

2nd MoFA Conference

September 23–24, 2021

Natural History Museum Vienna

The decision to organize the 2nd MoFA Conference as a hybrid meeting (physically and online) was by no means an easy one for us. The arguments for and against were strong because planning was uncertain due to unpredictable developments of the Covid19 pandemic and the efforts necessary were not easy to assess.

In the end, we enjoyed a small but fine conference with about 40 participants and 11 presentations. The people physically present appreciated being there, because in the past year we had built up a noticeable deficit in scientific and social meetings. On the other side, the virtual participants encouraged us, and feeling the scientific participation and personal sympathy we were encouraged to stick to this format: Those who can come personally are happy about it, and those who can't, still have the chance to be there online!

Purely “physical” was the excursion on Schneeberg mountain on the second day. Starting from the top station of the famous Schneeberg rack railway, we withstood a violent storm of up to 100 km/h. Anyhow, with remarkable light and weather conditions, the 11 participants enjoyed the autumnal mountains. Turning our attention to the alpine snail fauna, we met a lot of old acquaintances, e.g., *Cylindrus obtusus*. We will report on this in the next issue of ARIANTA.

Here, we once again heartily congratulate the winners of the MoFA student awards, **Hannah Schubert**, **Stephan Schulreich** and **Nikolaus Helmer**, and thank everyone who participated. We are convinced that in the end we all were winners!

On the top of Waxriegel, one of the peaks at Schneeberg massif.

From left to right: Martin Kapun, Stephan Schulreich, Nikolaus Helmer, Sonja Bamberger, Elisabeth Haring, Wilhelm Pinsker, Michael Mitschke. Photo: Helmut Sattmann



Abstracts of Talks (in alphabetical order of first author's surname)

**Species delimitation in the *Albinaria cretensis* complex (Clausiliidae)
using genetic data**

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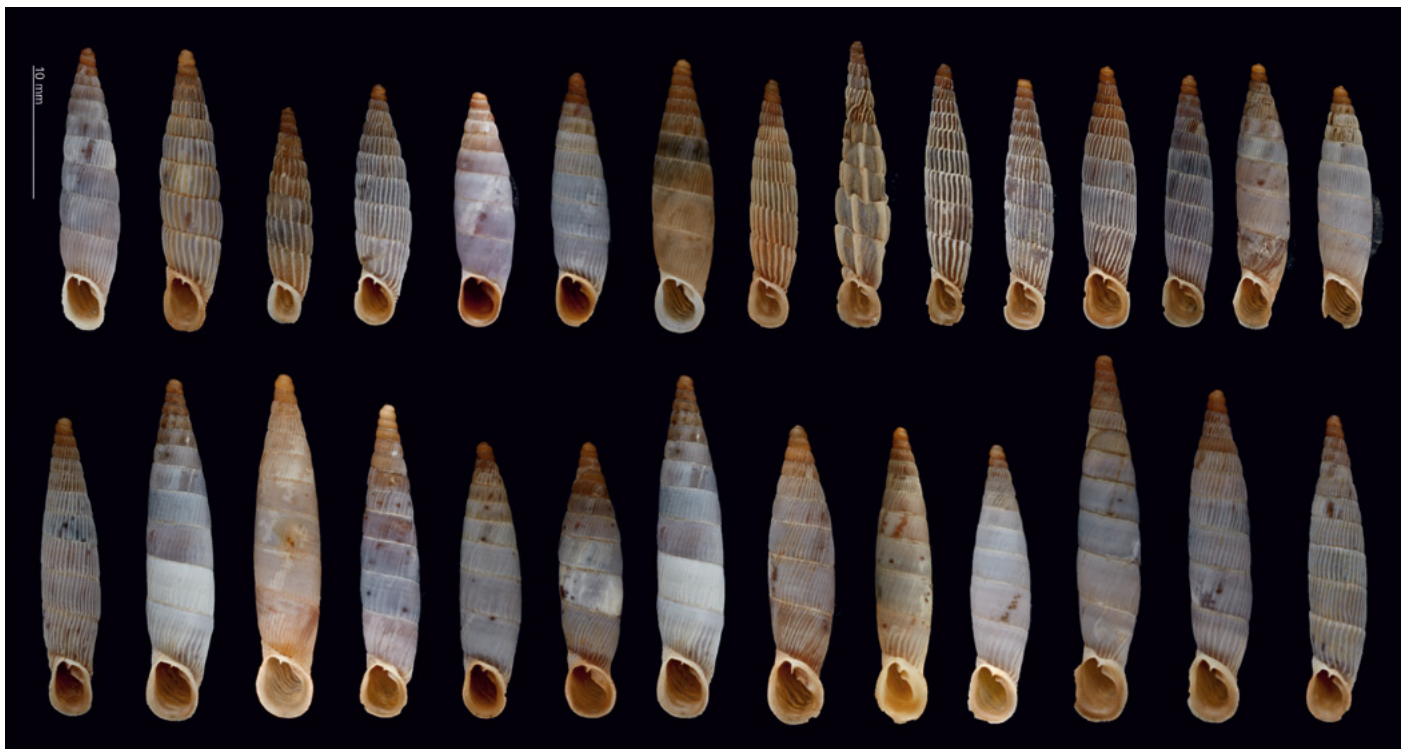
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The genus *Albinaria* is one of the most species-rich genera within the door snails (Clausiliidae). A particularly high number of endemic *Albinaria* species has evolved on the Greek island of Crete and surrounding islands. Currently, about 41 species are distinguished based on shell characteristics. The *Albinaria* species of Crete are so closely related that species delimitation and elucidation of their relationships by single gene analysis is not possible. Therefore, we generated double digest Restriction Site Associated DNA Sequencing (ddRADseq) data from 140 individuals from 48 populations of the *Albinaria cretensis* complex from western Crete. Competing classifications based on shell characteristics previously divided this complex into five to nine species with up to 18 additional subspecies. Various species delimitation methods using ddRADseq data suggested a division into 14 to 45 species. We consider the proposed subdivisions as candidate species and tested these hypotheses by combining a model-based

clustering method that indicates possible gene flow with a test for isolation by distance (IBD). Using the IBD test, we evaluated whether differentiation of allopatric candidate species is greater than would be expected based on the increase of genetic divergence between individuals with increasing geographic distance. Based on this criterion, a classification of the *Albinaria cretensis* complex into nine species is presented. Some of these correspond to morphologically delimited species. Some include several morphologically distinct species, and in two cases we were able to recognize cryptic species that have not been distinguished so far.

Further Reading

Bamberger S., Xu J. & Hausdorf B. (2021): Evaluating Species Delimitation Methods in Radiations: The Land Snail *Albinaria cretensis* Complex on Crete. Systematic Biology syab050. <https://doi.org/10.1093/sysbio/syab050>



Morphological variability within the *Albinaria cretensis* complex. Photo: S. Bamberger

Molluscs in the rural green belt of Vienna: continuities and changes

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The mollusc fauna in the rural green belt of Vienna belonging to areas subjected to the agricultural and rural development program of the EU has been studied from 2020–2021. Vienna potentially inhabits about 180 species of molluscs, 160 of them snails and slugs, another 20 are mussels. Samples were taken in about 190 sites. Additionally, literature and unpublished data of several working groups of the last 30 years were taken into consideration. The least mollusc diversity was found in arable land in the eastern and southern parts of Vienna. The mollusc fauna there consisted mainly in drought-tolerant euryoecious and invasive gastropods, the latter in some cases only introduced in the last decades, e.g., *Arion vulgaris*. In these areas a higher diversity was only located in semi-ruderal habitats like shelter belts and waysides. The Danubian floodplains, which once inhabited a species-rich mollusc fauna, suffered from massive changes in hydrology caused by stream control, climate change and invasive species.

Some typical aquatic and wetland species, e.g., *Theodoxus danubialis* therefore became extinct, while others, once widespread, are now limited to a few numbers of sites like *Viviparus contectus*. The gastropod fauna of the river Danube itself changed the most within the last 30 years, as invasive aquatic species like *Corbicula fluminea* became dominant. The highest diversity was found in various habitats of the Vienna Woods and adjacent areas. Within forests, typical east Alpine species like *Aegopis verticillus* could be detected. But also, more specialized species like spring snails, e.g., *Bythinella austriaca*, could be recorded. Diverse habitats, like wet meadows and spring fens as well as steppe areas and vineyards are home to several endangered species, e.g., *Chondrula tridens*. Therefore, the Vienna Woods and adjacent areas can be assumed as the area in Vienna with the highest continuity concerning its mollusc fauna.

The European Cenozoic land snail record: dispersals, extinctions and the dawn of the modern fauna

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The evolution of the European Cenozoic terrestrial gastropod faunas is characterized by a complex pattern of diversification, turnover, extinction, and immigration events. Based on a critical review of a literature-based dataset comprising 1640 species from 609 sites, we calculated net diversity through time (expressed as species, genus and family richness) and β -diversity (as species, genus and family turnover). Within this data, we recognize major disruptive phases, with turnover events at the Ypresian-Lutetian and the Eocene-Oligocene boundaries, as well as extinction events at the Oligocene-Miocene, Burdigalian-Langhian Pliocene-Pleistocene boundaries. Phases of diversification during the Lutetian, Burdigalian and Pliocene, in contrast, are linked to phases of relative climate

stability. At least five immigration events are reflected by the appearance of exotic elements in European faunas. Many of them correlate with the formation of terrestrial pathways and major migration events in mammals. The dataset gives reasonable estimates for the first appearance of modern genera and species, which thus may serve as anchors for molecular clock analyses.

Further Reading

Harzhauser M. & Neubauer T.A. (2021): A review of the land snail faunas of the European Cenozoic – composition, diversity and turnovers. *Earth-Science Reviews* 217: 103610. <https://doi.org/10.1016/j.earscirev.2021.103610>

The New System of Gastropoda: History, Facts, Hypotheses, Questions

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The classic system of Gastropoda was substantially constructed by German malacologists (Johannes Thiele, Wilhelm Wenz und Adolf Zilch) from 1925 to 1960 and was a so-called “Key-System”, i.e., a basal identification key for the very high diversity of the group. Accordingly, this system is still applied in the identification literature such as shell books. However, since 1980 various new data accumulated, which did not agree with the classic system. These data included (1) newly discovered taxa from the deep-sea hydrothermal vents (hot vents), (2) microanatomical and histological re-investigations of traditional taxa (e.g., Cocculinida and Lepetelloidea), and (3) data on the ultrastructure mainly of sensory organs and of sperm. Finally, the consequent application of the meanwhile established cladistic (Hennigian) methodology of phylogenetics resulted in a first new system of Gastropoda by Luitfried Salvini-Plawen, Gerhard Haszprunar, Winston F. Ponder, and David R. Lindberg. This first revolution mainly concerned the basal, formerly „prosobranch“ groups and was substantially based on morphological data. Main results were the non-monophyly and thus elimination of Prosobranchia, Archaeo-, and Mesogastropoda as well as the monophyly of Heterobranchia with several so-called allogastropod groups at the basis of Euthyneura.

Since 2010 mainly molecular data provided by Michael Schrödl, Katharina M. Jörger, Timea Neusser und Heike Wägele triggered a second revolution. Results included the polyphyly of both the former main euthyneuran groups, Opisthobranchia und Pulmonata. On the other hand, monophyletic Ringipleura (Ringiculida,

Pleurobranchomorpha, Nudibranchia), Tectibranchia, and Pan-Pulmonata (including Siphonariida, Sacoglossa, Pyramidellomorpha, Acochlidiomorpha, Hygrophila, and Eupulmonata) have been established. Although the deep phylogeny of Gastropoda currently appears largely resolved, internal systematics in particular of the species-rich taxa Caenogastropoda and Stylommatophora and of the allogastropod grade is still heavily discussed.

After the correction of classic erroneous data, the central phenomenon of gastropod torsion is currently understood as a primary larval adaptation. However, there are several evolutionary events, which still await explanation: The substantial modification of the buccal apparatus at the origin of Heterobranchia, the dramatic re-arrangement of the mitochondrial genome among basal Heterobranchia after the split off of Ectobranchia (Valvatoidea), any selection advantage of heterostrophy or giant nerve cells, or the reason of the loss of operculum in the pulmonate taxa. You may become famous by resolving any of these questions.

More leaves on the phylogenetic tree of Scaphopoda (Mollusca)

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Despite the global marine distribution, the taxon Scaphopoda is notoriously understudied. The evolutionary position of this infaunal group within the molluscs is as disputed as the internal systematics. The greatest diversity of scaphopod species is recorded from depths below 200 m and tropical shallow water habitats. Diagnoses of the 567 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 soft body characters. Although the subtaxa Dentaliida and Gadilida are well defined, the validity of many family- and genus-rank taxa remains unresolved. Molecular phylogenetic studies suffered from limited taxon sampling and agreed with the morphological studies on a robust support for Dentaliida and Gadilida only.

The present study is based on mostly Indo-Pacific dentaliid specimens from the National Museum of Natural His-

tory, Paris. Nuclear marker sequences (18S rRNA gene, 28S rRNA gene) and mitochondrial markers (16S rRNA gene, cytochrome c oxidase subunit 1 gene) were analysed with the aim to improve our knowledge on the phylogenetic signal of these markers and to gain new insights into the poorly studied phylogeny of this group.

The newly obtained trees are better resolved than those in preceding studies. Major taxa like Dentaliida, Gadilida, Entalimorpha, and Gadilimorpha are also well supported. However, monophyly of family-level taxa and most genera with more than two species in the dataset (e.g., *Dentalium* and *Antalis*) is not supported. This indicates that shell shape and sculpture, even if supplemented by radula morphology, are too homoplastic and, thus, not sufficient for diagnosing taxa at genus and higher levels.

Integrated taxonomic and ecological study on malacofauna of Lake Ohrid, Albania

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The present study has been carried out during 2019–2021 in the Albanian part of Lake Ohrid. Mollusc samples were taken from four sites, according to transect lines from 2 m to 17 m depth, using an Ekman grab from boat, whereas at the shoreline, until 0.5 m depth, samples were taken by hand and with a hand net. Habitat characteristics were recorded, including bottom typology (soft bottom, hard bottom, presence, or lack of macrovegetation, predominance of *Chara*, *Phragmites* or other macrovegetation). Molecular genetic analyses of molluscs were carried out for 16 species, out of 48 species collected in total. These analyses aimed at combined species determination via morphological characters and DNA barcoding using a part of the mitochondrial cytochrome c oxidase subunit 1 gene (CO1). Furthermore, comparisons between newly determined DNA sequences with DNA sequences published in public databases (GenBank; BOLD) were carried out. In particular sequences from the same species or genera originating predominantly from the Balkan region

were included. With these data sets, the establishment of phylogenetic trees helped to critically evaluate the identification based on morphological characters. For several genera, the phylogenetic trees illustrated a still unsettled taxonomy and show that published DNA sequences should be considered with caution. Nevertheless, the DNA barcoding analyses confirmed to some extent the morphological species assignment and, furthermore, enabled identification of several species that were impossible or very difficult to identify to species level solely by morphological characters. Specimens of the family Hydrobiidae and the genus *Gyraulus* were the most difficult taxa to identify, and DNA barcodes did not result in straightforward conclusions. This was due to the fact, that they are not well represented in GenBank and BOLD, which in turn reflects the need for extensive investigations of these groups. The preliminary results and findings from this study add new data to the malacofauna of Lake Ohrid and highlight its significance at regional and global level.

Hidden diversity – trematodes of freshwater snails in Austria

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Digenean trematodes as parasites of vertebrates are of medical, veterinarian and ecological importance. They use molluscs as obligatory first intermediate hosts. Among those with medical relevance are avian schistosomes of the family Schistosomatidae. Their final hosts are birds, but their infective larvae (cercariae) can cause dermatitis in humans, called cercarial dermatitis or swimmer's itch. Itchy skin inflammations and secondary bacterial infections are unpleasant symptomatic consequences. First intermediate hosts are freshwater snails. Our working group conducted a survey of trematodes in freshwater snails in Upper Austria, Lower Austria and Vienna, with the aim to record species diversity and build up DNA barcode reference data. Classical morphological data combined with molecular genetic analyses were used for classification of the trematodes recorded. Emphasis was put particularly on schistosomes. Until recently, only two species had been recorded for Austria: *Bilharziella polonica* and *Trichobilharzia szidati*. Within our study, evidence of two additional species in Austria was proven: *Trichobilharzia franki* from *Radix auricularia* snail host (Reier et al. 2020) and *Trichobilharzia physellae* from *Physella acuta* snail host (Fig. 1). The latter is probably the first record of Schistosomatidae in *Physa/Physella* in Europe (Helmer et al. 2021). A further task of the project was the detection of specific trematode DNA as well as mollusk DNA from water samples, so called eDNA. But this is another story and will be reported on another occasion.

record of *Trichobilharzia franki* Müller & Kimmig, 1994, from *Radix auricularia* (Linnaeus, 1758) for Austria. Parasitology Research 119: 4135–4141. <https://doi.org/10.1007/s00436-020-06938-3>



Fig. 1: Left: Cercaria of *Trichobilharzia physellae* from *Physella acuta* from lake Pleschinger See, Upper Austria; scale 100 µm. Right: *Physella acuta* specimen, which was infected with *T. physellae*, from lake Pleschingersee, Upper Austria; scale 5 mm. Both photos: NHMW

References

- Helmer N., Blatterer H., Hörweg C., Reier S., Sattmann H., Schindelar J., Szucsich N.U. & Haring E. (2021): First record of *Trichobilharzia physellae* (Talbot, 1936) in Europe, a possible causative agent of cercarial dermatitis. Pathogens 10: 1473. <https://doi.org/10.3390/pathogens10111473>
- Reier S., Haring E., Billinger F., Blatterer H., Duda M., Gorofsky C., Grasser H.-P., Heinisch W., Hörweg C., Kruckenhauser L., Szucsich N.U., Wanka A. & Sattmann H. (2020): First confirmed

Incorporating palaeogeography into ancestral area estimation can explain the disjunct distribution of land snails in Macaronesia and the Balearic Islands (Helicidae: Allognathini)

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The systematics and biogeographical history of the Western Mediterranean and Macaronesian land snail tribe Allognathini (Helicidae: Helicinae) is investigated based on mitochondrial and nuclear DNA sequence data. Our molecular phylogenetic analyses indicate that the genus-group systematics of the tribe needs to be revised. We show for the first time that the narrow-range endemics *Lampadia* and *Idiomela* from the Madeira Archipelago belong to Allognathini and represent together the sister group of the diverse Canary Island *Hemicycla* radiation. We therefore suggest synonymising Lampadiini with Allognathini. Sister to these Macaronesian genera was the Balearic Island *Allognathus* radiation. *Pseudotachea* was not recovered as a monophyletic group and the two currently recognised species clustered in *Iberus*. Similarly, *Adiverticula* was not recovered as a monophyletic group and clustered in *Hemicycla*. We therefore suggest synonymising *Pseudotachea* with *Iberus* and *Adiverticula* with *Hemicycla*. The six extant genera in Allognathini, which we distinguish here (*Cepaea*, *Iberus*, *Allognathus*, *Hemicycla*, *Idiomela* and *Lampadia*), originated in Western to South-western

Europe according to our ancestral area estimation and the fossil record. The disjunct distribution of the Balearic Islands and Macaronesian sister clades and the mainly Iberian *Iberus* clade that separated earlier can be explained by the separation of the Betic-Rif System from the Iberian Peninsula during the Late Oligocene to Early Miocene, along with independent Miocene dispersals to the Balearic Islands and Macaronesia from the Iberian Peninsula, where the ancestral lineage became extinct.

Further Reading

Neiber M.T., Chueca L.J., Caro A., Teixeira D., Schlegel, K.A., Gómez-Moliner B.J., Walther F., Glaubrecht M. & Hausdorf B. (2021): Incorporating palaeogeography into ancestral area estimation can explain the disjunct distribution of land snails in Macaronesia and the Balearic Islands (Helicidae: Allognathini). *Molecular Phylogenetics and Evolution* 162: 107196. <https://doi.org/10.1016/j.ympev.2021.107196>

Distribution and DNA barcoding of hydrobioids (Gastropoda) from the Kalkalpen National Park (Austria)

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The Kalkalpen National Park is situated in Upper Austria and contains more than 800 springs. The international importance of this park is, from the perspective of nature conservation directives, highly significant (European nature reserve Natura 2000, recognized wetland of the Ramsar convention). In the current study the hydrobioid fauna ('spring snails') of the Kalkalpen National Park was evaluated. These tiny snails are difficult to determine, however, their investigation is especially desirable, as several species are threatened and as they are important for water quality assessment. Snails collected in 39 selected springs were examined with classical morphological methods (shell and genital anatomy) and subsequently by DNA analysis. For this task, a partial sequence of the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene (~670 bp) was PCR amplified and sequenced. From 107 specimens, the *COI* barcoding sequence could be obtained and compared with already existing DNA sequences. The (sub)endemic species *Bythinella conica*, *Hauffenia kerschneri*, *Hauffenia wienerwaldensis* and *Belgrandiella aulaei* could be clearly identified. For *Bythiospeumnocki*, despite the ambitious collecting effort, only empty shells

were found in four springs (including the locus typicus spring) in the park and its surroundings. The genus *Bythinella* was detected in 36 springs. From 25 of these localities DNA barcodes could be created, which matches those of the species *Bythinella conica* (comparison data from ABOL). It is therefore concluded that the species occurs widely in the Kalkalpen National Park. The genus *Hauffenia* was sampled from 16 springs. From one the haplotype of the species *Hauffenia wienerwaldensis* could be identified (spring is 5 km outside the park) and from six the haplotype of the species *Hauffenia kerschneri*. The species *Belgrandiella aulaei* was found in three springs, which all lie outside the boundaries and are therefore not included in the protection measures of the national park. The data and analyses obtained contributes to the assessment of the taxonomic status of the species studied. The present study gives a good baseline for further monitoring of the hydrobioids in the Kalkalpen National Park, which is important to evaluate current as well as to decide on future protection measures for this group.

Mesoderm and muscle formation in the quagga mussel, *Dreissena rostriformis* (Deshayes, 1838)

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The mesoderm is a unique feature of bilaterians and gives rise to one of the prominent derivatives, the musculature. Developmental genes with commonly conserved expression during mesoderm and muscle formation are, e.g., Brachyury (*Bra*), even-skipped (*eve*), Mesoderm/Mesenchyme homeobox gene (*Mox*), and myosin heavy chain (*mhc*). Within the molluscs, the bivalves remain largely unstudied regarding mesodermal gene expression and data on myogenesis likewise remain scarce, given their global distribution and evolutionary significance. Here, myogenesis and developmental expression of *Bra*, *eve*, *Mox*, and *mhc* were investigated in the quagga mussel *Dreissena rostriformis* to contribute to questions concerning the bivalve larval muscular ground pattern and the putative involvement of these genes in mesoderm formation and myogenesis. The data show that all four genes are expressed during mesoderm formation but show additional, individual sites of expression. As such, *Mox* and *mhc* are additionally expressed in early myogenesis. *Eve* expression is present in the shell field, and *Bra* is expressed in the foregut. Comparative analysis suggests that *Mox* has an ancestral role in mesoderm and possibly muscle for-

mation in bilaterians, while *Bra* and *eve* have been conserved in mesoderm development of nephrozoans. The first F-actin-positive domains form in the trochophore larva of *D. rostriformis*. In the veliger larva, four pairs of velum retractors, a velum muscle ring, one pair of larval retractors, muscles of the pallial line, two pairs of mantle retractors, one pair of foot retractors and an initially two-partite anterior adductor are present. The data presently available suggest that the muscular ground pattern of autobranch bivalve larvae includes at least a velum muscle ring, three or four pairs of velum retractors, one or two pairs of larval retractors, two pairs of foot retractors together with the pedal plexus, possibly two pairs of mantle retractors, the muscles of the pallial line, the anterior and the posterior adductor.

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