

ARIANTA IV

REPORT ON THE WORKSHOP ALPINE LAND SNAILS 2014



Programme and abstracts

Editors

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Report of the Workshop Alpine Land Snails 2014

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The Workshop Alpine Land Snails took place in Johnsbach, Steiermark, Austria
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Alpine land snails extended

Workshop Alpine Land Snails 2014 is No. 18 in a series of similar workshops that were carried out by our working group under different titles and with different focus since the year 1992. In the majority of cases the workshops took place in Johnsbach, hosted by the famous alpine guest house “Köblwirt”. This year we meet again in Johnsbach and, as always, the group consists of numerous colleagues and friends from the Museum of Natural History Vienna and from other research institutes in Austria as well as other countries.

There is a high variety of topics and emphases that will be reported in our oral presentations and posters. Besides summarizing reports on sections of our successful FWF project (No. P19592 B17) on the “Phylogeography of alpine land snails” some talks refer to monitoring and research projects on snails of various regions in Austria, like Kalkalpen National Park, Biosphärenpark Wienerwald or the eastern foothills of the Alps. We also present our recently started FWF project (No. P26581 B25) dealing with *Montenegrina*, a very diverse clausiliid genus, endemic in the Balkan peninsular. From an Austrian point of view, apart from the Eastern Alps, one of the nearest hot spots of biodiversity is the Balkan Peninsula. The scientific questions concerning the basis of morphological and genetic diversity as well as problems of species delimitation are similar in organism groups of the Eastern Alps as well as the Balkan Peninsula. Additionally, we present topics on the evolution of snails, and on the diversity of some exotic land snails.

A major issue this year was the start of our important project “ABOL – Austrian barcoding of life”. The kickoff phase of this project includes a pilot project – led by members of our *Working Group Alpine Land Snails* – to document and characterize all mollusc species of Austria by DNA barcoding of voucher samples. An introducing talk provides an overview of the ABOL initiative, the plans for the forthcoming years and the importance of ABOL especially for nature conservation. Furthermore, some talks deal with topics of importance for the ABOL initiative, e.g., utilisation of museum material for genetic analyses and experiences and thoughts about scientific collections and legal collecting. These aspects are closing the circle to ABOL. We will also learn about a gripping arthropod story dealing with another Austrian DNA barcoding effort on millipedes. Although our workshops traditionally are dedicated to snails, we welcome also contributions from colleagues working with other organisms groups. Thus, we are convinced that “crossing taxonomic borders” always provides inseminating aspects.

As every year, one main task is to conduct various excursions. This time they are focused on the biodiversity of snails (and arthropods) with respect to our tasks within the ABOL initiative. Our aim is to cover as much of the species diversity as possible. It is a good start within the Gesäuse region which is extraordinary rich in species and endemics.

Last but not least, we will have some presentations for the public to demonstrate the importance of biodiversity research in all facets and the fascination of studying biodiversity of snails.

As always, we have a very mixed group here consisting of senior scientists, students and family members, which makes this a multi-experience week. Thanks to all for coming and contributing their knowledge and their good mood!

Vienna, August 2014
Elisabeth Haring
Helmut Sattmann

Programme

Monday August 18

14.00 Arrival and get-together

17.00 Poster presentations

Sandra Kirchner, Luise Kruckenhauser, Helmut Sattmann, Josef Harl, Michael Duda & Elisabeth Haring

Alpine populations of *Pyramidula pusilla*: A closer look into the species phylogeography (Gastropoda: Pulmonata: Pyramidulidae)

Luis Cadahía-Lorenzo, Josef Harl, Helmut Sattmann, Michael Duda, Luise Kruckenhauser, Zoltán Fehér, Laura Zopp & Elisabeth Haring

Phylogenetic relationships of *Cylindrus obtusus* (Pulmonata, Helicidae) deduced from nuclear and mitochondrial DNA marker sequences

Katharina Jaksch, Helmut Sattmann, Luise Kruckenhauser, Michael Duda, Josef Harl & Elisabeth Haring

Biodiversity homemade?! – *Clausilia dubia* and its various subspecies

Luise Kruckenhauser, Laura Zopp, Helmut Sattmann, Barbara Däubl, Luis Cadahía-Lorenzo & Elisabeth Haring

Snails do it – but sometimes they don't need to do it! Selfing in geographically separated populations of *Cylindrus obtusus* (Gastropoda: Pulmonata: Helicidae)

Oliver Macek, Daniela Bartel, Nikola Szucsich & Günther Pass

Cocktails and pills- A COI primer cocktail for Austrian pill millipedes

19.00 – 20.30 Talks

Anatoly A. Schileyko

On the early evolution of pulmonate molluscs

Barna Páll-Gergely & Takahiro Asami

Systematic revision of the neglected land snail family Plectopylidae

Zoltán Fehér, Katharina Jaksch, Virág Krízsik & Elisabeth Haring

Phylogeny of the rock-dwelling gastropod genus *Montenegrina* – preliminary results

Tuesday August 19

09.00 – 17.00 Collecting Excursion – Ennskirchen / Krumau / Weng / Hall / Langgriesgraben & Ödelsteinhöhle

19.00 – 20.30 Talks

Michael Duda, Katharina Jaksch & Anita Eschner

(Re)colonisation in a snail's pace: biodiversity monitoring of land gastropods in the biosphere reserve Wienerwald

Anita Eschner, Georg Bieringer, Michael Duda, Gwendolin Gobets, Katharina Jaksch & Alexander Mrkvicka

Small steppe land species - big questions: First attempts to clarify the situation of *Helicopsis striata* and ssp. in Eastern Austria

Jan Steger

A local hotspot of molluscan species richness - first impressions of the malacofauna of the Kalkalpen National Park (Austria, Upper Austria)

Oliver Macek, Michael Duda & Luise Kruckenhauser

Step across the border – do the two subspecies of *T. oreinos* hybridize?

Wednesday August 20

09.00 – 17.00 *Collecting Excursion* – Hartelsgraben/ Sulzkar/ Ebneralm

19.00 – 20.30 *Talks*

Katharina Jaksch, Anita Eschner & Elisabeth Haring

Genes in museum collections

Sattmann Helmut

Statutory provisions of collecting invertebrates in Austria

Nikola Szucsich on behalf of the ABOL-Team

DNA-Barcoding im Nationalpark Gesäuse: Biodiversität, ABOL und der Mehrwert für Schutzgebiete

Thursday August 21

09.00 – 17.00 *Collecting excursion* – Tamischbachturm / Schneckensafari, Hieflau

19.00 – 20.30 *Öffentlicher Abend – Public evening*

Öffentliche Präsentationen – Presentations for the public

19.00 Helmut Sattmann: **Schnecken, Sex und Rock'n'Roll**

19.30 Elisabeth Haring: **Was wir immer schon über Schnecken wissen wollten**

20.00 Johnsbacher Schneckentheater: **Schneckenabenteuer im Gesäuse (ein Schwank)**

20.15 Präsentation lebender Schnecken: **Schnecken zum Streicheln**

Friday August 22

10.00 – 14.00 *Excursion* – Ebneralm & Schneckenalm

Farewell and Departure



Photo: WG Alpine Landsnails

Abstracts: talks

arranged in alphabetical order of first authors

(Re)colonisation in a snail's pace: biodiversity monitoring of land gastropods in the "Biosphärenpark Wienerwald"

Michael Duda, Katharina Jaksch & Anita Eschner

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From April to October 2012 species diversity and abundances of land gastropods were analysed in 28 core forest areas which were put out of use a few years ago, as well as in 14 managed forests of the "Biosphärenpark Wienerwald". Aim of this study was to document the starting effect of abandoning forestry operation on quantity and quality of land snail communities and predicting their future development. In total 951 living individuals and 2588 empty shells of 49 species were recorded at the monitored plots. The samples from the core areas showed more individuals and a higher diversity of living species, but the differences were statistically not significant. These results can be explained by (1) the relatively short period since the core areas have been put out of use and (2) the rather smooth transition between the two types of management. Similar results have been recorded in other monitoring projects concerning land snails but as well as other forest dwelling organisms, concluding that significant changes are due to long-term development. Therefore, positive impacts on the gastropod fauna can be expected in the core areas in medium to long-term period, resulting in an increase of the snail species diversity and abundances.

This project is funded by "Biosphärenpark Wienerwald" and the EU

Small steppe land species - big questions: First attempts to clarify the situation of *Helicopsis striata* and ssp. in Eastern Austria

Anita Eschner, Georg Bieringer, Michael Duda, Gwendolin Gobets, Katharina Jaksch & Alexander Mrkvicka

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Three species of the genus *Helicopsis* - *Helicopsis striata* (O. F. MÜLLER 1774), *Helicopsis hungarica* (SOOS & WAGNER, 1935) and *Helicopsis austriaca* GITTENBERGER 1969 - were originally described in older malacological literature for Austria. The latter two were subsequently downgraded to subspecies of *Helicopsis striata*. All three taxa inhabit steppe land areas with patchy vegetation, an endangered type of landscape in Austria. The aim of this project was to make the first steps in clarifying the taxonomic assignment of the three subspecies, as *H. s. austriaca* is listed in the Annex II of the FFH directive and some authors question the validity of the subspecies *hungarica*. Preliminary results of a genetic analysis revealed an unambiguous differentiation of the three taxa on the basis of their mitochondrial DNA. Austrian specimens of *H. s. striata*, the nominate subspecies, cluster with individuals from northern and central Germany, the type locality of this taxon. The subspecies *H. s. austriaca* was confirmed to be an endemic of the southern Vienna basin and the eastern margins of the Alps in Lower Austria. Live specimens of *H. s. cf. hungarica* were detected for the first time in Austria. It forms an own clade, but so far a comparison with topotypic populations from Hungary is still missing. In the course of this project, populations of all three subspecies were found again in locations in which they were previously considered to be extinct; this reappearance might be a result of better management of dry meadows and steppe land areas in recent years.



Live specimen of *Helicopsis striata* & habitat / Burgenland, Photos: A. Mrkvicka



Live specimen of *Helicopsis striata austriaca* & habitat / Lower Austria; Photos: A. Mrkvicka

This project is funded by Burgenländische Landesregierung

Phylogeny of the rock-dwelling gastropd genus *Montenegrina* – preliminary results

Zoltán Fehér, Katharina Jaksch, Virág Krízsik & Elisabeth Haring

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One of the first steps towards „Understanding the origin of diversity using *Montenegrina* as a model system” is to reconstruct the phylogeny of this speciose group based on molecular markers. This should serve as a basis for further tasks like the systematic revision and the biogeographical history reconstruction of this genus.

Material from ca. 200 populations representing 81 of the 89 described *Montenegrina* taxa and several undescribed ones was collected for the investigations planned. Some taxa are available only as so called ‘mummies’, i.e. they were once collected alive and stored then dry. In many cases we succeeded in gaining DNA from those samples.

Preliminary analyses of mitochondrial COI and 16S sequences show considerable intra-generic variability (much higher than that we found in the related genus *Alopiia*): ca 45% of the COI sequence (655 bp) and ca. 57–58% of the 16S sequence (854–872 bp) proved to be variable.

Regarding the main clades’ composition, COI and 16S phylograms are essentially congruent and both trees infer that *Montenegrina* is a radiation in the sense that there were multiple speciation events within a relatively short period of time. We can thus distinguish 13 main clades that refer to ‘species

groups' in the taxonomical sense, but their branching order remains obscure. Whether this is due to insufficient phylogenetic information to resolve the tree topology or to a hard polytomy remains open.

Our result challenges the current *Montenegrina* system (e.g. Fauna Europaea) in several points. The mitochondrial markers confirmed our anticipation that many 'taxon-rich' species do not reflect real phylogenetic relationships and therefore e.g. *M. dofleini*, *M. perstriata*, *M. janinensis* and *M. irmengardis* need a thorough revision. At the same time, it seems that other species, like *M. subcristata*, *M. sattmanni*, *M. laxa* and *M. helvola* are monophyletic.

Another issue concerns the relationship between sympatric / parapatric / peripatric taxa. Preliminary results confirmed our hypothesis that parapatric taxon pairs (which are geographically isolated and differ only in one conspicuous character) are closely related. On the other hand, characteristically different sympatric taxa seem to be distinct species in a secondary contact (*M. minuscula* and *M. perstriata skipetarica*, *M. dofleini pindica* and *M. janinensis grammica*) but at the same time we found a close phylogenetic relationship between the mitochondrial sequences of *M. dofleini pinteri* and *M. stankovici*, which is an unexpected result. In order to reveal whether it reflects a real relationship, further analyses of nuclear markers (e.g., histone H3–H4 genes and microsatellites) are required.



Photo: Zoltan Feher

This project is funded by the Austrian Science fund (FWF P 26581-B25).

Genes in museum collections

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Museums harbour huge treasures of new or old, regular or odd, common or rare animals – and therefore also a big collection of genes. This is quite vital as the molecular genetic approach in scientific studies is very important, but sometimes it is not possible to gain fresh material, because the animal is e.g. extinct nowadays, difficult to find or not native in a special area anymore. In the framework of a Synthesys 2 - JRA 5 project, we tested the usage of museum material for DNA-analyses in molluscs with two different extraction methods. In total we analysed specimens from 72 glasses harboured in our mollusc collection. comprising 20 different taxa of 4 classes. The years of collection of the chosen samples ranged from 1877 to 2002. To test the success of the DNA extraction, we amplified two short sections of the mitochondrial genome (COI and 16S rRNA genes).

The gastropod taxa worked best with all tested primer sets, although the slug taxa yielded mostly bad results. The polyplacophors showed good results, the bivalves and the cephalopods worked sparsely. Concerning the age a higher percentage of the samples collected after 1900 showed positive results than the samples before 1900. Nevertheless, several samples aged about 120 years worked well, which is a quite surprising and important outcome of the project.

This project is funded by the European Synthesys 2, JRA 5 Program.



Museums collection; Photo: Katharina Jaksch

Step across the border – do the two subspecies of *T. oreinos* hybridize?

Oliver Macek, Michael Duda & Luise Kruckenhauser

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Trochulus oreinos oreinos and *T. oreinos scheerpeltzi* are two land snail taxa endemic to the Northeastern Austrian Alps, which have been proven to be clearly separated ecologically, morphologically and genetically from the highly variable, widespread land snail *T. hispidus*. In previous studies, which covered large parts of the distribution area of both subspecies, we analysed the two subspecies of *T. oreinos* conchologically, anatomically and genetically to evaluate whether delimitation between them is possible and, if so, to resolve their phylogenetic relationships.

Shell morphological investigations revealed high similarity between the two *T. oreinos* taxa. Duda et al. (2011, 2014) described small conchological and anatomical differences, however the shell difference (groove beneath the keel) is not always consistent and intermediate forms exist. In contrast, the mitochondrial data (sections of the COI, 16S rRNA and 12S rRNA genes) acquired so far, revealed high divergence between the two taxa. Even the nuclear ITS2 sequences, which proved to be not informative for several other species of the genus *Trochulus*, clearly separated the two subspecies. Hence, the two *T. oreinos* taxa appear as distantly related sister groups which indicate that they represent old lineages, well separated from all other species of the genus. However, the question whether *T. o. oreinos* and *T. o. scheerpeltzi* should be considered as separate species remained unsolved.

During 2013 we performed several collecting trips (also in the course of the Johnsbach Workshop 2013) in which we focused on the geographic area which might be a putative contact zone of the two subspecies. The aim was to investigate whether the genetic data indicate hybridization between the two subspecies. We sequenced a fragment of the COI gene from 162 individuals and, in addition, from 53 individuals the nuclear ITS2 section was sequenced. The additional COI sequences confirmed the deep split of the two lineages. Both lineages can be found on the mountain range of the Haller Mauern, with a clear geographic split: all the western sampling sites include only individuals found in the clade representing *T. o. scheerpeltzi* and all the eastern sampling sites include only individuals found in the clade representing *T. o. oreinos*. The analysis of the nuclear ITS2 sequences revealed, that from most sampling sites all individuals are found in the same clade as with the COI. The only exception is found at the sampling sites in the eastern Haller Mauern: At those sites a few individuals possess a COI sequence of the *T. o. oreinos* clade, while in the ITS2 they are either homozygous for the *T. o. scheerpeltzi* type or heterozygous for sequences of both clades. Hence, the genetic data show that only limited gene flow has occurred or still occurs between the two subspecies.

The next step will be to find out how far the hybridisation zone extends westwards to the Haller Mauern and to evaluate to which extent this hybridisation is also reflected in the morphological traits of the specimens analysed.



Natternriegel: sampling site in the eastern Haller Mauern;
Photo: WG Alpine Landsnails

Duda M, Kruckenhauser L, Haring E, Sattmann H (2010) Habitat requirements of the pulmonate land snails *Trochulus oreinos oreinos* and *Cylindrus obtusus* endemic to the Northern Calcareous Alps, Austria. *eco.mont*, **2**, 5–12.

Duda M, Sattmann H, Haring E *et al.* (2011) Genetic differentiation and shell morphology of *Trochulus oreinos* (Wagner, 1915) and *T. hispidus* (Linnaeus, 1758) (Pulmonata: Hygromiida) in the Northeastern Alps. *Journal of Molluscan Studies*, **77**, 30–40.

Duda M, Kruckenhauser L, Sattmann H *et al.* (2014) Differentiation in the *Trochulus hispidus* complex and related taxa (Pulmonata: Hygromiidae): morphology, ecology and their relation to phylogeography. *Journal of Molluscan Studies*; 1-17. DOI: 10.1093/mollus/eyu023

Kruckenhauser L, Duda M, Bartel D *et al.* (2014) Paraphyly and budding speciation in the hairy snail (Pulmonata, Hygromiidae). *Zoologica Scripta*, 43/3: 273-288; DOI: 10.1111/zsc.12046.

This work was funded by the Austrian Science Fund (FWF): P19592-B17.

Systematic revision of the neglected land snail family Plectopylidae

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The land snail family Plectopylidae is widely distributed in East Asia. Approximately 40% of the species are sinistral which is exceptionally high among all flat shelled snail families. These snails have peculiar armatures consisted of lamellae and plicae on both the parietal and palatal sides inside the whorls of shell. Taxonomic identification and description are mainly based on these traits.

Most of the approximately 100 taxa of this family were described between 1880 and 1910; almost no publication has dealt with this group in the last century. The system (generic subdivision) of the family was proposed in 1899 based on only a few shell characters.



Photo: Barna Páll-Gergely

We have examined all so-far available shell and alcohol materials from several countries. Observing the similarities of shell characters helped to understand the homologies of each shell characters, which were used in identifying hypothesized monophyletic groups. Every taxon is defined by unique combinations of morphological characters. We discovered more than 40 species and subspecies new to the science from China, Vietnam, Thailand, Malaysia, Nepal and Laos. We found 10 synonym names in the literature. The revision of the system revealed that six new genera and two tribes need to be described and more than 40% of the hitherto known species were wrongly placed in their genera.

This study was supported by scholarships from Japan Student Services Organization and Mitsubishi Corporation to Barna Páll-Gergely and Grants-in-Aid from Japan Society for the Promotion of Science to Takahiro Asami.

Statutory provisions of collecting invertebrates in Austria

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Collecting (and killing) free living native organisms is regulated in Austria, like in many other countries, by different laws. Vertebrates are concerned e.g. by game laws, pest control laws, animal welfare acts and conservation laws. Invertebrates (and plants) are mainly protected by nature conservation laws and decrees. In nature conservation the nine federal states (provinces) of Austria are autonomous legislators. Consequently, a puzzle of nine conservation laws exists in Austria, each of them with different structures. All of these laws specify environments and species underlying particular kinds of protection. Although most of these laws also provide exceptions or approvals for scientific research purposes, these exemptions are, unfortunately, issued, if any, in different provinces by different authorities. Consequently for scientists it is not always easy to obtain such permission, especially for scientists from other countries who are not familiar with the administrative infrastructure of the country.

While in former times collecting without any permission was practiced frequently, nowadays, many but not all scientists aim to collect legally. They are forced by international conventions and laws targeted to increase protection of environment and biodiversity, and by the accompanied practice of many high ranked scientific journals to demand official certificates and collecting permissions for specimens analysed. (Besides that, to avoid misunderstandings and duplication of work, it is strongly recommended to get in accordance with local working groups about collecting in “foreign hunting grounds”.)

To avoid depletion and exploitation of shrinking environments and collapsing biodiversity worldwide, it is reasonable to have stringent conservation rules. However, for biodiversity research, systematics and taxonomy collecting is essential. And research again is essential for reasonable conservation. Therefore, these national rules of protection and the procedures to apply for special permissions should be more transparent and visible, for the national and international research communities. One important step would be to make the rules and requirements globally visible and to publish main points in English language, in addition to the national language. It would be very helpful to provide an internet platform with information about the legal background and requisites for collecting and collecting permissions for researchers. This contribution intends to make one step to stimulate the establishment of such a Europe-wide network.

On the early evolution of pulmonate molluscs

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The most ancient findings of terrestrial pulmonate molluscs are dated to the Palaeozoic (Carboniferous-Permian). Already at that time the variety of shells was such that one can state that the exit of gastropods on land had taken place markedly earlier – probably in the Devonian. The variation of Palaeozoic shells concerns shell shape (flattened and elongated), sculpture (smooth and ribbed), structure of aperture (simple and toothed). However, all of them had a small size; e.g., the height of elongated shells (*Dendropupa*, *Anthracopupa*) does not exceeded 8 mm (*Dendropupa vetusta*) and usually is quite smaller (≤ 5 mm). The diameter of flattened shells (*Protodiscus*) is ≤ 2.5 mm.

The first steps of pulmonates from the sea onto dry land were made through littoral and farther through the splash zone. That time almost the only source of food was detritus, ejected by the sea. Thus, also for non-marine molluscs the initial type of nutrition was, probably, detritus and it can be assumed that with such a low-calorie food the snails could not reach large sizes. After that a huge gap of time follows during which terrestrial shells are totally unknown: from the Upper Carboniferous to Lower Cretaceous (not less than 160 million of years). This fact could be explained by at least two factors:

1. Food resource throughout all this time was not significantly changed.
2. Chemical composition of the Mesozoic soils did not favor the fossilization of calcareous shells; carbonate soils on land got a wide distribution only toward the end of Mesozoic.

The heyday of land pulmonates started in the Lower Cretaceous, when increased angiosperm plants emerged, which gave abundant and inexhaustible food resource. Modern pulmonates do neither feed on gymnosperms nor on ferns or mosses, which formed the basis of the flora of the planet throughout most of Mesozoic. In addition, in the Cretaceous period limestone had been formed, providing an additional stimulus for the development of land malacofauna.

Starting from the Upper Cretaceous a few recent families appeared as well as some groups, which subsequently became extinct again: Filholiidae (lower Oligocene – middle Eocene of Europe), Anadromidae (upper Cretaceous – middle Eocene of Europe and NW Africa), Grangerellidae (Paleocene of N America). Among Cretaceous and, especially, Tertiary pulmonates there are, inter alia, shells of large size, for example, *Hodopoeus* (Humboldtianidae-Lysinoeinae or perhaps, Pleurodontidae) with shell diameters up to 60 mm, which lived in Eocene or Paleocene of N America, or Filholiidae with shell height up to 100 mm.

Consequently, beginning in the Cretaceous, the limits for dimensional characteristics of a shell had disappeared – to the extent in which the sizes depended on the quality of food.

Recent Stylommatophora can be divided in accordance with their diet into seven groups:

1. Detritophages. Molluscs of small size.
2. Micromycophages. Feed on fungi hyphae. Snails of small size.
3. Macromycophages. Eat fruit bodies of macromycetes. Dimensions are not limited (slugs are not considered).
4. Lichenophages. Feed on lichens. Molluscs of small or middle size.
5. Phytophages. Feed on living tissues of angiosperms. Dimensions are not limited.
6. Predators. Feed on other molluscs (including cannibalism), soil oligochaetes and insect larvae with soft covers. Dimensions are not limited.
7. Polyphages. Eat everything, including rotting parts of the angiosperms and organic of animal origin. Dimensions are not limited.

Representatives of four of these seven groups can reach a considerable size.

As pulmonates descended from some marine Pectinibranchia, the eyes in the initial forms were located at the bases of the tentacles, i.e. as in Basommatophora. Nevertheless, the modern basommatophorans, nearly all of which are freshwater animals, are derived from terrestrial ancestors, otherwise it is impossible to explain the presence of the lung in them and, correspondingly, their aerial breathing.

Consequently, the very first land pulmonates were formally Basommatophora. Then some of them changed the structure of tentacles (retractable instead of contractile) and topography of the eyes (on the tips instead of at bases of tentacles), forming the superorder of Stylommatophora. Another part, changing the method of breathing, but retaining the original structure of the tentacles and eyes returned to the aquatic environment – these are the modern Basommatophora.

The earliest pulmonates were characterized at least by five characters. 1. They had contractile tentacles with eyes at their bases. 2. They had a small elongated shell. 3. They had a columellar fold extending along the entire length of the columella. 4. In the aperture they had, except the columellar fold, at least one tooth – parietal. 5. They had internal fertilization, because it is a prerequisite for land life independent of a return to water for reproducing.

This reconstruction is based on the following facts and considerations. There is nearly unanimous opinion that the ancestor of pulmonates was some ellobioid-like form, and all Ellobiidae (both fossil and modern) have elongated shells.

In a number of Ellobiidae the shell is heterostrophic, i.e. the direction of the growth axis of the initial whorls does not coincide with the direction of the later whorls growth. Obviously, when the direction of shell growth has changed, on the columella a fold arises, which then continues until the completion of growth.

The parietal lamella protects the organs that are located in the angular region of the aperture (rectum, distal part of the secondary ureter and pneumostome), from the pressure of the adjacent structures. Since the most ancient land pulmonates ate low-calorie food (detritus), they had to consume a relatively large amount of food. If so, their crop during intensive feeding might greatly swell and put the pressure on adjacent organs. This could cause some problems in the functioning of the ureter and rectum, as well as respiration. The presence of a hard parietal barrier solves this problem. This parietal barrier (opposite to palatal and basal teeth which appear at subadult age only) exists and works at all stages of postembryogenesis.

The absence of teeth is a secondary phenomenon because the problem of mutual position of the crop, pneumostome, rectum and ureter in such snails had been solved in more progressive way, without participation of any hard structures. If a rigid structure exists, it cannot be removed. Any hard structure in the aperture inevitably slows down the speed of withdrawing the snail into the shell: slowdown of withdrawing for small snails is connected with the risk of drying out and reduced shield against enemies. In the shells with a toothless aperture the problem of mutual arrangement is solved by a more progressive way – by creating of temporary partitions. Such partitions can arise through redistribution of hemolymph in the body sinuses together with coordinated contractions of some groups of muscles.

There is one more particular, but important question – why among Basommatophora the slugs are totally absent (and seemingly never existed). I think, because of at least three reasons.

1. On dry land an assortment of environmental conditions is much richer and more variable than in fresh water (I do not consider the situation in shallow water of sea). Moreover, in water there are no sharp temperature changes and no wind, i.e. the conditions of life in fresh water are much more constant and uniform than on land. Consequently, in the majority of freshwater invertebrates (especially molluscs with their limited mobility) there is much less incentives to intensive adaptive radiation. At the same time, the most strong stimulus to the loss of the shell, and, consequently, to replacing of method of a passive protection by an active method (like in slugs) is the acquisition of the “ability to think”, i.e. adequately assess the situation and the ability to choose the best strategy for the given case. So, in fresh water the stimulus to lose the shell is practically absent.

2. The relative volume of the lung. Terrestrial animals can breathe with any frequency and with any intensity, since the volume of their lung is not limited, while the lung volume of slugs is comparatively smaller than that of snails. This is due to the fact that under the reduction of shell the visceral sac undergoes profound changes, which results, in particular, to decreasing of lung volume. Water pulmonates, for sufficient breathing, cannot have a too small lung, as they are forced to rise periodically to the surface of the water for gas exchange.

3. For management of shell in the air environment some efforts are required, resulting in additional energy expenditures. Slugs do not need any mechanism of control of the shell. But for the snails that live in water the problem of management by the shell does not exist, because of the uplift. Not by chance the biggest and heaviest shells are found among just marine molluscs.

Living conditions in the water, as stated above, are much more stable than on dry land, so the number of families in Basommatophora is many times less than in Stylommatophora, but these families (Lymnaeidae, Planorbidae, Physidae) often have enormous areas, and, under a small number of genera include many species.

Concerning the mainstream of stylommatophoran evolution in the future, it is not exaggerated to state, that the shell created a mollusk. However, the shell, being an effective protection, at a certain stage of evolution has become a brake of biological progress. Indeed, among various branches of

Stylommatophora there are many taxa consisting of slugs or semislugs, i.e. mollusks that have lost the shell – partly or completely. A snail has only one response to different environmental conditions – to retreat into its shell while the slug must assess the situation more adequately – to crawl away, to stay in place, to hide in a shelter or something else. In other words, slugs are “cleverer” and more flexible than snails. This is confirmed by the fact, that the density of synapses in the procerebrum of a slug is much higher than in the brain of a snail. That is why it is not surprising that at the present geological time we observe a universal tendency to the loss of shell among many taxa (now there are more than 20 cases of independent reduction of shell within Stylommatophora).

Thus, speaking about the prospects of development of Stylommatophora, we can hypothesize that in several millions of years the malacofauna of the planet will be represented predominantly by slugs. Exceptions will be a few highly specialized and morphologically conservative families like Pupilloidea, Clausiliidae or Urocoptidae.

A local hotspot of molluscan species richness - first impressions of the malacofauna of the Kalkalpen National Park (Austria, Upper Austria)

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Established in 1997, the Kalkalpen National Park is located in the southeastern part of Upper Austria and consists of two main mountain ranges, the Sengsengebirge and the Reichraminger Hintergebirge. Its total area of almost 21000 hectares, ranging from 385 to 1963 m a.s.l. in altitude, comprises of a variety of different habitat types, including several of high conservational interest, such as springs and alpine habitats.

Until the last few years, only little was known about the malacofauna of that area, despite the fact the nonmarine molluscs are amongst the taxa most severely suffering from anthropogenic environmental changes and associated extinctions of species. A mere total of around 40 species and subspecies of continental molluscs had been reported to occur in the National Park as of the year 2007.

As a first step to increase the faunistic knowledge on local molluscs, the malacofauna of selected sampling sites was studied qualitatively in a three months period from June to August 2012. Different collecting methods, such as visual search, soil-, litter- and sediment sorting as well as Barber pitfall traps were used. In addition, recent data provided by Peter L. Reischütz and Alexander Reischütz were included in this study. Both, living specimens and empty shells were taken into consideration. A particular focus was on the assessment of the malacofauna of alpine habitats, areas of poorly impacted forest and springs.

A total of 86 molluscan taxa, more than twice as many as known before, were found within the borders of the Kalkalpen National Park. This represents around 19% of the known molluscan fauna of Austria in terms of the number of species. In addition, many endemic taxa as well as taxa listed as threatened in the Austrian Red List were among the species found.

Of particular importance was the first record of the rare endemic and only recently described hydrobiid snail *Bythiospeum nocki* (Haase, Weigand & Haseke, 2000) within the National Park. So far, this subterranean taxon is only known from a few specimens. A single shell was found in sediments of a tiny spring, suggesting that also living specimens of this critically endangered species might occur in the National Park. In the alpine habitats, large populations of endemic alpine landsnails, e. g. *Orcula pseudodolium* (A. J. Wagner, 1912), *Trochulus oreinos scheerpeltzi* (Mikula, 1957), *Arianta arbustorum styriaca* (Frauenfeld, 1868) and *Cylindrus obtusus* (Draparnaud, 1805) were present.

Combined with the apparent lack of introduced mollusc species, these results indicate the great importance of the national park for the conservation of autochthonous molluscan communities within Austria.



Plateau of the mountain Hoher Nock, which represents the highest elevation of the Sengsengebirge.
Photo: Erich Weigand.



Bythiospeum nocki, a rare endemic and poorly known subterranean aquatic snail.
Photo: Jan Steger

DNA-Barcoding in the Gesäuse National Park – Biodiversity, ABOL and added values for protected areas

Nikolaus U. Szucsich in behalf of ABOL

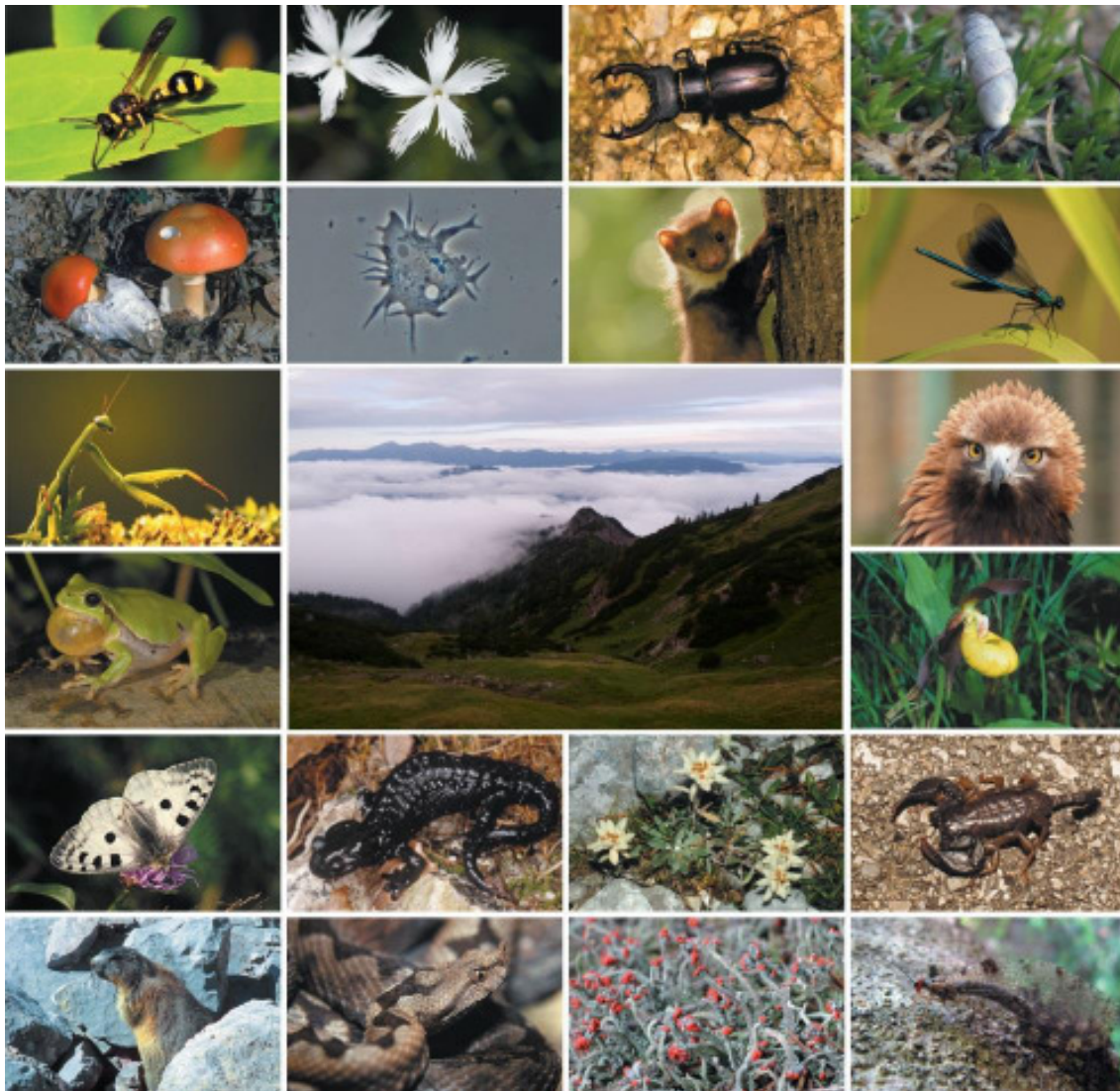
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A major part of the fascination of biodiversity is owed to its multi-faceted nature. Species diversity encompasses morphological and genetic variation, both of which are likewise present within species. All of this organismal diversity is dependent and interwoven with a biodiversity of biotopes. DNA-Barcoding is an approach to study biodiversity, which established itself rapidly in the past few years. This is partly due to a basic idea which is straightforward: A standardized fragment of the genome allows for identification of organism to species level and below, opening up new areas to biodiversity research. An ever growing number of national initiatives emerged in the past years (e.g. GBOL, German Barcode of Life), all following the quality standards of an international umbrella organization. The complete spectrum of the phenomenon biodiversity becomes available only in an integrative approach, where DNA-Barcoding is combined with traditional taxonomy, ecology and bio-geography.

Consequently, the recently launched Austrian initiative ABOL (Austrian Barcode of Life) will try to establish a platform for all institutions and experts dealing with biodiversity of Austria.

The pilot phase will be used to build up such a platform. In parallel, four pilot project will sample and study barcodes of vertebrates, butterflies and moths, molluscs and parasitic worms. The aim of the bigger following project DNA-Barcodes will be made available for all animals, plants, and fungi of Austria.

The open access policy of the barcoding initiative will provide data, valuable for many applied fields, especially conservation issues. Strict rules in vouchering and documentation, and the possibility to identify life stages, hard to determine by traditional approaches will allow institutions like National Parks to use the data for monitoring-projects, but likewise for public outreach.



ABOL and the Biodiversity of Austria; Photos: B. Däubel, P. Garcia Escobar, L. Kruckenhauser, R. Osterkorn, Österreichische Mykologische Gesellschaft, H. Sattmann, P. Sehnal, J. Walochnik, D. Zimmermann

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Abstracts: posters

arranged in alphabetical order of first authors

Phylogenetic relationships of *Cylindrus obtusus* (Pulmonata, Helicidae) deduced from nuclear and mitochondrial DNA marker sequences

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Throughout many years the systematic position of the Eastern Alpine endemic land snail *Cylindrus obtusus* has been discussed controversially. The species features interesting particularities in biology, ecology and anatomy. Its genital anatomy, highly consistent with the genus *Arianta*, has led to placing the species within the family Helicidae, subfamily Ariantinae. However, *C. obtusus* shows a conspicuous aberrant shell form within this group – all other representatives display globular or more or less depressed shells. Furthermore, *C. obtusus* has a geographically and ecologically rather restricted distribution. This raised questions about the age of the species, its evolutionary history and its relationships within Ariantinae. In this study we tested the presumed sister group relationship of *Arianta* and *Cylindrus* using phylogenetic methods, including additional genera of Ariantinae and of some other subfamilies of Helicidae.

Five molecular markers were used: partial sequences of three mitochondrial genes, cytochrome oxidase subunit 1 (COI), 12S rRNA and 16S rRNA, and two nuclear genes for histone 3 and histone 4. Phylogenetic trees calculated using Bayesian methods confirm that the sister group of *Cylindrus* is indeed the genus *Arianta*. The *p* distance in the COI gene between the two taxa is about 20%. The molecular systematic analysis provided insights regarding the phylogenetic relationships within Ariantinae. Unfortunately, no reliable dating of the age of the split between *Cylindrus* and *Arianta* is feasible. Furthermore, no fossils of *C. obtusus* have been found, except some rare and probably young findings within the extant distribution range in the Eastern Alps. Thus the “age” of the species remains enigmatic.

Our findings underline the relevance of genital anatomical characters for taxonomic issues, opening the question as to why shell morphology of *Cylindrus* is so different from its relatives, and whether its restricted distribution, habitat preferences and ecological requirements, along with its evolutionary history, can explain this phenotypic difference.

Reference: Cadahía, L., Harl, J., Duda, M., Sattmann, H., Kruckenhauser, L., Fehér, Z., Zopp, L. & Haring, E. (2014): New data on the phylogeny of Ariantinae (Pulmonata, Helicidae) and the systematic position of *Cylindrus obtusus* based on nuclear and mitochondrial DNA marker sequences. – Journal of Zoological Systematics and Evolutionary Research, 52/2: 163-169. doi:10.1111/jzs.12044.

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Biodiversity homemade?! – *Clausilia dubia* and its various subspecies

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Clausilids are one of the most species-rich groups among land snails. In Austria they are very abundant in the alpine region inhabiting different habitats. In this study we investigate *Clausilia dubia* Draparnaud 1805, a species which displays a broad variability in its morphology, and hence several subspecies were described. The subspecies classification was reassessed by morphological analyses (various shell characters) and for the first time by molecular analyses (partial sequences of the mitochondrial gene for COI). In Austria 17 subspecies of *C. dubia* are described, 13 of them occur in the investigated area of the Northern Calcareous Alps of eastern Austria. The individual distribution areas of these subspecies often overlap and some of them are occurring even syntopically.

The aim of our study was to evaluate whether the described subspecies are genetically and/or morphologically differentiated. Moreover we wanted to find out whether the morphological and the genetic results are in accordance. The determination of the subspecies proved to be difficult, but there are some subtle character combinations, which allowed distinguishing them. For an easier classification the subspecies were merged into four morphogroups. However, neither with the morphometric, nor with the molecular results a separation of any of the described subspecies or morphogroups was supported. Hence, the validity of the described subspecies appears doubtful.

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Alpine populations of *Pyramidula pusilla*: A closer look into the species phylogeography (Gastropoda: Pulmonata: Pyramidulidae)

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Even though the alpine land snail *Pyramidula pusilla* (VALLOT, 1801) is the most widespread species of the monotypic family *Pyramidulidae*, it often remains unnoticed, which might be due to its small and inconspicuous shell. Its distribution ranges from the Mediterranean area to Western and Central Europe where it inhabits sunlit limestone and feeds on endolithic lichens. Although it is quite common, nobody has taken a closer look into its phylogeography so far.

In the present study, genetic and morphometric analyses were performed to gather first insights into intraspecific diversity and phylogeography of *P. pusilla* with a special focus on its Eastern Alpine distribution. A total of 357 individuals were investigated genetically, whereof 143 were also used for morphometric analyses. The individuals were collected at 98 different sampling sites located mainly in the Eastern Alps. After DNA extraction, amplification and sequencing of a ~650 bp section of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene was performed. The phylogenetic trees obtained from the sequence data reveal, besides two clades with a distribution in the Balkans and Western Asia, at least two distinct Alpine clades that appear also differentiated in the multivariate morphometric analysis. However, the analysis of the geographic coordinates of the differentiated Alpine clades shows no specific geographic pattern. An additional task was to uncover potential refugia and postglacial migration-scenarios by combining the results of the genetic and morphometric analysis with information on the geographic occurrence of the

different clades. The results imply that the species most likely survived the last glacial period in several refugia. Considering the current distribution of *P. pusilla*, passive dispersal seems to have occurred frequently and has probably played an important role in the phylogeographic history of *P. pusilla*.

Whether the findings of these distinct clades indicate the division of *P. pusilla* into two separate species or merely express a high degree of genetic variation within Alpine populations of *P. pusilla* has to be investigated by further studies on gene flow and reproductive barriers.

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Snails do it – but sometimes they don't need to do it! – Selfing in geographically separated populations of *Cylindrus obtusus* (Gastropoda: Pulmonata: Helicidae)

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Cylindrus obtusus is a hermaphroditic land snail, endemic in the Austrian Alps, which is restricted to high elevations (1600 to 2500 m asl) and limestone. It has a quite patchy distribution area which may become even further reduced in the future due to global warming. Previous investigations revealed geographic differences in the genital apparatus: All specimens from the more western populations had one stylophore and two equally developed mucus glands more than twice the length of the stylophore. In contrast, in individuals from the eastern margin of the species distribution, one or two mucus glands were found and the size ratio between stylophore and mucus gland(s) was highly variable.

To find out whether these anatomical differences reflect a genetic differentiation, which might be an indication for distinct glacial refugia, we investigated a 650 bp fragment of the mitochondrial COI gene (200 individuals) and 9 microsatellite loci (500 individuals from 29 populations) from samples covering the whole distribution range of the species. The COI sequences showed a geographic differentiation between eastern, central and western populations. The microsatellite analysis revealed a high differentiation between the populations implying restriction of gene flow. Remarkable nearly all individuals from the eastern populations, which are variable in their genital morphology, are homozygous in all microsatellite loci (although different alleles were found within populations). The most plausible explanation for this finding is an altered mode of reproduction.

For further investigations quantitative measurements of the mucus glands and the stylophore were taken from more than 70 individuals collected from 16 populations. The results confirm the east-west division suggesting possible functional differences. To assess the functionality of the mucus glands, a comparative histological investigation of the mucus glands was performed.

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Cocktails and pills – a COI primer cocktail for pill millipedes from Austria

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Pill millipedes (Glomerida) represent an intricate order of Diplopoda, encompassing more than 400 described European variations which are presently assigned to ~280 species worldwide. This astonishing variability is an ideal set-up to test DNA-barcoding for its delimitation capability in species recognition. Our study focuses on species from Austria, allowing for a pilot study within the upcoming initiative ABOL (*Austrian Barcode of Life*). Due to the widely unexplored status of diplopods with respect to barcoding, we tested several methods at the beginning of our study. To avoid a probably inhibiting effect of the defensive secretions we used only the head of the specimens for DNA-extraction. Furthermore we applied a Non-Destructive-Extraction method (NDE), to preserve all cuticular structures for subsequent analysis by traditional taxonomy using morphological characters. Only in *Trachysphaera*, a genus lacking defence glands, with minute species below 5 mm body-length, entire specimens were used for NDE. To further optimize the protocol, a combination of primers was developed for both the PCR and the sequencing reaction. The used primer-cocktail GlomF1/R1 gave satisfying results for all investigated species. No specific adjustments were necessary for species newly added to the data set. To validate our results we sequenced a fragment of the nuclear 28S rRNA-gene (900 bp) in addition, spanning both conserved and variable regions. The obtained sequences resulted in clusters fully congruent with the COI based tree.

Up to now we generated from a total of 102 pill millipede individuals COI barcodes and 28S rRNA sequences. Both markers separated the Glomerida collected in Austria into 11 well defined clusters. In both trees distances are large among clusters, but small within each cluster. The recovered molecular clusters are congruent with currently accepted species. Establishing our protocol for COI and 28S rRNA genes, we are eager to include the remaining species collected in Austria into the newly launched ABOL-Initiative.

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Photo: WG Alpine Landsnails



WORKSHOP ALPINE LAND SNAILS 2013; Photos: WG Alpine Landsnails

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