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DISSERTATION

Titel der Dissertation

Trochulus oreinos (A.J. Wagner, 1915) and *T. hispidus* (Linnaeus, 1758) (Gastropoda: Pulmonata: Hygromiidae) in the eastern Alps and adjacent areas: Morphology, ecology and their context to phylogeography

(*Trochulus oreinos* (A. J. Wagner, 1915) und *T. hispidus* (Linnaeus, 1758) (Gastropoda: Pulmonata: Hygromiidae) in den Ostalpen und angrenzenden Gebieten: Morphologie, Ökologie und ihr Kontext zur Phylogeographie)

Verfasser

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Summary

Species delimitation and taxonomy within the genus *Trochulus* Chemnitz, 1786 (Gastropoda: Pulmonata: Hygromiidae; common name: Hairy snails) have always been subject to contradicting opinions. These have been intensified because the first descriptions of many taxa are minimal and only partially comprehensible. This doctoral thesis deals with certain poorly described representatives of the genus from the Austrian Alps and adjacent areas: (1) The widespread *T. hispidus* (Linnaeus, 1758) is a highly controversial taxon and one of the most problematic groups within European terrestrial gastropods. *T. hispidus* – together with other poorly described and likewise controversial species – is subsumed under the term *T. hispidus/sericeus* complex. (2) *Trochulus oreinos* (A. J. Wagner, 1915) with its two subspecies *Trochulus oreinos oreinos* and *T. oreinos scheerpeltzi* (Mikula, 1954) is an endemic of the northeastern Austrian Alps. Both subspecies were originally regarded as subspecies of the highly variable *T. hispidus*. In the present thesis the three taxa were analysed morphologically, anatomically and ecologically and the results were combined with genetic data.

Shell morphological and anatomical results revealed high similarity between the two *T. oreinos* taxa and their clear separation from the *T. hispidus/sericeus* complex. Two diagnostic traits of *T. oreinos* ssp. – the specific hair morphology and a constant pattern of penial folds – were documented for the first time. One diagnostic trait is hair length: *T. hispidus* has significantly longer hairs (> 0.21 mm) than both *T. oreinos* subspecies (0.03 - 0.09 mm). Additionally, hairs of the *T. oreinos* subspecies are often curled or strongly bent. Finally, the penis in *T. oreinos* has a single intrapapillar cavity interrupted by one disconnection, whereas representatives of the *T. hispidus/sericeus* complex show two separated intrapapillar cavities. Several additional characters such as shell measurements and structures distinguish the two species by trend, although there are overlaps in some cases. Within the two *T. oreinos* subspecies, a small but constant difference was found: *T. o. oreinos* shows a bulge attached to the penial fold, which occasionally has an additional small fold. This trait conforms to the results of the molecular analysis because it only occurs in specimens with the *T. o. oreinos* genotype. The groove beneath the keel, originally described as typical trait of *T. o. scheerpeltzi*, turned out to be an unreliable trait to separate this subspecies. The question whether the two *T. oreinos* subspecies might represent different species – as suggested by the high genetic distance between the two reciprocally

monophyletic clades in the genetic analysis – remains unresolved at the current state of knowledge.

An even more distinct separation between *T. oreinos* and the *T. hispidus/sericeus* complex was indicated by comparing ecology and habitat needs in the mountain massifs Hochschwab, Schneealpe, Rax and Schneeberg. These selected four areas provided adequate samples of the studied taxa, and exact vegetation maps were available. The subspecies of *T. oreinos* have the same habitat preferences, both being restricted to rocky high alpine areas in the northeastern Austrian Alps. They prefer open dry alpine grassland with diggable soil and/or stones and are restricted to subalpine and alpine boulder societies and Caricetum firmae. In contrast, representatives of the *T. hispidus/sericeus* complex are distributed over a wider altitudinal and geographic range, preferring moist areas and scrubby perennial herb vegetation near water bodies. Because *T. oreinos* occurs at naturally forest-free areas, it is not affected by structural changes of the habitat, such as reforestation caused by the abandonment of grazing and the shift of vegetation zones. Note, however, that *T. oreino*s is limited by microclimatic factors because it is adapted to cooler habitats. The mountains Schneealpe, Rax and Schneeberg, ca. 2000 m in height, represent the climatic limit of the species. This makes it vulnerable to the upward shift of climate zones. *T. oreinos* shows striking similarities in its habitat preference to the Swiss endemic *T. biconicus*, both taxa preferring the same dry alpine habitats. In this respect they differ considerably from other representatives of the genus, which prefer damp habitats.

While the results allow a clear morphological interspecific differentiation between the *T. hispidus/sericeus* complex and both *T. oreinos* taxa, the intraspecific differentiation remains ambiguous, especially in the *T. hispidus/sericeus* complex. Genetic investigations conducted in parallel with the same material as investigated in the present thesis revealed nine different mitochondrial clades within the *T. hispidus/sericeus* complex. These clades are intermingled with clades representing morphologically clearly defined species (*T. villosulus*, *T. striolatus*, *T. villosus*, *T. clandestinus*), thus rendering *T. hispidus* as currently defined paraphyletic. In contrast, *T. oreinos* is monophyletic and well separated from all the other clades. It splits from a basal node in the phylogenetic tree, after the split of *T. biconicus* and the outgroup taxon *Plicuteria lubomirskii*.

Despite the high genetic distances between the genetic clades of the *T. hispidus/sericeus* complex, the morphometric analysis did not reveal any differentiation among these clades. Moreover, no populations or geographic groups are differentiated in shell morphology, irrespective of their genetic affiliation. The problematic taxon *T. sericeus*, quite

sparsely defined by a “narrow umbilicus” in the literature, is differentiated neither in the morphological nor in the genetic analyses. Especially, the relation umbilicus width / shell width (often used as a diagnostic trait) is not a reliable predictor for *T. sericeus*: specimens with a low ratio of umbilicus width / shell width occur in four of seven morphologically investigated clades. There is no difference in the genital anatomy that discriminates any of the *T. hispidus/sericeus* clades or that is diagnostic for all of them. Thus, morphology and ecology fail to answer the question whether the differing genetic clades of *T. hispidus* or the subspecies of *T. oreinos* represent different species or not. The *T. hispisdus/sericeus* complex exemplifies the problematic praxis of DNA barcoding without detailed knowledge of phylogenetic relationships, phylogeographic patterns and species delimitation. Even for a comparably small region like the Eastern Alps and adjacent areas, one or even a few COI sequences for defining *T. hispidus* would definitely be insufficient. Our analyses show the border of species delimitation based on classical morphological as well as phylogenetic investigations. For defining hybridisation barriers in sibling (cryptic) land snail species, details of reproduction biology are indispensable. Moreover, crossing experiments and genetic analyses to test gene flow between clades are necessary. Independent from this, assessing the geographic distribution of clades within the *T. hispidus/sericeus* complex is relevant for conservation issues because the habitat of some of these clades is under pressure.

Zusammenfassung

Artabtrennung und Taxonomie der Gattung *Trochulus* Chemnitz, 1786 (Gastropoda: Pulmonata: Hygromiidae; Trivialname: Haarschnecken) waren seit je her Gegenstand widersprüchlicher Lehrmeinungen. Dies ist vor allem dadurch bedingt, dass die Erstbeschreibungen mancher Taxa sehr dürftig und oft nur teilweise nachvollziehbar sind. Die vorliegende Dissertation beschäftigt sich mit einigen teilweise nur unzureichend beschriebenen Vertretern dieser Gattung, welche in den Österreichischen Alpen und angrenzenden Gebieten vorkommen. Es sind dies (1) der weitverbreitete *T. hispidus* (Linnaeus, 1758), ein kontroversiell diskutiertes und problematischsten Taxon innerhalb europäischer Landgastropoden. *T. hispidus* wird in der vorliegenden Arbeit - zusammen mit einer anderen, sehr strittigen Art – unter dem Namen *T. hispidus*/*sericeus*-Komplex zusammengefasst. (2) *Trochulus oreinos* (A. J. Wagner, 1915) mit seinen zwei Unterarten *Trochulus oreinos oreinos* und *T. oreinos scheerpeltzi* (Mikula, 1954), ein Endemit der Österreichischen Nordostalpen. Die beiden Unterarten von *T. oreinos* wurden ursprünglich als Unterarten des höchst variablen *T. hispidus* betrachtet. In der vorliegenden Dissertation wurden alle drei Taxa in Hinblick auf Morphologie, Anatomie und Ökologie analysiert; die Ergebnisse wurden mit Daten aus molekularbiologischen Untersuchungen verknüpft.

Die Ergebnisse hinsichtlich Schalenmorphologie und innerer Anatomie zeigten eine hohe Ähnlichkeit zwischen den beiden Taxa von *T. oreinos* und ihre klare Abtrennung zu *T. hispidus*. Zwei diagnostische Merkmale beiden Unterarten von *T. oreinos* – die artspezifische Haarmorphologie und ein konstantes Muster der Penialfalten – wurden das erste Mal ausführlich dokumentiert. Das erste diagnostische Merkmal ist die Haarlänge: Die Periostrakumhaare von *T. hispidus* (Mindestlänge 0.21mm) sind signifikant länger als jene der beiden *T. oreinos* ssp. (0.03 bis 0.09 mm). Außerdem sind die Haare letzterer oft eingeringelt oder zumindest stark gebogen. Das zweite Merkmal ist im Querschnitt des Penis ersichtlich, welcher bei *T. oreinos* lediglich eine nur einmal durchbrochene intrapapilläre Höhlung aufweist, wohingegen bei Vetretern des *T. hispidus/sericeus*-Komplexes zwei getrennte, ca. gleichlange intrapapilläre Höhlungen vorhanden sind. Zusätzliche Merkmale wie Schalendimensionen und -strukturen können die beiden Arten tendenziell separieren, wobei es allerdings teilweise auch Überlappungen gibt. Zwischen den beiden *T. oreinos* Unterarten konnte ein kleines, aber konsistentes Unterscheidungsmerkmal nachgewiesen werden: und zwar eine Ausbuchtung, optional mit einer zusätzlichen, kleineren Penialfalte. Dieses Merkmal stimmt auch mit molekularbiologischen Ergebnissen überein, da es nur bei

Individuen mit *T. o. oreinos* Genotyp vorkommt. Die flache Rinne unter dem Kiel, welche ursprünglich als Erkennungsmerkmal von *T. o. scheerpeltzi* beschrieben wurde, stellte sich nicht als konstantes Merkmal zur Unterscheidung der beiden Unterarten heraus. Die Frage, ob die beiden *T. oreinos* Unterarten möglicherweise zwei verschiedene Arten repräsentieren – so wie es die hohen genetischen Distanzen zwischen den beiden monophyletischen Kladen vermuten lassen – kann zum gegenwärtigen Zeitpunkt nicht beantwortet werden.
Ein noch stärkerer Unterschied zwischen *T. oreinos* ssp. und dem *T. hispidus*/*sericeus*-Komplex zeigte sich durch Vergleiche der ökologischen Einnischung und der Lebensraumansprüche. Die beiden Unterarten von *T. oreinos* zeigen dieselben Habitatpräferenzen, da sie auf felsige Bereiche in den Hochlagen der nordöstlichen Kalkalpen beschränkt sind. Sie bevorzugen trockenes alpines Offenland mit lockerem, grabbarem Boden und/oder Steinen und sind auf (sub)alpine Fels- und Schuttgesellschaften sowie lockeres *Caricetum firmae* beschränkt. Im Gegensatz dazu ist der *T. hispidus*/*sericeus*-Komplex über weitere geographische bereiche sowie Höhenstufen verbreitet, wobei Feuchtgebiete und Hochstaudenbestände an Gewässern bevorzugt werden.

Da *T. oreinos* nur auf natürlich waldfreien Standorten vorkommt, ist er nicht durch Strukturänderungen des Lebensraumes – etwa eine durch Aufgabe der Weidewirtschaft oder Anhebung der alpinen Vegetationszonen bedingte Verbuschung oder Bewaldung – betroffen. Allerdings wird die Verbreitung von *T. oreinos* durch mikroklimatische Faktoren beschränkt, da die Art auf kühlere Lebensräume adaptiert ist. Die Habitatpräferenzen von *T. oreinos* ähneln auffallend jenen des Schweizer Endemiten *T. biconicus*, da beide Arten fast dieselben kühl-trockenen Habitate bewohnen, welche sich deutlich von denen anderer Arten der Gattung *Trochulus* unterscheiden, die zumeist feuchte Lebensräume bevorzugen.

Während die Ergebnisse eine klare interspezifische Abtrennung zwischen *T. oreinos* und dem *T. hispidus*/*sericeus*-Komplex zeigen, ist die intraspezifische Differenzierung vor allem innerhalb des *T. hispidus*/*sericeus*-Komplexes unklar. Molekularbiologische Untersuchungen an Vertretern des *T. hispidus*/*sericeus*-Komplexes, die parallel mit demselben Material durchgeführt wurden, ergaben neun unterschiedliche mitochondriale Kladen. Diese waren mit jenen von morphologisch klar definierten Arten (*T. villosulus*, *T. striolatus*, *T. villosus*, *T. clandestinus*) durchmischt, was den *T. hispidus*/*sericeus*-Komplex paraphyletisch erscheinen lässt. Im Gegensatz dazu ist *T. oreinos* monophyletisch und von allen anderen Kladen klar abgetrennt. Die Abspaltung erfolgt an der Basis des phylogenetischen Baumes, zusammen mit *T. biconicus* und *Plicuteria lubomirskii*, einer Art der Außengruppe.

Trotz der hohen genetischen Distanzen zwischen den Kladen des *T. hispidus*/*sericeus*-Komplexes konnten diese in morphologischen Analysen nicht eindeutig differenziert werden. Die Schalenmorphologie zeigte auch keine klaren Unterschiede hinsichtlich Populationen oder geographischer Gruppen, unabhängig von deren genetischer Zuordnung. Auch das problematische Taxon *T. sericeus*, welches in der Literatur zumeist aufgrund seines engen Nabels definiert wird, konnte weder in der morphologischen noch in der genetischen Analyse klar definiert werden. Außerdem konnten keine Unterschiede in der Genitalanatomie gefunden werden, welche eine der Kladen des *T. hispidus*/*sericeus*-Komplexes eindeutig von den anderen abgrenzt. Anhand des *T. hispidus/sericeus*-Komplexes werden problematische Aspekte des DNA-Barcodings aufgezeigt, sofern dieses ohne Hintergrundwissen betreffend phylogenetischer Verhältnisse, phylogeographischer Muster und Artenkenntnis erfolgt. Sogar für ein vergleichsweise kleines Untersuchungsgebiet wie die Ostalpen und daran angrenzende Gebiete wären einzelne oder wenige COI Sequenzen zur Definition von *T. hispidus* bzw. *T. sericeus* irreführend.

Die vorliegenden Analysen zeigen die Grenzen sowohl des klassischen morphologischen Ansatzes als auch moderner phylogenetischer Methoden zur Artabgrenzung in kritischen Fällen. Um Hybridisierungsbarrieren bei kritischen Arten zu erkennen, sind genaue Kenntnisse der Reproduktionsbiologie unerlässlich. Zusätzlich sind Kreuzungsexperimente und Analysen bezüglich des Genflusses zwischen den verschiedenen Kladen notwendig. Unabhängig davon bringt die Kenntnis der geographischen Verbreitung der unterschiedlichen genetischen Linien Zusatzinformationen für den Naturschutz, da einige dieser Linien in hauptsächlich in gefährdeten Lebensräumen vorkommen.

Preamble

Because of its geomorphological and climatic diversity, Austria is rich in endemic taxa compared to other central European countries. A main reason responsible for this richness is the specific role of the Austrian Alps providing potential refugia during the ice ages, which is confirmed especially for the last glaciation (Essl & Rabitsch 2009). In the last years a lot of affords have been made to concatenate several aspects of glacial refugia, species radiation and endemics in the Alps leading to the designation of potential glacial refugia in the peripheral regions of the Alps which are summarized by Tribsch & Schönswetter (2003) and Schönswetter et al. (2005) and Essl & Rabitsch (2009). Glacial refugia are characterized by higher numbers of endemic species but are also reflected by genetic patterns of species with wider distribution. This includes also land snails, which are predestined for studying inter- and intraspecific diversification because of their poor ability of active dispersal (Baur 1986, 1993; Bisenberger et al. 1999; Kleewein 1999). Reischütz and Reischütz (2007) count up to 442 species of Mollusca in Austria. Among these there are 38 endemic species of Gastropoda, mostly Hydrobiidae, which occur in springs and soil water systems (Reischütz & Reischütz 2007, 2009). The reliability of many European taxa of land gastropods has always been a point of contradicting discussions, as e.g. the species list of the CLECOM project of Falkner et al. (2001) was taken into doubt by other authors (e.g. Davis 2004). In the last years, the connection of "classic" gastropod determination by shell and genital traits in combination with "new" molecular biological methods opened new ways of species delimitation. The slug *Arion fuscus* (O. F. Müller, 1774) was regarded as conspecific synonym of the wide spread *Arion subfuscus* (Draparnaud, 1805) until the work of Pinceel et al. (2004). A broad-ranged analysis comparing mitochondrial clades with genital morphological traits revealed the existence of at least two species within central and northern Europe, *Arion subfuscus* (Draparnaud, 1805) in the northwest and *Arion fuscus* (O. F. Müller, 1774) in the southeastern parts of the continent (Pinceel et al. 2004). Besides high genetic distances between *A. fuscus* from the Balkans and populations from the rest of the continent the question was brought up, whether even more cryptic taxa are hidden within this species (Pinceel et al. 2005). Another example is the snail *Pupilla pratensis* (Clessin, 1871), which was considered to be a synonym of the common species *Pupilla muscorum* (Linnaeus, 1758) or misidentified as *Pupilla alpicola* (Charpentier, 1837) in Bohemia and Moravia (Horsák et al. 2010). An intensive investigation of collection material and newly collected material led to the revalidation of *P. pratensis* by Von Proschwitz (2009). Intensive morphometric and ecological work comparing

the three morpholocigal similar taxa *P. muscorum*, *P. alpicola* and *P. pratensis* revealed significant differences in the ecological niches and the historical development of habitats between the three species and a clear shell morphological separation of *P. muscorum* and *P. praticola*. The analysis led to a better knowledge of the distribution of the latter species (Horsák et al. 2010, Horsák et al. 2012).

A phenomenon described in the last years is the "hidden genetic diversity", which is frequently detected within land snails (e.g. Thomaz et al. 1996, Chiba 1999, Hayashi & Chiba 2000, Thacker & Hadfield 2000, van Riel et al. 2005). Several populations of one species, which cannot be separated by morphological or internal anatomical traits, show exceptional high genetic distances which are in the range or even higher than those found between well described species. Again *A. subfuscus* is an example for this phenomenon: Intraspecific sequence divergences in the mitochondrial 16S rDNA gene are extremely high reaching up to 21%. Despite this fact no morphological differences between representatives of these genetically distinct groups could be found (Pinceel et al. 2004).

In the genus *Trochulus* Chemnitz, 1786 previous results of Pfenninger et al. (2005) point in a similar direction – high mitochondrial (mt) sequence divergences combined with lacking morphological separation.

To elucidate the validity of some taxa and the connections between populations, species radiation and post-glacial colonization of Austrian land snails, the project "Phylogeography of Alpine land snails" (Austrian science foundation, FWF, No 19592 B17) was initialized in 2007. In this project a combined genetic and morphological approach was employed, to elucidate intraspecific variability and phylogeography of three Alpine snail species and their relatives: (1) *Cylindrus obtusus* (Draparnaud, 1801), an endemic of the Austrian Alps showing west-east differences among populations; (2) *Orcula dolium* (Draparnaud) with of its six subspecies presumably occurring in Austria, five of them endemics, and (3) *T. hispidus* (Linnaeus, 1756) a species widely distributed in Europe and its relationship to *Trochulus oreinos* (A.J. Wagner, 1915) another Austrian endemic Hygromiidae.

The last point includes the investigations of the present doctoral thesis. *T. hispidus* has been recognized as a polymorphic species, and its systematics has been for long in the focus of controversy. There had been several attempts to split it into different species (e.g. Forcart, 1965). Moreover, it is not clearly differentiated from other sometimes ill-described and morphologically similar taxa (e.g. *Trochulus sericeus*, *Trochulus coelomphala*). In this introduction only a very short sketch of the taxonomy of *T. hispidus* is given, as

comprehensive overviews are already provided by Falkner et al. (2002) and Proćków (2009). Furthermore, the issue is addressed and discussed in detail in the following papers.

Forcart (1965) suggested a division of *T. hispidus* into two distinct species, a "globular" form *T. hispidus* in the northeast of the distribution area and a "flat" form *T. concinnus* in the southeast. This suggestion was already rejected by Gittenberger et al. (1970), followed by various authors including Klemm (1974), Falkner (1982), Naggs (1985), and Proćków (2009). Not only *T. concinnus* appears to be a dubious taxon, but also *T. sericeus*, a name, which is in general applied to representatives of *Trochulus* sp. with very narrow umbilicus and globular shell. There was some confusion about the correct name for this form: Forcart (1965) claimed, that the name "*sericeus*" should be replaced by "*plebeius*", while authors like e.g. Falkner et al. (2001) claimed, that *T. plebeius* can only be applied to populations living in the Jura Mountains in France and Switzerland. Falkner (1973) stated, that the name "*sericeus*" is just a placeholder in a system that we do not understand yet. Finally, in the most recent publication about this topic Proćków (2009) recommended synonymizing all these taxa (*T. concinnus*, *T. plebeius* and *T. sericeus*) with the name *T. hispidus*. This opinion was partly supported by Depraz et al. (2009), who suggested subsuming this group of taxa under the term *T. hispidus*/*sericeus* complex, but also pointed out the possible existence of cryptic taxa by detecting reduced gene flow between two local populations of this species complex in Switzerland.

From the above mentioned controversies it becomes clear that taxonomy and systematics of *Trochulus* in general and especially the *T. hispidus*/*sericeus* complex deserved intensive research, both morphologically as well as genetically. The results of preliminary genetic analyses (Kruckenhauser et al. in prep.) indicate that there are several highly distinct mt clades all representing individuals matching the morphology of *T. hispidus.* Thus, until now, all genetic investigations of *T. hispidus* uncovered a "hidden diversity" (Pfenninger et al. 2005; Depraz et al. 2009), a phenomenon that also has been described for other gastropods (see above). This raised the question whether there are some cryptic species within the *T. hispidus*/*sericeus* complex or – more general - which of the mt lineages could be differentiated by morphological or anatomical features. Therefore, comprehensive analyses providing more data on morphology, ecology and recognition features appeared necessary.

The state of knowledge about *T. oreinos* at the beginning of this doctoral thesis was remarkably poor and the species was rather unknown by the broad scientific public: Originally it was described as a local subspecies of *T. hispidus* in the work of Wagner 1915 about land gastropods of the former Austro-Hungarian monarchy and the Balkans. The

original description consisted on a text describing outstanding shell traits of this form, but it contained no picture. Nearly 40 years later another East-Alpine subspecies of *T. hispidus* was described as *T. hispidus scheerpeltzi* (Mikula, 1954). Subsequently, Falkner (1981, 1995) separated *T. oreinos* as a different species from *T. hispidus* because of "different hair building" [original text: "unterschiedliche Haarbildung"] and assigned *T. o. scheerpeltzi* as a local subspecies of *T. oreinos*. The different hair-building led him to the conclusion, that *T. oreinos* is not even just a separate species, but also quite distantly related to *T. hispidus* and similar forms. Klemm (1974) outlined the distribution of these two high Alpine forms and provided the first distribution maps. Both taxa occur in elevations above 1400 m above sea level (asl). *T. o. oreinos* is restricted to a small mountain area in Lower Austria and Styria, while *T. o. scheerpeltzi* is an endemic of Upper Austria. This distribution goes conform with the typical pattern of Austrian endemics of the north-Eastern Alps (Essl & Rabitsch 2009). Reischütz & Reischütz (2009, in Essl & Rabitsch 2009) counted both *T. oreinos* taxa in a list of Austrian endemics of molluscs together with modified distribution maps of Klemm (1974) and basic habitat descriptions. However, no details about habitat and ecological requirements were known, except an unpublished manuscript of Falkner (1973), which denominates *scheerpeltzi* as an inhabitant of rocky high alpine areas. *T. oreinos* is registered in several red data books (e.g. Frank & Reischütz 1996 and Reischütz & Reischütz 2007) and the CLECOM list (Falkner et al., 2001). Moreover, it was mentioned in at least two sample lists of Sattmann et al. (2000) and Freitag & Desch (1996). Nevertheless, it remained absolutely unknown even by specialists. This might have been the reason, why it was not mentioned in the taxonomic review of the Genus *Trochulus* Chemnitz, 1786 by Proćków, 2009, which was published during the initial phase of this doctoral thesis.

In the case of *T. oreinos* several points demanded clarification: as mentioned above, no detailed and comprehensible traits were documented so far, how this Austrian endemic could be distinguished from *T. hispidus*. There was a lack of a substantial description of shell morphology, genital anatomy, habitat preferences and genetic differentiation. Additionally, almost nothing was known about intraspecific variability and differentiation of the two subspecies *T. o. oreinos* and *T. o. scheerpeltzi.* Therefore, morphological, anatomical and genetic analyses were needed to address these questions. Furthermore, the question arose, why and how *T. oreinos* became an endemic form of the Austrian North-Eastern Alps. It could be hypothesized that it represents a relic of a formerly widely distributed species that adapted to high Alpine environments in the course of Pleistocene cold phases. Another possibility is that it radiated in situ and survived the glaciations in more or less the same

regions as it exists now. These phylogeographic questions should be addressed by combining the results of the present doctoral thesis with the outcomes of the genetic analyses.

On the basis of the outlined facts and problems the following specific questions were adressed:

(1) Can *T. oreinos* and *T. hispidus* be differentiated unambiguously by morphological and anatomical characters?
(2) Is there an indication for an intraspecific subdivision in *T. oreinos* and *T. hispidus*? Can the suspected divergent mt clades of *T. hispidus* or some of the dubious taxa of the *T. hispidus/sericeus* complex be separated by morphological or ecological analyses? Are there diagnostic characters to delimitate *T. o. oreinos* and *T. o. scheerpeltzi*?
(3) Is there a difference of *T. oreinos* and *T. hispidus* in respect to habitat preferences? Which conclusions about distribution patterns and evolutionary history of these two species can be drawn from their ecological requirements?

In paper Nr. 1 a morphometric analysis of shell morphological characters in combination with genetic data are used to differentiate between *T. oreinos* (with its two subspecies *T. o. oreinos* and *T. o. scheerpeltzi*) and local adjacent populations of the *T. hispidus/sericeus* complex. Furthermore, it includes a rough analysis of ecological requirements of the two species and an extensive literature review on *T. oreinos*.

Paper Nr. 2 deals mainly with the development of the habitat of *T. oreinos* in comparison with *C. obtusus* another Alpine snail form restricted to high elevations. The main conclusion is, that both endemics radiated in situ on azonal rocky habitats. In the course of the Pleistocene climate changes they were able to react by migrating upwards and downwards on rocky slopes.

In paper Nr. 3 representatives of the taxonomically ambiguous genus *Trochulus* in Austria and adjacent areas are investigated with respect to morphology and ecology. In the centre are the genetically differentiated clades of the *T. hispidus*/*sericeus* complex as well as the related species *T. oreinos* and *T. striolatus.*

The list of individuals together with sample localities and the raw data of all analyses in paper 1 and 3 are listed in the Appendix.

Synopsis of the papers/manuscripts

Paper Nr. 1

Full Citation:

Duda, M., Sattmann, H., Haring, E., Bartel, D., Winkler, H., Harl, J. & Kruckenhauser, L. (2011): Genetic differentiation and shell morphology of *Trochulus oreinos* (WAGNER, 1915) and *T. hispidus* (LINNAEUS, 1758) (Pulmonata: Hygromiidae) in the Northeastern Alps. Journal of Molluscan Studies **77(1)**: 30-40

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http://mollus.oxfordjournals.org/content/77/1/30.full.pdf+html

Paper Nr. 2

Full Citation:

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 (2)**: 5-12

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GENETIC DIFFERENTIATION AND SHELL MORPHOLOGY OF *TROCHULUS OREINOS* (WAGNER, 1915) AND *T. HISPIDUS* (LINNAEUS, 1758) (PULMONATA: HYGROMIIDAE) IN THE NORTHEASTERN ALPS

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ABSTRACT

Trochulus oreinos oreinos and *T. oreinos scheerpeltzi* are two land snail taxa endemic to the Northeastern Austrian Alps, which have been regarded as subspecies of the highly variable, widespread land snail *T. hispidus*. We analysed these three taxa morphologically and genetically to evaluate whether a delimitation between them is possible and, if so, to resolve their phylogenetic relationships. Shell morphological results revealed high similarity between the two *T. oreinos* taxa, and that they are clearly separated from *T. hispidus*. Additionally, the *T. oreinos* subspecies concur with respect to their habitat preferences, as they are both restricted to rocky high alpine areas, whereas the local form of *T. hispidus* is distributed over a wider altitudinal range in moist areas and scrubby perennial herb vegetation near water bodies. While the morphological and ecological results allow clear differentiation between *T. hispidus* and *T. oreinos* only, analyses of the mitochondrial cytochrome *c* oxidase subunit I and 16S rRNA genes revealed high sequence divergences between all three taxa, which indicates that they represent old lineages. The two *T. oreinos* taxa appear as distantly related sister groups, well separated from *T. hispidus*. Whether *T. o. oreinos* and *T. o. scheerpeltzi* should be considered as species cannot be decided at the current state of knowledge.

INTRODUCTION

A large number of land snail species have been described from the Alpine region, including several endemics. Many species have been divided into different infra- or subspecific entities (races, forms etc.), mainly by minor shell morphological features. This differentiation has often been explained by glacial/postglacial events of isolation, displacement, dispersal and (re-)colonization (Adensamer, 1937; Klemm, 1974; Gittenberger, 1991). Moreover, the environment may have triggered special genetic adaptations and/or changes caused by phenotypic plasticity, e.g. smaller shells at higher elevations. Often, subspecific entities have been described from defined altitudinal zones or within the boundaries of particular mountain massifs. Different interpretations and opinions concerning the origin of described morphological variants often resulted in controversial taxonomic conclusions and systematic assignments.

The genus *Trochulus* Chemnitz, 1786 is one such example. The genus comprises small, pulmonate land snails with flattened or globular, particularly hairy shells, mainly distributed in Central and Western Europe. The CLECOM list (Falkner, Bank & von Proschwitz, 2000) gives up to 18 different species of *Trochulus s. s.* for central and northern Europe. Two species – *Trochulus waldemari* (Wagner, 1915) and *T. suberectus* (Clessin, 1878) – were not included by Falkner *et al.* (2000), because their distribution ranges are not covered by the CLECOM list. However, the number of recognized species varies among different authors. For example, one of the listed taxa, *T. alpicola* (Eder, 1921), has not been regarded as an independent species by some authors. Additionally, some authors (e.g. Davis, 2004; Cameron *et al.*, 2006) have expressed objections to some taxonomic decisions in the CLECOM list. Subsequently, Proćków (2009) enumerated 22 species in *Trochulus* by lumping *T. plebeius* and *T. hispidus* at species level and the (sub)genera *Petasina* and *Plicuteria* at generic level, based on morphological data.

This study is focused on the poorly known northeastern Alpine endemic *T. oreinos* (Wagner, 1915), which was originally regarded as regional subspecies of *T. hispidus* (Linnaeus, 1758), but was later considered as a separate species not closely related to *T. hispidus* (Falkner, 1982, 1995). According to the current taxonomic view, *T. oreinos* comprises two subspecies, *T. oreinos oreinos* (=*Fruticicola hispida oreinos*) and *T. oreinos scheerpeltzi* (Mikula, 1957) (=*T. hispidus scheerpelzi*), which are both endemic to the northeastern Alps in Austria (Fig. 1A, B). Falkner (1982, 1995) based his decision to treat *T. o. scheerpeltzi* as a subspecies of *T. oreinos* on 'differing hair morphology' of the two *T. oreinos* taxa. However, he did not provide any details. This is remarkable because the original description of Wagner (1915) characterized *T. o. oreinos* as hairless, and Mikula (1957) did not mention any hairs in *T. o. scheerpeltzi*. Because no clear descriptions and pictures are available, knowledge of *T. o. oreinos* and *T. o. scheerpeltzi* has been

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Figure 1. A. *Trochulus o. oreinos*, specimen from sample site 34. **B.** *Trochulus o. scheerpeltzi*, specimen from sample site 45. **C.** *Trochulus hispidus*, specimen from sample site 2. Scale bar = 2.0 mm.

restricted to a few specialists who have inspected specimens collected by the describers and deposited in scientific collections.

Trochulus o. oreinos (Fig. 1A) is found in Lower Austria and Styria at high altitudes (1,600–2,280 m). Its distribution extends from Schneeberg mountain to Totes Gebirge (Klemm, 1974). Reischütz & Reischütz (2009) mentioned rocky grass biotopes and duff as habitats. In the original description Wagner (1915) characterized it by a shiny, finely granular, hairless shell with coarse, irregular ridges. The shell was described as smaller than that of *T. hispidus*, but 'more stable'. As additional traits Wagner mentioned a strong lip inside the aperture, visible as a yellow structure from outside, with a tooth-like structure at the basal margin. The type locality is at Hochschwab mountain in Styria at elevations above 2,000 m. So far, no picture of *T. o. oreinos* has been published.

Trochulus o. scheerpeltzi (Fig. 1B) is found in the mountain ranges of Höllengebirge to Totes Gebirge and in parts of Haller Mauern (Klemm, 1974). Like *T. o. oreinos* this subspecies is found at high altitudes (1,600–2,300 m) and in a similar habitat – rocky grass biotopes and crevices with duff (Reischütz & Reischütz, 2009). In the original description Mikula (1957) mentioned a groove beneath a clearly visible keel as a trait distinguishing *T. o. scheerpeltzi* from *T. o. oreinos* and *T. hispidus*. The type locality of *T. o. scheerpeltzi* is 'Hauptkar' at the Hohe Nock Mountain in Upper Austria at elevations of 1,600–1,800 m. The only published pictures are those in the original description (Mikula, 1957).

Trochulus hispidus (Fig. 1C) has a wide distribution in Europe, occurring over a broad range of altitudes (up to 2,300 m) and habitats. The distribution covers large parts of Europe from Ireland and France to Kazan and St Petersburg in European Russia. In the north it reaches the Arctic Circle. It does not occur in southernmost parts of Europe (Ložek, 1956). According to Giusti & Manganelli (1987) records from Sardinia are very likely due to confusion with *Ichnusotricha berninii*. As *T. hispidus* is a polymorphic species, its systematics have long been the focus of controversy. Forcart (1965) suggested a division of *Trichia hispida* (nowadays *Trochulus hispidus*) into two distinct species, *Trichia hispida* and *Trichia concinna*. Subsequently, he assigned the two subspecies *oreinos* and *scheerpeltzi* to *T. concinna*. However, Gittenberger, Backhuys & Ripken (1970), followed by various authors including Klemm (1974), Falkner (1982) and Naggs (1985), rejected this theory because large clinal transition zones exist between *hispida* and *concinna* and the geographic distribution ranges are not clearly delimited. Shileyko (1978) raised doubts about the justification of the species status of several taxa of the '*T. hispida* group' (including *T. plebeia*, *T. sericea*, *T. septentrionalis* and *T. concinna*). Recent papers that have employed molecular biological methods (Pfenninger *et al.*, 2005; Dépraz, Hausser & Pfenninger, 2009) have provided an even more confusing picture, showing several distinct mitochondrial lineages in *T. hispidus*, and perhaps the occurrence of cryptic species. Proćków (2009) synonymized *T. plebeius* and *T. concinnus* with *T. hispidus* based on an extended morphological analysis.

The questions we wanted to clarify were: (1) Can the three taxa be differentiated morphologically? (2) Are the two *T. oreinos* taxa and the northeastern Alpine form of *T. hispidus* genetically differentiated? (3) Do the morphological and genetic data corroborate the species status of *T. oreinos*? (4) Do the three forms occupy different habitats, elevations and geographic ranges? Using samples covering the entire distribution range of *T. o. oreinos* and *T. o. scheerpeltzi* as well as the local forms of *T. hispidus* we performed morphological and genetic analyses to answer these questions.

The systematics of some species of *Trochulus* are problematic. This is especially true of *T. hispidus* with a number of divergent lineages (Pfenninger *et al.*, 2005; Dépraz *et al.*, 2009), among which there may be some cryptic species. However, since no comprehensive phylogenetic study of *Trochulus* has yet been carried out, we provisionally adopted the classification of the Austrian taxa provided by Reischütz & Reischütz (2007).

MATERIAL AND METHODS

Study area and sampling

The study area was located in the Northeastern Austrian Alps, including parts of the provinces Upper Austria, Lower Austria and Styria. This covers the distribution ranges of the two *T. oreinos* taxa (according to Klemm, 1974) and adjacent areas where *T. hispidus* occurs (Fig. 2). Most sample sites are situated on the limestone bedrock of the Northern Calcareous Alps, one on Palaeozoic limestone of the Grazer Bergland and four on metamorphic rocks of the Central Alps. Most of the study area is characterized by a cool humid Central European climate with heavy precipitation; only the easternmost and southeastern parts are influenced by the warmer and dryer Illyrian and Pannonian climates (Kilian, Müllner & Starlinger, 1994). Both *T. oreinos* subspecies occur down to an altitude of 1,300–1,450 m, which is also the lower limit of the subalpine zone. Like other marginal Alpine areas, the study area has been the focus of research projects seeking potential glacial refugia (e.g. Schönswetter *et al.*, 2005), because large parts of it remained ice-free during the last glaciations (Van Husen, 1997).

Sampling and habitat analysis were carried out at 45 sampling sites (Fig. 2, Table 1). Topotypes of *T. o. oreinos* (11 specimens from sample site 32) and *T. o. scheerpeltzi* (11 specimens from sample site 38) were included. Exact positions and elevations of collection sites were determined using GPS. The sampling period extended from May to July in 2007, 2008 and 2009. Adjacent water bodies, vegetation, habitat structure and dominant plant species were recorded. *Trochulus hispidus* was identified by morphological traits described in the literature (e.g. Ložek, 1956; Gittenberger *et al.*, 1970; Kerney, Cameron & Jungbluth, 1983). *Trochulus o. oreinos* and *T. o. scheerpeltzi* were identified using the original descriptions (Wagner, 1915; Mikula, 1957) as well as by comparison with reference specimens (paratypes and syntypes) in the collections of the Natural History Museum, Vienna (NHMW). In total, 327 specimens (225 living animals and 102 empty shells) of the three *Trochulus* taxa were included (Table 1).

Shell morphology

Four shell traits were measured in intact adult specimens (shell diameter, umbilicus diameter, shell height and height of last whorl) with a graduated eyepiece in a stereomicroscope. The definition of adulthood from shell apertural traits was problematic, as presumably adult specimens of *T. hispidus* often lack an outer lip (Geyer, 1915; Frömming, 1954; Cameron, 1982). Therefore, individuals were defined as 'adult' when their shells had a minimum diameter of 5.4 mm, as this was the size of the smallest individual seen with a fully developed lip. This might appear arbitrary, because the standard literature and some collections contain only 'typical' specimens with outer lip. However this analysis was intended to include all naturally occurring variants and therefore we had to define a size limit. Altogether 304 specimens (complete specimens and empty shells) from all 45 sample sites were measured and standard

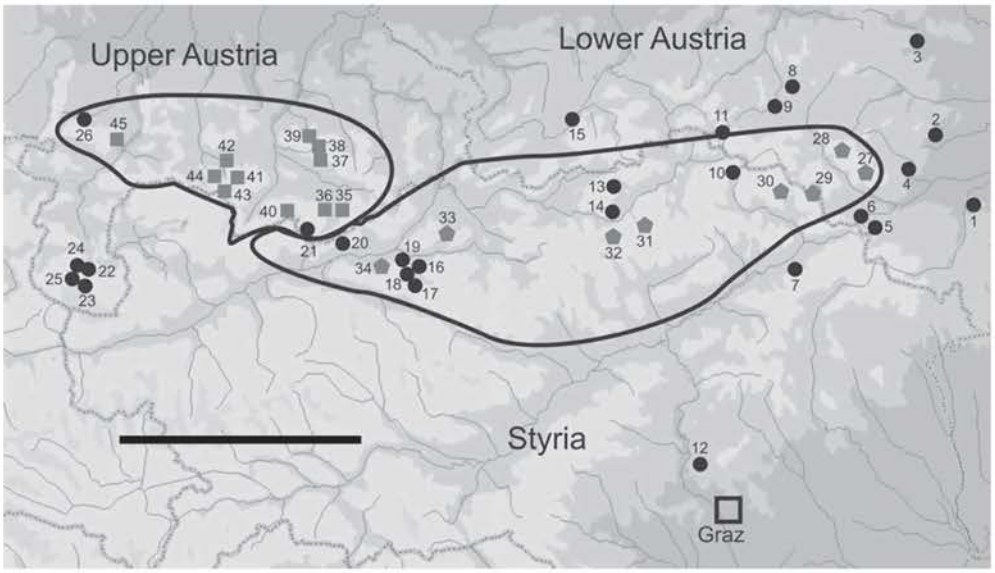

Figure 2. Sample localities: *Trochulus o. oreinos*, grey pentagons; *T. o. scheerpeltzi*, grey squares; *T. hispidus*, black circles. Numbers correspond to localities in Table 1. Distribution ranges of *T. o. oreinos* and *T. o. scheerpeltzi* (according to Klemm, 1974) are delineated with black lines. The scale bar represents 50 km.

Table 1. Sampling localities of *Trochulus* taxa.

Species	Number of locality	Name of locality	E	T	L	M	S	J	G	A
T. hispidus	1	Pittental-Schlattenbach	397	3	3	2	0	1	3	2
	2	Würflach-Johannesbachklamm	445	5	3	4	2	1	3	2
	3	Berndorf-Grabenweg	412	6	6	6	0	0	3	3
	4	Sierningtal-Stixenstein	470	9	4	9	5	0	3	3
	5	Semmering-Maria Schutz	871	3	3	2	0	1	3	2
	6	Breitenstein-Adlitzgraben	650	3	2	3	1	0	2	2
	7	Fischbacher Alpen-Hauereck	1,187	2	2	1	0	1	2	1
	8	Halbachtal-Rossbachklamm	649	5	5	5	0	0	3	3
	9	Tiefental-Ochbauer	739	10	10	10	0	0	3	3
	10	Frein-Freinbach	869	10	10	10	0	0	3	3
	11	Göller-Gscheid	914	10	10	10	0	0	3	3
	12	Grazer Bergland-Semriach	503	11	10	11	1	0	10	10
	13	Dürradmer-Kräuterin	1,100	3	3	3	0	0	3	3
	14	Salzatal-Weichselboden	660	3	3	2	0	1	3	3
	15	Dürrenstein-Lechnergraben	604	3	3	2	0	1	3	2
	16	Johnsbachtal-Wasserfallmauer	978	5	3	5	2	0	3	3
	17	Johnsbachtal-Kölblwirt	868	3	3	3	0	0	3	3
	18	Johnsbachtal-Kneippstation	865	3	3	2	0	1	3	2
	19	Johnsbachtal-Langriesmündung	652	3	3	2	0	1	3	2
	20	Großer Phyrgas-Arlingsattel	1,425	10	10	10	0	0	2	3
	21	Warscheneck-Wurzeralmbahn	810	1	1	1	0	0	1	1
	22	Hallstatt-Salzberg	942	3	3	3	0	0	3	3
	23	Hallstatt-Klausalm	796	4	3	3	1	1	3	2
	24	Hallstatt-Sportplatz	524	3	3	2	0	1	3	2
	25	Hallstatt-Waldbachstrub	806	4	4	4	0	0	4	4
	26	Hochlecken-Taferlklause	778	1	1	1	0	0	1	1
T. o. oreinos	27	Schneeberg-Waxriegel	1,873	41	3	41	38	0	3	3
	28	Schneeberg-Fadenwände	1,562	7	2	6	5	1	2	1
	29	Rax-Bismarksteig	1,787	36	6	31	30	5	6	1
	30	Schneealpe-Schauerkogel	1,664	11	9	11	2	0	3	3
	31	Hochschwab-Severinkogel	2,010	1*	1	0	0	0	1	0
	32	Hochschwab-Schiestlhaus	2,179	11	11	10	0	1	3	2
	33	Tamischbachturm	1,940	10	1	10	9	0	3	3
	34	Admonter Kalbling	2,026	9	6	9	3	0	6	6
T. o. scheerpeltzi	35	Großer Phyrgas-Haller Mauern	1,900	11	11	10	0	1	3	2
	36	Großer Phyrgas-Westgrat	2,000	3*	3	2	0	0	3	3
	37	Hohe Nock-Feichtausee	1,399	10	10	10	0	0	2	2
	38	Hohe Nock-Hauptkar	1,704	11	11	11	0	0	3	3
	39	Hohe Nock-Haltersitz	1,583	10	10	10	0	0	3	2
	40	Warscheneck-Toter Mann	2,028	2	1	2	1	0	1	1
	41	Großer Priel-Welser Hütte	1,747	9	9	8	0	1	3	2
	42	Großer Priel-Hinterer Ackergraben	1,564	2	2	2	0	0	2	2
	43	Großer Priel-Schlund	2,284	2	2	2	0	0	2	2
	44	Großer Priel-Fleischbanksattel	2,157	12	12	10	0	2	3	1
	45	Höllengebirge-Bledigupf	1,677	3	1	3	2	0	1	1
				327	225	304	102	21	132	111

Abbreviations: E, elevation (metres above sea level); T, total number of investigated specimens; L, total number of living specimens (adult and juvenile); M, total number of morphologically investigated specimens (adult; living and empty shells); S, total number of adult empty shells; J, total number of living juvenile specimens; G, total number of genetically investigated specimens (selected living adult and all juvenile specimens); A, total number of specimens investigated genetically and morphologically. *One adult living specimen of each of these samples was broken.

variables (mean, variance and standard deviation) were calculated. Hairs (which are present also in juveniles) were inspected in all 327 individuals. Another two adult specimens could be investigated only genetically, because their shells were broken. A total of 111 specimens were investigated using all methods (Table 1). The measurement error is too often neglected in measurements of small (<10 mm) globular shells. The main source of error is the lack of precise measurement points on the shell. Furthermore, the definition of the main axes is not very precise and the projection of the shell in two dimensions is problematic. Measurement error was determined by repeated measurements (10 times) of shell diameter, umbilicus diameter and shell height in 140 empty shells of *Trochulus* and examination of the distribution of residuals (total 4,200).

Four additional shell traits were recorded as presence/absence, because they were mentioned as typical for

T. o. oreinos in the original description (Wagner, 1915). Three of these were apertural traits: basal tooth, internal rib and paler area around the aperture (Fig. 3). The occurrence of coarse, irregular ridges was also recorded as presence/absence. Ridges were classified as 'coarse' if broad ridges (>0.5 mm) were immediately followed by smaller ones (Fig. 3). In *T. o. scheerpeltzi* a groove beneath the keel was described by Mikula (1957) and was recorded by us in three categories: well developed, partly developed and absent. If the groove was clearly visible with ×16 magnification and covered at least 50% of the circumference, it was characterized as well developed. If it covered less than this and was only weakly visible, it was characterized as partly developed. Measurements of shells were log-transformed. These data together with scores obtained with a correspondence analysis of presence/absence data were used in a canonical discriminant analysis (Tabachnick & Fidell, 1996). Morphometric analyses were performed with programs written by one of us (H.W.). Since we were interested in identifying group differences and the variables responsible for these differences, we used discriminant analysis rather than PCA or ordination techniques that deal with overall variation, which might be dominated by variables that do not contribute to, or even mask, the variation among groups.

To quantify hair length and structure, digital microscopic images were taken of five hairs of 15 specimens (five of each form). Hair lengths were measured by using TPSdig Version 2.14 (Rohlf, 2001). To proof the repeatability of measurements, all hairs were measured twice.

As measuring of hairs takes a lot of time and the different hair morphologies can be easily recognized (Fig. 3), the values from all specimens were assigned to three categories: long hairs (>0.2 mm), short hairs (<0.1 mm) and no hairs.

Genetic analysis

From 132 specimens (adults and juveniles) a partial region of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was sequenced. In addition, from representatives of each clade a partial region of the mitochondrial 16S rRNA (16S) gene was sequenced (altogether 38). As outgroup taxa *Monacha cantiana* and *Plicuteria lubomirskii* (one specimen each) were analysed for both fragments.

A piece of foot tissue was extracted with QIAgen Blood and Tissue Kit. Primers were based on those used by Gittenberger, Piel & Groenenberg (2004) for COI and by Pfenninger, Posada & Magnin, (2003) for 16S. Primers were optimized on the basis of alignments of published sequences of several snail species. Primer sequences for COI: COIfolmerfwd 5′-GGTCAACAATC ATAAAGATATTGG-3′ (LCO1490 modified from Folmer *et al.*, 1994) and COIschneckrev 5′-TATACTTCTGGATGACCA AAAAATCA-3′ (H2198-Alb modified from Gittenberger *et al.*, 2004). Primer sequences for 16S: 16Sfw 5′-CGCAGTAC TCTGACTGTGC-3′ (Pfenninger *et al.*, 2003) and 16S_sch_rev 5′-CG CCGGTCTGAACTCAGATC-3′ (16Srev modified from Pfenninger *et al.*, 2003). Resulting fragment sizes were 705 bp (COI) and about 395 bp (16S), respectively. PCR was performed on a Master gradient thermocycler (Eppendorf) in 50 µl with 1 U *Taq* DNA polymerase (Roche), 1 µM of each primer and 0.2 mM of each dNTP (Boehringer Mannheim). Each PCR comprised 35 reaction cycles with the following annealing temperatures: 50°C (COI) and 55°C (16S). Control reactions for both DNA extractions and PCR amplifications were carried out. PCR products were purified using the QIAquick PCR Purification kit (QIAGEN) and analysed with direct sequencing (both directions). Sequencing using the amplification primers was performed by AGOWA (Berlin, Germany).

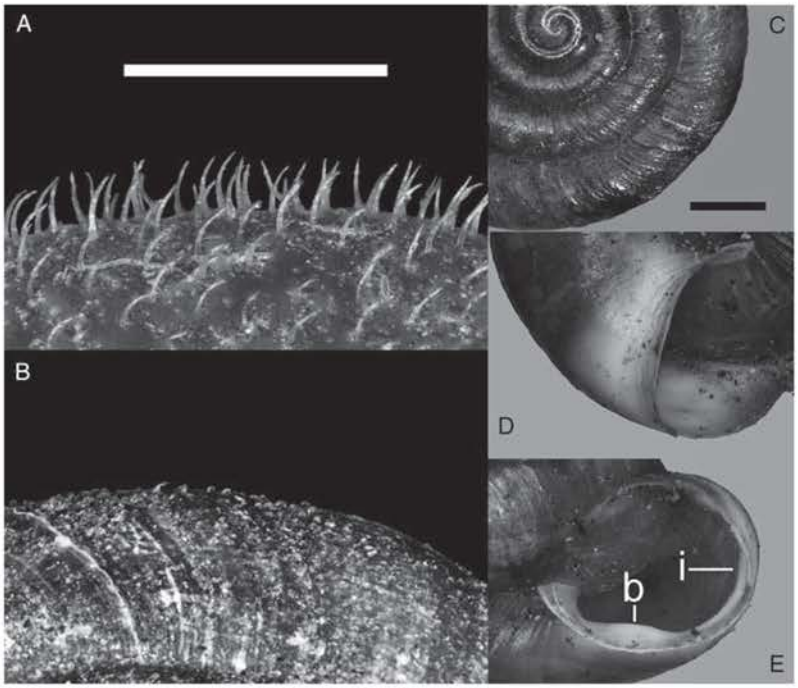

Figure 3. Shell traits. **A.** Hairs of *Trochulus hispidus* (specimen from sample site 17). **B.** Hairs of *T. o. scheerpeltzi* (specimen from sample site 37). **C–E.** *Trochulus o. oreinos* specimen from sample site 29. **C.** Coarse riffles. **D.** Pale area around aperture. **E.** Internal rib (i) and basal tooth (b). Scale bars = 1.0 mm.

For the COI sequences the alignment was straightforward as there were no insertions or deletions. Alignment of 16S sequences was performed with Tcoffee (Notredame, Higgins & Heringa, 2000) and adjusted manually. Neighbour-joining (NJ; Saitou and Nei, 1987) dendrograms were calculated with ClustalX v. 2.0.12 (Larkin *et al*., 2007) using *p*-distances. Bootstrap analyses were performed with 1,000 replicates. For calculation of models of sequence evolution the sequences were collapsed to haplotypes using Collapse1.2 (Posada, 2004). The resulting dataset was used applying the Akaike information criterion corrected for small sample size (AICc) as implemented in the jModelTest v. 0.1.1. (Posada, 2008); the selected models were HKY + I + G for the COI dataset, GTR + G for the 16S dataset and GTR + I + G for the combined (COI + 16S) dataset. Bayesian analyses (BI) were performed using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001). Runs were started with random trees and performed for 2 million generations each with four Markov chains, and a sampling frequency of every 100th generation. Those trees generated prior to stationarity were discarded as burn-in and were not included in the calculation of the consensus trees.

Numbers of haplotypes and haplotype diversity and average *p*-distances (gaps excluded) were calculated with ARLEQUIN v. 3.11 (Excoffier, Laval & Schneider, 2005). The sequences determined in this study are deposited in GenBank under the accession numbers HQ204370–HQ204503 (COI) and HQ204504–HQ204543 (16S).

RESULTS

Shell morphology

Trochulus hispidus shows higher variation in all shell measurements than the *T. oreinos* forms, which are at least 0.5 mm smaller (Table 2). Of the four measurements, shell width and umbilicus width differed between the two *T. oreinos* taxa. Although the *T. oreinos* subspecies are smaller and less variable than *T. hispidus*, ranges overlap. Small specimens of *T. hispidus* (e.g. those from sample site 17 at Sierningtal-Stixenstein) overlap the range of *T. oreinos*. Therefore, shell measurements alone are not a suitable discriminating character for the three taxa. Although the systematic measurement error was relatively high (with 1% error probability, from ±0.14 to ±0.18 mm), it did not compromise these results (see Table 2). Measures showed small differences between *T. o. oreinos* and *T. o. scheerpeltzi*, but large differences between both taxa and *T. hispidus*.

Table 2. Summary of shell measurements (mm) of *Trochulus* taxa.

		Range	ME	Mean	SD	SE
T. o. oreinos, 8 sample	WS	5.4–7.5	0.15	6.37	0.43	0.04
sites, *n* = 118	WU	0.8–1.6	0.14	1.24	0.15	0.01
	SH	2.8–4.2	0.18	3.42	0.28	0.03
	HW	2.0–2.8	0.17	2.40	0.19	0.02
T. o. scheerpeltzi, 11	WS	5.6–7.5	0.15	6.50	0.38	0.05
sample sites, *n* = 70	WU	0.9–1.5	0.14	1.16	0.13	0.02
	SH	2.8–4.0	0.18	3.43	0.25	0.03
	HW	2.0–2.9	0.17	2.39	0.18	0.02
T. hispidus, 26 sample	WS	5.5–8.4	0.15	7.18	0.65	0.06
sites, *n* = 116	WU	1.0–2.3	0.14	1.59	0.22	0.02
	SH	2.9–4.7	0.18	3.79	0.36	0.03
	HW	2.1–3.5	0.17	2.80	0.26	0.02

Abbreviations: WS, width of shell; WU, width of umbilicus; SH, shell height; HW, height of last whorl; ME, measurement error; SD, standard deviation; SE, standard error of the mean.

At first sight *T. hispidus* has remarkably longer hairs than both *T. oreinos* subspecies. Hair length of *T. hispidus* ranges from 0.21 to 0.31 mm (mean 0.27 mm), of *T. o. oreinos* from 0.03 to 0.09 mm (mean 0.06 mm) and of *T. o. scheerpeltzi* from 0.04 to 0.08 mm (mean 0.06 mm). Additionally, hairs of the *T. oreinos* subspecies are often curled or strongly bent, while those of *T. hispidus* are only slightly bent. The problem with this trait is that elder specimens and empty shells often lack hairs. For example, of the 118 specimens investigated in *T. o. oreinos*, 86 were hairless, but of these individuals 78 were empty shells. Among *T. o. scheerpeltzi* individuals 59 out of 70 were hairy, most of them collected alive. Among the 116 specimens of *T. hispidus* 106 showed the characteristic long hairs.

The *T. oreinos* taxa show strong development of the lip and aperture (internal rib, basal tooth, pale around aperture), while in *T. hispidus* these traits occur only occasionally and rarely in combination (Fig. 4). Most specimens of *T. hispidus* show only an internal rib or even none of these traits. Both *T. oreinos* forms consistently show strong irregular riffles, while only 8 of 116 specimens of *T. hispidus* have this character. The groove beneath the keel proved not to be a constant character of *T. o. scheerpeltzi* as two specimens were found in which this trait was virtually absent. On the other hand, nine specimens of *T. o. oreinos* were found in which a faint groove was present, five of them from site 34 (Tamischbachturm). At this same site three *T. o. oreinos* with a well-developed groove were found.

The occurrence of coarse riffles could not be included in the discriminant analysis, as it is a constant trait of both *T. oreinos* forms. The variables which most strongly influenced the results of the analysis were the qualitative characters (hair length, groove, internal rib, basal tooth, pale area), especially the apertural traits. The dominance of these factors on the first axis caused a visible 'horseshoe' effect (Fig. 5A). The results show a clear differentiation between *T. o. scheerpeltzi* and *T. hispidus*. *Trochulus o. oreinos* and *T. hispidus* are visibly differentiated but still close. The two *T. oreinos* taxa show an overlap in the discriminant analyses (polygons in Fig. 5A) mainly caused by the occurrence of a groove beneath the keel of some specimens of *T. o. oreinos*. The first axis explains 84% and the second 16% of the total variation. The first axis separates *T. hispidus* from the other populations, and correlates negatively with the scores associated with the first reciprocal ordering component (mainly defined by the groove beneath the keel and hair length), and positively with all linear measurements, indicating a general size difference. *Trochulus o. scheerpeltzi*

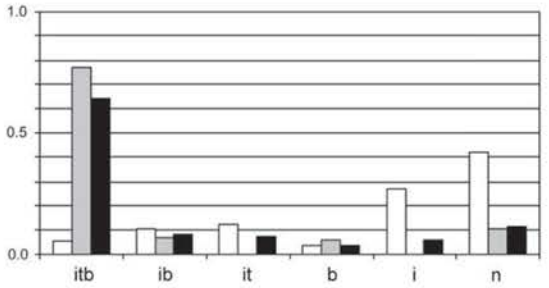

Figure 4. Combination of apertural traits of *Trochulus o. oreinos*, *T. o. scheerpeltzi* and *T. hispidus* in proportion values. White bars, *T. hispidus*; grey bars, *T. o. oreinos*; black bars, *T. o. scheerpeltzi*. itb: internal rib, basal tooth, pale area around aperture; ib: internal rib, pale area around aperture; b: pale area around aperture; i: internal rib; n: none of these traits.

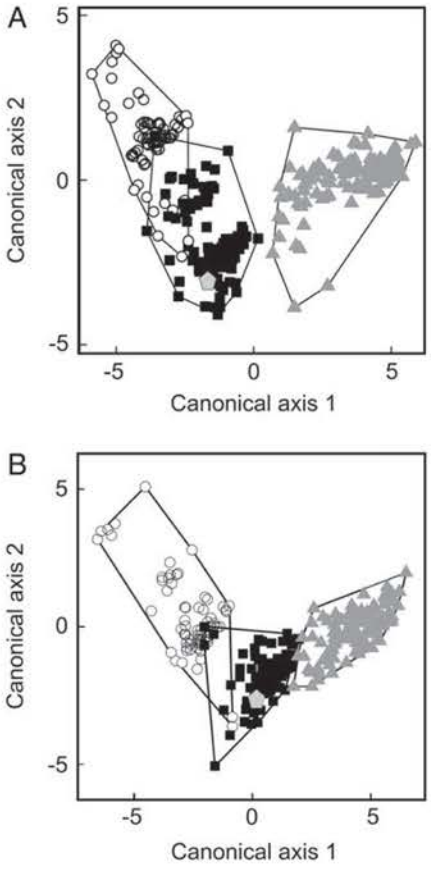

Figure 5. Discriminant analysis of *Trochulus o. scheerpeltzi*, *T. o. oreinos* and *T. hispidus*. The outlier was treated separately as we considered it a malformation (see text). **A.** Discriminant analysis including hair traits. Wilks' Lambda = 4.6328*E*−02, $F_{14,590} = 153.7$, $P < 0.00001$. **B.** Discriminant analysis excluding hair traits. Wilks' Lambda = 4.2035E−02, $F_{14,588} = 120.1$, $P < 0.00001$. Grey triangles, *T. hispidus*; white circles, *T. o. scheerpeltzi*; black squares, *T. o. oreinos*; grey pentagon, *T. hispidus* (outlier).

scores higher on the second axis, which is mainly characterized by a negative correlation with the scores derived from the second axis of the correspondence analysis (mainly defined by strong development of the groove beneath the keel) (Table 3). Hair length could not be analysed in several shells which had obviously lost the hairs (empty shells and some live-collected). However, as this is an essential discriminant trait, we included it in our analysis. An additional discriminant analysis excluding hair length is shown in Figure 5B. It reveals the same groups, although *T. o. oreinos* and *T. hispidus* are less clearly separated from each other.

There is one outlier of *T. hispidus*, a rather small, hairless, empty shell (site 4) which has an internal rib and a pale area around the aperture. This outlier was not included in the computation of the discriminant function, but was subsequently scored to show its position (Fig. 5).

Habitat selection and elevation

Trochulus o. oreinos was found at elevations from 1,562 to 2,179 m, *T. o. scheerpeltzi* from 1,399 to 2,157 m and *T. hispidus* from 397 to 1,425 m (Table 1). Both *T. oreinos* taxa were found to be restricted to rocky habitats, mostly among sparse alpine grass or in vegetation-free areas and mountain pine shrubbery, while *T. hispidus* preferred moist habitats, in particular tall perennial herbs, often near water bodies (Fig. 6). *Trochulus hispidus* inhabits a wider range of vegetation types and landscape structures than both *T. oreinos* forms.

Table 3. Correspondence analysis of *Trochulus* taxa: linear discriminant coefficients of linear discriminant.

Variable	Linear discriminant coefficients	
	1	2
WS	−0.35081	−0.26637
WU	0.57778	0.11054
SH	0.28020	0.69537
HW	0.47491	0.34116
ROC1	−0.44336	0.05416
ROC2	0.10844	−0.46297
ROC3	−0.17531	0.31563

Abbreviations: WS, width of shell; WU, width of umbilicus; SH, shell height; HW, height of last whorl; ROC1−3, reciprocal order components.

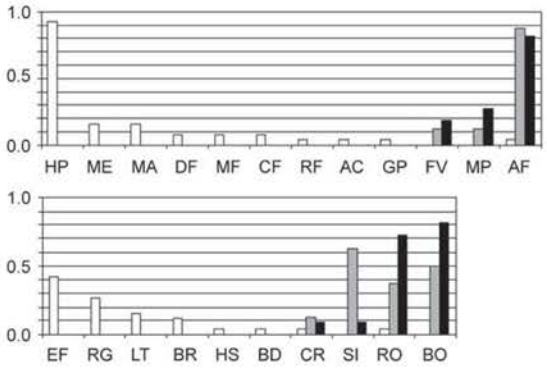

Figure 6. Habitat (top) and landscape structure (below) preferences of the three *Trochulus* taxa in proportion values. White bars, *T. hispidus*; grey bars, *T. o. oreinos*; black bars, *T. o. scheerpeltzi*. Abbreviations: HP, high perennial herbs; ME, meadow; MA, marsh; DF, deciduous forest; MF, mixed forest; CF, coniferous forest; RF, riparian forest; AC, alder carr; GP, garden/park; FV, free of vegetation; MP, mountain pine shrubbery; AG, alpine grassland; EF, edge of forest; RG, riverbank grove; LT, losse trees and shrubs; BR, boundary ridge; HS, hedgerows and shrubs; BD, bank/dam; CR, canyon/rock face; SI, single stones; RO, rocks; BO, boulders.

Molecular analysis

Among the 132 individuals analysed for COI, 51 different haplotypes were detected. In the 38 individuals from which 16S sequence was obtained, 19 different haplotypes were observed. In the BI tree based on the COI dataset (Fig. 7) three clearly differentiated groups are found: (1) *T. hispidus*, (2) *T. oreinos scheerpeltzi* and (3) *T. oreinos oreinos*. The same topology was obtained from both 16S and combined datasets, and with a different tree-building algorithm (NJ; data not shown). All three taxa are highly supported (maximum posterior probability) in analyses of all three datasets. The two *T. oreinos* taxa are well-supported sister groups in all trees. The genetic distances among the three clades are high. Average *p*-distances in

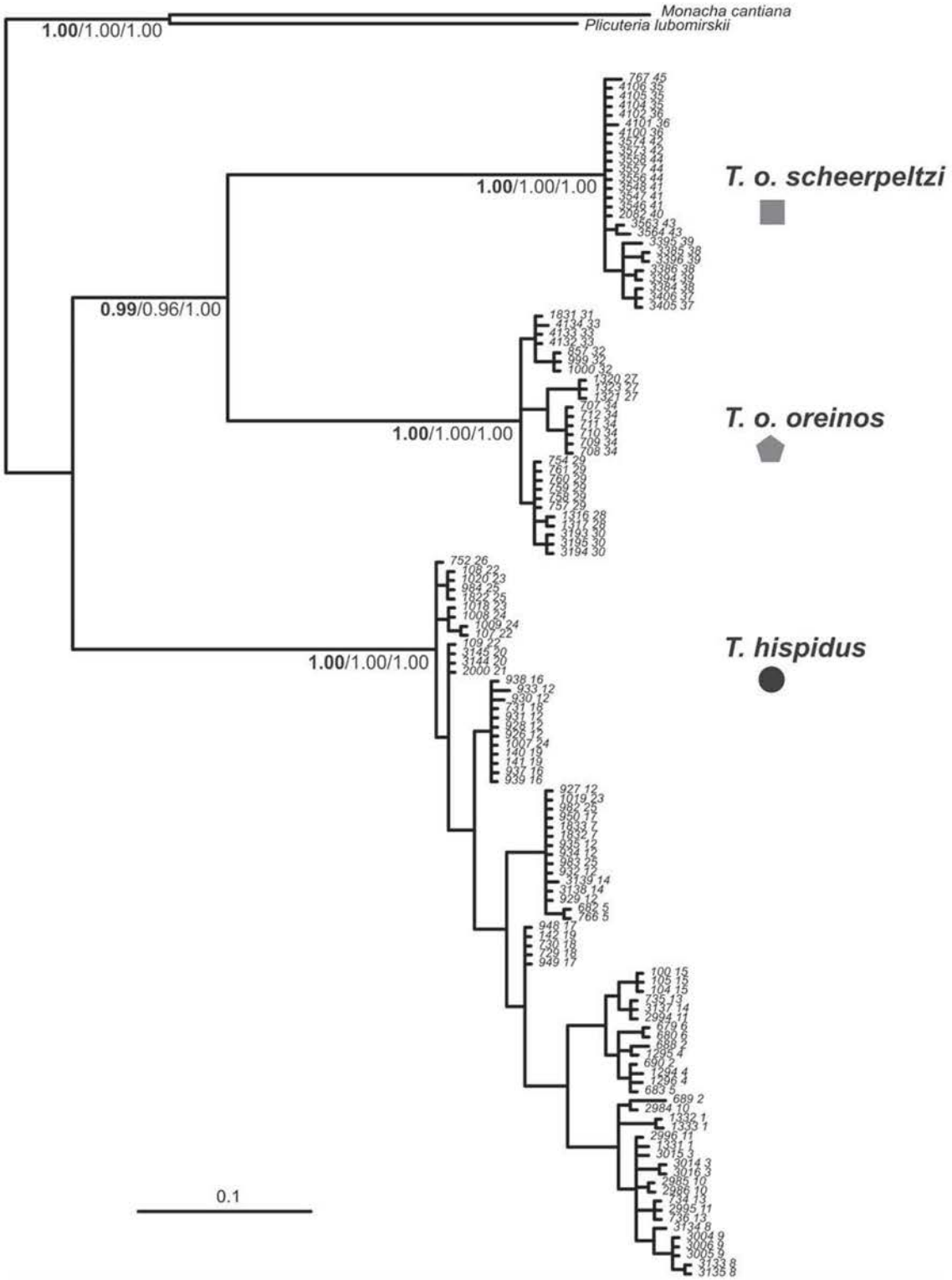

Figure 7. Bayesian tree of the COI sequences of *Trochulus* species. Posterior probabilities of the main groups are indicated at the nodes (COI/16S/COI + 16S). Posterior probabilities of the depicted tree (COI) are in bold. Labelling of the individuals include individual number and locality (before the space; as in Table 1). *Monacha cantiana* and *Plicuteria lubomirskii* were used as outgroups.

the COI sequences are 13.3% between the two *T. oreinos* taxa. Between *T. hispidus* and *T. o. oreinos*, and *T. o. scheerpeltzi*, the values are 14.4% and 16.0%, respectively. The average *p*-distance for the 16S sequences is 13.7% between the two *T. oreinos* taxa. Between *T. hispidus* and *T. o. oreinos* and *T. o. scheerpeltzi* the values are 18.0% and 17.4%, respectively. Both *T. oreinos* subspecies have a somewhat lower haplotype diversity (*T. o. scheerpeltzi*: 0.88 and *T. o. oreinos*: 0.70) compared to *T. hispidus* (0.95), but the number of sample sites is different in all three (*T. hispidus* 26, *T. o. oreinos* 8, *T. o. scheerpeltzi*, 11). In *T. oreinos* specimens from one locality had either the same or very similar haplotypes, while in *T. hispidus* quite distinct haplotypes coexist even within one locality (e.g. sample site 25).

DISCUSSION

The results obtained by the two different approaches (morphological and genetic) agree only in some aspects. The clear differentiation between *Trochulus hispidus* and the two *T. oreinos* taxa is supported by hair and shell morphology, genetic analysis and ecological preferences. In contrast, a clear differentiation between *T. o. oreinos* and *T. o. scheerpeltzi* is only found in the genetic analyses, but not in morphology or ecological preferences.

Differentiation between T. hispidus *and* T. oreinos

Trochulus o. oreinos and *T. o. scheerpeltzi* are very similar to each other shell morphology and ecology, while *T. hispidus* differs from these taxa in both respects and also appears to be more variable. Hair morphology discriminates the *T. oreinos* subspecies from *T. hispidus*, and this character is also useful for juvenile specimens. The basic pattern for hair length and distance between hairs in *Trochulus* is supposed to be a stable trait as it is determined by glands in the mantle (Kaiser, 1966). Other shell morphological traits also show clear differences between *T. oreinos* subspecies and *T. hispidus*. A constant trait of both *T. oreinos* subspecies are the coarse riffles. Furthermore, only 20% of both *T. oreinos* subspecies, but nearly 70% of *T. hispidus*, did not show fully developed apertural traits. Seasonality cannot explain this observation, as the snails were collected at similar times of the year. A lack of fully developed apertural traits in adult specimens of *T. hispidus* has been reported several times in the literature. Weinland (1883) mentioned that it is difficult to find specimens of *T. hispidus* with a fully developed lip. A similar finding was reported by Geyer (1915), who noticed that in Upper Austrian populations of *T. hispidus* many adult specimens did not show a lip.

Shell measurements alone are not enough for a reliable differentiation. Furthermore, these traits can also be influenced by the environment (Davis, 2004). Our results show that *T. hispidus* is more variable than both *T. oreinos* subspecies. Other authors have also described substantial shell variability in *T. hispidus*, which can result in difficulties in species recognition (Ložek, 1956; Forcart, 1965; Naggs, 1985; Von Proschwitz, 1993; Proćków, 2009). However, in contrast to previous studies, we found no specimens with pronounced globular shells.

With respect to habitat preferences a clear difference was revealed between *T. hispidus* and *T. oreinos*. Both *T. oreinos* subspecies are restricted to rocky areas with alpine vegetation at high elevations, whereas *T. hispidus* extends over a larger range, predominantly in lower areas, preferring moist habitats with a well-developed herb layer, often close to water bodies. The small overlap in the altitudinal distribution of *T. hispidus* and *T. oreinos* subspecies might be due to the geomorphological conditions of the northern Calcareous Alps. Springs and creeks usually discharge at the base of the mountain massifs, while plateaus and slopes at high elevation remain dry (Lieb, 1991). Where damp habitats extend up to higher elevation, *T. hispidus* can be found above 1,000 m, as at the sampling sites 'Fischbacher Alpen-Hauereck' (1,180 m) and 'Haller Mauern-Arlingsattel' (1,425 m). The wide distribution of *T. hispidus* even in formerly glaciated areas of Austria might be due to the fact that rivers and creeks act as linear corridors along which the snails can easily disperse (actively or passively via rafting). The occurrence of *T. o. scheerpeltzi* at 1,399 m at the site 'Hohe Nock-Feichtausee' is the lowest one ever recorded for this taxon, as the known range is 1,600–2,300 m (Klemm, 1974). Nevertheless, this site fits the habitat preferences of *T. o. scheerpeltzi* because it is an azonal deep habitat of high alpine vegetation resulting from the cool microclimate of a sunless shady slope with northern exposure.

Our genetic data are in accordance with the morphological and ecological differentiation between *T. hispidus* and *T. oreinos*. We cannot conclude that *T. oreinos* and *T. hispidus* are sister species, owing to our limited sampling of other taxa, and because the phylogenetic status and monophyly of *T. hispidus* are unclear (see above). This underlines the need for a complete phylogeny of the genus. The high genetic distances (mean distance for COI 15.2%) (only slightly higher than those between the two *T. oreinos* subspecies: mean distance for COI 13.3%) suggest that the differentiation between the lineages leading to these two species might have taken place before the Pleistocene glaciations. Also, the difference in the morphological variability in *T. oreinos* and *T. hipidus* is reflected in the haplotype variation, *T. hispidus* showing higher haplotype diversity than the other two taxa. The haplotype diversity found within the *T. oreinos* taxa is rather low (0.88 and 0.70). Direct comparison of the haplotype diversity is problematic, since the number of sampled localities is higher in *T. hispidus* than in the two *T. oreinos* taxa, but it is still meaningful since we sampled the whole distribution range of the two *T. oreinos* taxa. Hence, it cannot be expected that the diversity will increase much by including more localities and samples. Only a part of the distribution range was sampled *T. hispidus*, but well-differentiated haplotypes can be found within a single locality. The low haplotype diversity within the two *T. oreinos* taxa is remarkable, since the populations live on isolated mountain peaks. This suggests past extinction and recolonization events, perhaps during glacial periods. Other studies have reported highly divergent lineages within *T. hispidus* (Pfenninger *et al.*, 2005; Dépraz *et al.*, 2009). However, in our analysis all *T. hispidus* from the foothills of the northeastern Austrian Alps belong to a single haplogroup, distantly related to the haplogroups of *T. hispidus* published so far (Pfenninger *et al.*, 2005, Dépraz *et al.*, 2009). Thus, our analysis reveals yet another lineage that morphologically resembles *T. hispidus*.

Differentiation between T. o. oreinos *and* T. o. scheerpeltzi

While the morphological differentiation between *T. hispidus* and *T. oreinos* is straightforward, the distinction between *T. o. oreinos* and *T. o. scheerpeltzi* is difficult, as there are no apparent differences in hair and shell morphology, size and habitat selection. The only discriminating characters mentioned in the literature are the different geographical ranges and the groove beneath the keel. However, our results indicate that the groove is not a constant trait of *T. o. scheerpeltzi* as some specimens lack this characteristic, whereas specimens with a groove were found at the western sample sites of *T. o. oreinos*. The discriminant analysis (Fig. 5) showed overlap of the two taxa. These findings are in accordance with reports of intermediate forms between the two *T. oreinos* taxa (Mikula, 1957; Falkner, 1970, 1982) and suggest why the two forms have been classified as subspecies. However, from the

molecular results there is no indication of hybridization between *T. o. oreinos* and *T. o. scheerpeltzi*. The distribution of mitochondrial haplotypes is completely in accordance with the distribution ranges of the two taxa; no haplotypes of *T. o. oreinos* were found in the range of *T. o. scheerpeltzi* or *vice versa*. It could be hypothesized that the groove is just a result of phenotypic plasticity and/or connected with a yet unknown adaptation to climate or habitat conditions. As the distribution ranges of both subspecies belong to the same geological formation, it is unlikely that geological factors could be of importance. In this region there is a transition between two local climatic zones – western *vs* eastern part of the northern margin of the northeastern Alps (as defined by Kilian *et al.*, 1994). The transition between these two climatic zones covers the eastern part of the distribution area of *T. o. scheerpeltzi* as well as the western part of the *T. o. oreinos* distribution (Fig. 2), i.e. regions where some morphologically atypical individuals (*T. o. oreinos* possessing a groove, *T. o. scheerpeltzi* lacking a groove) were detected. Subtle climate differences might in some way influence the formation of a partially or fully developed groove. Another explanation for the presence of a groove in the specimens from Tamischbachturm is that they are malformations, although it is hard to explain why this should have happened coincidentally to many individuals.

The genetic data present a different picture. *Trochulus o. scheerpeltzi* and *T. o. oreinos* are well separated. The *p*-distance values for COI and 16S are surprisingly high. It has been suggested that terrestrial gastropods might have a substantially higher substitution rate in their mtDNA than that reported for other animal groups (Thomaz, Guiller & Clarke, 1996; Hayashi & Chiba, 2000; Davison, 2002; Van Riel *et al.*, 2005). However, this is not a plausible explanation for the surprisingly high distances found between the *Trochulus* taxa, since preliminary analysis of nuclear 5.8S rRNA, ITS2 and 28S rRNA sequences support the hypothesis of an old split (LK, unpublished results). It should be mentioned that many studies report moderate evolutionary rates in molluscs (e.g. Pfenninger & Magnin, 2001; Haase *et al.*, 2003; Ketmaier, Giusti & Caccone, 2006). Hence, our data suggest a long separation of *T. o. scheerpeltzi* and *T. o. oreinos*.

The shell morphological traits and ecological preferences do not provide strong arguments to raise the two subspecies *T. o. oreinos* and *T. o. scheerpeltzi* to species level. They also do not differ in the gross anatomy of the reproductive organs (Mikula, 1957). More detailed anatomical analyses (e.g. cross-sections of penis and vagina) should be performed to search for differences in these traits. Applying the ecological and phylogenetic species concepts, *T. o. oreinos* and *T. o. scheerpeltzi* are not unequivocally separated, as they inhabit the same habitat and no diagnostic morphological traits have been detected so far. The status of *T. oreinos* as an independent species separated from *T. hispidus* (Falkner, 1982) is corroborated by morphological (groove, hairs) and ecological (habitat preferences) arguments. The clear genetic separation corresponding to the geographical distributions did not provide any indication for interbreeding between the two taxa. Nevertheless, in the light of the biological species concept further analyses (e.g. ecophysiological, anatomical, breeding experiments) are needed to test the potential for interbreeding before a final decision can be made about species or subspecies status.

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Habitat requirements of the pulmonate land snails *Trochulus oreinos oreinos* and *Cylindrus obtusus* endemic to the Northern Calcareous Alps, Austria

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Keywords: Mollusca, Gastropoda, Cylindrus obtusus, Trochulus oreinos, *habitat preferences, Alpine endemics, climate change*

Abstract

The habitat needs and potential threats to *Trochulus oreinos oreinos* (Wagner 1915) and *Cylindrus obtusus* (Draparnaud 1805) were assessed by comparing vegetation maps and our own records. We selected four sites from which we had adequate samples and for which exact vegetation maps were available: the mountains Hochschwab, Schneealpe, Rax and Schneeberg. Both taxa prefer open dry alpine grassland with diggable soil and/or stones. *T. oreinos oreinos* is restricted to subalpine and alpine boulder societies and *Caricetum firmae*. While *C. obtusus* dwells on several communities of plants, it seems to be bound to unconsolidated stony ground. As both taxa prefer naturally forest-free areas, they are not affected by structural changes of the habitat, such as reforestation caused by the abandonment of grazing and the shift of vegetation zones. But it has to be considered that *T. oreinos oreinos* and *C. obtusus* are limited by microclimatic factors, as they prefer cooler habitats. The mountains Schneealpe, Rax and Schneeberg, reaching barely 2 000 m in height, are on the climatic limit of the species distribution. Therefore, the investigated taxa are vulnerable to the upward shift of climate zones. *T. oreinos oreinos* shows striking similarities in its habitat preference to the Swiss endemic *T. biconicus*, as both taxa prefer the same dry alpine habitats which are quite different to those of other representatives of the genus, which prefer damp habitats.

Profile

Mountain range

Alps

Country

Austria

Introduction

The north-eastern Calcareous Alps are a hotspot of endemic plants and animals (Rabitsch & Essl 2009). This is also the case for land snails, which have a maximum of endemics in this area. Because of their poor dispersal abilities, they are especially predestined to develop local forms in isolated areas. Two of the endemic pulmonate land snail species of the north-eastern Alps – *Trochulus oreinos* (Wagner 1915) and *Cylindrus obtusus* (Draparnaud 1805) – which are the focus of a larger project on the phylogeography of Alpine land snails – inhabit open, rocky areas from the subalpine ecotone upwards to the alpine zone (Klemm 1974; Rei-schütz & Reischütz 2009). *Trochulus oreinos* comprises two subspecies: *Trochulus oreinos oreinos* (Wagner 1915), subject of the present paper, distributed in the northern Calcareous Alps of Lower Austria and Styria from Schneeberg to Totes Gebirge, and *Trochulus oreinos scheerpeltzi* (Mikula 1954), restricted to the Northern Calcareous Alps of Upper Austria from Sengsengebirge to Höllengebirge. *Cylindrus obtusus* is distributed in the Northern Calcareous Alps from Schneeberg in Lower Austria, across the northern parts of Styria to Mt. Dachstein in Upper Austria and carbonate sites within siliceous bedrock of the Central Alps westwards to the Glocknergruppe. Both species are restricted to higher elevations: *Trochulus oreinos* from 1 400 to 2 200 m (Klemm 1974; Duda et al. 2010), *Cylindrus obtusus* from 1 100 to 2 680 m (Klemm 1974). The lower altitudinal range of the latter was already seen as atypical exception by Klemm (1974).

Figure 1 – Alpine boulders and screes with poor vegetation are a suitable habitat for both Trochulus oreinos *(left) and* Cylindrus obtusus *(right).* © *Helmut Sattmann*

For both species there are only sparse documentations on their habitat needs, as is the case for many Central European land snails. In *Cylindrus obtusus*, previous investigations focused on morphology, anatomy, phylogeography and on systematics (Adensamer 1937; Freitag & Desch 1996; Shileyko et al. 1997; Edlinger 1999), but only little information is available about the ecology and biology of the species (Kühnelt 1937; Freitag & Desch 1996; Bisenberger et al. 1999). For *Trochulus oreinos*, some taxonomic appraisals have been published (Falkner 1982, Falkner 1995). A first

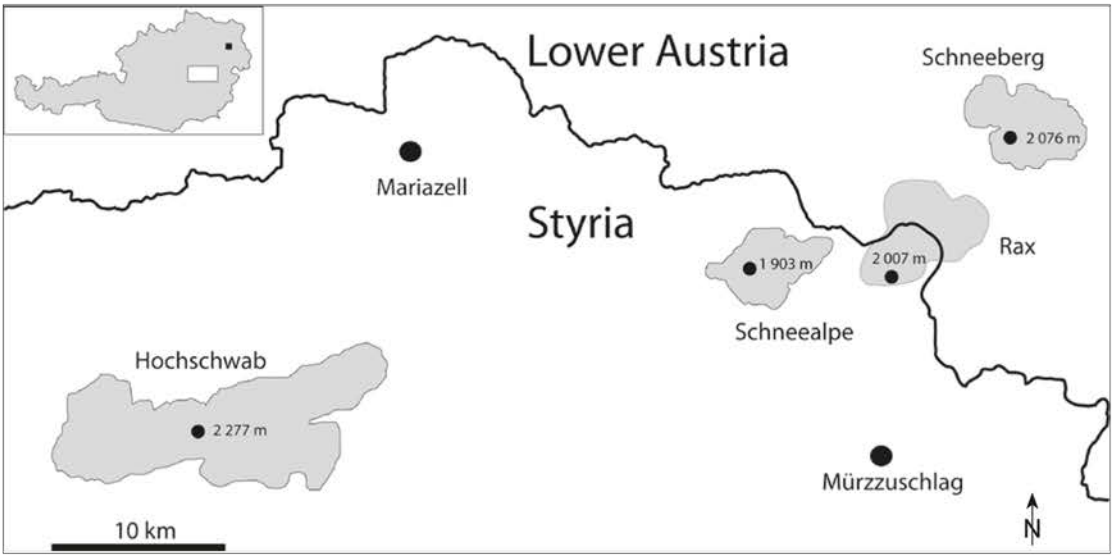

Figure 2 – Location (top left) and sketch map of study area. Elevation of the highest peaks in metres above sea level.

phylogenetic analysis, combined with more detailed morphological investigations, was performed recently (Duda et al. 2010) indicating that *T. oreinos* is an old phylogenetic lineage and presumably has a long independent history in the north-eastern Calcareous Alps. Also *C. obtusus*, which is the only representative of the genus *Cylindrus,* is considered an old endemic of this region. Yet, so far hardly any published information has been available on habitat preferences and population ecology of the two species (Reischütz & Reischütz 2009; Duda et al. 2010). From the literature it is known that both species are restricted to alpine and subalpine ecotones above 1 400 m and occur predominantly near calcareous gravel (Klemm 1974; Duda et al. 2010). As both species are endemics of the Eastern Alps with quite restricted distribution – concerning their ranges as well as their altitudinal distribution – their habitat requirements are of special interest, also in the light of human habitat destruction and climate change.

In the course of collecting trips in the years 2006 – 2009, we gathered a high number of specimens of these taxa throughout the Austrian Eastern Alps. As a result, detailed data on their occurrences are now available for further interpretations. In the present paper, we combine our data with published vegetation maps (and general climatic assessments) to address questions of ecological requirements and possible threats.

For our investigations we selected mountain ranges of the north-eastern margins of the distribution ranges (Hochschwab, Schneealpe, Rax and Schneeberg), because these regions are well surveyed in terms of vegetation mapping (Figure 2). The selected areas represent potential glacial refugia and thus allow interpretations concerning the Pleistocene history of the species. These northern margins of the north-eastern Calcareous Alps have been the focus of several research projects intended to determine potential glacial refugia (e.g. Tribsch 2004; Schönswetter et al. 2005), as several parts of it remained ice-free during the last glaciations (Van Husen 1997). Thus, they might play an important role for endemism.

We assumed that the proportion of primarily (postglacially) non-forested habitats compared to areas deforested by human activities like pasturing might be the most crucial factor and addressed the following questions: 1) How can the preferred habitat of *C. obtusus* and *T. oreinos* be characterized? 2) Do the species also occur at locations that are influenced by anthropogenic activities, like man-made mountain pastures on forest and krummholz sites or are they restricted to primarily forest-free sites? 3) Are they afflicted by recent changes of alpine agriculture? 4) Are the habitats and studied populations affected by climatic changes? The latter question is of particular interest as the influence of global warming on high-mountain organisms is a matter of debate and was discussed in several studies and projects.

Material and methods

Sampling areas and vegetation maps

The mountain ranges of Hochschwab, Schneealpe, Rax, and Schneeberg were selected, because, according to the available literature, these regions are well documented by vegetation mapping. On Rax and Schneealpe, large parts of alpine meadows are situated below the tree line. They are the result of human agricultural activities (traditional pasture management). As the pasture activities declined during the last hundred years, intrusion of krummholz has taken place in many of these areas (Dirnböck et al. 2003). On Schneealpe, only a few exposed peaks on the plateau and steep rock faces on the eastern slopes are thought to have been originally free of forest. Also on the Rax, large parts of the high plateau were primarily forested except for a few steep ridges and summit areas (Dullinger et al. 2001). Mt. Schneeberg as the easternmost high-alpine peak of the northern Calcareous Alps is

Table 1 – Sample sites on Schneeberg, Rax, Schneealpe and Hochschwab. Occurrences of C. obtusus *(C) and* T. oreinos *(T) and none of both species (–).*
Abbreviations: Vegetation type: AG: alpine grassland, AS: Alnus viridis *shrub; DF: deciduous forest; FV: free of vegetation; ME: meadow; MF: mixed forest; PM:* Pinus mugo *shrub*
Landscape structure: CA: canyon / rock face; BD: bank, dam; BO: boulders; EF: edge of forest; LT: loose tree stands and shrubs; RO: rocks; SI: single stones
Plant society (based on Greimler & Dirnböck 1996): BSC: boulder and scree societies, CFE: Caricetum ferruginae, *CFD:* Caricetum firmae, *dense, CFL:* Caricetum firmae, *loose, FSF: complex of* Firmetum, Seslerio-Sempervivetum *and* Festuca-Agrostis *meadow, SFO: subalpine forest societies, SCS:* Seslerio-Caricetum sempervirentis. *Additional societies in brackets.*

Mountain / Site	Taxa	Elevation (m)	Vegetation types	Landscape structures	Plant society
Schneeberg, highest peak: Klosterwappen 2076 m					
Schneeberg, Fadenwände_1393	C	1393	AG, FV	BO, RO	BSC
Schneeberg, Schneidergraben		1401	AG, FV	BO, RO	BSC
Schneeberg, Fadenwände_1525	–	1525	FV, AG	RO	BSC
Schneeberg, Fadenwände_1562	C, T	1562	FV, AG	BO, RO	BSC
Schneeberg, Fadenwände_1695	C	1695	FV, AG	RO	BSC
Schneeberg, Kaiserstein 1	C	1860	AG	SI, BO	CFL
Schneeberg, Waxriegel	C, T	1873	AG	SI	CFL
Schneeberg, Ochsenboden	C	1905	AG	SI	FSF
Schneeberg, Kaiserstein 2	C	1910	AG	SI, BO	CFL
Schneeberg, Klosterwappen 2	C	1994	AG	BO	FSF
Schneeberg, Klosterwappen 1	C	2024	AG	SI, RO	CFL
Rax, highest peak: Heukuppe 2007 m					
Rax, Preiner Gscheid	–	1249	MF	RO	SFO
Rax, Gsolhirn-Steig	–	1422	DF	LT, RO	SFO
Rax, Göbl-Kühn-Steig	–	1397	DF	RO	SFO
Rax, Thörlweg	C	1592	FV, AG	BO, RO	BSC
Rax, Schlangenweg	T	1600	AG	BO	BSC
Rax, Höllentalaussicht	C	1620	FV, AG	BO	BSC
Rax, Praterstern	–	1680	AG, PM	-	CFE
Rax, Jakobskogel - Große Kanzel	C	1734	FV, AG, PM	BO	CFL
Rax, Seehütte	C	1761	AG, PM	RO, BO	SCS
Rax, Bismarcksteig	C, T	1787	AG	BO	BSC
Rax, Karl Ludwig-Haus	C	1791	FV, AG	BO	CFD
Schneealpe, highest peak: Windberg 1903 m					
Schneealpe, Kohlebnerstand	–	1487	MF	EF,BD,RO	SFO
Schneealpe, Blarergraben-Weg	C, T	1664	AG	RO, CA	BSC
Schneealpe, Kutatsch-Huette	–	1673	AG, AS		SCS
Schneealpe, Schneealpenhaus	C, T	1742	AG	SI, BO	BSC (SCS)
Schneealpe, Windberg Osthang1	C	1810	AG	SI, RO	SCS (BSC)
Schneealpe, Windberg West	C	1891	FV, AG	BO	CFL
Schneealpe, Windberg Gipfelhang	C	1903	AG	SI, RO	SCS
Hochschwab, highest peak: Hochschwab 2277 m					
Hochschwab, Böser Wald	–	1076	DF, ME	EF, BO	SFO
Hochschwab, Staritzen Ostgipfel	–	1779	AG	RO	BSC
Hochschwab, Kleiner Ebenstein	C	1847	AG	RO	SCS
Hochschwab, Ochsenreichkar	C	1963	AG	BO, RO	BSC
Hochschwab, Severinkogel	C, T	2010	AG	RO	CFL
Hochschwab, Schiestlhaus	C, T	2179	AG,PM	SI, RO	CFL

strongly afflicted by the pannonic climate of the Vienna basin. Therefore it provides warmer and dryer conditions, but to some extent Rax and Schneealpe are also under this climatic influence (Kilian et al. 1993; Dirnböck & Greimler 1997; Dullinger et al. 2001). On Mt. Hochschwab, larger areas seem to be naturally forest-free, but it has also been influenced by human land use till today and large parts of currently open grassland would – without alpine farming – be covered by krummholz (Dirnböck et al. 1999).

The collecting trips were performed in the years 2006–2009. We sampled primarily the locations recorded in the literature (Klemm 1974) and in the collections of the Museum of Natural History Vienna. In addition, we sampled several new localities not previously recorded. For our comparisons, we included all collecting sites registered in our database (n = 35) that are located within the regions mentioned above and are within the altitudinal distribution of the species (Klemm 1974). Elevations of the localities ranged

Figure 3 – Complex of Firmetum, Seslerio-Sempervivetum *and* Festuca-Arostis *meadow is a result of strong structured relief and comprises elements of several communities of plants. In this habitat only* C. obtusus *is found. © Barbara Däubl*

from 1 076 – 2 179 m (Table 1). We included also localities at which both *C. obtusus* and *T. oreinos* were lacking, although they seemed to provide potential habitats of the species.

We tried to assess the habitat requirements of both species more precisely by combining the gathered data on the occurrences of the taxa as well as local factors (coarse vegetation types and landscape structures, see below) registered during the collection trips with published vegetation maps. The vegetations maps used for characterization of the areas are published in Greimler & Dirnböck (1996), Dirnböck & Greimler (1997), Dullinger et al. (2001) and Dirnböck et al. (2003).

Sampling

Animals were sampled manually in an area of approximately 100 – 300 m^2. In general, two to five collectors screened the area for the occurrence of snails for 15 – 30 min. A limited number of these snails were collected, other taxa were either noted or reference specimens taken. Elevation, inclination, and coordinates were determined by GPS (Geo_WGS_84). A coarse analysis of the habitat was performed by a presence / absence list of habitat structures and vegetation types, which were noted in a case report form developed for alpine land snails in general.

Results

The results are summarized in Table 1. Three vegetation types were found on sites with occurrence of the investigated species, i.e. free of vegetation, alpine grassland, *Pinus mugo* shrubbery. Both taxa were constantly found on alpine grassland (*T. oreinos*: 8 out of 8; *C. obtusus* 24 out of 24); *Pinus mugo* shrubbery was only found at the edge of the investigation sites. Of the landscape structures, stony or rocky formations were most frequent, such as single stones (*T. oreinos*: 4 out of 8; *C. obtusus* 10 out of 24), boulders (*T. oreinos*: 4 out of 8; *C. obtusus* 13 out of 24) and rocks (*T. oreinos*: 3 out of 8; *C. obtusus* 13 out of 24). The frequency data of the coarse vegetation types and landscape structures showed no striking difference between the two taxa. All points with findings of one of the two species were at primarily non-forested sites above the lowest limit of the lower subalpine ecotone (1 300 m) as defined by Kilian et al. (1993). No records of either species was found on man-made subalpine and alpine pastures. Four natural non-forested communities of plants were recorded as characteristic for at least one of the species concerned. 1) The *Caricetum firmae* is situated on exposed, carbonate sites, which are under heavy mechanical pressure and are often blown free of snow during winter season. 2) The *Seslerio-Caricetum sempervirentis* grows on former boulder areas and is characterized by loose, stony ground (Grabherr & Mucina 1993). 3) The complex of *Firmetum*, *Seslerio-Sempervivetum* and *Festuca pumila – Agrostis alpina* meadow (Figure 3) is a result of heavily structured relief and comprises elements of several communities of plants. 4) Alpine boulders and scree societies, as defined by Greimler & Dirnböck (1996), include areas with poor or no vegetation (see Figure 1) influenced by extreme mechanical pressure.

Trochulus oreinos was mostly found on alpine boulders and scree societies (5 out of 8 sample sites, most of all rock areas and boulders, but also on loose *Caricetum-firmae* (3 out of 8). Only one sample site was situated near *Seslerio-Caricetum sempervirentis* (see also Table 1). All sample sites were situated on natural forest-free parts of the mountain ranges, at altitudes from 1 562 to 2 179 m. Remarkably, half of these sites were definitely below the upper limit of the krummholz ecotone (about 1 900 m) on natural shrub- and forest-free vegetation (boulders, rocks).

Cylindrus obtusus in general was found more frequently than *T. oreinos* and the findings were situated on all plant communities mentioned above (Table 1). Elevations of sites ranged from 1 393 to 2 179 m. As with *T. oreinos*, about half of the sites were definitely located below the natural timberline, the others approximately at this limit or within the alpine ecotone.

Discussion

In summary, the basic questions formulated in the introduction can be answered as follows: 1) *C. obtusus* and *T. oreinos* prefer rocky habitats from the subalpine ecotone upwards. While *T. oreinos* is restricted to *Caricetum firmae* meadow and alpine boulder and scree societies, *C. obtusus* can inhabit several plant communities. 2) Both species are restricted to primarily forest-free sites. 3) Therefore they are not affected by the abandonment of man-made meadows, the most significant recent change within alpine agriculture. 4) As both


Figure 4 – Trochulus oreinos oreinos *(left) and* Cylindrus obtusus *(right). © Michael Duda and Helmut Sattmann respectively*

taxa are restricted to the subalpine and alpine ecotone, at least populations at the lower altitudinal distribution are potentially affected by climatic changes. Both taxa have few opportunities to enlarge their potential habitat upwards as they have already reached sites near the highest summit areas of the investigated mountain massifs. Below we discuss aspects of East-Alpine endemism on the basis of the ecology of *T. oreinos* and *C. obtusus.*

Vertical distribution and climatic impacts

Trochulus oreinos and *Cylindrus obtusus* are restricted to naturally non-forested sites, often also situated below the timberline in the subalpine ecotone. This is highlighted by the findings at Schneealpe and Rax, where both taxa occur at the lower limit of their altitudinal and climatic distribution. Therefore, both species seem neither to be primarily affected by the end of grazing and the intrusion of shrubbery, as it is evident in many subalpine and alpine plants (Dirnböck et al. 2003), nor by an upward shift of the tree line. Also excessive land use (e.g. winter tourism) does not seem to be a major threat as the steep and rocky slopes where *C. obtusus* and *T. oreinos* live are not of major interest for human activities. The main potential threat for both taxa can be seen in the rapid warming of the climate. Although no extensive studies on climatic preferences exist for either species, their restriction to a cooler climate can be inferred from their high altitudinal distribution (Klemm 1974; Duda et al. 2010). This fact is also in accordance with the only published ecophysiological data of Kühnelt (1937), who stated that *C. obtusus* is not sensitive to low humidity, but vulnerable to high temperatures. Therefore, the lower edge of the subalpine ecotone seems to be a natural lower limit for both taxa. It thus seems like they are limited by climatic change rather than by changes in the habitat structure through reforestation.

As pointed out by Dirnböck et al. (2003) and Ohlemüller et al. (2008), the altitudinal distribution and the smaller number of colonized habitats of endemics with a limited distribution make these species especially vulnerable to climate change. Essl et al. (2009) state that although currently the endemics of high-mountain areas in Austria are not threatened by extinction, this might change drastically in the future, as they have only few possibilities for moving upwards with incipient warming. This is very evident in our currently investigated area: some sites where both snail taxa occur are obviously already at the lower climatic limit of these species. This is corroborated by the fact that some populations of *C. obtusus* on lower sites in the Gesäuse area, located in northern Styria about 100 m west of Schneeberg and Rax, which Klemm listed in 1974, are now extinct (Sattmann et al. 2000). The current investigation also shows that *C. obtusus* cannot enlarge its potential habitat upwards as its highest records in the currently documented area are already near the highest summits on Schneeberg, Schneealpe and Hochschwab (see also Table 1). Also the highest record on Rax given by Klemm (1974) is directly on its highest peak. *T. oreinos* reaches the highest summit area only on Hochschwab, but, in addition to its potential climatic restriction, it is also limited by its habitat requirements (see below "Ecological niche"). Other populations of *C. obtusus* in the western High Alps of Austria (e.g. on the Glocknergruppe at the border of Salzburg, Carinthia and Tyrol) have the potential to move upwards while, within *T. oreinos*, only populations of the western subspecies *T. o. scheerpeltzi* in the Totes Gebirge (Upper Austria) have the possibility to enlarge their habitat to higher elevations.

The suboptimal climatic conditions caused by the influence of the Pannonian climate combined with low elevation on Schneeberg and Rax are perhaps reflected in some morphological features detected in *C. obtusus*: Adensamer (1937) stated that the average shell size on these easternmost populations tends to be smaller than in western populations, a result confirmed by Edlinger (1999). Shileyko et al. (1997) pointed out that *C. obtusus* has irregular and reduced mucus glands on Schneeberg and Rax, while those from the Gesäuse area showed mucus glands of even length.

Ecological niche

The results show that both taxa clearly prefer sites with loose vegetation structure. Nevertheless, the community of plants itself is no prerequisite for

their occurrence but rather vegetation stands in combination with specific abiotic factors, which enable their existence.

Several authors mention that *C. obtusus* prefers "alpine black earth" and tries to avoid "red loam" (Adensamer 1937; Kühnelt 1937; Freitag & Desch 1996). The term "black earth" refers to rendzic leptosol, which is the typical soil for the *Caricetum firmae*, while "red loam" describes humic cambisol, which represents a relict soil in the investigated area. A first clue to the habitat needs lies within the structure of these soil types. Rendzic leptosols do not provide high water retention (Dirnböck & Grabherr 2000), but their loose structure enables easy digging for snails. Freitag & Desch (1996) interpreted digging by *C. obtusus* as a survival strategy during adverse climatic events (e.g. drought) besides hiding under stones and in mouse holes. Humic cambisol exhibits high water retention (Dirnböck & Grabherr 2000) but offers poor digging opportunities because of its dense structure and is therefore not suitable as habitat for *C. obtusus*. The number of findings rises above 1 600 m, only two of our records of *C. obtusus* are situated below this line. This observation is similar to that of Freitag (1990), although it has to be mentioned that this author also reported findings as low as 1 100 m. Yet some of those records were questioned by Frank (2006) as they could be a result of displacement of the empty shells by water or erosion.

Trochulus oreinos seems to prefer even drier habitats than *C. obtusus*. In comparison to related forms like *T. hispidus* and *T. striolatus*, *T. oreinos* differs even more significantly as it does not prefer damp habitats (Proćków 2009). Only a few authors, like Falkner (1970) and Reischütz & Reischütz (2009), mention aspects of habitat choice. Another hairy snail, *T. biconicus* (Eder 1917), an endemic of Switzerland, shows striking parallels to *T. oreinos*, as it is restricted to the communities of plants composed of *Seslerio-Caricetum sempervirentis*, *Caricetum firmae* and alpine boulders in areas of Central Switzerland, which were potentially ice-free during Würm glaciation (Kerney et al. 1983; Baggenstos 2010). Within the investigated area, *T. oreinos* is not only restricted by climatic but also landscape-ecological factors. Only the Hochschwab massif, with its large areas of sparse vegetation on rocky ground, offers large suitable habitats for *T. oreinos*. In contrast, this species has very few opportunities to settle potential new habitats on Schneealpe, Rax and Schneeberg in case of climate warming.

Patterns of endemism

According to Ohlemüller et al. (2008), areas with high climatic gradients such as mountain ranges provide long-term stability for special ecotones. Additionally, the authors mention the occurrence of rare climates as factor, which enables the radiation of endemics. In our example, this matches perfectly the distribution of *T. oreinos* and *C. obtusus*. Both dwell in ecotones which are rare amongst the Central European landscapes because of their special abiotic factors, such as high elevation, steepness and extreme mechanical and climatic influence. The fact that some of these azonal occurrences often span wide ranges of elevations (e. g. boulders) provides the snails with the opportunity to move upwards or downwards in case of climate change. This fits well with the "climatic buffer hypothesis" of Ohlemüller et al. (2008). During the last glaciation, subalpine and alpine ecotones were situated approximately 1 000 m lower than today (Rabitsch & Essl 2009). Moreover, large parts of the north-eastern Alps were not glaciated (Van Husen 1997). As shown by Rabitsch & Essl (2009), most Austrian endemics occur in high-alpine boulders and grassland. Some vascular plants like *Noccea krantzii* and *Papaver alpinum subalpinum* show similar distribution patterns and habitat preferences like *T. oreinos* and *C. obtusus*. (Schönswetter et al. 2005; Rabitsch & Essl 2009). Therefore it seems plausible that *T. oreinos* and *C. obtusus* survived the ice ages at the northern edge of the Calcareous Alps. Accordingly, both snail taxa would represent remains of the pre-Quarterian alpine fauna, as has already been suggested by Adensamer (1937) for *C. obtusus*.

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Morphological and ecological investigations of the highly divergent mitochondrial clades within the *Trochulus hispidus/sericeus* species complex and related taxa (Gastropoda: Pulmonata: Hygromiidae)

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Keywords: Mollusca, Gastropoda, *Hygromiidae*, *Trochulus*, morphological diversity

Abstract

Introduction:

This study investigated selected representatives of the taxonomically ambiguous genus *Trochulus* in Austria and adjacent areas in respect to morphology and ecology. The main focus was on the *T. hispidus*/*sericeus* complex, which was represented by several genetically differentiated clades as determined in a parallel molecular genetics study. We also more closely examined the related species *T. oreinos* and *T. striolatus*.

Results:

There was no clear morphological differentiation among the genetic clades of the *T. hispidus/sericeus* complex, and the populations could not be distinguished based on their morphology, irrespective of their genetic affiliation. *T. sericeus* was differentiated neither in the morphological nor in the genetic analyses. No characters were detected in the genital anatomy that enabled discriminating any of the *T. hispidus/sericeus* clades or that were diagnostic for all of them. Within *T. striolatus* there was a slight morphological differentiation between the subspecies *T. s. striolatus*, *T. s. juvavensis* and *T. s. danubialis*. The two subspecies of *T. oreinos* could be discriminated by a small but consistent difference in the cross-section of the penis. Finally, certain morphological differences corresponded with ecological factors (habitat, altitude). Concerning interspecific delimitation, *T. hispidus/sericeus*, *T. striolatus* and *T. oreinos* were clearly differentiated by shell morphological and anatomical characters, e.g., shell structure and details of the penis. Shell dimensions of the *T. hispidus* clades were strongly correlated with altitude at least in the Austrian Alps. *T. oreinos* clearly inhabited different habitats than the other two species.

Conclusion: Morphological and ecological parameters failed to answer the question if the differing genetic clades of *T. hispidus*, respectively of *T. oreinos*, represented distinct species or not. This highlights the limitations of classical morphological investigations. For defining hybridisation barriers in sibling land snail species, details of the reproduction biology are indispensable. Moreover, the geographic distribution of genetic haplotypes is relevant for conservation because the habitat of some clades within the *T. hispidus*/*sericeus* complex is under pressure.

Background

The classification of species and subspecies in Central European terrestrial gastropods is still disputed in many cases. One reason is that reliable morphological characters differentiating the taxa are scarce. Moreover, varying species concepts have led to contradictory taxonomic classifications, which in some cases were also influenced by conservation aspects. Some authors (Falkner 1991; Reischütz 1999) suggested "moderate splitting" by describing morphologically slightly deviating forms as subspecies. This is a potentially useful argument to protect local populations threatened by habitat destruction. The introduction of molecular genetic methods in biological systematics has often failed to solve taxonomic problems. This approach has, however, revealed more complex patterns, e.g. by displaying hitherto unnoticed genetic variation and differentiation of mitochondrial (mt) clades.

One example is the genus *Trochulus* Chemnitz 1786. It has frequently been in the focus of taxonomic questions, which were addressed using morphological (Forcart 1965; Shileyko 1978; Falkner 1995; Gittenberger et al. 1970; Proćków 2009) and genetic data (Pfenninger et al. 2005; Depraz et al. 2009; Kruckenhauser et al., in prep.). The species with the widest distribution within the genus is *Trochulus hispidus* (Linnaeus 1758). It prefers moist habitats from the northern ends of Mediterranean Peninsulas (Iberian, Apennine, Balkans) northwards up to Scandinavia and eastwards to the Urals (Ložek 1956). Reports from Sardinia were likely based on confusions with *Ichnusotricha berninii* (Guisti & Manganelli 1987). Based on its high shell variability, several attempts have been undertaken to divide *T. hispidus* into different species/subspecies (Forcart 1965; Shileyko 1978). These, however, have been criticised and are not commonly accepted (Gittenberger 1970; Naggs 1985; Proćków 2009). Additionally, some conchologically similar species, particularly *Trochulus plebeius*, *Trochulus sericeus* and *Trochulus coeleomphola*, were considered as valid species by some authors (e.g. Falkner et al. 2000), while other authors suggested merging at least some of them with *T. hispidus* (e.g. Procków 2009). Finally, based on molecular analyses, some authors suggested splitting *T. hispidus* into several cryptic species (Pfenninger et al. 2005; Depraz et al. 2009). Due to the complicated taxonomic situation and the ambiguous differentiation of *T. hispidus* and *T. sericeus*, Depráz et al. (2009) suggested to subsume those taxa under the term *Trochulus hispidus/sericeus* complex, a view which we also follow in the present paper.

Beside *T. hispidus*, several related species occur in Austria and the surrounding countries, among them *Trochulus oreinos* (A. J. Wagner, 1915), *Trochulus striolatus* (C. Pfeiffer, 1828), *T. coelomphala* (Loccard, 1888), *Trochulus clandestinus* (Hartmann, 1821), *Trochulus villosus* (Draparnaud, 1805), *Trochulus villosulus* (Roßmässler, 1838) and *Trochulus biconicus* (Eder 1917).

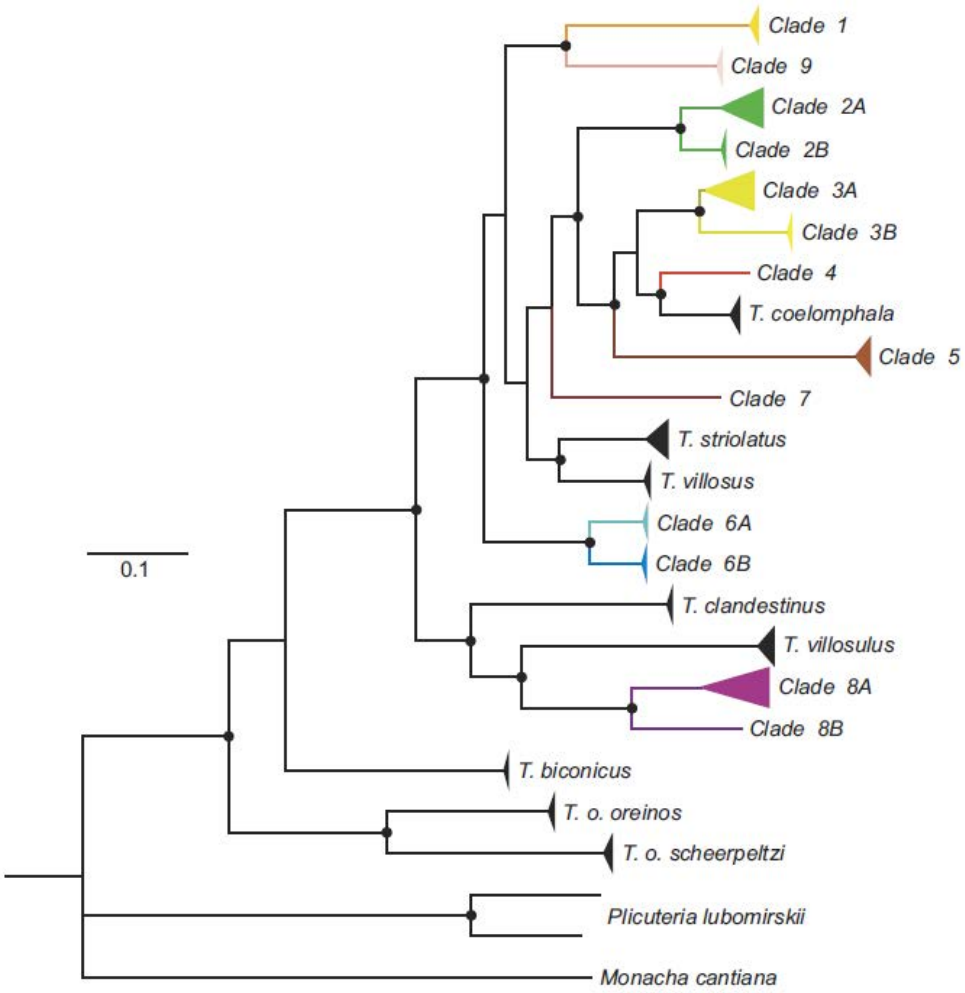

Figure 1. Schematic tree based on partial sequences of COI, 16S rRNA and 12S rRNA genes of *Trochulus* sp. and related taxa. (Kruckenhauser et al. in prep.). Clade 1-9: different genetic clades of the *T. hispidus/sericeus* complex.

The genetic analysis of Austrian populations of the *T. hispidus/sericeus* complex (Kruckenhauser et al., in prep.) revealed 17 mt clades separated by remarkably high distances (Fig. 1). Among these clades, eight represented morphologically more or less well-defined species/subspecies. Only *T. biconicus* and *T. oreinos* were clearly separated in the tree. The

other six clades were interspersed among nine clades containing individuals of "typical" *T. hispidus* appearance (flattened shell with wide umbilicus), as well as specimens with a more globular shell and narrow umbilicus. The latter appearance conforms to descriptions of the problematic taxon *T. sericeus*. Thus, both *T. hispidus* and *T. sericeus* were paraphyletic according to the tree and their assignment to specific clades remained ambiguous. Accordingly, we designated these nine clades as *T. hispidus/sericeus* complex.

These complicated relationships raised the question as to the status of *T. hispidus* and whether the clades or at least some of them might represent distinct species. To determine whether snails belonging to distinct mt clades were distinguishable by morphometric traits not visible by cursory inspection requires a comprehensive morphological investigation. This was the central aim of the present study, which was combined with analyses of habitat preferences, including the evaluation of the influence of ecological factors on the habitus of individuals.

Two of the related species, *T. oreinos* and *T. striolatus*, were available in sufficient numbers to be included in the morphological and ecological analyses. *T. oreinos*, an Austrian endemic of the northern calcareous Alps, is characterized by small flat shell and tiny curved hairs. It was originally considered to be a local subspecies of *T. hispidus* (A. J. Wagner, 1915), but was later separated as an independent species (Falkner 1982, 1995). The latter view was reconfirmed by genetic and morphological data (Duda et al. 2011; Kruckenhauser et al., in prep.) as well as ecological data (Duda et al. 2010). *T. oreinos* comprises two geographically separated subspecies, *T. o. oreinos* (Wagner, 1915) and *T. o. scheerpeltzi* (Mikula, 1954), which overlap in shell morphology but are genetically distinct (for details see Duda et al. 2011 and Kruckenhauser et al., in prep.).

T. striolatus has the second-widest distribution within the genus. It occurs from Ireland and Great Britain across France and Germany to Austria and along the Danube River in southern Slovakia and northern Hungary (Kerney et al. 1983; Proćków 2009). Its shell is characterized mainly by larger, stronger striation and a blunt keel at the last whorl (Kerney et al. 1983; Falkner 1989). *T. striolatus* comprises five subspecies that have been described based on small differences in shell and genital morphology: *T. s. striolatus* (Pfeiffer, 1828), *T. s. danubialis* (Clessin, 1874), *T. s. juvavensis* (Geyer, 1914), *T. s. austriacus* (Mahler, 1952) and *T. s. abludens* (Loccard, 1888).

The morphological and anatomical investigations presented here include populations representing the *T. hispidus/sericeus* complex as well as *T. oreinos* and *T. striolatus* (sample localities in Fig. 2). The following questions were addressed: (1) Are the clades of the *T.*

hispidus/*sericeus* complex differentiated with respect to shell morphology? (2) Are there any populations that are differentiated in shell morphology irrespective of their genetic affiliation? For example, is there any morphologically differentiated group that can be ascribed to *T. sericeus*? (3) Is there any difference in the genital anatomy that characterizes any of the clades within the *T. hispidus*/*sericeus* complex or that is diagnostic for all of them (compared to the close relatives) or that clearly separates *T. hispidus* from *T. sericeus*? (4) What are the morphological and anatomical characters differentiating the three species *T. hispidus*, *T. striolatus* and *T. oreinos* and their subspecies? (5) Are there any morphological differences that can be explained by ecological factors (e.g., habitat, altitude)?

In a final step, we discuss habitats and their history to consider the differentiation of mt clades of the various taxa (*T. hispidus*, *T. oreinos, T. striolatus*) regarding ecological and biogeographic aspects.

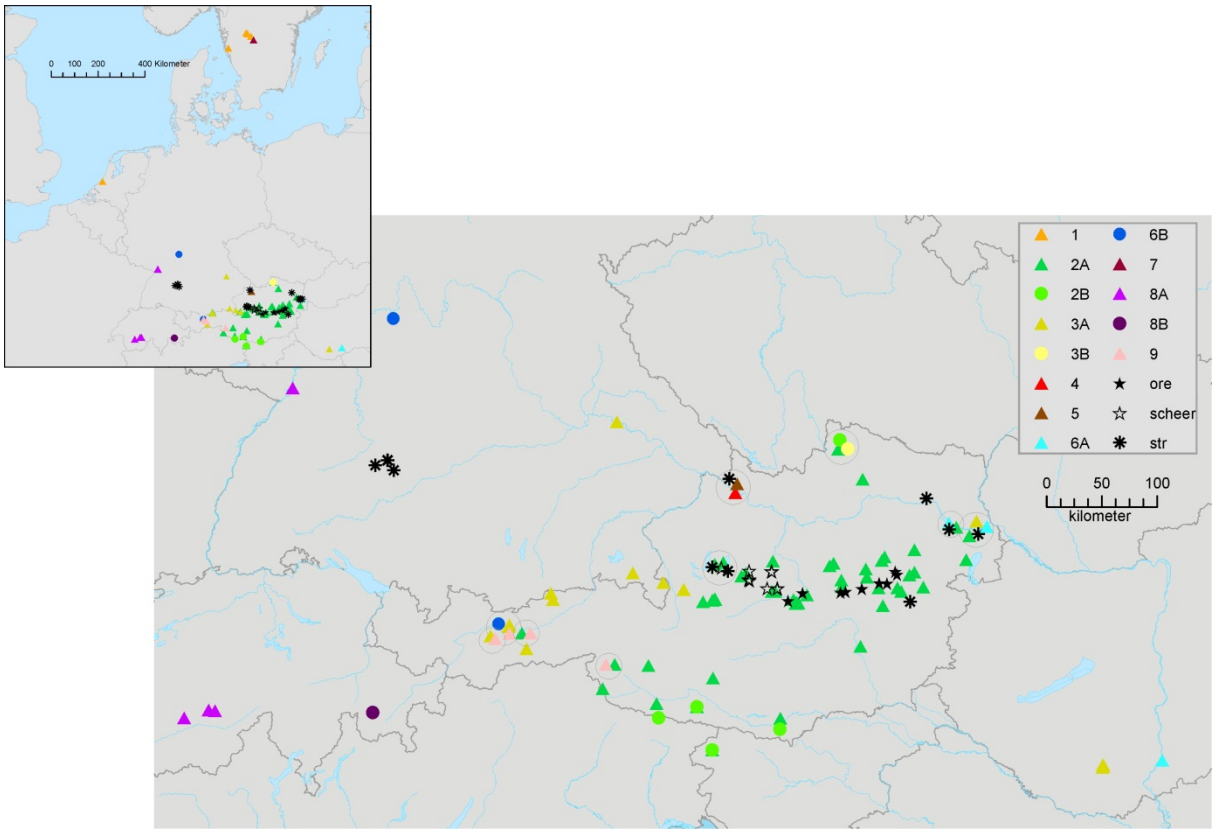

Figure 2. Distribution of investigated clades and taxa in Europe and Austria. 1-9: different genetic clades of the *T. hispidus/sericeus* complex; ore: *T. o. oreinos*; scheer: *T. o. scheerpeltzi*; str: *T. striolatus* ssp.

Overall, these analyses should explore the general possibilities and limitations of classical morphological analyses in snails. Furthermore, the combined genetic and morphological results should help to clarify the unresolved systematic issues. Finally, we discuss conservation aspects of populations belonging to different mt clades of the *T. hispidus/sericeus* complex in connection with landscape development. The number of sample sites, specimens and methodological approaches are summarized in table 1A and B.

Table 1A: Sample sites of the *T. hispidus/sericeus* complex. **Bold:** sample sites present in more than one mt clade, SNr: sample number, Alt = altitude (meters above sea level), H: habitat analysis (0/1 = no/yes), G: number of specimens investigated genetically, M: number of specimens included in analysis of shell morphology, A: number of specimens included in analysis of genital anatomy, EM: number of specimens included in the Mantel test

Country	Locality	spID	Alt	Clade	H	G	M	A	EM
The Netherlands	Leiden, Valkenburgske_Meer	418	-23	1	1	3	3	3	0
Sweden	Västra Götalands Iän, Kvänum	451	85	1	0	2	2	1	0
Sweden	Göteborg, Botanical garden	452	15	1	0	2	1	1	0
Sweden	Västra Götalands Iän, Falköping	454	217	1	0	1	1	1	0
Sweden	Västra Götalands Iän, Norra Vånga	455	110	1	0	2	2	2	0
Austria	**Donauauen, Orth, Altarm**	3	145	2a	0	4	4	0	4
Austria	Semmering, Maria Schutz	5	871	2a	1	3	2	0	2
Austria	Johnsbachtal, Langriesmündung	24	652	2a	1	3	2	0	2
Austria	Johnsbachtal, Kneippstation	32	865	2a	1	3	2	0	2
Austria	**Donauauen, Regelsbrunner Arm**	33	147	2a	1	3	3	1	3
Austria	Hochlecken, Taferlklause	42	778	2a	0	2	1	0	1
Austria	Würflach, Johannesbachklamm	50	445	2a	1	3	3	0	3
Austria	Breitenstein, Adlitzgraben	52	650	2a	1	2	2	0	2
Austria	**Sattnitz, Mieger**	60	408	2a	1	3	0	0	0
Austria	Gailtaler Alpen, Kreuzen	64	985	2a	1	6	2	1	2
Austria	Gurktaler Alpen	66	950	2a	0	4	2	0	2
Austria	**Achensee, Achenbachtal**	93	843	2a	1	2	0	0	0
Austria	Hallstatt, Salzberg	102	942	2a	1	3	3	0	3
Austria	Dürrenstein, Lechnergraben	104	604	2a	1	3	3	0	3
Austria	Dürradmer, Kräuterin	130	1100	2a	1	3	3	0	3
Austria	Grazer Bergland, Semriach	140	503	2a	1	10	10	1	10
Austria	Johnsbachtal, Kölblwirt	144	868	2a	1	3	3	0	3
Austria	Johnsbachtal, Wasserfallmauer	145	978	2a	1	3	3	0	3
Austria	Hallstatt, Waldbachstrub	157	806	2a	0	4	4	0	4
Austria	Hallstatt, sports field	158	524	2a	1	3	2	0	2
Austria	**Gmünd, Kurzschwarza**	159	551	2a	1	8	8	2	8
Austria	Hallstatt, Klausalm	160	796	2a	1	3	2	0	2
Austria	Pittental, Schlattenbach	167	397	2a	1	3	2	0	2
Austria	Sierningtal, Stixenstein	168	470	2a	1	3	3	0	3
Austria	Innervillgraten, Kalkstein	204	1620	2a	1	4	3	0	3
Austria	Gailtaler Alpen, Laas	205	920	2a	0	3	3	0	3
Austria	**Defereggen Gebirge, Obermauern**	207	1320	2a	0	1	0	0	0
Austria	Fischbacher Alpen, Hauereck	208	1187	2a	0	2	1	0	1
Austria	Seewaldtal, stream	215	1090	2a	1	1	1	0	1
Slovenia	**Soča valley, Soča**	223	435	2a	1	2	2	0	2

Country	Locality	SNr	Alt	Clade	H	G	M	A	EM
Austria	Donauinsel, Neue Donau	231	165	2a	1	3	2	0	2
Austria	Warscheneck, Wurzeralmbahn	237	810	2a	1	1	1	1	1
Austria	Salzkammergut, Hochalm	285	663	2a	0	1	1	1	1
Austria	Neusiedler See, west shore	286	124	2a	0	2	2	0	2
Austria	Frein, Freinbach	306	869	2a	0	3	3	0	3
Austria	Göller, Gscheid	311	914	2a	1	3	3	0	3
Austria	Tiefental, Ochbauer	313	739	2a	1	3	3	1	3
Austria	Berndorf, Grabenweg	315	412	2a	1	3	3	0	3
Austria	Halbachtal, Rossbachklamm	317	649	2a	1	3	3	1	3
Austria	Salzatal, Weichselboden	318	660	2a	1	3	2	0	2
Austria	Großer Phyrgas, Arlingsattel	319	1425	2a	1	2	1	0	1
Austria	Johnsbachtal, Kölblalm	323	1076	2a	1	2	2	0	2
Austria	Hieflau, Schneckensafari	327	523	2a	1	3	3	0	3
Austria	Lunz, Seehof	341	610	2a	0	2	2	0	2
Austria	Gosau, Talstation_Zwieselbahn	361	924	2a	1	3	2	0	2
Austria	Almtal, Almsee	380	593	2a	1	3	3	0	3
Austria	Straneggbachtal, Vordere_Hetzau	385	668	2a	1	3	3	0	3
Austria	Steyerlingtal, Schattseite	386	485	2a	1	2	1	0	1
Austria	Großglockner, Jungfernsprung	446	1148	2a	1	3	3	0	3
Austria	Gföhl, Neubau	534	550	2a	1	1	1	0	1
Austria	**Gmünd, Langschwarza**	545	552	2a	1	1	1	0	1
Austria	**Neu Götzens , Lufens**	548	820	2a	1	5	5	1	5
Austria	Gailtaler Alpen, Kreuzen	64	985	2b	1	3	2	1	2
Italy	Plöckenpass, Tischlbong	200	837	2b	1	3	2	2	2
Slovenia	**Soča valley, Soča**	223	435	2b	1	1	1	1	1
Austria	**Gmünd, Kurzschwarza**	159	551	2b	1	2	2	2	2
Austria	Hochobirmassiv, Freibach	402	733	2b	1	3	3	3	3
Austria	**Donauauen, Regelsbrunner Arm**	33	147	3a	1	1	1	0	1
Austria	**Achensee, Achenbachtal**	93	843	3a	1	1	0	0	0
Austria	Seewaldtal, stream	215	1090	3a	1	2	2	0	2
Austria	Seewaldtal, Seewaldmoor	217	1048	3a	1	6	5	2	5
Hungary	Mecsek	288	182	3a	1	2	2	1	2
Hungary	Komló, Sikonda Cementry	291	195	3a	1	3	3	1	3
Austria	**Sauwald, Schlögen**	476	293	4	1	1	0	0	0
Austria	**Sauwald, Schlögen**	476	293	5	1	2	1	0	1
Austria	**Donauauen, Orth, Altarm**	3	145	6a	0	4	3	3	3
Austria	**Donauauen, Regelsbrunner Arm**	33	147	6a	1	3	3	2	3
Switzerland	Kandersteg, Lötschbergpass	561	2195	8b	0	2	2	2	2
Austria	**Defereggen Gebirge, Obermauern**	207	1320	9	1	1	0	0	0
Austria	**Neu Götzens , Lufens**	548	820	9	1	8	5	5	5
Austria	**Inntal, Hatting**	549	599	9	1	1	1	1	1
	Total number					253	212	68	203

Table 1B: Sample sites of the *T. oreinos* and *T. striolatus*. **Bold:** sample sites with syntopical occurrence of *T. hispidus/ sericeus* complex and *T. striolatus* ssp.. SNr: sample number, Alt = altitude (meters above sea level), H: habitat analysis (0/1 = no/yes), G: number of specimens investigated genetically, M: number of specimens included in analysis of shell morphology, A: number of specimens included in analysis of genital anatomy

Country	Locality	SNr	Alt	Species	Subspecies	H	G	M	A
Austria	Admonter Kalbling	55	2026	*T. oreinos*	*oreinos*	1	6	6	2
Austria	Rax, Bismarksteig	79	1787	*T. oreinos*	*oreinos*	1	6	1	1
Austria	Hochschwab, Schiestlhaus	134	2179	*T. oreinos*	*oreinos*	1	3	2	1
Austria	Hochschwab, Severinkogel	165	2010	*T. oreinos*	*oreinos*	1	1	0	0
Austria	Schneeberg, Fadenwände	172	1562	*T. oreinos*	*oreinos*	1	2	1	0
Austria	Schneeberg, Waxriegel	178	1873	*T. oreinos*	*oreinos*	1	3	3	1
Austria	Schneealpe, Schauerkogel	338	1664	*T. oreinos*	*oreinos*	1	3	3	2
Austria	Tamischbachturm	399	1940	*T. oreinos*	*oreinos*	1	3	1	1
Austria	Rax, Schlangenweg	448	1600	*T. oreinos*	*oreinos*	0	2	1	0
Austria	Hohe Veitsch	588	1979	*T. oreinos*	*oreinos*	1	3	3	2
Austria	Höllengebirge, Bledigupf	12	1677	*T. oreinos*	*scheerpeltzi*	1	1	1	1
Austria	Warscheneck, Toter Mann	132	2028	*T. oreinos*	*scheerpeltzi*	1	1	1	1
Austria	Hohe Nock, Hauptkar	351	1704	*T. oreinos*	*scheerpeltzi*	1	3	3	1
Austria	Hohe Nock, Haltersitz	367	1583	*T. oreinos*	*scheerpeltzi*	1	3	2	2
Austria	Hohe Nock, Feichtausee	369	1399	*T. oreinos*	*scheerpeltzi*	1	2	2	1
Austria	Großer Priel, Hinterer Ackergraben	382	1564	*T. oreinos*	*scheerpeltzi*	0	2	2	1
Austria	Großer Priel, Welser Hütte	383	1747	*T. oreinos*	*scheerpeltzi*	1	3	2	1
Austria	Großer Priel, Fleischbanksattel	387	2157	*T. oreinos*	*scheerpeltzi*	1	3	1	0
Austria	Großer Priel, Schlund	389	2284	*T. oreinos*	*scheerpeltzi*	1	3	2	1
Austria	Großer Phyrgas, Haller Mauern	443	1900	*T. oreinos*	*scheerpeltzi*	1	3	2	1
Austria	Großer Phyrgas, Westgrat	444	2000	*T. oreinos*	*scheerpeltzi*	1	3	2	1
Austria	**Donauauen, Orth, Altarm**	**3**	**145**	***T. striolatus***	***danubialis***	**0**	**1**	**0**	**0**
Austria	**Donauauen, Regelsbrunner Arm**	**33**	**147**	***T. striolatus***	***danubialis***	**1**	**3**	**0**	**0**
Austria	Wechsel, Mariensee	71	800	*T. striolatus*	*danubialis*	0	1	1	1
Austria	Stockerau, Donau Auen	142	176	*T. striolatus*	*danubialis*	1	2	1	0
Austria	Fischamend-Altarm	298	154	*T. striolatus*	*danubialis*	0	2	2	2
Austria	Sauwald-Engelhartszell	469	282	*T. striolatus*	*danubialis*	1	3	3	2
Austria	Höllengebirge, Aurach Ursprung	41	857	*T. striolatus*	*juvavensis*	0	2	0	0
Austria	**Höllengebirge, Taferlklause**	**42**	**778**	***T. striolatus***	***juvavensis***	**1**	**1**	**0**	**0**
Austria	Höllengebirge, Steinkogel	43	1531	*T. striolatus*	*juvavensis*	1	3	2	0
Austria	Pledialm, Feuerkogel	45	1444	*T. striolatus*	*juvavensis*	0	3	3	0
Austria	Hochlecken, Höllengebirge	122	1574	*T. striolatus*	*juvavensis*	1	6	3	2
Germany	Alb-Donau Kreis, Laichingen	249	750	*T. striolatus*	*striolatus*	0	2	2	0
Germany	Schwäbische Alb, Filsursprung	414	414	*T. striolatus*	*striolatus*	1	3	3	2
Germany	Schwäbische Alb, Wiesensteih	415	594	*T. striolatus*	*striolatus*	1	3	3	0
Germany	Schwäbische Alb, Grabenstetten	416	675	*T. striolatus*	*striolatus*	1	3	3	1
				Total Number		21	73	48	24

Results

Morphological analysis and species delimitation

Shell morphology

The individuals of the mt clades representing the *T. hispidus*/*sericeus* complex (Kruckenhauser et al., in prep.) were subjected to a morphometric analysis of shell characters to evaluate potential differences not noticed by visual inspection. First, we analysed individuals of the *T. hispidus*/*sericeus* complex, then the complete data set, including individuals of other taxa as well. Individuals of the *T. hispidus/sericeus* complex (specifically clades 2, 3, 6, and 9) showed very variable shell measurements (Table 2). Especially umbilicus width ranged broadly from 0.4-2.5 mm (standard deviation, SD: 0.41), except in clade 9, where this width tended to be smaller and more homogeneous (range: 0.7-1.3 mm, SD: 0.17). Furthermore, the clades showed a large overlap in shell measurements. This first impression was reconfirmed in the discriminant analysis (DA) comprising individuals of the *T. hispidus/sericeus* complex: no differentiation was found, either in the DA based on measurement values only (Figure 3.1) or in the combined DA (measurements plus qualitative traits, Figure 3.2). Representatives of all clades form mostly overlapping clouds in the biplot of the first two axes.

Representatives of clade 1 (northern Europe) and clade 8 (Baden-Württemberg/Switzerland) tended to be more globular and have a narrower umbilicus, while those from other clades showed a broad variability. It was clearly not possible to distinguish the mt *T. hispidus/sericeus* clades detected by Kruckenhauser et al. (in prep.) or the problematic taxon *T. sericeus* based on shell width, umbilicus width, height of last whorl and shell height in the investigated area. Especially the relation umbilicus width/shell width (a diagnostic trait in Kerney et al. 1983) was not a reliable predictor for *T. sericeus* because specimens with a low ratio of umbilicus width/shell width occur in four out of eight clades. The “predict” function of the program r (R development Core team, 2012) based on a linear model object, in which we tried to predict the clade affiliation of specimens, also led to a high number (about 40%) of misidentifications in both analyses (measurements alone as well as measurements combined with qualitative traits) in clades 1, 3, 5, 6, 8 and 9. Some clades were even not recognized in the “predict” function, namely clades 1, 5 and 9 for both datasets and, additionally, clade 6 for the combined dataset. The high recognition number of clade 2 (about

90 %) reflects the disproportionately high number of individuals from this clade compared with the other clades. To override this bias, we used trained models with a reduced dataset (R Development core team); but this also failed to clearly separate the clades. To illustrate the enormous morphological variation within and among clades of the *T. hispidus*/*sericeus* complex, photographs of representative shells were compiled in Fig. 4 and 5 together with representatives of *T. striolatus* and *T. oreinos* spp.

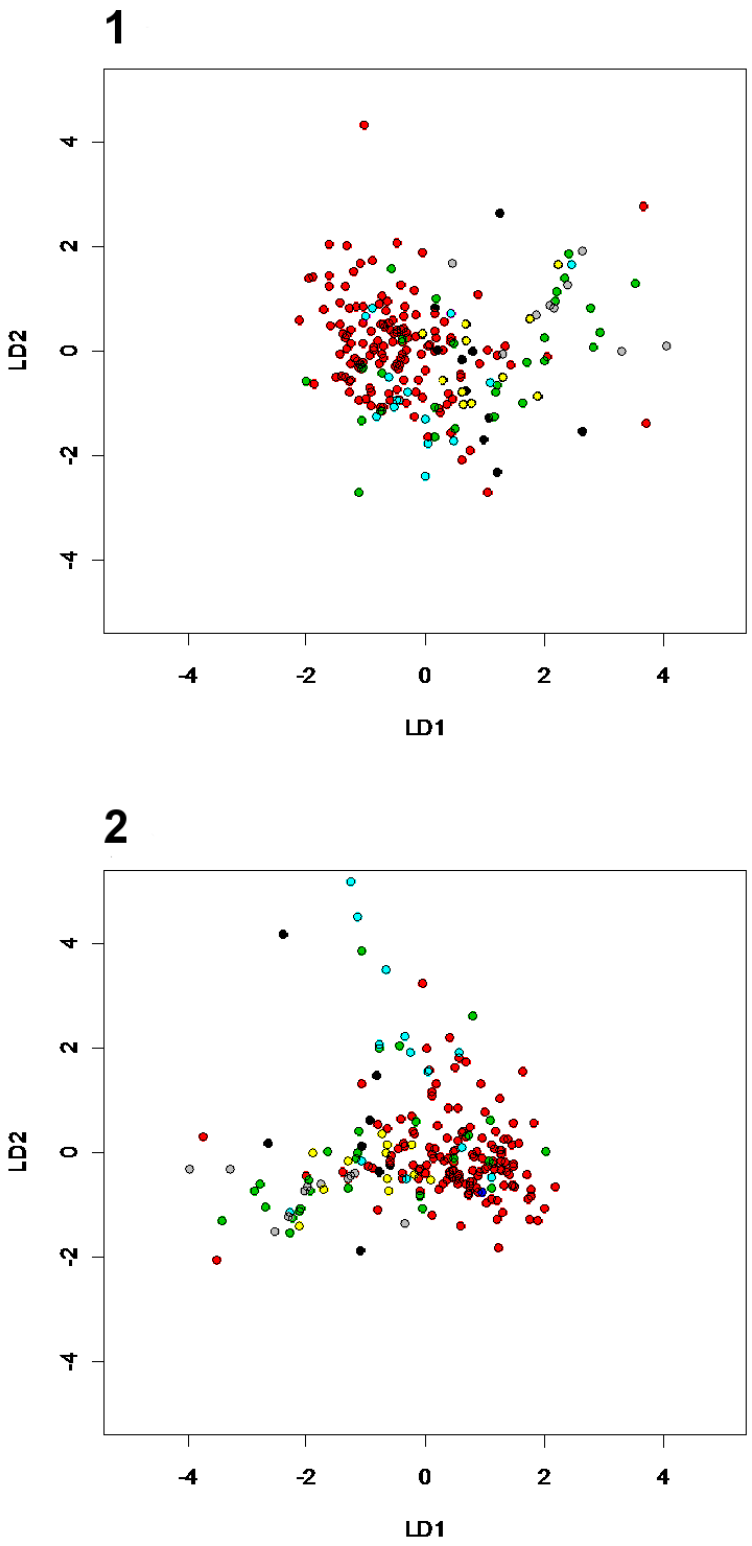

Figure 3. 1: Discriminant analysis of seven clades within the *T. hispidus/sericeus* complex based on measurements. Black: Clade 1, Red: Clade 2, Green: Clade 3, Dark blue: Clade 5, Light blue: Clade 6, Pink: Clade 8, Yellow: Clade 9; 2: Combined discriminant analysis of seven clades within the T. hispidus/sericeus complex based on measurements and the first three dimensions of a correspondence analysis concerning qualitative shell traits. Black: Clade 1, Red: Clade 2, Green: Clade 3, Dark blue: Clade 5, Light blue: Clade 6, Pink: Clade 8, Yellow: Clade 9

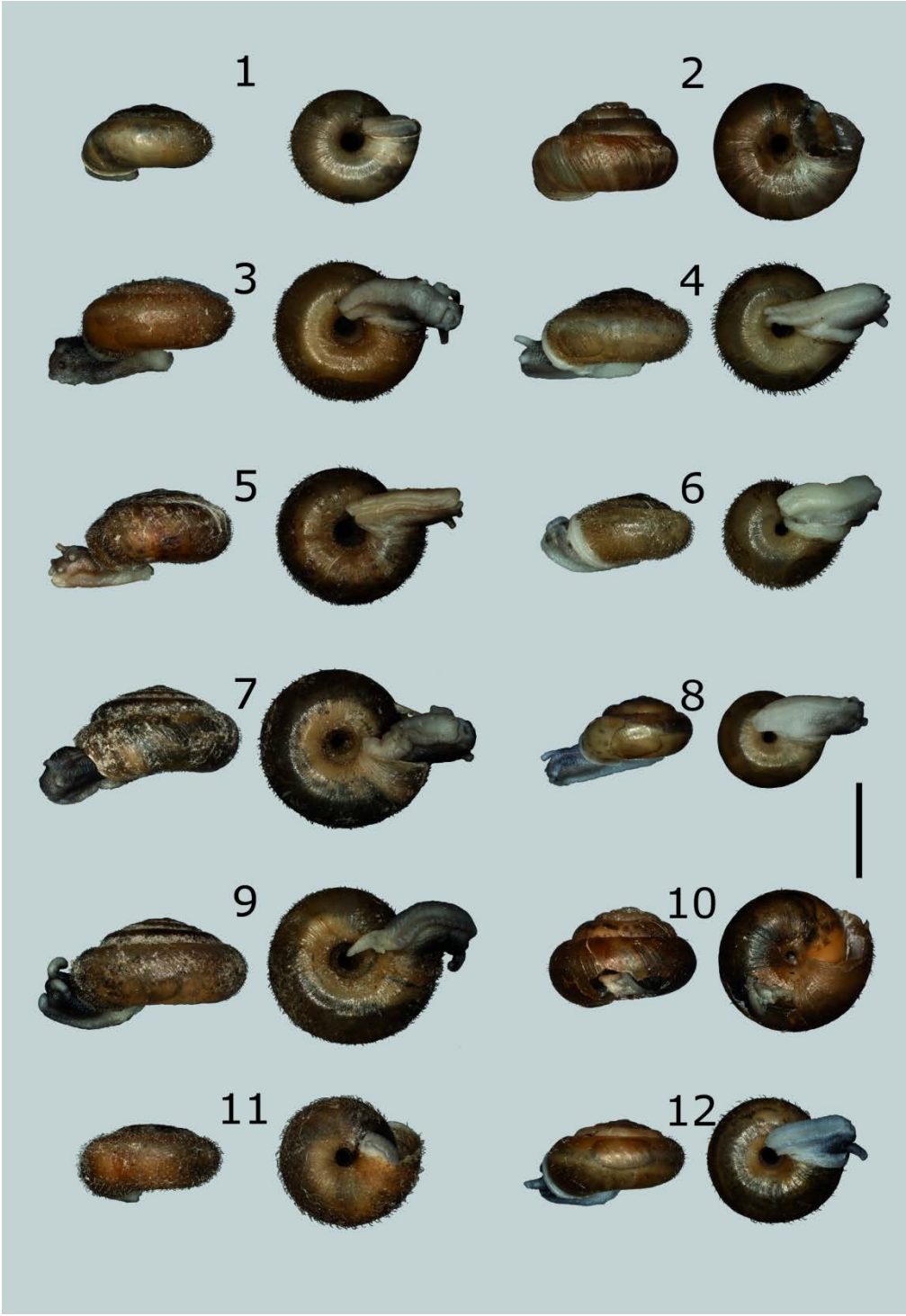

Figure 4. Selected representatives of the *T. hispidus*/*sericeus* complex, scale bar=5mm **1:** Clade 1, Ind. Nr. 4175: The Netherlands, Leiden: Sample Nr. 418; **2:** Clade 1, Ind. Nr. 4292 Sweden: Sample Nr. 454; **3:** Clade 2A, Ind. Nr. 3154 Austria: Sample Nr. 323; **4:** Clade 2A, Ind. Nr. 6236 Austria, Inntal: Sample Nr. 548; **5:** Clade 2B, Ind. Nr. 4155 Austria, Carinthia; **6:** Clade 3A, Ind. Nr. 4157: Germany, Sample Nr. 407; **7:** Clade 3B, Ind. Nr. 113 Austria, Donau-Auen: Sample Nr. 33; **8:** Clade 6A, Ind. Nr. 111, Austria, Donau-Auen: Sample Nr. 33; **9:** Clade 6B, Ind. Nr. 6234: Austria, Inntal: Sample Nr. 549; **10:** Clade 8A, Ind. Nr. 2079 Switzerland, Graubünden: Sample Nr. 284; **11:** Clade 8B, Ind. Nr. 6250 Switzerland, Lac de Tzeuzier: Sample Nr. 541; **12:** Clade 9, Ind. Nr. 6416: Austria, Inntal: Sample Nr. 650

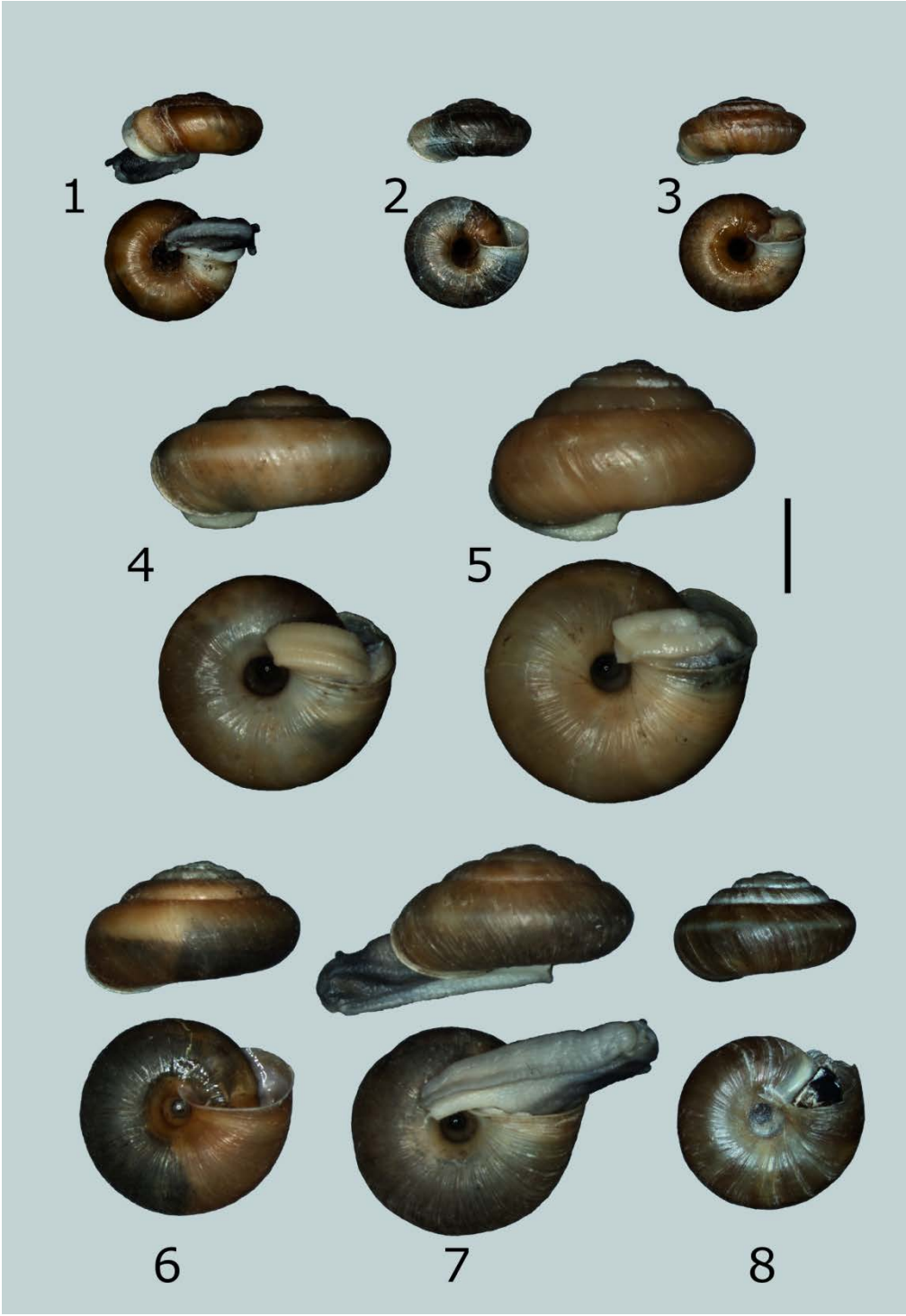

Figure 5. Selected representatives of *T. oreinos* ssp. and *T. striolatus* ssp, scale bar=5mm. **1:** *T. o. oreinos*, Ind. Nr. 6224: Typical shell phenotype. Austria, Hohe Veitsch : Sample Nr. 588; **2:** *T. o. oreinos*, Ind. Nr.1000: Intermediate shell phenotype *oreinos-scheerpeltzi*. Austria, Hochschwab: Sample Nr. 134 (Topotype); **3:** *T. o. scheerpeltzi*, Ind. Nr. 767: Typical shell phenotype. Austria, Höllengebirge, Sample Nr.12; **4:** *T. s. striolatus* , Ind. Nr. 4002 : Germany, Schwäbische Alb: Sample Nr. 414; **5:** *T. s. striolatus*, Ind. Nr. 4011 : Germany, Schwäbische Alb: Sample Nr. 415 ; **6:** *T. s. danubialis*, Ind. Nr. 687: Austria, Wechsel: Sample Nr. 71; **7:** *T. s. danubialis*, Ind. Nr. 2981 Austria, Fischamend: Sample Nr. 298; **8:** *T. s. juvavensis*, Ind. Nr. 718: Austria, Höllengebirge: Sample Nr. 122.

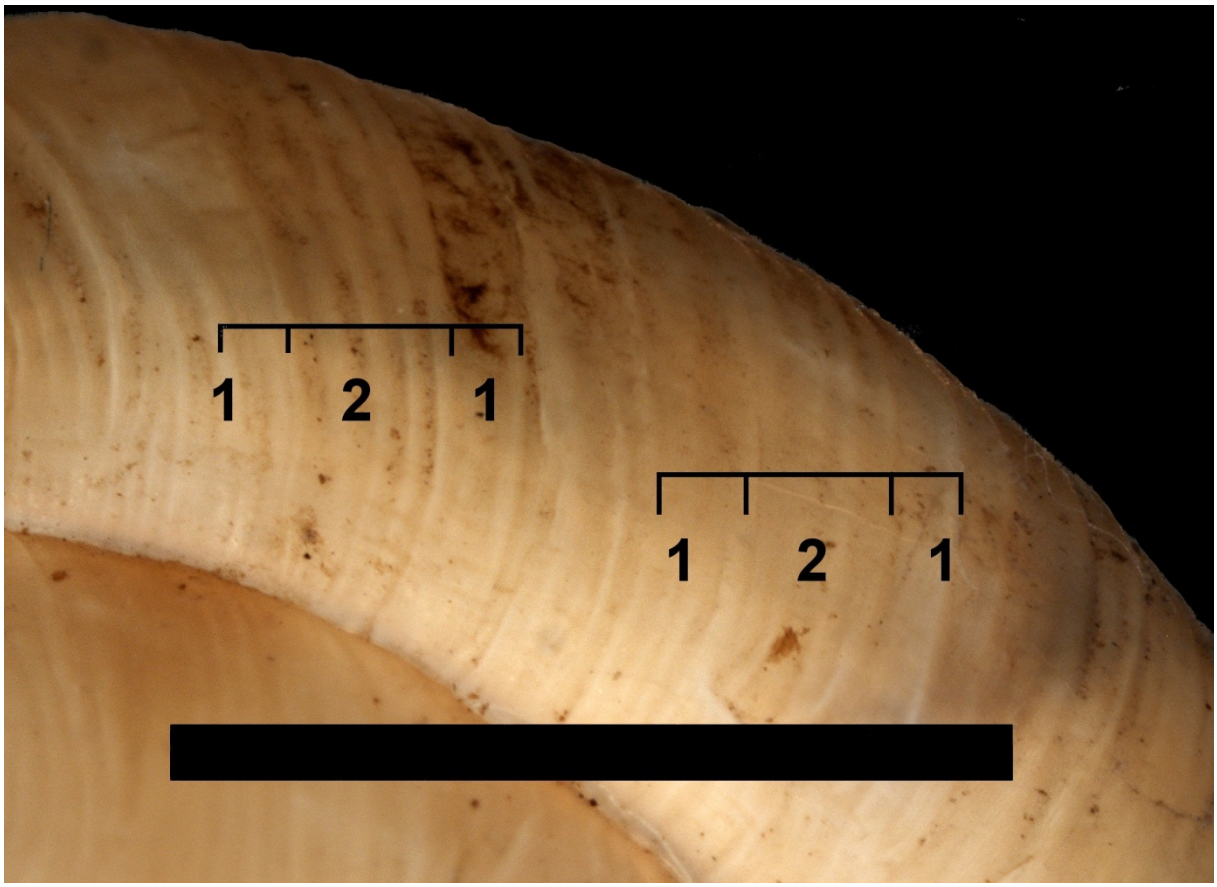

Figure 6. Characteristic riffle structures on the periostracum of *T. striolatus* ssp, shown at an individual of the nominate subspecies (Ind . Nr. 4043, sample site 416): coarse ribs (1) are followed by smooth ones (2). scale bar = 5 mm

The morphometric analysis including similar taxa (*T. striolatus* spp., *T. oreinos* spp.) revealed *T. striolatus* and *T. oreinos* ssp. as clearly distinct groups. This is already visible in the raw data: *T. oreinos* was on average smaller and tended to be less variable than the *T. hispidus/sericeus* complex, while *T. striolatus* tended to be bigger and slightly more variable than the other taxa. An exception was umbilicus width, which seemed to be more homogeneous in this taxon. The three groups were clearly differentiated even including only measurement values in the DA. The combined DA of measurements and the first three dimensions of qualitative characters led to an even better separation. The "predict" function (R development core team) applied to both datasets of interspecific comparison confirmed the clear separation of the three groups. There was only one outlier of *T. striolatus* predicted as a member of the *T. hispidus/sericeus* complex in the analysis based on measurements (Fig. 7.1). Another outlier of the *T. hispidus/sericeus* complex was predicted as *T. striolatus* (Fig. 7.2) in the combined analysis. For *T. striolatus*, the occurrence of "double riffles" and fields of coarse ribs followed by smooth ones (Fig. 6) appeared to be a discriminating trait separating it

from the *T. hispidus*/*sericeus* complex. Within *T. striolatus* there were only subtle shell morphological differences between the nominate form and the subspecies *T. s. danubialis* and *T. s. juvavensis*. The latter two, especially *T. s. juvavensis*, appeared to be smaller. Small sample size, however, precludes conclusive statements. Additionally, *T. s. juvavensis* showed stronger riffles on the shell surface than the other two subspecies, but this trait was apparently merely descriptive. More intense sampling would be necessary for final decisions about the validity of this subspecies.

Table 2: Summary of shell measurements (mm) of different *Trochulus* taxa and mt clades. Only clades/subspecies with more than 10 specimens are documented separately. T/C: taxon/clade, SD: standard deviation, SE: standard error of the mean, SW: shell width, WU: umbilicus width, SH: shell height, HW: height of last whorl

	SW	WU	SH	HW	SW	WU	SH	HW
T/C	*T. hispidus/sericeus*, all clades (n=213)				*T. hispidus/sericeus* clade 2 (n=140)			
Range	5.2-9.3	0.4-2.5	2.7-5.5	1.6-3.8	5.2-9.1	0.4-2.3	2.7-4.9	1.6-3.8
Mean	7.13	1.43	3.92	2.91	7.23	1.55	3.85	2.88
SD	0.88	0.41	0.50	0.34	0.76	0.30	0.44	0.34
SE	0.06	0.03	0.03	0.02	0.06	0.03	0.04	0.03
T/C	*T. hispidus/sericeus* clade 3 (n=28)				*T. hispidus/sericeus* clade 6 (n=13)			
Range	5.3-9.3	0.5-2.5	3.1-5.0	2.3-3.6	5.7-9	0.6-2	3.5-4.7	2.3-3.6
Mean	6.83	1.20	4.03	2.98	7.60	1.60	4.29	3.04
SD	1.21	0.59	0.57	0.35	1.01	0.40	0.52	0.36
SE	0.23	0.11	0.11	0.07	0.28	0.11	0.14	0.10
T/C	*T. hispidus/sericeus* clade 9 (n=12)				*T. oreinos,* both subspecies (n=42)			
Range	5.7-7.9	0.7-1.3	3.2-4.7	2.5-3.3	5.9-7.5	0.9-1.5	4.8-6.6	2.9-4.1
Mean	6.79	1.11	4.00	2.93	6.54	1.20	5.48	3.43
SD	0.59	0.17	0.41	0.20	0.41	0.13	0.49	0.32
SE	0.17	0.05	0.12	0.06	0.06	0.02	0.08	0.05
T/C	*T. o. oreinos* (n=21)				*T. o. scheerpeltzi* (n=21)			
Range	5.9-7.3	0.9-1.4	4.8-6.6	2.9-4.1	5.9-7.5	0.9-1.5	4.8-6.6	2.9-4.0
Mean	6.53	1.23	5.36	3.40	6.55	1.18	5.60	3.46
SD	0.43	0.12	0.42	0.35	0.39	0.14	0.53	0.28
SE	0.09	0.03	0.09	0.08	0.09	0.03	0.12	0.06
T/C	*T. striolatus,* three subspecies (n=26)				*T. s. striolatus* (n=11)			
Range	9.0-13.5	1.3-2.4	4.7-8.4	3.5-5.5	9.0-13.5	1.4-2.4	4.8-8.4	3.5-5.5
Mean	10.71	1.75	6.13	4.45	11.01	1.96	6.37	4.54
SD	1.20	0.36	0.85	0.51	1.54	0.42	1.11	0.63
SE	0.23	0.07	0.17	0.10	0.46	0.13	0.33	0.13

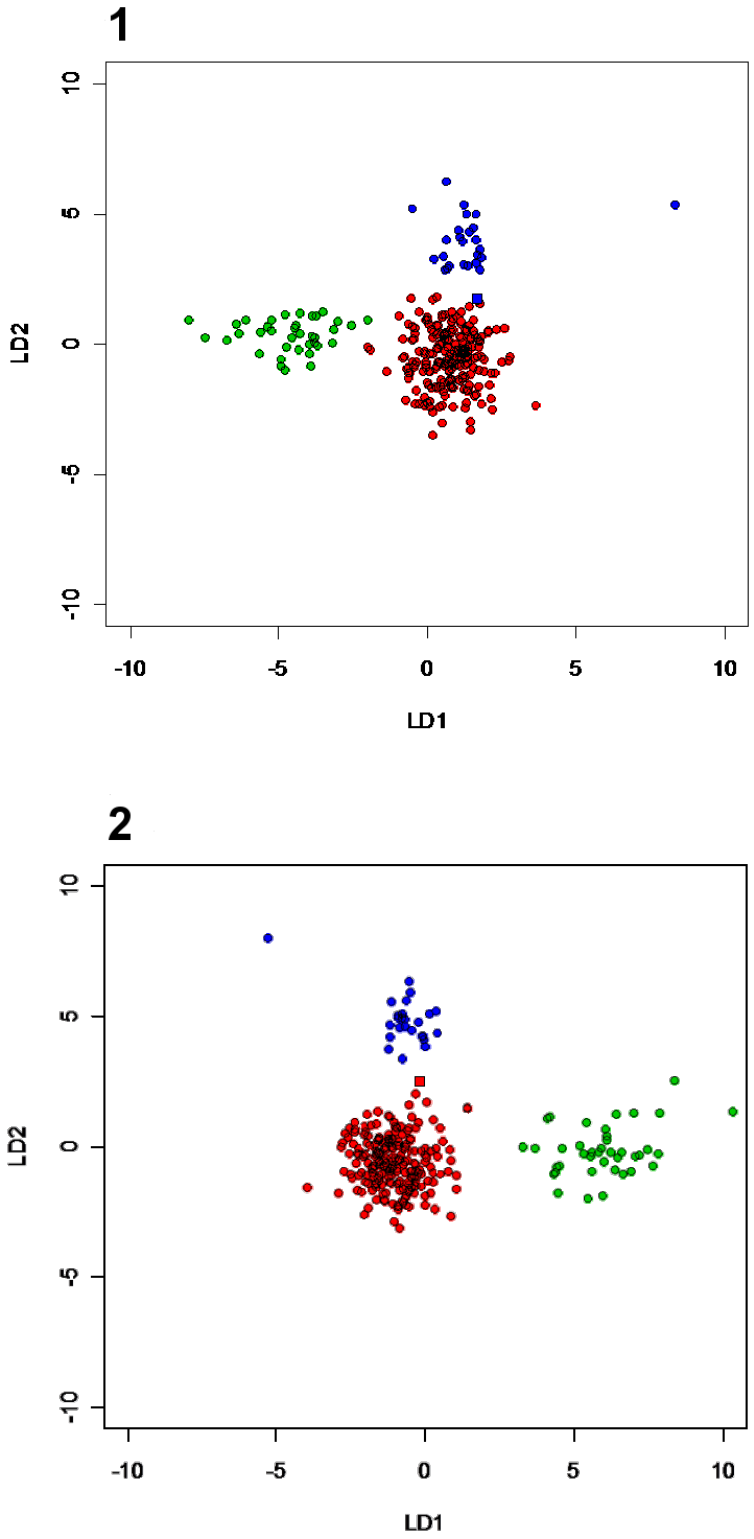

Figure 7. 1: Discriminant analysis of the three taxa/species groups based on measurements. Red: *T. hispidus/sericeus* complex; Blue: *T. striolatus* ssp; Green: *T. oreinos* ssp. Green square: Outlier of *T. striolatus* ssp., Blue square: Outlier of *T. striolatus* ssp. **2:** combined discriminant analysis of the three taxa/species groups based on measurements and the first three dimensions of a correspondence analysis concerning qualitative shell traits. Red: *T. hispidus*/*sericeus* complex; Blue: *T. striolatus* ssp.; Green: *T. oreinos* ssp. Red square: Outlier of *T. hispidus/sericeus* complex.

Anatomical analyses

In a next step, representatives of different clades and described taxa were investigated with respect to differences in genital anatomy. Among representatives of clades representing the *T. hispidus/sericeus* complex, no constant differences were found in the form of the bursa copulatrix, penis form and flagellum length: all those traits showing a high variability (some examples are shown in Figs. 8 and 9). Especially individuals showing the shell-morphotype of *T. sericeus* are not conspicuous in genital anatomy. Moreover, the consistent spherical (as long as broad) spermatheca – described as a typical trait for *T. sericeus* in Great Britain and mainland France by Anderson (2008) – could not be verified in our material. Moreover, the presence of three instead of four pairs of mucous glands, which was reported to be a discriminative trait for the poorly described and disputed taxon *T. suberectus*, occurred just occasionally in clades 2, 8 and 9. The pattern of folds in the cross section of the penis showed no variation, whereas the diameter differed somewhat (Figs. 12 and 14).

In contrast, the related species can be distinguished by specific differences in their genital anatomy, i.e., in the penis structure observed in cross section: In *T. oreinos*, the penis has a single intrapapillar cavity interrupted at one side (Figs. 13 and 14). One constant difference was detected between the two *T. oreinos* subspecies: *T. o. oreinos* has a bulge attached to the penial fold, which occasionally has an additional small fold, whereas *T. o. scheerpeltzi* lacks this trait (Figs. 13 and 14). *T. striolatus* could be distinguished from *T. hispidus/sericeus* in some cases by a penis with additional folds or modified folds with protuberances (Figs. 13 and 14). Nevertheless, in all seven specimens of *T. striolatus*, representing the subspecies *danubialis* and *juvavensis*, the arrangement of the penial folds was the same as in *T. hispidus*. Thus, this structure seems to be very variable in *T. striolatus.*

Besides these specific traits, the general genital anatomy of *T. oreinos*, *T. striolatus* and *T. hispidus* showed no constant differences (Fig. 8, 9, 10 and 11). Moreover, the relation of the lengths of the inner and outer dart sacks as well as the configuration of vaginal folds (in longitudinal section) did not appear to be informative.

Identification of other species

The identifications of *T. villosus*, *T. villosulus*, *T. clandestinus*, *T. biconicus* and *P. lubomirskii* based on shell morphological and anatomical traits described by Ložek (1956), Kerney et al. (1983) and Proćków (2009) were straightforward. *T. coelomphala* proved to be

problematic because two representatives of its clade resembled a *T. hispidus/sericeus* morphotype, while the other three specimens from Günzburg showed the expected *T. coelomphala* morphotype, i.e., a broad umbilicus (umbilicus width about ¼ of total shell width) and a slender upper vagina (for details see appendix 1-3).

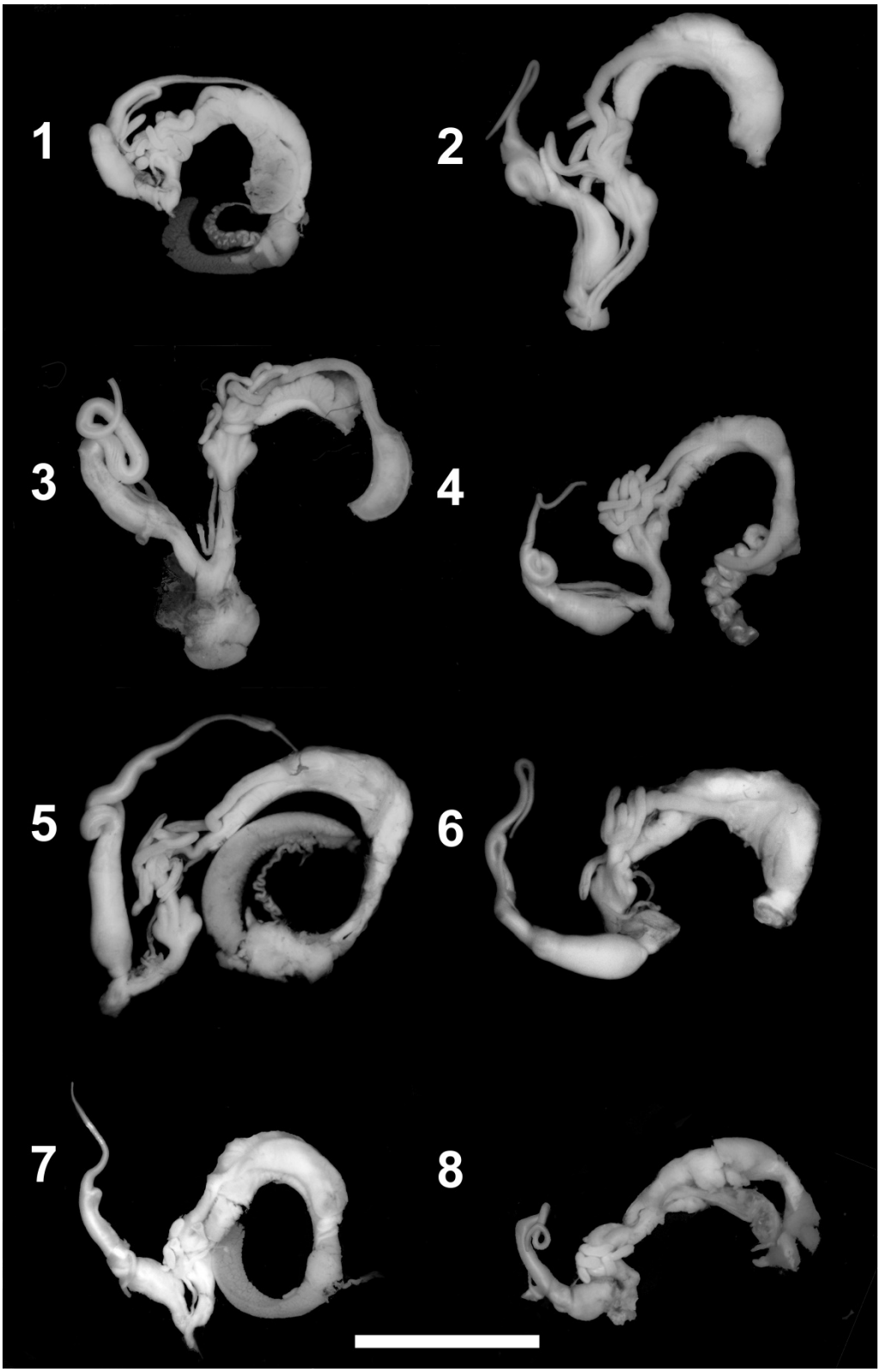

Figure 8. Examples of genital ducts of all *T. hispidus/sericeu*s clades: **1**: Clade 1, Ind. Nr. 4177: The Netherlands, Leiden: Sample Nr 418; **2**: Clade 2A, Ind. Nr 3154: Austria, Johnsbachtal: Sample Nr. 323; **3**: Clade 2B, Ind. Nr 1456: Italy, Plöckenpass: Sample Nr.200; **4**: Clade 3A, Ind. Nr. 6410: Austria, Hatting: Sample Nr. 549; **5**: Clade 3A, Ind Nr.113: Austria, Regelsbrunner Arm: Sample Nr. 33; **6**: Clade 6A, Ind Nr. 664: Austria, Orth: Sample Nr. 3; **7**: Clade 9, Ind. Nr. 6237: Austria, Neu Götzens: Sample Nr. 548; **8**: Clade 8A, Ind. Nr. 6249: Germany, Eggenstein: Sample Nr. 555. Scale bar = 5mm

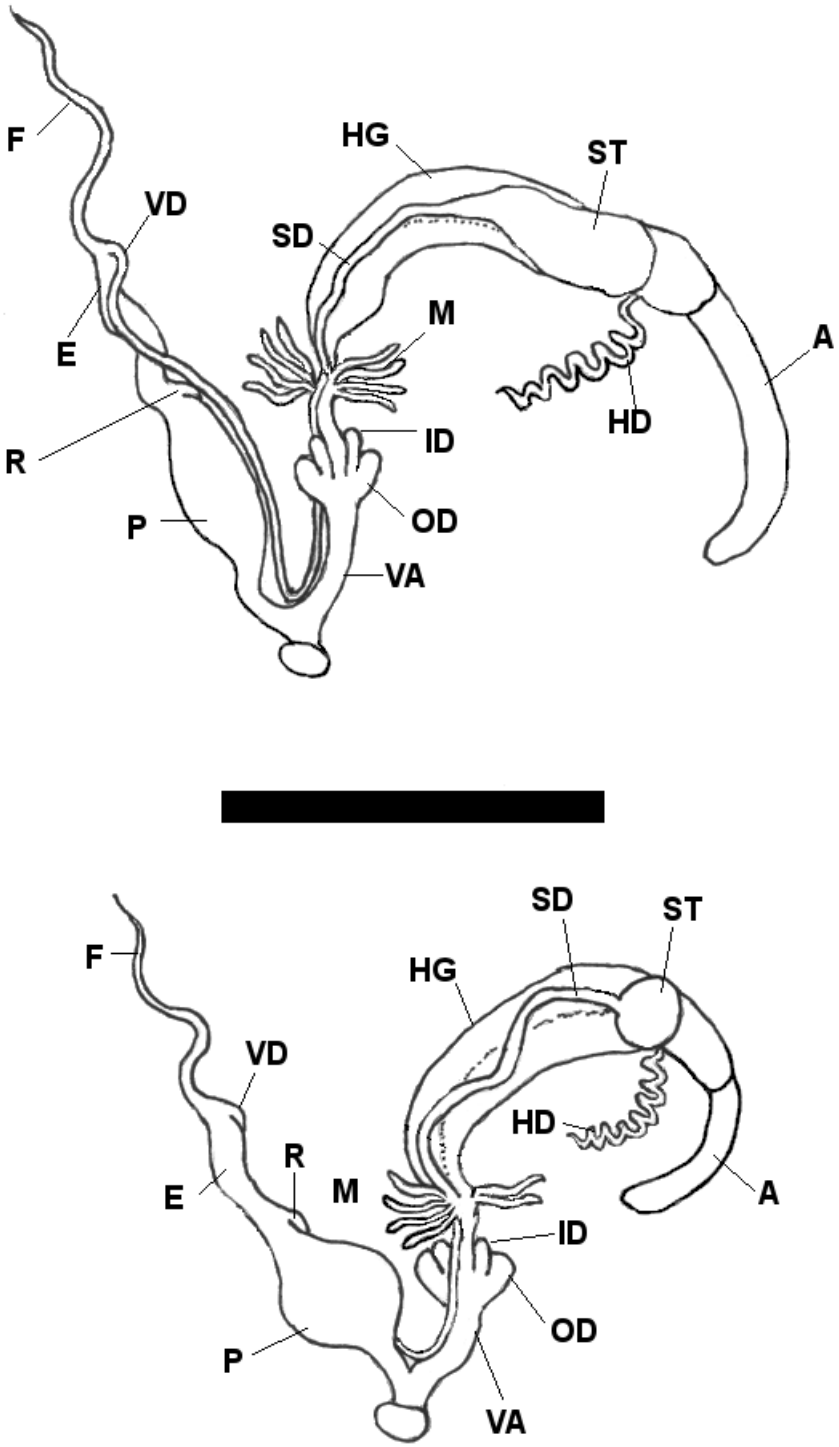

Figure 9. Sketch drawing of two variations of *T. hispidus* genitalia. The upper one represents a variation with fusiform penis, elongate spermatheca and four pairs of mucous glands, the lower one a variation with bulbous penis, round spermatheca and three pairs of mucous glands. Abbreviations: A=Albumen gland, E = Epiphallus, F = Flagellum, H = Hermaphroditic duct, ID = Inner dart sacks, M = Mucous glands, OD = Outer dart sacks, P = Penis, R = Retractor muscle, SD = Spermathecal duct, ST = Spermatheca, VA = Vagina, VD= Vas deferens. Scale bar = 5mm

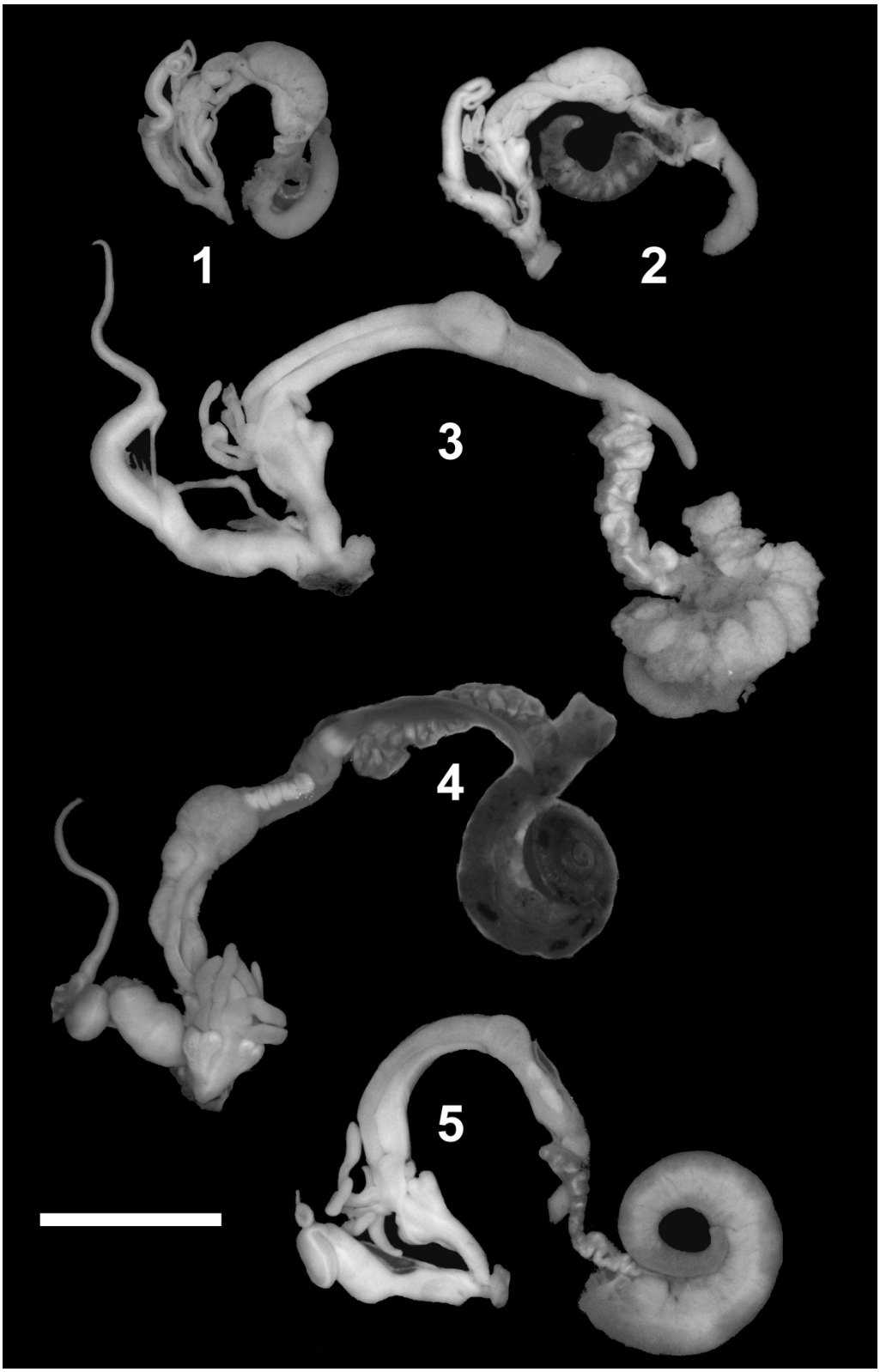

Figure 10. *T. oreinos* ssp and *T. striolatus* ssp.: Examples of genital ducts. **1**: *T. o. oreinos*: Ind. Nr 1321: Austria, Schneeberg: Sample site Nr. 178; **2**: *T. o. scheerpeltzi* : Ind Nr. 3384: Austria, Hohe Nock: Sample site Nr. 351; **3**: *T. s. striolatus*: Ind. Nr. 4011: Germany, Wiesensteig: Sample Nr. 415; **4**: *T. s. danubialis*: Ind. Nr 5528: Austria, Sauwald; Sample Nr. 468; **5**: *T. s. juvavensis*,: Ind. Nr. 717: Austria, Höllengebirge: Sample Nr. 122; Scale bar=5mm

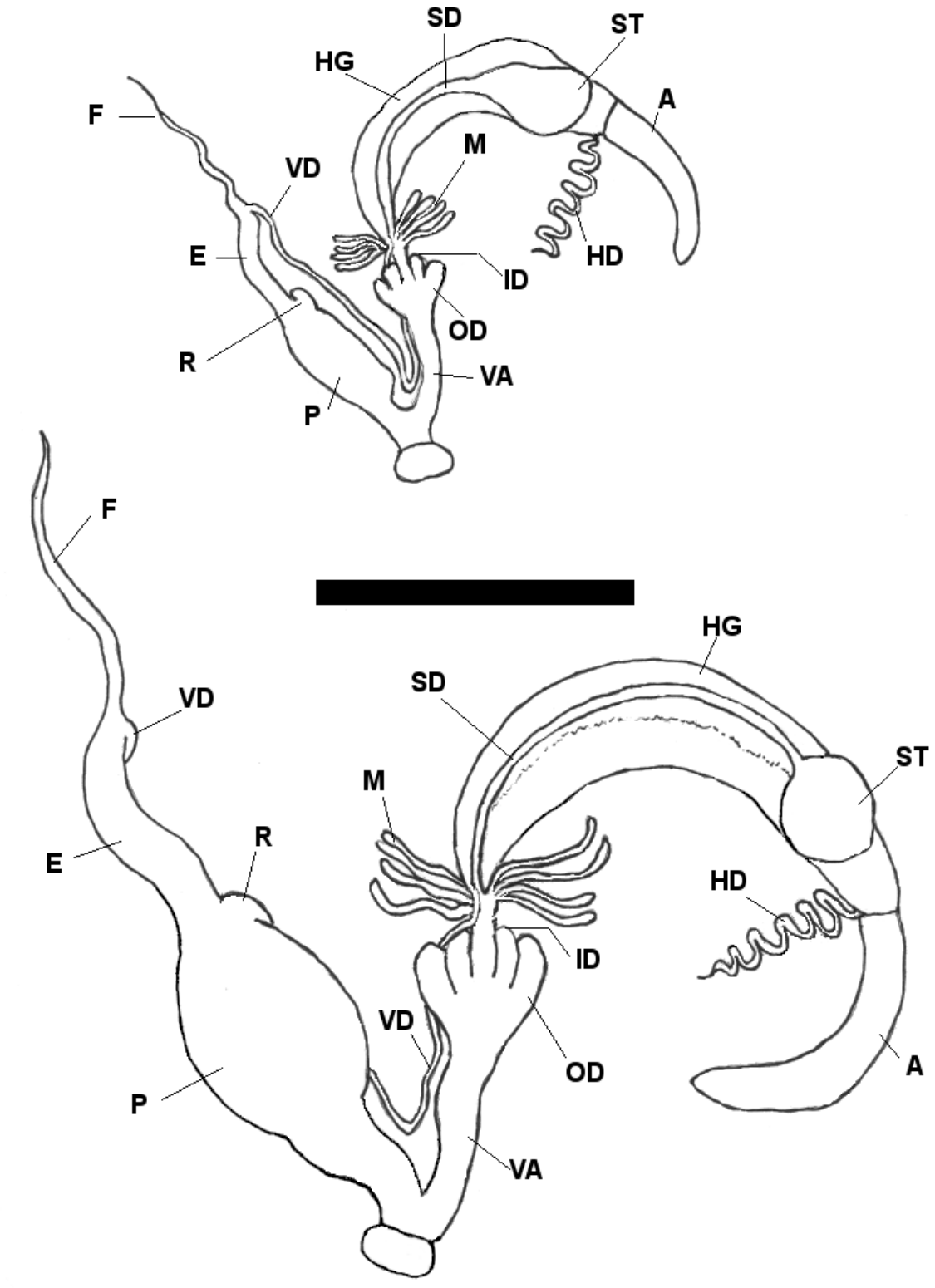

Figure 11. *T. oreinos* and *T. striolatus*: Sketch drawings of genital ducts of. Abbreviations: A = Albumen gland, E = Epiphallus, F = Flagellum, H = Hermaphroditic duct, ID = Inner dart sacks, M = Mucous glands, OD= Outer dart sacks, P= Penis, R= Retractor muscle, SD = Spermathecal duct, ST= Spermatheca, VA= Vagina, VD= Vas deferens.

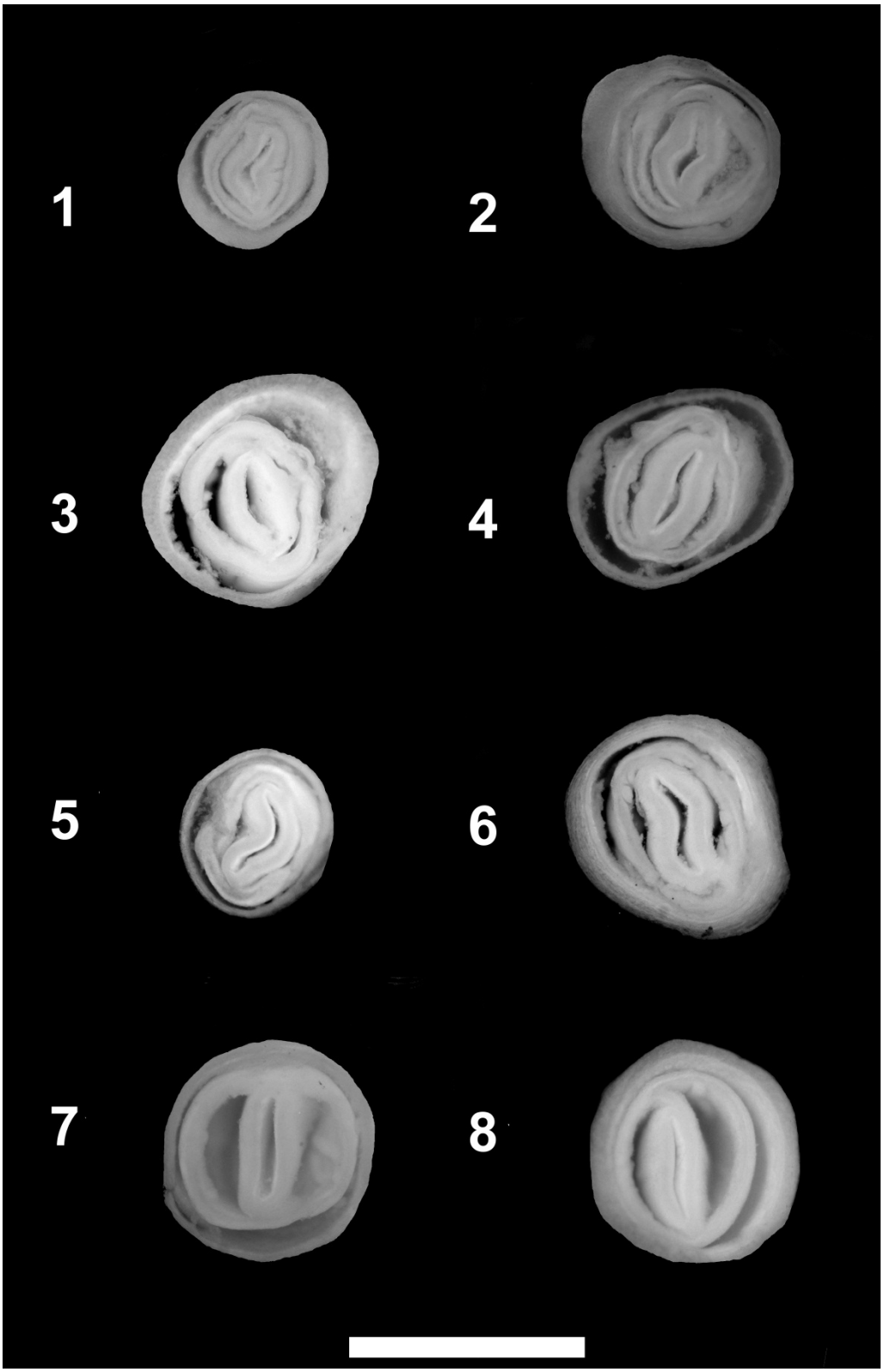

Figure 12: Penis cross sections of several *T. hispidus/sericeus* clades. **1**: Clade 1, Ind. Nr 4177: The Netherlands, Leiden: Sample Nr. 418 ; **2**: Clade 2A, Ind. Nr. 3004: Austria, Tiefental: Sample Nr. 313; **3**: Clade 2B : Ind Nr. 1455 : Italy, Plöckenpass: Sample Nr. 200 **4**: Clade 3A, Ind Nr. 2882, Hungary , Mecsek : Sample Nr.288 ; **5:** Clade 3B, Ind. Nr. 849 : Austria, Langschwarza: Sample Nr. 545, **6:** Clade 6A, Ind. Nr. 111: Austria, Regelsbrunner Arm: Sample Nr. 33; **7** Clade 8B, Ind Nr. 6250: Switzerland, Lac de Tseuzier: Sample Nr. 541; **8**: Clade 9, Ind. Nr. 6406: Austria, Neu Götzens: Sample Nr. 548. Scale bar = 1mm

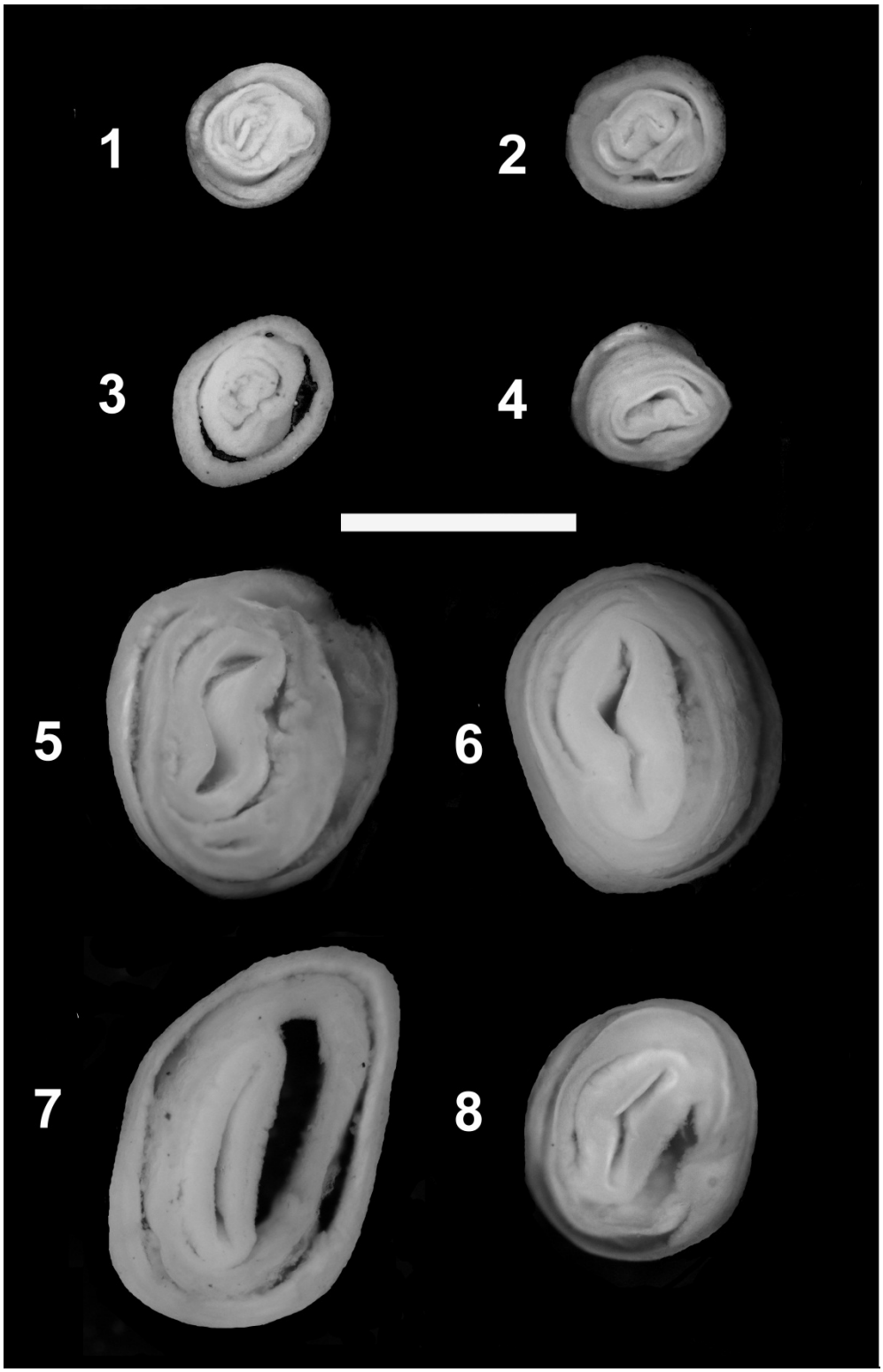

Figure 13. Penis cross sections of *T. oreinos* ssp. and *T. striolatus* ssp. Scale bar =1mm. **1**: *T. o. oreinos*: Ind. Nr. 1000: Austria, Hochschwab: Sample Nr. 134; **2**: *T. o. oreinos*: Ind. Nr. 1321, Austria: Schneeberg; Sample site Nr.178; **3**: *T. o. scheerpeltzi*: 3405, Austria, Feichtausee: Sample Nr. 369; **4**: *T. o. scheerpeltzi*: Ind Nr. 3396, Austria: Hohe Nock; **5**: *T. s. striolatus*: Ind. Nr. 4042: Germany, Grabenstetten: Sample Nr. 416; **6**: *T. s. danubialis*: Ind Nr. 2981: Austria, Fischamend: Sample site Nr. 298; **7**: *T. s. danubialis*: Ind. Nr. 687, Austria, Wechsel; Sample Nr. 7; **8**: *T. s. juvavensis*: Ind. Nr. 717: Austria, Höllengebirge: Sample Nr. 122. Scale bar = 1mm

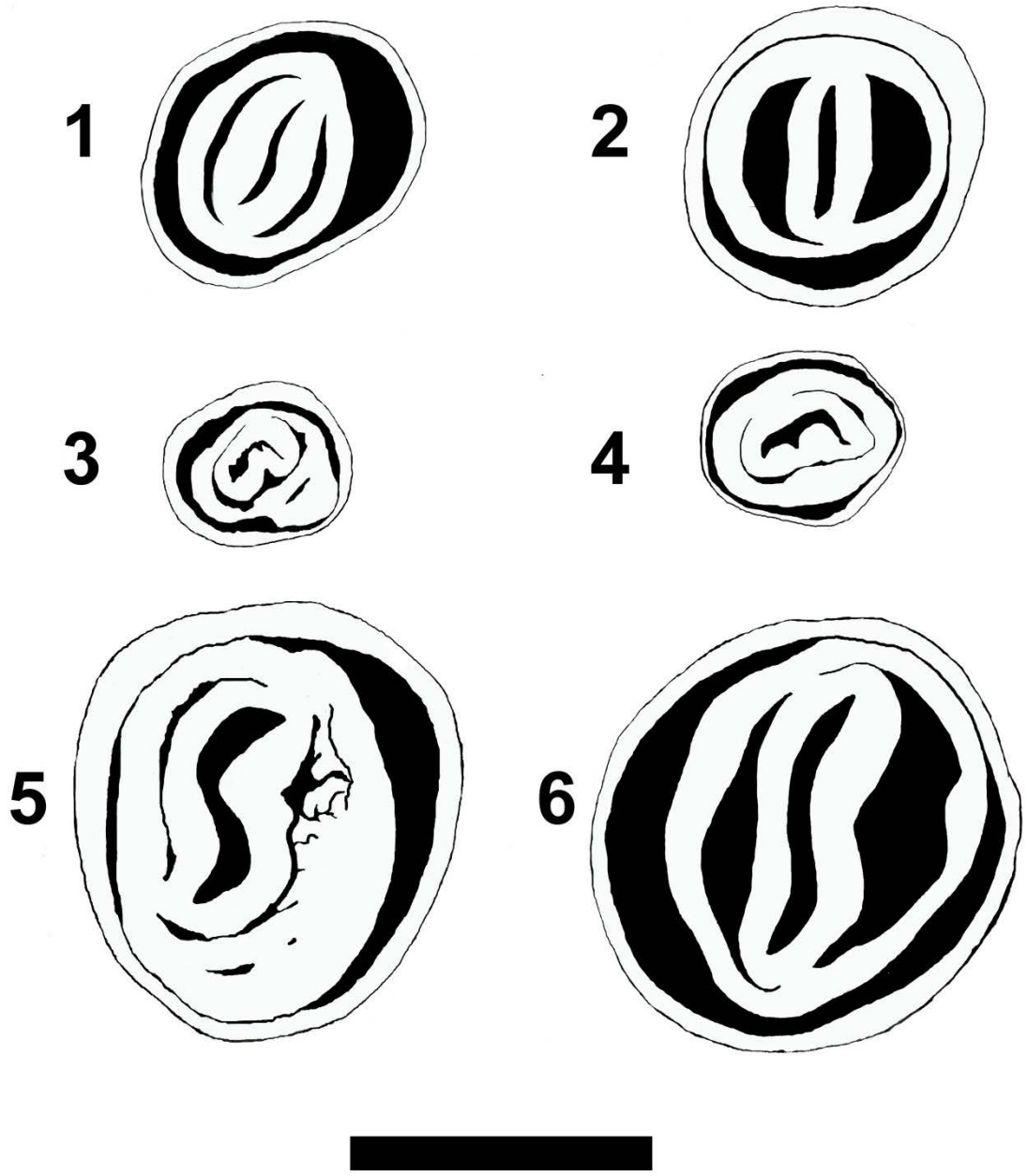

Figure 14. Sketch drawing of penis cross sections. Ground patterns of penis cross section in the *T. hispidus/sericeus* complex, *T. striolatus* ssp. and *T. oreinos* ssp. **1**: *T. hispidus*/*sericeus*, small folds, **2**: *T. hispidus*/*sericeus*, broad folds, **3**: *T. oreinos*: additional fold and bulge. Only found in *T. o. oreinos*. **4**: *T. oreinos*: No additional fold. Only found in *T. o. scheerpeltzi.* **5**: *T striolatus*, folds with protuberances. Mainly found at *T. s. striolatus*. **6**: *T. striolatus*, smooth folds, found at *T. s. danubialis* and *T. s. juvavensis*. Scale bar = 1mm.

Ecological influences

Shell size and altitude

Shell size is often influenced by microclimatic impacts such as temperature, moisture and nutrition. Altitude is a good estimator for such influences (see also Rieger et al. 2010) because, with increasing altitude, the temperature usually decreases and moisture increases. To test the influence of altitude, a Mantel test for all shell measures and their ratios was performed based on Austrian samples of the *T. hispidus/sericeus* complex. The results showed that the narrowness of the umbilicus is strongly correlated with increasing altitude and that there is a significant tendency toward bigger shell size at lower elevations (Table 2).

Table 3: Results of the Mantel test. SW: shell width, SH: shell height, WU: with of umbilicus, HB: height of body whorl; significance: Mantel p<0.05: correlation significant (1); Mantel p>0.05: correlation not significant (0).

	Mantel r	Mantel p	significance
Measurements			
SW	0.127	0.025	1
SH	0.172	0.023	1
WU	0.198	0.003	1
HB	0.042	0.227	0
Ratios			
SW/SH	0.093	0.089	0
SW/UW	0.252	0.008	1
SH/HB	0.014	0.334	0

Habitat preferences

In the correspondence analysis, we tested which taxa were separated according to their ecological preferences (for habitat and landscape structures see Table 3). This analysis showed a clear separation of *T. oreinos* from *T. hispidus* and *T. striolatus* (Fig. 15). The localities of the latter two species occupied a large space in the plot, with widely overlapping clouds and only a few sample sites lying close to the cloud representing localities of *T. oreinos*. This configuration reflects the broad ecological niche of *T. hispidus* and *T. striolatus*, which inhabit a wide variety of habitats, whereas *T. oreinos* is a stenoecious inhabitant of rocky alpine sites. The values responsible for separating *T. oreinos* from the two other taxa were “rocks”, “boulders”, “free of vegetation”, “*Pinus mugo* shrubbery” and “(sub)alpine

meadows". The space covered by *T. hispidus* and *T. striolatus* was vaguely subdivided into two clouds, each containing sample sites of both species. The cloud on the positive side of the first dimension represented mainly alpine or rocky habitat (dominating factors: rocks, boulders, alpine grassland), the other one located in the negative side represented the remaining habitats (dominating factors: high perennial herbs, meadow and boundary ridge). Additionally, the *T. hispidus/sericeus* complex and *T. striolatus* ssp. tended to preferentially occur adjacent to water bodies: this was the case in 44 of the 60 sample sites within the *T. hispidus*/*sericeus* complex and six of 10 sites within records of *T striolatus*, but only in one of 19 sites with records of *T. oreinos* ssp. Within the clades of the *T. hispidus/sericeus* complex, no differences with regard to ecological preferences were detected.

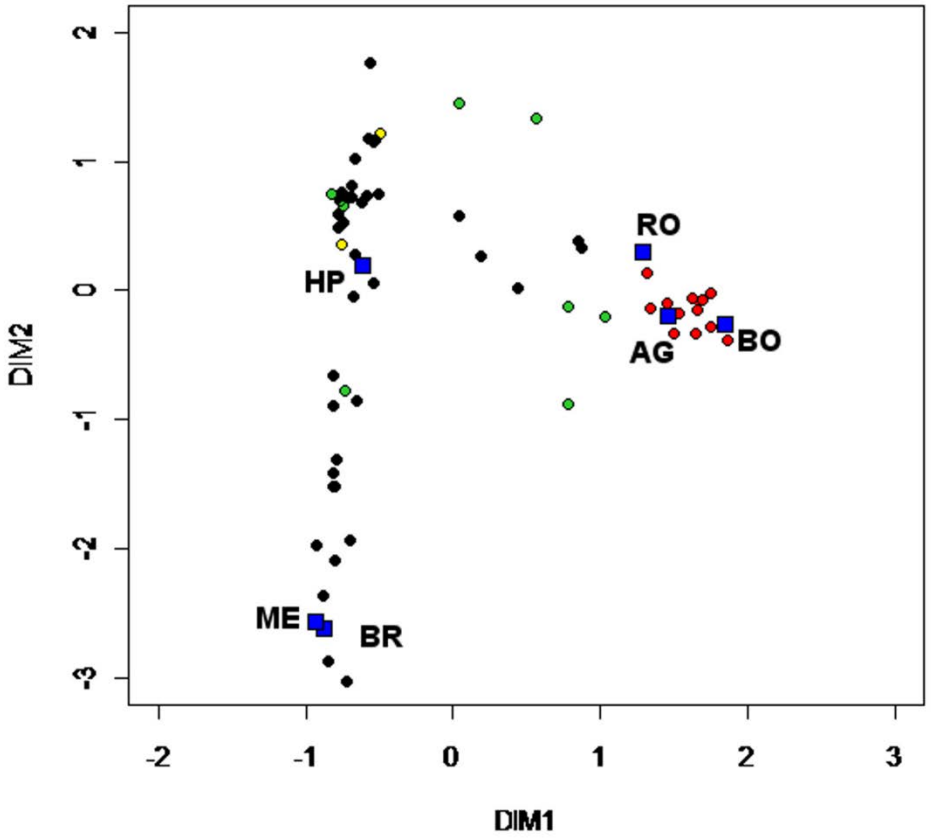

Figure 15. Correspondence analysis based on habitat types and landscape structures of 86 sample sites: biplot of the first two dimensions. Black points: sample sites of *T. hispidus*/*sericeus* complex (n=56). Yellow points: Sample sites with co-occurrence of *T. hispidus*/*sericeus* complex and *T. striolatus* ssp.(n=2). Green points: sample sites of *T. striolatus* ssp. (n=8). Red points: sample sites of *T. oreinos* ssp. (n=19). Blue squares: habitat types and landscape structures with the highest impact on the first two dimensions: BR: boundary ridge, ME: Meadow, HP: High perennial herbs, AG: Alpine grassland; BO: boulders

Discussion

Differentiation of clades and taxa by morphological and anatomical characters

Variation within the *T. hispidus/sericeus* complex

The clades of the *T. hispidus/sericeus* complex were separated from each other by unexpectedly high genetic distances ranging up to 18.9% (p distances of COI sequences; Kruckenhauser et al. in prep.). Nevertheless, they could not be differentiated based on the morphological and anatomical characters investigated. The highly variable shell morphology even within populations supports the results of Proćków (2009). In view of these results, and with no information about gene flow, the taxonomic status of the clades of the *T. hispidus/sericeus* complex remains debatable. Accordingly, as long as no unequivocal evidence for the species status for these clades exists, they should be considered as members of a single species. This approach was already used by Pinceel et al. (2005), who found highly divergent mt clades within the slug *Arion subfuscus* but treated them as one species because there were no morphological traits to separate them.

Concerning the poorly described form *T. sericeus*, the results agree with those of Naggs (1985) and Proćków (2009), who also were unable to delimit this taxon. On the other side, preliminary results from Czech Republic indicate a separation of *T. sericeus* (assigned as *T. plebeius* in that work) and two clades of *T. hispidus* in Bohemia and Moravia (Hrabaková et al. 2006). All clades that included the *T. sericeus* morphotype (narrow umbilicus and more or less globular shells) in our study also contained flat-shelled specimens with broad umbilicus. As the Mantel test showed, the frequency of narrow umbilici is strongly correlated with altitude in the investigated snails and therefore perhaps reflects phenotypic plasticity. In contrast to the general tendencies of these results, note that four members of clade 8B from south-western Germany (sample sites 555 and 556, see table 1) showed a typical *sericeus* morphotype, although they originated from elevations below 200 m asl. For Austrian populations of *T. sericeus* reported by Klemm (1974), it is remarkable that the distribution maps show a range of *T. sericeus* including areas adjacent to the range of *T. oreinos* in the north-eastern Alps. Although this specific area was quite intensively sampled for a previous and the present investigation, no specimens with pronounced globular shells and narrow umbilicus were found. Unfortunately, the mollusc collection of the Museum of Natural

History Vienna does not harbour specimens from this area. Thus, these reports by Klemm (1974) remain unconfirmed. In general, our findings add evidence that umbilicus and shell width are larger at lower elevations; this is because *T. hispidus* predominately occurred below 1000 m asl in this area due to specific geomorphological conditions (for details see also Duda et. al. 2011).

T. suberectus, another poorly described taxon, could not be verified by our results. As mentioned in the anatomical analysis, the occurrence of three instead of four pairs of mucous glands, which is the discriminating trait for this dubious species (Proćków 2009), occurred occasionally in several clades. This supports Turner et al. (1998), who set *T. suberectus* to the synonymy of *T. sericeus*.

Concerning *T. coelomphala*, the present data are insufficient to decide whether it is an independent species or a subspecies of *T. hispidus.* In Kruckenhauser et al. (in prep), five individuals forming a separate clade were tentatively assigned to this taxon based on their geographic origin. In this study they were not tested as a separate group due to the small sample size (five individuals from two localities). Three of them correspond to the "classical" morphotype of *T. coelomphala* because they resemble the comparably big (shell width >8 mm), flat *Trochulus* sp. with a very broad umbilicus. Moreover, they were collected in Günzburg, a locality well known for this form (Falkner 1973). However, two specimens originating from Taubertal in northern Bavaria resembled a typical *T. hispidus* morphotype (see also photographs in Annex). There are three possible explanations for these results (which remain preliminary due to the small sample size): (1) *T. coelomphala* displays a high phenotypic variation similar to that observed in *T. hispidus*. (2) The two morphologically overlapping specimens are the result of hybridisation or introgression or (3) *T. coelomphala* is definitely not a separate taxon, but merely representsanother lineage of the highly variable *T. hispidus* complex. In any case, the suggestion of Proćków (2009) that *T. coelomphala* is a synonym of *T. caelatus* has to be rejected: The latter is an endemic of dry carbonate rocks in the Swiss Jura and has completely different habitat preferences (Ursenbacher et al. 2009), while *T. coelomphala* inhabits moist habitats along the river banks of the northern Danube area (Ringler & Dingler 2005). Those authors, who classified it as separate species, described it as characteristic of silting swamps in floodplain forests, which represent highly endangered habitats (Ringler & Dingler 2005). Proćków's opinion was perhaps influenced by a supposed misidentification (Clessin 1874) claimed by Falkner (1973). Additionally, there is confusion about the French populations of a very flat *Trochulus* sp. with broad umbilicus from the Rhone valley, which is sometimes assigned to *T. coelomphala* (e. g., by Falkner 1989). In

any case, further investigations are urgently required for *T. coelomphala* before it becomes extinct.

Differentiation of *T. striolatus* and *T. oreinos*

The differentiation of *T. striolatus* and *T. oreinos* from the *T. hispidus*/*sericeus* complex was straightforward by constant diagnostic traits (see above). Furthermore, some characters such as shell measurements sometimes allowed separating the species based on trend, although there were overlaps. The status of the Austrian endemic *T. oreinos* as an independent species was already shown by shell morphological, genetic and ecological analyses (Duda et al. 2010, 2011; Kruckenhauser et al., in prep.). The present study found the cross-section of the penis to be an additional stable character of *T. oreinos*; its pattern totally differed from the *T. hispidus*/*sericeus* complex, but was similar to *T. biconicus* (see also Proćków 2009). Concerning the two subspecies of *T. oreinos* (*oreinos* and *scheerpeltzi*), their overlapping shell traits were already shown in a more extensive data set (Duda et al. 2011). The present study detected a small but constant anatomical difference in the cross-section of the penis. These findings are interesting in comparison with the clades of the *T. hispidus*/*sericeus* complex: they genetically diverged to a similar or even higher degree, but could be differentiated neither in conchological characters nor in genital anatomical traits. The assumption is that the two subspecies of *T. oreinos* evolved independently in isolation over a long period; the genetic data indicate that each underwent bottlenecks (Duda et al. 2011; Kruckenhauser et al., in prep).

T. striolatus is clearly differentiated from the *T. hispidus*/*T. sericeus* complex by its specific riffle pattern on the shell surface and its genetic traits. Other morphological or anatomical traits such as shell measurements, structure of genitalia or of penial plicae separated only some individuals from the *T. hispidus/sericeus* complex. Moreover, the bulky penis was not a constant trait in *T. striolatus*, as claimed by Shileyko (1978) and Proćków (2009), because individual 4011 in Fig. 10 had a fusiform penis. Similar difficulties in separating *T. striolatus* from the *T. hispidus/sericeus* complex were pointed out by Naggs (1985) and Turner (1998). In comparing our data with Pfenninger et al. (2005), we conclude that from the *striolatus*-lineages reported in that study, only lineage A corresponds to *T. striolatus* as defined in our genetic analysis (Kruckenhauser et al., in prep). The *T. striolatus* clade in our tree covered a wide geographic area from south-western Germany to eastern Austria and contained individuals unambiguously determined as *T. striolatus* according to the description above. Concerning infraspecific classification, some authors suggested not

accepting subspecies within *T. striolatus* (Anderson 2008; Proćków 2009). For the area we investigated, at least the separation of two subspecies – *T. s. striolatus* and *T. s. danubialis* – seems to be supported by subtle morphological and anatomical differences, which correspond to a west-eastern geographic separation. *T. s. danubialis* showed no additional penial plicae (see Figs. 13, 14) and somewhat smoother riffles on the shell surface. Furthermore, *T. s. juvavensis* was characterized by ambiguous traits, such as smaller shell dimensions (see Fig. 5) and coarser riffles. It was not differentiated from *T. s. danubialis* in the genetic analysis. This raises the question whether the subspecific classification of *T. s. juvavensis* is justified. At the same time, it is apparently geographically restricted to the Salzkammergut area in the Northern Calcareous Alps in Austria, a fact that supports its state as subspecies. This calls for additional research to reach a final conclusion.

Problems of morphological determination, character selection and species delimitation

The detection of diagnostic traits is important to distinguish species. Shell measurements can be ambiguous in discriminating land snail species in general, as they may be affected by environmental conditions such as climate and nutrition (Davis 2004). Nevertheless, a few species can only be separated based on shell measurements, e.g., *Pupilla pratensis* from *Pupilla muscorum* (Horsák et al. 2010). Nonetheless, land pulmonates are sometimes defined by weak discriminators even in field guides (e.g., Kerney et al. 1983; Falkner 1989) with descriptions such as “umbilicus a little more narrow than” or “shell more slender than”. While skilled malacologists are able to determine taxa based on trends, such descriptions may confuse less experienced persons and lead to incorrect determinations. Therefore, beyond detecting genetically distinct entities, whether such entities can be correlated with morphologically or anatomically differentiated groups is crucial. A major question for the present study was whether taxa and/or clades can be distinguished by morphometric analyses of such characters. For example, several species could be clearly classified and were distinctly differentiated in our tree and in previous publications (Pfenninger et al. 2005). In the current case, the taxonomic designation and mtDNA sequence data of *Plicuteria lubomirskii*, *T. biconicus*, *T. clandestinus*, *T. villosus* and *T. villosulus* were conform with those of Pfenninger et al. (2005). Additionally, these species can be unambiguously determined by shell morphology and anatomical characters (compare the photos in the annex with the figures in Kerney et al. 1983 and Proćków 2009). *T. coelomphala* (not investigated by Pfenninger et al. 2005) remained problematic, whereas or results on *T. striolatus* contradict those of Pfenninger et al. (2005). In this respect, the present study

provides important information about the usefulness of specific characters for discriminating the various taxa around the *T. hispidus/sericeus* complex.

Another point we underline here is that investigations (qualitative or quantitative) of animals from only a few localities have no taxonomic value. Moreover, the use of measurements alone without discriminating traits can lead to ambiguous results. For example, Naggs (1985) pointed out the case of a British *Trochulus* population whose shell and genitalia dimensions were intermediate between *T. hispidus* and *T. striolatus*. First attempts in the direction of diagnostic values in *Trochulus* were made by Shileyko (1978, 2006), but his studies often included only very few specimens; intraspecific variation could therefore not be recognized, as pointed out by the author himself (Shileyko 1978). Similarly, reports that found major differences in genital measurements between *T. hispidus* and related species (Klöti-Hauser 1901) must be interpreted with caution because those data are based only on single or very few sampling sites. The variation in shell dimensions within populations as well as within mt clades of the *T. hispidus/sericeus* complex is extremely high. This necessitates including individuals from many localities, representative for the whole distribution area, and finding stable traits. In this respect, even our comprehensive data are preliminary because they are concentrated on Austria and surrounding regions. Nonetheless, the data available on populations outside Austria (this study as well as Pfenninger et al. 2005) indicate that our results are representative for *T. hispidus* in general. Yet, a multinational mapping project with intense sampling of *T. hispidus* over the whole distribution area is needed to complement the available data and to assess the status of related problematic taxa (e.g., *T. coelomphala*, *T. plebeius*, *T. sericeus*).

It remains open whether (or which of) the clades among the *T. hispidus/sericeus* complex shown in Fig. 1 represent species or not. The issue of potential cryptic species within the *T. hispidus/sericeus* complex can only be answered by testing for hybridisation barriers and gene flow. This could be accomplished by studying reproduction biology and by breeding experiments, as well as by genetic analyses of nuclear markers. The *T. hispidus/sericeus* complex exemplifies the problematic practice of DNA barcoding without detailed knowledge of phylogenetic/phylogeographic relationships and species delimitation. Even for a comparably small area like the eastern Alps and adjacent regions, a few COI sequences for defining *T. hispidus* are clearly misleading.

Ecological separation

Our results show that the *T. hispidus*/*T. sericeus* complex and *T. striolatus* accept a wide range of habitats which in some cases resemble the niche of *T. oreinos.* This, however, this is true only if the data are based on few and simple categories. With a more detailed analysis including vegetation societies, it is possible to separate *T. oreinos* unambiguously from the others. This confirms our former study (Duda et. al. 2010) in which *T. o. oreinos* was characterized as an inhabitant of cool dry *caricetum firmae* meadow and boulder societies with sparse vegetation. A more detailed analysis including Ellenberg values might show even more differences in the habitat needs of the three taxa by characterizing them with quantitative biotic and abiotic factors (see also Horsák et al. 2007). Interestingly, for the *T. hispidus*/*T. sericeus* complex in the investigated area, the western populations in mountainous regions inhabit slightly different habitats than the eastern lowland populations. The former are less confined to adjacent water bodies and often found at sites without high perennial herbs, but rather on rocks and subalpine meadows. This may reflect climatic conditions, as the Atlantic climate in this area is more humid. Populations in the eastern Austrian flatlands are strictly bound to wetlands adjoining water bodies. Čejka et al. (2008) report similar results for land snail faunas in Slovak Danubian floodplain forests. That study also indicates that *T. hispidus* and *T. striolatus* share overlapping habitat preferences, whereby *T. hispidus* has a moister and *T. striolatus* a drier optimum.

Phylogenetic and phylogeographic implications

Besides pointing to possibilities and limitations of species delimitations, the grouping in the genetic tree (Fig. 1) shows a big clade of "*Trochulus* s. str.", which is divided into two geographic subclades: an eastern subclade comprising *T. hispidus*/*sericeus* clades 1-7 and 9, *T. coelomphala*, *T. villosulus* and *T. striolatus*, and a western one consisting of *T. hispidus/sericeus* clade 8, *T. clandestinus* and *T. villosus*. *P. lubomirskii* (assigned as a member of *Trochulus lubomirskii* by some authors, e.g., Proćków 2009), *T. biconicus* and *T. oreinos* apparently belong neither to the eastern nor to the western group of "*Trochulus* s. str.". This agrees with the hypotheses of Shileyko (1978: *P. lubomirskii*), Falkner (1982: *T. oreinos*) and Turner (1998: *T. biconicus*), who considered these species to be only distantly related to *Trochulus* s. str.. Conspicuously, these taxa show either extremely short hairs <0.1 mm (evident in *P. lubomirskii* and *T. oreinos*, see also Duda et al. 2011) or no hairs at all (*T. biconicus*). This confers plausibility to Proćków (2009), who considered short hairs or the

general lack of hairs on the periostracum within the tribus Trochulini as a plesiomorphic trait. All the mentioned taxa branch off from basal nodes in the genetic tree. These implications are only preliminary because final conclusions or a taxonomical review of European Trochulini require more data on all known taxa including the (sub)genera *Petasina* and *Edentiella*. We can, however, definitively reject a possible sister-group relationship of the *T. hispidus/sericeus* complex to both *T. oreinos* ssp., an issue addressed as unresolved by Duda et al. (2011).

Comparing divergences of genetic clades (Fig. 1) and habitat preferences (Fig. 15) reveals that, in both cases, the *T. hispidus/sericeus* complex varies considerably more than *T. oreinos* ssp. Members of the *T. hispidus/sericeus* complex inhabit a broad range of often dynamic or anthropogenically influenced habitats bound to rivers and wetland areas. This enables quick dispersal, either actively (along river valleys acting as corridors) or passively (drift by flood, anthropogenic transport). In addition, the broad range of possible habitats and the tolerance to different climatic conditions facilitate the colonization of new areas. All these facts explain the extensive range of the *T. hispidus/sericeus* complex, reaching from the north of the Mediterranean peninsulas to Scandinavia and even the colonization of North America as neobiont (see e.g. Hotopp et al. 2010). This also makes plausible the survival of several climatically suboptimal periods in isolated refugia followed by expansion during warm interglacial periods during the Pleistocene. Together with the high age of the *T. hispidus*/*sericeus* complex (according to Ložek (1956) and Frank (1996) – the first record from Central Europe is dated to the early Pleistocene – these facts might help explain the great morphological and mt variability. In contrast, *T. oreinos* has an entirely different evolutionary history. According to Duda et al. (2010), it was always a stenoecious inhabitant of a very narrow ecological niche consisting of cool, primarily treeless and slightly azonal habitats such as boulders, rocks and *Caricetum firmae* meadows with patchy structure. Clearly, *T. oreinos* has restricted dispersal and colonization abilities, considering that suitable habitats like *Caricetum firmae* with patchy structure exist all across the Northern Calcareous Alps. However, only a very small, restricted area is populated, probably corresponding to habitats that remained ice-free during the last glaciation (van Husen 1986). In summary, all these factors led to a comparably low genetic and morphological variation within each *T. oreinos* subspecies, which was further reduced by bottleneck effects (Duda et al. 2011). Compared to the previous species, *T. striolatus* seems to have an intermediate position: it is variable in habitat choice and morphology, but quite homogeneous in mt variation. This might

reflect a quick dispersal from a single refuge over large parts of Europe after the last glaciation.

Irrespective of taxonomic status and of morphological and genetic variation, however, the geographic distribution of clades and morphotypes is relevant from the conservation perspective. The habitats of some clades within the *T. hispidus/sericeus* complex and several local populations of *T. striolatus* are under pressure. Two regions impacted by landscape degradation should be pointed out. (1) Wetlands and even the big riverine forests in the northern and very eastern flatlands of Lower Austria were heavily influenced by intensive agriculture, construction activity and hydraulic engineering in the last decades of the 20th century. As these habitats are the only ones in which both the *T. hispidus/sericeus* clade 6B and *T. striolatus danubialis* occur, both taxa might be affected by such anthropogenic impact. The latter taxon is even classified as "critically endangered" in the red data book of Austria (Reischütz & Reischütz 2007). (2) The inner-alpine valleys of Tyrol and Salzburg are under heavy pressure of settlement development due to the reduced space on the valley plains. Therefore, suitable habitats such as moist meadows have already become extremely rare. This concerns populations of clades 3A and 9. *T. sericeus* and *T. hispidus* (assigned as separate species by Reischütz & Reischütz 2007) are classified as "Least Concern" in the current red data book of Austria, with slight tendencies of decline. Nevertheless, even if none of the clades represents a cryptic species, the extinction of geographically restricted clades would clearly affect intraspecific diversity. Therefore, new conservation policies are required that also protect phylogenetically diverged clades irrespective of their taxonomic state.

Conclusion

The existence of many different mt clades in the *T. hispidus*/*sericeus* complex and the lack of diagnostic traits to differentiate them reveal general problems and limitations of classical (morphology-based) taxonomy in land snails, especially in so-called "critical taxa". Nevertheless, our morphological analyses, together with habitat data, provide valuable information about the morphological and genetic plasticity in the *T. hispidus/sericeus* complex. Moreover, our analyses yielded several new diagnostic traits for interspecific separation as well as for some subspecies of *T. striolatus* and *T. oreinos*.

Materials and methods

In species delimitation of *Trochulus*, the selection of both shell and genital traits is problematic (Naggs 1985). Nevertheless, in some cases, combinations of these traits distinguish species by trend (Pawłowska-Banasiak 2008; Duda et al. 2011). Among shell traits, especially external traits such as conspicuously distinct hair lengths and constant shell structure allow reliable recognition in some species (Gittenberger & Neuteboom 1991; Pawłowska-Banasiak 2008; Duda et al. 2011). Among anatomical traits, the basic patterns of plicae in the penis and vagina proved to be useful to differentiate species within the tribus Trochulini Lindholm, 1929 (Shileyko 1978, 2006; Proćków 2009), although this cannot be assumed for all Hygromiidae (see also Pawłowska-Banasiak 2008). Moreover, conspicuous formations within the genital apparatus occurring in single species, such as the extremely prolonged inner dart sacks of *Petasina unidentata*, may provide reliable species recognition (Shileyko 1978, 2006; Proćków 2009). Measurements of genitalia lengths can lead to ambiguous results: they can be biased by differences within populations, by seasonal differences, retraction state of the soft body, by stretching or different positioning during measuring, or by the preservation method (Emberton 1985, 1989; Jordaens et al. 2002). We therefore also sought qualitative traits (e.g., the basic patterns of plicae in penis) that are constant even in geographically separated populations.

Data sampling and documentation

Data collected by the authors themselves were documented in the following way: Exact positions and elevations of sampling sites were determined using GPS and recorded together with habitat and landscape structures (see also appendix 3 and 4 for exact definitions). Animals were drowned in heated water as described by Kruckenhauser et al. (2011) and stored in 80% ethanol. Specimens collected by colleagues were directly fixed in 96% ethanol.

All photographs were taken with a Nikon digital sight D3-Fi1 camera fixed on different stereomicroscopes. Photos of shells and complete genital tracts were taken using a Wild M420 stereomicroscope (*T. hispidus/sericeus*, *T. oreinos* ssp.) or a Leica MZ 12 5 (*T. striolatus* ssp.) at lowest magnification. Penis cross sections of all taxa were examined under a Wild M420 stereomicroscope at highest magnification. Selected shells of different species and genetic clades were pictured from the back side and underpart to document at least parts of the morphological variation. This extraordinary way of record was necessary as some

shells were particularly broken on the front and upper surface due to tissue extraction for genetic analyses. All photographs were created as extended depth of field images with CombineZ software.

Shell morphology

Seven parameters of shell morphology described by Duda et al. (2011) were recorded. The four quantitative shell traits were measured in intact adult specimens with a graduated eyepiece under a stereomicroscope: shell diameter, umbilicus diameter, shell height, and height of last whorl. These values were log10 transformed for subsequent analyses. Furthermore, three qualitative aperture traits were recorded: basal tooth, internal rib and paler area around the aperture. The quantitative measurements were subjected to a discriminant analysis (DA). In a next step, measurements were merged in a combined DA including the first three dimensions of a correspondence analysis performed with the qualitative data (Tabachnik & Fidell 1986). This combination should better separate different groups and was performed as an operative tool of descriptive statistics. The analyses included individuals of the *T. hispidus/sericeus* complex only and, subsequently, the complete data set, including individuals of other taxa as well. The software r (R Development Core Team 2012) was used for all calculations.

Genital anatomical traits

We followed the approach already used by other authors for *Trochulus* sp. (Shileyko 1978, 2006; De Winter 1991; Proćków 2009) and produced internal sections of the genital tract, cross sections of the penis as well as longitudinal sections of the vagina to record the basic patterns of plicae in penis and vagina. Our aim was to compare the results with those from previous studies. Ten individuals of each mt clade were analysed. If fewer individuals were available from a particular clade, all specimens were analysed. Specimens were selected to represent differing regions as much as possible. A total of 92 individuals were dissected; all were photographed before sectioning.

Ecological influences

With increasing altitude, the temperature generally decreases and moisture increases. This makes altitude a good indicator for the influences of microclimatic conditions on shell size (see also Rieger et al. 2010). To test the correlation between altitude and shell size, a Mantel test for all shell dimensions and their ratios was performed with all Austrian samples

of the *T. hispidus/sericeus*-clade. For this test, the averages of these values were calculated for populations (i.e., localities); the test was performed with the software r (R Development Core Team 2012). The above averages were calculated from all individuals of each locality, irrespective of the mt clades to which the specimens belonged.

A correspondence analysis was performed to evaluate whether habitat parameters such as vegetation type and landscape structure (defined in Appendix 4 and 5) reveal different habitat requirements of species (software r). Only ecological data evaluated by the authors themselves were used in the analysis.

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Authors' contributions

All authors contributed substantially to the field collections. MD conducted all measurements, sections, analyses, drawings and photos, and drafted the manuscript. LK provided the genetic background, designed Figures 1 and 2 and contributed to the manuscript. HS conceived the project, supervised the analysis and contributed to the manuscript. JH contributed to the manuscript and provided substantial help for layout and arrangement of the photos. EH conceived the project, supervised the analyses and contributed to the manuscript. All authors red and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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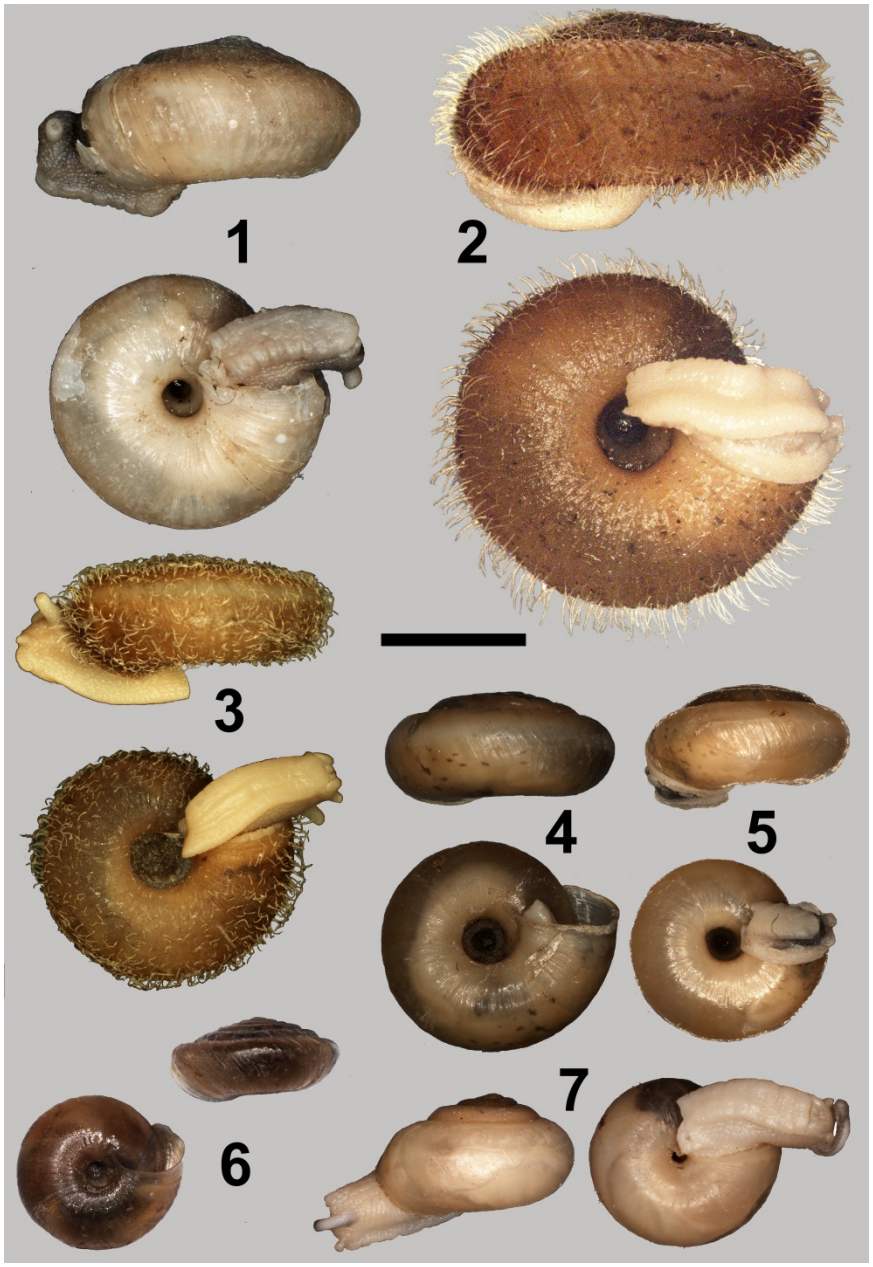
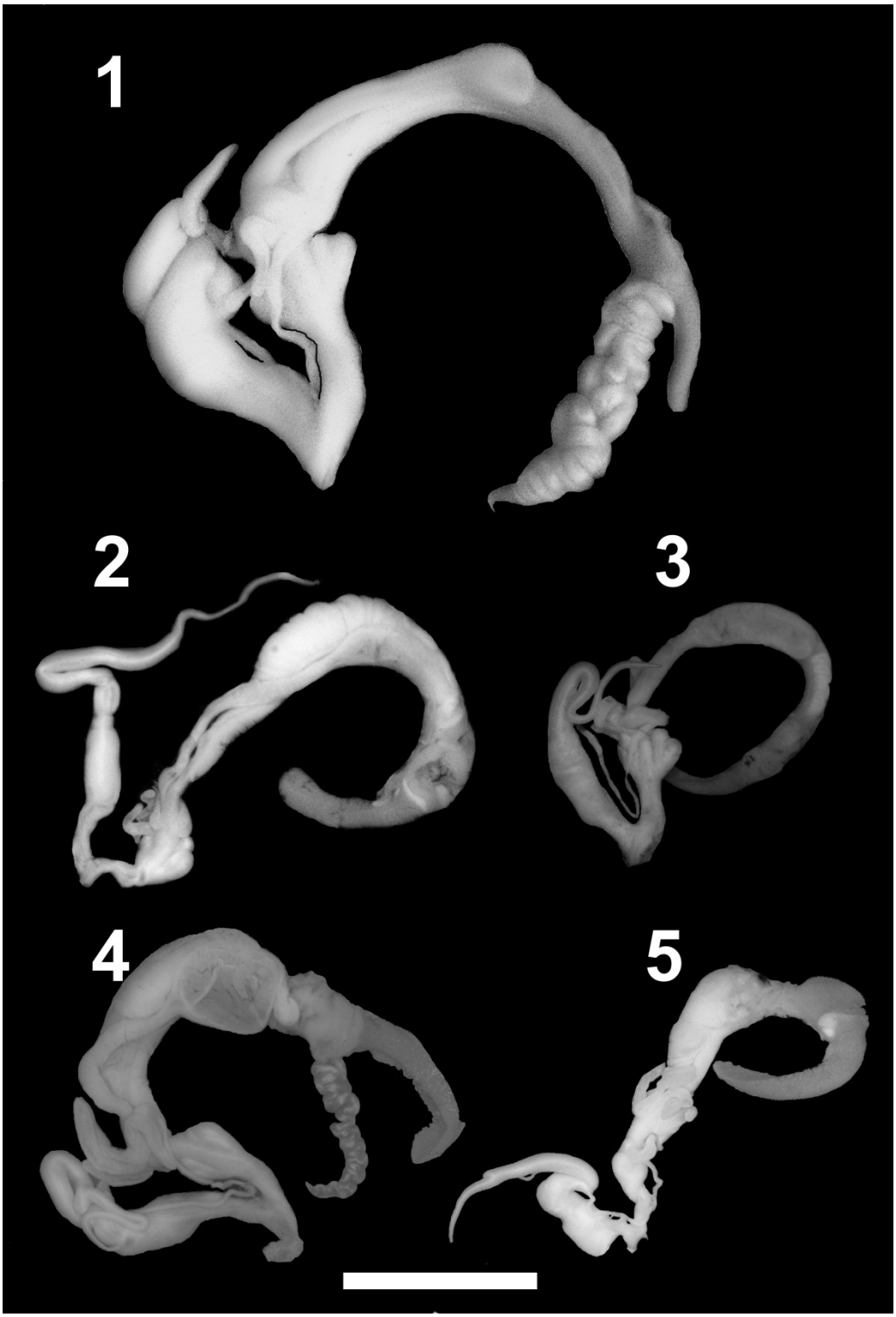
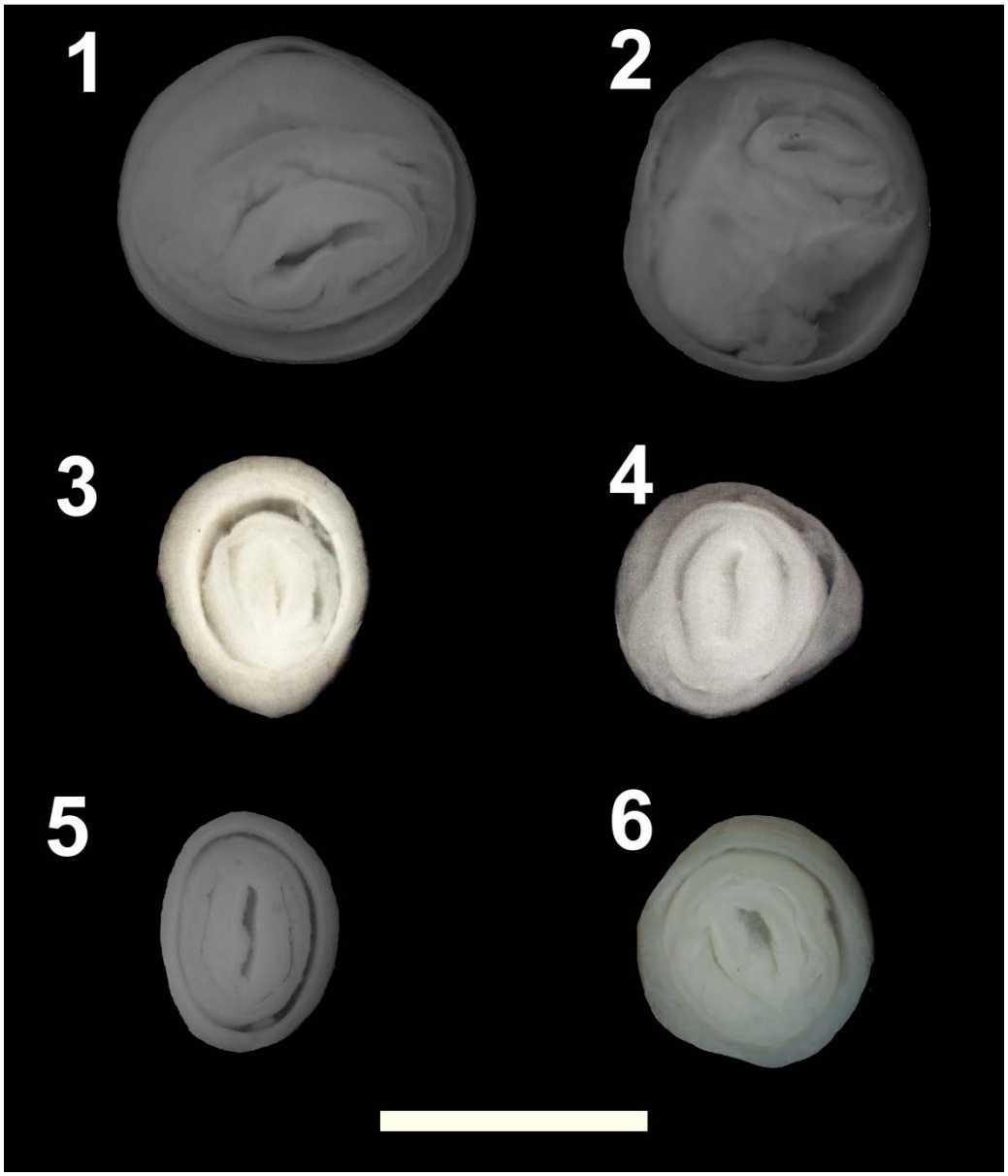
Appendix



Appendix 1. Selected representatives of *Trochulus* sp. and *Plicuteria lubomirski* specimens which were included in the genetic tree in Fig. 1. **1:** *T. clandestinus*, Ind. Nr. 1381: Switzerland, Graubünden: Sample Nr. 199; **2:** *T. villosus*, Ind. Nr. 4202: Germany, Tuttlingen: Sample Nr. 417; **3:** *T. villosulus*, Ind. Nr. 1291: Slovakia, Čičmany: Sample Nr. 193; **4:** *T. coelomphala*: typical specimen with flat shell and broad umbillicus, Ind. Nr. 4046: Germany, Günzburg: Sample Nr. 483; **5:** *T. coelomphala* : specimen resembling the T. *hispidus/sericeus* complex, Ind. Nr. 5592: Germany, Regensburg: Sample Nr. 413; **6:** *T. biconicus*, Ind. Nr. 6358: Switzerland, Bannalp: Sample Nr. 559; **7:** *Plicuteria lubomirski*, Ind. Nr. 2690 Slovakia, Strážovské vrchy: Sample Nr. 190. Scale bar = 5mm



Appendix 2. Examples of genital ducts of *Trochulus* sp. and *Plicuteria lubomirskii* specimens which were included in the genetic tree in Fig. 1: **1**: *T. villosus*, Ind. Nr.: 4202: Germany, Tuttlingen: Sample Nr. 417; **2**: *T. coelomphala* – typical specimen with prolonged penis and vagina, Ind. Nr 4046: Germany, Günzburg: Sample Nr. 413; **3**: *T. coelomphala* – specimen resembling the *T. hispidus/sericeus* complex, Ind. Nr 5592: Germany, Regensburg: Sample Nr. 413; **4**: *T. clandestinus*, Ind. Nr. 1381: Switzerland, Graubünden: Sample Nr. 191; **5**: *Plicuteria lubomirskii*, Ind. Nr. 2690 Slovakia, Strážovské vrchy: Sample Nr. 190. Scale bar = 5mm



Appendix 3: Examples of penis cross sections of *Trochulus* sp. and *Plicuteria lubomirskii* specimens which were included in the genetic tree in Fig. 1. **1**: *T. villosus*, Ind. Nr.: 4202: Germany, Tuttlingen: Sample Nr. 417; **2**: *T. clandestinus*, Ind. Nr. 1381: Switzerland, Graubünden: Sample Nr. 191; **3** *T. coelomphala* – typical specimen, Ind. Nr 4046: Germany, Günzburg: Sample Nr. 413; **4**: *T. coelomphala* – specimen resembling the *T. hispidus/sericeus* complex, Ind. Nr 5592: Germany, Regensburg: Sample Nr. 413; **5:** *T. villosulus*, Ind. Nr. 1291: Slovakia, Čičmany: Sample Nr. 193; **6:** *Plicuteria lubomirskii*, Ind. Nr. 2690 Slovakia, Strážovské vrchy: Sample Nr. 190. Scale bar = 1mm

Appendix 4: Definition of Habitat types

Habitat type	Definition
Open areas	
Free of vegetation (FV)	Natural or anthropogenically influenced areas with no vegetation
Meadow (ME)	Medium dry grassland, more or less intensively farmed, below the subalpine ecotone
Marsh (MA)	Wet grassland vegetated by grasses, reeds and sedges, can be farmed or not
High perennial herbs (HP)	Dense populations of high perennial herbs like *Urtica* sp. and *Petasite*s sp.
Forests	
Riparian forest (RF)	Central European inundation forests along rivers, at least particularly periodically flooded
Alder carr (AC)	Forest on permanent wet locations dominated by alders (*Alnus* sp.). No periodical flood, but consistently high soil water level
Deciduous forest (DF)	Central and northern European forests dominantly vegetated by deciduous trees, on medium moist to dry locations
Mixed forest (MF)	Central and northern European forests vegetated by deciduous and coniferous trees, on medium moist to dry locations
Coniferous forest (CF)	Central and northern European forests vegetated by coniferous trees, on medium moist to dry locations
(sub) Alpine habitats	
(sub) Alpine grassland (AG)	Natural and anthropogenically influenced meadows above the lower border of subalpine ecotone on medium moist to dry places
Mountain pine shrubbery (MP)	Subalpine areas vegetated by shrubberies of mountain pines (*Pinus mugo*). Represents the highest community of closed woody vegetation in the Alps together with green alder (*Alnus viridis*) shrubbery
Habitats with strong anthropogenic interference	
Garden/park (GP)	Intensively cultivated areas dominated by lawn and ornamental plants, occasionally with fruit-bearing plants, woody and herbaceous as well, situated within or adjacent to settlement areas
Ruderal Area (RA)	Areas with intensive anthropogenic disturbance but without direct cultivation or land use like construction sites or abandoned fields

Appendix 5: Definition of Landscape structures

Landscape structure	Definition
Edge of forest (EF)	Gradational or abrupt change of forest to open vegetation like meadows
Loose trees and shrubs (LT)	Expanded cover of trees and shrubs in patchy formation
Hedgerows and shrubs (HS)	Lines or small covers of shrubs which can vary in density and structure
Boundary ridge (BR)	Narrow lines of extensive Greenland between meadows, fields or along streets and ways
Single trees and shrubs (ST)	Single, isolated specimens of trees and shrubs
Riverbank grove (RG)	Groups or rows of trees beneath a riverbank
Single stones (SI)	Stones lying on the surface with no contact to each other
Bank/Dam (BD)	Earth walls caused by artificial change of one territory level to another, e.g. batters and levees
Boulders (BO)	Stones with contact to each other, not covered by earth or vegetation
Rocks (RO)	Compact, solid in-situ aggregation of minerals occurring naturally
Canyon/Rock face (CR)	Steep, extended walls of rocks

Concluding discussion

1) The first point that was solved in the course of this doctoral thesis was the finding of unambiguous discriminating traits for *T. oreinos*. The original assumption that *T. oreinos* was a subspecies of *T. hispidus* reflects the fact that the description was based solely on superficial shell traits. These can separate *T. oreinos* by trend from *T. hispidus*, but do not provide unambiguous discrimination. As shown in papers 1 and 3, *T. oreinos* can be unambiguously recognized by its differing pattern of the cross section of the penis and by its extremely short and dense hairs. The last finding refers to the comments of Falkner (1981, 1995), who mentioned "unterschiedliche Haarbildung" [different hair morphology] as a discriminating trait, without further explanation. This rather imprecise description was probably used because elder specimens and empty shells often lack hairs, and hence this trait might have appeared ambiguous. Parallel to and independent of the current study, the hair form in land gastropods was documented by Allgaier (2011). That study showed that the patterns and size of the hairs also affect the shell surface. Accordingly, it can be concluded that, if the hair morphology is clearly differentiated, then the microstructures on the shell surface must also be quite different. The distinctness of the *T. oreinos* subspecies from the *T hispidus*/*sericeus* group was unambiguously confirmed. As estimated by Falkner (1985), *T. oreinos* ssp. is definitely distantly related to the *T. hispidus*/*sericeus* complex and its closer relatives such as *T. striolatus* or *T. villosulus*.

(2) The intra-specific separation of the extremely divergent mt clades within *T. hispidus* detected in the current investigations remains problematic because no morphological differentiation between the mt clades were found. Also, a clear separation of *T. hispidus* from another poorly described critical taxon – *T. sericeus* – could not be confirmed based on the morphological and genetic data presented in this doctoral thesis. Although some specimens resembled the *T. sericeus* morphotype described in literature, they could not be clearly separated from others with a *T. hispidus* morphotype, neither morphologically nor genetically. Hence, these two forms were subsumed as *T. hispidus/ sericeus* complex in paper Nr. 3. In a concatenated tree of several mt genes, the various clades of the T. *hispidus*/*sericeus* complex cluster in two groups, both of which are mixed up with other morphological well-defined species (*T. striolatus*, *T. villosulus*, *T. clandestinus* and *T. villosus*). The eastern clades of the T. *hispidus*/*sericeus* complex mix with *T. striolatus* and *T. villosulus*, whereas the western clades are related to *T. clandestinus* and *T. villosus*. Within the Eastern Alps, *T. sericeus* (defined as *Trochulus* sp. with narrow umbilicus and globular shell) can be found in different

clades (Nr. 2, 3, 6 and 9 in paper Nr. 3) besides "*T. hispidus*-like" morphs (defined as *Trochulus* sp. with broad umbilicus and flat shell). Thus, *T. sericeus* is merely one form of an immense morphological variation of the *T. hispidus*/*sericeus* complex. A narrow umbilicus seems to be connected with cooler and moister climate as related to elevation. This, however, does not apply for the western clade (Clade 8 in paper Nr. 3), which includes some individuals with a narrow umbilicus collected both in flatlands and in Alpine areas., In the current doctoral thesis no constant anatomical and morphological trait was found to separate the two main clades visually. Hence, if *T. hispidus* and *T. sericeus* are separate species, their differentiation has to be based on other traits (e. g. interspecific separation by airborne pheromones). In contrast, the intraspecific separation of the *Trochulus oreinos* ssp. is much more straightforward. Originally, they were separated by shell morphology, in particular the groove beneath the keel, which was the original trait used to discriminate *T. o. scheerpeltzi* from the nominate form. Although this trait proved to be ambiguous in paper Nr. 1, another consistent trait was found: the cross section of the penis revealed a bulky structure and an additional fold in *T. o. oreinos*, which enables separating at least adult specimens. This additional trait also conforms to the strict geographical and genetic separation of the two subspecies. The extremely high genetic distances between the two subspecies clearly raise the question whether they could be elevated to vicariant separate species. Before making a decision about this topic, breeding experiments should be performed to evaluate the interbreeding success of both taxa.

(3) The *T. hispidus*/*sericeus* complex and *T. oreinos* ssp. do not only show a strikingly different ecological niche, but their differing habitat choice might even be the main reason why they are so divergent: Representatives of the *T. hispidus/sericeus* complex inhabit a broad range of very often dynamic or anthropogenically influenced habitats bound to rivers and wetland areas. This enables quick dispersal, which can be active, e.g. along river valleys, or passive by drift caused by flood events or even anthropogenic transport. Good evidence for the latter possibility is the fact that *T. hispidus* occurs as a neozoon in North America (Hottopp et al. 2010). Additionally, the broad range of possible habitats, reaching from open areas to forests, and the tolerance to different climatic conditions (from boreal to northern Mediterranean and from planar to alpine ecotone) makes it easier to colonize new areas. The great flexibility of the T. *hispidus*/*sericeus* complex also enabled its survival during several climatic changes within the Pleistocene, surviving in refugia during bad times and re-colonizing areas in good times. This might explain the great morphological and mitochondrial variability. Also, certain taxa closely related to the *T. hispidus*/*sericeus* complex, such as *T.*

striolatus, show a broad niche of suitable habitats and climate zones and sometimes even anthropochorous dispersal (Forsyth 2008). As opposed to *T. hispidus*, *T. striolatus* merely shows ecological and morphological, but no extremely high genetic variability.

T. oreinos exhibits a quite different phylogeographic history. It was probably always a stenoecious dweller of a very narrow ecological niche. Today, it inhabits cool, dry and primarily treeless habitats such as *caricetum-firmae* meadows with patchy structure; it is also found in boulder fields, which appear to be slightly azonal because they occur from below the timberline in subalpine up to the generally treeless alpine ecotone as well. This specific habitat might have been available throughout the Pleistocene, as it might have shifted up and down the slopes according to climate amplitudes. One restriction for this species was that large parts of its habitat were covered by an ice-shield during the glaciation, and the adjacent flatland areas apparently not did offer suitable conditions. Clearly, *T. oreinos* has restricted dispersal and colonization abilities due to its stenoecious habitat choice and the lack of overland corridors. Suitable habitats such as *Caricetum-firmae* with patchy structure exist all across the Northern Calcareous Alps, although only a very small, restricted area, which remained ice-free during the last glaciation (van Husen 1986), is actually settled. A similar distribution pattern can also be found in the endemic Helicidae land snail *Cylindrus obtusus* and several other invertebrates and plants (Essl & Rabitsch 2009). Within the genus *Trochulus*, the Swiss endemic *Trochulus biconicus* shows a similar ecological niche on a periglacial area (Baggenstoss & Niederer 2010).

Besides the main questions, the current study also sheds light on the evolution of hairs, a characteristic trait of the genus *Trochulus.* A tree derived from concatenated mt sequences pointed out the relationships within the genus *Trochulus*, showing a group of *Trochulus* s. str. consisting of nine *T. hispidus*/*sericeus* clades (including *T. coelomphala*), *T. villosus*, *T. villosuslus*, *T. clandestinus* and *T. striolatus*. *T. oreinos* and *T. biconicus* appear to be very basic to this species group, and *Plicuteria lubomirskii* was reconfirmed as being only distantly related to *Trochulus* s. str.. The suggestion of Proćków (2009) to consider the occurrence of very short hairs (<0.1mm, evident in *T. oreinos* and *P. lubomirskii*) or no hairs (evident in *T. biconicus*) as a plesiomorphic trait therefore appears plausible although the incompleteness of the current data situation has to be considered. Further investigations are required before drawing final conclusions about the relations within the Trochulini Lindholm, 1927 and the reliability of certain (sub)genera such as *Petasina* and *Edentiella*, which are viewed as separate units to *Trochulu*s s. str. by some authors (e. g. Falkner et al. 2001; Reischütz & Reischütz 2007).

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Appendix

Raw data 1: Shell measurements and shell traits of all morphologically investigated specimens in Paper 1 and 3. inID: individual identification numbers of specimens. spID: identification numbers of sample sites. Appearance in papers (1 = presence / 0 = absence): P1: paper 1, P2: paper 2; C: genetic clade, 1-9: Clades of the *T. hispidus /sericeus* complex, 10: *T. o. oreinos*, 11: *T. o. scheerpeltzi*; 12. *T. striolatus* ssp., #: empty shell - no genetical investigation; TA: taxonomic assignment, his: *T. hispidus/sericeus* complex, oor: *T. o. oreinos*, osc: *T. o. scheerpeltzi*, sst: *T. s. striolatus*, sda: *T. s. danubialis*, sju: *T. s. juvavensis*; Shell measurements (in millimetres): SH: shell height, WU: width of umbilicus, SH: shell height, HL: height of last whorl; shell traits (1 = presence / 0 = absence): IR: internal rib, BT: basal tooth, PA: pale area around aperture, G2: groove beneath the keel clearly visible with x16 magnification covering at least 50% of the circumference, G1: groove beneath the keel covered less than 50% and is only weakly visible, G0: no groove beneath the keel visible, R: coarse riffles; H2: hair longer than 0,2 mm, H2: hair shorter than 0,1mm, H0: hairless specimen

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
4175	418	0	1	1	his	6,8	1,2	4,0	3,1	1	0	1	0	0	1	0	1	0	0
4176	418	0	1	1	his	6,1	1,1	3,9	3	1	0	0	0	0	1	0	1	0	0
4177	418	0	1	1	his	6	0,7	3,2	2,7	0	0	0	0	0	1	0	1	0	0
4285	451	0	1	1	his	6	1,1	3,6	2,5	0	0	0	0	0	1	0	1	0	0
4286	451	0	1	1	his	7,3	1,2	4,2	3	0	0	0	0	0	1	0	1	0	0
4287	452	0	1	1	his	7,6	1,3	4	3	0	0	0	0	0	1	0	1	0	0
4292	454	0	1	1	his	7,8	1,4	5	3,5	1	0	0	0	0	1	1	0	0	1
4293	455	0	1	1	his	7,5	1,2	4,5	3,6	0	0	0	0	0	1	0	0	0	1
4294	455	0	1	1	his	7,5	0,9	5	3,8	0	0	0	0	0	1	0	1	0	0
100	104	1	1	2a	his	6,4	1,6	3,1	2,6	0	0	0	0	0	1	0	1	0	0
104	104	1	1	2a	his	6,7	1,5	3,5	2,8	0	0	0	0	0	1	0	1	0	0
107	102	1	1	2a	his	6,5	1,3	3,5	2,7	0	0	0	0	0	1	0	1	0	0
108	102	1	1	2a	his	6	1,2	2,9	2,6	0	0	0	0	0	1	0	1	0	0
109	102	1	1	2a	his	6,3	1,2	3,4	2,6	0	0	0	0	0	1	0	1	0	0
140	24	1	1	2a	his	8	1,6	4,2	3	1	0	1	0	0	1	0	1	0	0
141	24	1	1	2a	his	7,4	1,8	3,8	2,9	1	0	1	0	0	1	0	1	0	0
679	52	1	1	2a	his	7,5	1,6	3,9	2,8	1	1	1	0	0	1	1	1	0	0
680	52	1	1	2a	his	6,7	1,4	3,6	2,6	0	0	1	0	0	1	0	1	0	0
682	5	1	1	2a	his	6,8	1,7	3,5	2,6	0	0	0	0	0	1	0	1	0	0
683	5	1	1	2a	his	8	2	4	2,7	1	0	0	0	0	1	0	0	0	1
688	50	1	1	2a	his	7,8	1,8	3,9	2,8	1	0	0	0	0	1	0	1	0	0
689	50	1	1	2a	his	6,7	1,4	3,2	2,7	0	0	0	0	0	1	0	1	0	0
729	32	1	1	2a	his	6,6	1,4	3,6	2,6	0	0	0	0	0	1	0	1	0	0
730	32	1	1	2a	his	5,7	1,1	3,3	2,5	0	0	0	0	0	1	0	1	0	0
734	130	1	1	2a	his	7	1,7	3,5	2,8	0	0	0	0	0	1	0	1	0	0
735	130	1	1	2a	his	8,3	1,7	4,3	3,4	1	1	1	0	0	1	0	1	0	0
736	130	1	1	2a	his	7,6	1,3	4,1	3	1	1	0	0	0	1	0	1	0	0
752	42	1	1	2a	his	7,2	1,5	4,1	3	1	1	0	0	0	1	0	0	0	1

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
926	140	1	1	2a	his	7,1	1,8	3,6	2,6	0	0	0	0	0	1	0	1	0	0
927	140	1	1	2a	his	6,7	1,7	3,6	2,9	0	0	0	0	0	1	0	1	0	0
928	140	1	1	2a	his	7,8	1,7	4,4	3,2	1	0	0	0	0	1	0	1	0	0
929	140	1	1	2a	his	6,9	1,6	3,8	2,9	1	0	0	0	0	1	0	1	0	0
930	140	1	1	2a	his	6,2	1,6	3,2	2,6	0	0	0	0	0	1	0	1	0	0
931	140	1	1	2a	his	7,1	1,7	3,8	3	1	0	0	0	0	1	0	1	0	0
932	140	1	1	2a	his	7,6	1,7	3,9	3	1	0	0	0	0	1	0	1	0	0
933	140	1	1	2a	his	6,8	1,8	3,6	2,7	0	0	0	0	0	1	0	1	0	0
934	140	1	1	2a	his	7,2	1,6	3,8	2,9	1	0	0	0	0	1	0	1	0	0
935	140	1	1	2a	his	6,6	1,5	3,3	2,6	1	0	1	0	0	1	0	1	0	0
937	145	1	1	2a	his	7,8	1,5	4,2	3,1	1	0	0	0	0	1	0	0	0	1
938	145	1	1	2a	his	7,9	1,7	4	2,9	0	0	0	0	0	1	0	1	0	0
939	145	1	1	2a	his	7,7	1,5	4,4	3,3	1	0	0	0	0	1	0	1	0	0
948	144	1	1	2a	his	7,8	1,8	4	3	0	0	1	0	0	1	0	1	0	0
949	144	1	1	2a	his	6,3	1,7	3,4	2,5	0	0	0	0	0	1	0	1	0	0
950	144	1	1	2a	his	7,7	1,6	4	3	0	0	0	0	0	1	0	1	0	0
982	157	1	1	2a	his	7,8	1,7	4,2	2,8	1	0	1	0	0	1	0	1	0	0
983	157	1	1	2a	his	7,5	1,3	4,1	3,2	1	0	0	0	0	1	0	1	0	0
984	157	1	1	2a	his	8,2	2,3	4	3,2	1	0	1	0	0	1	0	1	0	0
1007	158	1	1	2a	his	7,6	1,8	3,6	3,1	1	0	0	0	0	1	0	1	0	0
1008	158	1	1	2a	his	6,5	1,3	3,4	2,4	0	0	1	0	0	1	0	1	0	0
1019	160	1	1	2a	his	6,4	1,3	3,3	2,6	0	0	0	0	0	1	0	1	0	0
1020	160	1	1	2a	his	7	1,4	3,5	2,8	1	0	0	0	0	1	0	1	0	0
1294	168	1	1	2a	his	6	1	3,1	2,5	0	0	1	0	0	1	0	1	0	0
1295	168	1	1	2a	his	6,7	1,6	3,3	2,4	1	0	0	0	0	1	0	1	0	0
1296	168	1	1	2a	his	7	1,7	3,4	2,1	1	0	1	0	0	1	0	1	0	0
1331	167	1	1	2a	his	6,3	1,7	3,4	1,9	1	0	0	0	0	1	0	1	0	0
1332	167	1	1	2a	his	7,1	1,5	3,8	2,4	1	1	1	0	0	1	0	1	0	0
1582	140	1	0	#	his	7,8	1,9	4,4	3,1	1	0	1	0	0	1	0	0	0	1
1738	145	1	0	#	his	6,7	1,5	3,6	2,9	1	1	0	0	0	1	0	0	0	1
1739	145	1	0	#	his	6,2	1,2	3,7	2,8	0	0	0	0	0	1	0	1	0	0
1747	168	1	0	#	his	6,3	1,5	3,7	2,6	1	1	1	0	0	1	0	1	0	0
1748	168	1	0	#	his	7,0	1,9	3,7	2,7	1	0	1	0	0	1	1	1	0	0
1749	168	1	0	#	his	6,5	1,7	3,5	2,5	1	0	1	0	0	1	1	1	0	0
1750	168	1	0	#	his	6,6	1,7	3,7	2,5	1	1	1	0	0	1	0	1	0	0
1751	168	1	0	#	his	5,5	1,2	3,0	2,1	1	0	1	0	0	1	1	0	0	1
1752	160	1	0	#	his	6,8	1,7	4,1	2,9	0	0	0	0	0	1	0	0	0	1
1755	50	1	0	#	his	7,8	2,0	4,0	3,1	1	1	1	0	0	1	1	0	0	1
1757	50	1	0	#	his	7,6	1,7	4,2	3,2	0	0	0	0	0	1	0	1	0	0
1759	52	1	0	#	his	7,5	1,6	4,1	3,0	1	0	0	0	0	1	0	0	0	1
1822	157	1	1	2a	his	6,7	1,4	3,8	2,8	0	0	0	0	0	1	0	1	0	0
1832	208	1	1	2a	his	5,8	1,2	3,2	2,7	0	0	0	0	0	1	0	1	0	0
2000	237	1	1	2a	his	7,7	1,9	4	2,9	1	1	0	0	0	1	0	1	0	0
2984	306	1	1	2a	his	6,7	1,4	3,5	2,7	0	0	0	0	0	1	0	1	0	0
2985	306	1	1	2a	his	7,3	1,6	3,7	2,9	1	0	0	0	0	1	0	1	0	0
2986	306	1	1	2a	his	8,3	1,6	4,7	3,5	1	0	0	0	0	1	0	1	0	0
2987	306	1	0	#	his	8,1	1,5	4,6	3,2	1	0	0	0	0	1	0	1	0	0
2988	306	1	0	#	his	7,6	1,5	4,2	3,0	0	0	0	0	0	1	0	1	0	0
2989	306	1	0	#	his	7,7	1,6	4,0	2,9	0	0	0	0	0	1	0	1	0	0
2990	306	1	0	#	his	7,1	1,5	3,8	2,6	0	0	0	0	0	1	0	1	0	0
2991	306	1	0	#	his	7,1	1,7	3,7	2,8	0	0	0	0	0	1	0	1	0	0
2992	306	1	0	#	his	8,4	1,8	4,3	3,3	0	0	0	0	0	1	0	1	0	0
2993	306	1	0	#	his	6,4	1,4	3,5	2,9	0	0	0	0	0	1	0	1	0	0
2994	311	1	1	2a	his	8	1,5	4,3	3,1	0	0	0	0	0	1	0	1	0	0
2995	311	1	1	2a	his	7,6	1,9	3,9	3,2	1	0	0	0	0	1	0	1	0	0
2996	311	1	1	2a	his	7,5	1,4	4,1	2,8	0	0	0	0	0	1	0	1	0	0
2997	311	1	0	#	his	7,4	1,5	3,8	2,7	0	0	0	0	0	1	0	1	0	0
2998	311	1	0	#	his	7,4	1,7	3,6	3,0	0	0	0	0	0	1	0	1	0	0

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
2999	311	1	0	#	his	7,7	1,7	3,9	2,8	0	0	0	0	0	1	0	1	0	0
3000	311	1	0	#	his	7,5	2,0	3,5	2,7	0	0	0	0	0	1	0	1	0	0
3001	311	1	0	#	his	7,5	1,6	4,0	2,8	0	0	0	0	0	1	0	1	0	0
3002	311	1	0	#	his	8,0	1,3	4,4	3,0	1	0	0	0	0	1	0	1	0	0
3003	311	1	0	#	his	8,0	1,6	4,0	2,9	1	0	0	0	0	1	0	1	0	0
3004	313	1	1	2a	his	7,5	1,5	3,6	2,7	1	0	0	0	0	1	0	1	0	0
3005	313	1	1	2a	his	8,4	2	4,1	3,2	1	1	0	0	0	1	0	1	0	0
3006	313	1	1	2a	his	7,7	1,8	3,9	2,9	1	0	0	0	0	1	0	1	0	0
3007	313	1	0	#	his	7,6	1,8	3,6	2,6	1	0	0	0	0	1	0	1	0	0
3008	313	1	0	#	his	7,5	1,7	3,8	2,7	1	0	0	0	0	1	0	1	0	0
3009	313	1	0	#	his	6,9	1,6	3,2	2,5	0	0	0	0	0	1	0	1	0	0
3010	313	1	0	#	his	7,2	1,9	3,8	2,7	1	0	0	0	0	1	0	1	0	0
3011	313	1	0	#	his	7,2	1,7	3,4	2,6	0	0	0	0	0	1	0	1	0	0
3012	313	1	0	#	his	8,0	1,6	4,0	3,2	1	0	1	0	0	1	0	1	0	0
3013	313	1	0	#	his	8,0	1,7	4,1	3,0	1	0	0	0	0	1	0	1	0	0
3014	315	1	1	2a	his	7,2	1,5	3,9	2,9	1	1	0	0	0	1	0	1	0	0
3015	315	1	1	2a	his	7,5	1,6	4	3	1	1	0	0	0	1	0	1	0	0
3016	315	1	1	2a	his	7,4	1,6	4	2,9	1	0	0	0	0	1	0	1	0	0
3019	315	1	0	#	his	6,2	1,4	3,4	2,4	0	0	0	0	0	1	0	1	0	0
3020	315	1	0	#	his	7,4	1,2	4,6	2,6	1	1	0	0	0	1	0	1	0	0
3022	315	1	0	#	his	7,0	1,6	4,0	2,9	1	0	0	0	0	1	0	1	0	0
3133	317	1	1	2a	his	7,8	1,9	4	3	1	1	0	0	0	1	0	1	0	0
3134	317	1	1	2a	his	7,8	1,9	4,1	3,2	1	1	0	0	0	1	1	1	0	0
3135	317	1	1	2a	his	7,6	1,8	3,9	3	1	0	0	0	0	1	1	1	0	0
3136	317	1	0	#	his	8,0	2,1	3,9	3,1	1	0	0	0	0	1	0	1	0	0
3138	318	1	1	2a	his	7	1,7	3,5	2,5	0	0	0	0	0	1	0	1	0	0
3139	318	1	1	2a	his	7,5	1,6	4,2	3	1	1	0	0	0	1	0	1	0	0
3145	319	1	1	2a	his	7	1,3	4	2,6	0	0	0	0	0	1	0	1	0	0
3146	319	1	0	#	his	6,7	1,6	3,6	2,7	0	0	0	0	0	1	0	1	0	0
3147	319	1	0	#	his	7,2	1,5	4,1	3,2	1	0	1	0	0	1	0	1	1	0
3148	319	1	0	#	his	6,3	1,3	3,3	2,5	0	0	0	0	0	1	0	1	0	0
3149	319	1	0	#	his	6,9	1,4	3,7	2,9	1	1	0	0	0	1	0	1	0	0
3150	319	1	0	#	his	6,2	1,3	3,4	2,6	0	0	0	0	0	1	0	1	0	0
3151	319	1	0	#	his	6,4	1,3	3,7	2,8	0	0	0	0	0	1	0	1	0	0
3152	319	1	0	#	his	6,1	1,3	3,6	2,7	0	0	0	0	0	1	0	0	0	1
7039	319	1	0	#	his	7,7	1,6	4,2	2,1	0	0	0	0	0	1	0	1	0	0
7040	319	1	0	#	his	7,7	1,5	4,0	3,0	0	0	0	0	0	1	0	1	0	0
102	33	0	1	2a	his	8,7	1,9	4,5	3,8	1	0	1	0	0	1	0	1	0	0
103	33	0	1	2a	his	9,1	1,8	4,9	3,3	1	0	0	0	0	1	0	1	0	0
105	104	0	1	2a	his	5,2	1,1	2,8	2,3	0	0	0	0	0	1	0	1	0	0
112	33	0	1	2a	his	8,1	1,5	4,9	3,6	0	0	0	0	0	1	0	1	0	0
659	3	0	1	2a	his	8,2	1,6	4,1	3,2	1	0	1	0	0	1	0	1	0	0
663	3	0	1	2a	his	8,1	1,6	4	3,3	1	0	1	0	0	1	0	1	0	0
666	3	0	1	2a	his	8,1	1,9	4,4	3,1	1	0	0	0	0	1	0	1	0	0
667	3	0	1	2a	his	8,1	1,4	4,5	3,7	1	0	0	0	0	1	0	1	0	0
690	50	0	1	2a	his	6	1,2	3,2	2,7	0	0	0	0	0	1	0	1	0	0
726	94	0	1	2a	his	6,1	0,7	4,7	2,7	0	0	0	0	0	1	0	1	0	0
763	64	0	1	2a	his	5,7	1,4	2,7	2,4	0	0	0	0	0	1	0	1	0	0
765	64	0	1	2a	his	6,9	1,6	3,9	2,8	1	1	0	0	0	1	0	1	0	0
768	66	0	1	2a	his	6,9	1,4	4,2	3	1	0	0	0	0	1	0	1	0	0
769	66	0	1	2a	his	6	1,3	3,2	2,5	0	0	0	0	0	1	0	1	0	0
851	545	0	1	2a	his	6,3	1,4	3,6	2,2	1	0	0	0	0	1	0	1	0	0
852	159	0	1	2a	his	8,2	2,2	4,2	2,9	1	0	0	0	0	1	0	1	0	0
853	159	0	1	2a	his	7,2	1,8	3,6	2,7	1	0	0	0	0	1	0	1	0	0
925	159	0	1	2a	his	7,9	1,8	3,6	2,9	1	0	0	0	0	1	0	1	0	0
993	159	0	1	2a	his	7,5	2	4,1	2,6	1	0	1	0	0	1	0	1	0	0
994	159	0	1	2a	his	8	2	3,9	2,8	1	0	1	0	0	1	0	1	0	0
1460	204	0	1	2a	his	6,9	1,3	4,1	2,7	0	0	0	0	0	1	0	1	0	0

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
1462	205	0	1	2a	his	7,5	1,6	4,2	3	0	0	0	0	0	1	0	1	0	0
1463	205	0	1	2a	his	7	1,3	4,1	3,1	0	0	0	0	0	1	0	1	0	0
1464	205	0	1	2a	his	8	1,6	4,1	3,1	0	0	0	0	0	1	0	1	0	0
1472	223	0	1	2a	his	7,7	1,9	3,9	3	0	0	0	0	0	1	0	1	0	0
1473	223	0	1	2a	his	7,7	1,7	4	2,9	1	1	0	0	0	1	0	1	0	0
1481	204	0	1	2a	his	7,3	1,1	4,3	3,1	0	0	0	0	0	1	0	1	0	0
1482	204	0	1	2a	his	7	1	4,2	3,2	0	0	0	0	0	1	0	1	0	0
1802	215	0	1	2a	his	5,4	0,4	3,4	2,6	0	0	0	0	0	1	0	1	0	0
1834	231	0	1	2a	his	7,4	1,2	4,3	3,1	0	0	0	0	0	1	0	1	0	0
1836	231	0	1	2a	his	5,5	1	3,1	2,4	0	0	0	0	0	1	0	1	0	0
2879	286	0	1	2a	his	8,2	1,7	4,3	3,3	0	0	0	0	0	1	0	1	0	0
2880	286	0	1	2a	his	8,3	1,5	4,4	3,3	1	0	0	0	0	1	0	1	0	0
2884	285	0	1	2a	his	7,4	1,3	4	3,1	1	0	0	0	0	1	0	1	0	0
2982	299	0	1	2a	his	7,3	1,7	3,5	2,8	1	1	0	0	0	1	0	1	0	0
2983	299	0	1	2a	his	7,5	1,8	3,8	3	1	0	0	0	0	1	0	1	0	0
3154	323	0	1	2a	his	7,8	1,5	4	3	1	0	0	0	0	1	0	1	0	0
3155	323	0	1	2a	his	8,7	2,2	4,3	3,3	1	0	0	0	0	1	0	1	0	0
3163	327	0	1	2a	his	7,5	1,8	4,1	3,1	1	0	0	0	0	1	0	1	0	0
3164	327	0	1	2a	his	7,7	1,7	4	3	1	0	0	0	0	1	0	1	0	0
3165	327	0	1	2a	his	8,4	2	4,4	3,4	1	1	0	0	0	1	0	1	0	0
3307	341	0	1	2a	his	6,4	1,5	3	2,3	0	0	0	0	0	1	0	1	0	0
3308	341	0	1	2a	his	7	1,4	3,7	2,8	1	0	0	0	0	1	0	1	0	0
3309	344	0	1	2a	his	8	1,8	4,1	2,9	1	1	0	0	0	1	0	1	0	0
3414	361	0	1	2a	his	8,3	1,7	4,4	3,4	1	1	0	0	0	1	0	0	0	1
3416	361	0	1	2a	his	7,3	1,5	3,8	2,9	0	0	0	0	0	1	0	1	0	0
3575	386	0	1	2a	his	5,5	1	2,8	2,3	0	0	0	0	0	1	0	1	0	0
3907	385	0	1	2a	his	7,2	1,6	3,7	2,5	1	0	0	0	0	1	0	1	0	0
4055	380	0	1	2a	his	6,5	1,5	3,5	2,7	1	0	0	0	0	1	0	1	0	0
4056	380	0	1	2a	his	6,8	1,2	4,1	3,8	0	0	0	0	0	1	0	1	0	0
4057	380	0	1	2a	his	7,9	1,6	4,5	3,6	1	1	0	0	0	1	0	1	0	0
4135	385	0	1	2a	his	7,2	1,4	3,8	2,5	1	0	0	0	0	1	0	1	0	0
4136	385	0	1	2a	his	7,2	1,5	3,9	2,8	1	0	0	0	0	1	0	1	0	0
4263	446	0	1	2a	his	7,5	1,3	4,3	3,2	0	0	0	0	0	1	0	1	0	0
4264	446	0	1	2a	his	5,7	1	3,3	2,6	0	0	0	0	0	1	0	1	0	0
4265	446	0	1	2a	his	5,7	0,8	3,2	2,8	0	0	0	0	0	1	0	0	0	1
6236	548	0	1	2a	his	7,3	1,1	4,2	3,2	1	0	0	0	0	1	0	1	0	0
6403	534	0	1	2a	his	7,3	1,6	3,4	1,6	1	0	0	0	0	1	0	1	0	0
6404	548	0	1	2a	his	6,9	1	4,1	3	0	0	0	0	0	1	0	1	0	0
6407	548	0	1	2a	his	6,5	1,3	3,9	2,8	0	0	0	0	0	1	0	1	0	0
6409	548	0	1	2a	his	7,1	0,9	4,4	3,2	0	0	0	0	0	1	0	1	0	0
764	64	0	1	2b	his	7,1	1,5	4,8	2,9	1	0	0	0	0	1	0	1	0	0
1455	200	0	1	2b	his	7,7	1,6	4,2	3,4	1	0	0	0	0	1	0	1	0	0
1456	200	0	1	2b	his	7,8	1,7	4,2	3,2	1	0	0	0	0	1	0	1	0	0
1471	223	0	1	2b	his	8	2,1	4	3,1	1	0	0	0	0	1	0	1	0	0
2065	64	0	1	2b	his	7,9	1,9	4,3	3,1	1	1	0	0	0	1	0	1	0	0
3310	344	0	1	2b	his	7,3	1,7	3,4	2,8	1	0	1	0	0	1	0	1	0	0
3311	344	0	1	2b	his	8	2	3,6	2,8	1	0	1	0	0	1	0	1	0	0
4145	402	0	1	2b	his	7,2	1,7	3,7	3	0	0	0	0	0	1	0	1	0	0
4146	402	0	1	2b	his	7,3	1,8	3,8	2,8	1	0	0	0	0	1	0	1	0	0
4147	402	0	1	2b	his	6,9	1,8	3,2	2,9	0	0	0	0	0	1	0	1	0	0
1303	168	1	0	#	his	6,8	1,6	3,8	2,4	1	1	0	0	0	1	0	1	0	0
3137	318	1	0	#	his	7,6	1,8	3,9	2,8	1	0	0	0	0	1	1	1	0	0
113	33	0	1	3a	his	9	2,2	4,8	3,3	0	0	0	0	0	1	0	1	0	0
1474	217	0	1	3a	his	7,5	1,1	4,4	3,6	1	0	0	0	0	1	0	1	0	0
1475	217	0	1	3a	his	6,5	1,1	4,1	3	1	0	0	0	0	1	0	1	0	0
1476	217	0	1	3a	his	6,6	1,1	3,7	3	1	1	0	0	0	1	0	1	0	0
1803	215	0	1	3a	his	5,3	0,6	3,3	2,7	0	0	0	0	0	1	0	1	0	0
1804	215	0	1	3a	his	5,5	0,6	3,4	2,6	0	0	0	0	0	1	0	1	0	0

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
1812	217	0	1	3a	his	6	0,5	3,9	3	0	0	0	0	0	1	0	1	0	0
1813	217	0	1	3a	his	6,8	1	4,3	3,2	0	0	0	0	0	1	0	1	0	0
2850	292	0	1	3a	his	6,8	2	3,4	2,8	0	0	0	0	0	1	0	1	0	0
2851	292	0	1	3a	his	8,5	2,2	4,5	3,3	1	0	0	0	0	1	0	1	0	0
2881	288	0	1	3a	his	8,6	1,9	5	2,9	1	0	0	0	0	1	0	1	0	0
2882	288	0	1	3a	his	8,4	1,7	5	3,2	1	0	1	0	0	1	0	1	0	0
2899	291	0	1	3a	his	9,3	2	4,7	3,6	1	1	0	0	0	1	0	1	0	0
2900	291	0	1	3a	his	8,4	1,8	4,9	3,3	1	0	1	0	0	1	0	1	0	0
2901	291	0	1	3a	his	8,6	2,5	4,8	3,5	1	0	1	0	0	1	0	1	0	0
4155	407	0	1	3a	his	5,3	0,6	3,3	2,6	0	0	0	0	0	1	0	1	0	0
4156	407	0	1	3a	his	5,7	0,6	3,8	2,8	0	0	0	0	0	1	0	1	0	0
4157	407	0	1	3a	his	6	0,6	3,8	3	0	0	0	0	0	1	0	1	0	0
4165	412	0	1	3a	his	5,5	0,6	3,7	2,8	0	0	0	0	0	1	0	1	0	0
4166	412	0	1	3a	his	5,8	0,7	3,6	3	0	0	0	0	0	1	0	1	0	0
4167	412	0	1	3a	his	5,7	0,7	3,6	3,1	0	0	0	0	0	1	0	1	0	0
5591	483	0	1	3a	his	6,2	1,1	3,4	2,5	0	0	0	0	0	1	0	1	0	0
6233	549	0	1	3a	his	6,1	0,6	3,6	2,9	0	0	0	0	0	1	0	1	0	0
6410	549	0	1	3a	his	7	1	4,5	3,1	0	0	0	0	0	1	0	1	0	0
6411	549	0	1	3a	his	7,2	1,1	4,3	3,4	0	0	0	0	0	1	0	1	0	0
6414	550	0	1	3a	his	6,3	1	4,1	2,4	1	1	0	0	0	1	0	1	0	0
849	545	0	1	3b	his	6,7	1,5	3,7	2,4	1	0	0	0	0	1	0	1	0	0
850	545	0	1	3b	his	6	1,2	3,1	2,3	0	0	0	0	0	1	1	1	0	0
992	159	0	1	3b	his	7,1	1,7	3,7	2,9	0	0	0	0	0	1	0	1	0	0
5539	476	0	1	5	his	6	1,3	3,1	2,5	0	0	0	0	0	1	0	0	0	0
101	33	0	1	6a	his	8,7	2	4,7	3,3	0	0	0	0	0	1	1	1	0	0
111	33	0	1	6a	his	9	1,8	5,3	3,5	1	0	1	0	0	1	0	1	0	0
249	33	0	1	6a	his	8,5	1,8	4,5	3,6	1	0	0	0	0	1	1	1	0	0
660	3	0	1	6a	his	8,5	1,8	4,1	3,2	1	0	0	0	0	1	0	1	0	0
664	3	0	1	6a	his	8,1	1,7	4,4	3,3	1	0	1	0	0	1	0	1	0	0
665	3	0	1	6a	his	7,1	1,6	4,2	2,8	1	0	1	0	0	1	0	1	0	0
2889	296	0	1	6a	his	8	1,9	4,8	2,9	1	0	1	0	0	1	0	1	0	0
2890	296	0	1	6a	his	7,4	1,9	4,1	2,9	1	0	1	0	0	1	0	1	0	0
2891	296	0	1	6a	his	8,2	1,9	4,9	3,4	1	0	1	0	0	1	1	1	0	0
6234	549	0	1	6a	his	5,7	0,6	3,5	2,6	1	0	0	0	0	1	0	1	0	0
6413	549	0	1	6a	his	6,2	1	3,8	2,9	0	0	0	0	0	1	0	1	0	0
5587	482	0	1	6b	his	6,3	1,6	3,6	2,3	0	0	0	0	0	1	0	0	0	1
5589	482	0	1	6b	his	7,1	1,2	3,9	2,8	1	0	0	0	0	1	0	0	0	1
6238	556	0	1	8a	his	6	0,7	3,7	2,8	1	0	0	0	0	1	0	0	0	1
6240	556	0	1	8a	his	6,1	0,6	3,7	2,7	0	0	0	0	0	1	0	1	0	0
6245	561	0	1	8a	his	5,7	0,6	3,5	2,8	0	0	0	0	0	1	0	1	0	0
6246	561	0	1	8a	his	5,3	0,8	3,3	2,6	0	0	0	0	0	1	0	1	0	0
6248	555	0	1	8a	his	6,2	0,8	3,8	2,7	1	0	0	0	0	1	0	1	0	0
6249	555	0	1	8a	his	6,5	0,8	4	2,7	1	0	0	0	0	1	0	1	0	0
6250	541	0	1	8a	his	7,7	1,1	3,9	3,2	0	9	0	0	0	1	0	1	0	0
2079	248	0	1	8b	his	8,1	0,9	5,5	2,9	0	0	0	0	0	1	0	1	0	0
2080	248	0	1	8b	his	6,5	0,6	4,7	2,8	1	0	0	0	0	1	0	1	0	0
6229	550	0	1	9	his	6,1	1,1	3,7	2,8	0	0	0	0	0	1	0	1	0	0
6230	550	0	1	9	his	5,7	1,1	3,2	2,5	0	0	0	0	0	1	0	1	0	0
6231	550	0	1	9	his	6,4	1	3,6	2,9	0	0	0	0	0	1	0	1	0	0
6235	548	0	1	9	his	6,7	1,3	3,9	2,8	1	0	0	0	0	1	0	1	0	0
6237	548	0	1	9	his	6,5	1,2	4	2,8	1	0	0	0	0	1	0	1	0	0
6405	548	0	1	9	his	7,9	1,2	4,7	3,3	0	0	0	0	0	1	0	1	0	0
6406	548	0	1	9	his	7	1	4,5	3,1	0	0	0	0	0	1	0	1	0	0
6408	548	0	1	9	his	7,6	1	4,5	3	0	0	0	0	0	1	0	1	0	0
6412	549	0	1	9	his	6,7	0,7	3,9	3	0	0	0	0	0	1	0	1	0	0
6415	550	0	1	9	his	6,6	1,1	3,8	2,8	0	0	0	0	0	1	0	1	0	0
6416	550	0	1	9	his	7	1,3	4,2	3	0	0	0	0	0	1	0	1	0	0
6417	550	0	1	9	his	7,3	1,3	4	3,1	0	0	0	0	0	1	0	1	0	0

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
707	55	1	1	10	oor	6,6	1,3	5,1	3,2	1	1	1	0	0	1	1	0	1	0
708	55	1	1	10	oor	7,3	1,4	5,2	3,5	1	1	1	0	0	1	1	0	0	1
709	55	1	1	10	oor	7,1	1,3	5,5	3,9	1	1	1	0	1	0	1	0	0	1
710	55	1	1	10	oor	5,9	0,9	6,6	3	0	0	0	0	0	1	1	0	1	0
711	55	1	1	10	oor	6	1,1	5,5	3	0	0	0	0	0	1	1	0	1	0
712	55	1	1	10	oor	7	1,3	5,4	4,1	1	1	1	0	0	1	1	0	1	0
754	79	1	1	10	oor	5,9	1,2	4,9	3,1	1	1	1	0	0	1	1	0	1	0
999	134	1	1	10	oor	5,9	1	5,9	3,4	0	0	0	0	0	1	1	0	0	1
1000	134	1	1	10	oor	6,3	1,3	4,8	3,4	1	1	1	0	1	0	1	0	0	1
1317	172	1	1	10	oor	6,9	1,4	4,9	4	0	0	1	0	0	1	1	0	1	0
1320	178	1	1	10	oor	6,7	1,3	5,2	3,5	1	1	1	0	0	1	1	0	1	0
1321	178	1	1	10	oor	6	1,1	5,5	3	1	1	1	0	0	1	1	0	1	0
1323	178	1	1	10	oor	6,5	1,1	5,9	3,3	1	1	1	0	0	1	1	0	1	0
1585	79	1	0	#	oor	7,5	1,5	3,8	2,8	1	1	1	0	0	1	1	0	0	1
1586	79	1	0	#	oor	6,1	1,2	3,1	2,3	1	1	1	0	0	1	1	0	0	1
1587	79	1	0	#	oor	5,7	0,8	3,0	2,3	0	0	1	0	0	1	1	0	0	1
1590	79	1	0	#	oor	6,0	1,3	3,5	2,4	1	1	1	0	0	1	1	0	0	1
1591	79	1	0	#	oor	6,5	1,3	3,8	2,4	1	1	1	0	0	1	1	0	0	1
1593	79	1	0	#	oor	6,9	1,4	3,7	2,4	1	1	1	0	0	1	1	0	0	1
1594	79	1	0	#	oor	6,6	1,1	3,5	2,5	1	1	1	0	0	1	1	0	0	1
1595	79	1	0	#	oor	6,2	1,3	3,2	2,3	1	1	1	0	0	1	1	0	0	1
1599	79	1	0	#	oor	6,1	1,1	3,5	2,4	0	0	0	0	0	1	1	0	0	1
1600	79	1	0	#	oor	7,1	1,6	3,7	2,5	1	1	1	0	0	1	1	0	0	1
1603	79	1	0	#	oor	5,6	1,1	3,4	2,0	1	0	1	0	0	1	1	0	0	1
1604	79	1	0	#	oor	6,5	1,1	3,7	2,4	1	1	1	0	0	1	1	0	0	1
1605	79	1	0	#	oor	6,4	1,3	3,6	2,4	1	1	1	0	0	1	1	0	0	1
1607	79	1	0	#	oor	6,7	1,3	3,8	2,6	1	1	1	0	0	1	1	0	0	1
1608	79	1	0	#	oor	6,3	1,1	3,8	2,3	1	1	1	0	0	1	1	0	0	1
1610	79	1	0	#	oor	7,2	1,4	3,8	2,5	1	0	1	0	0	1	1	0	0	1
1612	79	1	0	#	oor	5,2	0,9	3,0	2,2	0	0	0	0	0	1	1	0	1	0
1613	79	1	0	#	oor	6,5	1,4	3,9	2,5	1	1	1	0	0	1	1	0	0	1
1615	79	1	0	#	oor	6,3	1,2	3,5	2,3	1	1	1	0	0	1	1	0	0	1
1618	79	1	0	#	oor	6,9	1,3	4,0	2,4	1	1	1	0	0	1	1	0	0	1
1619	79	1	0	#	oor	5,8	1,1	3,2	2,2	1	1	1	0	0	1	1	0	0	1
1624	79	1	0	#	oor	6,5	1,2	3,6	2,5	1	1	1	0	0	1	1	0	0	1
1625	79	1	0	#	oor	6,1	1,3	3,3	2,2	1	1	1	0	0	1	1	0	0	1
1626	79	1	0	#	oor	6,4	1,3	3,3	2,3	1	0	1	0	0	1	1	0	0	1
1627	79	1	0	#	oor	6,4	1,3	3,3	2,3	1	1	1	0	0	1	1	0	0	1
1628	79	1	0	#	oor	7,0	1,4	4,2	2,6	1	1	1	0	0	1	1	0	0	1
1631	79	1	0	#	oor	6,5	1,5	3,4	2,3	1	1	1	0	0	1	1	0	0	1
1632	79	1	0	#	oor	6,5	1,5	3,6	2,6	1	1	1	0	0	1	1	0	0	1
1633	79	1	0	#	oor	6,1	1,3	3,2	2,3	0	0	0	0	0	1	1	0	1	0
1634	79	1	0	#	oor	6,3	1,1	3,2	2,5	0	0	1	0	0	1	1	0	0	1
1635	172	1	0	#	oor	6,6	1,3	3,4	2,6	1	1	1	0	0	1	1	0	0	1
1636	172	1	0	#	oor	6,3	1,1	3,4	2,3	0	0	0	0	0	1	1	0	0	1
1637	172	1	0	#	oor	6,6	1,4	3,4	2,5	1	1	1	0	0	1	1	0	0	1
1641	172	1	0	#	oor	6,5	1,3	3,8	2,4	1	1	1	0	0	1	1	0	0	1
1642	172	1	0	#	oor	5,5	1,1	3,0	2,1	0	0	0	0	0	1	1	0	0	1
1643	55	1	0	#	oor	6,8	1,5	3,7	2,4	1	1	1	0	0	1	1	0	0	1
1646	55	1	0	#	oor	7,0	1,6	3,8	2,7	1	0	1	0	0	1	1	0	0	1
1647	55	1	0	#	oor	6,6	1,2	3,6	2,6	1	1	1	0	0	1	1	0	0	1
1651	178	1	0	#	oor	6,0	1,1	3,2	2,5	0	0	1	0	0	1	1	0	1	0
1652	178	1	0	#	oor	6,3	1,4	3,5	2,3	1	1	1	0	0	1	1	0	0	1
1653	178	1	0	#	oor	6,4	1,3	3,6	2,5	1	1	1	0	0	1	1	0	0	1
1654	178	1	0	#	oor	6,5	1,3	3,7	2,5	1	1	1	0	0	1	1	0	0	1
1655	178	1	0	#	oor	6,1	1,2	3,7	2,5	1	1	1	0	0	1	1	0	0	1
1657	178	1	0	#	oor	6,1	1,2	3,2	2,3	1	1	1	0	0	1	1	0	1	0
1658	178	1	0	#	oor	6,3	1,2	3,3	2,4	1	1	1	0	0	1	1	0	0	1

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
1659	178	1	0	#	oor	6,8	1,4	3,6	2,6	1	1	1	0	0	1	1	0	0	1
1661	178	1	0	#	oor	5,8	1,1	3,2	2,3	0	0	1	0	0	1	1	0	0	1
1662	178	1	0	#	oor	6,5	1,2	3,5	2,5	1	1	1	0	0	1	1	0	0	1
1664	178	1	0	#	oor	6,4	1,3	3,3	2,5	1	1	1	0	0	1	1	0	0	1
1665	178	1	0	#	oor	5,9	1,5	3,0	2,4	1	1	1	0	0	1	1	0	0	1
1667	178	1	0	#	oor	6,9	1,5	3,5	2,6	1	1	1	0	0	1	1	0	0	1
1668	178	1	0	#	oor	6,5	1,2	3,6	2,4	1	0	1	0	0	1	1	0	0	1
1669	178	1	0	#	oor	6,6	1,4	3,8	2,5	1	1	1	0	0	1	1	0	0	1
1671	178	1	0	#	oor	6,8	1,4	3,6	2,7	1	1	1	0	0	1	1	0	0	1
1672	178	1	0	#	oor	6,3	1,3	3,5	2,3	1	0	1	0	0	1	1	0	0	1
1673	178	1	0	#	oor	5,9	1,1	3,2	2,5	0	0	1	0	0	1	1	0	0	1
1674	178	1	0	#	oor	6,5	1,5	3,3	2,4	1	1	1	0	0	1	1	0	0	1
1676	178	1	0	#	oor	5,8	1,1	3,2	2,2	0	0	0	0	0	1	1	0	0	1
1677	178	1	0	#	oor	6,6	1,5	3,3	2,5	1	1	1	0	0	1	1	0	0	1
1678	178	1	0	#	oor	6,2	1,2	3,2	2,3	1	1	1	0	0	1	1	0	0	1
1679	178	1	0	#	oor	6,5	1,2	3,5	2,4	1	1	1	0	0	1	1	0	0	1
1680	178	1	0	#	oor	5,4	0,9	3,3	2,4	1	1	1	0	0	1	1	0	0	1
1683	178	1	0	#	oor	6,2	1,4	3,5	2,3	1	1	1	0	0	1	1	0	0	1
1685	178	1	0	#	oor	6,0	1,4	3,2	2,4	1	1	1	0	0	1	1	0	0	1
1686	178	1	0	#	oor	6,7	1,2	3,7	2,7	1	1	1	0	0	1	1	0	0	1
1688	178	1	0	#	oor	7,0	1,4	3,8	2,6	1	1	1	0	0	1	1	0	0	1
1693	178	1	0	#	oor	6,2	1,2	3,4	2,3	1	1	1	0	0	1	1	0	0	1
1694	178	1	0	#	oor	5,6	1,1	3,0	2,3	0	0	0	0	0	1	1	0	1	0
1696	178	1	0	#	oor	5,9	1,2	3,2	2,4	1	1	1	0	0	1	1	0	0	1
1697	178	1	0	#	oor	6,1	1,2	3,2	2,4	1	1	1	0	0	1	1	0	0	1
1698	178	1	0	#	oor	6,1	1,1	3,2	2,5	0	0	1	0	0	1	1	0	1	0
1699	178	1	0	#	oor	6,1	1,3	3,3	2,3	1	1	1	0	0	1	1	0	0	1
1700	178	1	0	#	oor	6,1	1,2	3,6	2,3	1	1	1	0	0	1	1	0	0	1
1702	178	1	0	#	oor	6,1	1,2	3,4	2,4	1	1	1	0	0	1	1	0	0	1
1704	178	1	0	#	oor	5,6	1,2	3,3	2,0	1	1	1	0	0	1	1	0	0	1
1705	178	1	0	#	oor	5,8	1,0	3,2	2,3	1	0	1	0	0	1	1	0	0	1
3193	338	1	1	10	oor	6,6	1,3	5,1	3,3	1	1	1	0	0	1	1	0	1	0
3194	338	1	1	10	oor	6,2	1,3	4,8	2,9	1	1	1	0	0	1	1	0	1	0
3195	338	1	1	10	oor	6,6	1,3	5,1	3,3	1	1	1	0	0	1	1	0	1	0
3196	338	1	0	10	oor	6,7	1,3	3,3	2,5	1	1	1	0	0	1	1	0	1	0
3197	338	1	0	10	oor	6,1	1,1	3,3	2,2	1	1	1	0	0	1	1	0	1	0
3198	338	1	0	10	oor	6,3	1,2	3,1	2,3	1	1	1	0	0	1	1	0	1	0
3199	338	1	0	10	oor	6,7	1,4	3,2	2,4	1	1	1	0	0	1	1	0	1	0
3202	338	1	0	10	oor	5,7	1,0	2,8	2,0	0	0	0	0	0	1	1	0	1	0
4132	399	1	1	10	oor	6,3	1,2	5,3	3,2	1	1	1	0	1	0	1	0	1	0
7031	134	1	0	#	oor	6,8	1,3	3,7	2,8	1	1	1	0	0	1	1	0	0	1
7032	134	1	0	#	oor	6,1	1,1	3,3	2,5	1	1	1	0	0	1	1	0	0	1
7033	134	1	0	#	oor	6,5	1,3	3,4	2,3	1	0	1	0	0	1	1	0	1	0
7034	134	1	0	#	oor	6,8	1,2	3,8	2,5	1	1	1	0	1	0	1	0	1	0
7035	134	1	0	#	oor	6,6	1,3	3,5	2,8	1	1	1	0	1	0	1	0	0	1
7036	134	1	0	#	oor	7,4	1,4	4,0	2,8	1	1	1	0	0	1	1	0	0	1
7037	134	1	0	#	oor	6,9	1,4	3,5	2,5	1	1	1	0	0	1	1	0	1	0
7038	134	1	0	#	oor	6,5	1,2	3,4	2,7	1	1	1	0	0	1	1	0	0	1
7041	338	1	0	10	oor	6,5	1,3	3,2	2,3	1	1	1	0	0	1	1	0	0	1
7042	338	1	0	10	oor	6,3	1,1	3,4	2,3	1	1	1	0	0	1	1	0	0	1
7068	399	1	0	10	oor	6,4	1,2	3,5	2,3	1	1	1	0	0	1	1	1	0	1
7069	399	1	0	10	oor	6,4	1,1	3,5	2,3	1	1	1	0	1	0	1	0	0	1
7070	399	1	0	10	oor	6,5	1,3	3,1	2,4	1	1	1	1	0	0	1	0	0	1
7071	399	1	0	10	oor	5,5	1,0	2,8	2,2	0	0	0	0	1	0	1	0	1	0
7072	399	1	0	10	oor	6,7	1,1	3,6	2,5	1	1	1	1	0	0	1	0	1	0
7073	399	1	0	10	oor	6,4	1,1	3,4	2,3	1	1	1	0	1	0	1	0	1	0
7074	399	1	0	10	oor	6,2	1,0	3,2	2,1	1	1	1	0	1	0	1	0	1	0
7075	399	1	0	10	oor	6,7	1,3	3,2	2,4	1	1	1	0	1	0	1	0	0	1

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
7076	399	1	0	10	oor	6,2	1,2	3,2	2,3	1	1	1	1	0	0	1	0	0	1
4262	448	0	1	10	oor	6,7	1,2	5,6	3,2	1	1	1	0	1	0	1	0	1	0
6224	588	0	1	10	oor	7,2	1,3	5,4	3,5	1	1	1	0	0	1	1	0	1	0
6226	588	0	1	10	oor	6,7	1,3	5,2	3,4	1	1	1	0	0	1	1	0	1	0
6227	588	0	1	10	oor	6,8	1,2	5,7	4,1	1	1	1	0	0	1	1	0	1	0
767	12	1	1	11	osc	6,7	1,4	4,8	3,6	1	1	1	1	0	0	1	0	1	0
1927	12	1	0	#	osc	5,8	1,2	3,1	2,2	0	0	0	1	0	0	1	0	0	1
1928	12	1	0	#	osc	6,6	1,3	3,5	2,5	1	1	1	1	0	0	1	0	0	1
2081	132	1	0	#	osc	6,9	1,3	3,8	2,6	1	1	1	1	0	0	1	0	1	0
2082	132	1	1	11	osc	7,2	1,3	5,5	4	1	1	1	1	0	0	1	0	1	0
3384	351	1	1	11	osc	6,2	1	6,2	3,2	1	1	0	1	0	0	1	0	1	0
3385	351	1	1	11	osc	6,5	1	6,5	3,3	1	1	1	1	0	0	1	0	1	0
3386	351	1	1	11	osc	6,6	1,3	5,1	3,3	1	1	1	1	0	0	1	0	1	0
3388	351	1	0	#	osc	6,8	1,4	3,6	2,3	1	1	1	1	0	0	1	0	1	0
3389	351	1	0	#	osc	6,8	1,3	3,4	2,4	1	1	0	1	0	0	1	0	1	0
3390	351	1	0	#	osc	6,6	1,3	3,4	2,4	1	1	0	1	0	0	1	0	1	0
3394	367	1	1	11	osc	6,7	1,2	5,6	3,5	1	0	1	1	0	0	1	0	1	0
3396	367	1	1	11	osc	7	1,2	5,8	3,8	1	1	1	1	0	0	1	0	1	0
3397	367	1	0	#	osc	6,7	1,1	3,7	2,5	1	1	1	1	0	0	1	0	1	0
3398	367	1	0	#	osc	6,3	1,0	3,2	2,2	0	0	1	1	0	0	1	0	1	0
3399	367	1	0	11	osc	6,5	1,0	3,6	2,6	0	0	0	1	0	0	1	0	1	0
3400	367	1	0	#	osc	6,6	1,1	3,5	2,3	1	0	1	1	0	0	1	0	1	0
3401	367	1	0	#	osc	7,0	1,1	3,9	2,6	1	1	1	1	0	0	1	0	1	0
3402	367	1	0	#	osc	6,4	0,9	3,5	2,0	1	0	0	1	0	0	1	0	1	0
3403	367	1	0	#	osc	6,5	1,0	3,3	2,2	1	1	1	1	0	0	1	0	1	0
3405	369	1	1	11	osc	6,4	1,2	5,3	3	1	1	1	1	0	0	1	0	1	0
3406	369	1	1	11	osc	6,5	1,1	5,9	3,5	0	0	0	1	0	0	1	0	1	0
3407	369	1	0	#	osc	7,0	1,2	3,5	2,4	1	1	1	1	0	0	1	0	1	0
3408	369	1	0	#	osc	6,3	1,0	3,3	2,2	1	1	1	1	0	0	1	0	1	0
3409	369	1	0	#	osc	6,5	1,0	3,2	2,3	1	1	1	1	0	0	1	0	1	0
3410	369	1	0	#	osc	6,9	1,1	3,6	2,5	0	0	0	1	0	0	1	0	1	0
3411	369	1	0	#	osc	6,2	0,9	3,4	2,4	0	0	0	0	1	0	1	0	1	0
3413	369	1	0	#	osc	6,7	1,1	3,3	2,4	0	0	0	1	0	0	1	0	1	0
3547	383	1	1	11	osc	6,7	1,1	6,1	3,5	1	0	1	1	0	0	1	0	1	0
3548	383	1	1	11	osc	6,3	1	6,3	3,5	1	1	1	1	0	0	1	0	1	0
3549	383	1	0	#	osc	5,7	0,9	3,0	2,3	0	0	0	1	0	0	1	0	1	0
3551	383	1	0	#	osc	6,7	1,2	3,4	2,5	1	1	1	1	0	0	1	0	1	0
3552	383	1	0	#	osc	6,7	1,2	3,6	2,4	1	1	1	1	0	0	1	0	1	0
3553	383	1	0	#	osc	6,3	1,1	3,3	2,2	1	0	0	1	0	0	1	0	1	0
3554	383	1	0	#	osc	6,3	1,1	3,3	2,4	1	1	0	1	0	0	1	0	1	0
3555	383	1	0	#	osc	6,5	1,1	3,4	2,5	1	1	1	1	0	0	1	0	1	0
3556	387	1	1	11	osc	5,9	1,2	4,9	2,9	0	0	0	1	0	0	1	0	1	0
3563	389	1	1	11	osc	5,9	0,9	6,6	3,9	0	0	1	1	0	0	1	0	0	1
3564	389	1	1	11	osc	6	1,2	5	3,2	1	1	1	1	0	0	1	0	1	0
3573	382	1	1	11	osc	6,8	1,2	5,7	3,5	1	1	1	1	0	0	1	0	1	0
3574	382	1	1	11	osc	6,7	1,2	5,6	3,2	1	1	1	1	0	0	1	0	1	0
4100	444	1	1	11	osc	7,5	1,5	5	3,8	1	1	1	1	0	0	1	0	1	0
4101	444	1	1	11	osc	6,5	1,3	5	3,5	1	1	1	1	0	0	1	0	1	0
4104	443	1	1	11	osc	6,3	1,1	5,7	3,6	1	1	1	1	0	0	1	0	1	0
4105	443	1	1	11	osc	6,5	1,2	5,4	3,4	1	1	1	1	0	0	1	0	1	0
7043	351	1	0	#	osc	7,2	1,4	4,0	2,7	1	1	1	1	0	0	1	0	1	0
7044	351	1	0	#	osc	6,7	1,2	3,5	2,6	1	1	1	1	0	0	1	0	1	0
7045	351	1	0	#	osc	6,4	1,2	3,4	2,4	1	1	1	1	0	0	1	0	1	0
7046	351	1	0	#	osc	6,4	1,2	3,6	2,4	1	1	1	1	0	0	1	0	1	0
7047	351	1	0	#	osc	6,5	1,1	3,5	2,3	1	1	1	1	0	0	1	0	1	0
7048	367	1	0	#	osc	6,3	1,0	3,4	2,3	1	1	1	1	0	0	1	0	0	1
7049	369	1	0	#	osc	6,8	1,1	3,6	2,3	1	1	1	0	1	0	1	0	1	0
7050	369	1	0	#	osc	7,5	1,4	3,7	2,9	1	1	1	1	0	0	1	0	1	0

inID	spID	P1	P3	C	TA	SW	WU	SH	HL	IR	BT	PA	G2	G1	G0	R	H2	H1	H0
7051	387	1	0	#	osc	6,2	1,2	3,4	2,3	1	0	1	1	0	0	1	0	1	0
7052	387	1	0	#	osc	6,2	1,2	3,2	2,4	1	0	0	1	0	0	1	0	1	0
7053	387	1	0	#	osc	6,1	1,0	3,3	2,3	1	1	1	1	0	0	1	0	1	0
7054	387	1	0	#	osc	6,2	1,2	3,0	2,2	1	1	1	1	0	0	1	0	1	0
7055	387	1	0	#	osc	6,0	1,1	3,1	2,3	1	0	1	1	0	0	1	0	1	0
7056	387	1	0	#	osc	6,1	1,2	3,1	2,2	1	1	0	1	0	0	1	0	1	0
7057	387	1	0	#	osc	5,6	1,2	2,8	2,1	1	1	1	1	0	0	1	0	0	1
7058	387	1	0	#	osc	6,3	1,2	3,3	2,4	1	1	1	1	0	0	1	0	0	1
7059	387	1	0	#	osc	6,4	1,1	3,6	2,3	1	1	1	1	0	0	1	0	0	1
7060	443	1	0	#	osc	6,2	1,1	3,4	2,4	1	0	0	1	0	0	1	0	1	0
7061	443	1	0	#	osc	6,9	1,3	3,8	2,8	1	1	1	1	0	0	1	0	1	0
7062	443	1	0	#	osc	7,0	1,3	3,6	2,8	1	1	1	1	0	0	1	0	1	0
7063	443	1	0	#	osc	6,1	1,1	3,4	2,3	1	1	1	1	0	0	1	0	1	0
7064	443	1	0	#	osc	6,8	1,3	3,5	2,6	1	1	1	1	0	0	1	0	1	0
7065	443	1	0	#	osc	6,1	1,1	3,4	2,4	1	0	1	1	0	0	1	0	1	0
7066	443	1	0	#	osc	6,4	1,2	3,3	2,4	1	1	1	1	0	0	1	0	1	0
7067	443	1	0	#	osc	6,7	1,3	3,4	2,6	1	1	1	1	0	0	1	0	1	0
687	71	0	1	12	sda	10,9	1,4	6,4	4,6	1	0	0	0	0	1	1	0	0	1
1343	142	0	1	12	sda	11,3	1,9	6,3	4,3	1	0	0	0	0	1	1	1	0	0
2980	298	0	1	12	sda	9,7	1,4	5,9	4,3	1	0	0	0	0	1	1	0	0	1
2981	298	0	1	12	sda	12	1,8	6,8	5	1	0	0	0	0	1	1	0	0	1
5528	469	0	1	12	sda	10,5	1,8	5,6	4,3	1	0	0	0	0	1	1	0	0	1
5529	469	0	1	12	sda	11,3	1,8	6,4	4,7	1	0	0	0	0	1	1	0	0	1
5530	469	0	1	12	sda	11,4	1,9	6,6	4,7	1	0	0	0	0	1	1	0	0	1
253	122	0	1	12	sju	9,2	0,2	4,7	3,5	0	0	0	0	0	1	1	0	0	1
717	122	0	1	12	sju	11,3	2,1	6,3	4,8	1	0	1	0	0	1	1	0	0	1
718	122	0	1	12	sju	10	1,9	5,8	4	0	0	0	0	0	1	1	0	0	1
737	45	0	1	12	sju	10,1	1,4	5,6	4,5	0	0	0	0	0	1	1	0	0	1
738	45	0	1	12	sju	9,6	1,4	6	4,2	0	0	0	0	0	1	1	0	0	1
739	45	0	1	12	sju	10,1	1,4	6,1	4,8	0	0	0	0	0	1	1	0	0	1
748	43	0	1	12	sju	10,3	1,9	5,5	3,9	1	0	0	0	0	1	1	0	0	1
749	43	0	1	12	sju	9,7	1,4	5,4	4,2	0	0	0	0	0	1	1	0	0	1
2077	249	0	1	12	sst	9,5	1,3	5,5	4	0	0	0	0	0	1	1	0	0	1
2078	249	0	1	12	sst	9,0	1,6	4,8	3,5	0	0	0	0	0	1	1	0	0	1
4001	414	0	1	12	sst	10	1,4	5,6	4,3	0	0	0	0	0	1	1	0	0	1
4002	414	0	1	12	sst	12,4	2,3	8,1	5	1	0	0	0	0	1	1	0	0	1
4003	414	0	1	12	sst	9,8	1,6	5,8	4	0	0	0	0	0	1	1	0	0	1
4011	415	0	1	12	sst	13,5	2,1	8,4	5,5	1	0	0	0	0	1	1	1	0	0
4012	415	0	1	12	sst	11,5	2,4	6,2	4,8	1	0	0	0	0	1	1	0	0	1
4013	415	0	1	12	sst	10	1,9	5,7	4,2	0	0	0	0	0	1	1	0	0	1
4041	416	0	1	12	sst	12,7	2,4	7,1	5	1	0	1	0	0	1	1	0	0	1
4042	416	0	1	12	sst	12,9	2,4	7,2	5,5	1	0	1	0	0	1	1	0	0	1
4043	416	0	1	12	sst	9,8	1,5	5,6	4,1	0	0	0	0	0	1	1	0	0	1

Raw data 2: Vegetation types. spID: identification number of each sample site; Appearence in papers: P1: paper 1, P3: paper 3; TA: taxa occurring at the respective sample site; his: *T. hispidus/sericeus* complex, oor: *T. o. oreinos*, osc: *T. o. scheerpeltzi*, sst: *T. s. striolatus*, sda: *T. s. danubialis*, sju: *T. s. juvavensis*; FV: free of vegetation, ME: meadow: Ma: marsh, HP: high perennial herbs RF: Riparian forest, AC: Alder carr, DF: Deciduous forest, MF: Mixed forest; CF: Coniferous forest; AC: (sub)alpine grassland, MP: Mountain pine shrubbery, GP: Garden/Park, RA: ruderal area; 1 = presence / 0 = absence

SpID	P1	P3	TA	FV	ME	MA	HP	RF	AC	DF	MF	CF	AG	MP	GP	RA
5	1	1	his	0	0	0	1	0	0	0	0	0	0	0	0	0
24	1	1	his	0	0	0	1	1	0	0	0	0	0	0	0	0
32	1	1	his	0	0	1	0	0	1	1	0	0	0	0	0	0
50	1	1	his	0	0	0	1	0	0	0	0	0	0	0	0	0
52	1	1	his	0	0	0	1	0	0	0	0	0	0	0	0	0
60	0	0	his	0	0	0	1	1	0	0	0	0	0	0	0	0
64	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
86	0	0	his	0	1	0	0	0	0	0	0	0	0	0	0	0
93	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
102	1	1	his	0	0	0	1	0	0	0	1	0	0	0	0	0
104	1	1	his	0	1	0	1	0	0	0	0	0	0	0	0	0
130	1	1	his	0	1	0	1	0	0	0	0	0	0	0	0	0
140	1	1	his	0	0	0	1	0	0	0	0	0	0	0	0	0
144	1	1	his	0	1	0	0	0	0	0	0	0	0	0	0	0
145	1	1	his	0	0	0	1	0	0	0	0	0	0	0	0	0
158	1	0	his	0	0	0	1	0	0	0	0	0	0	0	1	0
159	1	0	his	0	0	0	0	0	0	0	0	0	0	0	1	0
160	1	0	his	0	0	0	1	0	0	0	0	1	0	0	0	0
167	1	0	his	0	1	0	1	0	0	0	0	0	0	0	0	0
168	1	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
200	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
204	0	0	his	0	0	0	0	0	0	0	0	0	1	0	0	0
215	0	0	his	0	0	1	1	0	0	0	0	0	0	0	0	0
217	0	0	his	0	0	1	1	0	0	0	0	0	0	0	0	0
223	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
231	0	0	his	0	0	1	1	1	0	0	0	0	0	0	0	0
237	1	0	his	0	0	0	1	0	0	0	0	0	0	0	0	1
288	0	0	his	0	0	0	1	1	0	0	0	0	0	0	0	0
291	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
292	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
296	0	0	his	0	0	0	1	1	0	0	0	0	0	0	0	0
306	1	0	his	0	0	0	1	0	0	0	1	0	0	0	0	0
311	1	0	his	0	0	1	1	0	0	0	0	0	0	0	0	0
313	1	0	his	0	0	1	1	0	0	0	0	0	0	0	0	0
315	1	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
317	1	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
318	1	0	his	0	0	1	1	0	0	0	0	0	0	0	0	0
319	1	0	his	0	0	0	1	0	0	0	0	0	1	0	0	0
323	0	0	his	0	1	0	1	0	0	0	0	0	0	0	0	0
327	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
361	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
380	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
385	0	0	his	0	0	0	1	0	0	0	1	0	0	0	0	0
386	0	0	his	0	0	0	1	0	0	0	1	0	0	0	0	0

SpID	P1	P3	TA	FV	ME	MA	HP	RF	AC	DF	MF	CF	AG	MP	GP	RA
402	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
407	0	0	his	0	0	1	1	0	0	0	0	0	0	0	0	0
412	0	0	his	0	0	1	1	0	0	0	0	0	0	0	0	0
418	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
446	0	0	his	0	0	0	1	0	0	1	0	0	0	0	0	0
476	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	1
483	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
534	0	0	his	0	1	1	0	0	0	0	0	0	0	0	0	0
541	0	0	his	0	0	0	0	0	0	0	1	0	1	0	0	0
545	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
548	0	0	his	0	1	0	1	0	0	0	0	0	0	0	0	0
549	0	0	his	0	0	0	1	1	0	0	0	0	0	0	0	0
550	0	0	his	0	0	0	1	0	0	0	0	0	0	0	0	0
33	0	0	his/sda	0	0	0	1	1	0	0	0	0	0	0	0	0
42	1	1	his/sju	0	0	0	1	0	0	1	0	0	0	0	0	0
43	0	0	sju	0	0	0	1	0	0	0	0	0	1	1	0	0
45	0	0	sju	0	0	0	0	0	0	0	0	0	1	0	0	0
122	0	0	sju	0	0	0	1	0	0	0	0	0	1	0	0	0
142	0	0	sda	0	0	0	0	1	0	0	0	0	0	0	0	0
469	0	0	sda	0	0	0	0	1	0	0	0	0	0	0	0	0
414	0	0	sst	0	0	0	1	0	0	0	0	0	0	0	0	0
415	0	0	sst	0	0	0	0	0	0	1	0	0	0	0	0	1
416	0	0	sst	0	0	0	0	0	0	1	0	0	0	0	0	0
55	1	0	oor	0	0	0	0	0	0	0	0	0	1	0	0	0
79	1	0	oor	0	0	0	0	0	0	0	0	0	1	0	0	0
134	1	0	oor	0	0	0	0	0	0	0	0	0	1	1	0	0
165	1	0	oor	0	0	0	0	0	0	0	0	0	1	0	0	0
172	1	0	oor	1	0	0	0	0	0	0	0	0	0	0	0	0
178	1	0	oor	0	0	0	0	0	0	0	0	0	1	0	0	0
338	1	0	oor	0	0	0	0	0	0	0	0	0	1	0	0	0
399	1	0	oor	0	0	0	0	0	0	0	0	0	1	0	0	0
588	0	0	oor	0	0	0	0	0	0	0	0	0	1	0	0	0
12	1	0	osc	0	0	0	0	0	0	0	0	0	1	0	0	0
132	1	0	osc	0	0	0	0	0	0	0	0	0	1	0	0	0
351	1	0	osc	0	0	0	0	0	0	0	0	0	1	0	0	0
367	1	0	osc	0	0	0	0	0	0	0	0	0	0	1	0	0
369	1	0	osc	0	0	0	0	0	0	0	0	0	0	1	0	0
383	1	0	osc	0	0	0	0	0	0	0	0	0	1	1	0	0
387	1	0	osc	1	0	0	0	0	0	0	0	0	1	0	0	0
389	1	0	osc	0	0	0	0	0	0	0	0	0	1	0	0	0
443	1	0	osc	1	0	0	0	0	0	0	0	0	1	0	0	0
444	1	0	osc	0	0	0	0	0	0	0	0	0	1	0	0	0

Raw data 3: Landscape structures. spID: identification number of each sample site; Use in papers: P1: paper 1, P3: paper 3; TA: taxa occurring on the respective sample site; his: *T. hispidus/sericeus* complex, oor: *T. o. oreinos*, osc: *T. o. scheerpeltzi*, sst: *T. s. striolatus*, sda: *T. s. danubialis*, sju: *T. s. juvavensis*; EF: Edge of forest, LT: loose trees and shrubs, HS: hedgerows/shrubs, BR: Boundary ridge, ST: Single trees and shrubs, RG: Riverbank grove, SI: Single stones, BD: Bank/dam, BO: Boulders, RO: Rocks, CR: canyon/Rock face; 1 = presence / 0 = absence

SpID	P1	P3	TA	EF	LT	HIS	BR	ST	RG	SI	BD	BO	RO	CR
5	1	1	his	0	1	0	0	0	0	0	0	0	0	0
24	1	1	his	0	0	0	0	0	1	0	0	0	0	0
32	1	1	his	1	0	0	0	0	0	0	0	0	0	0
50	1	1	his	0	0	0	0	0	1	0	0	0	0	0
52	1	1	his	1	0	0	0	0	0	0	0	0	0	0
60	0	0	his	0	0	0	0	0	0	0	0	0	0	0
64	0	0	his	0	0	0	0	0	0	0	0	0	0	0
86	0	0	his	0	0	0	0	0	0	0	0	0	0	0
93	0	0	his	0	0	0	0	0	1	0	0	0	0	0
102	1	1	his	1	0	0	0	0	0	0	0	0	0	0
104	1	1	his	0	0	1	1	0	0	0	0	0	0	0
130	1	1	his	0	1	0	1	0	0	0	0	0	0	0
140	1	1	his	0	0	0	0	0	1	0	0	0	0	0
144	1	1	his	0	1	0	1	0	0	0	0	0	0	0
145	1	1	his	1	0	0	0	0	0	0	0	0	1	0
158	1	0	his	0	1	0	0	0	0	0	0	0	0	0
159	1	0	his	0	1	0	0	0	0	0	0	0	0	0
160	1	0	his	1	0	0	0	0	0	0	0	0	0	0
167	1	0	his	0	0	0	0	0	1	0	0	0	0	0
168	1	0	his	0	0	0	0	0	1	0	0	0	0	0
200	0	0	his	0	0	0	0	0	0	0	1	0	0	0
204	0	0	his	1	0	0	0	0	0	0	0	0	1	1
215	0	0	his	0	0	0	0	0	0	0	0	0	0	0
217	0	0	his	0	0	0	0	0	0	0	0	0	0	0
223	0	0	his	1	0	0	0	0	1	0	0	0	0	0
231	0	0	his	0	0	0	0	0	0	0	0	0	0	0
237	1	0	his	0	0	0	0	0	0	0	0	0	0	0
288	0	0	his	1	1	0	0	0	0	0	0	0	0	0
291	0	0	his	0	1	0	0	0	0	0	0	0	0	0
292	0	0	his	0	0	0	0	0	1	0	0	0	0	0
296	0	0	his	1	1	0	0	0	0	0	0	0	0	0
306	1	0	his	0	0	0	0	0	1	0	0	0	0	0
311	1	0	his	1	0	0	0	0	0	0	0	0	0	0
313	1	0	his	1	0	0	0	0	0	0	0	0	0	0
315	1	0	his	1	0	0	0	0	0	0	0	0	0	0
317	1	0	his	0	0	0	0	0	0	0	0	0	0	1
318	1	0	his	0	0	0	0	0	0	0	0	0	0	0
319	1	0	his	0	0	0	0	0	0	0	0	0	0	0
323	0	0	his	0	0	0	0	0	0	0	0	0	0	0
327	0	0	his	1	0	0	0	0	0	0	0	0	0	0
361	0	0	his	0	0	0	0	0	0	0	1	0	0	0
380	0	0	his	0	0	0	0	1	0	0	0	0	0	0
385	0	0	his	1	1	0	0	0	0	0	0	0	0	0
386	0	0	his	1	0	0	0	0	0	0	0	0	0	0
402	0	0	his	1	0	0	0	0	0	0	0	0	0	0

SpID	P1	P3	TA	EF	LT	HIS	BR	ST	RG	SI	BD	BO	RO	CR
407	0	0	his	1	0	0	0	0	0	0	0	0	0	0
412	0	0	his	0	0	0	0	0	1	0	0	0	0	0
418	0	0	his	0	0	1	0	0	0	0	0	0	0	0
446	0	0	his	1	0	0	0	0	1	0	0	0	0	0
476	0	0	his	1	0	0	0	0	0	0	0	0	0	0
483	0	0	his	0	0	0	0	0	1	0	0	0	0	0
534	0	0	his	0	0	0	1	0	0	0	1	0	0	0
541	0	0	his	0	0	0	0	0	0	0	0	0	1	0
545	0	0	his	0	0	0	1	0	0	0	0	0	0	0
548	0	0	his	1	0	0	1	0	0	0	0	0	0	0
549	0	0	his	1	0	0	0	0	1	0	0	0	0	0
550	0	0	his	1	0	0	0	0	1	0	0	0	0	0
33	0	0	his/sda	0	0	0	0	0	0	0	0	0	0	0
42	1	1	his/sju	1	0	0	0	0	0	0	0	0	0	0
43	0	0	sju	0	0	0	0	0	0	1	0	0	0	0
45	0	0	sju	0	1	0	0	0	0	1	0	0	0	0
122	0	0	sju	0	0	0	0	0	0	1	0	0	0	0
142	0	0	sda	0	1	0	0	0	0	0	0	0	0	0
469	0	0	sda	0	0	0	0	0	1	0	0	0	0	0
414	0	0	sst	0	0	0	0	0	1	0	0	0	0	0
415	0	0	sst	1	0	0	0	0	0	0	0	0	1	0
416	0	0	sst	0	0	0	0	0	0	0	0	0	1	0
55	1	0	oor	0	0	0	0	0	0	1	0	1	0	0
79	1	0	oor	0	0	0	0	0	0	0	0	1	0	0
134	1	0	oor	0	0	0	0	0	0	1	0	0	1	0
165	1	0	oor	0	0	0	0	0	0	1	0	0	1	0
172	1	0	oor	0	0	0	0	0	0	0	0	1	1	0
178	1	0	oor	0	0	0	0	0	0	1	0	0	0	0
338	1	0	oor	0	0	0	0	0	0	1	0	0	0	1
399	1	0	oor	0	0	0	0	0	0	0	0	1	0	0
588	0	0	oor	0	0	0	0	0	0	0	0	1	0	0
12	1	0	osc	0	0	0	0	0	0	0	0	0	1	1
132	1	0	osc	0	0	0	0	0	0	1	0	0	1	0
351	1	0	osc	0	0	0	0	0	0	0	0	1	0	0
367	1	0	osc	0	0	0	0	0	0	0	0	1	0	0
369	1	0	osc	0	0	0	0	0	0	0	0	1	0	0
383	1	0	osc	0	0	0	0	0	0	0	0	1	1	0
387	1	0	osc	0	0	0	0	0	0	0	0	1	1	0
389	1	0	osc	0	0	0	0	0	0	0	0	1	1	0
443	1	0	osc	0	0	0	0	0	0	0	0	1	1	0
444	1	0	osc	0	0	0	0	0	0	0	0	1	1	0

Authors contributions

The following authors contributed to the three studies of this doctoral thesis:

• Michael Duda (MD) [1,3]
• Daniela Bartel (DB) [2,3]
• Elisabeth Haring (EH) [2,3]
• Josef Harl (JH) [2,3]
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Genetic differentiation and shell morphology of *Trochulus oreinos* (Wagner, 1915) and *T. hispidus* (Linnaeus, 1758) (Pulmonata: Hygromiidae) in the Northeastern Alps

MD conducted the morphological analysis, compiled the tables, conducted the literature research and drafted the paper (except the genetic parts). LK conducted the genetic analysis and drafted the corresponding text parts. HW and MD performed the statistical analysis. EH and HS conceived and supervised the project, and contributed to the manuscript. JH contributed to collecting and processing of samples and contributed to the manuscript. DB contributed to the genetic analyses and to the processing and archiving of animals.
Personal contribution of MD: 60%

Habitat requirements of the pulmonate land snails *Trochulus oreinos oreinos* and *Cylindrus obtusus* endemic to the Northern Calcareous Alps, Austria

MD performed the analyses, designed the figures, conducted the literature research and drafted the manuscript. LK contributed to the manuscript. EH and HS conceived the project and contributed to the manuscript.

Personal contribution of MD: 85%

Morphological and ecological investigations of the highly divergent mitochondrial clades within the *Trochulus hispidus/sericeus* species complex and related taxa (Gastropoda: Pulmonata: Hygromiidae)

All authors contributed substantially to the field collections. MD conducted all measurements, sections, analyses, drawings and photos, and drafted the manuscript. LK provided the genetic background, designed Figures 1 and 2 and contributed to the manuscript. HS conceived the project, supervised the analysis and contributed to the manuscript. JH contributed to the manuscript and provided substantial help for layout and arrangement of the photos. EH conceived the project, supervised the analyses and contributed to the manuscript. All authors red and approved the final manuscript.

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Professional career:

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