

Abstracts: posters

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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.

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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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