More leaves on the phylogenetic tree of Scaphopoda (Bronn, 1862): Work in progress

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Despite the global marine distribution, the taxon of Scaphopoda is notoriously understudied. The evolutionary position of this infaunal group within the molluscs is as disputed as the internal systematics. Due to the uncertain assignment of earlier fossils to the Scaphopoda, this taxon is listed as the youngest major group of molluscs (~360 mya). The greatest diversity of scaphopod species is recorded from depths below 200 m and tropical shallow water habitats. Diagnoses of the 576 recent species are mostly based on shell characters. Only in some cases the description is supplemented by radula features. Scaphopod systematics was investigated in several morphological studies based on softbody characters. Although the major subtaxa are well defined, the validity of many family and genus-rank taxa remains unresolved. Two molecular phylogenetic studies suffered from limited taxon sampling and agreed with the morphological studies on a robust support for the subtaxa Dentaliida and Gadilida only. The present study is mostly based on Indo-Pacific dentaliid

specimens from the National Museum of Natural History, Paris. Nuclear marker sequences of the *18S* rRNA and *28S* rRNA genes and the internal transcribed spacer (*ITS*) as well as mitochondrial markers, i.e., *16S* rRNA gene and *cytochrome c oxidase subunit 1* gene (*CO1*) were analysed. The study will improve our knowledge on the phylogenetic signal of these markers, which, once established, will allow to increase taxon sampling considerably. We will test the monophyly of some of the doubtful genus-level taxa and the questionable shell characters they are based on and eventually obtain new insights into the poorly studied phylogeny of this group.

European mollusks: Systematic, description, distribution, determination key, images and literature: Short project presentation

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A project aiming at the development of a comprehensive database for European mollusks, including a determination tool, is presented. An overview on the present situation is given, analyzing the strengths/weaknesses of existing works (books, checklists, databases, webpages, determination tools), in terms of completeness regarding known taxa, availability / quality of descriptions, data on distribution, completeness and quality of images, availability/usefulness of a determination key/tool, clearness of terminology, availability of a glossary, literature list / availability of links. The results demonstrate that fairly complete and useful determination tools exist for very few areas (classes / geographic ranges / habitats and combinations thereof) only. The goals of the project regarding the criteria mentioned above are illustrated and compared with existing works. The benefit of such a project is critically discussed. A brief insight in the functions and design of the tool is given. Database and determination tool are intended to be published for free use.

DNA Barcoding of Austrian molluscs – a short update

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Within the Austrian Barcode of Life (ABOL) initiative, we took over the ambitious task to establish DNA barcodes for all Austrian molluscs: Molluscs are indicator species and therefore suitable for evaluation of habitat quality. Living in vulnerable habitats leads to a high risk of extinction: about 35% of the snail species and 37 % of the Austrian mussel species are endangered. The number of endemics is guite impressive (19.3 %) and approximately 30 % of the 400 native species are divided into subspecies. At the NHM Vienna several projects on snail species in Austria are conducted, hence pre-conditions (collected specimens and experiences) for successful DNA barcoding are given. However, genetic investigations in land pulmonates often showed extreme high intraspecific diversities. Hence, there is no standard value for genetic distances, which marks taxonomic delimitations. Due to the frequently observed overlap of intra- and interspecific variation no barcoding gap can be found, which has to be con-

sidered in data analysis. For the ABOL project molluscs we mainly used material specifically preserved for DNA analyses, which was collected during concerted field trips to different parts of Austria or collected from collaboration partners. In addition, we also used older material from the collections of the Natural History Museum Vienna, the "Haus der Natur" (Salzburg), and the Biology Centre of Linz. Until now, from 230 mollusc species (about 60 % of the Austrian species) 569 DNA-barcodes with all relevant metadata were established and uploaded to the BOLD database. From our results, we find that many species are placed within a BIN (Barcode Index Number) or at least one well supported clade, but we also see high genetic differentiation within species described by morphological characters, implying the existence of cryptic species. On the contrary, also different morphologically described species that cluster within one BIN can be found.

A fresh look at Melanopsidae (Caenogastropoda: Cerithioidea): evolutionary systematics, biogeography and conservation genetics

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Melanopsidae occur in subtropical and temperate regions of the Western Palearctic and are also reported from Zealandia, which is an unparalleled disjunction among freshwater animals. Melanopsidae also have a rich fossil record. We sequenced nuclear and mitochondrial markers of a representative sample of species/genera covering the entire geographic range of the group. We compare the timeframe for the evolution of major melanopsid lineages with geologic events and elucidate scenarios that may have shaped distribution patterns. Our phylogenetic analyses suggest that Melanopsidae are not monophyletic. Holandriana is more closely related to Pleuroceridae and Semisuclospiridae than to Melanopsidae s.str. The Zealandian taxa (Zemelanopsidae) were recovered as the sister group of a clade including Melanopsis, Microcolpia and Esperiana (Melanopsidae s.str.). Esperiana branches off first, while Microcolpia represents the sister group

of *Melanopsis*, which is comprised of three lineages: (1) western Mediterranean region, (2) eastern Mediterranean region and (3) Italy. While the Italian populations show little shell variability, the eastern and western Mela*nopsis* lineages are highly variable. Several geographically coherent groups in *Melanopsis* can be ranked as species. However, in a number of cases morphologically distinct forms were not recovered as monophyletic units. The Italian narrow-range endemic M. etrusca was known historically from twelve populations, half of which have already gone extinct. We used AFLP markers to assess the genetic diversity in the remaining populations, which showed that the westernmost populations are genetically distinct from the remaining populations. Recovery plans, which are urgently needed to prevent a further decline of the species, ought to take population structuring into account to preserve the genetic diversity.

Expression of mesodermal marker genes in the polyplacophoran mollusk Acanthochitona crinita

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The mesoderm is argued to be the youngest of the three germ-layers. It may either form by cells that immigrate from the blastopore margin into the blastocoel as in many protostomes or by detachment from the archenteron wall (in deuterostomes and some protostomes). Although its morphogenesis is well studied for numerous metazoans, the molecular components underlying this process remain largely unresolved. Gene expression studies in the mesoderm-lacking cnidarians showed that genes that contribute to mesoderm formation in bilaterians are expressed both in the cnidarian ectoderm and endoderm. This leads to the assumption that these genes were co-opted into mesoderm development in bilaterians. Mesodermal gene expression has yet to be studied in many protostomes, particularly in molluscs, where little is known about key genes involved in mesoderm formation. In our ongoing study, expression of common mesodermal marker genes belonging to the hairy and enhancer of split (HES) and Mox family are studied in the polyplacophoran mollusk

Acanthochitona crinita. One copy of AcMox was found in the A. crinita transcriptome, while seven putative AcHES genes were found. HES genes are fast evolving genes, which may lead to many species-specific duplications. Our findings support the monophyly of HES genes but it remains unclear how the individual HES genes of different species are related to each other. AcMox groups well with orthologs of other metazoans. Based on comparative data of other lophotrochozoans, expression of AcMox is expected to start early in larval development. This is in accordance with Mox expression of other lophotrochozoans such as Terebratalia transversa (brachiopod), Alitta virens (annelid) or Haliotis asinina (gastropod). HES genes are involved in many developmental processes such as partitioning of morphological territories or neurogenesis. Expression patterns of genes of this family differ in various bilaterians, currently hampering a clear hypothesis concerning its functions during early molluscan development.

Looking on the snails' side of life - Molluscs as intermediate hosts of digenean trematodes

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Many parasitic worms have life cycles, which include intermediate hosts. Digenea have a development, which consistently includes molluscs as first intermediate hosts and vertebrates as final hosts. The development of the larvae in the mollusc intermediate host involves an asexual propagation. Between the first and the final host, further intermediate hosts may be needed. Phylogenetically, Digenea are the sister group of Aspidogastrea. The latter parasitize and reproduce in both vertebrates and molluscs, but they lack an asexual generation. Somehow they resemble a simple digenean cycle. Therefore, it is assumed that the ancestors of trematodes primary were parasites either of molluscs or of vertebrates and later a life cycle including two hosts was achieved. From an ecological point of view, digeneans are important factors, which influence the growth, fecundity, life span and behaviour of their hosts. In the mollusc host, they may cause castration and behavioural changes that makes them easy prey (to final hosts). They influence the development of populations, the structure of biocoenoses and processes

of biodiversity and evolution. Economically, Digenea have strong impact in animal husbandry. Some digenean trematodes are also important as causes of human diseases. Especially in tropical environments, still many people suffer from fluke diseases. Merely the schistosomes affect worldwide more than 200 million people. In Central Europe e.g. digenean trematode infections in humans occur occasionally. Despite of the low medical relevance of trematodes in Central Europe, the number of species especially in snails - is considerable, including species of potential medical/veterinarian relevance as well as many wildlife parasites. The ecology, systematics and biology of many species has not been well studied- especially the snail side; unless this is an intimate relationship, which affects ecology and evolution of hosts and other organisms. In times of enormous environmental transformations, it will be of growing significance to increase the knowledge about digenean trematodes, their biology and their role in communities.

Linking the ecological species concept with systematics of trochid gastropods in the Northern Adriatic Sea

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The genera *Gibbula*, *Phorcus* and *Steromphala* represent a clade of marine gastropods of the family Trochidae (Vetigastropoda). More than 25 species of these genera live along rocky coasts of the Mediterranean Sea and the Atlantic Ocean feeding on biofilms on rocks and seagrass leaves. The use of DNA-barcoding, shell morphometrics, and radula morphology improved species delineation sufficiently to put the ecological species concept to the test. Although some of these closely related species occur in sympatry, little is known about their ecological niche differentiation. Abiotic parameters such as exposure to wave action, air and temperature, as well as biotic factors like predation and different reproductive strategies are potential key factors for this and may result in different vertical distribution patterns of these snails in the intertidal and shallow sublittoral zones. Preliminary observations indicate different depth preferences when two or more species of these genera cooccur. To quantify this observation, snails were collected at several sites along transects from the midwater line to about 4 m depth in Rovinj and on Brijuni Islands, Croatia. Specimens were identified by their shell morphology, and additionally with DNA barcoding to aid species identification, especially of juvenile specimens. Twelve species were identified by this integrative approach. Results show that, although depth ranges of species may overlap, the structure of the trochid species assemblages change from shallow to deep habitats. The genus *Phorcus* is predominant along the waterline and replaced by the genus *Steromphala* in the middle range of the study area. *Clanculus* spp. were exclusively found in the deepest parts of the study area.

Achatschnecken in der tierärztlichen Praxis

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Achatschnecken sind Landlungenschnecken, die ursprünglich in Afrika beheimatet sind. In Europa werden Achatschnecken immer beliebtere Haustiere. Sie werden in Kindergärten und Schulen gehalten und in der tiergestützten Therapie eingesetzt. Wenn Achatschnecken krank werden, werden vor allem "Exoten-Tierärzte" aufgesucht, um Hilfe zu finden. Derzeit existiert jedoch noch sehr wenig Literatur zu Diagnostik und Therapie von Erkrankungen bei Landschnecken. Einen kurzen Überblick über Biologie, Haltung, Handling, Erkrankungen, Anästhesie und Euthanasie geben Cooper und Knowler (1991). Genauere Anatomiestudien über Achatina fulica (A. fulica) wurden von Van Benthem Jutting (1951) und Srivastava (1992) durchgeführt. Um nun herauszufinden, welche Untersuchungsverfahren zur Diagnostik von gastrointestinalen und urogenitalen Erkrankungen bei Achatschnecken eingesetzt werden können und um gleichzeitig Referenzen für die radiologische, sonographische und computertomographische Untersuchung von Achatschnecken zu etablieren, wurde eine Studie mit 5 Achatschnecken (2 A. fulica, 3 A. albopicta albicans) gestartet. Das Schneckengehäuse, das Herz und Eier konnten bereits mittels Röntgenbildern dargestellt werden, während die Anatomie des Gastrointestinaltraktes am besten auf kontrastmarkierten Röntgenbildern demonstriert werden konnte. Computertomographische Untersuchungen lieferten zusätzliche Informationen zum Respirationstrakt, der Niere, der Eiweiss- und der Verdauungsdrüse. Mittels

Ultraschalluntersuchungen konnten der Fuß, das Herz und der vordere Gastrointestinaltrakt dargestellt werden. Weiters wurde das zoonotische Potential von ausgewählten Darmpathogenen oder Darm assoziierten Bakterien wie Enterobacteriales und Campylobacter sp. aus dem Gastrointestinaltrakt und der Haut und zusätzlich auf Methicillinresistenten Staphylococcus sp. (MRS) auf der Haut von 30 Achatschnecken untersucht. In keiner der untersuchten Proben konnte Campylobacter sp., Salmonella sp. oder MRS isoliert werden. Ein Isolat wies die höchste Sequenzähnlichkeit des 16S rRNA Gens zu Citrobacter freundii auf. Dieses Isolat zeigte ein erweitertes Spektrum von B-Lactamase (ESBL) und Plasmid-mediierter β-Lactamase-Produktion und war Träger von Resistenzgenen. Diese Studie wurde von der Ethik- und Tierschutzkommission der Veterinärmedizinischen Universität geprüft und befürwortet sowie in Übereinstimmung mit der Good Scientific Practice und unter Beachtung der einschlägigen nationalen Rechtsvorschriften durchgeführt (ETK-01/08/2016, ETK-07/09/2015).

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Mesoderm and muscle formation in the quagga mussel, Dreissena rostriformis

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Myogenesis involves modification, reduction and de novo formation of muscular tissue and is particularly dynamic in molluscs. Only few studies on myogenesis in bivalves using state-of-the-art methods such as fluorescence labelling and confocal microscopy are currently available, with the most detailed ones stemming from the scallop Nodipecten nodosus and the shipworm Lyrodus pedicellatus. The quagga mussel Dreissena rostriformis is a small, mytiliform freshwater mussel with an indirect life cycle that includes a trochophore and a veliger larva. Dreisse*na rostriformis* is an invasive species in Europe and in the USA. Herein, we describe the dynamics of myogenesis and larval myoanatomy in the quagga mussel. The first visible F-actin positive cells are found in the gastrula and in the trochophore larva. In the early veliger larva there are two anterior adductors, a ventral larval retractor, a velum ring musculature and a dorsal and ventral velum retractor. Subsequent muscle development includes a median velum retractor and an accessory velum, foot and mantle retractors. A comparative analysis suggests that the ground pattern of bivalve larvae includes a velum ring, velum retractors, a ventral larval retractor and anterior adductors. Unfortunately, we have so far not been able to produce postmetamorphic quagga mussels in the lab, thus hampering reconstruction of the fate of larval muscles and emergence of the adult muscular bodyplan. Very little is known about gene expression during mesoderm formation (the germ layer from which the musculature forms) in molluscs. Therefore, we investigate expression patterns of some well-known mesodermal marker genes (e.g., Hes, myosin heavy chain, even skipped, brachyury) during Dreissena rostriformis development. Preliminary results suggest that a Hes gene is expressed during early mesoderm formation at gastrulation. We found myosin heavy chain expression from the late gastrula until the veliger stage, where it is colocalized with developing muscle cells.

Confocal views of the nervous system of Scaphopoda

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Scaphopoda is a notoriously under-studied major molluscan taxon, even though its evolutionary links to the other groups of this phylum are elusive. Morphological information on most organ systems is histology-based and has not been confirmed or supplemented by recently established methods such as immuno-cytochemical stainings. We present initial results on the central and peripheral nervous systems of two gadilid species, Pulsellum lofotense and Cadulus subfusiformis, using serotonin, FMRF-amid, and acetylated alpha-tubulin stainings, visualized by confocal laser-scanning microscopy. The cerebro-pleural and buccal ganglia of both species have strong serotonergic and anti-FRMF-amidergic signals, as do the commissures and connectives emerging from these ganglia. Nerves and ganglia in the captacula, the unique scaphopod food capture organs, contain strong signals for

these neurotransmitters as well. Pedal and visceral ganglia do not stain as readily. The anterior pallial nerves of the cerebro-pleural ganglia are one of the few nervous elements with an alpha-tubulin signal. The posterior part of the peripheral nervous system has been insufficiently resolved so far. The present methods reveal a neural plexus on each side of the ring of cilia at the posterior mantle aperture that are connected with the visceral ganglia. We also visualize the serotonergic innervation of the dorsoventral musculature. These preliminary investigations reveal fine details of the scaphopod nervous system that are not easily assessable by other methods and encourage further studies. With additional data from other scaphopod taxa, a comparative approach will likely yield valuable systematically informative characters.

Presence and prevalence of non-indigenous molluscs in a *Posidonia oceanica* meadow in southern Crete

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Because of its geographic position, Crete hosts an increasing number of Lessepsian non-indigenous species which entered the Levantine Basin, the easternmost Mediterranean Sea, after the opening of the Suez Canal in 1869. For example, multiple non-indigenous fishes such as Siganus rivulatus, Siganus luridus, Fistularia commersonii have established abundant populations. Very little information is available for molluscs, notwithstanding the large number of species that has entered the Levantine Basin from the Red Sea. Seagrass meadows are very important marine ecosystem worldwide and the species Posidonia oceanica is essential in the Mediterranean Sea. It is an endemic species, identified as a priority habitat for conservation under the European Union Habitats Directive and under serious anthropogenic pressure: 46 % of the meadows in the Mediterranean have experienced reduction in range, density and/or coverage, and 20% have severely regressed since the 1970s. The objective of this

study was to describe the molluscan community structure and composition of a Posidonia oceanica meadow in Plakias Bay, south-western Crete, with a specific attention to the presence and abundance of non-indigenous species. Sampling took place in May and September 2017 at four different depths (5 m, 10 m, 15 m and 20 m). The leaves and rhizomes were sampled separately with a net and airlift sampling, respectively. Furthermore, the plant shoot density was quantified to assess its status. We identified 110 different living mollusc species and only three are aliens. The shoot density data indicates a very good condition of the Posidonia oceanica meadow. The state and the overall complexity of this habitat may explain the low number of non-indigenous species in accordance with the biotic resistance hypothesis which posits that ecosystems with high biodiversity are more resistant to invaders than ecosystems with low biodiversity.

Phylogeography and morphological variation of freshwater spring snails (*Bythinella*), along a west–east transect in Austria

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The small and dioecious spring snails of the genus Bythinella Moquin-Tandon, 1856 inhabit groundwater and caves, but typically are endemic to cold springs. They are spread from northern Germany to Sicily and from Spain to Turkey. Presently, more than 83 allopatrically occurring species and subspecies are classified. Nevertheless, it is not certain that these previously described species are distinct since their morphologic characteristics display a wide variation. The aim of this study is to gain insights into the genetic and the morphological variation of the central European species Bythinella austriaca (Frauenfeld, 1857) and Bythinella conica (Clessin, 1910) in Austria. The research is based on an integrated approach combining genetics and morphology. For the study, 100 specimens from 25 locations along a west-east transect in Austria were examined. The molecular investigation was done by using DNA sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene from the specimens to delimit the species. Additionally, the internal transcribed spacer 2 between the nuclear 18S and 28S rRNA genes (ITS2) was investigated to search for further variation that is congruent to the ones from the COI analysis. The morphometrical analysis based on Landmarks was performed to compare the overall shell shape and to check for characteristics that show comparable patterns to the genetic analysis. The molecular analysis, however, revealed a clear separation of Bythinella conica located in the west and Bythinella austriaca located in the east of Austria. Even though the separation is not a strict vertical one, it roughly follows the Hochkar mountain ridge. The morphological features are still being processed. So far, the analysis did not show a distinct pattern. Instead, these morphologic characteristics are linked to the prevailing abiotic parameters at the spring.

How does the lucinid clam maintain its symbiosis with chemosynthetic bacteria?

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Clams from the family Lucinidae are the most speciose group of bivalves associated with chemosynthetic symbionts (approximately 500 species). This symbiosis underpins the ecological and evolutionary success of these clams and has allowed them to colonise diverse marine environments across the globe. Lucinids form a highly stable one-to-one symbiosis with sulphur-oxidising bacteria that they harbour exclusively in their gills. The symbionts use reduced sulphur compounds in the environment to autotrophically synthesise sugars that are transferred to the clam. Hosting a large population of symbionts and meeting their metabolic demands imposes multiple physiological challenges upon the lucinid host, but we still have little understanding of how lucinids regulate the symbiosis at the molecular level. We are using the mediterranean lucinid Loripes orbiculatus in combination with next generation sequencing approaches to unravel the strategies underlying the maintenance of the lucinid symbiosis. To investigate this clam's physiological adaptations to a chemosymbiotic lifestyle, we compared the transcriptomes of four L. orbiculatus organs; the gills, mantle, foot and visceral mass. Our analyses reveal that innate immunity and metabolite transport are important processes in the symbiont housing gills. Although the symbionts are restricted to the gills, our findings suggest that the non-symbiotic organs also contribute to regulating the symbiosis by detecting and acquiring resources from the environment to meet the metabolic demands of chemosynthesis. These findings provide a detailed picture of the potential molecular and cellular processes involved in maintaining a beneficial relationship with bacteria, and form the basis of future efforts to understand the establishment of this highly successful symbiosis during animal development.

A multilevel approach to understanding nervous system development in the chiton Acanthochitona crinita

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Mollusca is a large animal phylum that includes simplebuilt worm-like representatives and polyplacophorans (chitons), the well-known bivalves (mussels, cockles) and gastropods (snails, slugs) as well as the complex cephalopods (squids, octopuses, cuttlefish). Consistent with their strikingly variable body plans, molluscs exhibit very different neuroanatomical organizations and degrees of nervous system centralization. For instance, polyplacophorans show little neuronal condensations, while conchiferans possess multiple pairs of ganglia that can be more or less fused and, especially within cephalopods, may even form highly complex brains. Our research focus is on the developmental and evolutionary emergence of these distinct neural architectures. Using morphological, bioinformatic and gene expression analyses, we currently study neurogenesis in Acanthochitona crinita, a member

of the Polyplacophora that probably represent the best living proxy for the ancestral molluscan condition. We are particularly interested in well-conserved regulators of bilaterian neural progenitor specification, proliferation and differentiation, such as *Sox* and pro-neural *bHLH* genes. In order to assess the expression of these genes in relation to the developing nervous system, we use double labelling with EdU for cell proliferation and with antibodies against selected molluscan neuropeptides and neuronal markers. The data obtained in this study should allow us to reveal key aspects of nervous system formation in *A. crinita* and, through comparison with data available from other better-studied taxa, provide new insights into the evolution of diverse nervous system features within Mollusca.

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Digitale Literatur/Digital Literature

Zeitschrift/Journal: Arianta

Jahr/Year: 2019

Band/Volume: 7

Autor(en)/Author(s): diverse

Artikel/Article: <u>More leaves on the phylogenetic tree of Scaphopoda (Bronn, 1862):</u> <u>Work in progress 3-9</u>