone.0093088.

[72] P. Piras, G. Sansalone, L. Teresi, M. Moscato, A. Profico, R. Eng, T.C. Cox, A. Loy, P. Colangelo, T. Kotsakis, Digging adaptation in insectivorous subterranean eutherians. The enigma of *Mesoscalops montanensis* unveiled by geometric morphometrics and finite element analysis, J Morphol. 276(10) (2015) 1157–1171. https://doi:10.1002/jmor.20405.

[73] S. Serrano-Fochs, S. de Esteban-Trivigno, J. Marcé-Nogué, J. Fortuny, R.A. Fariña, Finite Element Analysis of the Cingulata Jaw: An Ecomorphological Approach to Armadillo's Diets, PLoS One 10(6) (2015) e0120653. https://doi:10.1371/journal.pone.0129953.

[74] A.C. Sharp, Comparative finite element analysis of the cranial performance of four herbivorous marsupials, J Morphol. 276(10) (2015) 1230–1243. https://doi:10.1002/jmor.20414.

[75] M.E.H. Jones, F. Gröning, H. Dutel, A.C. Sharp, M.J. Fagan, S.E. Evans, The biomechanical role of the chondrocranium and sutures in a lizard cranium, J R Soc Interface 14(137) (2017) 20170637. https://doi:10.1098/rsif.2017.0637.

[76] O. Panagiotopoulou, J. Iriarte-Diaz, S. Wilshin, P.C. Dechow, A.B. Taylor, H. Mehari Abraha, S.F. Aljunid, C.F. Ross, In vivo bone strain and finite element modeling of a rhesus macaque mandible during mastication, Zoology 124 (2017) 13–29. https://doi:10.1016/j.zool. 7.08.010.

[77] A.A. Farke, Frontal sinuses and head-butting in goats: a finite element analysis, J Exp Biol. 211(19) (2008) 3085–3094. https://doi:10.1242/jeb.019042.

[78] Z.J. Tseng, Cranial function in a late Miocene *Dinocrocuta gigantea* (Mammalia: Carnivora) revealed by comparative finite element analysis, Biol J Linn Soc. 96(1) (2009) 51–67. https://doi:10.1111/j.1095-8312.2008.01095.x.

[79] W.C.H. Parr, S. Wroe, U. Chamoli, H.S. Richards, M.R. McCurry, P.D. Clausen, C. McHenry, Toward integration of geometric morphometrics and computational biomechanics: New methods for 3D virtual reconstruction and

quantitative analysis of Finite Element Models, J Theor Biol. Elsevier 301 (2012) 1–14. https://doi:10.1016/j.jtbi.2012.01.030.

[80] P. Aquilina, U. Chamoli, W.C.H. Parr, P.D. Clausen, S. Wroe, Finite element analysis of three patterns of internal fixation of fractures of the mandibular condyle, Br. J. Oral. Maxillofac. Surg. 51(4) (2013) 326–331. https://doi:10.1016/j.bjoms.2012.08.007.

[81] B. Figueirido, Z.J. Tseng, F.J. Serrano-Alarcon, A. Martin-Serra, J.F.F. Pastor, Three-dimensional computer simulations of feeding behaviour in red and giant pandas relate skull biomechanics with dietary niche partitioning, Biol Lett. 10(4) (2014) 20140196. https://doi:10.1098/rsbl.2014.0196.

[82] J.F. Fish, C.T. Stayton, Morphological and mechanical changes in juvenile red-eared slider turtle (*Trachemys scripta elegans*) shells during ontogeny, J Morphol. 275(4) (2014) 391–397. https://doi:10.1002/jmor.20222.

[83] J.M. Neenan, M. Ruta, J.A. Clack, E.J. Rayfield, Feeding biomechanics in *Acanthostega* and across the fish-tetrapod transition, Proc R Soc B Biol Sci. 281(1781) (2014). https://doi:10.1098/rspb.2013.2689.

[84] C.A. Brassey, J.D. Gardiner, A.C. Kitchener, Testing hypotheses for the function of the carnivoran baculum using finite-element analysis, Proc R Soc B Biol Sci. 285(1887) (2018) pii: 20181473. https://doi:10.1098/rspb.2018.1473.

[85] S. Lautenschlager, P.G. Gill, Z.-X. Luo, M.J. Fagan, E.J. Rayfield, The role of miniaturization in the evolution of the mammalian jaw and middle ear, Nature 561 (2018) 533–537. https://doi:10.1038/s41586-018-0521-4.

[86] P. Piras, L. Maiorino, L. Teresi, C. Meloro, F. Lucci, T. Kotsakis, P. Raia, Bite of the cats: Relationships between functional integration and mechanical performance as revealed by mandible geometry, Syst Biol. 62(6) (2013) 878–900. https://doi:10.1093/sysbio/syt053.

[87] L. Maiorino, A.A. Farke, T. Kotsakis, L. Teresi, P. Piras, Variation in the shape and mechanical performance of the lower jaws in ceratopsid dinosaurs (Ornithischia, Ceratopsia), J Anat. 227(5) (2015) 631–646. https://doi:10.1111/joa.12374.

[88] J. Marcé-Nogué, T.A. Püschel, T.M. Kaiser, A biomechanical approach to understand the ecomorphological relationship between primate mandibles and diet, Sci. Rep. 7 (2017) 8364. https://doi.org/10.1038/s41598-017-08161-0.

[89] J. Soons, A. Genbrugge, J. Podos, D. Adriaens, P. Aerts, J. Dirckx, A. Herrel, Is Beak Morphology in Darwin's Finches Tuned to Loading Demands?, PLoS One 10(6) (2015) e0129479. https://doi:10.1371/journal.pone.0129479.

[90] S. Miura, R. Saito, V. Parque, T. Miyashita, Design factors for determining the radula shape of *Euhadra* Peliomphala, Sci. Rep. 9(1) (2019) 749. https://doi:10.1038/s41598-018-36397-x.

[91] E.C. Aifantis, On the role of gradients in the localization of deformation and fracture, Int. J. Eng. Sci. 3 (1992) 1279–1299. https://doi:10.1016/0020-7225(92)90141-3.

[92] N. De Jager, M.D. Kler, J.M. Zel, The influence of different core material on the FEA-determined stress distribution in dental crowns, Dent. Mater. 22 (2006) 234–242. https://doi:10.1016/j.dental.2005.04.034.

[93] A. Bingbing, R. Wang, D. Arola, D. Zhang, The role of property gradients on the mechanical behavior of human enamel, J. Mech. Behav. Biomed. Mater. 9 (2012) 63–72. https://doi:10.1016/j.jmbbm.2012.01.009.

[94] J. Michels, J. Vogt, S.N. Gorb, Tools for crushing diatoms — opal teeth in copepods feature a rubber-like bearing composed of resilin, Sci. Rep. 2 (2012) 465. https://doi:10.1038/srep00465.

[95] Z. Liu, Y. Zhu, D. Jiao, Z. Weng, Z. Zhang, R.O. Ritchie, Enhanced protective role in materials with gradient structural orientations: Lessons from nature, Acta Biomater. 44 (2016) 31–40. https://doi:10.1016/j.actbio.2016.08.005.

[96] S. Büsse, S.N. Gorb, Material composition of the mouthpart cuticle in a damselfly larva (Insecta: Odonata) and its biomechanical significance, Royal Soc. Open Sci. 5 (2018) 172117. https://doi:10.1098/rsos.172117.

[97] B.D. Saltin, Y. Matsumura, A. Reid, J.F. Windmill, S.N. Gorb, J.C. Jackson, Material stiffness variation in mosquito antennae, J. R. Soc. Interface 16(154) (2019) 20190049. http://doi:10.1098/rsif.2019.0049.

[98] L.-Y. Wang, M. Jafarpour, C.-P. Lin, E. Appel, S.N. Gorb, H. Rajabi, Endocuticle sclerotisation increases the mechanical stability of cuticle, Soft Matter 15 (2019) 8272. http://doi:10.1039/c9sm01687b.

[99] E.J. Rayfield, Finite Element Analysis and Understanding the Biomechanics and Evolution of Living and Fossil Organisms, Annual Review of Earth and Planetary Sciences 35(1) (2007) 541–576. http://doi:10.1146/annurev.earth.35.031306.140104.

[100] S.P. Woodward, On some new freshwater shells from Central Africa, Proc. Zool. Soc. Lond. 27 (1859) 348-351.

[101] K.J. Boss, On the evolution of gastropods in ancient lakes, in: V. Fretter, J. Peake (Eds.), Pulmonates Systematics, Evolution and Ecology, vol. 2a, Academic Press, London, 1978, 385–428.

[102] M.R. Johnston, A.S. Cohen, Morphological divergence in endemic gastropods from Lake Tanganyika: implications for models of species flock formation, Palaios 2 (1987) 413–425. https://doi:10.2307/3514613.

[103] G.W. Coulter, Lake Tanganyika and its Life, Oxford University Press, Oxford, 1991.

[104] E. Michel, A.S. Cohen, K. West, M.R. Johnston, P.W. Kat, Large African lakes as natural laboratories for evolution: examples from the endemic gastropod fauna of Lake Tanganyika, Mitt. Internat. Verein. Limnol. 23 (1992) 85–99. https://doi:10.1080/05384680.1992.11904012.

[105] E. Michel, Why snails radiate: a review of gastropod evolution in long-lived lakes, both recent and fossil, in: K. Martens, B. Goddeeris, G.W. Coulter (Eds.), Speciation in ancient lakes, Advances in Limnology, E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, 1994, 285–317.

[106] E. Michel, Phylogeny of a gastropod species flock: exploring speciation in Lake Tanganyika in a molecular framework, Adv. Ecol. Res. 31 (2000) 275–302. https://doi:10.1016/S0065-2504(00)31016-9.

[107] K. Martens, Speciation in ancient lakes, Trends. Ecol. Evol. 12(5) (1997) 177–182. https://doi:10.1016/S0169-5347(97)01039-2.

[108] K. West, E. Michel, The dynamics of endemic diversification; molecular phylogeny suggests an explosive origin of the thiarid gastropods of Lake Tanganyika, Adv. Ecol. Res. 31 (2000) 331–354. https://doi:10.1016/S0065-2504(00)31018-2.

[109] A.B. Wilson, M. Glaubrecht, A. Meyer, Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika, Proc. Royal Soc. Lond. B 271(1538) (2004) 529–536. https://doi:10.1098/rspb.2003.2624.

[110] E. Leloup, Exploration Hydrobiologique du Lac Tanganika (1946–1947), Bruxelles, 1953.

[111] D.S. Brown, G. Mandahl-Barth, Living molluscs of Lake Tanganyika: a revised and annotated list, J. Conchol. 32 (1987) 305–327.

[112] D. Brown, Freshwater Snails of Africa and their Medical Importance, Taylor and Francis, 1994.

[113] C.E. Kehl, J. Wu, S. Lu, D.M. Neustadter, R.F. Drushel, R.K. Smoldt, H.J. Chiel, Soft-surface grasping: radular opening in *Aplysia californica*, J. Exp. Biol. 222 (2019) jeb191254. https://doi:10.1242/jeb.191254.

[114] T.P. Neusser, M. Heβ, G. Haszprunar, M. Schrödl, Computer-based three-dimensional reconstruction of the anatomy of *Microhedyle remanei* (Marcus, 1953), an interstitial acochlidian gastropod from Bermuda, J. Morphol. 267(2) (2006) 231–247. https://doi:10.1002/jmor.10398.

[115] R.E. Golding, A.S. Jones, Micro-CT as a novel technique for 3D reconstruction of molluscan anatomy, Molluscan Res. 27(3) (2007) 123–128.

[116] W. Holznagel, A nondestructive method for cleaning gastropod radulae from frozen, alcohol-fixed, or dried material, Am. Malacol. Bull. 14(2) (1998) 181–183.

[117] D.M. Ebenstein, L.A. Pruitt, Nanoindentation of biological materials, Nano Today 1 (2006) 26–33. https://doi:10.1016/S1748-0132(06)70077-9. [118] A.L. Smith, S. Benazzi, J.A. Ledogar, K. Tamvada, L.C.P. Smith, G.W. Weber, M.A. Spencer, P.W. Lucas, S. Michael, A. Shekeban, K. Al-Fadhalah, A.S. Almusallam, P.C. Dechow, I.R. Grosse, C.F. Ross, R.H. Madden, B.G. Richmond, B.W. Wright, Q. Wang, C. Byron, D.E. Slice, S. Wood, C. Dzialo, M.A. Berthaume, A. van Casteren, D.S. Strait, The Feeding Biomechanics and Dietary Ecology of *Paranthropus boisei*, Anat. Rec. 298(1) (2014) 145–167. https://doi:10.1002/ar.23073.

[119] D. Montroni, X. Zhang, J. Leonard, M. Kaya, C. Amemiya, G. Falini, M. Rolandi, Structural characterization of the buccal mass of *Ariolimax californicus* (Gastropoda; Stylommatophora), PLoS One 14(8) (2019) e0212249. https://doi:10.1371/journal.pone.0212249.

[120] J. Marcé-Nogué, J. Fortuny, L. Gil, M. Sánchez, Improving mesh generation in Finite Element Analysis for functional morphology approaches, Spanish J. Palaeontol. 31 (2015) 117–132.

[121] J. Marcé-Nogué, S. de Esteban-Trivigno, C. Escrig, L. Gil, Accounting for differences in element size and homogeneity when comparing Finite Element models: Armadillos as a case study, Palaeontol. Electron. 19(2) (2016) 1–22. https://doi:10.26879/609.

[122] D.G. Reid, Systematics and Evolution of *Littorina*, Ray Society, London, 1996.

[123] S.A. Herrera, L. Grunenfelder, E. Escobar, Q. Wang, C. Salinas, N. Yaraghi, J. Geiger, R. Wuhrer, P. Zavattieri, D. Kisailus, Stylus support structure and function of radular teeth in *Cryptochiton stelleri*, 20<sup>th</sup> International Conference on Composite Materials Copenhagen, 19–24<sup>th</sup> July 2015, 2015.

[124] M.-C.G. Klein, J.K. Deuschle, S.N. Gorb, Material properties of the skin of the Kenyan sand boa *Gangylophis colubrinus* (Squamata, Boidae), J. Comp. Physiol. A 196 (2010) 659–668. https://doi:10.1007/s00359-010-0556-y.

[125] M.-C. G. Klein, S.N. Gorb, Epidermis architecture and material properties of the skin of four snake species, J. R. Soc. Interface 9 (2012) 3140–3155. https://doi:10.1098/rsif.2012.0479.

[126] J.A. Shaw, L.R. Brooker, D.J. Macey, Radula tooth turnover in the chiton, *Acanthopleura hirtosa* (Blainville, 1825) (Mollusca: Polyplacophora). Molluscan Res. 22 (2002) 93–99.

[127] N.W. Runham, P.R. Thornton, Mechanical wear of the gastropod radula: a scanning electron microscope study. J. Zool. 153 (1967) 445–452.

[128] F. Haas, S.N. Gorb, R.J. Wootton, Elastic joints in dermapteran hind wings: materials and wing folding, Arthropod Struc. Dev. 29 (2000a) 137–146. https://doi:10.1016/S1467-8039(00)00025-6.

[129] F. Haas, S.N. Gorb, R. Blickhan, The function of resilin in beetlewings, Proc. R. Soc. Lond. B 267 (2000b) 1375–1381. https://doi:10.1098/rspb.2000.1153.

[130] H. Rajabi, A. Darvizeh, A. Shafiei, D. Taylor, J.H. Dirks, Numerical investigation of insect wing fracture behaviour. J. Biomech. 48 (2015) 89–94. https://doi:10.1016/j.jbiomech.2014.10.037.

[131] H. Rajabi, A. Shafiei, A. Darvizeh, S.N. Gorb, Resilin microjoints: a smart design strategy to avoid failure in dragonfly wings, Sci. Rep. 6 (2016a) 39039. https://doi:10.1038/srep39039.

[132] H. Rajabi, A. Shafiei, A. Darvizeh, J.-H. Dirks, S.N. Gorb, Effect of microstructure on the mechanical and damping behaviour of dragonfly wing veins, R. Soc. open sci. 3 (2016b) 160006. https://doi:10.1098/rsos.160006.

[133] A. Miserez, Y. Li, J.H. Waite, F. Zok, Jumbo squid beaks: inspiration for design of robust organic composites. Acta Biomater. 3 (2007) 139–149.

[134] A. Miserez, T. Schneberk, C. Sun, F.W. Zok, J.H. Waite, The transition from stiff to compliant materials in squid beaks. Science 319 (2008) 1816–1819.

[135] A. Miserez, D. Rubin, J.H. Waite, Cross-linking Chemistry of Squid Beak, J. Biol. Chem. 285 (49), (2010) 38115–38124.

[136] Y.P. Tan, S. Hoon, P.A. Guerette, W. Wei, A. Ghadban, C. Hao, A. Miserez, J.H. Waite, Infiltration of chitin by protein coacervates defines the squid beak mechanical gradient. Nat. Chem. Biol. 11 (2015) 488–495.

https://doi:10.1038/nchembio.1833.

[137] V. Fretter, A. Graham, British prosobranch mollusca, their functional anatomy and ecology, Ray Society, London. 1962.

[138] A. Graham, The anatomical basis of function in the buccal mass of prosobranch and amphineuran molluscs, J. Zool. Lond. 169 (1973) 317–348.

[139] P.J.W. Jüch, G.J. Boeksehoten, Trace fossils and grazing traces produced by *Littorina* and *Lepidochitona*, Dutch Wadden Sea, Geologie en Mijnbouw 59 (1980) 33–42.

## <u>Supplements</u>

# Tab. 1: Values of the stress and strain, in MPa, for certain defined areas (Fig. 8a 1-11) on the CT, LT, and OMT with different condition of the model.

Tooth type	ario	membrane	Material properties of teeth	Stress for homologous points										Strain for homologous points											
	Scen	Embedding		1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11
	11	Hard	Homogeneous									0.174560000000	0.04836000000	0.13984000000									0.000077671000	0.000021499000	0.000063500000
Central tooth	12		Heterogeneous									0.25996000000	0.059710000000	0.13984000000									0.000389980000.0	0.000389980000.0	0.000017661000
	6	m-hard	Homogeneous									0.174560000000	0.048360000000	0.139840000000									0.000776710000	0.000214990000	0.00063500000
	10	Mediu	Heterogeneous									0.441200000000	0000006902000	0.13984000000									0.000066178000	0.000008740800	0.000017661000
	7	Soft	Homogeneous									0.174560000000	0.04836000000	0.139840000000									0.007767100000	0.002149900000	0.00635000000
2	1	4	3	9																					
----------------	-----------------	----------------	----------------	----------------																					
Sc	oft	Mediur	m-hard																						
Heterogeneous	Homogeneous	Heterogeneous	Homogeneous	Heterogeneous																					
15.19100000000	12.160000000000	13.64100000000	12.12600000000	12.12800000000																					
7.08160000000	7.134800000000	7.00630000000	7.13480000000	6.904900000000																					
1.21600000000	1.22120000000	1.21600000000	1.22120000000	1.21600000000																					
0.297320000000	0.28590000000	0.29732000000	0.28590000000	0.297330000000																					
0.51226000000	0.507190000000	0.51225000000	0.50719000000	0.51225000000																					
0.006251400000	0.53893000000	0.005613200000	0.053893000000	0.004990700000																					
0.002879200000	0.317110000000	0.002848600000	0.031711000000	0.002807400000																					
0.000268620000	0.05504500000	0.000268620000	0.005504500000	0.000268620000																					
0.000064677000	0.012714000000	0.000064679000	0.001271400000	0.000064681000																					
0.000111390000	0.022543000000	0.000111390000	0.002254300000	0.000111390000																					

# Chapter 4. Trophic specialization

# *4. 1 Trophic specialisation reflected by radular tooth material properties in an 'ancient' Lake Tanganyikan gastropod species flock*

Wencke Krings<sup>1</sup>\*, Marco T. Neiber<sup>1</sup>, Alexander Kovalev<sup>2</sup>, Stanislav N. Gorb<sup>2</sup>, Matthias Glaubrecht<sup>1</sup>

<sup>1</sup> Center of Natural History (CeNak), Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

<sup>2</sup> Functional Morphology and Biomechanics, Zoological Institute of the Christian-Albrechts-Universität zu Kiel, Am Botanischen Garten 9, 24118 Kiel, Germany

\*corresponding author: wencke.krings@uni-hamburg.de

#### <u>Abstract</u>

Lake Tanganyika belongs to the East African Great Lakes and is well known for harbouring a high proportion of endemic and morphologically distinct genera, in cichlids but also in paludomid gastropods. With about 50 species these snails form a flock of high interest because of its diversity, the question of its origin and the evolutionary processes that might have resulted in this elevated amount of taxa. While earlier debates centred on these paludomids to be a result of an intralacustrine adaptive radiation, there are strong indications for the existence of several paludomid lineages before the lake formation. In order to evaluate hypotheses on the evolution and radiation the detection of actual adaptations is crucial. Since the Tanganyikan gastropods show distinct radular tooth morphologies hypotheses about potential trophic specializations are at hand. Here, based on a phylogenetic tree of the paludomid species from Lake Tanganyika and adjacent river systems, the mechanical properties of their teeth were evaluated by nanoindentation, a method measuring the hardness and elasticity of a structure, and related with the gastropods' specific feeding substrate (soft, solid, mixed). Results identify mechanical adaptations in the tooth cusps to the substrate and, with reference to the tooth morphology, assign distinct functions (scratching or gathering) to tooth types. Pure morphology does not consistently reflect ecological specializations, but the mechanical properties allow the establishment of eco-morpho-propo-types. In almost every lineage we discovered adaptations to different substrates leading to the hypothesis that one main engine of the flock's evolution is trophic specialization establishing distinct ecological niches and allowing the coexistence of taxa.

Keywords: Functional morphology, nanoindentation, mechanical properties, Gastropoda, trophic specialisation, adaptive radiation

## <u>Background</u>

Hypotheses on how biodiversity relates with the temporal and spatial 'filling' of available habitats and annidation itself, i.e. the actual formation of ecological niches as a combined process of internal and external factors, is paramount for understanding how species evolve under geographical and ecological conditions [1–3]. Addressing this topic has seen various approaches based on vertebrates, such as the studies on Darwin finches [4–9], the cichlid fishes in the East African lakes [10–13] and Nicaragua [14–16], or *Anolis* lizards [17–19]. These species flocks [*cf.* 20] are usually regarded as examples of adaptive radiations, the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage, thus linking speciation and ecology [21–26].

However, even though the majority of all known animals are invertebrates [27–28] fewer model systems were on focus (with exceptions, e.g. [29–30] on crickets). There are however spectacular examples of invertebrate species flocks exhibiting a great diversity, especially among molluscs [see also 31–32] as the lacustrine and riverine freshwater gastropods on Sulawesi [33–38], Madagascar [39], in the Thai rivers [40–41], or 'ancient' Lake Tanganyika [42–44]. The latter is well known for its unique assemblage of endemic species and has been a natural laboratory for research on the drivers of evolution for decades. It harbours paludomid gastropods (Fig. 1) which triggered many expeditions and subsequently malacological descriptions [e.g. 45–60] and a long-lasting controversy about the origin and evolution of the lake and its fauna. Due to their marine-like appearance (termed 'thalassoid' by [45] and 'halolimnic' by [61]) many earlier authors addressed the possibility of a marine origin of the Lake Tanganyikan fauna and discussed the causes of the thalassoid appearance of its endemic mollusks. However, this eventually led to the refutation of Moore's controversial hypothesis [61–63] of the lake being once directly connected to the ocean [see e.g. in 64–68].

For a long time the idea that this largest and deepest of the African lakes has supplied its gastropod fauna with a stable inland environment and offered unique opportunities for within-lake diversification ('ancient endemic radiation' see [65, 69]) resulting in a truly 'adaptive' radiation *cf.* [70–71] was common [e.g. 72–86]. However, strong evidence for an ancient origin of disparity and diversity in this flock has been presented indicating the existence of major gastropod lineages before

the formation of the lake itself or its proto-lakes. The oldest formation estimates are 9–12 Mya [87– 89], while more recent studies date the pre-rift formation to 4–11 Mya and the earliest onset of a true rifting activity to 5.5 Mya [90–93]. With a molecular clock approach in support [42] this alternative hypothesis of the former existence of several originally riverine paludomid lineages later inhabiting the lake and bringing possible adaptations to former river environments with them was suggested by [43–44] and was summarized in [Krings et al. unpublished data, here chapter 2.1].

In order to allocate hypotheses about paludomid evolution and radiation - especially in the context of adaptive radiations - the identification of actual adaptations is crucial. Morphological structures associated with feeding, such as e.g. bills or skulls in birds, vomer bones in cichlid fishes or teeth in mammals, can exhibit adaptations and indicate trophic specialization. They serve as an interface between the organism and its ingesta (food, minerals) and, as they provided insights into functional adaptations and hence evolution, are of high research interest in various groups [e.g. 94–97 on Darwin's finches, 98 on oviraptorosaurian dinosaurs, 99 on cichlid fish].

The gastropods radula, one important synapomorphy of the Mollusca, acts as such an interface, mechanically processing ingesta and directly linking the organism with its food. Various muscles control the motion of this feeding organ pulling the chitinous radular membrane with rows of small embedded radular teeth [100] across an odontophoral cartilage (Fig. 2a). As teeth are in direct contact with the ingesta their morphology can often be directly linked with the animal's ecology and can reflect various transitions from zoovorous to herbivorous traits [101–106]. Form together with the tooth's position and chemical composition is widely considered adaptive to food and are hence closely associated with feeding strategies, competitor avoidance and thus trophic specialization [107–117].

Strong indications for trophic specialization in the context of gastropod adaptive radiations have been described for the radular tooth morphologies of lacustrine *Tylomelania* from Sulawesi [33, 35, 38] and riverine gastropods from Kaek River [40]. For the Lake Tanganyikan paludomids hypotheses on the potential influence of trophic specialization on the evolution of this flock are consequential since these species show an extraordinarily high interspecific diversity in tooth morphologies [e.g. 44, 118]. Most characteristic features were recently evaluated and related with the gastropods' specific feeding substrates (soft, mixed or solid) since teeth as highly functionate interfaces do not only interact with the food but also with the substrate the food is attached to [Krings et al. unpublished data, here chapter 2.1].

In addition to morphology the structural composition also influences functionality. For reconstructing the evolutionary history of the African Paludomidae and in order to develop hypotheses on the role of trophic specialisation in their evolution we here identified the hardness and elasticity of taenioglossan radular teeth from 24 species belonging to this flock utilising nanoindentation, a technique previously employed to identify local mechanical properties of various biological materials [e.g. 119–125]. Results, with reference to morphology, allow the assignment of distinct functions to certain tooth types. The identification of mechanical adaptations to the preferred feeding substrate in the tooth cusps allowed the establishment of eco-morphopropo-types. Our results strongly indicate that one main engine of the flock's evolution is trophic specialization allowing the coexistence of species.

Fig. 1. Shells of examined species. Black scale bars: from Lake Tanganyika. Blue scale bar: from adjacent river systems. Forms next to the letters a-x indicate preferred feeding substrate (circle = mud, hexagon = sand, square = plant surface, triangle = rock). *a. Bridouxia ponsonbyi* ZMB 220.137-1, *b. B. grandidieriana* BMNH 1889.6.23.57-61, *c. B. rotundata* ZMB 220.063-3, *d. B. praeclara* DBL 19-4, *e. Spekia zonata* ZMB 220.007-2, *f. Leloupiella minima* ZMB 220.008-3, *g. Reymondia horei* ZMB 220.007-1, *h. Cleopatra johnstoni* ZMB 220.102, *i. Stanleya neritinoides* ZMB 102.624-1, *j. Tanganyicia rufofilosa* ZMB 102.621-1, *k. Martelia tanganyicensis* ZMB 220.134-1, *l. Anceya giraudi* ZMB 220.000-1, *m. Syrnolopsis lacustris* ZMB 220.046-1, *n. Chytra kirkii* SMF 290543-1, *o. Limnotrochus thomsoni* SMF 290542-1, *p. Tiphobia horei* SMF 290550-1, *q. Paramelania iridescens* SMF 290538, *r. P. damoni* SMF 290531-2, *s. P. crassigranulata* SMF 290528-1, *t. Mysorelloides multisulcata* DBL without number, *u. Lavigeria spinulosa* ZMB 220114, *v. L. grandis* SMF 292827-1, *w. L. nassa* ZMB 220.172-1, *x. L. livingstoniana* ZMB 220116; Scale bars: a-d, f, k, l, m = 2.5 mm; e = 5 mm; g, i-j, n, o, v-w = 10 mm; h = 5 mm; p-s = 20 mm, t = 0.75 mm; u, x = 6 mm.





Fig. 2. *a.* Schematic drawing of the radula when feeding, *b.* taenioglossan radula of *Spekia zonata* (ZMH 150008/999-2), black line = area of nanoindentation for central, lateral and marginal teeth, *c.* radula (ZMB220.143-2) embedded in epoxy resin and polished for nanoindentation (longitudinal section along the radula), *d-f.* magnification of some tested areas (*e.* with nanoindentation mark, *f.* crosses indicate points of indentation), *g.* representative results of nanoindentation measurement curve for basis, stylus, and cusps of central teeth, elastic modulus, GPa, versus displacement into tooth material. The values for the cusps within the indentation depth of 480-520 nm were used for further calculation. Scale bars: b = 100  $\mu$ m; c = 250  $\mu$ m; d, e = 30  $\mu$ m; f = 60  $\mu$ m. CT = central tooth, LT = lateral tooth, MRT = mature radular teeth, O = odontophore, OMT = outer marginal tooth, RM = radular muscles, RT = radular teeth, WZ = working zone.

#### <u>Results</u>

Tooth's morphologies (Fig. 3–8) correlate with the substrate-preference [see also Krings et al. unpublished data, here chapter 2.1]. Grazing on stones usually correlates with certain morphologies of the central tooth, either involving a spatulate, prominent central denticle (Nassopsini and *Reymondia*; Fig. 4, 8), or few or no denticles (*Bridouxia ponsonbyi*, *B. rotundata*, *B. praeclara*, *Spekia*, and *Leloupiella*; Fig. 3, 4), and sometimes with laterals bearing a prominent denticle (Nassopsini, *Reymondia*, *Bridouxia praeclara*, *B. ponsonbyi*, *B. rotundata*, *Spekia*, and *Tanganyicia*; Fig. 3, 4, 5, 7, 8). Few solid-substrate feeders (*Stanleya* and *Tanganyicia*; Fig. 5) display central and lateral teeth with long denticles of the same size. Here teeth are rather similar in their morphology to teeth of gastropods foraging on sand, possessing central, lateral, and marginal teeth with small or finger-like denticles at each cusp (Fig. 4–7). All mixed substrate feeders (*Paramelania damoni*, *Limnotrochus thomsoni*, and *Bridouxia grandidieriana*) display small, finger-like denticles as well (Fig. 3, 6, 7).

Nanoindentation experiments provided the Young's modulus (E) as a measure of the stiffness of a solid material describing the relationship between mechanical stress and indentation depth, and the hardness (H), the measure of the resistance to local plastic deformation. Statistical analysis of these parameters revealed normal distribution for both. Significant differences between all tooth cusps (Fig. 9) of the separate substrate feeder groups (solid, soft, and mixed) regarding both E and H were detected (p<0.0001, F-ratio: 2, df: 59578.92 for E, df: 20833.04 for H). Paludomids feeding on sand have comparatively soft and flexible tooth cusps (mean  $\pm$  std. deviation; E = 4.57  $\pm$  0.45 GPa, H = 0.18  $\pm$  0.07 GPa), species foraging on stone have the stiffest and hardest tooth cusps (E = 6.08  $\pm$  1.52 GPa, H = 0.26  $\pm$  0.11 GPa), and mixed substrate feeders are intermediate (E = 4.94  $\pm$  0.99 GPa, H = 0.20  $\pm$  0.09 GPa).

Significant differences were found between all central, lateral, and marginal tooth cusps (Fig. 9) (p<0.0001, F-ratio: 2, df: 70177.01 for E, df: 24978.35 for H). Marginal teeth are comparable soft and flexible (E =  $4.68 \pm 0.63$  GPa, H =  $0.19 \pm 0.08$  GPa), the central teeth are comparatively hard and stiff (E =  $6.48 \pm 1.84$  GPa, H =  $0.28 \pm 0.13$  GPa), and the lateral one are intermediate (E =  $5.54 \pm 0.92$  GPa, H =  $0.24 \pm 0.09$  GPa).

Comparing the mechanical properties within each species we consistently detect significant differences (p<0.0001, F-ratio: 2) between central, lateral, and marginal tooth cusps (see Table 1 for all E and H values, df, and homologous groups). All species feeding on solid substrate clearly display gradients in their radular properties, the stiffest and hardest parts are always the central tooth cusps, followed by the lateral ones; the softest and most flexible parts are the marginal cusps (see additionally Fig. 10, 11). In the mixed substrate feeders there's a similar situation, central cusps are hard and stiff, lateral ones intermediate, and marginals soft and flexible; but central and lateral tooth cusps are not as distinct as in the solid substrate feeders. Species foraging on sand have quite similar mechanical properties in their tooth cusps and are more homogenous (Table 1, Fig. 10, 11).

When comparing E and H of each tooth type between all species significant differences were detected (p<0.0001, F-ratio: 71, df: 20217.82 for E, df: 2734.555 for H; see Fig. 10, 11 and Table 1 for homologous groups).



Fig. 3. Radular teeth of: *a-b. Bridouxia ponsonbyi* ZMB 220.137-1, *a.* overview, *b.* marginals; *c-d. B. grandidieriana* ZMB 220.139-4, *c.* overview, *d.* centrals and laterals; *e-f. B. rotundata* ZMB 220063-1, *e.* overview, *f.* marginals; *g-h. B. praeclara* ZMB 220.063-2, *g.* overview, *h.* marginals. Scale bars:  $a = 40 \mu m$ ; b, d, f,  $h = 10 \mu m$ ; c, e,  $g = 20 \mu m$ . Forms indicate preferred feeding substrate (circle = mud, hexagon = sand, square = plants, triangle = rock).



Fig. 4. Radular teeth of: *a-b. Spekia zonata* ZMH 150008/999-2, *a.* overview, *b.* marginals; *c-d. Leloupiella minima* ZMB 220.135, *c.* overview, *d.* marginals; *e-f. Reymondia horei* ZMB 220.147-1, *e.* centrals and laterals, *f.* marginals; *g-h. Cleopatra johnstoni* ZMB 220.102-1, *g.* overview, *h.* marginals. Scale bars:  $a = 100 \mu m$ ;  $b = 50 \mu m$ ;  $c, d = 10 \mu m$ ;  $e, f = 30 \mu m$ ;  $g = 40 \mu m$ ;  $h = 20 \mu m$ . Forms indicate preferred feeding substrate (circle = mud, hexagon = sand, square = plants, triangle = rock).



Fig. 5. Radular teeth of: *a-b. Stanleya neritinoides* MRAC without number, *a.* centrals and laterals, *b.* marginals; *c-d. Tanganyicia rufofilosa, c.* centrals and laterals, *d.* marginals; *e-f. Martelia tanganyicensis* ZMB 220.133-1, *e.* overview, *f.* laterals and marginals; *g-h. Anceya giraudi* ZMB 220.132, *g.* overview, *h.* centrals and laterals. Scale bars: a, b, c, e, f, h = 10  $\mu$ m; d, g = 30  $\mu$ m. Forms indicate preferred feeding substrate (circle = mud, hexagon = sand, square = plants, triangle = rock).



Fig. 6. Radular teeth of: *a-b. Syrnolopsis lacustris* ZMB 220.131, *a.* overview, *b.* marginals; *c-d. Chytra kirkii* IRSNB no. 63, *c.* overview, *d.* centrals and laterals; *e-f. Limnotrochus thomsoni* ZMB 107.102, *e.* overview, *f.* centrals and laterals; *g-h. Paramelania iridescens* ZMB 220.053, *g.* overview, *h.* centrals. Scale bars:  $a = 30 \mu m$ ;  $b, h = 10 \mu m$ ;  $c = 100 \mu m$ ;  $d, f = 20 \mu m$ ;  $e = 50 \mu m$ ;  $g = 120 \mu m$ . Forms indicate preferred feeding substrate (circle = mud, hexagon = sand, square = plants, triangle = rock).



Fig. 7. Radular teeth of: *a-b. Paramelania damoni* ZMH without number, *a.* centrals and laterals, *b.* marginals; *c-d. P. crassigranulata* ZMB 220.037-1, *c.* overview, *d.* centrals and laterals; *e-f. Mysorelloides multisulcata* IRSNB no. 126, *e.* centrals and laterals, *f.* marginals; *g-h. Lavigeria spinulosa* ZMB 220.051, *g.* overview, *h.* marginals. Scale bars: a, f = 10 µm; b, d, h = 30 µm; c, g = 100 µm; e = 20 µm. Forms indicate preferred feeding substrate (circle = mud, hexagon = sand, square = plants, triangle = rock).



Fig. 8. Radular teeth of: *a-b. Lavigeria grandis* ZMH 154657/999, *a.* overview, *b.* laterals and marginals; *c-d. L. nassa* ZMB 220.074, *c.* overview, *d.* laterals and marginals; *e-f. L. livingstoniana* ZMB 220.117-1, *e.* overview, *f.* marginals and laterals. Scale bars:  $a = 100 \mu m$ ;  $b = 50 \mu m$ ; c, d, f = 30  $\mu m$ ; e = 100  $\mu m$ . Forms indicate preferred feeding substrate (circle = mud, hexagon = sand, square = plants, triangle = rock).



Fig. 9. Results of nanoindentation, hardness (GPa) and Young's modulus (GPa). (*Left*) Comparing all tooth cusps of species feeding on mixed, soft and solid substrate; (*right*) comparing all central to all lateral and marginal tooth cups. Letters represent homologous groups. N = number of tested tooth cusps.

					Yo	ungʻs	s Mod	dulus,	GPa		Hardness, G	Pa			
			ω	4 о	6	7	8	9	10.	11.	0 0 0 0 0 0	0	0	0	-
			00	8 8	00	00	00	00	00	00	.40 .20	70	80	90	00
		CT N = 102	H		4										
Anceya giraudi		LT N = 99		$\vdash \Box \vdash$											
N = 5		MT N = 171 CT N = 66													
Chytra kirkii		LT N = 61	1	ш— ЮН											
N = 3	-	MT N = 120		НШН											
<i>ci</i>		CT N = 151		Н	-										
Cleopatra johnstoni		MT N = 123													
N = 0		CT N = 81		нар-											
Martelia tanganyicensis		LT N = 75	⊢												
N = 4		MT N = 152 CT N = 41	L		-										
Mysorelloides multisulcata		LT N = 39		' <u>ц</u>	н										
N = 2	-	MT N = 74		μ											
	_	CT N = 59	1		-										
Paramelania crassigranulata		MT N = 03													
N = 5		CT N = 76		нар-	-										
Paramelania iridescens		LT N = 78		ншн											
N = 4		MIN = 144 CTN = 127													
Svrnolopsis lacustris		LT N = 132		НШН											
N = 9	-	MT N = 273													
Tinhahiahawai	_	CT N = 45													
N – 2	•	MT N = 78	<u>⊢</u>												
N – 2		CT N = 167		нс	-		-								
Bridouxia grandidieriana		LT N = 176				-									
N = 9		MIN = 297 CTN = 132													
Limnotrochus thomsoni		LT N = 142		μΩ											
N = 8		MT N = 290													
Paramalania damoni		CT N = 68													
N = 4		MT N = 137	<u>н</u>												
		CT N = 101			-		_			-					
Bridouxia ponsonbyi N = 6		LT N = 98 MT N = 228			<u> </u>	н									
N = 0		CT N = 71			-										
Bridouxia praeclara		LT N = 66			нш	н									
N = 4		MT N = 142	+	$-\Box$	-										
Bridouxia rotundata		LT N = 95													
N = 6		MT N = 109			<u> </u>	·									
lidi-		CT N = 84			H										
Lavigeria granais N – 6		LI N = 111 MT N = 208													
N=0		CT N = 81	'		-										
Lavigeria livingstoniana		LT N = 74		1	C										
N = 4		MT N = 155 CT N = 178			-			_							
Lavigeria nassa		LT N = 176		F		ъ-Г			1						
N = 9		MT N = 342													
Laviaoria chinuloca		CT N = 106													
N = 6		MT N = 219													
		CT N = 97			- · ·		1								
Reymondia horei		LT N = 102		. –	н										
N = 6		MIN = 216 CTN = 110		<u>н</u> -ц								_			
Spekia zonata		LT N = 112													
N = 7		MT N = 111	⊢ ⊦		—										
Stanleva neritinoides		LT N = 59		-		_ F		. <u> </u>	-						
N = 4		MT N = 106	Η			<i>.</i>									
		CT N = 92						<u> </u>							
Leioupiella minima		LT N = 84 MT N = 177			<u> </u>										
C – VI		CT N = 121			. ' +				-						
Tanganyicia rufofilosa		LT N = 101		HD											
N = 7		MT N = 242	H		-										

Fig. 10. Results of nanoindentation. Hardness (GPa) and Young's modulus (GPa) for all cusps (central, lateral, marginal, with N = number of measured cusps) for each species (N = number of measured specimens) correlated with the preferred feeding substrate (circle = mud, hexagon = sand, square = plants, triangle = rock). Letters represent homologous groups. N = number of tested specimens and tooth cusps.

Table 1. Mean  $\pm$  Std. Dev. for Modulus E (GPa) and hardness H (GPa) for each species and tooth types. Homologous groups for comparing the tooth types within single species and homologous groups for comparing all species and structures as well as the preferred feeding substrate are listed. CT = central tooth, LT = lateral tooth, MT = marginal tooth.

	Species and Structure	Modulus, GPa Mean±Std. Dev.	Hardness, GPa Mean± Std. Dev.	Homologous groups in each species for E	Homologous groups in each species for H	Homologous groups for E, all species; p<0.0001; F-ratio: 71; df: 20217.82	Homologous groups for H, all species; p<0.0001; F-ratio: 71; df: 2734.555	Feeding Substrate		Species and Structure	Modulus, GPa Mean± Std. Dev.	Hardness, GPa Mean±Std. Dev.	Homologous groups for E, all species; p<0.0001; F-ratio: 71; df: 20217.82	Homologous groups for H, all species; p<0.0001; F-ratio: 71; df: 2734.555	E: p<0.0001; F-ratio: 71; df: 20217.82	H: p<0.0001; F-ratio: 71; df: 2734.555	Feeding Substrate
Bridouxia grandidieriana	CT Cus P LT Cus P MT Cus P	5.81 ± 0.81 5.55 ± 0.66 4.47 ± 0.51	0.27 ± 0.08 0.25 ± 0.10 0.19 ± 0.08	p<0.000 1 F-ratio: 2 df: 9409.88 7 A B C	p<0.000 1 F-ratio: 2 df: 1623.20 3 A B C	S T M1	JK QR D1E1	Mixed	Bridouxia ponsonbyi	CT Cus P LT Cus P MT Cus P	7.81 ± 0.99 5.93 ± 0.52 4.81 ± 0.56	0.35 ± 0.08 0.26 ± 0.08 0.19 ± 0.07	p<0.000 1 F-ratio: 2 df: 24848.3 5 A B C	p<0.000 1 F-ratio: 2 df: 6166.87 3 A B C	G Q A1B1C1	CDE NOP B1C1D1	Solid
Limnotrochus thomsoni	CT Cus P LT Cus P MT Cus P	5.87 ± 0.80 5.31 ± 0.42 3.83 ± 0.42	0.23 ± 0.09 0.21 ± 0.08 0.14 ± 0.05	p<0.000 1 F-ratio: 2 df: 25875.1 4 A B C	p<0.000 1 F-ratio: 2 df: 3296.83 0 A B C	R X T1	T VWX M1	Mixed	Bridouxia praeclara	CT Cus P LT Cus P MT Cus P	7.92 ± 1.03 6.11 ± 0.35 4.77 ± 0.55	0.35 ± 0.08 0.24 ± 0.07 0.20 ± 0.06	p<0.000 1 F-ratio: 2 df: 20873.7 9 A B C	p<0.000 1 F-ratio: 2 df: 4929.30 7 A B C	F O C1D1E1	CDEF RS ZA1B1C 1	Solid
Paramelania damoni	CT Cus P LT Cus P MT Cus P	6.00 ± 0.81 5.48 ± 0.45 4.03 ± 0.42	0.26 ± 0.12 0.21 ± 0.08 0.14 ± 0.07	p<0.000 1 F-ratio: 2 df: 11870.7 1 A B C	p<0.000 1 F-ratio: 2 df: 1684.68 0 A B C	P UV S1	MN VWX M1	Mixed	Bridouxia rotundata	CT Cus p LT Cus MT Cus p	8.04 ± 1.14 6.50 ± 0.52 4.78 ± 0.70	0.36 ± 0.09 0.28 ± 0.09 0.20 ± 0.08	p<0.000 1 F-ratio: 2 df: 20354.7 3 A B C	p<0.000 1 F-ratio: 2 df: 4417.62 7 A B C	DE L A1B1C1D 1	BC J A1B1C1	Solid
Anceya giraudi	CT Cus p LT Cus MT Cus p	4.30 ± 0.69 4.67 ± 0.32 4.03 ± 0.59	0.19 ± 0.05 0.22 ± 0.05 0.18 ± 0.07	p<0.000 1 F-ratio: 2 df: 1598.90 1 A B C	p<0.000 1 F-ratio: 2 df: 560.181 7 A B C	P1Q1 F1 S1	E1F1 TU G1H111	Soft	Lavigeria grandis	CT Cus p LT Cus MT Cus p MT	$8.07 \pm 1.11$ $6.45 \pm 0.46$ $4.50 \pm 0.36$	0.34 ± 0.13 0.29 ± 0.09 0.19 ± 0.08	p<0.000 1 F-ratio: 2 df: 38217.9 5 A B C	p<0.000 1 F-ratio: 2 df: 3899.47 9 A B C	D LM L1M1	EF I D1E1F1	Solid
Chytra kirkii	CT Cus P LT Cus P	4.17 ± 0.26 4.38 ± 0.12	0.17 ± 0.06 0.17 ± 0.06	p<0.000 1 F-ratio: 2 df: 8124.83 7 C B	p<0.000 1 F-ratio: 2 df: 157.486 7 C B	R1 N101	J1K1 11J1	Soft	Lavigeria livingstoniana	CT Cus P LT Cus P	7.74 ± 0.97 6.69 ± 0.45	0.33 ± 0.09 0.27 ± 0.07	p<0.000 1 F-ratio: 2 df: 20914.6 1 A B	p<0.000 1 F-ratio: 2 df: 3411.92 3 A B	н	Н	Solid

	MT Cus p	4.66 ± 0.21	0.19 ± 0.07	А	А	F1G1	E1F1			MT Cus p	4.62 ± 0.48	0.20 ± 0.04	с	с	G1H1	ZA1B1	
Cleopatra johnstoni	CT Cus P LT Cus P MT Cus P	4.65 ± 0.42 4.56 ± 0.18 4.68 ± 0.31	0.21 ± 0.08 0.18 ± 0.06 0.19 ± 0.06	p<0.000 1 F-ratio: 2 df: 252.994 2 B C A	p<0.000 1 F-ratio: 2 df: 205.980 1 A C B	F1G1H1  1K1  F1	VWXY F1G1H1 D1E1F1	Soft	Lavigeria nassa	CT Cus P LT Cus P MT Cus P	8.40 ± 0.85 6.19 ± 0.53 5.53 ± 0.50	0.36 ± 0.11 0.27 ± 0.08 0.24 ± 0.07	p<0.000 1 F-ratio: 2 df: 44599.7 6 A B B	p<0.000 1 F-ratio: 2 df: 4134.60 3 A B C	B N TU	B KL S	Solid
Martelia tanganyicensis	CT Cus P LT Cus MT Cus P	4.55 ± 0.49 4.35 ± 0.48 4.52 ± 0.53	0.20 ± 0.05 0.18 ± 0.05 0.17 ± 0.07	p<0.000 1 F-ratio: 2 df: 116.877 6 A B A	p<0.000 1 F-ratio: 2 df: 244.291 4 A B C	11.11K1L1 01P1 K1L1	XYZA1 E1F1G1H 1 J1K1	Soft	Lavigeria spinulosa	CT Cus P LT Cus MT Cus P	7.88 ± 0.68 6.42 ± 0.40 5.01 ± 0.63	0.33 ± 0.10 0.26 ± 0.08 0.21 ± 0.07	p<0.000 1 F-ratio: 2 df: 29162.2 1 A B C	p<0.000 1 F-ratio: 2 df: 3134.94 8 A B C	F M Z	GH MN WXY	Solid
Mysorelloides multisulcata	CT Cus P LT Cus P MT Cus P	5.06 ± 0.43 5.39 ± 0.13 5.12 ± 0.35	0.26 ± 0.10 0.28 ± 0.03 0.25 ± 0.06	p<0.000 1 F-ratio: 2 df: 337.223 6 C A B	p<0.000 1 F-ratio: 2 df: 109.809 4 B A C	YZ VW Y	LMNO IJ PQR	Soft	Leloupiella minima	CT Cus P LT Cus MT Cus P	7.99 ± 1.22 6.06 ± 0.39 5.30 ± 0.37	0.34 ± 0.16 0.27 ± 0.08 0.25 ± 0.09	p<0.000 1 F-ratio: 2 df: 15762.0 0 A B B	p<0.000 1 F-ratio: 2 df: 739.739 6 A B C	E OP X	F KLM OPQ	Solid
Paramelania crassigranulata	CT Cus p LT Cus p MT Cus p	$4.76 \pm 0.36 + 4.61 \pm 0.21 + 4.67 \pm 0.24$	0.22 ± 0.08 0.19 ± 0.07 0.19 ± 0.07	p<0.000 1 F-ratio: 2 df: 212.766 7 A C B	p<0.000 1 F-ratio: 2 df: 221.373 6 A B B	B1C1D1E1 F1G1 G1H1I1J1	TU C1D1E1F 1 D1E1F1	Soft	Reymondia horei	CT Cus P LT Cus P MT Cus P	8.21 ± 1.34 6.48 ± 0.85 4.99 ± 0.65	0.35 ± 0.15 0.29 ± 0.11 0.20 ± 0.09	p<0.000 1 F-ratio: 2 df: 17244.1 9 A B C	p<0.000 1 F-ratio: 2 df: 2694.07 4 A B C	C L Z	BCD I YZ	Solid
Paramelania iridescence	CT Cus P LT Cus P MT Cus P	4.65 ± 0.38 4.55 ± 0.18 4.73 ± 0.24	0.22 ± 0.08 0.18 ± 0.06 0.18 ± 0.07	p<0.000 1 F-ratio: 2 df: 401.331 1 B C A	p<0.000 1 F-ratio: 2 df: 218.949 2 A B B	F1G1H1 K1L1 E1	UV E1F1G1H 1 E1F1G1	Soft	Spekia zonata	CT Cus P LT Cus P MT Cus P	8.09 ± 0.65 5.78 ± 0.42 4.74 ± 0.50	0.34 ± 0.07 0.27 ± 0.09 0.21 ± 0.07	p<0.000 1 F-ratio: 2 df: 11499.2 2 A B C	p<0.000 1 F-ratio: 2 df: 1089.91 8 A B C	D S D1E1	DEFG JKLM VW	Solid
Syrnolopsis lacustris	CT Cus P LT Cus P MT Cus P	$4.82 \pm 0.29 \\ 4.64 \pm 0.26 \\ 4.41 \pm 0.21$	0.18 ± 0.06 0.16 ± 0.06 0.14 ± 0.06	p<0.000 1 F-ratio: 2 df: 5510.11 0 A B C	p<0.000 1 F-ratio: 2 df: 842.068 2 A B C	A1 F1G1H1 N1	G1H1I1 L1 M1	Soft	Stanleya neritinoides	CT Cus P LT Cus P MT Cus P	9.32 ± 1.09 6.96 ± 0.55 4.83 ± 0.53	0.51 ± 0.18 0.29 ± 0.12 0.19 ± 0.06	p<0.000 1 F-ratio: 2 df: 26627.2 8 A B C	p<0.000 1 F-ratio: 2 df: 5043.36 7 A B C	A J A181	A I D1E1F1	Solid

				p<0.000 1	p<0.000 1								p<0.000 1	p<0.000 1			
				F-ratio:	F-ratio:								F-ratio:	F-ratio:			
				2	2 df:				sa				2	2 df:			
				298.381	695.601				filo.				24804.2	420.153			
ore				6	4				loj				3	1			
a h	СТ	4.97	0.26					Ŧ	ı rı	СТ	7.27	0.24					id
iqc	Cus	±	±	А	А	Z	KLMN	So	icia	Cus	±	±	А	А	1	R	Sol
hha	р	0.65	0.08						'n	р	0.80	0.14					
ΪĹ	LT	4.26	0.17						ıgc	LT	5.40	0.21					
	Cus	±	±	С	в	Q1	H1I1J1K1		lar	Cus	±	±	В	В	w	VWX	
	р	0.42	0.07							р	0.29	0.07					
	MT	4.78	0.16			A101C101F				MT	4.61	0.19					
	Cus	±	±	В	С	AIDICIDIE	K1L1			Cus	±	±	С	С	H1J1	D1E1	
	р	0.86	0.08			1				р	0.60	0.08					



Fig. 11. Results of nanoindentation. Median of Young's modulus (GPa) of the central, laterals and marginal tooth cusps for each species correlated with the preferred feeding substrate (circle = mud, hexagon = sand, square = plants, triangle = rock) against the background of a phylogenetic tree (Bayesian). Taxa without molecular information were allocated to groups based on morphological analyses from relevant literature.

### <u>Discussion</u>

The gastropods in Lake Tanganyika have limited options regarding their habitat as they occur below the surf zone down to 200 m, with the deeper parts of the lake containing little oxygen and are toxic [44]. Sharing habitats might result in considerable inter- and intraspecific competition, but [44, Krings et al. unpublished data, here chapter 2.1] found strong evidences for the avoidance or the reduction of resource competition by clear substrate-specificity in most paludomid groups. In Group 1, Spekiini Ancey, 1906 [58], Reymondia Bourguignat, 1885 [45] and riverine Cleopatrini Pilsbry & Bequaert, 1927 [60], Group 2, Hauttecoeuriini Bourguignat, 1885 [45], Syrnolopsini, Bourguignat, 1890 [47], and Group 3, Tiphobiini Bourguignat, 1886 [46], (groups in accordance with [42, 44, Krings et al. unpublished data, here chapter 2.1]) some species feed on biofilm that covers stones (solid substrate), some select algae from sandy and muddy surfaces (soft substrate) and few (mixed) feed either on both (Paramelania damoni, Limnotrochus thomsoni) or on algae attached to plants and covering sand (Bridouxia grandidieriana). Group 4, containing Lavigeria and riverine Potadomoides, consist probably of species that exclusively feed on solid substrate. Unfortunately, reliable data on preferred substrate is not available for Potadomoides which has not been found again in the last decades. Its localities, the Malagarasi River and the Congo River drainage, are characterized by swampy areas as well as rapids with rocks. But since its radular tooth characters are similar to Lavigeria species [43], we rather conclude that Potadomoides also feeds on algae from solid substrates [see also Krings et al. unpublished data, here chapter 2.1].

The mechanical properties (E, H) of the paludomid radular teeth correlate with the preferred substrate and reflect different eco-morpho-propo-types (Fig. 11). All species foraging on stones, viz. *Bridouxia ponsonbyi, B. rotundata, B. praeclara, Leloupiella minima, Spekia, zonata, Reymondia horei, Stanleya neritinoides, Tanganyicia rufofilosa, Lavigeria spinulosa, L. livingstoniana, L. nassa, and L. grandis, show gradual and distinct differences in their stiffness and hardness between the tooth types which can be explained by different functional loads (Fig. 10, 11). The material properties certainly influence the mechanics of structures, the Young's Modulus E is, for example, directly linked with the ability of a structure to transfer forces [e.g. 132–135] and correlates with the structures mechanical behaviour while puncturing and probably the resistance of structures to* 

failure [e.g. 136–137]. We anticipate here that the stiff central and lateral teeth are rather used for scratching across the solid feeding substrate removing food items attached to it [see also 124] transferring higher force from the radular muscles via the tooth cusps onto the ingesta. The softer and more flexible marginal teeth have a smaller ability to transfer forces necessary to loosen a tightly attached biofilm. But their elasticity allows the reduction of the stress concentration for example in case of hitting the substrate asperities. Their mechanical properties enable them to catapult back to place, possibly without fractures or ruptures, after hitting an obstacle. We would hence deduce that the marginal teeth are rather functionally different from the central and lateral teeth, possibly harvesting like a broom food items or particles that had been loosened from the substrate by grinding action of the central and lateral teeth [see also 108, 124, 138]. This type of radula is considered to be a multifunctional tool.

For all species foraging on sand or mud, viz. *Cleopatra johnstoni, Martelia tanganyicensis, Anceya giraudi, Syrnolopsis lacustris, Chytra kirkii, Tiphobia horei, Paramelania iridescens, P. crassigranulata*, and *Mysorelloides multisulcata*, we found similar mechanical properties in all tooth types as well as comparably soft and flexible tooth cusps (Fig. 10, 11). Their hardness and elasticity values are comparable to the mechanical properties of the solid substrate feeders' marginal teeth. We would hence propose that these species rather possess a monofunctional radula with each tooth serving as broom collecting algae from the surface.

The mechanical properties of the mixed substrate feeders, viz. *Bridouxia grandidieriana*, *Limnotrochus thomsoni*, and *Paramelania damoni*, are intermediate, as they have slightly softer and more flexible central and lateral tooth cusps compared to the gastropods loosening algae from stones but stiffer and harder ones than the species selecting biofilm from sand (Fig. 10, 11). Even though the gradients between the tooth cusps are not as distinct as in the gastropods foraging on stone, the existence of the gradual differences leads to the conclusion that, despite of softer and more flexible tooth cusps, the mixed substrate feeders also possess a multifunctional radula. Central and lateral teeth are rather used for loosening food items whereas the softer marginal tooth cusps, showing similar properties to the marginal teeth of solid feeders and to each tooth type of species selecting algae from sand, serve as brooms.

Tooth's morphologies also correlate in most cases with the feeding substrate [see also Krings et al. unpublished data, here chapter 2.1]. Central teeth displaying either a prominent denticle (Nassopsini and Reymondia; Fig. 4, 8), or few or no denticles (Bridouxia ponsonbyi, B. rotundata, B. praeclara, Spekia, and Leloupiella; Fig. 3, 4), as well as laterals bearing a prominent denticle (Nassopsini, Reymondia, Bridouxia praeclara, B. ponsonbyi, B. rotundata, Spekia, Tanganyicia; Fig. 3, 4, 5, 7, 8), allow a large interaction surface between tooth cusps and ingesta directly transferring force. Additionally, these teeth are rather short and broad, probably leading to the reduction of deformation when tensile and compressive stresses appear in the structure during this action. However, some solid feeders (Stanleya and Tanganyicia; Fig. 5) as well as the mixed substrate feeders (Paramelania damoni, Limnotrochus thomsoni, Bridouxia grandidieriana; Fig. 3, 6, 7) display an alternative morphology, rather similar to teeth of gastropods foraging on sand. Soft substrate feeders possessing central, lateral, and marginal teeth with small or finger-like denticles at each cusp (Fig. 4–7) which probably enables them to rake between the grains gathering the food particles. We hypothesize that radular tooth performance in Stanleya, Tanganyicia, and all mixed substrate feeders is ensured by mechanical properties rather than morphology. Thus, pure morphology does not consistently reflect adaptations, but the morphology fostered by mechanical properties allows the establishment of tooth eco-morpho-propo-types.

Adaptations to distinct substrates, solid as well as soft and mixed, are present in most taxonomic paludomid lineages (Fig. 11). This in turn leads to the hypothesis that one main engine of their evolution is trophic specialization to feeding substrates establishing distinct ecological niches and allowing the coexistence of taxa. Only Group 4 (Nassopsini Kesteven, 1903 [139], contain *Lavigeria*) is exceptional because it contains exclusively solid substrate feeders (Fig. 11). *Lavigeria* is a paludomid group containing a plethora of named species [see e.g. 79, 131] that had been treated as result of an exclusively intralacustrine adaptive radiation. Unfortunately, a systematic revision identifying evolutionary entities is still lacking hindering hypotheses on their evolution. However, when comparing *Lavigeria* adult shells their distinct sizes are apparent which are interpreted as result of annidation through different body sizes [Krings et al. under revision in Scientific Reports]. Also, *Lavigeria* radular teeth are of distinct tooth sizes. *Lavigeria grandis* displaying the largest teeth, followed by *L. nassa, L. spinulosa*, and finally *L. livingstoniana* possessing the smallest teeth (Fig. 7,

8). This could be an indication that *Lavigeria* species avoid competition by trophic specialization, albeit not to different feeding substrates but rather to different algae or biofilm types growing on solid substrates. However, in order to determine whether species have distinct food preferences, it would be necessary to collect and gather feeding substrates and biofilms directly *in situ*. The only available collectors' comments on Lake Tanganyikan biofilms, however, suggest that paludomids feed on soft algae, overcasting either rocks or plant structures or covering sandy or muddy substrates. This could explain the relatively soft and elastic tooth cusp of these gastropods in comparison with published hardness and elasticity measurements on teeth of hard calcified algae feeders, such as e.g. Polyplacophora and Patellogastropoda (with E ranging from 16 GPa over 90– 125 GPa up to 52–140 GPa [140–144] and H ranging from 9–12 GPa [140, 142, 144]).

Molecular clock approaches [42, Krings et al. unpublished data, here chapter 2.1] support an ancient origin of diversity and disparity, long before the formation of Lake Tanganyika or a proto-lake. After the rifting of the African continent and the formation of the lake several independent colonialization events of already distinct riverine paludomid lineages succeeded from the surrounding river systems. We additionally reconstructed the ancestral riverine feeding substrate as of soft nature, and the ancestral tooth morphologies, involving long and slender teeth with numerous denticles of equal size, as monomorphic and indicative of a preadaptation sensu strictu [145] to the riverine substrate. Within the rivers, probably in rapids, two paludomid lineages (ancestor of (i) Spekiini and Reymondia as well as (ii) Nassopsini and Potadomoides) evolved central and lateral tooth morphologies adapted to solid substrates [Krings et al. unpublished data, here chapter 2.1]. This was probably accompanied by the evolution of harder and stiffer central and lateral tooth cusps in the Spekiini, Reymondia, and Lavigeria. Unfortunately, we lack biomaterial property information for Potadomoides since to date specimens have not been collected again in the lake's surrounding river systems. Additionally, after the origin and the colonialization of Lake Tanganyika, two other lineages (viz. the ancestor of (i) Bathanalia as well as (ii) Stanleya and Tanganyicia) have adapted convergently to solid substrate [Krings et al. unpublished data, here chapter 2.1]. Nevertheless, Bathanalia [see images of radula in 129], Stanleya, and Tanganyicia display (Fig. 5), as described above, rather monomorphic radular teeth similar to the soft substrate feeders. These taxa probably still carry their ancestral riverine morphological characters. We were not able to receive Bathanalia

radula for indentation, but the hard and stiff tooth cusps of *Stanleya* and *Tanganyicia* suggest that here adaptation to solid substrate involved only the change in material properties hardness and elasticity.

*Bridouxia* is probably a case of an exclusively intralacustrine adaptive radiation, strongly indicated by molecular clock approaches [Krings et al. unpublished data, here chapter 2.1, Krings et al. accepted for publication in Plos One]. Here we were able to detect secondary adaptation to mixed feeding substrate for *B. grandidieriana*. This taxon became probably adapted through changes in morphology as well as material properties, evolving softer and more flexible central and lateral teeth with small denticles serving as a broom (Fig. 3). The mixed substrate feeders *Paramelania damoni* and *Limnotrochus thomsoni* retained their ancestral tooth morphologies (Fig. 6, 7), but temporary solid substrate feeding is probably enabled by the evolution of stiffer and harder central and lateral tooth cusps. The evolution of this might have taken place in the riverine environment as the molecular clock approach indicates [Krings et al. unpublished data, here chapter 2.1]. All other lacustrine species (*Martelia, Anceya, Syrnolopsis, Chytra, Tiphobia, P. iridescens, P. crassigranulata, Mysorelloides*) are adapted to foraging on soft substrates carrying their ancestral riverine tooth morphologies as well as displaying soft and flexible teeth (Fig. 10, 11).

#### **Conclusion**

Here we present the first comparative study on the mechanical properties, hardness and elasticity, of taenioglossan radular teeth from African paludomid gastropods from Lake Tanganyika and surrounding river systems based on a large sample size and in a phylogenetic and ecological context. The tested paludomid teeth correlate with their preferred feeding substrate and reflect different tooth eco-morpho-propo-types accompanying morphology. Our identification of adaptations allows to put forward a new perspective on the evolution of this species flock. Accordingly, we here hypothesize that the originally riverine paludomid gastropods were essentially adapted to soft substrate. In some lineages the adaptations to solid and mixed feeding substrate evolved in riverine rapids while in other groups changes in ecology are part of an intralacustrine specialization, within

Lake Tanganyika. We postulate that trophic specialisation resulting in niche partitioning has played a major role in the evolution and radiation of this flock.

#### <u>Methods</u>

As basis we used paludomid gastropods (Fig. 1) collected in earlier studies [see in 44], supplemented by additional material of taxa collected by Heinz Büscher, Basel. Specimens stored in ethanol are inventoried at the Museum für Naturkunde Berlin (ZMB), the Musée Royal L'Afrique Centrale, Tervuren, Belgium (MRAC), the Royal Belgian Institute of Natural Sciences, Brussels, Belgium (IRSNB) and the Zoological Museum (ZMH) of the Center of Natural History (CeNak) in Hamburg (for details on sampling locality see Supplementary Table 1). Specimens were identified based on shell morphology in comparison with type material following essentially [44] and literature referred to therein.

For scanning electron microscopy (SEM) one radula per species (Supplementary Table 1) was extracted, digested with proteinase K according to the protocol of [126], cleaned for a few seconds in an ultrasonic bath, mounted on an aluminium stub, coated with carbon and visualized either with a SEM Zeiss LEO 1525 (One Zeiss Drive, Thornwood, NY) or a Tabletop SEM TM4000Plus (Hitachi, Tokyo, Japan) (see Fig. 3–8 for SEM images).

For nanoindentation [detailed descripting of method in 124–125] overall 124 radulae belonging to 23 species (Supplementary Table 1), accompanied by data on 7 specimens from *Spekia zonata* taken from [124], were manually extracted from adult specimens, freed from surrounding tissues, dried and, laying on its side, tapped with double-sided adhesive tape to a glass object slide. The tape ensured that the radulae were accurately arranged, with marginal teeth at the bottom, followed by lateral, central, lateral, and on the top marginal teeth. This procedure ensured that after polishing only one tooth type was superficial at the plain surface. Each radula was surrounded by a small metallic ring resulting an almost parallel sample after polishing necessary for an almost error-free nanoindentation. Rings were filled with epoxy resin (RECKLI®EPOXIWST, elasticity modulus of the epoxy is 1 GPa), known to not infiltrate the teeth, polymerizing at room temperature. Object slide and tape were removed, radulae were polished with gradual diamond pastes (Buehler MetaDi Ultra Paste 6  $\mu$ m 3  $\mu$ m, 1  $\mu$ m) and smoothened with a polishing machine (Buehler MataServ 250 with Struers OP-U, 0.04  $\mu$ m suspension with 250 rpm) for a plain surface displaying the longitudinal section of teeth (Fig. 2c-f). After performing nanoindentation, employing a Nanoindenter SA2 (MTS

Nano Instrument, Oak Ridge, TN, USA; CSM) equipped with Berkovich indenter tip, on the superficial tooth row (marginals) samples were again smoothened until the next tooth row (laterals) was on display (Fig. 2b). Steps were repeated until all teeth were measured. The indents for this study were made at the tooth cusps with each indentation curve controlled manually for correct surface finding. In each specimen, 20 tooth rows of the outer wear zone were tested, resulting in more than 9027 measured tooth cusps. We focussed on this specific radular locality to exclude not matured teeth from this study. Elasticity moduli (E) and hardness (H) of materials were either determined at penetration depths of 480–520 nm (for larger teeth, in Spekia, Reymondia, Lavigeria, Chytra) or at penetration depth 450–500 nm (for smaller teeth, in Bridouxia, Leloupiella, Cleopatra, Stanleya, Tanganyicia, Martelia, Anceya, Syrnolopsis, Limnotrochus, Paramelania, Mysorelloides) with about 30 values per indentation. These indentation depths were targeted, because at low depths E and H strongly fluctuated due to surface roughness both (Fig. 2g), and at higher depths the side effects of the epoxy could not be excluded. All statistical analyses were performed with JMP® Pro, Version 14 (SAS Institute Inc., Cary, NC, 1989–2007), calculating mean values and standard deviations; Shapiro-Wilk-W-test for testing of normality and one-way ANOVA followed by a Tukey-Kramer test for detecting homogenous groups were carried out. Mechanical properties were compared between the preferred feeding substrates (i), all central, lateral, and marginal tooth cusps (ii), within each species (iii), between the species (iv).

In order to establish a hypothesis on the evolutionary history of the African paludomids in the context of trophic specialisation based on biomechanical properties, we used all available DNA sequences of tested species, here sequences of the mitochondrial 16S rRNA (16S) and the cytochrome c oxidase subunit I (COI) gene from previous studies [42, 85, Krings et al. unpublished data, here chapter 2.1] (Supplementary Table 1); additionally, *Melanoides tuberculata* and *Paludomus siamensis* sequences were used as outgroup. Sequences were aligned with MAFFT 7 [127] employing the Q-INS-I algorithm, the 1PAM/ $\kappa$  = 2 option for the scoring matrix for nucleotide sequences and otherwise default settings. Bayesian inference with MrBayes 3.2.6 [128] were used to reconstruct phylogenetic relationship. For some species, *Bridouxia praeclara, B. rotundata, Paramelania crassigranulata, Mysorelloides multisulcata*, no molecular information could be obtained from various previous approaches. These taxa were placed tentatively in the resulting

phylogeny comparing morphological characters and identifying synapomorphies as suggested by [44, 129, Krings et al. accepted for publication in Plos One], resulting in a systematization [see 130 for further details of this term].

The information on the preferred feeding substrate was summarized in [Krings et al. unpublished data, here chapter 2.1] which is based on the relevant literature [44, 79, 118, 131] supplemented by notes from the collectors of individual samples in the field (Heinz Büscher, Matthias Glaubrecht).

### <u>Declarations</u>

Ethics approval and consent to participate. This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication. Not applicable.

Availability of data and materials. All data is available.

Competing interests. We have no competing interest.

Funding. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. The original material was collected by M.G. during an earlier research project funded by the Deutsche Forschungsgemeinschaft (DFG GL 297/5-1).

Authors' contributions. WK wrote the manuscript, with contributions by MG, drew the figures, performed nanoindentation and analysed the data. AK supported the experiments and discussed data. SNG discussed the manuscript and the figures; his expertise was critical for understanding the results and the functional morphology. MG, in addition to writing parts of the manuscript, initialized and planed the study and discussed the data his expertise was critical for understanding the evolution of the paludomid gastropods. All authors contributed to the manuscript.

Acknowledgements. We like to thank Peter Stutz from the Mineralogical-Petrographic Institute of the University of Hamburg for the great support in the sample preparation. Heinz Büscher (Basel) collected part of the specimens at Lake Tanganyika. Renate Walter (Zoological Institute of Universität Hamburg) helped with SEM of the radulae. Thomas M. Kaiser (CeNak) helped with the discussion of results. We thank the anonymous reviewers for their helpful comments.

#### <u>Literature</u>

- 1 Terborgh JW. Toward a trophic theory of species diversity. PNAS 2015;112(37):11415–22. https://doi.org/10.1073/pnas.1501070112.
- 2 Pocheville A. The Ecological Niche: History and Recent Controversies. In: Heams T, Huneman P, Lecointre G, Silberstein M, editors. Handbook of Evolutionary Thinking in the Sciences. Dordrecht: Springer; 2015.
- 3 Hendry AP. Eco-evolutionary dynamics. Princeton: Princeton University Press; 2017.
- 4 Lack D. Darwin's Finches. Cambridge: Cambridge University Press; 1947.
- 5 Grant PR. Ecology and Evolution of Darwin's Finches. Princeton: Princeton University Press; 1986.
- 6 Grant PR, Weiner J. Ecology and Evolution of Darwin's Finches. Princeton: Princeton University Press; 1999.
- 7 Grant PR, Grant BR. How and Why Species Multiply. The Radiation of Darwin's Finches. Princeton: Princeton University Press; 2008.
- 8 Grant PR, Grant BR. 40 Years of Evolution: Darwin's Finches on Daphne Major Island. Princeton: Princeton University Press; 2014.
- 9 Farrington HL, Lawson LP, Clark CM, Petren K. The Evolutionary History of Darwin's Finches: Speciation, Geneflow, and Introgression in a Fragmented Landscape. Evolution 2014;68(10):2932–44. https://doi.org/10.1111/evo.12484.
- 10 Barlow GW. The Cichlid Fishes: Nature's Grand Experiment in Evolution. Cambridge: Perseus Publishing; 2000.
- 11 Seehausen O. African cichlid fish: A model system in adaptive radiation research. Proc. R. Soc. Lond. B 2006;273(1597):1987–98. https://doi.org/10.1098/rspb.2006.3539).
- 12 Malinsky M, Salzburger W. Environmental context for understanding the iconic adaptive radiation of cichlid fishes in Lake Malawi. PNAS 2016;113(42):11654–56. https://doi.org/10.1073/pnas.1614272113.
- 13 Ronco F, Büscher HH, Indermaur A, Salzburger W. The taxonomic diversity of the cichlid fish fauna of ancient Lake Tanganyika, East Africa. J. Great Lakes Res. 2019; doi:10.1016/j.jglr.2019.05.009.
- 14 Elmer KR, Kusche H, Lehtonen TK, Meyer A. Local variation and parallel evolution: morphological and genetic diversity across a species complex of Neotropical crater lake cichlid fishes. Philos. Trans. R. Soc. Lond. B 2010;365:1769–82.
- 15 Elmer K, Fan S, Kusche H et al. Parallel evolution of Nicaraguan crater lake cichlid fishes via non-parallel routes. Nat. Commun. 2014;5:5168. https://doi.org/10.1038/ncomms6168.
- 16 Gaither MR, Greaves S, Amirthalingam P. The physiology of rapid ecological specialization: A look at the Midas cichlids. Mol. Ecol. 2020;29(7):1215–18. https://doi.org/10.1111/mec.15408.
- 17 Jaffe AL, Campbell-Staton SC, Losos JB. Geographical variation in morphology and its environmental correlates in a widespread North American lizard, *Anolis carolinensis* (Squamata: Dactyloidae). Biol. J. Linn. Soc. 2016;117(4): 760–74. https://doi.org/10.1111/bij.12711.
- 18 Stroud JT, Losos JB. Bridging the Process-Pattern Divide to Understand the Origins and Early Stages of Adaptive Radiation: A Review of Approaches With Insights From Studies of *Anolis* Lizards. J. Hered. 2020;111(1):33–42. https://doi.org/10.1093/jhered/esz055.
- 19 Velasco JA, Villalobos F, Diniz-Filho JA, Poe S, Flores-Villela O. Macroecology and macroevolution of body size in *Anolis* lizards. Ecography 2020; https://doi.org/10.1111/ecog.04583.
- 20 Greenwood PH. The cichlid fishes of Lake Victoria, east Africa: The biology and evolution of a species flock. Bull. Br. Mus. Nat. Hist. Zool. 1974;6:1–134.
- 21 Schluter D. Ecological causes of adaptive radiation. Am. Nat. 1996;148:40–63. https://doi.org/10.1086/285901.

- 22 Schluter D. The Ecology of Adaptive Radiation. Oxford: Oxford University Press; 2000.
- 23 Schluter D. Ecological character displacement in adaptive radiation. Am. Nat. 2000;156:4–16. https://doi.org/10.1086/303412.
- 24 Schluter D. Evidence for ecological speciation and its alternative. Science 2009;323(5915):737–41. https://doi.org/10.1126/science.1160006.
- 25 Glor RE. Phylogenetic insights on adaptive radiation. Annu. Rev. Ecol. Evol. Syst 2010;41:251–70. https://doi.org/10.1146/annurev.ecolsys.39.110707.173447.
- 26 Losos JB, Mahler DL. Adaptive radiation: The interaction of ecological opportunity, adaptation, and speciation. In: Bell M, Futuyma D, Eanes W, Levinton J, editors. Evolution Since Darwin: The First 150 Years. Sunderland: Sinauer; 2010. p. 381–420.
- 27 Chapman AD. Numbers of living species in Australia and the world. 2nd edition. Toowoomba: Australian Biodiversity Information Services; 2009.
- 28 Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B. How Many Species Are There on Earth and in the Ocean? PLOS 2011;9(8): e1001127. https://doi.org/10.1371/journal.pbio.1001127.
- 29 Mullen SP, Mendelson TC, Schal C, Shaw KL. Rapid evolution of cuticular hydrocarbons in a species radiation of acoustically diverse Hawaiian crickets (Gryllidae: Trigonidiinae: Laupala). Evolution 2007;61:223–31.
- 30 Knope ML, Bellinger MR, Datlof EM, Gallaher TJ, Johnson MA. Insights into the evolutionary history of the Hawaiian Bidens (Asteraceae) adaptive radiation revealed through phylogenomics. J. Hered. 2020;111(1):119–37.
- 31 Glaubrecht M. On "Darwinian Mysteries" or molluscs as models in evolutionary biology: From local speciation to global radiation. Am. Malacol. Bull. 2009;27:3–23. https://doi.org/10.4003/006.027.0202.
- 32 Glaubrecht M. Toward solving Darwin's "mystery": speciation and radiation in freshwater gastropods. Am. Malacol. Bull. 2011;29(1/2):187–216. https://doi.org/10.4003/006.029.0211.
- 33 Rintelen Tv, Wilson AB, Meyer A, Glaubrecht M. Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. Proc. R. Soc. Lond. 2004;271(1557):2541–9. https://doi.org/10.1098/rspb.2004.2842.
- 34 Rintelen Tv, Bouchet P, Glaubrecht M. Ancient lakes as hotspots of diversity: a morphological review of an endemic species flock of *Tylomelania* (Gastropoda: Cerithioidea: Pachychilidae) in the Malili lake system on Sulawesi, Indonesia. Hydrobiologia 2007;592(1):11–94.
- 35 Rintelen Tv, Rintelen Kv, Glaubrecht M. The species flocks of the viviparous freshwater gastropod *Tylomelania* (Mollusca: Cerithioidea: Pachychilidae) in the ancient lakes of Sulawesi, Indonesia: The role of geography, trophic morphology and color as driving forces in adaptive radiation. In: Glaubrecht M, editor. Evolution in Action. Case Studies in Adaptive Radiation, Speciation and the Origin of Biodiversity. Heidelberg: Springer; 2010. p. 485–512.
- 36 Rintelen Tv, Glaubrecht M. Anatomy of adaptive radiation: a unique reproductive strategy in the endemic freshwater gastropod *Tylomelania* (Cerithiodea: Pachychilidae) on Sulawesi, Indonesia and its biogeographical implications. Biol. J. Linn. Soc. 2005;85(4):513–42. https://doi.org/10.1111/j.1095-8312.2005.00515.x.
- 37 Glaubrecht M, Rintelen Tv. The species flocks of lacustrine gastropods: *Tylomelania* on Sulawesi as models in speciation and adaptive radiation. Proceedings of the "Speciation in Ancient Lake IV" Symposium, Berlin. Hydrobiologia 2008;615:181–99.
- 38 Hilgers L, Hartmann S, Pfaender J, Lentge-Maaß N, Rintelen Tv, Hofreiter M. Radula diversification promotes ecomorph divergence in an adaptive radiation of freshwater snails. bioRxiv 2020.01.17.910034; doi:10.1101/2020.01.17.910034.
- 39 Köhler F, Glaubrecht M. Uncovering an overlooked radiation: morphological and mitochondrial DNA differentiation in endemic freshwater snails on Madagascar (Caenogastropoda: Pachychilidae) and their biogeography. Biol. J. Linn. Soc. 2010;99(4):867–94.

- 40 Glaubrecht M, Köhler F. Radiating in a river: systematics, molecular genetics and morphological differentiation of viviparous freshwater gastropods endemic to the Kaek River, central Thailand. Biol. J. Linn. Soc. 2004;82(3):275–311. https://doi.org/10.1111/j.10958312.2004.00361.x.
- 41 Köhler F, Panha S, Glaubrecht M. Speciation and Radiation in a River: Assessing the Morphological and Genetic Differentiation in a Species Flock of Viviparous Gastropods (Cerithioidea: Pachychilidae). In: Glaubrecht M, editor. Evolution in Action. Case Studies in Adaptive Radiation, Speciation and the Origin of Biodiversity. Heidelberg: Springer; 2010.
- 42 Wilson AB, Glaubrecht M, Meyer A. Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika. Proc. Royal Soc. Lond. B 2004;271(1538):529–36. https://doi.org/10.1098/rspb.2003.2624.
- 43 Glaubrecht M, Strong EE. Ancestry to an endemic radiation in Lake Tanganyika? Evolution of the viviparous gastropod *Potadomoides* Leloup, 1953 in the Congo River system (Cerithioidea, Paludomidae). Biol. J. Linn. Soc. 2007;92(2):367–401. https://doi.org/10.1111/j.1095-8312.2007.00867.x.
- 44 Glaubrecht M. Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology and systematization of a paludomid species flock in an ancient lake. Zoosystematics Evol. 2008;84:71–122. https://doi.org/10.1002/zoos.200700016.
- 45 Bourguignat MJR. Notice prodromique sur les mollusques terrestres et fluviatiles. Paris: Savy; 1885.
- 46 Bourguignat MJR. Nouveautés Malacologiques: Unionidæ et Iridinidæ du Lac Tanganika. Paris: Savy; 1886.
- 47 Bourguignat MJR. Histoire Malacologique du Lac Tanganika (Afrique Equatoriale). Ann. Sci. Nat., Zool. Biol. Anim. 1890;10:1–267.
- 48 Pelseneer P. Mollusques recueillis par M. Le Capitaine Storms dans la region du Tanganyika. Bull. Mus. R. Hist. Nat. Belg. 1886;4:103–15.
- 49 Moore JES. The molluscs of the Great African Lakes. I. Distribution. Quarterly Journal of Microscopic Sciences (new series) 1898;41:159–80.
- 50 Moore JES. The molluscs of the Great African Lakes. II. The anatomy of the *Typhobias*, with a description of the new genus (*Bathanalia*). Quarterly Journal of Microscopic Sciences (new series) 1898;41:181–204.
- 51 Moore JES. The molluscs of the Great African Lakes. III. *Tanganyikia rufofilosa*, and the genus *Spekia*. Quarterly Journal of Microscopic Sciences (new series) 1899;42:155–85.
- 52 Moore JES. The molluscs of the Great African Lakes. IV. *Nassopsis* and *Bythoceras*. Quarterly Journal of Microscopic Sciences (new series) 1899;42:187–201.
- 53 Smith EA. Diagnoses of new shells from Lake Tanganyika and East Africa. Ann. Mag. Nat. Hist. 1880;6:425–30.
- 54 Smith EA. On the shells of Lake Tanganyika and of the neighbourhood of Ujiji, Central Africa. Proc. Zool. Soc. Lond. 1880;1880:344–52.
- 55 Smith EA. Descriptions of two new species of shells from Lake Tanganyika. Proc. Zool. Soc. Lond. 1881;49(3):558–61.
- 56 Smith EA. Diagnosis of new shells from Lake Tanganyika. Ann. Mag. Nat. Hist. 1889;6:173–5.
- 57 Smith EA. Some remarks on the Mollusca of Lake Tanganyika. Proc. Malacol. 1904;6(2):77–104.
- 58 Ancey C-F. Reflexions sur la fauna malacologique du Lac Tanganika et cataloque des mollusques de ce lac. Bull. Biol. Fr. Bel. 1906;5(9):229–70.
- 59 Germain L. Mollusques du Lac Tanganyika et de ses environs. Extrait des resultats secientifiques des voyages en Afrique d'Edouard Foa. Bull. Mus. Natl. Hist. Nat. 1908;14:1–612.
- 60 Pilsbry HA, Bequaert J. The aquatic mollusks of the Begian Congo. With a geographical and ecological account of Congo malacology. Bull. Am. Mus. Nat. Hist. 1927;53:69–602.
- 61 Moore JES. On the zoological evidence for the connection of Lake Tanganyika with the sea. Proc. Roy. Soc. 1898;62:451–8.
- 62 Moore JES. The fresh-water fauna of Lake Tanganyika. Nature 1897;56:198–200.
- 63 Moore JES. The Tanganyika problem. London: Burst and Blackett; 1903.
- 64 Hudleston WH. On the origin of the marine (halolimnic) fauna of Lake Tanganyika. Geol. Mag., Suppl. 1904;1904:337–82.
- 65 Cunnington WA. The fauna of the African Lakes: a study in comparative limnology with special reference to Tanganyika. Proc. Zool. Soc. Lond. 1920;1920:507–622.
- 66 Yonge CM. The prosobranchs of Lake Tanganyika. Nature 1938;142:464–6.
- 67 Schwetz J, Dartevelle E. Sur l'origine des mollusques thalassoides du Lac Tanganika. Revue historique et analytique. Memoires de la Institut Royal Colonial Belge, Sciences Naturelles et Medicales 1948;16(7):1–58.
- 68 Hubendick B. On the evolution of the so-called thalassoid molluscs of Lake Tanganyika. Arkiv för Zoologi 1952;3:319–23.
- 69 Brooks JL. Speciation in ancient lakes. Q. Rev. Biol. 1950;25:30-60;131-176.
- 70 Mayr E. Animal, species, and evolution. Cambridge: Harvard University Press; 1963.
- 71 Mayr E, Ashlock PD. Principles of systematic zoology. New York: McGraw-Hill; 1991.
- 72 Boss KJ. On the evolution of gastropods in ancient lakes. In: Fretter V, Peake J, editors. Pulmonates, Vol. 2a. Systematics, Evolution and Ecology. London: Academic Press; 1978. p. 385–428.
- 73 Johnston MR, Cohen AS. Morphological divergence in endemic gastropods from Lake Tanganyika: implications for models of species flock formation. Palaios 1987;2:413–25.
- 74 Brown DS, Mandahl-Barth G. Living molluscs of Lake Tanganyika: a revised and annotated list. J. Conchol. 1987;32:305–27.
- 75 Berthold T. Intralacustrine speciation and the evolution of shell sculpture in gastropods of ancient lakes application of Günther's niche concept. Abhandlungen des Naturwissenschaftlichen Vereins Hamburg (NF) 1990;31/32:85–118.
- 76 Coulter GW. Lake Tanganyika and its Life. Oxford: Oxford University Press; 1991.
- 77 Gorthner A. Bau, Funktion und Evolution komplexer Gastropodenschalen in Langzeit-Seen. Mit einem Beitrag zur Paläobiologie von *Gyraulus "multiformis"* im Steinheimer Becken. Stuttgarter Beiträge zur Naturkunde, Serie B 1992;190:1–173.
- 78 Michel E, Cohen AS, West K, Johnston MR, Kat PW. Large African lakes as natural laboratories for evolution: examples from the endemic gastropod fauna of Lake Tanganyika. Mitteilungen der Internationalen Vereinigung für Limnologie 1992;23:85–99.
- 79 Brown D. Freshwater Snails of Africa and their Medical Importance. London: Taylor and Francis; 1994.
- 80 Michel E. Why snails radiate: a review of gastropod evolution in long-lived lakes, both Recent and fossil. In: Martens K, Goddeeris B, Coulter G, editors. Speciation in ancient lakes. Stuttgart: Advances in Limnology; 1994. p. 285–317.
- 81 Michel E. Phylogeny of a gastropod species flock: exploring speciation in Lake Tanganyika in a molecular framework. In: Rossiter A, editor. Biology of Ancient Lakes. Advances in Ecological Research. London: Academic Press; 2000. p. 275–302.
- 82 Michel E. *Vinundu*, a new genus of Gastropod (Cerithioidea: 'Thiaridae) with two species from Lake Tanganyika, east Africa, and its molecular phylogenetic relationships. J. Molluscan Stud. 2004;70:1–19. https://doi.org/10.1093/mollus/70.1.1.

- 83 West K, Cohen A. Shell microstructure of gastropods from Lake Tanganyika, Africa: adaptation, convergent evolution, and escalation. Evolution 1996;50(2):672–81. https://doi.org/10.1111/j.1558-5646.1996.tb03877.x.
- 84 Martens K. Speciation in ancient lakes. Trends Ecol. Evol. 1997;12(5):177-82.
- 85 West K, Michel E. The dynamics of endemic diversification; molecular phylogeny suggests an explosive origin of the thiarid gastropods of Lake Tanganyika. Adv. Ecol. Res. 2000;31:331–54. https://doi.org/10.1016/S0065-2504(00)31018-2.
- 86 Van Damme D, Pickford M. The late Cenozoic Thiaridae (Mollusca, Gastropoda, Cerithioidea) of the Albertine Rift Valley (Uganda-Congo) and their bearing on the origin and evolution of the Tanganyikan thalassoid malacofauna. Hydrobiologia 2003;498:1–83. https://doi.org/10.1023/A:1026298512117.
- 87 Tiercelin JJ, Mondeguer A. The geology of the Tanganyika trough. In: Coulter GW, editor. Lake Tanganyika and Its Life. New York: Oxford University Press; 1991. p. 7–48.
- 88 Cohen AS, Soreghan MJ, Scholz CA. Estimating the age of formation of lakes: an example from Lake Tanganyika, East African Rift system. Geology 1993;21:511–4. https://doi.org/10.1130/0091-7613(1993)021<0511:ETAOFO>2.3.CO;2.
- 89 Cohen AS, Talbot MR, Awramik SM, Dettman DL, Abell P. Lake level and paleoenvironmental history of Lake Tanganyika, African, as inferred from late Holocene and modern stromatolithes. Geol. Soc. Am. Bull. 1997;109(4):444–60. https://doi.org/10.1130/00167606(1997)109<0444:LLAPHO>2.3.CO;2.
- 90 Lezzar KE, Tiercelin JJ, Le Turdu C, Cohen AS, Reynolds DJ, Le Gall B, Scholz CA. Control of normal fault interaction on the distribution of major Neogene sedimentary depocenters, Lake Tanganyika, East African rift. AAPG Bull. 2002;86:1027–59. https://doi.org/10.1306/61EEDC1A-173E-11D7-8645000102C1865D.
- 91 Spiegel C, Kohn BP, Belton DX, Gleadow AJW. Morphotectonic evolution of the Central Kenya rift flanks: implications for late Cenozoic environmental change in East Africa. Geology 2007;35:427–30. https://doi.org/10.1130/G23108A.1.
- 92 Bauer FU, Glasmacher UA, Ring U, Schumann A, Nagudi B. Thermal and exhumation history of the central Rwenzori Mountains, Western rift of the east African rift system, Uganda. Int. J. Earth Sci. 2010;99(7):1575–97.
- 93 Roller S, Hornung J, Hinderer M, Ssemmanda I. Middle Miocene to Pleistocene sedimentary record of rift evolution in the southern Albert rift (Uganda). Int. J. Earth Sci. 2010;99:1643–61.
- 94 Herrel A, Podos J, Huber SK, Hendry AP. Bite performance and morphology in a population of Darwin's finches: implications for the evolution of beak shape. Funct. Ecol. 2005;19(1):43–8. https://doi.org/10.1111/j.0269-8463.2005.00923.x.
- 95 Herrel A, Podos J, Huber SK, Hendry AP. Evolution of bite force in Darwin's finches: a key role for head width. J. Evol. Biol. 2005;18(3):669–75. https://doi.org/10.1111/j.1420-9101.2004.00857.x.
- 96 Soons J, Genbrugge A, Podos J, Adriaens D, Aerts P, Dirckx J, Herrel A. Is Beak Morphology in Darwin's Finches Tuned to Loading Demands? PLoS One 2015;10(6): e0129479. https://doi.org/10.1371/journal.pone.0129479.
- 97 Tokita M, Yano W, James HF, Abzhanov A. Cranial shape evolution in adaptive radiations of birds: comparative morphometrics of Darwin's finches and Hawaiian honeycreepers. Philos. Trans. R. Soc. B. 2016;372(1713):20150481. https://doi.org/10.1098/rstb.2015.0481.
- 98 Ma W, Brusatte SL, Lü J, Sakamoto M. The skull evolution of oviraptorosaurian dinosaurs: the role of niche partitioning in diversification. J. Evol. Biol. 2020;33(2):178–88. https://doi.org/10.1111/jeb.13557.
- 99 Pérez-Miranda F, Mejía O, González-Díaz AA, Martínez-Méndez N, Soto-Galera E, Zúñiga G, Říčan O. The role of head shape and trophic variation in the diversification of the genus *Herichthys* in sympatry and allopatry. J. Fish Biol.2020;96(6):1370–8. https://doi.org/10.1111/jfb.14304.
- 100 Guralnick R, Smith K. Historical and biomechanical analysis of integration and dissociation in molluscan feeding, with special emphasis on the true limpets (Patellogastropoda: Gastropoda). J. Morphol. 1999;241:175–95. https://doi.org/10.1002/(SICI)1097-4687(199908)241:2<175::AID-JMOR7>3.0.CO;2-0)

- 101 Solem A. Patterns of radular tooth structure in carnivorous land snails. Veliger 1974;17:81–8.
- 102 Walsby JR. Feeding and the Radula in the Marine Pulmonate Limpet, *Trimusculus reticulatus*. Veliger 1975;18:139–45.
- 103 Burch JB, Jeong KH. The Radula Teeth of selected Planorbidae. Malacological Review 1984;17(1–2):67–84.
- 104 Hawkins SJ, Watson DC, Hill AS, Harding SP, Kyriakides MA, Hutchinson S, Norton TA. A comparison of feeding mechanisms in microphagous, herbivorous, intertidal, prosobranchs in relation to resource partitioning. J. Molluscan Stud. 1989;55(2):151–65. https://doi.org/10.1093/mollus/55.2.15.
- 105 Duda TF, Kohn AJ, Palumbi SR. Origins of diverse feeding ecologies within *Conus*, a genus of venomous marine gastropods. Biol. J. Linn. Soc. 2001;73(4):391–409. https://doi.org/10.1006/bijl.2001.0544.
- 106 Ukmar-Godec T, Kapun G, Zaslansky P, Faivre D. The giant keyhole limpet radular teeth: A naturally-grown harvest machine. J. Struct. Biol. 2015;192(3):392–402. https://doi.org/10.1016/j.jsb.2015.09.021.
- 107 Breure ASH, Gittenberger E. The rock-scraping radula, a striking case of convergence (Mollusca). Neth. J. Zool. 1981;32(3):307–12. https://doi.org/10.1163/002829681X00347.
- 108 Steneck RS, Watling L. Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. Mar. Biol. 1982;68:299–319. https://doi.org/10.1007/BF00409596.
- 109 Kesler DH, Jokinen EH, Munns WR Jr. Trophic preferences and feeding morphology of two pulmonate snail species from a small New England pond, USA. Can. J. Zool. 1986;64(11):2570–5. https://doi.org/10.1139/z86-377.
- 110 Black R, Lymbery A, Hill A. Form and Function: size of radular teeth and inorganic content of faeces in a guild of grazing molluscs at Rottnest Island, Western Australia. J. Exp. Mar. Biol. Ecol. 1988;121:23–35. https://doi.org/10.1016/0022-0981(88)90021-4.
- 111 Blinn W, Truitt RE, Pickart A. Feeding ecology and radular morphology of the freshwater limpet *Ferrissia fragilis*. J. N. Am. Benthol. Soc. 1989;8(3):237–42. https://doi.org/10.2307/1467327.
- 112 Iken K. Feeding ecology of the Antarctic herbivorous gastropod *Laevilacunaria antarctica* Martens. J. Exp. Mar. Biol. Ecol. 1999;236(1):133–48. https://doi.org/10.1016/S0022-0981(98)00199-3.
- 113 Jensen KR. A review of sacoglossan diets, with comparative notes on radular and buccal anatomy. Malacological Review 1980;13:55–77.
- 114 Jensen KR. Observations on feeding methods in some Florida ascoglossans. J. Molluscan Stud. 1981;47(2):190–9. https://doi.org/10.1093/oxfordjournals.mollus.a065567.
- 115 Jensen KR. Factor affecting feeding selectivity in herbivorous Ascoglossa (Mollusca: Opisthobranchia). J. Exp. Mar. Biol. Ecol. 1983;66(2):135–48. https://doi.org/10.1016/0022-0981(83)90035-7.
- 116 Trowbridge CD. Diet specialization limits herbivorous sea slug's capacity to switch among food species. Ecology 1991;72(5):1880–8. https://doi.org/10.2307/1940985.
- 117 van der Wal P, Giesen H, Videler J. Radular teeth as models for the improvement of industrial cutting devices. Mater. Sci. Eng. C 2000;7:129–42. https://doi.org/10.1016/S0928-4931(99)00129-0.
- 118 Leloup E. Exploration Hydrobiologique du Lac Tanganika (1946–1947). Bruxelles; 1953.
- 119 Arzt E, Enders S, Gorb S. Towards a micromechanical understanding of biological surface devices. Z. Metalkd. 2002;93:345–51. https://doi.org/10.3139/146.020345.
- 120 Enders S, Barbakadze N, Gorb SN, Arzt E. Exploring biological surfaces by nanoindentation. J. Mater. Res. 2004;19:880–7. https://doi.org/10.1557/jmr.2004.19.3.880.
- 121 Barbakadze N, Enders S, Gorb S, Arzt E. Local mechanical properties of the head articulation cuticle in the beetle *Pachnoda marginata* (Coleoptera, Scarabaeidae). J. Exp. Biol. 2006;209:722–30. https://doi.org/10.1242/jeb.02065.
- 122 Klein M-CG, Gorb SN. Epidermis architecture and material properties of the skin of four snake species. J. Roy. Soc.

Interface 2012;9:3140–55. https://doi.org/10.1098/rsif.2012.0479.

- 123 Schulz-Kornas E, Braune C, Winkler DE, Kaiser TM. Does silica concentration and phytolith ultrastructure relate to phytolith hardness? Biosurf. Biotribol. 2017;3:135–43. https://doi.org/10.1016/j.bsbt.2017.12.004.
- 124 Krings W, Kovalev A, Glaubrecht M, Gorb SN. Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods. Zoology 2019;137:125713. https://doi.org/10.1016/j.zool.2019.125713.
- 125 Krings W, Faust T, Kovalev A, Neiber MT, Glaubrecht M, Gorb SN. In slow motion: radula motion pattern and forces exerted to the substrate in the land snail *Cornu aspersum* (Mollusca, Gastropoda) during feeding. R. Soc. Open Sci. 2019;6(7):2054–5703. https://doi.org/10.1098/rsos.190222.
- 126 Holznagel W. A nondestructive method for cleaning gastropod radulae from frozen, alcohol-fixed, or dried material. Am. Malacol. Bull. 1998;14(2):181–3.
- 127 Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 2013;30(4):772–80. https://doi.org/10.1093/molbev/mst010.
- 128 Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 2012;61(3),539–42. https://doi.org/10.1093/sysbio/sys029.
- 129 Strong EE, Glaubrecht M. Anatomy of the Tiphobiini from Lake Tanganyika (Cerithioidea, Paludomidae). Malacologia 2010;52(1):115–53. https://doi.org/10.4002/040.052.0108.
- 130 Ax P. Das Phylogenetische System: Systematisierung der lebenden Natur aufgrund ihrer Phylogenese. Stuttgart: Gustav Fischer Verlag; 1984.
- 131 West K, Michel E, Todd J, Brown D, Clabaugh J. The Gastropods of Lake Tanganyika: Diagnostic key, classification and notes on the fauna. Special publications: Societas Internationalis Limnologiae Int. Assoc. of Theoretical and Applied Limnology; 2003.
- 132 Bendsøe MP, Kikuchi N. Generating optimal topologies in structural design using a homogenization method. Comput. Methods Appl. Mech. Eng. 1988;71:197–224.
- 133 Bendsøe MP. Optimal shape design as a material distribution problem. Struct. Optim. 1989;1:193–202.
- 134 Bendsøe MP. Optimization of Structural Topology, Shape and Material. Berlin: Springer; 1995.
- 135 Dumont ER, Grosse IR, Slater GJ. Requirements for comparing the performance of finite element models of biological structures. J. Theor. Biol. 2009;256:96–103.
- 136 Freeman PW, Lemen CA. The trade-off between tooth strength and tooth penetration: predicting optimal shape of canine teeth. J. Zool. 2007;273:273–80.
- 137 Anderson PSL. Making a point: shared mechanics underlying the diversity of biological puncture. J. Exp. Biol. 2018;221: jeb187294. https://doi:10.1242/jeb.187294.
- 138 Mackenstedt U, Märkel K. Radular structure and function. In: Barker GM, editor. The Biology of Terrestrial Molluscs. Oxon: CABI Publishing; 2001. p. 213–36.
- 139 Kesteven L. Notes on Prosobranchiata. No. ii. Proc. Linn. Soc. N.S.W. 1903;27(4):620–36.
- 140 Weaver JC, Wang Q, Miserez A, Tantuccio A, Stromberg R, Bozhilov KN, Maxwell P, Nay R, Heier ST, Di Masi E. Analysis of an ultra hard magnetic biomineral in chiton radular teeth. Mater. Today 2010;13:42–52. https://doi.org/10.1016/S1369-7021(10)70016-X.
- 141 Lu D, Barber AH. Optimized nanoscale composite behaviour in limpet teeth. J. Royal Soc. Interface 2012;9:1318–24. https://doi.org/10.1098/rsif.2011.0688.
- 142 Grunenfelder LK, de Obaldia EE, Wang Q, Li D, Weden B, Salinas C, Wuhrer R, Zavattieri P, Kisailus D.

Biomineralization: Stress and damage mitigation from oriented nanostructures within the radular teeth of *Cryptochiton stelleri*. Adv. Funct. Mater. 2014;24/39:6085. https://doi.org/10.1002/adfm.201401091.

- 143 Barber AH, Lu D, Pugno NM. Extreme strength observed in limpet teeth. J. Royal Soc. Interface 2015;12:20141326. https://doi.org/10.1098/rsif.2014.1326.
- 144 Ukmar-Godec T, Bertinetti L, Dunlop JWC, Godec A, Grabiger MA, Masic A, Nguyen H, Zlotnikov I, Zaslansky P, Faivre D. Materials nanoarchitecturing via cation-mediated protein assembly: Making limpet teeth without mineral. Adv. Mater. 2017;29:1701171. https://doi.org/10.1002/adma.201701171.

145 Osche G. Das Praeadaptationsphänomen und seine Bedeutung für die Evolution. Zool. Anz. 1962;169:14–49.

#### <u>Supplementary</u>

# Supplementary Table 1. Specimens, collection number, locality, and preparation technique (SEM, nanoindentation, molecular, GenBank accession numbers of COI and 16S sequences).

Groups	Species	Collection number	Locality	Preparation or method	Molecular data, GenBank accessions		
					СОІ	16S	Reference of molecular data
	Bridouxia grandidieriana (Bourguignat, 1885)	ZMH 119367/999_1–9	Zambia, 08°43'25''S, 31°09'00''E	Nanoindentation		·	
		ZMB 220.009x	Zambia, Mpulungu Field Station	Molecular	AY456533	AY456587	[42]
		ZMB 220.139_4	Burundi	SEM			
		ZMB 220.137_1	Tanzania, Kigoma	SEM			
	Bridouxia ponsonbyi	ZMB 220.137_2– 6, _9		Nanoindentation			
	(Smith, 1889)	ZMB 220.137		Molecular		Will be added	Krings et al. unpublished data here chapter 2.1
	<i>Bridouxia praeclara</i> Bourguignat, 1885	ZMB 220.010_1- 4	Zambia, 08°45'258'S, 31°05'116'E	Nanoindentation			
		ZMB 220.063_2	Tanzania, Kigoma	SEM			
	Bridouxia rotundata (Smith, 1904)	ZMB 220.063_1	Tanzania, Kigoma	SEM			
Group 1		ZMB 220.063_3- 8		Nanoindentation			
	Cleopatra johnstoni Smith, 1893	ZMB 220.102_1	Zambia, 09°20'866'S, 28°43'886'E	SEM			
		ZMB 220.102b_1–8		Nanoindentation			
		ZMB 220.102a		Molecular	AY456536	AY456590	[42]
		ZMB 220.008_1	Zambia, 08°45'258'S, 31°05'116'E	Nanoindentation			
	l elouniella minima	ZMB 220.008x		Molecular	AY456576	AY456630	[42]
	(Smith, 1908)	ZMB 220.135	Tanzania, Kigoma	SEM			
		ZMH 150017/999_1–4	Zambia, 08°42'10''S, 30°55'21''E	Nanoindentation			
	<i>Reymondia horei</i> (Smith, 1880a)	ZMB 220.147_1	Tanzania, Kigoma	SEM			
		ZMB 220.147_10		Nanoindentation			
		ZMB 220.013a	Zambia, Kasenga Point	Molecular	AY456568	AY456622	[42]
		ZMH 150007/999_1–5	Zambia, 08°37'23''S, 31°12'01''E	Nanoindentation			

	<i>Spekia zonata</i> (Woodward, 1859)	ZMB 220.077_1- 2	Zambia, 08°45'547'S, 31°05'825'E	Nanoindentation, data taken from [124]			
		ZMB 220.143_1- 4	Tanzania, Kigoma	Nanoindentation, data taken from [124]			
		ZMH 150008/999_1	Zambia, 08°36'31''S, 31°11'42''E	Nanoindentation, data taken from [124]			
		ZMH 150008/999_2		SEM			
		ZMB 220.026a	Zambia, Kumbula Island	Molecular	AY456569	AY456623	[42]
		ZMB 220.132	Tanzania, Kigoma	SEM			
	Anceya giraudi Bourguignat, 1885	ZMB 220.000b	Zambia, Kasenga Point	Molecular	AY456529	AY456529	[42]
	bourguigiliat, 1003	ZMH 150015/999_1–5	Zambia, 08°35'45''S, 30°48'27''E	Nanoindentation			
	<i>Martelia tanganyicensis</i> Dautzenberg, 1907	ZMB 220.133_1	Tanzania, Kigoma	SEM			
		ZMB 220.133_2- 5		Nanoindentation			
		ZMB 220.006x	Zambia, Kumbula Island	Molecular	AY456578	AY456632	[42]
	<i>Stanleya neritinoides</i> (Smith, 1880a)	MRAC without number	'Lake Tanganyika'	SEM			
Group 2				Molecular	AY213146		[85]
Group 2		ZMB 102.624_1- 4	Tanzania, Kiranda	Nanoindentation			
	<i>Syrnolopsis lacustris</i> Smith, 1880a	ZMB 220.045_1- 9	Zambia, 08°42'887'S, 31°08'476'E	Nanoindentation			
		ZMB 220.131	Tanzania, Kigoma	SEM			
		ZMB 220.046a	Zambia, Mpulungu Field Station	Molecular	AY456574	AY456628	[42]
	Tanaanvicia rufofilosa	ZMH 150009/999_1–7	Zambia, 08°46'48''S, 31°00'22''E	Nanoindentation			
	(Smith 1880a)		'Lake Tanganyika'	SEM			
		ZMB 220.039a		Molecular	AY456580	AY456634	[42]
Group 3	<i>Chytra kirkii</i> (Smith, 1880a)	IRSNB no. 63	Tanzania, Malagarasi delta	SEM			
		ZMB 220.155_1- 3	Russago	Nanoindentation			
			Zambia, Wonzye Point	Molecular	AY213142		[85]
	Limnotrochus thomsoni Smith, 1880a	ZMB 107.102	Zambia, 08°47'52''S, 31°01'11''E	SEM			
		ZMB 220.038a	Zambia, Kumbula Island	Molecular	AY456558	AY456612	[42]

		ZMH 119371/999_1–8	Zambia, 08°47'50''S, 31°01'02''E	Nanoindentation			
	Mysorelloides multisulcata (Bourguignat, 1888)	IRSNB no. 126	Tanzania, Karema	SEM			
		ZMH without number_1–2	Zambia, 08°43'25''S, 31°09'00''E	Nanoindentation			
	Paramelania crassiaranulata (Smith	ZMB 220.037_1	Tanzania, Kigoma	SEM			
	1881)	ZMB 220.037_2- 4		Nanoindentation			
		ZMH without number	'Lake Tanganyika'	SEM			
	Paramelania damoni			Molecular	AY213152		[85]
	(Smith, 1881)	ZMH 150023/999_1–4	Zambia, 08°34'09''S, 31°45'02''E	Nanoindentation			
	Paramelania iridescens (Moore, 1898c)	ZMB 220.053	Burundi, Busumbura	SEM			
		ZMB 220.099_1- 4	Zambia, 08°41'700'S, 31°08'952'E	Nanoindentation			
		ZMB 220.099a		Molecular	AY456566	AY456620	[42]
	<i>Tiphobia horei</i> Smith, 1880b	ZMB 220.095_1- 2	Zambia, 08°41'700'S, 31°08'952'E	Nanoindentation			
		ZMB 220.095		Molecular	AY456582	AY456636	[42]
	<i>Lavigeria grandis</i> (Smith, 1881)	ZMB 220.018a	Zambia,	Molecular	AY456538	AY456592	[42]
		ZMB 220.018_1	31°08'476'E	Nanoindentation			
		ZMH 150020/999_1–5	Zambia, 08°43'25''S, 31°09'00''E	Nanoindentation			
		ZMH 154657/999	Zambia, Mibwebwe	SEM			
	Lavigeria livingstoniana (Bourguignat, 1885)	ZMB 220.117_1- 5	Tanzania, Kigoma	SEM			
Group 4		ZMB 107.097	Tanzania, Utinta	Molecular	Will be added	Will be added	Krings et al. unpublished data, here chapter 2.1
	<i>Lavigeria nassa</i> (Woodward, 1859)	ZMB 220.074	Zambia, 08°45'547'S, 31°05'825'E	SEM			
		ZMH 119369/999_1-9	Zambia, 08°29'23''S, 30°28'46''E	Nanoindentation			
		ZMB 220.019a	Zambia, Kasenga Point	Molecular	AY456548	AY456602	[42]
	<i>Lavigeria spinulosa</i> (Bourguignat, 1885)	ZMB 220.051	Zambia, 08°42'258'S, 31°05'116'E	SEM			
		ZMB 220.014a	Zambia, Kumbula Island	Molecular	AY456555	AY456609	[42]
		ZMH 150012/999_1-6	Zambia, 08°42'10''S, 30°55'21''E	Nanoindentation			

Outgroup	Paludomus siamensis Blanford, 1903	ZMB 200.231		Molecular	AY456560	AY456614	[42]
Outgroup	<i>Melanoides tuberculata</i> (Müller, 1774)	ZMB 220.060	Malawi	Molecular	AY456562	AY456616	[42]

# Chapter 5. Summary and Discussion

# Morphology, mechanical properties, and ecology

#### The role of morphology (Objectives 1, 2, 4, 5; chapter 2.1, 2.2, 3.2)

In most examined species the feeding ecology (feeding substrate preference) is reflected by morphologies of the central and lateral tooth whereas the inner and the outer marginal tooth, exhibiting a slender shape with many or numerous denticles confined to the distal tip, are rather similar in all examined species (Fig. 1).

Feeding on soft substrate usually involves a long, slender central tooth with numerous denticles of equal size and a lateral tooth with one long and few smaller denticles, both teeth are rather similar in morphology to the marginal teeth (yellow boxes in Fig. 1). Their slender shape and the possessing of fine denticles probably enables them to rake between the sand grains gathering food particles. These morphologies were found in *Chytra kirkii, Mysorelloides multisulcata, Tiphobia horei, Paramelania iridescence, P. crassigranulata, P. imperialis, Cleopatra johnstoni, C. bulimoides, Martelia tanganyicensis, Anceya giraudi, Syrnolopsis lacustris, and S. minuta.* 

Feeding on solid substrate can go along with laterals bearing one thicker and more prominent denticle (red boxes in Fig. 1; found in *Lavigeria* species, *Reymondia* species, *Bridouxia praeclara, B. ponsonbyi, B. rotundata, Spekia zonata, and Bathanalia howesii*), probably allowing a large interaction surface between tooth cusp and ingesta directly transferring force. The central teeth exhibit three morphologies: (a) a long and slender shape with a spatulate or prominent central denticle (red boxes in Fig. 1; found in *Lavigeria* and *Reymondia* species), (b) a wide and broad shape with few or no denticles along the outer edges (green boxes in Fig. 1; found in *Bridouxia praeclara, B. ponsonbyi, B. rotundata, S. zonata,* and *Leloupiella minima*), and (c) a central tooth with long denticles of the same size (yellow boxes in Fig. 1; found in *Stanleya neritinoides, Bathanalia howesii,* and *Tanganyicia rufofilosa*). In the first and second case a large interaction area probably leads to an increased ability to transfer force onto the ingesta. Additionally, these teeth are rather short and

broad presumably leading to the reduction of deformation when tensile and compressive stresses appear in the structure during this action.

The basic morphology of the tooth's attachment with the membrane seems to be phylogenetically relevant but can also provide an ecological signal since the anchorage area and fibre type corresponds to the width of the teeth. Most species that feed on solid substrate (exception is *Lavigeria*) have broader central and lateral teeth and hence larger tooth attachment areas than species feeding on soft (*Cleopatra johnstoni*) or mixed substrate (*Paramelania damoni*, *Limnotrochus thomsoni*, *Bridouxia grandidieriana*). The broader tooth anchorages probably prevent teeth from breaking loose while feeding on solid substrates. Only the solid substrate feeders *Lavigeria* have a small attachment area in the central teeth; but here teeth are stabilized by the fibres of the Type 2 connecting the tooth to all sides with the membrane and presumably reducing the ability of the tooth to flip during feeding. We can thus conclude that by examining the fibre anchorage angle in connection with the attachment area ecological specialisations can be determined. Even in a closer phylogenetic context we find differences in attachment areas that can be related with feeding ecology: *B. grandidieriana* is the only known species within *Bridouxia* feeding on softer substrates, plant surfaces and sand (Glaubrecht 2008); here the smaller attachment area of the central tooth reflects the possible trophic specialization of this species.

However, as already proposed by Padilla (2004) a deeper understanding of radular tooth function awaits an approach that addresses the exact 3-dimensional shape of the tooth, the interaction between the teeth, their material composition, and the interface between tooth and ingesta (see also Hickman 1980, 1983, 1984, 1985; Morris & Hickman 1981). Hypotheses on functionality can be based on the biomechanical behaviour of structures which can be simulated in form of a finiteelement-analysis (FEA). This approach involves standard statistical methods and results in visual representations of stress and strain distributions in structures, valuable for comparisons and contributing to eco-morphological and macroevolutionary objectives (e.g. feeding behaviour of: early tetrapods, Fortuny et al. 2011; mammals, Piras et al. 2013, Attard et al. 2014, Figueirido et al. 2014, Serrano-Fochs et al. 2015, Sharp 2015, Marcé-Nogué et al. 2017; reptiles: Jones et al. 2017; Darwin's finches: Soons et al., 2015). Not only the tooth's shape, its attachment with the membrane and interaction with other teeth, its interface but also the mechanical properties (see below) that are known to be of high functional importance (for the influence of mechanical properties on function see e.g. Aifantis 1992; De Jager et al. 2006; Bingbing et al. 2012; Michels et al. 2012; Liu et al. 2016; Büsse & Gorb 2018; Saltin et al. 2019; Wang et al. 2019) can be included in such an analysis (see also Rayfield 2007). In Malacology FEA had been applied in order to gain insight into the functionality of docoglossan (*Patella* and Polyplacophora, van der Wal et al. 2000) and '*Helix*' radular teeth (*Euhadra peliomphala*, Miura et al. 2019), but since van der Wal et al. (2000) analysed 2D models and Miura et al. (2019) did not include material gradients, we here provide the first FEA approach including all of these properties for our model species *Spekia zonata*.

The above stated hypotheses on functionality based on the interpretation of morphology are supported by the results of the FEA: short, broad morphologies do not deform as much as taller, thinner ones (Fig. 2). The long marginal teeth consisting both of a rather slender and thin stylus before terminating in a cusp containing small denticles always experience higher stress and strain than the rather short and broad central and lateral teeth. Additionally, central and lateral teeth exhibit thick rounded bulges at their basis, contributing to the reinforcement of the tooth structure and presumably support a force transmission to the radular membrane (see also Padilla 2004; Herrera et al. 2015). In contrast to one hypothesis stating that long radular teeth are more effective in removing algae tissue (Reid 1996), we found that the marginal teeth of *Spekia zonata*, because they are more affected by stress and strain in our model (Fig. 2), largely deform when in contact with the substrate but additionally also have an enlarged risk of breaking off.

Fig. 1. Schematic illustrations of taenioglossan radular teeth from representative paludomid species from Lake Tanganyika and surrounding rivers systems (*Cleopatra johnstoni*) sorted to the preferred feeding substrate (circle with: grey triangles = solid substrate, with green boxes and brown circles = plant surface and soft substrate [mixed feeders], with grey triangles and brown circles = solid and soft substrate [mixed feeders], with brown circles = soft substrate). Similar morphologies of teeth are highlighted with coloured boxes: yellow = long, slender teeth with numerous denticles of more or less equal size, red = teeth displaying one thick and more prominent denticle, green = wide and broad tooth with few or no denticles along the outer edges.



Fig. 2. Results of the FEA (stress and strain distribution) with proposed best embedment condition (hard membrane and modelled material property gradients) for A. inner and B. outer marginal tooth, C. lateral tooth, and D. central tooth of solid substrate feeder *Spekia zonata*. The scaling of the stress distributions in each tooth type and the scaling of the strain distribution in each tooth type are identical for comparison. Blue color = low strain or stress, (green and yellow as transition), red color = high stress and strain values.

The large and thick cutting edges of the central and lateral teeth seem to be highly functionate as well. The area of the interface can define the structures function since it



determines the force over area, the mechanical stress (for the relation of stress and [bite] force see e.g. van der Meji & Bout 2004; Huber et al. 2005; Wroe at al. 2005; Davis et al. 2010), which is exerted onto the ingesta surface. Thus, small forces can create high stresses when the contact area of the structures is also small. The morphology of the tool, its sharpness and hence the contact area and angle between tool and target material, is as high importance for its function (e.g. for shark teeth: Frazzetta 1988; Whitenack & Motta 2010; mammal teeth: Popowics & Fortelius 1997; Freeman & Weins 1997; Evans & Sanson 1998; Evans et al. 2005; Freeman & Lemen 2007; tools: Shergold & Fleck 2005; conodont teeth: Jones et al. 2012; review on puncture mechanics Anderson 2018). The effects of some radular tooth shapes on the ingesta (e.g. puncturing and cutting of the cell wall with sabot-shaped teeth in Sacoglossan species) were highlighted by Jensen (1993) and Padilla (2004) emphasized the importance of this contact area. This had also been supported by a study on large land snails; here the forces that were exerted in vivo by the radula on a glass capillary were recorded (Krings et al. 2019). The small contact area of the pointy tooth cusps (in this specific case it is an 'Helix' radular type) with the target surface was identified and the local pressure (max. 4698.7 bar) calculated, resulting in the hypothesis that radular teeth, even though comparatively soft, can cut or pierce harder materials by high punctual pressure. This in turn leads to fracturing of

the ingesta, but with probably also to high abrasion on the radular teeth. So overall, pointy radular teeth can exhibit a stronger pressure at their tooth cusps making them more effective at piercing and tearing (Krings et al. 2019), whereas blunt teeth are presumably more effective for loosening material from larger substrate surfaces (see also Padilla 1985, 1989, 2004; Jensen 1993) as the central and lateral teeth of *S. zonata*.

Reconstructing the 3D-model gave us insight into the precise position of the teeth. Their arrangement on the radular membrane results in the interaction and interlocking between them. This effect in turn presumably aids in the force transmission from the single tooth to the neighbouring teeth as had been previously postulated by Hickman (1980) and Padilla (2004). The central teeth from adjacent rows support each other by the interaction of their bulky bases with the rounded bulges on the styli. The rounded and broad bases of the lateral teeth from adjacent rows fit perfectly together, thus stabilizing each other while interacting with the substrate surface. The marginal teeth support each other as well, the inner and the outer marginal teeth can interlock tightly. Here, the outer, larger marginal tooth II embraces the inner, smaller marginal tooth I, leading to a single functionally unit (see also Hickman 1984). The performance of single teeth are of high interest as well (e.g. van der Wal et al. 2000, Miura et al. 2019), but in order to link morphology and function it is utterly necessary to consider the radula with all its teeth as a complex unit with mechanically interacting, non-independent structures (see also Morris & Hickman 1981; Hickman 1984; Padilla 2004).

The attachment areas connecting the tooth basis with the membrane seems to influence function as well (see also above). For e.g. *Spekia zonata* central and lateral teeth have quite large attachment whereas the marginal teeth display a relatively small area. For the central and lateral teeth forces can thus easily be transmitted from the tooth tip to the underlying membrane resulting in less stress and strain in the tooth structure itself whereas the marginal teeth have limited options transferring stress to the surrounding membrane. In previous studies based on pure morphology it has been hypothesized that taenioglossan central teeth are only used for gathering food (Fretter & Graham 1962; Jüch & Boeksehoten 1980; Steneck & Watling 1982), but our feeding simulations depict a different picture. The marginal teeth however seem to have a rather stronger range of deflection due to their small attachment area and their thin and slender stylus in connection with the material properties (see below); this enables this tooth type to be less prone to failure and fraction but does not allow the direct transfer of forces from the radula onto the ingesta. Steneck & Watling (1982) proposed the possibility of marginal teeth to gather food from a greater surface area by 'inward raking' as the teeth converge to the central axis of the radula during retraction and our results, that marginal teeth are less affected from stress and strain when the force is applied along the anterior-medial axis, comply.

Overall, we would thus conclude based on the FEA (Fig. 2) for *Spekia* zonata that marginal teeth are not capable of loosening algae from the substrate but rather gather ingesta whereas central and lateral teeth have the ability to scratch across the solid substrate. Since the marginal teeth of the soft and mixed substrate feeders as well as the central and lateral teeth of all soft substrate feeders display a similar morphology (Fig. 1) to the marginal teeth of the solid substrate feeders (e.g. *S. zonata*) they presumably exhibit a similar mechanical behaviour and thus function.

The third morphology of the central tooth can be observed in the solid-substrate feeders *Stanleya neritinoides, Bathanalia howesii,* and *Tanganyicia rufofilosa*, but also in all mixed substrate feeders (*Paramelania damoni, Limnotrochus thomsoni, Bridouxia grandidieriana*); here central as well as lateral teeth are rather similar in their morphology to teeth of gastropods foraging on sand and to the marginal teeth (Fig. 1). But since the solid substrate feeders *S. neritinoides* and *T. rufofilosa* (we lack data on *B. howesii*) have similar mechanical properties than all other solid substrate feeders (see below), we would hypothesize that here radular tooth performance is ensured by mechanical properties rather than morphology.

The mixed substrate feeders, however, are probably enabled to gather algae from soft substrate by the rather fine central and lateral tooth morphology possessing small denticles. But, the – at least temporary – foraging on harder (plant surface for *B. grandidieriana*) or solid surfaces (stone for *P. damoni* and *L. thomsoni*) is possibly ensured by possessing harder and stiffer central and lateral tooth cusps (see below). Thus, pure morphology does not consistently reflect adaptations, but the

morphology fostered by mechanical properties allows the establishment of radular tooth ecomorpho-propo-types (Fig. 3).

# The role of mechanical properties (functional gradients) in combination with morphology (Objectives 3, 4, 5; chapter 3.1, 3.2, 4.1)

Biological materials as a result of a long-lasting evolution are generally composites with heterogeneities as well as property gradients playing an important role on the structure's functionality or multifunctionality. This may include load bearing or support, interfacial strengthening, and contact damage resistance (for review see Liu et al. 2017). This material complexity complements morphology and gives rise to the profound understanding of functions including the interaction of structures or surfaces (e.g. Aifantis 1992; De Jager et al. 2006; Bingbing et al. 2012; Michels et al. 2012; Liu et al. 2016; Büsse & Gorb 2018; Saltin et al. 2019; Wang et al. 2019). These functional gradients and heterogeneities have been investigated in all kind of biological structures and are caused by chemical constituents as well as structure (for review see Liu et al. 2017). Examples for these principles include chemical composition or constituent gradients as in the chiton radular teeth caused by mineralization (e.g. Weaver et al. 2010; Wang et al. 2013), the tarsal setae of ladybird beetle induced by the distribution of the biomolecule resilin (Gorb et al. 2002; Peisker et al. 2013), the squid's beak resulting from the regionalization of histidine-rich proteins and the degree of hydration (Miserez et al. 2007, 2008, 2010; Tan et al. 2015). But the structural arrangement as in abalone shells (Nassif et al. 2005; Espinosa et al. 2009; Heinemann et al. 2011), the distribution as found in the cephalopods sucker ring (Miserez et al. 2009; Hiew & Miserez 2017), the dimension as in crustacean exoskeletons (Raabe et al. 2005; Boßelmann et al. 2007; Chen et al. 2008), and the orientations as found in pangolin scales (Liu et al. 2016; Wang et al. 2016), are also responsible. In many cases multiple gradient types in combination are the basis for the measured heterogeneities as in mammal teeth or bones (Lin et al. 1993; Marshall et al. 2001; Imbeni et al. 2005; Benjamin et al. 2006; Chan et al. 2011; Lu & Thomopoulos 2013; Tertuliano & Greer 2016).

For most teeth of the examined paludomid species we discovered (functional) gradients in hardness and elasticity reflecting ecology. Additionally, the mean values of both parameters relate with the preferred feeding substrate and, together with morphology, indicate different radular tooth ecomorpho-propo-types (Fig. 3):

All examined species foraging on solid substrate (Bridouxia ponsonbyi, B. rotundata, B. praeclara, Leloupiella minima, Spekia zonata, Reymondia horei, Stanleya neritinoides, Tanganyicia rufofilosa, Lavigeria spinulosa, L. livingstoniana, L. nassa, and L. grandis) show gradual and distinct differences in stiffness and hardness at their cusps (central tooth cusps as the hardest and stiffest, followed by the lateral tooth cusps, and finally marginal tooth cusps as the softest and most flexible cusps), which can be explained by different functional loads since the Young's Modulus can be linked with the ability of a structure to transfer forces (e.g. Bendsøe 1989, 1995; Bendsøe & Kikuchi 1988; Dumont et al. 2009). We would anticipate that the stiff central and lateral tooth cusps are used for scratching across the solid substrate loosening food items attached to it by transferring higher force from the radular muscles via the tooth cusps onto the ingesta. These teeth can be compared to scrapers. The softer and more flexible marginal tooth cusps probably have a smaller ability to transfer forces necessary to loosen a tightly attached biofilm, but their elasticity allows the reduction of the stress concentration in case of e.g. hitting on obstacle. Additionally, especially the marginal teeth of solid substrate feeders exhibit functional gradients with the basis as the softest and most flexible part, followed by the stylus, and finally the cusps as the hardest and most stiff part (for Spekia zonata see chapter 3.2; for other species it is referred to unpublished data; see also Fi. 3), which possibly enables them to catapult back to place. We would hence deduce that the marginal teeth are rather functionally different from the central and lateral teeth, possibly harvesting like a broom or rake food items or particles that had been loosened from the substrate by grinding action of the central and lateral teeth (see also Mackenstedt & Märkel 2001). This type of radula is here defined as multifunctional tool (Fig. 3) possessing two scrapers (central and lateral tooth) and two rakes (inner and outer marginal tooth).

These hypotheses about tooth functionality are supported by the FEA on Spekia zonata (Fig. 2): the modelling with integrated material gradients resulted in higher values of stress in the teeth, with both central and lateral teeth being less effected than the marginal ones. This already indicates different functions; the central and lateral teeth are capable of resisting to higher stresses and rather loosen food from the substrate whereas the marginal teeth are not. The higher stress values of the models have their origin in the stiffness of the tooth cusps. As mentioned above, a higher Young's modulus is linked with the ability of a structure, in this case of the tooth cusps, to transfer force into the ingesta (onto algae attached to a rocky surface). This system is analogous to other biological systems, such as mouthparts in Arthropods showing sclerotized and sometimes strongly mineralised cutting edges in their mouthparts enabling the crushing of food with a resistance to wear and the avoidance of structural failure (e.g. Michels et al. 2012; Büsse & Gorb 2018). However, the transmittance of higher stresses onto the ingesta could lead to natural wear at the cusps, which had been documented for some Molluscan taxa (Runham & Thornton 1967; Mackenstedt & Märkel 1987; Franz 1990; Padilla et al. 1996; Shaw et al. 2002, 2010), but might also result in a risk of fracture when teeth are exposed to higher stresses. However, since teeth continuously enter the wearing zone they can be replaced.

Additionally, the incorporation of material properties has a strong effect on the values of the strain in our FEA (Fig. 2): the modelled strain is much higher in homogenous teeth than in heterogeneous ones showing that homogenous teeth deform more. But the tooth regions are affected differently by strain: the basis and the stylus are more affected than the tooth cusps. This is a direct results of specific material properties: the relatively stiff and hard cusp is not affected by deformation (strain), but the flexible and soft basis and the stylus are. This pattern is exaggerated in the marginal teeth, but not as pronounced for the central and lateral teeth showing a more uniform distribution of strain. This behaviour can again be explained with distinct functions: in central and lateral teeth deformation would be very problematic because the teeth must maintain their shape while acting on ingesta. The marginal teeth however are provided by a flexible stylus and basis serving as shock absorption against mechanical impacts (see also Herrera et al. 2015) resulting in the reduction of structural failure while scratching across the substrate surface. The marginal teeth can also flip and rotate due to their ability to deform (this is not possible for the lateral and central teeth). This behaviour, the combination of different material properties in a complex network, appears to be functionally analogous to resilin-dominated areas in Arthropods e.g. wings or mouthparts (Haas et al. 2000a, 2000b; Michels et al. 2012; Rajabi et al. 2015, 2016a, 2016b; Büsse & Gorb 2018), reptile skins (Klein et al. 2010; Klein & Gorb 2012), or squid beaks resulting from the regionalization of cross-linking and the degree of hydration (Miserez et al. 2007, 2008, 2010; Tan et al. 2015); Combining the stiffness/hardness of the surface with the flexibility of underlying layers enables a structure to be less prone to failure. This again leads to the conclusions that teeth have different functions: the central and lateral teeth loosen food from the substrate, whereas the marginal teeth gather the algae from the substrate like brooms (Fig. 3).

All species foraging on sand or mud (*Cleopatra johnstoni*, *Martelia tanganyicensis*, *Anceya giraudi*, *Syrnolopsis lacustris*, *Chytra kirkii*, *Tiphobia horei*, *Paramelania iridescens*, *P. crassigranulata*, and *Mysorelloides multisulcata*) exhibit soft and flexible tooth cusps with similar mechanical properties as the solid substrate feeders' marginal teeth. But, in contrast to the marginal teeth of the species foraging on rock exhibiting functional gradients, all teeth of the soft substrate feeders show similar mechanical properties in every tooth regions (unpublished data; see also Fig. 3). This probably leads to an increased ability of all teeth to deform, bend and twist. We would this propose that these species rather possess a monofunctional radula (Fig. 3) with each tooth type (central, lateral, inner and outer marginal tooth) serving as broom or rake collecting algae from the sand grains.

The mechanical properties of the mixed substrate feeders (*Bridouxia grandidieriana, Limnotrochus thomsoni*, and *Paramelania damoni*) are intermediate, as they have slightly softer and more flexible central and lateral tooth cusps compared to the species loosening algae from stones, but stiffer and harder ones than the species gathering biofilm from sand. We found gradients in mechanical properties: between the tooth cusps, but also within each tooth type with the basis as the softest and most flexible part, followed by the stylus, and finally the cusps as the hardest and most stiff part (unpublished data; see also Fig. 3). These gradients are not as pronounced as in the gastropods foraging on stone, but the existence of these gradual differences leads to the conclusion that, despite of softer and more flexible tooth cusps, the mixed substrate feeders also possess a

multifunctional radula. Central and lateral teeth are probably at least temporarily enabled by their mechanical properties to loose food items attached to harder (plant surface in *B. grandidieriana*) or solid surfaces (rock in *L. thomsoni, P. damoni*), whereas their morphology involving small denticles allows the gathering of algae from soft substrates (see above). The lower hardness and elasticity values could be considered a compromise; building stiff and hard structures is probably a highly energy-consuming process and might only be profitable when feeding exclusively on solid substrate. The occasional foraging with more flexible tooth cusps might lead to more wear, but continuously new radular tooth rows are formed and teeth can hence be replaced. In contrast, scratching exclusively across solid substrate might result in the failure of teeth or a complete loss in those species. The softer inner and outer marginal teeth showing similar property gradients to the marginal teeth of solid feeders are probably used for gathering algae from sand and serve as brooms or rakes, whereas we would compare the central and lateral teeth to scrapers with tines (Fig. 3).

The found heterogeneities and (functional) gradients could be, as mentioned above, either the result of different chemical constituents, inorganic as well as organic, or fibre arrangement. Radular teeth are well known to contain high quantities of incorporated minerals like Fe, possibly leading to a higher wear resistance of tooth cusps. But this has only been reported for the molluscan class Polyplacophora and for Patellogastropoda, a basal clade of Gastropoda (e.g. van der Wal et al. 2000; Brooker et al. 2003; Lee et al. 2003; Wealthall et al. 2005; Shaw et al. 2009, 2010; Weaver at al. 2010; Saunders et al. 2011; Han et al. 2011; Brooker & Shaw 2012; Lu & Barber 2012; Nemoto et al. 2012; Wang et al. 2014; Barber et al. 2015). Most gastropod species, except Patellogastropoda, seem to lack substantial proportions of Fe (e.g. Simone 2011). Other elements like Si, Ca, and Mg were however found to be abundant in gastropod teeth (e.g. Sollas 1907; Jones et al. 1935; Lowenstam 1962; Liddiard et al. 2004; Hua & Li 2007). But the here examined paludomid radular teeth all lack such incorporations and are presumably only composed of chitinous fibres as first elemental analyses conducted via Energy-dispersive X-ray spectroscopy (EDX) indicate (unpublished data). So we would preliminarily rather conclude, that the measured gradients in hardness and elasticity are either based on the organic composition, e.g. the grade of tanning as it has been examined in cephalopod beaks (Miserez et al. 2007, 2008, 2010; Tan et al. 2015), or on the ultrastructure including the arrangement and quantity of the chitinous fibres as in insect cuticles (e.g. Neville 1975; Hepburn & Chandler 1976; Banerjee 1988). Both parameters await further investigation.



Fig. 3. Schematic illustrations of representative taenioglossan radulae (from left to right: outer marginal, inner marginal, lateral, and central tooth) categorized as either multifunctional or monofunctional; these functional hypotheses are either based morphology and mechanical properties, or mechanical properties; the measured Young's modulus (median, in GPa) is assigned to the certain tooth region (data on *Spekia zonata* from chapter 3.1; data on tooth cusps from chapter 4.1; data on other styli and bases from unpublished data); radulae are sorted to the preferred feeding substrate (circle with: grey triangles = solid substrate, with grey triangles and brown circles = solid and soft substrate [mixed feeder], with green boxes and brown circles = plant surface and soft substrate [mixed feeder], with brown circles = soft substrate); teeth are attributed to certain tool types (scraper, rake, scraper with tines). A. *Spekia zonata*, B. *Paramelania damoni*, C. *Bridouxia grandidieriana*, D. *Stanleya neritinoides*, E. *Anceya giraudi*.

# *The role of trophic specialization - from paludomid case study to Molluscan evolution?*

#### Hypotheses on paludomid evolution (Objective 6; chapter 2.1, 2.2, 4.1)

Our study resulted in a phylogenetic hypothesis for African paludomids (Fig. 4); this in connection with the results of the molecular clock approach supporting the existence of major gastropod lineages before the formation of the lake or its proto-lakes (Krings et al. unpublished data; see also Wilson et al. 2004) identify distinct lineages of gastropod groups that have inhabited Lake Tanganyika from the surrounding river systems independently. This is congruent with the hypotheses on paludomid evolution as proposed by Glaubrecht & Strong (2007) and Glaubrecht (2008). Our results do rather not support the idea of one single colonialization event followed by an 'ancient endemic radiation' (Cunnington 1920; Brooks 1950) resulting in a truly 'adaptive' radiation (cf. Mayr 1963; Mayr & Ashlock 1991) for the Paludomidae as it has been postulated (e.g. Boss 1978; Johnston & Cohen 1987; Brown & Mandahl-Barth 1987; Berthold 1990; Coulter 1991; Michel et al. 1992; Michel 1994, 2000, 2004; West & Cohen 1996; Martens 1997; West & Michel 2000; Van Damme & Pickford 2003).

The gastropods in Lake Tanganyika have a restricted area of distribution in Lake Tanganyika and occur between the surf zone down to 200 m as the deeper parts of the lake contain little oxygen and are toxic (Glaubrecht 2008). Sharing habitats might result in considerable inter- and intraspecific competition, but the clear substrate-specificity (either biofilm that covers stones [solid substrate], algae from sandy and muddy surfaces [soft substrate], algae from soft and solid surfaces [mixed], algae attached to plant surfaces and sand [mixed]) of paludomid species strongly indicates an avoidance or reduction of resource competition. In most groups (Group 1 containing: Spekiini, *Reymondia*, riverine Cleopatrini; Group 2 containing: Hauttecoeuriini and Syrnolopsini; Group 3 containing: Tiphobiini; groups in accordance with Wilson et al. 2004, Glaubrecht 2008; see also Fig. 4) we were able to identify soft, solid and mixed substrate feeders, only Group 4 (*Lavigeria* and riverine *Potadomoides*) contains probably only solid substrate feeders. We lack reliable data for *Potadomoides*, but since its radular tooth characters are similar to *Lavigeria* species (Glaubrecht & Strong 2007), we could propose that *Potadomoides* also feeds on algae from solid substrates.

In the course of this study we were able to relate the feeding substrate with the morphology and the mechanical properties of radular teeth; this allowed us to put forward hypotheses about the evolution of these gastropod lineages (Fig. 4). We propose that one main engine in the evolution of African Paludomidae included the (trophic) specialization to different feeding substrates (soft, mixed, and solid ones) allowing the coexistence of several taxa in the same environment by the formation of different niches. Morphology in concert with mechanical properties are here tentatively considered as adaptations. However, if found radular tooth characters/properties can be considered as true adaptations awaits further investigations, e.g. in form of feedings experiments involving paludomid species with distinct radular tooth morpho-propo-types foraging in competition on e.g. solid or soft substrate; here the individual fitness could be examined. In order to deeply understand paludomid tooth function and to allocate adaptations, data on the biofilms are also necessary. Not only the preferred algae species or algae conglomerate, but also the mechanical properties of the intaken biofilms should be examined. For this biofilm in front of the snail and the remaining biofilm behind the snail could be collected, which would certainly be quite difficult on the bottom of Lake Tanganyika. The biofilm's mechanical properties could be examined by establishing an experimental set-up: a small hooked needle (in similar size to a radular tooth) could be connected to a force transducer and be scratched across the biofilm in order to detect the force that is needed to detach the algae from e.g. the rock. However, since we found similar mechanical properties and tooth morphologies in each species regardless the date of collection or locality we would propose that both parameters are maintained over generations and contribute to the individual fitness and would rather conclude that teeth show adaptations.

This allows us to put forward hypotheses about the paludomid evolutionary history (Fig. 4): the reconstructed ancestral feeding substrate is of soft nature, and the proposed ancestral tooth morphologies involve long and slender teeth (small attachment area) with numerous denticles of equal size (monofunctional radula). Within the rivers, probably in rapids, two paludomid lineages (ancestor of (i) Spekiini and *Reymondia* as well as (ii) Nassopsini and *Potadomoides*) evolved convergently central and lateral tooth morphologies adapted to solid substrates. Additionally to tooth shape, Spekiini and *Reymondia* possibly evolved larger attachment area whereas Nassopsini and *Potadomoides* a stabilisation in form of Fibre Type 2. This was probably accompanied by the

parallel evolution of harder and stiffer central and lateral tooth cusps as well as functional gradients. Additionally, after the origin and the colonialization of Lake Tanganyika, two other lineages (viz. the ancestor of (i) Bathanalia as well as (ii) Stanleya and Tanganyicia) were adapted convergently to solid substrate (Fig. 4). Nevertheless, Bathanalia howesii (see images of radula in Strong & Glaubrecht 2010), Stanleya neritinoides, and Tanganyicia rufofilosa possess, as described above, radular teeth similar in morphology to the soft substrate feeders. These taxa probably still carry their ancestral riverine morphological characters (here interpreted as preadaptation sensu strictu Osche 1962 to the riverine substrate). Thus, their adaptation to solid substrate involved only the change in material properties hardness and elasticity (unfortunately we lack hardness/elasticity data for B. howesii). The mixed substrate feeders Paramelania damoni and Limnotrochus thomsoni retained their ancestral tooth morphologies (and small attachment areas) as well, but temporary solid substrate feeding is probably enabled by the convergent evolution of stiffer, harder central and lateral tooth cusps as well as functional gradients. This has presumably evolved in the river systems. With regard to S. neritinoides, T. rufofilosa, P. damoni, and L. thomsoni, we could hypothesize that pure tooth morphology might be more genetically fixated than the mechanical properties hardness and elasticity and that the latter could be adapted faster and easier to the ingesta.

*Bridouxia* is probably a case of an exclusively intralacustrine adaptive radiation (see also Krings et al. accepted for publication in Plos One) indicated by molecular clock approaches. Here we were able to detect secondary adaptation to mixed feeding substrate for *B. grandidieriana* (Fig. 4). This taxon became probably adapted through changes in morphology (also in a decrease in tooth attachment area) as well as material properties, evolving softer and more flexible central and lateral teeth with small denticles serving as scraper with tines (Fig. 3). *Lavigeria* could be another example of an intralacustrine radiation, since this genus contains a plethora of named species (see e.g. Brown 1994; West et al. 2003). Unfortunately, a systematic revision identifying evolutionary entities is still lacking hindering hypotheses on their evolution. However, when comparing *Lavigeria* adult shells their distinct sizes are apparent which are interpreted as result of annidation through different body sizes (Krings et al. under revision in Scientific Reports). Also, *Lavigeria* radular teeth are of distinct sizes, this could be an indication that *Lavigeria* species avoid competition by trophic specialization,

albeit not to different feeding substrates but rather to different algae or biofilm types growing on solid substrates. However, in order to determine whether species have distinct food preferences, it would be necessary to collect and gather feeding substrates and biofilms directly in situ.

All other lacustrine species (*Martelia, Anceya, Syrnolopsis, Chytra, Tiphobia, P. iridescens, P. crassigranulata, Mysorelloides*) are still adapted to foraging on soft substrates carrying their ancestral riverine tooth morphologies as well as displaying soft and flexible teeth.

Overall, we find evidences for parallel evolution in this species flock, e.g. adaptations in the central tooth for loosening algae from solid substrates evolved at least five times resulting in different general tooth morphologies (with a spatulate or prominent central denticle, broad and long denticles of the same size, or a wide and broad central tooth). As proposed by Muschick et al. (2012) for cichlid fish from Lake Tanganyika, stating that the found parallel phenotypes result from the combination of coexistence in the same habitat and the limited number of available niches, we can detect a similar pattern for the lacustrine paludomids.

The here examined paludomid species flock served as a good model system to gain insight into the relationship between tooth morphology, mechanical properties, function, feeding ecology, and evolution. This approach could be used for identifying the influence of ingesta in the evolution of other gastropod species flocks (e.g. *Biwamelania* gastropods in Lake Biwa, known to be substrate-specific: see Miura et al. 2018; *Tylomelania* from Sulawesi feeding on different substrates and possessing teeth with distinct morphologies: see Rintelen et al. 2004, 2007, 2010; Rintelen & Glaubrecht 2005; Glaubrecht & Rintelen 2008; Hilgers et al. 2020; *Brotia* in Keak River: see e.g. Glaubrecht & Köhler 2004; Madagascan Pachychilidae: see e.g. Köhler & Glaubrecht 2010) but could also contribute to a deeply understanding of the relationship between ingesta and Mollusc providing an integrated view of habitat requirements and possibly macroevolution in Mollusca as a whole.



Fig. 4. Proposed evolutionary history of the African Paludomidae (some species that were not tested by nanoindentation were excluded from this figure since the focus is on the mechanical properties, for the phylogenetic relationship and tooth morphologies of those species see chapter 2.1) depicting the four major gastropod lineages/groups; in the centre Bayesian 50% majority-rule consensus tree with Melanoides tuberculata and Paludomus siamensis as outgroup, Potadomoides species were placed in this tree based on morphology (see chapter 2.1); white background colour (Potadomoides and *Cleopatra*) outlines riverine species outside Lake Tanganyika, blue background colour outlines the colonialization and inhabitation of Lake Tanganyika; pattern indicate the preferred feeding substrate and at which point (ancestral feeding substrate = soft) a shift regarding this ecological parameter could have taken place: circle with brown circles = soft substrate, with grey triangles = solid substrate, with grey triangles and brown circles = solid and soft substrate [mixed feeder], with green boxes and brown circles = plant surface and soft substrate [mixed feeder]); schematic illustration of radular tooth types (central, lateral, marginal I and II) for each species with Young's moduli (mean, GPa) assigned to the tooth cusps; attachment areas of central and lateral teeth are depicted below the schematic illustration of the specific tooth type and the assigned fibre type (see chapter 2.2); circles indicate if the ecology is reflected by either tooth morphology (black circle) and/or mechanical properties (white circle); tooth function is assigned to certain tools (scraper, rake, scraper with tines).

# Outlook

## Evolutionary history and radular tooth functionality of Lake Tanganyikan paludomids

We here proposed hypotheses about paludomid evolution, but in order to gain a deeper insight into the species' evolutionary history and the role of trophic specialisation the subsequent topics should be addresses:

A) The systematic sampling of fresh genetic material could allow to (i) bridge existing genetic data gaps (lack of 16S and COI sequences, especially data on *Potadomoides* would be essential, see also chapter 2.1), (ii) analyse more genetic sequences and could thus contribute to a more profound picture on paludomid phylogeny.

B) In order to verify the results of the molecular clock approach fossils should be included (African museum collections need to be scanned for fossil Paludomidae).

C) A systematic sampling of paludomids, especially *Lavigeria* and *Bridouxia*, is necessary in order to revise these genera and to delimitate taxonomic uncertainties.

D) A sampling of the biofilm and algae cover in Lake Tanganyika is essential in order to understand if teeth show not only adaptations to substrate but also to certain algae or biofilm conglomerates, but (i) data on biofilm and algae distribution in Lake Tanganyika is lacking, (ii) if this data was accessible we need data on the algae species that is taken in by the specific gastropod species, (iii) if this data was accessible we need to measure the mechanical properties of the biofilm and algae networks in experiments (see also above), (iv) the documentation of the precise interaction of the tooth with the algae is also essential but difficult, because for this the natural algae covers must be gathered from the lake's bottom and sheltered unaltered in aquariums.

E) Individual fitness experiments involving different paludomid species foraging together on a defined feeding substrate (e.g. rock) should be conducted (see above).

F) Phenotypic plasticity of teeth needs to be examined (specimens from different localities, from different algae covers, etc.).

G) The feeding substrate needs to be tested (e.g. hardness/elasticity of substrate, diameter of mud or sand particles, and adhesion between particles).

H) FEA should be employed on more species' teeth in order to build substantial hypotheses on radular tooth functions and to allocate adaptations.

I) Data on the chemical composition of teeth needs to be collected (by employing e.g. EDX) possibly allowing the relating of tooth hardness/elasticity values with a potential chemical origin.

J) Radular teeth could be rebuilt by 3D-printing with different materials or by printing inverse models serving as moulds which could be filled with e.g. silicone. The radular apparatus including membrane could be imitated and by integrating 3D teeth with different mechanical properties the tooth's behaviour (including of interaction between teeth, range of deflection, etc.) could be simulated. This could not only lead to a profound understanding of the role of functional gradients in radular teeth but could also contribute to the invention of robots. So far radulae were used as inspiration for soft gripping devices (Mangan et al. 2005); in the here presented case further research could add to the development of multifunctional robotic systems.

## Understanding radular function

The here established workflows (FEA, nanoindentation) could be applied to different radular types and gastropods with distinct ecological niches. Important is our finding that in some cases morphology does not consistently reflect ecology, but material properties do relate with ingesta. This gives the opportunity to potentially find adaptations in radular teeth of species which occur in the same habitats but have similar radular tooth morphologies (e.g. species of land snails, Stylommatophora, they have quite similar radular tooth morphologies). The comprehension of the interface radula but also radular tooth function could lead to a profound understanding of the organisms' ecology, their niches, and habitat requirements but could also contribute to the quest for the origins of Molluscan biodiversity and success.

#### <u>References</u>

- Aifantis, E.C., 1992. On the role of gradients in the localization of deformation and fracture. Int. J. Eng. Sci. 3, 1279–1299.
- Anderson, P.S.L., 2018. Making a point: shared mechanics underlying the diversity of biological puncture. J. Exp. Biol. 221, jeb187294.
- Attard, M.R.G., Parr, W.C.H., Wilson, L.A.B., Archer, M., Hand, S.J., Rogers, T.L., Wroe, S., 2014. Virtual reconstruction and prey size preference in the mid cenozoic thylacinid, *Nimbacinus dicksoni* (Thylacinidae, Marsupialia). PLoS One 9(4), e93088.
- Banerjee, S., 1988. Organisation of wing cuticle in *Locusta migratoria* Linnaeus, *Tropidacris cristata* Linnaeus and *Romalea microptera* Beauvis (Orthoptera: Acrididae). Int. J. Insect. Morphol. Embryol. 17, 313–326.
- Barber, A. H., Lu, D., Pugno, N. M., 2015. Extreme strength observed in limpet teeth. J. Royal Soc. Interface 12, 20141326.
- Bendsøe, M.P., 1989. Optimal shape design as a material distribution problem. Struct. Optim. 1, 193–202.
- Bendsøe, M.P., 1995. Optimization of Structural Topology, Shape and Material. Springer, Berlin.
- Bendsøe, M.P., Kikuchi, N., 1988. Generating optimal topologies in structural design using a homogenization method. Comput. Methods Appl. Mech. Eng. 71, 197–224.
- Benjamin, M., Toumi, H., Ralphs, J.R., Bydder, G., Best, T.M., Milz, S., 2006. Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. J. Anat. 208, 471–490.
- Berthold, T., 1990. Intralacustrine speciation and the evolution of shell sculpture in gastropods of ancient lakes application of Günther's niche concept. Abhandlungen des Naturwissenschaftlichen Vereins Hamburg (NF) 31/32, 85–118.
- Bingbing, A., Wang, R., Arola, D., Zhang, D., 2012. The role of property gradients on the mechanical behavior of human enamel. J. Mech. Behav. Biomed. Mater. 9, 63–72.
- Boss, K.J., 1978. On the evolution of gastropods in ancient lakes. In: Pulmonates Systematics, Evolution and Ecology, Vol. 2a (Eds. V. Fretter and J. Peake). Academic Press, London.
- Boßelmann, F., Romano, P., Fabritius, H., Raabe, D., Epple, M., 2007. The composition of the exoskeleton of two crustacea: the American lobster *Homarus americanus* and the edible crab *Cancer pagurus*. Thermochim. Acta 463, 65–68.
- Brooker, L., Lee, A., Macey, D., Van Bronswijk, W., Webb, J., 2003. Multiple-front iron-mineralisation in chiton teeth (*Acanthopleura echinata*: Mollusca: Polyplacophora). Marine Biology 142, 447– 454.
- Brooker, L.R., Shaw, J.A., 2012. The chiton radula: a unique model for biomineralization studies. Intech Open Access Publisher.

Brooks, J.L., 1950. Speciation in ancient lakes. Q. Rev. Biol. 25, 30–60, 131–176.

- Brown, D., 1994. Freshwater Snails of Africa and their Medical Importance. Taylor and Francis, London.
- Brown, D.S., Mandahl-Barth, G., 1987. Living molluscs of Lake Tanganyika: a revised and annotated list. J. Conchol. 32, 305–327.
- Büsse, S., Gorb, S.N., 2018. Material composition of the mouthpart cuticle in a damselfly larva (Insecta: Odonata) and its biomechanical significance. R. Soc. Open Sci. 5, 172117.
- Chan, Y.L., Ngan, A.H.W., King, N.M., 2011. Nano-scale structure and mechanical properties of the human dentine-enamel junction. J. Mech. Behav. Biomed. Mater. 4, 785–795.
- Chen, P.-Y., Lin, A.Y.M., McKittrick, J., Meyers, M.A., 2008. Structure and mechanical properties of crab exoskeletons. Acta Biomater. 4, 587–596.
- Coulter, G.W., 1991. Lake Tanganyika and its life. Oxford University Press, Oxford.
- Cunnington, W.A., 1920. The fauna of the African Lakes: a study in comparative limnology with special reference to Tanganyika. Proc. Zool. Soc. Lond. 1920, 507–622.
- Davis, J.L., Santana, S.E., Dumont, E.R., Grosse, I.R., 2010. Predicting bite force in mammals: twodimensional versus three-dimensional lever models. J. Exp. Biol. 213, 1844–1851.
- De Jager, N., Kler, M.D., Zel, J.M., 2006. The influence of different core material on the FEAdetermined stress distribution in dental crowns. Dent. Mater. 22, 234–242.
- Dumont, E.R., Grosse, I.R., Slater, G.J., 2009. Requirements for comparing the performance of finite element models of biological structures. J. Theor. Biol. 256, 96–103.
- Espinosa, H.D., Rim, J.E., Barthelat, F., Buehler, M.J., 2009. Merger of structure and material in nacre and bone perspectives on de novo biomimetic materials. Prog. Mater. Sci. 54, 1059–110.
- Evans, A.R., Hunter, J., Fortelius, M., Sanson, G.D., 2005. The scaling of tooth sharpness in mammals. Ann. Zool. Fennici 42, 603–613.
- Evans, A.R., Sanson, G.D., 1998. The effect of tooth shape on the breakdown of insects. J. Zool. 246, 391–400.
- Figueirido, B., Tseng, Z.J., Serrano-Alarcon, F.J., Martin-Serra, A., Pastor, J.F.F., 2014. Threedimensional computer simulations of feeding behaviour in red and giant pandas relate skull biomechanics with dietary niche partitioning. Biol. Lett. 10(4), 20140196.
- Fortuny, J., Marcé-Nogué, J., de Esteban-Trivigno, S., Gil, L., Galobart, Á., 2011. Temnospondyli bite club: ecomorphological patterns of the most diverse group of early tetrapods. J. Evol. Biol. 24(9), 2040–2054.
- Franz, C.J., 1990. Feeding patterns of *Fissurella* species on Isla de Margarita, Venezuela: use of radulae and food passage rates. J. Molluscan Stud. 56, 25–35.
- Frazzetta, T.H., 1988. The mechanics of cutting and the form of shark teeth (Chondrichthyes, Elasmobranchii). Zoomorphology 108, 93–107.
- Freeman, P.W., Lemen, C.A., 2007. The trade-off between tooth strength and tooth penetration:

predicting optimal shape of canine teeth. J. Zool. 273, 273–280.

- Freeman, P.W., Weins, W.N., 1997. Puncturing ability of bat canine teeth: the tip. In: Life Among the Muses: Papers in Honor of J. S. Findley (Ed. T.L. Yates, W.L. Gannon & D.E. Wilson). NM: Museum of Southwestern Biology, Albuquerque.
- Fretter, V., Graham, A., 1962. British prosobranch mollusca, their functional anatomy and ecology. Ray Society, London.
- Glaubrecht, M., Köhler, F., 2004. Radiating in a river: systematics, molecular genetics and morphological differentiation of viviparous freshwater gastropods endemic to the Kaek River, central Thailand. Biol. J. Linn. Soc. 82(3), 275–311.
- Glaubrecht, M., 2008. Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: morphology and systematization of a paludomid species flock in an ancient lake. Zoosystematics Evol. 84, 71–122.
- Glaubrecht, M., Rintelen, T.v., 2008. The species flocks of lacustrine gastropods: *Tylomelania* on Sulawesi as models in speciation and adaptive radiation. Proceedings of the "Speciation in Ancient Lake IV" Symposium, Berlin. Hydrobiologia 615, 181–199.
- Glaubrecht, M., Strong, E.E., 2007. Ancestry to an endemic radiation in Lake Tanganyika? Evolution of the viviparous gastropod *Potadomoides* Leloup, 1953 in the Congo River system (Cerithioidea, Paludomidae). Biol. J. Linn. Soc. 92(2), 367–401.
- Gorb, S.N., Beutel, R.G., Gorb, E.V., Jiao, Y., Kastner, V., Niederegger, S., Popov, V.L., Scherge, M., Schwarz, U., Vötsch, W., 2002. Structural design and biomechanics of friction-based releasable attachment devices in insects. Integr. Comp. Biol. 42, 1127–1139.
- Haas, F., Gorb, S.N., Blickhan, R., 2000b. The function of resilin in beetlewings. Proc. R. Soc. Lond. B 267, 1375–1381.
- Haas, F., Gorb, S.N., Wootton, R.J., 2000a. Elastic joints in dermapteran hind wings: materials and wing folding. Arthropod Struc. Dev. 29, 137–146.
- Han, Y., Liu, C., Zhou, D., Li, F., Wang, Y., Han, X., 2011. Magnetic and structural properties of magnetite in radular teeth of chiton *Acanthochiton rubrolinestus*. Bioelectromagnetics 32, 226– 233.
- Heinemann, F., Launspach, M., Gries, K., Fritz, M., 2011. Gastropod nacre: structure, properties and growth biological, chemical and physical basics. Biophys. Chem. 153, 126–153.
- Hepburn, H.R., Chandler, H.D., 1976. Material properties of arthropod cuticles: the arthrodial membranes. J. Comp. Physiol. 109, 177–198.
- Herrera, S.A., Grunenfelder, L., Escobar, E., Wang, Q., Salinas, C., Yaraghi, N., Geiger, J., Wuhrer, R.,
  Zavattieri, P., Kisailus, D., 2015. Stylus support structure and function of radular teeth in
  *Cryptochiton stelleri*. 20th International Conference on Composite Materials Copenhagen, 19-24th July 2015.
- Hickman, C.S., 1980. Gastropod radulae and the assessment of form in evolutionary paleontology. Paleobiology 6, 276–294.

- Hickman, C.S., 1983. Ecological and phylogenetic implications of the unusual radula of *Laevinesta atlantica* (Mollusca, Gastropoda). Veliger 25, 323–325.
- Hickman, C.S., 1984. Implications of radular tooth-row functional-integration for archaeogastropod systematics. Malacologia 25, 143–160.
- Hiew, S.H., Miserez, A., 2017. Squid sucker ring teeth: multiscale structure–property relationships, sequencing, and protein engineering of a thermoplastic biopolymer. ACS Biomater. Sci. Eng. 3 (5), 680–693.
- Hilgers, L., Hartmann, S., Pfaender, J., Lentge-Maaß, N., Rintelen, T.v., Hofreiter, M., 2020. Radula diversification promotes ecomorph divergence in an adaptive radiation of freshwater snails. bioRxiv.01.17.910034.
- Hua, T.-E., Li, C.-W., 2007. Silica biomineralization in the radula of a limpet *Notoacmea schrenckii* (Gastropoda: Acmaeidae). Zoological Studies 46, 379–388.
- Huber, D.R., Eason, T.G., Hueter, R.E., Motta, P. J., 2005. Analysis of the bite force and mechanical design of the feeding mechanism of the durophagous horn shark *Heterodontus francisci*. J. Exp. Biol. 208, 3553–3571.
- Imbeni, V., Kruzic, J.J., Marshall, G.W., Marshall, S.J., Ritchie, R.O., 2005. The dentin-enamel junction and the fracture of human teeth. Nat. Mater. 4, 229–232.
- Jensen, K.R., 1993. Morphological adaptations and plasticity of radular teeth of the Sacoglossa (= Ascoglossa) (Mollusca: Opisthobranchia) in relation to their food plants. Biol. J. Linn. Soc. 48(2), 135–155.
- Johnston, M.R., Cohen, A.S., 1987. Morphological divergence in endemic gastropods from Lake Tanganyika: implications for models of species flock formation. Palaios 2, 413–425.
- Jones, D., Evans, A.R., Siu, K.K.W., Rayfield, E.J., Donoghue, P.C.J., 2012. The sharpest tools in the box? Quantitative analysis of conodont element functional morphology. Proc. R. Soc. B 279, 2849–2854.
- Jones, E.I., McCance, R.A., Shackleton, L.R.B., 1935. The role of iron and silica in the structure of the radular teeth of certain marine molluscs. J. Exp. Biol. 12, 59–64.
- Jones, M.E.H., Gröning, F., Dutel, H., Sharp, A.C., Fagan, M.J., Evans, S.E., 2017. The biomechanical role of the chondrocranium and sutures in a lizard cranium. J. R. Soc. Interface 14(137), 20170637.
- Jüch, P.J.W., Boeksehoten, G.J., 1980. Trace fossils and grazing traces produced by *Littorina* and *Lepidochitona*, Dutch Wadden Sea. Geologie en Mijnbouw 59, 33–42.
- Klein, M.-C.G., Deuschle, J.K., Gorb, S.N., 2010. Material properties of the skin of the Kenyan sand boa *Gangylophis colubrinus* (Squamata, Boidae). J. Comp. Physiol. A 196, 659–668.
- Klein, M.-C.G., Gorb, S.N., 2012. Epidermis architecture and material properties of the skin of four snake species. J. R. Soc. Interface 9, 3140–3155.
- Köhler, F., Glaubrecht, M., 2010. Uncovering an overlooked radiation: morphological and mitochondrial DNA differentiation in endemic freshwater snails on Madagascar

(Caenogastropoda: Pachychilidae) and their biogeography. Biol. J. Linn. Soc. 99(4), 867–894.

- Krings, W., Faust, T., Kovalev, A., Neiber, M.T., Glaubrecht, M., Gorb, S.N., 2019. In slow motion: radula motion pattern and forces exerted to the substrate in the land snail *Cornu aspersum* (Mollusca, Gastropoda) during feeding. R. Soc. Open Sci. 6(7), 20545703.
- Lee, A.P., Brooker, L.R., Macey, D.J., Webb, J., van Bronswijk, W., 2003. A new biomineral identified in the cores of teeth from the chiton *Plaxiphora albida*. J. Biol. Inorg. Chem. 8, 256–262.
- Liddiard, K.J., Hockridge, J.G., Macey, D.J., Webb, J., van Bronswijk, W., 2004. Mineralisation in the teeth of the limpets *Patelloida alticostata* and *Scutellastra laticostata* (Mollusca: Patellogastropoda). Molluscan Res. 24, 21–31.
- Lin, C.P., Douglas, W.H., Erlandsen, S.L., 1993. Scanning electron microscopy of type I collagen at the dentin-enamel junction of human teeth. J. Histochem. Cytochem. 41, 381–388.
- Liu, Z., Meyers, M.A., Zhang, Z., Ritchie, R.O., 2017. Functional gradients and heterogeneities in biological materials: Design principles, functions, and bioinspired applications. Progress in Materials Science 88, 467–498.
- Liu, Z., Zhu, Y., Jiao, D., Weng, Z., Zhang, Z., Ritchie, R.O., 2016. Enhanced protective role in materials with gradient structural orientations: Lessons from nature. Acta Biomater. 44, 31–40.
- Liu, Z.Q., Jiao, D., Weng, Z.Y., Zhang, Z.F., 2016. Structure and mechanical behaviors of protective armored pangolin scales and effects of hydration and orientation. J. Mech. Behav. Biomed. Mater. 56, 165–174.
- Lowenstam, H.A., 1962. Goethite in radular teeth of recent marine gastropods. Science 137, 279–280.
- Lu, D., Barber, A.H., 2012. Optimized nanoscale composite behaviour in limpet teeth. J. Royal Soc. Interface 9, 1318–1324.
- Lu, H.H., Thomopoulos, S., 2013. Functional attachment of soft tissues to bone: development, healing, and tissue engineering. Annu. Rev. Biomed. Eng. 15, 201–226.
- Mackenstedt, U., Märkel, K., 1987. Experimental and comparative morphology of radula renewal in pulmonates (Mollusca, Gastropoda). Zoomorphology 107, 209–239.
- Mackenstedt, U., Märkel, K., 2001. Radular structure and function. In: The Biology of Terrestrial Molluscs (Ed. G.M. Barker), pp. 213–236. CABI Publishing, Oxon.
- Mangan, E.V., Kingsley, D.A., Quinn, R.D., Sutton, G.P., Mansour, J.M., Chiel, H.J., 2005. A biologically inspired gripping device. Industrial Robot 32, 49–54.
- Marcé-Nogué, J., Püschel, T.A., Kaiser, T.M., 2017. A biomechanical approach to understand the ecomorphological relationship between primate mandibles and diet. Sci. Rep. 7, 8364.
- Marshall, G.W., Balooch, M., Gallagher, R.R., Gansky, S.A., Marshall, S.J., 2001. Mechanical properties of the dentinoenamel junction: AFM studies of nanohardness, elastic modulus, and fracture. J. Biomed. Mater. Res. 54, 87–95.
- Martens, K., 1997. Speciation in ancient lakes. Trends Ecol. Evol. 12(5), 177–182.
Mayr, E., 1963. Animal, species, and evolution. Harvard University Press, Cambridge.

Mayr, E., Ashlock, P.D., 1991. Principles of systematic zoology. McGraw-Hill, New York.

- Michel, E., 1994. Why snails radiate: a review of gastropod evolution in long-lived lakes, both Recent and fossil. In: Speciation in ancient lakes (Eds. K. Martens, B. Goddeeris, G. Coulter). Advances in Limnology, Stuttgart.
- Michel, E., 2000. Phylogeny of a gastropod species flock: exploring speciation in Lake Tanganyika in a molecular framework. In: Biology of Ancient Lakes. Advances in Ecological Research. (Ed. A. Rossiter). Academic Press, London.
- Michel, E., 2004. *Vinundu*, a new genus of Gastropod (Cerithioidea: 'Thiaridae) with two species from Lake Tanganyika, east Africa, and its molecular phylogenetic relationships. J. Molluscan Stud. 70, 1–19.
- Michel, E., Cohen, A.S., West, K., Johnston, M.R., Kat, P.W., 1992. Large African lakes as natural laboratories for evolution: examples from the endemic gastropod fauna of Lake Tanganyika. Mitteilungen der Internationalen Vereinigung für Limnologie 23, 85–99.
- Michels, J., Vogt, J., Gorb, S.N., 2012. Tools for crushing diatoms opal teeth in copepods feature a rubber-like bearing composed of resilin. Sci. Rep. 2, 465.
- Miserez, A., Li, Y., Waite, J.H., Zok, F., 2007. Jumbo squid beaks: inspiration for design of robust organic composites. Acta Biomater. 3, 139–149.
- Miserez, A., Rubin, D., Waite, J.H., 2010. Cross-linking Chemistry of Squid Beak. J. Biol. Chem. 285(49), 38115–38124.
- Miserez, A., Schneberk, T., Sun, C., Zok, F.W., Waite, J.H., 2008. The transition from stiff to compliant materials in squid beaks. Science 319, 1816–1819.
- Miserez, A., Weaver, J.C., Pedersen, P.B., Schneeberk, T., Hanlon, R.T., Kisailus, D., Birkedal, H., 2009. Microstructural and biochemical characterization of the nanoporous sucker rings from *Dosidicus gigas*. Adv. Mater. 21, 401–406.
- Miura, O., Urabe, M., Nishimura, T., Nakai, K., Chiba, S., 2018. Recent lake expansion triggered the adaptive radiation of freshwater snails in the ancient Lake Biwa. Evolution Letters 3(1), 43–54.
- Miura, S., Saito, R., Parque, V., Miyashita, T., 2019. Design factors for determining the radula shape of *Euhadra peliomphala*. Sci. Rep. 9(1), 749.
- Morris, T.E., Hickman, C.S., 1981. A method for artificially protruding gastropod radulae and a new model of radula function. Veliger 24, 85–89.
- Muschick, M., Indermaur, A., Salzburger, W., 2012. Convergent evolution within an adaptive radiation of cichlid fishes. Curr. Biol. 22, 2362–2368.
- Nassif, N., Pinna, N., Gehrke, N., Antonietti, M., Jager, C., Colfen, H., 2005. Amorphous layer around aragonite platelets in nacre. PNAS 102, 12653–12655.
- Nemoto, M., Wang, Q., Li, D., Pan, S., Matsunaga, T., Kisailus, D., 2012. Proteomic analysis from the mineralized radular teeth of the giant Pacific chiton, *Cryptochiton stelleri* (Mollusca). Proteomics

12, 2890–2894.

Neville, A.C., 1975. Biology of the arthropod cuticle. Springer Verlag, Berlin.

- Osche, G., 1962. Das Praeadaptationsphänomen und seine Bedeutung für die Evolution. Zool. Anz. 169, 14–49.
- Padilla, D.K., 1985. Structural resistance of algae to herbivores. A biomechanical approach. Mar. Biol. 90, 103–109.
- Padilla, D.K., 1989. Algal structural defenses: form and calcification in resistance to tropical limpets. Ecology 70, 835–842.
- Padilla, D.K., 2004. Form and function of radular teeth of herbivorous molluscs: Focus on the future. Am. Malacolog. Bull. 18, 163–168.
- Padilla, D.K., Dittman, D.E., Franz, J., Sladek, R., 1996. Radular production rates in two species of *Lacuna* Turton (Gastropoda: Littorinidae). J. Molluscan Stud. 62, 275–280.
- Peisker, H., Michels, J., Gorb, S.N., 2013. Evidence for a material gradient in the adhesive tarsal setae of the ladybird beetle *Coccinella septempunctata*. Nat. Commun. 4, 1661.
- Piras, P., Maiorino, L., Teresi, L., Meloro, C., Lucci, F., Kotsakis, T., Raia, P., 2013. Bite of the cats: Relationships between functional integration and mechanical performance as revealed by mandible geometry. Syst. Biol. 62(6), 878–900.
- Popowics, T.E., Fortelius, M., 1997. On the cutting edge: tooth blade sharpness in herbivorous and faunivorous mammals. Ann. Zool. Fennici 34, 73–88.
- Raabe, D., Sachs, C., Romano, P., 2005. The crustacean exoskeleton as an example of a structurally and mechanically graded biological nanocomposite material. Acta Mater. 53, 4281–4292.
- Rajabi, H., Darvizeh, A., Shafiei, A., Taylor, D., Dirks, J.H., 2015. Numerical investigation of insect wing fracture behaviour. J. Biomech. 48, 89–94.
- Rajabi, H., Shafiei, A., Darvizeh, A., Dirks, J.-H., Gorb, S.N., 2016b. Effect of microstructure on the mechanical and damping behaviour of dragonfly wing veins. R. Soc. Open Sci. 3, 160006.
- Rajabi, H., Shafiei, A., Darvizeh, A., Gorb, S.N., 2016a. Resilin microjoints: a smart design strategy to avoid failure in dragonfly wings. Sci. Rep. 6, 39039.
- Rayfield, E.J., 2007. Finite Element Analysis and Understanding the Biomechanics and Evolution of Living and Fossil Organisms. Annual Review of Earth and Planetary Sciences 35(1), 541–576.
- Reid, D.G., 1996. Systematics and Evolution of *Littorina*. Ray Society, London.
- Rintelen, T.v., Bouchet, P., Glaubrecht, M., 2007. Ancient lakes as hotspots of diversity: a morphological review of an endemic species flock of *Tylomelania* (Gastropoda: Cerithioidea: Pachychilidae) in the Malili lake system on Sulawesi, Indonesia. Hydrobiologia 592(1), 11–94.
- Rintelen, T.v., Glaubrecht, M., 2005. Anatomy of adaptive radiation: a unique reproductive strategy in the endemic freshwater gastropod *Tylomelania* (Cerithiodea: Pachychilidae) on Sulawesi, Indonesia and its biogeographical implications. Biol. J. Linn. Soc. 85(4), 513–542.

- Rintelen, T.v., Rintelen, K.v., Glaubrecht, M., 2010. The species flocks of the viviparous freshwater gastropod *Tylomelania* (Mollusca: Cerithioidea: Pachychilidae) in the ancient lakes of Sulawesi, Indonesia: The role of geography, trophic morphology and color as driving forces in adaptive radiation. In: Evolution in Action. Case Studies in Adaptive Radiation, Speciation and the Origin of Biodiversity (Ed. M. Glaubrecht). Springer, Heidelberg.
- Rintelen, T.v., Wilson, A.B., Meyer, A., Glaubrecht, M., 2004. Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. Proc. R. Soc. Lond. 271(1557), 2541–2549.
- Runham, N.W., Thornton, P.R., 1967. Mechanical wear of the gastropod radula: a scanning electron microscope study. J. Zool. 153, 445–452.
- Saltin, B.D., Matsumura, Y., Reid, A., Windmill, J.F., Gorb, S.N., Jackson, J.C., 2019. Material stiffness variation in mosquito antennae. J. R. Soc. Interface 16(154), 20190049.
- Saunders, M., Kong, C., Shaw, J.A., Clode, P.L., 2011. Matrix-mediated biomineralization in marine mollusks: A combined transmission electron microscopy and focused ion beam approach. Microsc. Microanal. 17, 220–225.
- Serrano-Fochs, S., de Esteban-Trivigno, S., Marcé-Nogué, J., Fortuny, J., Fariña, R.A., 2015. Finite Element Analysis of the Cingulata Jaw: An Ecomorphological Approach to Armadillo's Diets. PLoS One 10(6), e0120653.
- Sharp, A.C., 2015. Comparative finite element analysis of the cranial performance of four herbivorous marsupials. J. Morphol. 276(10), 1230–1243.
- Shaw, J.A., Brooker, L.R., Macey, D.J., 2002. Radula tooth turnover in the chiton, *Acanthopleura hirtosa* (Blainville, 1825) (Mollusca: Polyplacophora). Molluscan Res. 22, 93–99.
- Shaw, J.A., Macey, D.J., Brooker, L.R., Clode, P.L., 2010. Tooth use and wear in three ironbiomineralizing mollusc species. Biol. Bull. 218, 132–144.
- Shaw, J.A., Macey, D.J., Brooker, L.R., Stockdale, E.J., Saunders, M., Clode, P.L., 2009. The chiton stylus canal: An element delivery pathway for tooth cusp biomineralization. J. Morphol. 270, 588–600.
- Shergold, O.A., Fleck, N.A., 2005. Experimental investigation into the deep penetration of soft solids by sharp and blunt punches, with application to the piercing of skin. Trans. ASME 127, 838–848.
- Simone, L., 2011. Phylogeny of the Caenogastropoda (Mollusca), based on comparative morphology. Arq. Zool. 42(4), 161–323.
- Sollas, I.B.J., 1907. The molluscan radula: Its chemical composition, and some points in its development. J. Cell Sci. s2-51, 115–136.
- Soons, J., Genbrugge, A., Podos, J., Adriaens, D., Aerts, P., Dirckx, J., Herrel, A., 2015. Is Beak Morphology in Darwin's Finches Tuned to Loading Demands? PLoS One 10(6), e0129479.
- Steneck, R.S., Watling, L., 1982. Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. Marine Biology 68, 299–319.

- Strong, E.E., Glaubrecht, M., 2010. Anatomy of the Tiphobiini from Lake Tanganyika (Cerithioidea, Paludomidae). Malacologia 52(1), 115–153.
- Tan, Y.P., Hoon, S., Guerette, P.A., Wei, W., Ghadban, A., Hao, C., Miserez, A., Waite, J.H., 2015. Infiltration of chitin by protein coacervates defines the squid beak mechanical gradient. Nat. Chem. Biol. 11, 488–495.
- Tertuliano, O.A., Greer, J.R., 2016. The nanocomposite nature of bone drives its strength and damage resistance. Nat. Mater. 15, 1195–1202.
- Van Damme, D., Pickford, M., 2003. The late Cenozoic Thiaridae (Mollusca, Gastropoda, Cerithioidea) of the Albertine Rift Valley (Uganda-Congo) and their bearing on the origin and evolution of the Tanganyikan thalassoid malacofauna. Hydrobiologia 498, 1–83.
- van der Meij, M.A.A., Bout, R.G., 2004. Scaling of jaw muscle size and maximal bite force in finches. J. Exp. Biol. 207, 2745–2753.
- van der Wal, P., Giesen, H., Videler, J., 2000. Radular teeth as models for the improvement of industrial cutting devices. Mater. Sci. Eng. C 7, 129–142.
- Wang, B., Yang, W., Sherman, V.R., Meyers, M.A., 2016. Pangolin armor: overlapping, structure, and mechanical properties of the keratinous scales. Acta Biomater. 41, 60–74.
- Wang, C., Li, Q.Y., Wang, S.N., Qu, S.X., Wang, X.X., 2014. Microstructure and self-sharpening of the magnetite cap in chiton tooth. Mater. Sci. Eng. C 37, 1–8.
- Wang, L.-Y., Jafarpour, M., Lin, C.-P., Appel, E., Gorb, S.N., Rajabi, H., 2019. Endocuticle sclerotisation increases the mechanical stability of cuticle. Soft Matter 15, 8272.
- Wang, Q., Nemoto, M., Li, D., Weaver, J.C., Weden, B., Stegemeier, J., Bozhilov. K.N., Wood, L.R.,
  Milliron, G.W., Kim, C.S., DiMasi, E., Kisailus, D., 2013. Phase transformations and structural
  developments in the radular teeth of *Cryptochiton stelleri*. Adv. Funct. Mater. 23, 2908–2917.
- Wealthall, R.J., Brooker, L.R., Macey, D.J., Griffin, B.J., 2005. Fine structure of the mineralized teeth of the chiton *Acanthopleura echinata* (Mollusca: Polyplacophora). J. Morphol. 265, 165–175.
- Weaver, J.C., Wang, Q., Miserez, A., Tantuccio, A., Stromberg, R., Bozhilov, K.N., Maxwell, P., Nay, R., Heier, S.T., Di Masi, E., 2010. Analysis of an ultra hard magnetic biomineral in chiton radular teeth. Mater. Today 13, 42–52.
- West, K., Cohen, A., 1996. Shell microstructure of gastropods from Lake Tanganyika, Africa: adaptation, convergent evolution, and escalation. Evolution 50(2), 672–681.
- West, K., Michel, E., 2000. The dynamics of endemic diversification; molecular phylogeny suggests an explosive origin of the thiarid gastropods of Lake Tanganyika. Adv. Ecol. Res. 31, 331–354.
- West, K., Michel, E., Todd, J., Brown, D., Clabaugh, J., 2003. The Gastropods of Lake Tanganyika: Diagnostic key, classification and notes on the fauna. Special publications: Societas Internationalis Limnologiae - Int. Assoc. of Theoretical and Applied Limnology.
- Whitenack, L.B., Motta, P.J., 2010. Performance of shark teeth during puncture and draw: implications for the mechanics of cutting. Biol. J. Linn. Soc. Lond. 100, 271–286.

Wilson, A.B., Glaubrecht, M., Meyer, A., 2004. Ancient lakes as evolutionary reservoirs: evidence

from the thalassoid gastropods of Lake Tanganyika. Proc. Royal Soc. Lond. B 271(1538), 529–536.

Wroe, S., McHenry, C., Thomason, J., 2005. Bite club: comparative bite force in big biting mammals and the prediction of predatory behaviour in fossil taxa. Proc. R. Soc. Lond. Ser. B 272, 619–625.

## Chapter 6. List of Publications

### Published or accepted:

- Krings, W., Kovalev, A., Glaubrecht, M., Gorb, S.N., 2019. Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods. Zoology 137, 125713. https://doi.org/10.1016/j.zool.2019.125713.
- Krings, W., Brütt, J.-O., Gorb, S.N., Glaubrecht, M., 2020. Tightening it up: Diversity of the Chitin Anchorage of Radular-Teeth in paludomid Freshwater-Gastropods. Accepted for publication in Malacologica.
- Krings, W., Marcé-Nogué, J., Karabacak, H., Glaubrecht, M., Gorb, S.N., 2020. Finite element analysis of individual taenioglossan radular teeth (Mollusca). Accepted for publication in Acta Biomaterialia.

#### Under review:

Krings, W., Neiber, M.T., Kovalev, A., Gorb, S.N., Glaubrecht, M., Trophic specialisation reflected by radular tooth material properties in an 'ancient' Lake Tanganyikan gastropod species flock. Under review in BMC Evolutionary Biology.

## To be submitted soon:

Krings, W., Neiber, M.T., Glaubrecht, M., Filling the lake: tracing convergent trophic specialization in the evolution of paludomid gastropods in Lake Tanganyika.

# Beiträge/Contributions

*Filling the lake: tracing convergent trophic specialization in the evolution of paludomid gastropods in Lake Tanganyika:* 

Matthias Glaubrecht initiated and designed the study, Wencke Krings and Marco Neiber compiled and analysed the data. Wencke Krings drew the figures and wrote drafts of the manuscript together with the other two authors who contributed equally to the final version of the paper.



Prof. Dr. Stanislav Gorb



### Tightening it up:

## Diversity of the Chitin Anchorage of Radular-Teeth in paludomid Freshwater-Gastropods:

Matthias Glaubrecht and Wencke Krings conceived this study. Jan-Ole Brütt and Wencke Krings prepared the samples including histology, analysed the data, and worked on the manuscript. Wencke Krings analysed the data, drew the figures, and wrote the manuscript. Stanislav N. Gorb contributed to the overall question in the context of functional morphology and worked on the manuscript. Matthias Glaubrecht as malacologist is an expert in the Tanganyikan gastropod fauna and collected most specimens. All authors contributed to the manuscript and approved the final version of the manuscript.



Prof. Dr. Stanislav Gorb

Susanne DN: cn=Susanne Dobler

Digital unterschrieben von Susanne Dobler Dobler, o, ou, email=susanne.dobler@ uni-hamburg.de, c=DE Datum: 2020.07.27 10:20:21 +02'00'

# Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods:

Wencke Krings wrote the manuscript, drew the figures, performed nanoindentation and analysed the data. Alexander Kovalev is an expert in nanoindentation; he also discussed the data. Matthias Glaubrecht initialized the project and its design, discussed and planed; his expertise in malacology contributed greatly to the connection to molluscan biology. Stanislav N. Gorb designed the experiments and discussed the data, the manuscript, the figures; his expertise was critical for understanding the results and the functional morphology. All authors contributed to the manuscript.



Prof. Dr. Stanislav Gorb

Dobler

Digital unterschrieben von Susanne Dobler Susanne DN: cn=Susanne Dobler, o, ou, email=susanne.dobler@ uni-hamburg.de, c=DE Datum: 2020.07.27 10:20:49 +02'00'

### Finite element analysis of individual taenioglossan radular teeth (Mollusca):

Wencke Krings wrote the manuscript, drew the figures, generated data for the model conditions and analysed the FEA data. Jordi Marcé-Nogué is an expert in FEA and conducted all analyses, discussed the data and wrote the manuscript. Hasan Karabacak provided the 3D model for this analysis in the context of his bachelor thesis and discussed results. Matthias Glaubrecht helped to connect the biomechanical results to molluscan biology. Stanislav N. Gorb initiated, designed and planned this study, discussed the data, the manuscript, the figures; his expertise was critical for understanding the results and the functional morphology. All authors contributed to the final version of the manuscript.

Prof. Dr. Stanislav Gorb

Susanne DN: cn=Susanne Dobler, Dobler

Digital unterschrieben von Susanne Dobler o, ou, email=susanne.dobler@ uni-hamburg.de, c=DE Datum: 2020.07.27 10:21:27 +02'00'

Trophic specialisation reflected by radular tooth material properties in an "ancient" Lake Tanganyikan gastropod species flock:

Wencke Krings wrote the manuscript, with contributions by Matthias Glaubrecht, drew the figures, performed nanoindentation and analysed the data. Alexander Kovalev supported the experiments and discussed data. Stanislav N. Gorb discussed the manuscript and the figures; his expertise was critical for understanding the results and the functional morphology. Matthias Glaubrecht, in addition to writing parts of the manuscript, initialized and planed the study and discussed the data his expertise was critical for understanding the evolution of the paludomid gastropods. All authors contributed to the manuscript.



Prof. Dr. Stanislav Gorb

Susanne Dobler DN: cn=Susanne Dobler, Dobler

Digital unterschrieben von Susanne Dobler o, ou, email=susanne.dobler@ uni-hamburg.de, c=DE Datum: 2020.07.27 10:21:58 +02'00'

# Eidesstattliche Versicherung/Declaration on oath

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than acknowledged resources and aids.

Nip

Hamburg, den 13.07.2020

-

# Danksagung

An dieser Stelle möchte ich mich bei allen bedanken, die mich bei meinem Studium und bei der Vollendung meiner Promotion unterstützt haben:

Zuallererst muss ich herzlich meinen Betreuern Stanislav Gorb und Susanne Dobler sowie den Mitgliedern der Kommission danken, ohne Eure Unterstützung, Eure Zeit und Eure Energie wäre die vorliegende Arbeit nicht möglich gewesen. Danke, dass ihr das Thema Radula-Funktion so unermüdlich und tatkräftig unterstützt habt. Bei Matthias Glaubrecht möchte ich mich dafür bedanken, dass er mich den Paludomidae des Tanganjikasees vorgestellt und mir die Möglichkeit gegeben hat, dieses Thema zu bearbeiten. Ich habe diese Tiere und auch das Thema sehr zu schätzen gelernt.

Meine Hamburger Arbeitsgruppe hat mich in all den Jahren immer unterstützt, sowohl bei der gemeinsamen Forschung aber auch bei privaten Themen. Eine herzliche Umarmung an Marco Neiber, Benedikt Wiggering, Simon Bober und Nora Lentge-Maaß – ihr hattet immer ein offenes Ohr für mich. Bei meinen Koautoren möchte ich mich auch überaus herzlich bedanken, bei Cennet Gersthage, Alexandra Tobiasch und Jordi Marcé-Nogué. Renate Walter, Peter Stutz, Frederick Jessen, Alexander Daasch, Lena Schwinger, Katharina Gebauer, Stephanie Köhnk – ihr wart immer eine großartige Unterstützung, ob am REM, beim Einbetten der Proben, beim 3D-Druck, bei Färbereihen oder im Molekularlabor. Bernhard Hausdorf, Frank Walther, Sonja Bamberger, schön, dass wir uns immer viel über Schnecken austauschen konnten und dass ich Euch immer um Hilfe fragen durfte. Das Studienbüro war immer direkt zur Stelle, "Danke" an Petra Ziemer, Rene König und Markus Brändel. Hiermit möchte ich mich auch herzlich bei Alexander Haas dafür bedanken, dass er mir die 3D-Visualisierung beigebracht hat, die Masterarbeit hat meine wissenschaftlichen Interessen stark beeinflusst und gefördert.

Ein besonderer Dank gilt den Studenten und den FÖJlern, die meinen Weg gekreuzt haben: Taissa Faust, Hasan Karabacak, Carolin Scheel, Ole Brütt, Christine Hempel, Charlotte Neumann, Lisa Siemers, Nicole Martins, danke, dass ihr in all den Jahren Radula-Projekte mit vorangetrieben habt und diese dann zu unseren gemeinsamen Interessen geworden sind. Liebe CeNaker und Institutler, ihr habt mir meine Arbeit im Alltag, entweder in der Lehre oder auf dem Flur, überaus vergnüglich und angenehm gestaltet: Frank Friedrich, Alexandra Kerbl, Andreas Schmidt-Rhaesa, Ilka Sötje, Eva Zimmermann, Amanda Bauzá, Cordula Bracker, Karolin Engelkes, Oliver Hawlitschek, Rossen Kavrakov, Henrik Kusche, Martin Kubiak, Julian Glos, Caroline Stolter, Martina Mistera, Oliver Hallas – schön, dass wir so viel zu lachen hatten. Im erweiterten CeNak-Kreis gilt mein Dank Ellen Schulz-Kornas, Daniela Winkler, Heinrich Mallison – ihr habt für viele Denkanstöße und neue Einblicke gesorgt.

Ganz besonders möchte ich das Museumsteam hervorheben, Daniel Bein, Marie Rahn, Dennis Nitz, Laura Marrero Palma, Jan Wollenberg, Matthias Preuß, Levke Peters, Nadja Dahdouli, Linda Kuçi, Jakob Hallermann, Lara Wöhlkens, Holger Krohn, Timo Zeimet, Katrin Hoyer, Laura Jürgens, Imke Petersen, Rina Geiger, Pauline Falkenberg, Jana Hinners – mit Euch zu arbeiten war immer großartig!

Bei den Kielern möchte ich mich nicht nur dafür bedanken, dass ihr mich im Labor an Eure Geräte gelassen habt und mir immer mit Rat und Tat zur Seite standet, – ihr habt mich überaus herzlich aufgenommen. Einen herzlichen Dank an Yoko Matsumura, Alexander Kovalev, Thies Büscher, Sebastian Büsse, Jan Michels, Emre Kizilkan, Yun Ma, Hamed Rajabi, Clemens Schaber, Angela Veenendaal, Esther Appel, Joachim Oesert, Nienke Bijma, Mohsen Jafarpour, Chuchu Li, Dennis Petersen, Alexander Köhnsen, Fabian Bäumler – ach, eigentlich an alle.

Lieber Heinz Büscher, über Deine Päckchen mit Schnecken oder USB-Sticks mit Habitat-Bildern und –Videos habe ich mich immer sehr gefreut. Das waren aufregende Momente an der Pförtnerloge.

Und ganz besonders möchte ich mich bei meiner Familie und meinen Freunden bedanken – bei Thomas, Philip, Elisabeth, meinen Eltern, Frauke, Tobias, Maredon, Jens – danke, dass ihr mir in der ganzen Zeit den Rücken freigehalten habt und immer geduldig und verständnisvoll wart.