### Abstracts: Project reports: Alpine and other Land Snails

arranged in chronological order of the program

## Genetic variability and invasion routes of *Arianta arbustorum* in St. Petersburg and Leningrad area

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Biological invasions may be considered as "natural experiments", offering unique insights into ecological and evolutionary processes occurring in real-time (Lee, 2002; Sax *et al.*, 2007). The terrestrial snail *Arianta arbustorum* L., 1758 demonstrates a case of extremely successful invasion of regions in north-western Russia during a short period. According to the literature and personal observations snail numbers dramatically increased in the last 20 years and the species very rapidly extended its distribution in eastern and northeastern direction. The aim of our study was to find the source of this invasion using a section (284 bp) of the mitochondrial *cytochrome oxidase subunit* 1 gene *CO1* as molecular marker. We tried to test several hypotheses of invasion modes: (1) independent multiple introductions of the species from its native range resulting in a genetically diverse invasive population, (2) a "step and stone" scenario with declining genetic diversity along the colonization route, (3) a "bridgehead effect", which means that a particularly successful invasive population with small genetic diversity is the source for extensive secondary invasions in other regions.

In a first step we studied variation within and between populations of Leningrad region and compared the results with variation in snail populations at other territories. Specimens of *A. arbustorum* were collected at seven sites located on opposite shores of the Gulf of Finland with maximum distance of 100 km between collection sites. Among 47 sequences from snails gathered at this territory, we discovered only 4 haplotypes with 6 variable sites. All discovered haplotypes were unique as compared to Western Europe. Then we enlarged the study area and included snails collected in Denmark, Sweden, Finland, Baltic countries and in Pskov region of Russia.

The final alignment of sequences of 385 specimens contained 138 haplotypes, with 118 variable and 95 informative sites. At the territory surrounding the Baltic countries, Scandinavia and the north-west of Russia only 9 haplotypes were detected among 75 sequences. These haplotypes had only 3 informative and 19 variable sites. Thus, mollusks from this vast territory demonstrate extremely low genetic diversity as compared for instance with Austria, where 119 haplotypes were detected at the territory of 5 km<sup>2</sup> (Haase, Misof, 2009). The haplotype network shows very close connections of haplotypes from Russia, Baltic countries and Scandinavia in contrast to haplotypes of mollusks from Western Europe that scattered all over the network. Some haplotypes from Austria and The Netherlands cluster with the Russian samples, but differ by 7 and 10 nucleotide substitutions, respectively. All haplotypes from West European countries differ from the Russian ones by more than 15 substitutions. Despite small nucleotide and haplotype diversity within the Baltic countries, Scandinavia and the north-west of Russia, it is noteworthy that all discovered haplotypes are unique as compared to Western Europe. The data obtained suggest the 'bridgehead' model of the land snail invasion in the region, as all haplotypes recovered in the studied area were unique, differing from the closest haplotype from Denmark by several mutations. This fact together with extremely low haplotype and nucleotide diversity implies that snails accumulated mutations at a small



newly populated territory during a rather long period. Neutrality tests are in accordance with balancing selection.



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#### What do nuclear markers tell about Montenegrina phylogeny?

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*Montenegrina* is among the most taxon-rich obligate rock-dwelling gastropod taxa. In the door-snail family (Clausiliidae) it is the second most speciose genus after *Albinaria*. The geographic range of *Montenegrina* extends to ca. 400–450 km in NW–SE direction and includes the coastal regions of Montenegro south of the Bay of Kotor, Albania, western Macedonia, and northwestern Greece. Within this relatively narrow range, there are almost 400 known populations that are classified in 29 species (including subspecies 106 taxa altogether) according to the current morphology-based system (Fehér and Szekeres 2016). The range size and the number of populations can be deemed large enough, but still accessible to almost comprehensive sampling, rendering this hyperdiverse genus an attractive system to study and to better understand the general mechanisms of speciation and spatial distribution of rock-dwelling gastropods.

To obtain an overall phylogenetic framework we conducted a phylogenetic analysis based on the mitochondrial genes for *cytochrome c oxidase subunit 1, 16S rRNA*, and *12S rRNA* (*COI, 16S, 12S*). The reconstructed DNA-based tree confirmed by and large the traditional classification of the genus, but there were some discordant or at least noteworthy points.



Figure 12. Statistical parsimony network, based on the spacer region between *histone H3* and *H4* genes of *Montenegrina*. Sequences / taxa mentioned in the text are highlighted: *M. dofleini kastoriae* and *M. hiltrudae* robusta – green; *M. fuschi fuchsi* – red; *M. rugilabris golikutensis* – orange; *M. stankovici* and *M. dofleini* pinteri – navy blue; *M. perstriata drimica* specimens belonging to different mitochondrial haplogroups – yellow; three different sequences from the same *M. hiltrudae desaretica* specimen – cyan

Some morphologically different but geographically nearby located taxon pairs like *M. dofleini kastoriae* and *M. hiltrudae robusta*, *M. fuchsi fuchsi* and *M. rugilabris golikutensis* or *M. stankovici* and *M. dofleini* 

*pinteri* are closely positioned in the mitochondrial tree. At the same time, we have found two highly different mitochondrial haplogroups in *M. perstriata drimica* populations. Although these populations along the Drin River Valley are somewhat heterogeneous, no distinct morphotypes could be delimited and therefore, *M. perstriata drimica* was up to now considered one polymorphic taxon. Divergent mitochondrial genes might indicate the presence of a cryptic species, but introgression is also a plausible explanation for this phenomenon.

In order to resolve the above contradictions, we analysed two nuclear DNA markers, the *histone H3* and *H4* genes. Apart from the specific interest in the cases mentioned above, we also hoped to see how concordant the trees based on mitochondrial and nuclear genes are. As we have amplified a section of the histone cluster that involves the *H3* and *H4* genes and a spacer region in between (*H3–H4*) (see also Harl *et al.* 2014 a,b), our second objective was to test how suitable this spacer region is in the phylogenetic reconstruction of *Montenegrina*. We obtained 211 *histone H3–H4* sequences from 199 specimens. In several cases it was necessary to clone the amplified DNA fragments due to double peaks in the electropherograms. In those cases 3 clones were sequenced per specimen, resulting in two or sometimes three different sequences.

Unsurprisingly, as *histone* genes are under strong purifying selection (Nei and Rooney 2005), there was little variation in the coding regions, while the spacer section revealed considerable variation: 98 different haplotypes were found with at most 53 pairwise differences between aligned sequences (if gaps are excluded). To some extent, the *histone* tree reflects the mitochondrial one, but in some cases the spacer sequences of the same species, subspecies, population, or even the same specimen are positioned strikingly far from each other in the reconstructed tree or network. This possibly indicates hybridization events. Due to this phenomenon, this marker can only be used for phylogenetic inference in *Montenegrina* with some restrictions and care.

Nevertheless, the H3–H4 sequence served as a useful supplementary tool in the above mentioned cases of incongruent mitochondrial phylogeny and morphology. In the cases of *M. dofleini kastoriae / M. hiltrudae robusta, M. fuschi fuchsi / M. rugilabris golikutensis* as well as *M. stankovici / M. dofleini pinteri* identical or very similar spacer sequences indicate close relationship (conspecificity or sister taxon status)



Figure 13. Cliffs along the shore of Lake Ohrid, north of Trpejca, Macedonia. This is one of the known sites where two different *Montenegrina* species can be found at the same locality. *M. stankovici* (on the right) prefers the lowest regions directly above the water surface, while *M. dofleini pinteri* inhabits higher parts of the same cliff; © Z. Feher

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and confirm the 'rapid morphological alteration' scenario against the 'mitochondrial introgression'. In contrast, the spacer sequence similarity of *M. perstriata drimica* specimens that belong to different mitochondrial haplogroups renders the 'introgression' scenario more likely for this taxon than that of 'cryptic species'.

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# Subgeneric Taxonomy of the limestone rock-dwelling Clausiliidae: has the genital anatomy been neglected until now? A preliminary overview about *Montenegrina*, *Siciliaria*, *Charpentieria* and *Medora* (Pulmonata: Clausiliidae)

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The systematics of Clausiliidae at generic level is mainly based upon shell features even if differences in the genital apparatus are known to partially define each genus-group. The whole picture is still far from clear, and what is currently known is almost entirely due to the work of H. Nordsieck (*Zur Anatomie and Systematick der Clausilien* series).

Nonetheless, in his series and related papers, Nordsieck gave large relevance priority to a systematics based upon "shell-taxonomy", considering the morphology of genitalia of substantial importance only at generic level.

Genital morphology features are, on the other hand, considered of great importance at species-level in most of the Pulmonata families (e.g.: Zonitidae, Hygromiidae, Limacidae, Milacidae etc.) and in many Architaenioglossa (i.e. Hydrobiidae, Cochostomatidae etc).

The remarkable lack in literature of genital data at specific level in Clausiliidae does not necessarily mean that comparative genital morphology could not play an important role as a complementary tool, as well as together with molecular genetic analysis, in the complicated taxonomy and systematics of this family. It is worth stressing that the "shell-taxonomy" alone already proved to have clear limits in many groups (i.e. Zonitidae and Cochlostomatidae).

The genital anatomy of the genus *Montenegrina* is currently being investigated, having processed approximately 20 species until now. Not only the outer features of genitalia (mainly shape and proportions)

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have been investigated but also the inner ornamentation of the main sections that already revealed to be of fundamental importance in other groups: atrium, penial complex, penial papilla, epiphallus, vagina, free oviduct and complex of bursa copulatrix. Except for the penial papilla, most of the above-mentioned features have been never or only seldom considered, and never in an overall comparative frame.

As for many hygromiid and zonitid groups, specific and stable differences have been found among *Montenegrina* species and also in what is currently regarded as conspecific subspecies.

These differences sometimes follow and confirm the current systematic position of the species. For instance, as in *Montenegrina subcristata* and its subspecies where a general common arrangement can be detected, or as other cases, like *Montenegrina laxa*, where remarkable differences are observed in taxa currently considered as subspecies (*M. laxa laxa* and *M. laxa iba*).

In the wake of this new taxonomical approach to the Clausiliidae, other genera with a similar distributional pattern with isolated (or alleged so) populations, restricted along limestone-island, were investigated to test if this new model could be extended and validated.

*Siciliaria, Charpentieria* and *Medora* have been evaluated, providing results commensurate with those of *Montenegrina*, revealing specific and stable differences among the species so far investigated. More investigations are required to better evaluate the real taxonomical value of the genital morphology investigations on the systematics of Clausiliidae. Considering the huge extent of this family as regards its





Figure 14. Comparison of genital structures and shells of *Montenegrina laxa laxa* (left) & *Montenegrina laxa iba* (right); © W. de Mattia

diversity and distribution, the possible new taxonomical approach will require a huge amount of work.

In a frame of a global overview of the systematics and taxonomy of the Clausiliidae, only extending the classic shell morphology to a global "holistic" approach that includes both genital morphology and molecular genetic data could be the way for a better comprehension and definition of this family.

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#### Adaptations of pulmonate Molluscs to life on land

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Representatives of only one class (out of 7) of the phylum Mollusca, namely Gastropoda, were able to come from the sea onto the dry land.

The principal difference between life in water and life on the land is that water represents a much more conservative environment. First, for aquatic animals, the problem of saving of water does not exist while for many land invertebrates this problem is, probably, the most important. Second, changes in water temperature usually are more buffered and do not shift instantaneously, and not so extreme, while animals living on land often have to cope with fast temperature changes.

Although initially terrestrial pulmonates are moisture-loving animals, many of them have mastered almost all types of biotopes including zones with arid climate.

A principal and permanent danger threatening land mollusks living in conditions of moisture deficit is the danger of dehydration. Nevertheless, in arid areas a large number of species live and even prosper well. Accordingly, terrestrial mollusks elaborated a number of peculiarities which minimize this danger (Schmidt-Nielsen et al., 1971; Yom-Tov, 1971). Moreover, a majority of xerophilic snails cannot live in wet conditions.

All the diversity of adaptations may be assigned to four categories:

1. **Morphological** (shell shape and size, coloration, sculpture, apertural barriers); 2. **Biological** (reproduction); 3. **Ethological** (behavioral); 4. **Physiological** (breathing, water balance). As we will see, these categories are somewhat conditional, they often are combined with each other, and sometimes it is difficult to attribute this or that character to a particular category.

#### 1. Morphological adaptations

The aperture is the main channel of communication of a mollusk with the outside world and thus, any elements of the apertural structure are related, directly or indirectly, with the adaptation of the animals to the environment. It is obvious that different teeth in the aperture play a significant role in the regulation of water balance and speed of withdrawal of a snail into the shell.

A more or less swollen last whorl is characteristic for helicoid shells. This whorl is a container for withdrawal of the cephalopodium: the larger relative volume the cephalopodium occupies, the more effectively the animal can burrow into the soil for aestivation or hibernation. At the same time, the presence of a relatively bulky cephalopodium means that the animal is able to store a sufficiently large volume of water in its tissue, which is essential for the snails inhabiting regions with a dry season.

Coloration in some cases may be considered as adaptation to survival during the dry season. Snails living openly often form aggregations (clusters) on grass or bush branches and the shells of such snails often have white color (usually with dark bands or radial stripes). White surface reflects sun rays which reduces evaporation. Besides, such snails usually have a toothless aperture; the absence of teeth promotes faster withdrawal of the snail into the shell.

To reduce evaporation of water through the aperture, terrestrial prosobranchs use an operculum; since pulmonates have no operculum, they have acquired a special structure, the epiphragm, which is formed by dried mucus.

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#### 2. Biological adaptations

Various specializations of the life cycle reflect mainly adaptations of animals to life in conditions of seasonal changing. In climates without seasonal changes reproduction of mollusks takes place throughout the year. In areas where the wet season gives way to dry, the snails usually survive the dry season, falling into estivation and closing the aperture by an epiphragm. In the inactive state all life processes, especially loss of fluid, are strongly slowed down. In the state of estivation some obligatory xerophiles (for example, Sphincterochilidae, Fig. 15) can remain viable for several years (Yom-Tov, 1971).

#### 3. Ethological adaptations

To the category of behavioral adaptations one can, with some reservations, attribute the ability of many snails to stick to the substrate by means of mucus. This is an effective way to slow down the evaporation, but it is fraught with a certain danger: the complete sticking around the perimeter of the aperture would mean that the aperture is hermetically sealed which prevents gas exchange. A way out of this situation is possible in two ways. In some snails the aperture edges are bent, hence they do not lie in the same plane. As a result, a full adjoining of the aperture to the substrate cannot occur (some Enidae, many Helicodontidae). Another way to avoid complete sealing are small tubercles formed on the aperture edges, which also prevent a complete adhesion of the aperture to the substrate (some Chondrinidae and Lauriinae). This is a good example of interdependence of morphological and behavioral adaptations.

In many of mesophilous and hygrophilous snails the cephalopodium during mating is out of the shell, which imposes significant limitations on the duration of copulation. At the same time xerophilic snails, due to some anatomic reconstructions, can copulate, almost entirely hiding in shells, thus deminishing evaporation. In this case, there is also a combination of morphological and behavioral adaptations.

At last, xerophilic species with white shell usually have a behavioral adaptation – they often create dense clusters. Temperature within the cluster is lower than the ambient temperature.

#### 4. <u>Physiological adaptations</u>

To this category one can attribute, in particular, the problem of overheating and evaporation. Any structural elements that are found on the cephalopodium surface are connected, directly or indirectly, with the problem of expenditure of water since an animal with soft integuments when it is in an active condition, is in permanent danger of dehydration. If the weather is hot, there is also a risk of overheating, which can be reduced by evaporation from the surface of the foot, but again this is connected with expenditure of water.

In terrestrial pulmonates (especially in slugs) gas exchange occurs not only through the lung, but also through the skin. However, skin respiration demands additional water loss. Thus, one of the most important tasks which a land mollusk must decide is searching for optimal balance between expenditure and economy of water. There are two ways of saving water: either to reduce water consumption, or to re-utilize it. The first method is the most common and is carried out due to: (1) the presence of the shell, (2) the ability to estivation and/or (3) using of various shelters. The second way is observed in some Helicarionoidea and Arionoidea.

As stated above, one of the most important factors of life of land snails is the water regime. The main, and often the constant danger is the risk of dehydration. Water loss performs mainly through the aperture. Obviously, improving mechanisms that slow down evaporation – is, perhaps, the most urgent problem, which strongly depends on habitat conditions.

There are three methods to slow down evaporation rate: 1) the narrowing of the last whorl toward the aperture; 2) the formation of a lip and some teeth; 3) the formation of a protective film (epiphragm). The third method usually supplements the first two.

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The snails usually survive dry seasons by one of two ways.

1. Snails bury themselves in the soil or hide deep in the crevices of rocks. These snails lack traits listed above, but in fact they exist under the conditions where the temperature is lower and humidity is always high enough.

2. Snails live openly, glued to stones or other flat surfaces by the mucus. Such species are often characterized by three features: a toothless aperture, white shell color as well as by a behavioral feature – they often form dense aggregations. All three characters can be regarded as adaptations to life under arid conditions: lack of apertural teeth allows fast retraction of the snail into the shell; due to the white shell the risk of overheating is reduced and the temperature within the cluster, as has been said, is lower than the temperature of the ambient air.

Also the problem of water saving during movement of the snails is of importance. Movement itself is associated with a significant expenditure of liquid. When the animal is crawling, from the pedal gland, the orifice of which is located beneath the mouth, the mucus (the base of which is water) becomes excreted, and the snail moves upon this mucuous path by using cilia that cover the sole. Reducing water loss is achieved due to the fact that the sole in many mollusks not all the time adheres to the substrate, but during crawling it undulates wave-like in the vertical plane. Mollusk crawling on a dry surface, often show that the wet trace when moving, is not continuous, but consists of a series of spots, thus achieving water saving.

There is another way to save water when a snail is moving, for example, in many Helicarionidae, Zonitidae, Vitrinidae, etc. The sole in these mollusks is divided by grooves into three longitudinal zones, but the cilia cover only the middle zone. Only this zone contacts with dry surface and just it is responsible for the progressive movement of the snail.



Figure 15. *Sphincterochila boissieri* shell; © A. Schileyko

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### Testing gene flow between the subspecies of *Trochulus oreinos* in their supposed contact zone

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Trochulus oreinos is an endemic land snail species occurring in the Northeastern Austrian Alps at elevations above tree line (1,600 - 2,300 m a. s. l., Klemm 1974). Previous studies found T. oreinos to be clearly separated ecologically, morphologically and genetically from the highly polymorphic and widespread T. hispidus. Within T. oreinos, two morphologically highly similar subspecies have been distinguished. The easterly distributed T. o. oreinos features an additional penial fold whereas the westerly distributed T. o. scheerpeltzi differs by a groove beneath the shell keel. This morphological trait, however, appears only sometimes and occurs in intermediate forms (Duda et al. 2011, 2014). First genetic analysis of three mitochondrial marker sequences, cytochrome c oxidase subunit 1 (COI), 16S rRNA gene (16S), and 12S rRNA gene (12S) has revealed a high genetical divergence between the two subspecies. In addition, the nuclear marker sequence ITS2 (internal transcribed spacer), albeit not informative to distinguish several other species of the genus Trochulus, separated the two taxa clearly (Kruckenhauser et al. 2014). Focussing on the potential contact zone of the two subspecies, an expanded sample set of COI sequences (178 individuals) and nuclear ITS2 sequences (70 individuals) revealed evidence of a deep genetical split between the two lineages. Both taxa occur within the Haller Mauern mountain range, but a clear geographic split was found: all the western samples were part of the clade representing T. o. scheerpeltzi while all the eastern samples clustered with T. o. oreinos. The only exception were the sampling sites in the eastern Haller Mauern from which a few individuals possessed a COI sequence matching the T. o. oreinos clade, but the ITS2 was found either homozygous for a sequence of the T. o. scheerpeltzi clade or heterozygous for both taxa. As these results are based on one nuclear marker only, no decision could be made on whether T. o. oreinos and T. o. scheerpeltzi should be considered as separate species. Therefore, in a next step, potential gene flow between the two subspecies of T. oreinos within the contact zone was investigated using Amplified Length Fragment Polymorphisms (AFLPs), a DNA fingerprinting technique. A set of 200 individuals including samples from the whole distributional range was selected. First preliminary results of the nuclear AFLP marker set show a clear (geographic) separation of the two subspecies with no indication of gene flow among them. The results of the AFLP analysis will help to gain further insights into the delimitation of the two taxa and the clarification of the taxonomic status of the two subspecies of T. oreinos.

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Figure 16. Trochulus oreinos ssp.; © K. Jaksch



Figure 17. Natternriegel: sampling site in the eastern Haller Mauern; ©WG Alpine Land Snails

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### The case of *Cylindrus obtusus*: indication for selfing in geographically separated populations and evidence for Pleistocene survival within the Alps

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Cylindrus obtusus (Helicidae) is a hermaphroditic land snail, endemic in the Austrian Alps, which is restricted to high elevations (1600 to 2500 m asl) and limestone. As a specialist of high alpine rocky habitats, C. obtusus has a quite patchy distribution area (Klemm 1974). 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 (Schileyko, 1996; 1997; Zopp et al., in prep). 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 cytochrome oxidase subunit 1 gene (COI) (280 individuals) and 9 microsatellite loci (487 individuals from 29 populations) from samples covering the whole distribution range of the species. The COI sequences show a geographic differentiation between eastern, central and western populations, indicating at least three refugia during the last ice ages. The most western localities which were covered under the ice sheet are characterized by harbouring only single haplotypes. Overall genetic distances among all individuals are small (max. 1.7 %), which implies that C. obtusus has experienced severe bottlenecks in the past. The microsatellite analysis reveals a high differentiation between populations implying restriction of gene flow. The highest genetic variability was found in the central populations. Remarkably, nearly all individuals from the eastern populations (Veitsch to Schneeberg), 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. It was shown by Chase and Blanchard (2006) in Cornu aspersum that the mucus from the mucus glands, that covers the love dart before injection, is the reason for the increased probability for paternity of the dart shooting partner. This feature would, of course, not be necessary in a selfing organism. Hence the cooccurrence of the deformation of the mucus glands and the high excess of homozygosity point towards a high degree of selfing in the eastern populations. This finding is also supported by the calculated selfing rates between 0.82 and 0.96 in the eastern populations.

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Figure 18. Network showing the three mitochondrial clades of *Cylindrus obtusus* and distribution of haplogroups.

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### The efficiency of landscape management on selected thermophilous land snails – a small-scale case report from the vineyard area in northern Vienna

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Direct implications of landscape management measures, such as clearing and grubbing, on snails are only sparsely published. In this study, the impact of management on two xerothermophilous terrestrial gastropod species, *Zebrina detrita* and *Caucasotachea vindobonensis*, and on land snails in general, was evaluated in the vineyard area of Northern Vienna. This area belongs to the buffer zone of the UNESCO Wienerwald Biosphere Reserve in Austria.

A total of 18 sites were investigated, including vineyard embankments and dry meadows with different intensity levels of clearing and grubbing in recent years. Occurrence of both target species and their ability to recolonize newly created habitats were assessed. Both target species are only able to colonize new created habitats in numbers above detection level areas in direct vicinity of existing populations, a fact also

![](_page_12_Picture_11.jpeg)

described in other studies (Boschi & Baur 2008; Knop et al. 2011). Only annually repeated clearing of meadows and embankments with originally strong shrub coverage resulted in a visible effect. Continuous clearing efforts over 10 years (2002-2013) were associated with a dense population of *Z. detrita* on a formerly unsuited bush-covered meadow. In contrast, vineyard embankments that were cut free just once within two years (2012 and 2013) before the study harboured only a few specimens of *Z. detrita*. A similar effect was found in the composition of ecological groups of land snail species at the different sites. Only on the long-term managed sites the majority of species (>75%) were definitely open-land dwellers, while on the short-term managed vineyard embankments the number of forest dwellers and euryoecious species increased.

In general, landscape management suitable for xerothermophilous snails should be first applied at sites with existing populations of the targeted species and then extended to neighbouring sites. Also the former

occurrence of empty shells of grassland species should be taken into consideration when planning landscape management, because they can provide information on the potential success of restoring open grassland. For the long-term success of landscape management, a sustainable maintainability of new created habitats avoiding both too less but also too much management activity (Boschi & Baur 2008) must be guaranteed.

![](_page_13_Picture_6.jpeg)

![](_page_13_Picture_7.jpeg)

Figure 19. Mukental (Vienna) & Caucasotachea vindobonensis; © M. Duda

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![](_page_13_Picture_15.jpeg)

#### ABOL Mollusca – status

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Molluscs are in different ways appropriate as pilot group for barcoding the Austrian organisms (ABOL/ Austrian Barcode of Life). There are important indicator species among native snails and mussels, which are suitable for answering conservation-related issues, like the evaluation of habitat quality. Also the number of endemics is quite impressive with 19.3% of all native molluscs (Rabitsch & Essl 2009). Accordingly high is the threat of extinction of many molluscs: about 35% of the snails (especially freshwater species) and 37% of the mussel species are endangered (A. &. P.L. Reischütz 2007). All these facts increase the interest to investigate the approximately 400 different Austrian mollusc species also genetically.

Due to the large number of subspecies – approximately one-third (30%) of all native species is divided into subspecies (Cuttelod, Seddon & Neubert 2011) – and the high genetic diversity in some molluscs, interesting results can be expected.

Genetic investigations in land pulmonates showed extreme high intraspecific diversity (up to 30% pdistance in *COI*). Hence there is no standard value for the genetic distance which marks taxonomic delimitations in molluscs. In many cases no barcoding gap can be found due to the overlap of intra- and interspecific variation (Davison et al. 2009).

Previously collected data and experiences from past and running projects – see homepage of "Alpine land snails" (snails.nhm-wien.ac.at) and "Monitoring in the Biosphere Reserve Wienerwald" (Eschner et al. 2014) – provide good pre-conditions for a successful DNA-barcoding.

Up to now material of approximately 234 Austrian mollusc species are available in the collections of the Natural History Museum Vienna. This includes material from the above mentioned projects which was collected and preserved for DNA analyses, but also older material from the museum collection. DNA that is extracted from such material can be fragmented and of low concentration, therefore we will choose it only, if there is no other material from this species available, which is the case for 26 species. Up to now we established from 105 different species 180 barcodes with all relevant metadata.

![](_page_14_Picture_11.jpeg)

Figure 20. Apparatus for photo documentation (left) and the finalized photo of a *Viviparus acerosus* ABOL-voucher (right); © ABOL

![](_page_14_Picture_13.jpeg)

![](_page_15_Figure_3.jpeg)

Abbildung 18. Numbers of DNA barcodes obtained from species and individuals per family

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![](_page_15_Picture_12.jpeg)

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