# More than what meets the eye: cryptic speciation and hidden diversity in land snails

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

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Leiter der Arbeit:
Prof. Dr. Christian Kropf
Naturhistorisches Museum Bern

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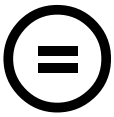

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Von der Philosophisch-naturwissenschaftlichen Fakultät angenommen.

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Der Dekan
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# Introduction

Following arthropods, molluscs are the second largest phylum of invertebrate animals with about 110,000 accepted living species (MolluscaBase, July 2022). They inhabit almost every habitat on earth, ranging from geothermal vents of the deep sea to shallow lagoons, freshwater lakes and rivers, tropical forests, deserts, high mountain peaks (e.g., Purchon 1977) and caves as deep as 980 m (Weigand *et al.* 2013). Due to their calcareous shells, they show a rich fossil record, dating to the late Cambrium (ca. 534 mya) (Maloof *et al.* 2010). Although 25,000 accepted fossil-only species are known (MolluscaBase, July 2022), another 100,000 yet undiscovered species are expected (Schneider, pers. comm. 2022). Molluscs have always been important ecosystem engineers, helping to structure aquatic bottom environments while providing protection, food and habitat to a wide range of other species (Fortunato 2015). Throughout human history, they have played an important role as food source (e.g., Khan & Liu 2019), tools, currency, decoration and in palliative care (i.e., *Conus* toxins); they are used in biomonitoring programs and in biomedical research (e.g., Bingham *et al.* 2010). Despite their many benefits, certain molluscs can also be pests or invasive species, or act as intermediate hosts of dangerous parasites (Lindberg 2001). The diversity in size, shape and colour of molluscs have always fascinated people. Especially the class Gastropoda displays an enormous variability in shell morphology. Shells of molluscs are mainly made of calcium carbonate and protect the animals against predation, dehydration and mechanical damage (Vermeij 1993). On the one hand, shell size is genetically inherited, but on the other hand, it is influenced by environmental factors. For example, humidity, substrate or population density can have an influence on the effective size of a land snail shell (Goodfriend 1986). Living in certain habitats favours different shell shapes. For example, flat species are more likely to be found on low-angle or horizontal surfaces and high-spired species are more likely to be found on high-angle or vertical surfaces and intermediate species are found on a wide variety of surfaces (Goodfriend 1986). Close relationship and/or similarly colonized habitats with similar environmental conditions can lead to species having similar shells (e.g., Goodfriend 1986, Gemmell *et al*. 2018).

The shell is a hard, resistant component and persists well beyond the death of the animal (Pearce *et al.* 2008). This is one of the main reasons, why historic taxonomic descriptions of most snail species are based on morphological characteristics of the shell, omitting others such as chemical, anatomical or genetic characteristics. By considering only morphological features, the diversity of cryptic species remains unrevealed (Bickford *et al.* 2007). A species is called cryptic, when two or more distinct species are classified as one species based on morphological similarity (e.g., Bickford *et al.* 2007; Pfenninger & Schwenk 2007). A literature survey by Struck *et al.* (2018) showed, that cryptic species have been defined based on

taxonomic history and molecular data, without quantifying morphological disparity, which wrongly leads to lumping of species. It is not clear from the definitions of numerous studies how many characters are needed to speak of cryptic species (see Struck *et al.* 2018). The prevalence of cryptic species skews biodiversity estimation and depends on how species are defined (Bickford *et al.* 2007; Adams *et al*. 2014; Struck *et al.* 2018). The unified species concept of de Queiroz (2007) contains two elements: the general concept of species as separately evolving metapopulation lineages and second, it treats this property as the only necessary property of species. Under a unified species concept, there should no longer be any disagreement about the numbers and boundaries of species that merely result from disagreements about the definition of the species category. Disagreements about species delimitation should result only from differences in e.g., the reliability of certain methods or the relevance of certain data (de Queiroz 2007). Therefore, taxonomists must discriminate characters according to their suitability and quality for species delineation (Jörger & Schrödl 2013). In order to distinguish and quantify cryptic species, information on morphological disparity has to be related with levels of gene flow, reproductive isolation and genetic divergence. For young species with a short divergence time, it could be, that the time was too short to accumulate considerable morphological differences (Struck *et al.* 2018). Another hypothesis is, that cryptic species appear to differ either by non-visual mating signals and/or are under selection that causes morphological stasis (Bickford *et al.* 2007). Cryptic diversity can also be a consequence of parallel or convergent morphological evolution under similar selection pressure (Trontelj & Fišer 2009). These themes elude our visual perception in the morphological description of species and require consideration of the evolutionary history of the taxa (e.g., Sites & Marshall 2003; Bickford *et al*. 2007, Struck *et al*. 2018). Accurate species identifications are crucial for the identification of invasive and pest species, diagnosis and prevention of disease and for the implementation of biological control (e.g., Bickford *et al*. 2007; Pfenninger & Schwenk 2007). Also, for conservation and natural resource management and protection, the correct identification and description of species is essential (e.g., Bickford *et al* 2007; Fišer *et al*. 2018; Horsáková *et al.* 2019).

Today, many integrative taxonomic studies are carried out, which involve more than just morphological characteristics to improve species discovery and description (Padial *et al.* 2010). In integrative taxonomy, different data sets are investigated, such as for example morphology, genetic data, mating signals, behavioural traits, bioacoustics data, and biogeography (e.g., Bickford *et al*. 2007; Jörger & Schrödl 2013). Numerous population genetic studies, phylogenetic studies or biogeographic studies in any organism groups (e.g., Hebert *et al.* 2004; Funk *et al*. 2011; Cruse *et al*. 2022) have discovered cryptic species in recent years. Especially within snails, there are frequently discrepancies when animals are sequenced and

compared to species that are based only on the description of an empty shell (e.g., Johnson *et al*. 2014; Horsáková *et al*. 2019; Hofman *et al*. 2022).

In my doctoral work, I used integrative taxonomy to unravel cryptic species in different terrestrial snail groups. With the inclusion of mitochondrial and nuclear DNA, shell morphological features, histological sections, and the investigation of the morphology of the radula, it was possible to describe the new genus *Iberozospeum* Jochum, Kneubühler, Prieto & Neubert, 2022 and a new species from Spain (Chapter 1). The focus of my doctoral work was on the resolution of the species complex within the hairy snail genus *Trochulus* Chemnitz, 1786. A rich sampling from the entire distribution area of the genus made it possible to clarify the complicated taxonomy of *Trochulus* (Chapters 2 and 3). In addition, I resolved phylogenetic problems within the ellobiid micro snail genera *Zospeum* Bourguignat, 1856 and *Carychium* O.F. Müller, 1773 (Chapter 4).

## Chapter 1

The micro snails of the ellobiid genus *Zospeum* are known to inhabit a wide network of caves underlying the Pyrenean-Cantabrian region of northern Spain. Due to their tiny size (< 2 mm) and their transparent shell, it is difficult to find them alive. Recent studies of northern Spanish *Zospeum* shells (Jochum *et al.* 2015) have provided valuable but limited insights towards understanding the evolutionary processes in this genus. The species within *Zospeum* are difficult to distinguish based on their shells, as they show great intraspecific variability with smooth transitions to other species. Recent collection efforts have provided significant finds and enabled the investigation of 57 populations in an integrative study. Two mitochondrial (COI and 16S) and two nuclear (H3 and 5.8S rRNA+ITS2) markers were examined, which revealed that the northern Spanish species form a radiation separate from the Dinaric *Zospeum* clades, for which the new genus *Iberozospeum* was proposed. In this work, several species were confirmed by genetic data, and one new species was described based on morphological and genetic evidence. However, the widespread species, *I. vasconicum* (Prieto, de Winter, Weigand, Gómez & Jochum, 2015), was found to consist of three cryptic clades. In this study, morphological evidence acquired via histological analysis of *I. vasconicum* (collected at the type locality Cueva de la Ermita de Sandaili, Spain) and scanning electron microscope (SEM) analyses of radulae of eastern Alpine, Dinaric and Iberian congeners, underscored the separation of these members to the new genus. This study revealed that the columellar muscle in *Iberozospeum* shows prominent humps containing vasculature extending to the tips of these humps, matching up perfectly to corresponding microstructure on the abutting lamellae. Additionally, radula ribbons harbour more and smaller teeth per transverse row and are longer and broader in *Iberozospeum* compared to those of Dinaric taxa.

## Chapter 2

*Trochulus biconicus* (Eder, 1917) is an endemic land snail known from only a few localities in the Central Swiss Alps. This species is classified as “vulnerable” by Swiss law (Federal Office of Environment) and is protected. It is also considered “endangered” (EN) by the International Union for Conservation of Nature (IUCN). Although some studies (e.g., Kruckenhauser *et al.* 2014; Proćków *et al*. 2021) in the past examined specimens of *T. biconicus*, the phylogenetic affiliation remained unclear. In my work here, living specimens from large populations were collected at 11 sites of the known distribution area, enabling a contemporary integrative study, incorporating molecular, anatomical and morphological shell data. Phylogenetic analyses revealed that *T. biconicus* forms a monophyletic clade, clearly separated from all known genera within the tribe of Trochulini. These findings are supported by morphological investigations of the genital organs, which revealed only four instead of six or eight mucous glands and a very long flagellum when compared to *Trochulus*. Therefore, the new monotypic genus *Raeticella* was introduced. *Raeticella biconica* lives on mountain peaks on the right and left side of the Engelberger valley between 1890 and 2575 m a.s.l. With its flat shell, it is perfectly adapted to live under or between stones. Low genetic diversity within the analysed populations indicates, that this species underwent a bottleneck event during the Pleistocene and the Last Glacial Maximum, whereby, some isolated populations survived this icy period on neighbouring nunataks (glacial islands). This stenoecious species is prone to extinction because of climate change. It already reached the summits of the mountains and there is no more alternative to avoid disagreeable climate conditions. If climate change proceeds at its current rapid pace, this species could become extinct in a few years.

## Chapter 3

Most of the species’ descriptions of the hairy snail genus *Trochulus* are based on morphological and anatomical characters. Unfortunately, they fail to address the diversity of *Trochulus* by leaving cryptic species unrevealed. Previous phylogenetic studies have included various specimens, but only from a small part of the distribution range and therefore, could not resolve the actual phylogenetic relationships within the genus. The enormous number of available names for an unknown number of biological entities led to a chaotic taxonomic situation. Until today, weak conception and widespread contradiction in the application of these names precluded a well-grounded species identification. Recent, intense collecting efforts and the examination of collection specimens from various museum collections, provided a remarkably rich sampling from the entire distribution range of the genus (ca. 570 specimens). Additional sequences from genetic data bases were included. Maximum Likelihood and Bayesian Inference based phylogenetic analyses, comprising two mitochondrial (COI and 16S) and one nuclear (5.8S rRNA+ITS2) marker, revealed 21 stable clades within *Trochulus*.

Additionally, the shell morphology was analysed using landmarks- and semi landmarks-based geometric morphometric methods (GMM). In contrast to traditional linear measurement methods, the shape variations of morphologies are covered in GMM. Considering the GMM data with the occurrence of hairs and the shell size, some clades could be morphologically well separated, while some cryptic species could not.

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ORGANISMS DIVERSITY & EVOLUTION

ORIGINAL ARTICLE

# Molecular investigation and description of *Iberozospeum* n. gen., including the description of one new species (Eupulmonata, Ellobioidea, Carychiidae)

**Jeannette Kneubühler[1,2] · Adrienne Jochum[1,2,3] · Carlos E. Prieto[4] · Eike Neubert[1,2]**

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**Abstract**
The subterranean realm of the Cantabrian-Pyrenean region of northern Spain harbours a rich diversity of *Zospeum*. Due to their tiny size and the difficulty of finding them alive, scarce animal material has been available for scientific investigation. Recent investigations of *Zospeum* shells have provided valuable, but limited insights towards our understanding of the evolutionary processes occurring within this taxon in northern Spain. In an integrative study, we investigate 57 populations of *Zospeum* from northern Spanish caves using two mitochondrial (COI and 16S) and two nuclear markers (H3 and 5.8 S rRNA + ITS2). Revealed is a separate radiation of the northern Spanish species for which the new genus, *Iberozospeum,* is proposed. The independent radiation of Dinaric *Zospeum* from that of northern Spain justifies the designation of *Iberozospeum* n. gen. Morphological evidence is provided via histological analysis of *Iberozospeum vasconicum* and SEM analyses of radulae of eastern Alpine, Dinaric and Iberian species. Important differences in morphological structure and character states are presented, including the first view of the sexually mature female and the presence of the giant albumen gland in an individual of the subterranean, troglobitic Carychiidae. Significant differences are revealed in superficial crystallographic structure of the columellar lamellae, the morphology of the columellar muscle and in the radula. Radular ribbon length, ribbon broadness, straightness of the ribbon base and cusp configuration are distinctive in the Iberian species. One new species is described corroborated by genetic and morphological characters.

**Keywords** Cave-dwelling species · Cryptic diversity · Histology · Intraspecific variability · Microgastropods · Subterranean land snail

## Introduction

Tiny subterranean snails of the genus *Zospeum* (Bourguignat, 1856), are known to inhabit the broad network of caves underlying the Pyrenean-Cantabrian region of northern Spain (Fig. 1). Although recent collection efforts have provided significant finds, many new discoveries have remained parked in the lab due to doubts and complications regarding the taxonomic status of the oldest-described species from Spain, *Iberozospeum schaufussi* (von Frauenfeld, 1862; Jochum et al., 2019). Only until recently were these taxonomic issues clarified such that the description of new species could proceed unhindered in a region known to harbour rich "*Zospeum*" diversity (Jochum et al., 2019). We emphasise that northern Spanish material molecularly assessed by Weigand et al. (2013) as cf. *Z. suarezi* (Gittenberger, 1980) and deposited in the BOLD data base under this name was revised (Jochum et al., 2019) and is now in sync with this investigation, *I. schaufussi* (von Frauenfeld, 1862). Caves harbouring two to three different morphotypes are considered not unusual (Alonso et al., 2018). Despite this suggested richness, only eight species have been formally described, including *I. schaufussi* (von Frauenfeld, 1862), *I. bellesi* (Gittenberger, 1973), *I. biscaiense* (Gómez & Prieto, 1983), *I. vasconicum* (Prieto et al., 2015 in Jochum et al., 2015a), *I. zaldivarae* (Prieto et al.,

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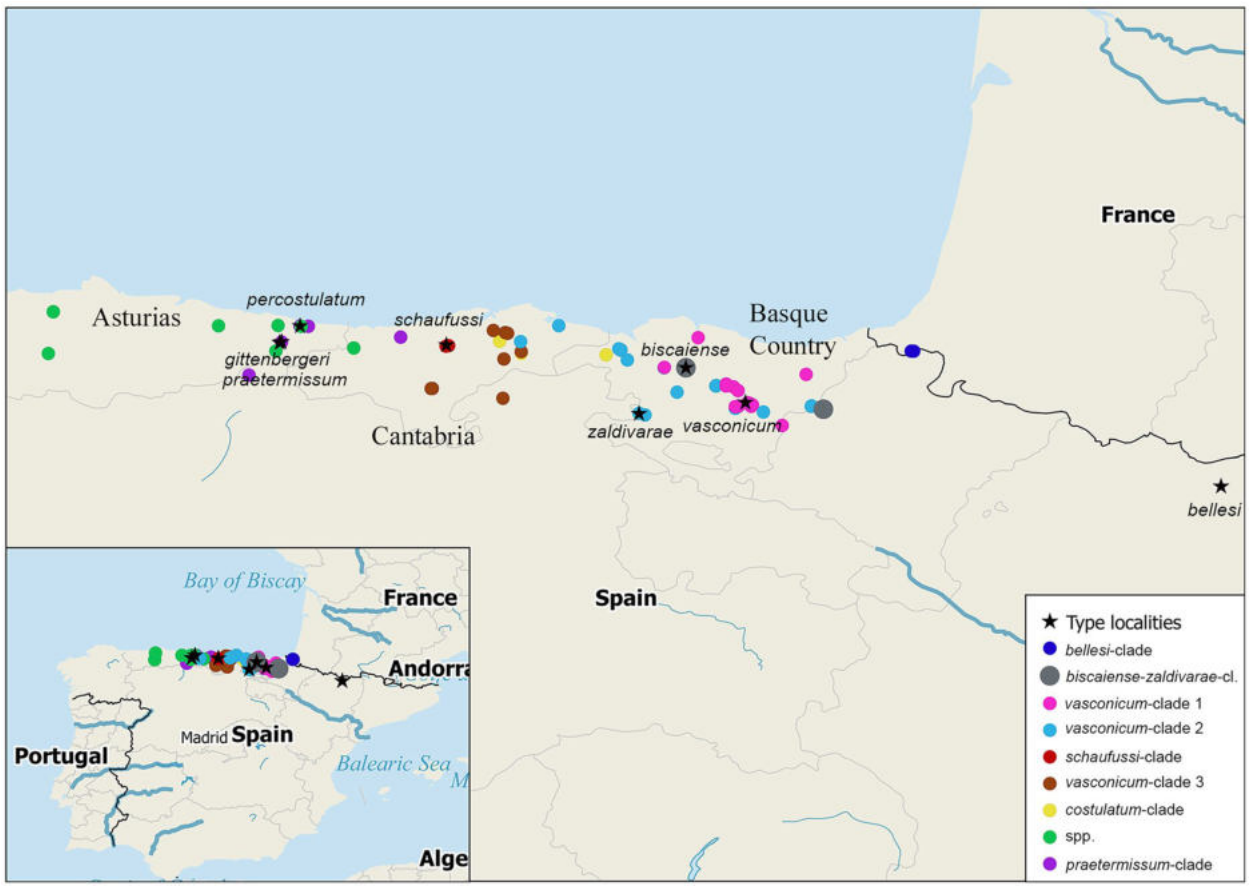


**Fig.1** Sampling locations of *Iberozospeum* specimens from northern Spanish caves grouped into clades named by province of origin and designated by coloured circles; stars represent the type localities of the hitherto known species

2015 in Jochum et al., 2015a), *I. percostulatum* (Alonso et al., 2018) and recently, *I. gittenbergeri* (Jochum et al., 2019) and *I. praetermissum* (Jochum et al., 2019).

Weigand et al. (2013) presented the first phylogenetic study incorporating Iberian "*Zospeum*" species, whereby nine populations of "*Zospeum*" are clustered into six evolutionary lineages (EL), with those from the Cantabrian Mountains being monophyletic. This study and additional phylogenetic investigations have since demonstrated the high incidence of intraspecific variability and cryptic diversity in Iberian, Eastern Alpine (Kruckenhauser et al., 2019; Weigand et al., 2011) and Dinaride (Inäbnit et al., 2019) *Zospeum* species.

In order to further understand zospeid evolutionary history in caves of northern Spain, we molecularly assess recent finds encompassing 57 populations. Anatomical perspectives of zospeid radulae and organ systems remain rare and few in number. Seven studies are known so far (see Jochum, 2011; Jochum et al., 2015b; Inäbnit et al., 2019). By studying histological sections of topotypic *I. vasconicum,* topotypic *Z. isselianum* (Pollonera, 1887) and *Z. amoenum* (von Frauenfeld, 1856) (see Jochum et al. 2015b) and *Z. spelaeum* (Rossmässler, 1839), we compare the presence of specific structures and character states in the visceral mass of one northern Spanish and three Dinaride taxa. We additionally describe significant differences in radular morphology and crystallographic structure on the columellar lamellae using scanning electron microscopy (SEM). Together, these investigations support the erection of the new genus, *Iberozospeum*.

## Material and methods

Material is housed in the following collections:

AJC: Adrienne Jochum Collection, Kelkheim, Germany.
CAA: Collection of Alvaro Alonso, Spain.
CSQS: Collection of Sergio Quiñonero-Salgado, Spain.
MHNG: Muséum histoire naturelle Gèneve, Geneva, Switzerland.

MNCN: Museo Nacional de Ciencias Naturales, Madrid, Spain.
MZB: Museu de Ciències Naturals (Zoologia) de Barcelona, Barcelona, Spain.
NHMW: Naturhistorisches Museum, Wien, Vienna, Austria.
NMBE: Naturhistorisches Museum der Burgergemeinde Bern, Bern, Switzerland.
RMNH: Naturalis Biodiversity Center (formerly Rijks-Museum van Natuurlijke Historie), Leiden, The Netherlands.
ZUPV/FC: Colección de Fauna Cavernícola (Departamento de Zoología) de la Universidad del País Vasco-Euskal Herriko Unibertsitatea, Bilbao, Spain.

## Individuals investigated in the molecular study

Individuals used for DNA sequence analyses were collected primarily by Carlos Prieto, Alvaro Alonso Suárez and Sergio Quiñonero-Salgado and additional collectors in caves from Asturias province to as far east as Navarra during the years 1987–2018. A total of 67 individuals were included. Sampling locations and the GenBank accession numbers for the obtained mtDNA (COI, 16S) and nDNA (H3, 5.8 S rRNA + ITS2) sequences are given in Table 1. Molecular analyses include the outgroup species *Zospeum spelaeum* (Rossmässler, 1839), *Zospeum exiguum* (Kuščer, 1932), *Zospeum manitaense* (Inäbnit et al., 2019), *Zospeum robustum* (Inäbnit et al., 2019), *Zospeum frauenfeldii* (Freyer, 1855), *Zospeum obesum* (von Frauenfeld, 1854) and *Zospeum pretneri* (Bole, 1960) from the Dinaric Alps (Inäbnit et al., 2019; Weigand et al., 2013). Additionally, sequences of *I. vasconicum* (No. 151 in Weigand et al., 2013), *I. schaufussi* (as *I. suarezi*, No. 140 in Weigand et al., 2013) and *I. zaldivarae* (No. 162 and 163 in Weigand et al., 2013) from their type localities were included. All images and DNA extracts of the specimens investigated in this study are housed in the Natural History Museum Bern (NMBE), Bern, Switzerland.

## DNA extraction, PCR amplification, and sequence determination

Live specimens were preserved in 80% ethanol. Before DNA extraction, every specimen was imaged under sterile conditions from frontal and apical view with a Leica DFC425 microscope camera using the image-processing program (IMS Client V15Q4, Imagic, Switzerland). For total DNA extraction, the Qiagen Blood and Tissue Kit (Qiagen; Hilden, Germany) was used. Each specimen was placed in a mix of 180 µl ATL buffer and 20 µl Proteinase K. It was then incubated for ca. 2 h at 56 °C in a heater (Labnet, Vortemp 56, witec AG, Littau, Switzerland). For subsequent DNA extraction, 200 µl Buffer AL was added. The mixture was vortexed and incubated at 56 °C for 10 min. Ethanol of 200 µl (100%) was added and vortexed again. The mixture was pipetted in a DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at 8000 rpm (Centrifuge 5424, Eppendorf) for 1 min. The spin column was placed in a new 2 ml collection tube and 500 µl Buffer AW1 was added. It was centrifuged for 1 min at 8000 rpm. The spin column was placed in a new 2 ml collection tube, and 500 µl Buffer AW2 was added. It was centrifuged for 3 min at 14,000 rpm. Afterwards, the spin column was transferred to a new 1.5 ml Eppendorf tube. The DNA was then eluted by adding 200 µl Buffer AE. It was incubated for 1 min at room temperature (ca. 23 °C) and centrifuged for 1 min at 8000 rpm.

In this study, two mitochondrial (mtDNA) markers (COI and 16S) and two nuclear (nDNA) markers (H3 and 5.8 S rRNA + ITS2) were investigated. PCR mixtures consisted of 12.5 µl GoTaq G2 HotStart Green Master Mix (Promega M7423), 4.5 µl dd$H_2O$, 2 µl forward and reverse primer each and 4 µl DNA template. In Table 2 the respective primer pairs for the PCR are listed. The following PCR cycles were used as follows: for COI the admixture was heated 2 min at 94 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 40 °C and 1 min at 72 °C and, finally, 5 min at 72 °C; for 16S the admixture was heated 5 min at 95 °C, followed by 45 cycles of 30 s at 95 °C, 30 s at 48 °C and 45 s at 72 °C and, finally, 5 min at 72 °C; for H3 the admixture was heated 3 min at 95 °C, followed 40 cycles of 1 min at 95 °C, 1 min at 42 °C and 1 min at 72 °C and, finally, 10 min at 72 °C; and for 5.8 S rRNA + ITS2 the admixture was heated 1 min at 96 °C, followed by 45 cycles of 30 s at 94 °C, 30 s at 50 °C and 1 min at 72 °C and, finally, 10 min at 72 °C (SensoQuest Tabcyclet and Techne TC-512, witec AG, Littau, Switzerland). The purification and sequencing of the PCR products were performed by LGC (LGC Genomics Berlin, Germany).

Specimens collected in 2011 or later could be successfully sequenced. Since the DNA content in older specimens was too low for any analyses, sequences of those specimens could not be included in our study. Long-term storage in ethanol proved to be problematic for the successful sequencing of such tiny snails (< 1.5 mm).

## Phylogenetic analyses

The software package Geneious v9.1.8 (Biomatters Ltd) was used for sequence processing and editing. Maximum likelihood (ML) topology was estimated with the Geneious RAxML plug-in (Stamatakis, 2006) using rapid bootstrapping setting to compute the best scoring ML tree and 1500 bootstrapping replicates. The protein-coding gene fragments of COI was defined in two data blocks. The first and third codon positions were defined as one block and the second

**Table 1** Sampled northern Spanish caves with their geographic location and the GenBank accession numbers for the analysed mtDNA (COI, 16S) and nDNA (H3, 5.8 S rRNA + ITS2) sequences

| NMBE-No | ZUPV-No | Cave and Province | Latitude | Longitude | Altitude [m] | GenBank accession number COI | GenBank accession number 16S | GenBank accession number H3 | GenBank accession number ITS2 |
|---|---|---|---|---|---|---|---|---|---|
| 540551_1 | 1847 | Akaitz Txiki (Gipuzkoa) | 42.9818 | −2.0925 | 824 | MW626838 | MW621911 | MW622169 | MW621272 |
| 540552_1 | 2039 | Azkillar (Bizkaia) | 43.0882 | −2.5912 | 1030 | MW626839 | MW621915 | MW622173 | MW621275 |
| 540552_3 | 2039 | Azkillar (Bizkaia) | 43.0882 | −2.5912 | 1030 | MW626841 | MW621917 | MW622175 | MW621277 |
| 540553_1 | 1066 | Elorrea (Bizkaia) | 43.0538 | −2.7956 | 1059 | - | MW621919 | MW622177 | - |
| 540554_4 | 1309 | Arrikrutz (Gipuzkoa) | 42.9988 | −2.4265 | 495 | - | MW621920 | MW622181 | MW621282 |
| 540555_2 | 937 | Basotxo (Navarra) | 42.8798 | −2.246 | 705 | - | - | MW622183 | - |
| 540556_1 | 874 | Hatxondo (Bizkaia) | 43.1835 | −2.862 | 300 | - | - | MW622184 | - |
| 557136 | 4885 | Herreria (Asturias) | 43.39784 | −4.75967 | 50 | - | MW621921 | MW622188 | MW621284 |
| 557138 | 4702 | Baja (Cantabria) | 43.40163 | −3.41371 | 51 | MW626842 | MW621922 | MW622189 | MW621285 |
| 557140 | 4862 | Collubina (Asturias) | 43.39841 | −4.72264 | 45 | MW626843 | MW621923 | MW622190 | MW621286 |
| 557142 | 3671 | Cumbre (Asturias) | 43.26893 | −4.89248 | 1761 | - | MW621924 | MW622191 | - |
| 557144 | 4136 | Udias (Cantabria) | 43.34163 | −4.23989 | 109 | MZ620729 | MW621925 | MW622192 | MW621287 |
| 557146 | 3820 | Montosas (Cantabria) | 43.22731 | −3.70042 | 725 | MW626844 | MW621926 | MW622193 | MW621288 |
| 557150 | 3405 | Txomenkoba (Gipuzkoa) | 42.98131 | −2.47352 | 810 | MW626845 | MW621927 | MW622194 | MW621289 |
| 557152 | 3758 | San Valerio (Gipuzkoa) | 43.08203 | −2.50105 | 444 | MW626846 | MW621928 | MW622195 | MW621290 |
| 557154 | 3849 | Penpelin (Gipuzkoa) | 43.062 | −2.47655 | 690 | MW626847 | MW621929 | MW622196 | MW621291 |
| 557156 | 3856 | Artegi (Bizkaia) | 43.09628 | −2.53847 | 540 | MW626848 | MW621930 | MW622197 | MW621292 |
| 557158 | 4601 | Saiturri-2 (Gipuzkoa) | 42.96902 | −2.49111 | 1063 | MW626849 | MW621931 | MW622198 | MW621293 |
| 557160 | 4757 | Urkoba (Gipuzkoa) | 42.97802 | −2.49103 | 1020 | - | - | MW622199 | MW621294 |
| 557162 | 4821 | Perusaroi-1 (Gipuzkoa) | 42.95026 | −2.34416 | 1156 | - | MW621932 | - | MW621295 |
| 557166 | 4990 | Aizkirri (Gipuzkoa) | 42.99568 | −2.41728 | 600 | MW626850 | MW621933 | MW622200 | MW621296 |
| 557168 | 5005 | Arlaban (Gipuzkoa) | 43.08629 | −2.53928 | 382 | MW626851 | MW621934 | MW622201 | MW621297 |
| 557174 | 1008 | Eskatxabel-2 (Bizkaia) | 43.27509 | −3.0888 | 565 | - | - | MW622202 | MW621298 |
| 557178 | 1607 | Paules (Burgos) | 42.94287 | −2.99363 | 830 | MW626852 | MW621935 | MW622203 | MW621299 |
| 557180 | 1740 | Lejazar (Araba) | 42.93484 | −2.96285 | 836 | - | MW621936 | MW622204 | MW621300 |
| 557182 | 874 | Hatxondo (Bizkaia) | 43.18162 | −2.86335 | 300 | - | MW621937 | - | - |

**Table 1** (continued)

| NMBE-No | ZUPV-No | Cave and Province | Latitude | Longitude | Altitude [m] | GenBank accession number COI | GenBank accession number 16S | GenBank accession number H3 | GenBank accession number ITS2 |
|---|---|---|---|---|---|---|---|---|---|
| 557187 | 1847 | Akaitz Txiki (Gipuzkoa) | 42.97995 | −2.09384 | 824 | MW626853 | MW621938 | MW622205 | MW621301 |
| 557188 | 1847 | Akaitz Txiki (Gipuzkoa) | 42.97995 | −2.09384 | 824 | MW626854 | MW621939 | MW622206 | MW621302 |
| 557189 | 2039 | Azkillar (Bizkaia) | 43.08632 | −2.59251 | 1030 | MW626855 | MW621940 | MW622207 | MW621303 |
| 557198 | no number | Fuente de Estragueña (Cantabria) | 43.2997 | −4.6066 | - | - | - | MW622208 | MW621304 |
| 557200 | 854 | San Juan-9 (Bizkaia) | 43.28078 | −3.1001 | 637 | MW626856 | MW621941 | MW622209 | - |
| 557207 | 3861 | Azkonar Zulueta (Gipuzkoa) | 42.98749 | −2.40171 | 790 | - | MW621942 | - | MW621305 |
| 557209 | 3904 | Iritegi (Gipuzkoa) | 42.9798 | −2.40768 | 527 | MW626857 | MW621943 | MW622210 | MW621306 |
| 557211 | 4864 | Grazal (Bizkaia) | 43.22227 | −3.05541 | 148 | MW626858 | MW621944 | MW622211 | MW621307 |
| 557213 | 5006 | Garcia (Burgos) | 43.022 | −3.7057 | 935 | MW626859 | MW621945 | MW622212 | MW621308 |
| 557215 | 5091 | Arrigueras (Cantabria) | 43.07316 | −4.07478 | 660 | - | - | MW622213 | MW621309 |
| 557217 | 4930 | Mendikute (Gipuzkoa) | 43.14687 | −2.12069 | 710 | MW626860 | MW621946 | MW622214 | MW621310 |
| 557219 | 5087 | Munarri Arrola (Bizkaia) | 43.33892 | −2.68541 | 63 | MW626861 | MW621947 | MW622215 | MW621311 |
| 557221 | 2874 | Irutxin (Navarra) | 42.966 | −2.02518 | 1095 | - | - | MW622216 | MW621312 |
| 557223 | 3728 | Otxas (Bizkaia) | 43.18599 | −2.74773 | 488 | MW626862 | MW621948 | MW622217 | MW621313 |
| 557225 | 4102 | Toyo (Cantabria) | 43.28468 | −4.48524 | 190 | MW626863 | MW621949 | MW622218 | MW621314 |
| 557226 | 4102 | Toyo (Cantabria) | 43.28468 | −4.48524 | 190 | MW626864 | MW621950 | MW622219 | MW621315 |
| 557227 | 4714 | Cuvias Negras (Cantabria) | 43.25668 | −3.60984 | 250 | - | - | MW622220 | - |
| 557229 | 4723 | San Juan de Socueva (Cantabria) | 43.26569 | −3.60993 | 430 | - | - | MW622221 | MW621316 |
| 557231 | 3078 | Valdebeci (Bizkaia) | 43.24918 | −3.1663 | 188 | - | - | MW622222 | MW621317 |
| 557232 | 4017 | Princesa (Bizkaia) | 43.2738 | −3.08898 | 620 | MW626865 | MW621951 | MW622223 | MW621318 |
| 557234 | 3371 | Lexotoa-2 (Navarra) | 43.26819 | −1.55843 | 215 | MW626866 | MW621952 | MW622224 | MW621319 |
| 557236 | 3323 | Lezea (Navarra) | 43.26853 | −1.57164 | 210 | MW626867 | MW621953 | MW622225 | MW621320 |
| 557238 | 2875 | Irutxin (Navarra) | 42.96476 | −2.03077 | 1095 | MW626868 | MW621954 | MW622226 | MW621321 |
| 557240 | 3727 | Otxas (Bizkaia) | 43.18165 | −2.74923 | 488 | - | MW621955 | MW622227 | MW621322 |
| 557242 | 4057 | Cubija (Cantabria) | 43.31812 | −3.61307 | 269 | MW626869 | MW621956 | MW622228 | MW621323 |

**Table 1** (continued)

| NMBE-No | ZUPV-No | Cave and Province | Latitude | Longitude | Altitude [m] | GenBank accession number COI | GenBank accession number 16S | GenBank accession number H3 | GenBank accession number ITS2 |
|---|---|---|---|---|---|---|---|---|---|
| 557244 | 559 | Paules (Burgos) | 42.94287 | −2.99363 | 840 | - | - | MW622229 | MW621324 |
| 557246 | 5210 | Valdemora (Asturias) | 43.47417 | −6.0556 | 310 | MW626870 | MW621957 | MW622230 | MW621325 |
| 557247 | 5302 | Valdemora (Asturias) | 43.47417 | −6.0556 | 310 | - | - | MW622231 | MW621326 |
| 557249 | 4915 | Llagar (Asturias) | 43.25613 | −6.08141 | 990 | MW626871 | MW621958 | MW622232 | - |
| 557251 | 5211 | Caleru (Asturias) | 43.40077 | −5.1919 | 80 | MW626872 | MW621959 | MW622233 | MW621327 |
| 557253 | 5209 | Herreria (Asturias) | 43.39987 | −4.76584 | 45 | MW626873 | MW621960 | MW622234 | MW621328 |
| 557255 | 5208 | Busecu (Leon) | 43.14277 | −5.03276 | 770 | MW626874 | MW621961 | MW622235 | - |
| 559620 | 5257 | Puente Inguanzo (Asturias) | 43.31626 | −4.86252 | 225 | MW626875 | MW621962 | MW622236 | MW621329 |
| 559622 | 5258 | Zurra (Asturias) | 43.40259 | −4.88078 | - | MW626876 | MW621963 | MW622237 | MW621330 |
| 559624 | 5203 | Iglesia (Cantabria) | 43.36562 | −3.69331 | 65 | MW626877 | MW621964 | MW622238 | MW621331 |
| 559626 | 3807 | Cesareo (Cantabria) | 43.32034 | −3.72279 | 258 | MW626878 | MW621965 | MW622239 | MW621332 |
| 559628 | 5175 | Soldados (Cantabria) | 43.29725 | −3.9894 | 370 | MW626879 | MW621966 | MW622240 | MW621333 |
| 559630 | 5171 | Buho (Cantabria) | 43.29427 | −4.0028 | 420 | MW626880 | MW621967 | MW622241 | MW621334 |
| 559632 | 5213 | Arrigueras (Cantabria) | 43.07284 | −4.0806 | 660 | MW626881 | MW621968 | MW622242 | MW621335 |
| 559634 | 5200 | Murcielagos (Cantabria) | 43.36159 | −3.68115 | 70 | MW626882 | MW621969 | MW622243 | MW621336 |
| 559636 | 5196 | Prementera (Cantabria) | 43.37676 | −3.75574 | 125 | - | MW621970 | MW622244 | MW621337 |

codon position as a second block. The non-coding regions from 16S and 5.8 S rRNA + ITS2 were defined as a single data block. The nucleotide model Gamma GTR I was used. Partitionfinder-2.1.1 (Lanfear et al., 2016) was applied for searching optimal evolutionary models for the partitions using the corrected Akaike Information Criterion (cAIC).

**Table 2** Primer designs used for PCR reactions

| Gene | Primer | Sequence | Sequence length (bp) | Reference |
|---|---|---|---|---|
| COI | LCO1490 | 5'-GGTCAACAAATCATAAAGATATTGG-3' | 680 | Folmer et al. (1994) |
| | HCO2198 | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' | | |
| 16S | 16S ar | 5'-CGC CTG TTT ATC AAA AAC AT-3' | 440 | Simon et al. (1994) |
| | 16S br | 5'- CCG GTC TGA ACT CTG ATC AT -3' | | |
| H3 | H3AD | 5'-ATGGCTCGTACCAAGCAGACVGC-3' | 380 | Colgan et al. (1998) |
| | H3BD | 5'-ATATCCTTRGGCATRATRGTGAC-3' | | |
| ITS2 | ITS2ModA | 5'-GCTTGCGGAGAATTAATGTGAA-3' | 900 | Bouaziz-Yahiatene et al. (2017) |
| | ITS2ModB | 5'-GGTACCTTGTTCGCTATCGGA-3' | | |

Bayesian Inference (BI) was performed using Mr. Bayes v3.2.6 × 64 (Altekar et al., 2004; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) through the HPC cluster from the University of Bern (http://www.id.unibe.ch/hpc). For the concatenated data set, Partitionfinder-2.1.1 was used for finding the optimal evolutionary models for each subset with the model = all function. The Monte Carlo Markov Chain (MCMC) parameter was set as follows: starting with four chains and four separate runs for 20 million generations with a tree sampling frequency of 1000 and a burn in of 25%.

### Species delimitation

The species delimitation method, Automatic Barcoding Gap Discovery (ABGD) was applied via the web browser (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html). The ABGD method (Puillandre et al., 2012) sorts the sequences into hypothetical species based on the barcode gap. ABGD was conducted using the COI alignment of the investigated *Iberozospeum* specimens. The input variables were chosen according to Weigand et al. (2013) and Inäbnit et al. (2019). The parameters were set as follows: Pmin = 0.001; Pmax = 0.1; Steps = 10; X = 1; Nb bins = 20; and distance: Jukes-Cantor.

### Morphological analyses using scanning electron microscopy (SEM)

Radulae of the eastern Alpine and Dinaride *Zospeum* used in the comparative analyses are presented in Inäbnit et al. (2019). For reasons of space, only images of the radular ribbons of *Z. exiguum* and *Z. pretneri* are presented here (Fig. 14). Topotypic *I. vasconicum* and *I. zaldivarae* were collected by AJ for radular investigation. Individuals from the westernmost sampled caves (Asturias Province), derived from the collection of Jos Notenboom, housed at the Naturalis Biodiversity Center, Leiden, NL: RMNH.MOL.234108 Cueva de Torcona (exception from Burgos); RMNH.MOL.234147 Cueva de la Huertas; RMNH.MOL.234109 Cueva de la Foz; RMNH.MOL.234116 Cueva a Sul; RMNH.MOL.234144 Cueva de Rales.

Radulae were prepared according to Holznagel (1998), preserved in 96% ethanol and mounted onto prepared SEM aluminium stubs. The radulae were sputtered with gold (1–2 × for 60 s) in the Agar Sputter Coater (Agar Scientific, Stanstead, UK) and viewed in the high vacuum mode of the Hitachi S-4500 scanning electron microscope (15 kV, probe current 20–100 pA) using the secondary electron detector. Photographs were taken with DISS—Digital Image Scanning System 5 (Point Electronic, Halle, Germany). All processing of radulae was conducted at the Goethe University (Frankfurt, Germany).

SEM images of all Spanish shells showing crystallographic structure were made at the Naturalis Biodiversity Center (Leiden, NL) using the JEOL JSM-6480LV scanning electron microscope. Aluminium stubs were coated with gold–palladium using the Polaron Equipment LTD-E5100 SEM auto-coating sputter system. Shells of three potential new species, awaiting further investigation beyond our purposes here, derive from the J. Notenboom Collection: RMNH.MOL.234104 Cueva del Comediante; RMNH.MOL.234141 Cueva a Sul; and RMNH.MOL.234120, Cueva Refugio, Basinagre, Trucios. The former two shells were preserved in 75% ethanol by the collector (Notenboom & Meijers, 1985), and, thus, finer morphological structure is consequently eroded in these shells.

Histological sectioning and light microscopy follow Jochum et al. (2015b). Three individuals used in the comparative morphology include *Z. isselianum* (AJC 2287), Turjeva jama, Slovenia (46.2435, 13.5046); *Z. spelaeum* (AJC 848), Betalov Spodmol cave, Slovenia (45.7922, 14.1877); and *I. vasconicum* (AJC 1848), Cueva de la Ermita de Sandaili (42.9994, 2.4381).

## Results

The maximum likelihood and Bayesian inference tree (Fig. 2) shows the concatenated data set (COI, 16S, H3 and 5.8 S rRNA + ITS2) of 67 specimens of species from the west European radiation from 55 caves and seven Dinaride outgroup taxa. The dataset was supplemented with genetic data from Weigand et al. (2013) of topotypic specimens of *I. vasconicum* (No. 151 in Weigand et al., 2013), *I. zaldivarae* (No. 162 and 163 in Weigand et al., 2013) and *I. schaufussi* (designated as *I. suarezi*, No. 140 in Weigand et al., 2013). The tree was rooted by two species of *Carychium* from the collection of AJ (in the NMBE). The topology reveals nine larger clades, which follow a geographic pattern (cf. Fig. 1).

The basic node separates Dinaride *Zospeum* taxa from taxa inhabiting northern Spanish caves. This node has a full ML and BI support and justifies the designation of the western *Zospeum* radiation to a separate new genus, *Iberozospeum* n. gen. The p-distances which show the numbers of base differences per site between sequences were calculated (Kumar et al., 2018). The mean p-distance from *Iberozospeum* n. gen. and *Zospeum* is 0.0767 (Table S1 in the supplementary material). The mean p-distance from *Zospeum* and *Carychium* is 0.1285. The mean p-distance of *Iberozospeum* n. gen. and *Carychium* is 0.1177.

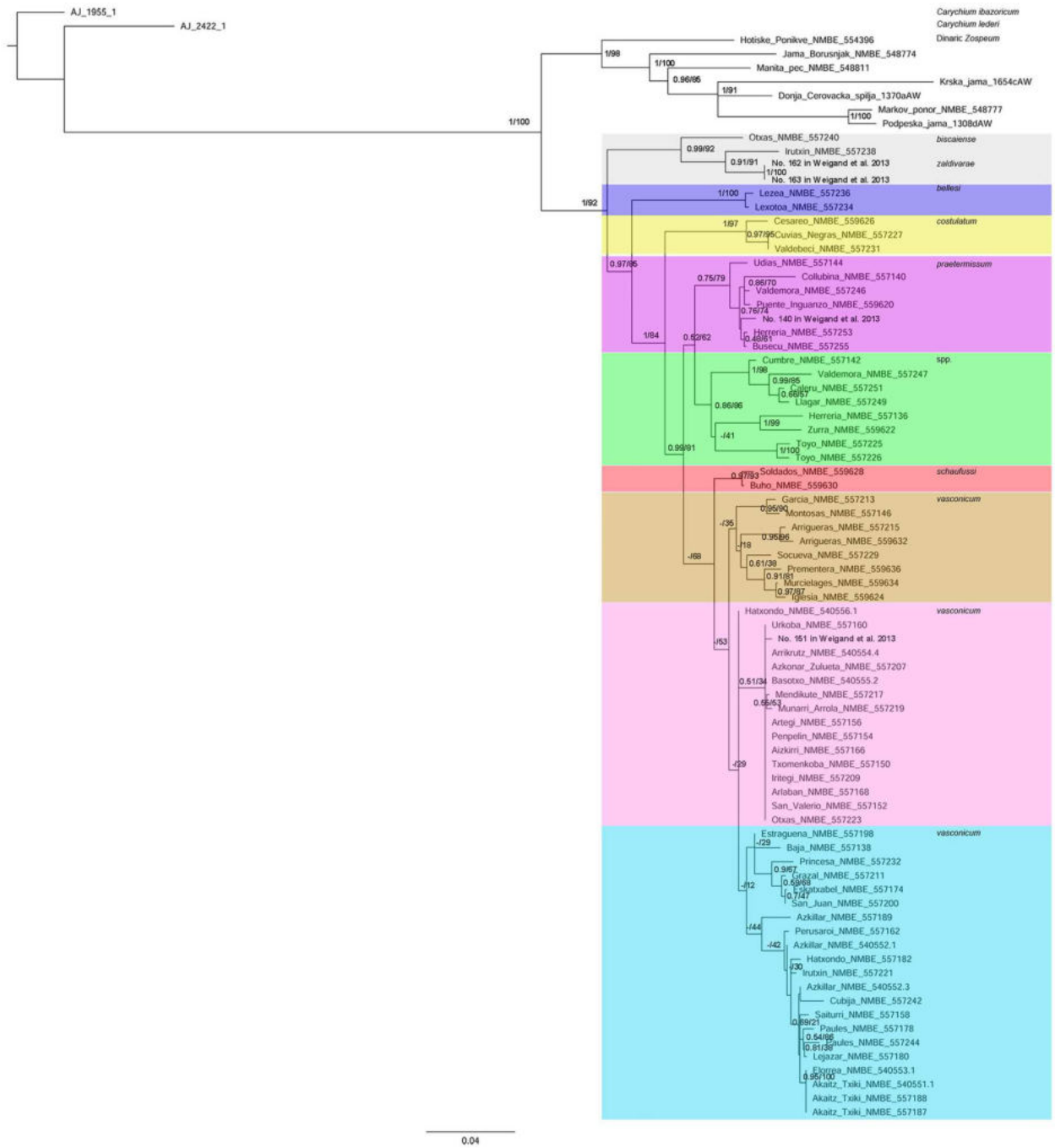


**Fig. 2** Combined maximum likelihood (RAxML) and Bayesian inference (BI) tree based on concatenated data set of COI, 16S, H3 and 5.8 S rRNA + ITS2. The analysis comprises 67 individuals of northern Spanish *Iberozospeum* from 55 caves and seven Dinaride outgroup taxa. The tree was rooted by two species of *Carychium* from the collection of AJ (in the NMBE). Additionally, sequences of *I. schaufussi*, *I. vasconicum* and *I. zaldivarae* from their type localities are included. The numbers at the nodes represent the Bayesian posterior probabilities (left) and the bootstrap support values (right). The dash (-) means that the node is not supported

In order to study the effect of incomplete genetic data, a reduced set comprising 42 individuals was computed (Fig. 3). Here, only specimens were used with a complete record of all four markers. The bootstrap support values and the Bayesian posterior probabilities are over all higher in the tree with complete marker sets (Fig. 3).

The *biscaiense-zaldivarae*-clade (grey clade in Figs. 2 and 3) harbours congeners from two different caves (Otxas

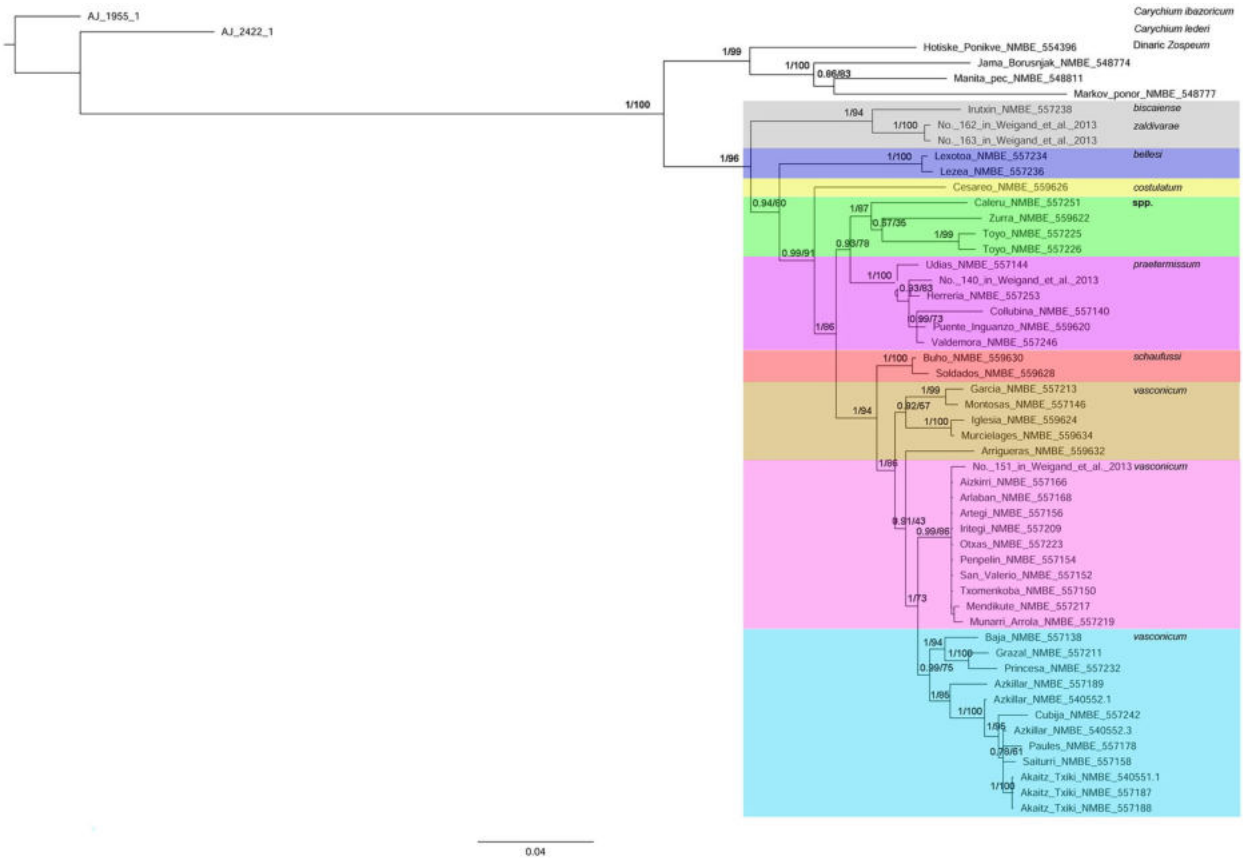


**Fig. 3** Combined maximum likelihood (RAxML) and Bayesian inference (BI) tree based on concatenated data set of COI, 16S, H3 and 5.8 S rRNA + ITS2. The analysis consists of 42 individuals of northern Spanish *Iberozospeum* from 37 caves and four Dinaride outgroup taxa. Only specimens with complete marker sets are included. The tree was rooted by two species of *Carychium* from the collection of AJ (in the NMBE). Additionally, sequences of *I. schaufussi*, *I. vasconicum* and *I. zaldivarae* from their type localities are included. The numbers at the nodes represent the Bayesian posterior probabilities (left) and the bootstrap support values (right)

and Irutxin) which cluster with specimens of *zaldivarae* from the type locality (No. 162 and 163 in Weigand et al., 2013). The caves, Otxas and Irutxin, are about 100 km apart from each other and are non-contiguous. Congeners from these two caves are also found in the low-resolution *vasconicum*-clade 1 (pink clade in Figs. 2 and 3) and *vasconicum*-clade 2 (light blue clade in Figs. 2 and 3). From the external perspective, the investigated specimen (NMBE 557240) in the grey clade shows a typical *biscaiense* morph (Figs. 4a). It is found in the cave Otxas, which is the type locality of *I. biscaiense*. The specimen from the cave Irutxin (NMBE 557238) displays typical character states of *zaldivarae*.

The specimens from the *bellesi*-clade (dark blue clade in Figs. 2 and 3) were collected in the caves Lezea and Lexotoa at the border of Spain and France. The caves are 1 km apart from each other and probably contiguous. This node is supported in our investigation (posterior probability of 0.97 and bootstrap value of 85 in Fig. 2, respectively, 0.94 and 80 in Fig. 3). In both phylogenetic trees (Fig. 2 and 3), the two investigated specimens have a bootstrap value of 100. The p-distance is 0.0039. From a morphological perspective, they have a similarly high-spired, conical shell form, but the aperture is clearly different. The aperture of the specimen from Lexotoa (NMBE 557234) (Fig. 5b) is oblique and has a moderately angular parietal shield. On the other hand, the parietal shield of the specimen from Lezea (NMBE 557236) (Fig. 5a) is compact and consists of thick callus, which is seemingly fused onto the body whorl. The peristome of the Lezea specimen is thick and roundish in form.

The Basque clade (Figs. 6 and 7) splits into two: a narrow, more centrally distributed *vasconicum*-clade 1 (pink clade in Figs. 2 and 3; Fig. 6) and a broader, western-reaching distribution comprising the *vasconicum*-clade 2 (light blue clade in Figs. 2 and 3; Fig. 7). The node of the split of these two clades has a low support (bootstrap value of 29 in Fig. 2, respectively 73 in Fig. 3). The split of these two clades is

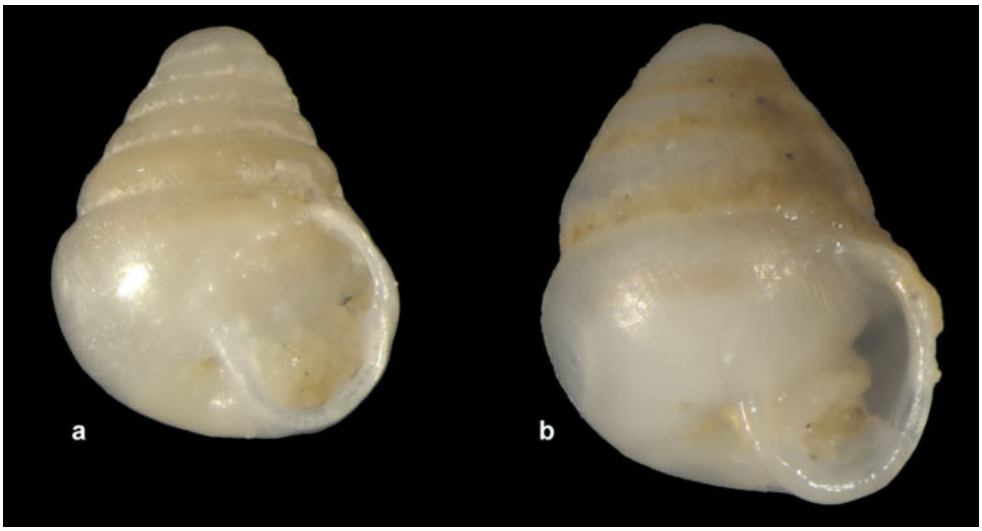


**Fig. 4** Grey clade; (**a**) *Iberozospeum biscaiense* NMBE 557240, Durang, Igorre, Cueva Otxas, 23.3.2016, sh: 1.37 mm; (**b**) *Iberozospeum zaldivarae* NMBE 557238, Aralar, Arbizu, Cueva Irutxin, 20.6.2015, sh: 1.59 mm. — All phot. × 40

not supported by the Bayesian posterior probability. The topotypic specimen of *I. vasconicum* (No. 151 in Weigand et al., 2013) clusters within the pink clade. Morphologically, shells from caves clustering in the pink *vasconicum*-clade 1 (Fig. 6) showed a commensurate similarity in shell shape and apertural configuration with noticeable differences in spire height in shells from Basotxo cave (Ton de Winter, unpubl. data 2015). The two westernmost specimens NMBE 557138 and NMBE 557242 (cf. Fig. 1 and Fig. 7b, j) in the light blue *vasconicum*-clade 2 differ morphologically from the remaining *vasconicum*-like specimens in the clade.

The *vasconicum*-clade 3 (brown clade in Figs. 2 and 3) has high posterior probability and bootstrap support only in the terminal nodes. The deep nodes in the brown clade are not supported in the ML analysis (bootstrap support of 53, respectively, 57 in Fig. 3). Morphologically, they resemble typical *I. vasconicum* due to shell shape and the apertural configuration. The specimen in Fig. 8a is about 1.5 times bigger than the smallest specimen from the brown clade, but the shell shape is very similar to the other specimens in this clade. Figure 8g is remarkable due to its broad conical form, the deeply pronounced suture and the obvious, broadly deepened umbilicus. We consider it a new species.

Support values in the *schaufussi*-clade (red clade in Figs. 2 and 3) are high. In Fig. 9a, a specimen from the cave Búho, Puente Viesgo, is illustrated, which is the type locality of *I. suarezi* (Gittenberger, 1980). However, comparing our specimen with the holotype of *I.*

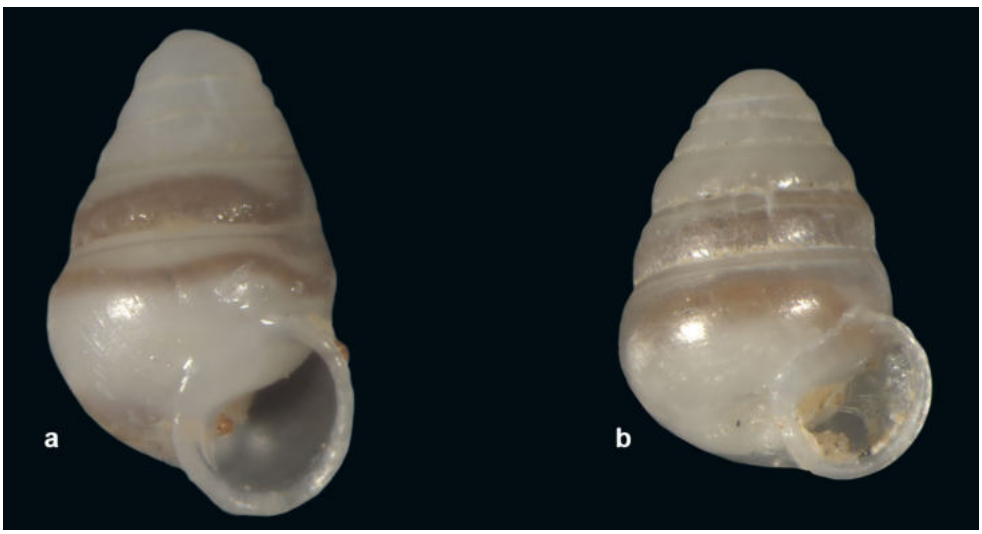


**Fig. 5** *Iberozospeum bellesi.* Dark blue clade; (**a**) NMBE 557236, Zugarram, Sare, Lezea, 13.12.2015, sh: 1.67 mm. (**b**) NMBE 557234, Zugarram, Zugarramurdi, Lexotoa-2, 20.12.2015, sh: 1.38 mm. — All phot. × 40


**Fig. 6** *Iberozospeum vasconicum*. Pink clade; (**a**) NMBE 557207, Aizkorri, Onati, Azkonar Zulueta, 28.5.2016, sh: 1.31 mm; (**b**) NMBE 557160, Aizkorri, Eskortatza, Urkoba, 8.3.2017, sh: 1.18 mm; (**c**) NMBE 557217, Ernio, Tolosa, Mendikute, 23.9.2017, sh: 1.5 mm; (**d**) NMBE 557219, Bust-Lea, Forua, Munarri Arrola, 9.12.2017, sh: 1.56 mm; (**e**) NMBE 557209, Aizkorri, Onati, Cueva Iritegi, 28.5.2016, sh: 1.43 mm; (**f**) NMBE 557150, Aizkorri, Onati, Txomenkoba Goikoa, 16.12.2015, sh: 1.27 mm; (**g**) NMBE 557156, Udalaitz, Elorrio, Cueva Artegi, 17.5.2016, sh: 1.48 mm; (**h**) NMBE 557168, Aizkorri, Onati, Arlaban, 11.2017, sh: 1.27 mm; (**i**) NMBE 557154, Udalaitz, Aretxabaleta, Cueva Penpelin, 26.4.2016, sh: 1.34 mm; (**j**) NMBE 557166, Aizkorri, Onati, Aizkirri, 11.2017, sh: 1.59 mm; (**k**) NMBE 557223, Durang, Igorre, Otxas, 23.3.2016, sh: 1.24 mm; (**l**) NMBE 557152, Udalaitz, Arrasate, San Valerio (Galarra), 23.3.2016, sh: 1.25 mm. No picture of NMBE 540554.4, 540555.2, 540556.1 (shell destroyed). — All phot. × 40


a
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p

◂**Fig. 7** *Iberozospeum vasconicum*. Light blue clade; (**a**) NMBE 557198, Cueva de la Fuente de Estragueña, 16.6.2011, sh: 1.26 mm; (**b**) NMBE 557138, Castro, Laredo, La Baja, 12.4.2017, sh: 1.11 mm; (**c**) NMBE 557232, Triano, Galdames, Mina Princesa (superior), 3.7.2016, sh: 1.51 mm; (**d**) NMBE 557211, Triano, Güenes, Grazal, 2.10.2016, sh: 1.49 mm; (**e**) NMBE 557174, Triano, Galdames, Escachabel-2 (Urallaga), 1.4.2013, sh: 1.44 mm; (**f**) NMBE 557200, Triano, Galdames, Bitzkaia, Cueva de la San Juan, 10.6.2012, sh: 1.23 mm; (**g**) NMBE 557189, Durang, Atxondo, Azkillar (=Galtaikoba), 16.3.2014, sh: 1.19 mm; (**h**) NMBE 557182, Durang, Zeberio, Cueva Hatxondo, 30.12.2012, sh: 1.28 mm; (**i**) NMBE 557221, Aralar, Arbizu, Cueva Irutxin, 20.6.2015, sh: 1.54 mm; (**j**) NMBE 557242, Ason, Matienzo, Cueva Cubija (=Marcos), 19.7.2016, sh: 1.34 mm; (**k**) NMBE 557178, Salvada, Berberana, Las Paules, 21.6.2011, sh: 1.3 mm; (**l**) NMBE 557180, Salvada, Izarra, Torca Lejazar, 28.12.2013, sh: 1.29 mm; (**m**) NMBE 557244, Salvada, Berberana, Las Paules, 9.11.2013, sh: 1.28 mm; (**n**) NMBE 557158, Aizkorri, Eskoriatza, Saiturri-2, 22.2.2017, sh: 1.47 mm; (**o**) NMBE 557187, Aralar, Ataun, Cueva Akaitz Txiki, 14.1.2014, sh: 1.47 mm; (**p**) NMBE 557188, Aralar, Ataun, Cueva Akaitz Txiki, 14.1.2014, sh: 1.32 mm. NMBE 557162, Aizkorri, Parzoneria, Perusaroi-1, 15.5.2017 (subadult shell, not shown on plate). No pictures of NMBE 540551.1, 540552.1, 540552.3, 540553.1 (shells destroyed). — All phot. ×40

*suarezi* (RMNH.MOL.55383) (Jochum et al., 2019: 72, Fig. 4a–c), it is evident that this is the same species. The specimens from the two Cantabrian clades have a more elliptical aperture compared with the *vasconicum*-clade specimens (pink, light blue and brown clades in Figs. 2, 3, 6, 7, and 8).

The Asturian clade splits into two subclades (purple and green clades in Figs. 2 and 3). Support values within these clades are moderate to high. Weigand et al. (2013: Fig. 5) sequenced two specimens from the Cueva del Bosque/Cueva Inguanzo, which cluster within the purple clade (Figs. 2 and 3). Moreover, Weigand et al. (2013) identified these two specimens as *I. suarezi* and considered them "evolutionary lineage Z14" (No. 140 in Weigand et al., 2013 in our trees). Jochum et al. (2019: 83–84, Fig. 15) explained that this cave is situated 1 km opposite of the cave, Cueva del Puente de Inguanzo, which is the cave Gittenberger (1980) considered to be inhabited by *I. schaufussi* sensu Gittenberger (1980: Fig. 1) and *I. suarezi* Gittenberger (1980: Fig. 2). These two taxa were recognised by Jochum et al. (2019) to be misidentifications and were subsequently described as new species, viz., *I. gittenbergeri* Jochum (Jochum, Prieto & De Winter, 2019 in Jochum et al., 2019) and *I. praetermissum* Jochum (Jochum, Prieto & De Winter, 2019 in Jochum et al., 2019). Since *I. gittenbergeri* is known only from a single shell and no clearly definable, live adult snail was found, it could not be included in this study. In Figs. 2 and 3, a specimen from the "evolutionary lineage Z14" (Weigand et al., 2013) and another species (Fig. 10f) from Cueva del Puente Inguanzo are included. The specimen in Fig. 10f was found at the type locality of *I. praetermissum*. This specimen is a juvenile and the identification is not clear since the aperture is not fully grown. The specimens in Figs. 10a–e have a high, conical spire and a barely developed lamella on the columella (Fig. 10d) and are considered to be *I. praetermissum*. The p-distance from the specimen in Fig. 10f and the topotypic specimen No. 140 from Weigand et al. (2013) is 0.0086. The mean p-distance from the investigated specimens in the purple clade (Figs. 2 and 3, Fig. 10a–f) and the specimen, No. 140 from Weigand et al. (2013), is 0.006.

The green clade contains various morphs. The topotype specimen of *I. percostulatum* (Fig. 10h) from the cave Herrería is a juvenile. In this cave, another species, here considered to be *I. praetermissum*, (Fig. 10b) lives in syntopy or at least in sympatry with *I. percostulatum*. Another well-supported lineage is visible in Fig. 10i and j, with both specimens originating from the same population in El Toyo in Cantabria; the two shells are morphologically quite different, illustrating shell variability within the lineage. The shell of the specimen from the Picos (Fig. 10k) is broadly conical, with a remarkably narrow-stepped coiling pattern of the teleoconch whorls and a large elliptical aperture. The group comprising Fig. 10l–n, forms another lineage in the green clade, which is characterised by broad conical shells resembling the specimen from the Picos, but with high and well-rounded teleoconch whorls.

The yellow clade (Figs. 2, 3, and 11) contains ribbed specimens with varying degrees of superficial striation. Although the specimen in Fig. 11a has less-pronounced ribs, they are clearly visible in apical view. This shell bears a deep suture and its aperture is elliptical and reverted. The specimen in Fig. 11b is a juvenile but the ribs are clearly visible. Although the shell morphology is different, it clusters together with specimen 11c, with a bootstrap support of 95 (Fig. 2). The p-distance of these individuals is 0 since only the nuclear marker H3 could be sequenced for both specimens and this sequence is identical. On the other hand, the strongly ribbed specimen in Fig. 10c constitutes the new species, *Iberozospeum costulatum* n. sp.

The ABGD assigned the 45 *Iberozospeum* COI sequences into 22 groups. The Barcode gap distance is 0.012. In Table 3 are the groupings of the ABGD analysis. Since we do not have the COI sequences of all investigated animals, not all specimens could be assigned to a group. The two animals from the same population from Toyo Cave (NMBE 557225 and 557226) were divided into different groups. Using ABGD, all animals from the pink *vasconicum*-clade were classified into group 1. Group 2 consists exclusively of animals from the light blue *vasconicum*-clade. Some animals from the light blue *vasconicum*-clade were classified into other groups (Table 3). The two animals from the *schaufussi*-clade (NMBE 559628 and 559630) were placed in group 6. The specimens from the *praetermissum*-clade were placed in group 7. The two specimens from the *bellesi*-clade (NMBE 557234 and 557236) were assigned in group 15. The remaining groups each contain only one animal.

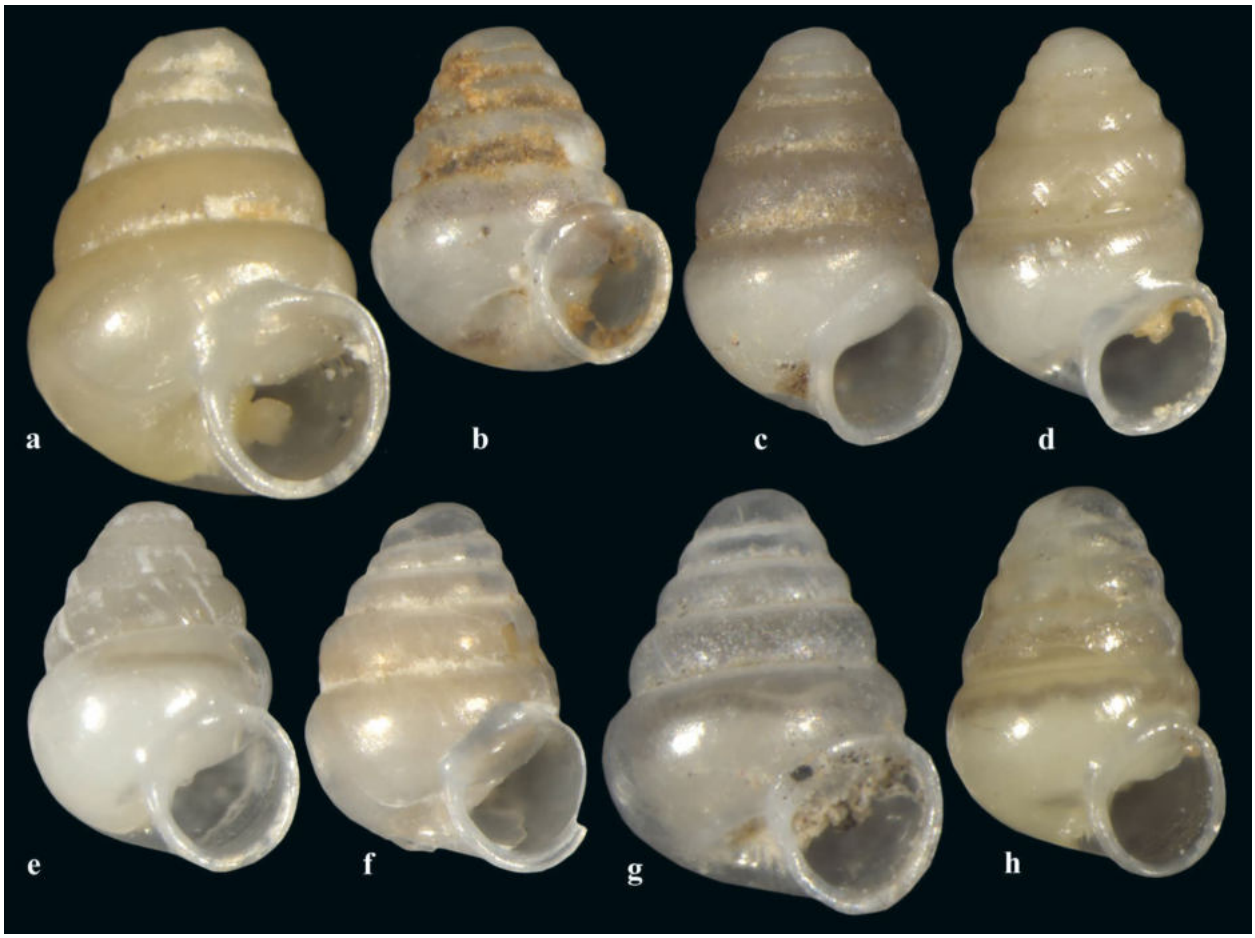



**Fig. 8** *Iberozospeum vasconicum*. Brown clade; (**a**) NMBE 559634, Cantabria, Entrambasaguas, Murcielagos, 28.3.2018, sh: 1.56 mm; (**b**) NMBE 559624, Cantabria, Entrambasaguas, Iglesia, 28.3.2018, sh: 1.14 mm; (**c**) NMBE 557215, Cantabria, Santiurde de Reinosa, Las Arrigueras, 20.1.2018, sh: 1.39 mm; (**d**) NMBE 559632, ditto, 29.3.2018, sh: 1.36 mm; (**e**) NMBE 557213, Guareña, Merindad de Sotoscueva, Garcia, 26.11.2017, sh: 1.23 mm; (**f**) NMBE 557146, Miera, Soba, Las Montosas, 20.2.2016, sh: 1.23 mm; (**g**) NMBE 557229, Ason, Arredondo, San Juan de Socueva, 12.4.2017, sh: 1.41 mm; (**h**) NMBE 559636, Cantabria, Entrambasaguas, Prementera, 28.3.2018, sh: 1.3 mm. — All phot. ×40

## Morphology

Although only one member of *Iberozospeum* is histologically sectioned here, sections of the upper visceral complex of topotypic *I. vasconicum* (AJC 1848) show that the anatomy follows the general carychiid design (Morton, 1955; Harry, 1997–1998) and that described for *Zospeum* in Maier (1982), Dörge (2010), and Jochum et al. (2015b) as well as in Barker (2001) for aspects of the Ellobiidae (Fig. 12). The cross-sections reveal no phylogenetic differences but rather, seasonal differences in the specific individuals. The *I. vasconicum* individual, collected in June 2011, is aphallate. Euphally was detected by Jochum et al. (2015b) in *Z. amoenum* (von Frauenfeld, 1856) (species revised from "*Zospeum* sp." in Inäbnit et al., 2019), collected in August 2008 from Konečka zijalka, Slovenia. The histological sections of the upper visceral complex of individuals of topotypic *Z. isselianum* (Pollonera, 1887) (AJC 2287) and *Z. spelaeum* (Rossmässler, 1839) (AJC 848) from the Dinarides (Fig. 12a–b) in this work also show that these snails were aphallate during time of collection in June and October, respectively. The same upper visceral region of the snail (cuts 14–22) in our histological sections (Fig. 12) shows different perspectives due to the generally larger size of the two Dinaride individuals in this study. Visible in clockwise rotation from the outside in, *Z. isselianum* shows the mantle gland (mag), the cerebral ganglion (cg), the mantle cavity (mc), the pleural ganglion (pg), the foot (f), the pharynx (ph), the oesophagus (oe), the statocyst containing otoliths and the columellar muscle (cm) (Fig. 12a). For *Z. spelaeum*, the same clockwise perspective shows the mantle gland (mag), the foot (f), some haemolymph (hl), the pharynx (ph), the oesophagus (oe), a hump shaped upper, non-vascularized columellar muscle (cm) and a moderately long genital opening (go) (Fig. 12b). For *I. vasconicum,* a remarkably huge, glandular albumen gland (ag) is clearly

visible (Fig. 12c). This is a compound, sac-like enlargement of the oviduct and is responsible for producing albumen for the formation of the eggs (Luchtel et al., 1997). The albumen gland is a female accessory sex gland, which enlarges with sexual maturation of the animal. The size of this gland is considered to determine the maximum number of eggs that can be produced at any one time (Barker, 2001). Due to the presence and large size of the albumen gland (indicative of the female phase) here, modification in existing structures and superposition of others on the fundamental carychiid pattern is clearly the case. Also, strikingly apparent is the large, well-developed, mucous gland (stained reddish violet) (mg). Additionally, visible in the clockwise perspective of the upper visceral complex of *I. vasconicum* is the kidney (k) with a prominent renopericardial passage and the heart (h) located at the base of the kidney, haemolymph (hl) at the foot section (f), the contractile pneumostome (p), the mantle gland (mag), the pharynx (ph) and oesophagus (oe), the vascularized, 2-humped columellar muscle (cm) and the long genital opening (go) (Fig. 12c). We remark that deeper analysis of anatomical aspects and individual structural analyses of *Iberozospeum* is beyond the scope of this paper and will be presented in a future work.

Significant however, for *Iberozospeum* n. gen., is that the columellar muscle shows prominent humps, which contain vasculature extending to the tips of these humps (Fig. 12c). In our investigation of the columellar lamellae of *Iberozospeum*, the dense, scaley crystallographic structure on localised zones of the lamellae correlates perfectly with the corresponding points of contact of these two hump-like elevations of the columellar muscle (Fig. 13). In sync with this observation, Barker (2001) emphasized there is a trend amongst the Ellobiidae that the columellar muscle becomes detached from the body wall from its origin on the columella and has become largely free in the haemocoel. The columellar muscle, thus, runs forward to attach on the cephalic organs and anterior body wall (Barker, 2001). In this case, rather than just simply retracting, the cephalopedal mass becomes inverted when the animal retracts into the shell (Barker, 2001). In *Iberozospeum*, the columellar muscle is indeed detached except at these two points of contact. In the Alpine and Dinaride species of *Zospeum*, crystallographic structure is not overlapping and not restricted to specific points. Moreover, it is comprised of low shoals of crystallographic structure (seen also in fossil Carychiidae in Jochum et al., 2015c, Fig. 5), randomly interspersed over the columellar lamellae deep in the shells of *Z. spelaeum* from two separate caves in Slovenia (MCBI CSR SASA 37049a, Velika Pasica and AJC 847, Betalov Spodmol Grotte) (Fig. 13a–b) here. Remarkable are the dense, localised, overlapping wedges of crystallographic structure on the complementary points of the columellar lamellae in the empty shells of the Iberian taxa: *Iberozospeum* sp. RMNH.MOL.234120 (Cueva Refugio, Trucios) (Fig. 13c–d), on the upper part of the lamella of *Iberozospeum* sp. RMNH.MOL.234104 (Cueva del Comediante) and on the lamella of *Iberozospeum* sp. RMNH.MOL.234141 (Cueva a Sul, Oviedo) (Fig. 13e–f) as well as on that of *I. vasconicum* (AJC 1849, Cueva Arrikrutz) (Fig. 13g–h). The lower lamella of *I.* sp. RMNH.MOL.234120 (Cueva Refugio, Trucios) clearly shows the specific locality of the contact point of the columellar muscle (Fig. 13c–d). Shells of RMNH.MOL.234104 (Cueva del Comediante) and RMNH.MOL.234141 (Cueva a Sul, Oviedo) (Fig. 13e–f), which were initially preserved in 75% ethanol, still show the characteristic, rough crystallographic structure despite deterioration by the ethyl-alcohol treatment subjected to them by the collector (Notenboom & Meijers, 1985).

Our morphological investigations also included the radulae of four Dinaride and eastern Alpine taxa described and imaged in Inäbnit et al. (2019, Supplementary Figs. S17–21) including *Z. exiguum* (Kuščer, 1932) (NMBE 553384) (Inäbnit et al., 2019, Fig. S17a–d); *Z. obesum* (von Frauenfeld, 1854) (NMBE 553409) (Inäbnit et al., 2019, Fig. S18f–h); *Z. pretneri* (Bole, 1960) (NMBE 553290) Inäbnit et al., 2019, Fig. S19a–d); and *Z. spelaeum* (Rossmässler, 1839) (NMBE 553311) (Inäbnit et al., 2019, Fig. S20e–f). These were compared with radulae extracted from individuals collected from the westernmost-sampled caves by Oviedo (Asturias) and radulae from topotypic material of *Z. vasconicum* (Prieto et al., 2015 in Jochum et al., 2015a) (AJC 1847) and *Z. zaldivarae* (Prieto et al., 2015 in Jochum et al., 2015a) (AJC 1876).

In this comparative study, the narrow radular ribbons of Dinaride *Zospeum* are tapered to an obtuse or to a straight base as in *Z. exiguum* (Fig. 14a) or a straight base as in *Z. pretneri* (Fig. 14b). The Iberian radulae have a tapered

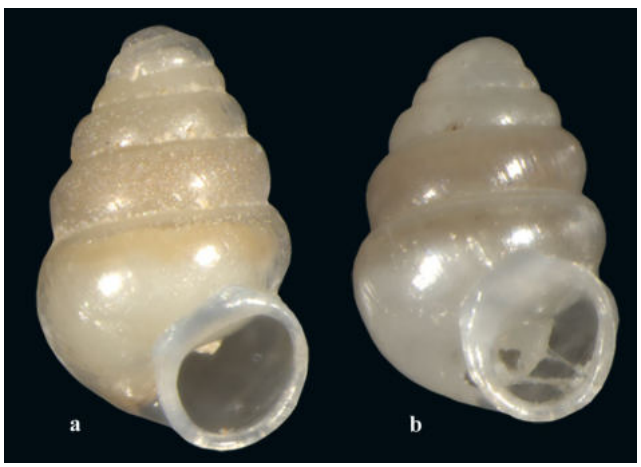



**Fig. 9** *Iberozospeum schaufussi*. Red clade; (**a**) NMBE 559630, Cantabria, Puente Viesgo, Búho, 27.3.2018, sh: 1.41 mm; (**b**) NMBE 559628, Cantabria, Puente Viesgo, Soldados, 27.3.2018, sh: 1.28 mm. — All phot. ×40


a
b
c
d
e
f
g
h
i
j
k
l
m
n

◂**Fig. 10** *Iberozospeum praetermissum*. Purple clade; a–f. (**a**) NMBE 557144, Cantabria, Cabezon-MS, Udias, Udias (Cobijon), 28.8.2016, sh: 1.25 mm; (**b**) NMBE 557253, Asturias, Llanes, Cueva La Herrería, 23.12.2017, sh: 1.34 mm; (**c**) NMBE 557255, Leon, Soto de Sajambre, Cueva Busecu, 10.3.2018, sh: 1.37 mm; (**d**) NMBE 557246, Asturias, Candamo, Cueva de la Peñona de Valdemora, 5.3.2018, sh: 1.22 mm; (**e**) NMBE 557140, Asturias, Llanes, Collubina, 18.7.2017, sh: 1.35 mm; (**f**) NMBE 559620, Cantabria, Cueva Puente Inguanzo, 6.4.2018, sh: 1.29 mm. — *Iberozospeum* spp. Green clade; g–n. (**g**) NMBE 559622, Cantabria, Cueva La Zurra, 6.4.2018, sh: 1.31 mm; (**h**) NMBE 557136, Asturias, Llanes, La Herrería, 18.7.2017, sh: 1.14 mm; (**i**) NMBE 557226, Cantabria, Lamason, El Toyo, 11.7.2015, sh: 1.7 mm; (**j**) NMBE 557225, Cantabria, Lamason, El Toyo, 11.7.2015, sh: 1.33 mm; (**k**) NMBE 557142, Asturias, Picos, Cabrales, Torca Cumbre, .8.2016, sh: 1.45 mm; (**l**) NMBE 557251, Asturias, Parres, Cueva El Caleru, 10.3.2018, sh: 1.41 mm; (**m**) NMBE 557249, Asturias, Yernes y Tameza, Cueva Llagar, 2.2.2018, sh: 1.49 mm; (**n**) NMBE 557247, Asturias, Candamo, Cueva de la Peñona de Valdemora, 5.3.2018, sh: 1.48 mm. — All phot. ×40

anterior end (velum), followed by a well-defined adhesive zone leading to a remarkably straight, carpet-like swath of longitudinal rows of smaller teeth and more of them per transverse row (Figs. 14c–h, 15c). The rachidian and lateral teeth of *I. vasconicum* demonstrate remarkable similarity to those of *Carychium ibazoricum* Bank & Gittenberger, 1985, imaged in Martins (2007, Fig. 138) (Fig. 15e). The rachidian and lateral teeth of the radula of topotypic *I. vasconicum* (AJC 1848) show long endo- and ectocones flanking the mesocone by one half–three fourths the length of the mesocone (Fig. 15e). The radula of topotypic *Z. pretneri* (NMBE 553290), *I. vasconicum*'s externally most similar phenotypic relative from the Dinarides, shows endo- and ectocones that are one third–one half the length of the mesocone (Fig. 15a). Medial grooves are visible on the mesocones of *Z. pretneri* and *Z. isselianum* (AJC 874, Turjeva jama, Sovenia) here (Fig. 15a–b) as well as in a number of other eastern Alpine and Dinaride species (see Inäbnit et al., 2019, Supplementary Figs. S17–21). The basal plates are generally more compact and shorter in the radulae of Iberian taxa (Figs. 15c–e, h) versus the longer and thinner versions of those of the eastern Alpine and Dinaride *Zospeum* species (Fig. 15a–b) (see also Inäbnit et al., 2019, Supplementary Figs. S17–21). Basal plates maintain the spacing of teeth and support them in the feeding process (Luchtel et al., 1997). The radular ribbon of topotypic *I. zaldivarae* (AJC 1876) shows a rachidian tooth with a long pointed mesocone and very short endo- and ectocones that are about one fourth the length of the mesocone (Fig. 15g). The lateral teeth bear very short mesocones flanked by long, fang-like endo- and

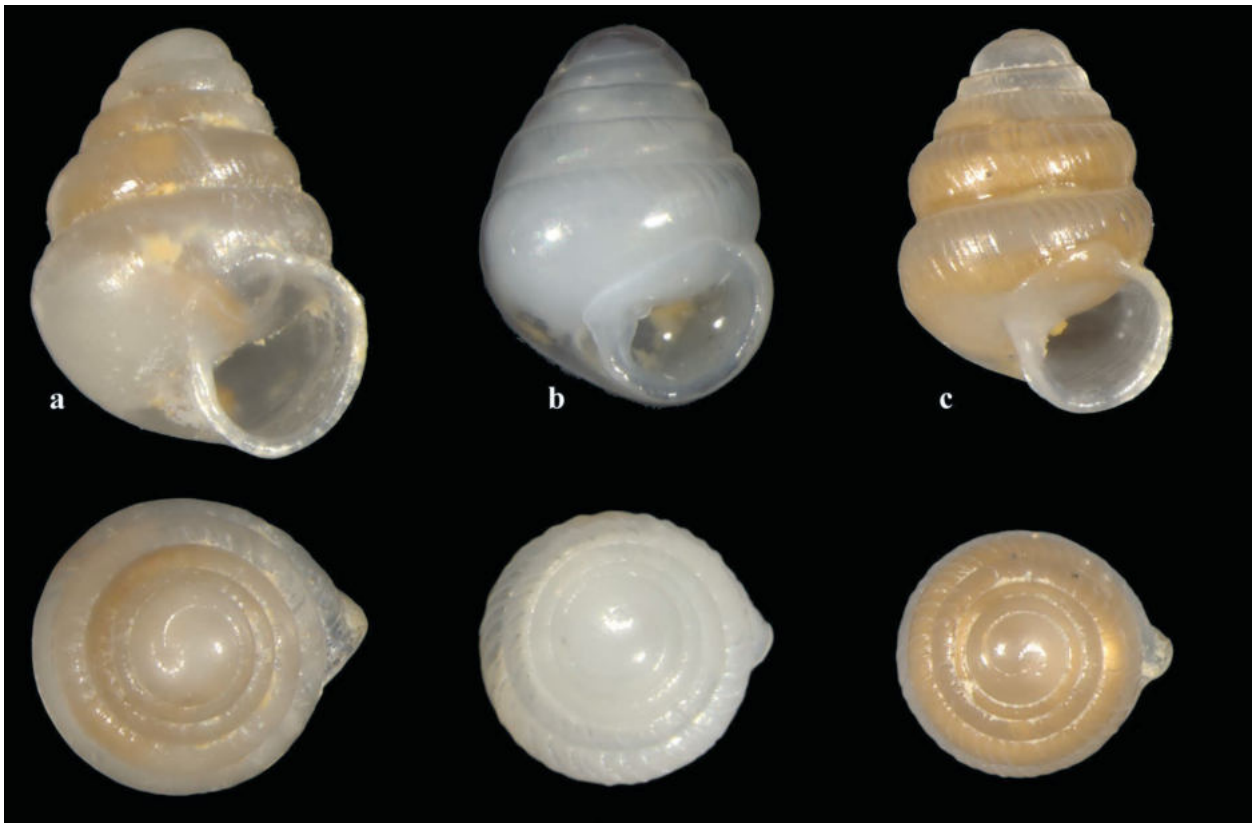



**Fig. 11** *Iberozospeum costulatum*. Yellow clade; (**a**) NMBE 559626, Cantabria, Lierganes, Cesareo, 21.3.2016, sh: 1.42 mm; (**b**) NMBE 557227, Cantabria, Ason, Soba, Cuvias Negras, 12.4.2017, sh: 1.27 mm; (**c**) NMBE 557231, Alen, Sopuerta, Valdebeci, 20.10.2015, sh: 1.27 mm. — All phot. ×40

**Table 3** Results of the species delimitation method ABGD

| NMBE-No | ZUPV-No | Cave and Province | ABGD group | clade |
|---|---|---|---|---|
| 557150 | 3405 | Txomenkoba (Gipuzkoa) | 1 | *vasconicum* (pink) |
| 557152 | 3758 | San Valerio (Gipuzkoa) | 1 | *vasconicum* (pink) |
| 557154 | 3849 | Penpelin (Gipuzkoa) | 1 | *vasconicum* (pink) |
| 557156 | 3856 | Artegi (Bizkaia) | 1 | *vasconicum* (pink) |
| 557166 | 4990 | Aizkirri (Gipuzkoa) | 1 | *vasconicum* (pink) |
| 557168 | 5005 | Arlaban (Gipuzkoa) | 1 | *vasconicum* (pink) |
| 557209 | 3904 | Iritegi (Gipuzkoa) | 1 | *vasconicum* (pink) |
| 557217 | 4930 | Mendikute (Gipuzkoa) | 1 | *vasconicum* (pink) |
| 557219 | 5087 | Munarri Arrola (Bizkaia) | 1 | *vasconicum* (pink) |
| 557223 | 3728 | Otxas (Bizkaia) | 1 | *vasconicum* (pink) |
| 540551_1 | 1847 | Akaitz Txiki (Gipuzkoa) | 2 | *vasconicum* (light blue) |
| 540552_1 | 2039 | Azkillar (Bizkaia) | 2 | *vasconicum* (light blue) |
| 540552_3 | 2039 | Azkillar (Bizkaia) | 2 | *vasconicum* (light blue) |
| 557178 | 1607 | Paules (Burgos) | 2 | *vasconicum* (light blue) |
| 557187 | 1847 | Akaitz Txiki (Gipuzkoa) | 2 | *vasconicum* (light blue) |
| 557188 | 1847 | Akaitz Txiki (Gipuzkoa) | 2 | *vasconicum* (light blue) |
| 559632 | 5213 | Arrigueras (Cantabria) | 3 | *vasconicum* (brown) |
| 557189 | 2039 | Azkillar (Bizkaia) | 4 | *vasconicum* (light blue) |
| 557138 | 4702 | Baja (Cantabria) | 5 | *vasconicum* (light blue) |
| 559628 | 5175 | Soldados (Cantabria) | 6 | *schaufussi* (red) |
| 559630 | 5171 | Buho (Cantabria) | 6 | *schaufussi* (red) |
| 557140 | 4862 | Collubina (Asturias) | 7 | *praetermissum* (purple) |
| 557246 | 5210 | Valdemora (Asturias) | 7 | *praetermissum* (purple) |
| 557253 | 5209 | Herreria (Asturias) | 7 | *praetermissum* (purple) |
| 557255 | 5208 | Busecu (Leon) | 7 | *praetermissum* (purple) |
| 559620 | 5257 | Puente Inguanzo (Asturias) | 7 | *praetermissum* (purple) |
| 557249 | 4915 | Llagar (Asturias) | 8 | spp. (green) |
| 557251 | 5211 | Caleru (Asturias) | 8 | spp. (green) |
| 559626 | 3807 | Cesareo (Cantabria) | 9 | *costulatum* (yellow) |
| 557242 | 4057 | Cubija (Cantabria) | 10 | *vasconicum* (light blue) |
| 557213 | 5006 | Garcia (Burgos) | 11 | *vasconicum* (brown) |
| 557200 | 854 | San Juan-9 (Bizkaia) | 12 | *vasconicum* (light blue) |
| 557211 | 4864 | Grazal (Bizkaia) | 12 | *vasconicum* (light blue) |
| 559624 | 5203 | Iglesia (Cantabria) | 13 | *vasconicum* (brown) |
| 559634 | 5200 | Murcielagos (Cantabria) | 13 | *vasconicum* (brown) |
| 557238 | 2875 | Irutxin (Navarra) | 14 | *biscaiense* (grey) |
| 557234 | 3371 | Lexotoa-2 (Navarra) | 15 | *bellesi* (dark blue) |
| 557236 | 3323 | Lezea (Navarra) | 15 | *bellesi* (dark blue) |
| 557146 | 3820 | Montosas (Cantabria) | 16 | *vasconicum* (brown) |
| 557232 | 4017 | Princesa (Bizkaia) | 17 | *vasconicum* (light blue) |
| 557158 | 4601 | Saiturri-2 (Gipuzkoa) | 18 | *vasconicum* (light blue) |
| 557225 | 4102 | Toyo (Cantabria) | 19 | spp. (green) |
| 557226 | 4102 | Toyo (Cantabria) | 20 | spp. (green) |
| 557144 | 4136 | Udias (Cantabria) | 21 | *praetermissum* (purple) |
| 559622 | 5258 | Zurra (Asturias) | 22 | spp. (green) |

ectocones on compact basal plates. The size and shape of teeth vary from row to row (Fig. 15h). As it is known for many ellobiids, the transition from the lateral teeth to the pectinate marginal teeth is abrupt (Fig. 15e) or gradual via a few intermediate, transitional teeth (Fig. 15c, h) (Martins, 1996, 2007). Within the central longitudinal rows of the radulae, the rachidian teeth of the individuals from Asturias (Fig. 15c–d) and Burgos (Fig. 15f) show a long mesocone

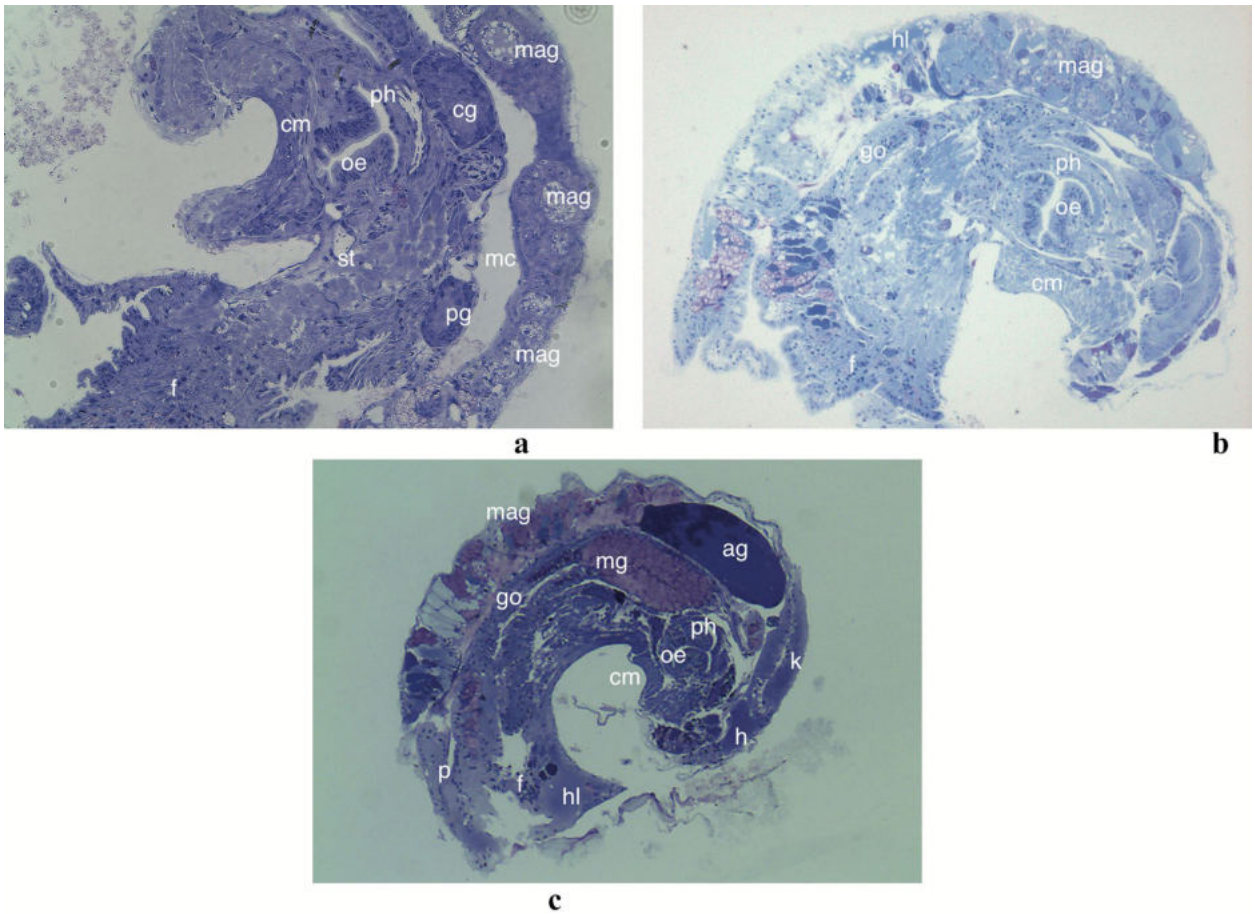



**Fig. 12** Light micrograph showing histological overview of the upper visceral mass of *Zospeum* and *Iberozospeum.* Visible structures vary due to the season of collection, degree of sexual maturity of the individual and the presence of certain structures and the superposition of others. Labels proceed clockwise rotating from the outside in; (**a**) topotypic *Z. isselianum* (AJC 2287), Turjeva jama, Slovenia (46.2435, 13.5046): mantle gland (mag), foot (f), mantle cavity (mc), cerebral ganglion (cg), pleural ganglion (pg), pharynx (ph), oesophagus (oe), statocyst (st), pedal ganglion (pg), columellar muscle (cm); (**b**) *Z. spelaeum* (AJC 848), Betalov Spodmol jama, Slovenia (45.7922, 14.1877): mantle gland (mag), foot (f), genital opening (go), pharynx (ph), oesophagus (oe), statocyst (st), columellar muscle (cm); (**c**) topotypic *I. vasconicum* (AJC 1848), Cueva de la Ermita de Sandaili, Spain (42.9994, -2.4381): mantle gland (mag), giant albumen gland (ag), kidney with renopericardial passage (k), heart (h), foot with haemolymph (f, hl), long genital opening (go), large mucus gland (mg), pharynx (ph), oesophagus (oe), two humps of vascularised columellar muscle (cm). scale: 500 µm. — All images taken by Dorian Dörge, Goethe University Frankfurt am Main

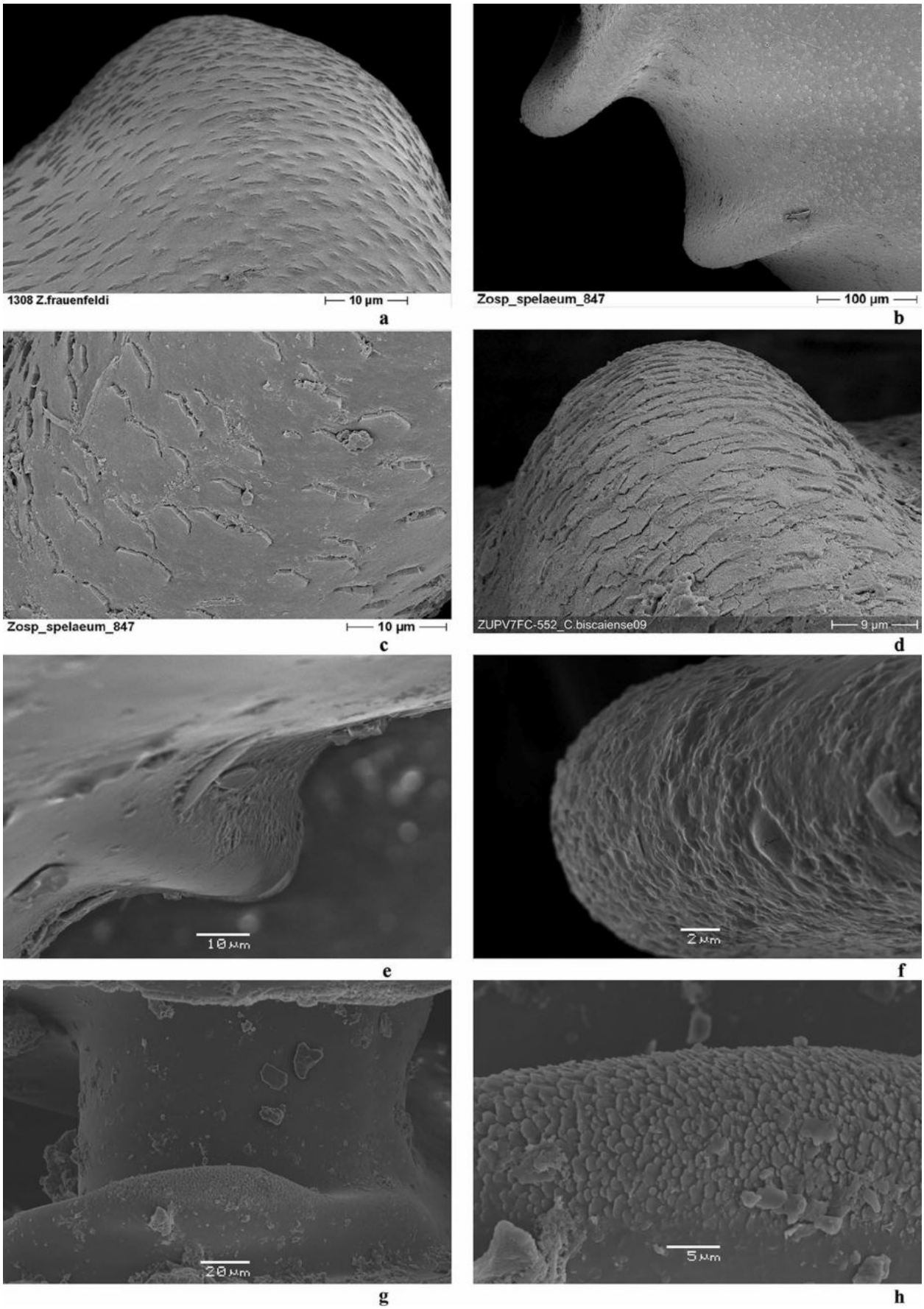
flanked by endo- and ectocones of varying lengths from short to long. A hint of a median groove is present on some of the lopsided transitional teeth of *I. zaldivarae* (AJC 1876) (Fig. 15h). In the transverse rows, there is an increased incidence of bi-cuspid, fang-like lateral teeth (i.e. RMNH.MOL. 234,116, Cueva a Sul, Oviedo) flanking both sides of the rachidian tooth (Figs. 15c–d). As is considered for pulmonates (Luchtel et al., 1997), the teeth show constant form in the longitudinal rows but vary considerably in the transverse direction on the radular ribbon.

Individuals of *I. vasconicum* are generally smaller (Jochum et al., 2015a) than the Alpine and Dinaride species of *Zospeum*, except those comprising the *Z. pretneri* clade, which are about the same size and sometimes slightly larger (Inäbnit et al., 2019).

## Discussion

In the molecular assessment, the topology generally did not change in the concatenated tree with all investigated specimens (Fig. 2) nor in the concatenated tree with complete marker sets (Fig. 3). In the latter, the support values are slightly higher and corroborate our interpretation of the phylogenetic and morphological findings. In order to understand the morphological traits in *Iberozospeum* n. gen., a figure is added here showing the type specimens of all hitherto described species (Fig. 16).

The *biscaiense-zaldivarae*-clade (grey clade in Figs. 2 and 3) contains sequences of two specimens (No. 162 and 163) from Weigand et al. (2013), who identified these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◂**Fig. 13** Crystallographic structure on the columellar lamellae of Dinaride *Zospeum* and *Iberozospeum* shells; (**a**) *Zospeum spelaeum*, (AJC 847), Betalov Spodmol jama, Slovenia (45.7922 14.1877), pattern of low, non-overlapping, wedges of crystallographic structure on the lamella; (**b**) *Zospeum spelaeum*, (MCBI CSR SASA 37049a), Velika Pasica, Slovenia (N45.9189 E14.4934), non-overlapping wedges of crystallographic structure on lamella in old shell; (**c**) *Iberozospeum* sp., (RMNH.MOL. 234,120), Cueva Refugio, Trucios, overview of dense, overlapping, scale-like wedges of localized, crystallographic structure on upper part of the lower lamella; (**d**) ibid., closeup view of c; (**e**) *Iberozospeum* sp., (RMNH.MOL. 234,104), Cueva del Comediante, Santander, upper part of the lamella of chemically treated shell showing dense, overlapping wedges of localized, crystallographic structure; (**f**) *Iberozospeum* sp., (RMNH. MOL. 234,141), Cueva a Sul, Oviedo, localized, overlapping wedges of crystallographic structure on lamella of chemically treated shell; (**g**) *Iberozospeum vasconicum*, (AJC 1849), Cueva Arrikrutz, overview of dense, localized, crystallographic structure on lower part of the lamella; h, ibid., closeup view of g. — Magnification varies for each perspective, see scale bars; Figs. a–b, g–h) imaged by M. Ruppel, (ret.) Goethe University Frankfurt am Main; Figs. c–f imaged by Dirk Vendermarel, Naturalis Biodiversity Center

specimens as lineage Z18. In 2015, Jochum et al. described the lineage Z18 as *I. zaldivarae*. The sequences of the two specimens of *I. zaldivarae* (Weigand et al., 2013) cluster with one specimen from the cave Irutxin. The cave, Otxas, is the type locality of *I. biscaiense* (Gomez & Prieto, 1983). We consider both species and supported entities; morphologically, the species differ by the mode of shell coiling (tighter in *I. biscaiense* when compared with *I. zaldivarae*) and presence of a small palatal denticle in *I. biscaiense,* which is lacking in *I. zaldivarae*.

The *bellesi*-clade (dark blue clade in Fig. 2) is not supported, although the complete marker set for both investigated specimens could be sequenced (dark blue clade in Fig. 3). A possible explanation for this finding is that *I. bellesi* belongs to a young radiation. The species is morphologically separated from the other known species in the Pyrenees, but our selected genetic markers cannot reveal the genetic differentiation of the species. The known distribution of *I. bellesi* spans the western Pyrenean region, including the French Basque Country and the Navarrese Pyrenees with the Sare-Zugarramurdi massif constituting an isolated enclave (Prieto & Zuazu, 2018). While *I. bellesi* is a unique Pyrenean species, all other known *Iberozospeum* taxa derive from the Basque-Cantabrian Mountains. Genetically, *I. bellesi* is clearly separated from the *biscaiense-zaldivarae*-clade but close to the other known species in Spain. Maybe other genetic markers are needed to reveal the genetic differentiation of *I. bellesi* from the other known species in Spain.

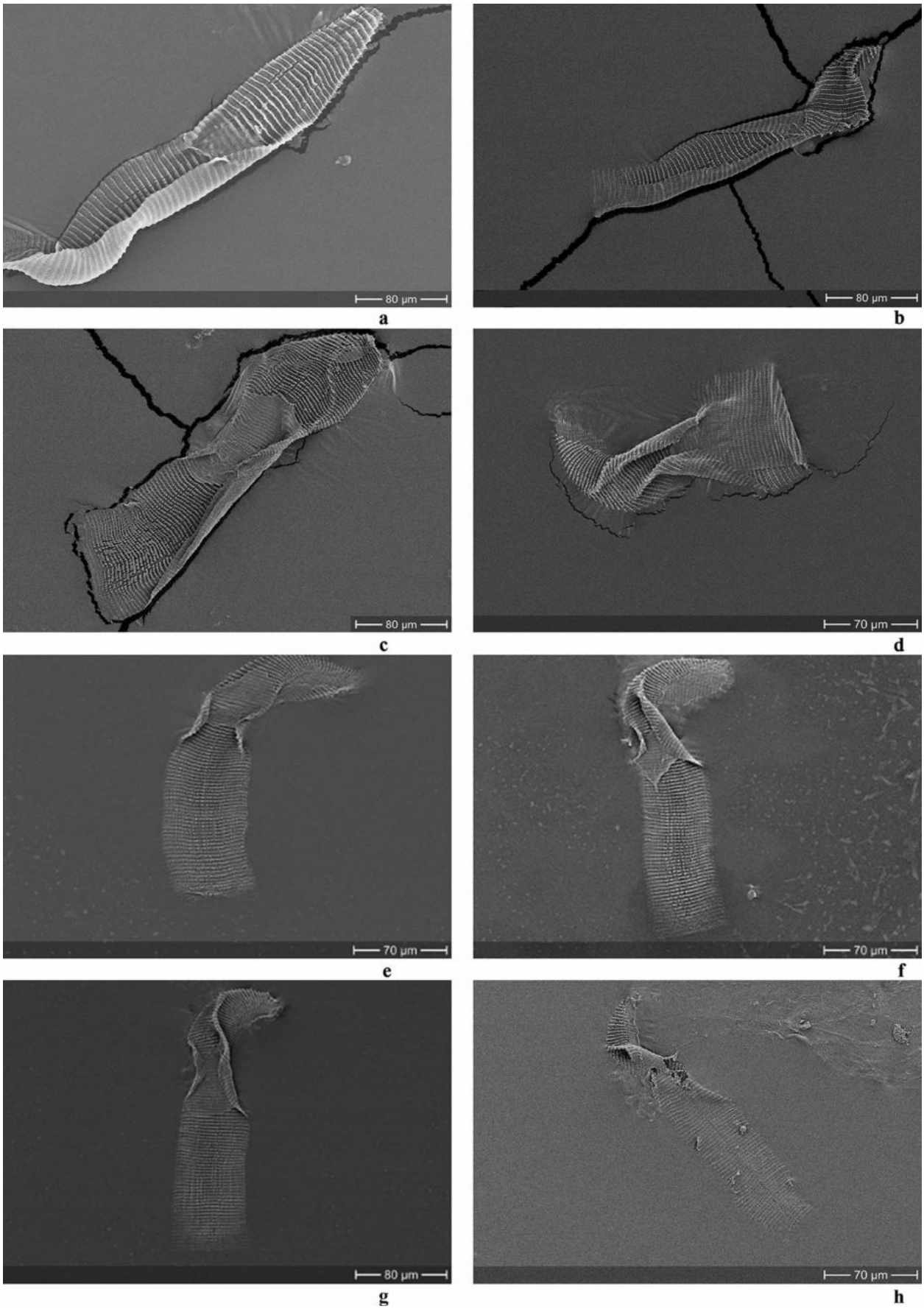
Within the Basque clades (pink and light blue clades in Figs. 2 and 3), the bootstrap support values and Bayesian posterior probabilities are low. This could be due to some missing markers in the concatenated tree (Fig. 2), because in the maximum likelihood tree, with complete marker sets (Fig. 3), the support values in the pink and light blue clades are much higher. The pink clade forms a polytomy with low support values. A possible explanation for this finding could be that these individuals form a young radiation. The selected genetic markers used in this study could not reveal the genetic differentiation of the specimens. The individuals in the light blue clade are genetically more differentiated than in the pink clade, but, bootstrap support values are also low. We included two specimens, NMBE 557178 and NMBE 557244 (Fig. 7k and m), which syntopically inhabit Las Paúles cave with *I. zaldivarae*. Both specimens cluster within the *vasconicum*-clade 2 (light blue clade in Fig. 2 and 3). On the morphological front, they resemble individuals in Fig. 7b and j.

The brown clade is the sister clade to the two *vasconicum*-clades (pink and light blue in Figs. 2 and 3) with high support (posterior probability of 1 and bootstrap value of 86 in Fig. 3). The support values in the deep nodes are low, maybe due to missing mitochondrial markers for some specimens. Due to genetic and morphological similarities to the *vasconicum*-clades 1 and 2 (Figs. 2, 3, 6, and 7), the specimens in the brown clade are considered to be *I. vasconicum*. We remain conservative with this clade because the subclades are not well supported. However, the type population of *I. vasconicum* (Weigand et al., 2013) is included in the pink subclade. For this reason, we consider all three subclades as *I. vasconicum*. Additional molecular methods like Next Generation Sequencing (NGS) are needed to resolve this group properly.

The *schaufussi*-clade (red clade in Figs. 2 and 3) contains a specimen from Cueva del Buho, which is the type locality for *I. suarezi* (Gittenberger, 1980). The investigated specimens from the red clade have an elongate-conical shell, moderately tightly coiled whorls and a roundish-lunate aperture typical for *I. schaufussi* (Jochum et al., 2019).

The purple clade (Figs. 2 and 3) contains the specimen No. 140 from Weigand et al. (2013), which was described by Jochum et al. (2019) as *I. praetermissum*. Morphologically, the individuals in the purple clade (Fig. 10a–f) show a uniform shell shape, except the specimen in Fig. 10f, which is a juvenile. The individual in Fig. 10d contains a clearly visible parietal tooth, which is typical for *I. praetermissum* and differentiates this species from *I. gittenbergeri* (Jochum et al., 2019). The individual in Fig. 10f cannot be clearly assigned to a species since the aperture is not fully grown. We remark, however, that this individual was found at the type locality of *I. praetermissum* (Jochum et al., 2019). The genetic analyses revealed that it clearly belongs to the *praetermissum*-clade even if its morphology is not typical for *I. praetermissum*, leading us to suggest that a certain spectrum of morphological variability within *I. praetermissum* is the case.

The lineages within the green clade (Figs. 2 and 3) have moderate to high support values but differ morphologically. Each lineage is represented by a different morph. The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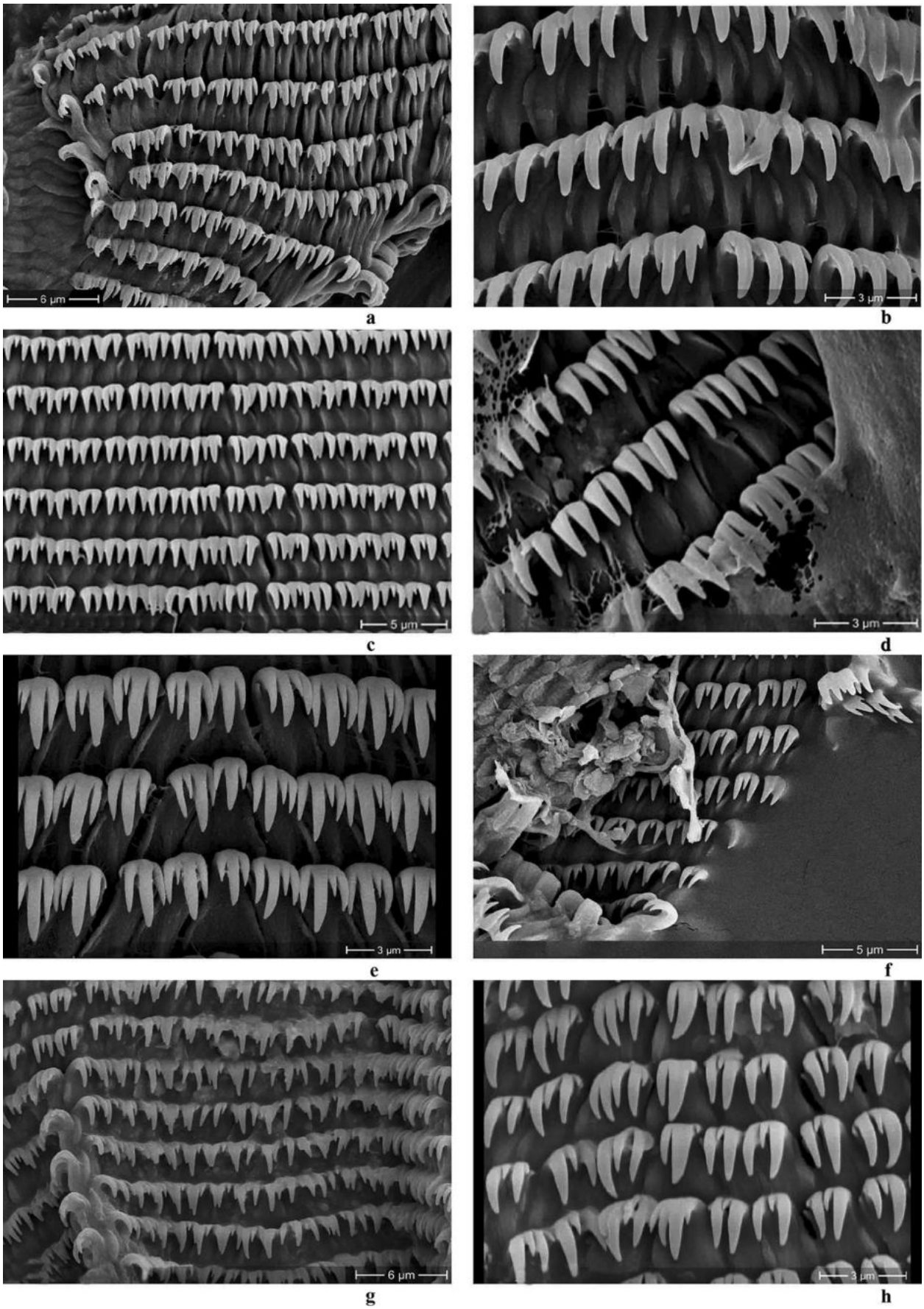
◂**Fig. 14** Scanning electron micrographs (SEM) showing radular ribbon form, middle adhesive zone (az) and rows of dentition (rd) of Dinaride and Iberian individuals (notation denotes aspects on one Dinaride *Zospeum* and one *Iberozospeum* ribbon); (**a**) *Z. exiguum* (NMBE 553384), Križna jama, Slovenia (45.7452, 14.4673), long and narrow, tapered anterior end (tae), short adhesive zone (az), bottom furled with narrow obtuse or straight base (nosb); (**b**) *Z. pretneri*, (NMBE 553290), Gornja Cerovačka pećina, Croatia (44.2701, 15.8855), ibid., with straight base; (**c**) *I. vasconicum*, (AJC 1848), Cueva Ermita de Sandaili (42.9994, -2.4381), moderately long and broad, tapered anterior end (tae), prominent adhesive zone (az), straight base (sb); (**d**) *I. zaldivarae*, (AJC 1876), Cueva de Las Paúles (43.1282, -2.7362), ibid.; (**e**) *Iberozospeum* sp. (RMNH.MOL. 234,109), Cueva de la Foz, long and broad, ibid; (**f**) *Iberozospeum* sp., (RMNH.MOL. 234,144), Cueva de Rales, very long and broad, ibid; (**g**) *Iberozospeum* sp., (RMNH.MOL. 234,116), Cueva a Sul, long and broad, ibid; (**h**) *Iberozospeum* sp., (RMNH.MOL. 234,108), Cueva de Torcona, very long and broad, ibid. — Magnification varies for each perspective, see scale bars; all Figs imaged by M. Ruppel, (ret.) Goethe University Frankfurt am Main

individual in Fig. 10h is considered to be *I. percostulatum* since it was collected at the type locality in Herrería cave and has strongly developed ribs on the teleoconch whorls, the namesake character typical for *I. percostulatum* (Alonso et al., 2018). Although it clusters together with the individual in Fig. 10g, a specimen from Cueva la Zurra, Cantabria, the specimen in Fig. 10g is considered a new species, i.e. *Iberozospeum* sp.1. It differs from the remaining specimens in the green clade due to the five loosely coiled convex whorls of the spire, an apparent parietal and basal angularity of the thick peristome, the presence of a very low parietal denticle (or thickening) in the lowermost region of the parietal part of the peristome and by the presence of a tiny denticle at the base of the columella. The specimens from El Toyo are considered a new species because they have a characteristically high, narrowly tapering, tightly coiled spire in conjunction with a thickly callused, rounded peristome fused onto the body whorl. It is not possible to recognise any dentition in the aperture in these full-bodied specimens. This combination of characters is not seen in other known Spanish taxa; therefore they are considered as *Iberozospeum* sp.2. The shells of the individuals in Fig. 10k–n resemble that of *I. gittenbergeri* by their relatively large, broad conical shape, their bearing 5.5 regularly coiled whorls and the typical, straight, long angular, thick parietal callus of the peristome (Jochum et al., 2019). The newly discovered species *Iberozospeum* sp.1 and *Iberozospeum* sp.2 are described in separate publications. The species delimitation method (ABGD) (Table 3) yielded four genetically different groups, a finding, which is not fully supported from the shell morphology. Probably, we deal here with a species complex with several cryptic species present.

The yellow clade (Figs. 2 and 3) is genetically clearly separated from the remaining clades, albeit that the complete marker set could be sequenced in only one specimen (Table 1 and Fig. 3). Morphologically, the three investigated individuals are completely different compared with the other known taxa in Spain. The specimen in Fig. 11b is a juvenile but resembles the specimen in Fig. 11c. Both specimens contain strong ribs. A new, ribbed species is described from this clade.

The caves of northern Spain show a high incidence of multiple species sympatry in contrast to those reported for the eastern Alpine and Dinaride regions (Inäbnit et al., 2019). In context, we remark here that Inäbnit et al.'s (2019) molecular investigation showed little congruency with Bole's (1974) earlier morphological interpretation of the eastern Alpine and Dinaride taxa, revealing that the incidence of multiple sympatric species is the exception, and not the rule in Dinaride caves. In Table 4, all investigated sympatric *Iberozospeum* species are listed.

The radulae of Iberian taxa show significant differences in radular ribbon form and dental morphology from those of eastern Alpine and Dinaride *Zospeum*. These differences reflect both odontophore and ribbon constitution in how it flattens out on the dorsal and lateral sections of the muscular odontophore. The ellobiid radula changes with age, with the very young specimens usually showing strongly denticulate crowns (Martins, 2007). Inäbnit et al. (2019) described four ribbon morphologies in eastern Alpine and Dinaride taxa, including those with an attenuated triangular base (*Z. isselianum, Z. frauenfeldii*) and those tapered to an obtuse to straight base as in *Z. exiguum* (Fig. 14a) or a straight base as in *Z. pretneri* (Fig. 14b) here. The form and composition of radular teeth vary according to their position on the radular membrane (referred in prepared SEM form here as ribbon) as well as diet, mineral composition of the environment, temperature and other factors (Luchtel et al., 1997). Within the ellobiids, Martins (1996, 2007) considered radular morphology a useful, distinguishing character at the generic level. For *Iberozospeum* here, the radular ribbons are clearly distinguishable from those of the Dinaride taxa. They are long and broad in form and very straight below the zone of the adhesive layer. They also bear more and smaller teeth per transverse row (Fig. 14c–h). Like those in their eastern Alpine and Dinaride relatives, they have a tapered anterior end (velum) above the adhesive layer. However, while the narrower ribbons of the latter taxa are tapered and sometimes straight at the base (i.e. *Z. pretneri*), all those of *Iberozospeum* are so far completely straight at the base, showing a flatter surface area aligned with very straight rows of teeth. We emphasize that this situation is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◂**Fig. 15** Scanning electron micrographs (SEM) showing transverse rows of dentition on Dinaride *Zospeum* and *Iberozospeum* radulae; (**a**) *Z. pretneri*, (NMBE 553290), Gornja Cerovačka pećina, Croatia, transverse rows of teeth on long, slender basal plates (bp), rachidian (r) and lateral teeth (l), arrows indicate medial grooves on mesocones of individual teeth; (**b**) *Z. isselianum*, NMBE 553389, Turjeva jama, Slovenia, ibid.; (**c**) *Iberozospeum* sp. (RMNH.MOL.234,116), Cueva a Sul, straight transverse rows of small, seemingly bi-cuspid lateral teeth (l) with reduced mesocones on compact basal plates; (**d**) ibid., close up view of rachidian teeth (r), lateral fang-like teeth (l) and transitional teeth (t); (**e**) *I. vasconicum*, (AJC 1848), Cueva Ermita de Sandaili, rachidian teeth (r) flanked by 4-cuspid lateral teeth (l), *C. ibazoricum*-like in form; (**f**) *Iberozospeum* sp. (RMNH.MOL.234108), Cueva la Torcona, lateral teeth showing reduced mesocones (me) flanked by long, fang-like endo- and ectocones (e), rachidian tooth (r) (flipped over in upper righthand corner of image); (**g**) *I. zaldivarae* (AJC 1876a), Cueva de Las Paúles, transverse rows of teeth showing varying cusp lengths; (**h**) ibid., close up view (left to right) of marginal (m) and transitional teeth (t) on short, compact basal plates (bp). — Magnification varies for each perspective, see scale bars; all Figs taken by M. Ruppel, (ret.) Goethe University Frankfurt am Main

enhanced by the tendency towards increased length (i.e. double the ribbon length below the adhesive zone in those of the Pyrenean-Cantabrian individuals), the further westward (i.e. Asturias) the individuals were found, albeit the exception found in Burgos (RMNH.MOL.234108) (Fig. 14h). The radula of topotypic *I. vasconicum* (Fig. 14c) from the more eastern province of Gipuzkoa, for example, shows a moderately long ribbon in comparison to the exceptionally long ribbons extracted from individuals from Asturias and the one from Burgos. Moreover, since the radula is tensioned and moved over the muscular odontophore by muscles that derive from the buccal mass, radular ribbon form is a reflection of these muscles operating the odontophore and radula in concert. In addition, since the odontophore is moved by muscles extending from the body wall and the columellar muscle (in shelled pulmonates) (Luchtel et al., 1997), it is apparent here that *Iberozospeum* has a different muscle constitution (i.e. muscles and modified muscle cells operating the odontophore, the odontophore itself and the columellar muscle) than *Zospeum*. This situation is revealed here by the presence of crystallographic structure on specific points of muscle adhesion on the columellar lamellae in shells of *Iberozospeum* (Fig. 13). Our observation is in sync with the trend in the Ellobiidae (Barker, 2001) that the columellar muscle becomes detached from the body wall from its origin on the columella and has become largely free in the haemocoel. Moreover, the dense, localized surface structure most probably provides traction for the columellar muscle. On a side note, but worth mentioning in context, is that during the manual preparation for SEM, ribbons from *Iberozospeum* mounted especially easily, like laying a carpet, onto the prepared SEM stub. Those of the eastern Alpine and Dinaride taxa, as well as from some *Carychium* species (AJ unpubl. data), tended to be generally more difficult to mount due to their flopping over and furling at the sides during the mounting procedure. This difference may be due to mineral composition or quality of the flexible chitin comprising the ribbon or to potential material thinness due to different or enhanced muscular action on the radular membrane in situ. In contrast to the long and broadly straight version of the Iberian taxa, the radular ribbons of the studied Alpine and Dinaride species are long and narrow and tapered on the anterior end and sometimes, on both ends (i.e. *Z. isselianum* and *Z. frauenfeldii*) (Inäbnit et al., 2019). We consider the consistency in shape, length, broadness and the straight-edged base of the radular ribbon systematically significant in *Iberozospeum*.

Ecologically, the westernmost *Iberozospeum* in our radula study were collected from caves characterised by certain aquatic biotopes (Notenboom & Meijers, 1985). These biotopes included influent caves (Cueva de Rales, Prov. Oviedo (now Asturias)) and temporal effluent caves (Cueva de la Foz, Cueva a Sul, Prov. Oviedo (now Asturias)), and caves with pools, puddles or gours (Cueva la Torcona, Prov. Burgos) or a combination of the latter two (Cueva la Torcona). These ecological situations play a role in diet and substrate composition as well as in the density of mud comprising *Iberozospeum* habitats (Jochum et al., 2012). As for the observed differences in *Iberozospeum*'s radular morphology, Jochum et al. (2015b) considered that the cusps of the radular teeth in *Z. isselianum* (Fig. 15b) interacted with substrate composition and structure (i.e. grain), causing adaptive moderations of morphological detail to evolve and correlate with substrate grain. In the case of *Iberozospeum*, it is probable that the longer, seemingly bi-cuspid lateral teeth of the westernmost sampled populations may well reflect environmentally induced adaptive factors in caves of the westernmost part of *Iberozospeum*'s range. We remark that these westernmost populations were not included in the molecular analyses comprising our study, but rather, collected by Jos Notenboom in 1983–1984 during a study of groundwater fauna in caves of northern Spain (Notenboom & Meijers, 1985).

The radiation of *Iberozospeum* aligns into the bigger context of Iberian geological and evolutionary considerations for which the southern peninsulas of Europe served as major refugia during the Pleistocene glaciation (Hewitt, 2004). Moreover, the Iberian Peninsula has been associated with climate stability over geologic time and is considered to be a historically climate-stable region encompassing high species diversity and endemism (Abellán & Svenning, 2014). Specifically, our study underscores the considerations of Abellán and Svenning (2014), in that the repeated range contractions and expansions experienced during the Pleistocene climatic oscillations may have resulted in the generation of multiple isolated lineages of fauna (in this case, *Iberozospeum*) in


**Fig. 16** Type specimens of species of *Iberozospeum*; (**a**) holotype *I. vasconicum* (Prieto et al., 2015 in Jochum et al., 2015a), Gipuzkoa, Cueva de la Ermita de Sandaili, MNCN15.05/60147H, sh: 1.2 mm; (**b**) paratype *I. biscaiense* (Gómez & Prieto, 1983), RMNH.MOLL.334913, Urkizu, Yurre, Cueva de Otxas, sh: ca. 1.23 mm (imaged by Ton de Winter); (**c**) holotype *I. percostulatum* (Alonso et al., 2018), Asturias, Llanes, Cueva de La Herrería, MNCN 15.05/200017H, sh: 1.58 mm; (**d**) lectotype *I. schaufussi* (von Frauenfeld, 1862), "Spain", NHMW 71,837, sh: 1.3 mm; (**e**) holotype *I. praetermissum* (Jochum et al., 2019), Asturias, Cueva del Puente de Inguanzo, RMNH.MOL.55391, sh: 1.08 mm; (**f**) paratype *I. bellesi* (Gittenberger, 1973), Huesca, Cueva del Molino de Aso, RMNH.Mol.234,137, sh: 1.49 mm (imaged by Ton de Winter); (**g**) holotype *I. zaldivarae* (Prieto et al., 2015 in Jochum et al., 2015a), Burgos, Cueva de Las Paúles, MNCN 15.05/60148H, sh: 1.52 mm; (**h**) holotype *I. gittenbergeri* (Jochum et al., 2019), Asturias, Cueva del Puente de Inguanzo, RMNH.MOL.234166, sh: 1.49 mm

different refugial areas. The Cantabrian and Pyrenees Mountains, harbouring labyrinthine cave systems, well serve as current refugial areas for *Iberozospeum* species that may have been more widespread during colder periods during the Pleistocene. Moreover, with the Pyrenees-Cantabrian Mountains running east–west together with the main rivers flowing along a north–south axis, the following scenario can be proposed considering climatic, geological and physiographical characteristics of this geologically younger part of Spain (Notenboom & Meijers, 1985): *Iberozospeum* could have survived and spread via altitudinal shifts as cave systems gave way and sank into each other due to geologic processes and groundwater mechanisms, allowing the distribution of species and sympatry of species to occur over time.

## Taxonomic implications

We found a well-supported, two-clade system in the genus *Zospeum* (Figs. 2 and 3) and propose here a new genus encompassing the northern Spanish radiation.

Genus ***Iberozospeum*** Jochum, Kneubühler, Prieto and Neubert, n. gen.

**Type species** *Zospeum zaldivarae* (Prieto et al., 2015 in Jochum et al., 2015a).

**Differential diagnosis** *Iberozospeum*, n. gen., differs from *Zospeum* by the generally smaller shell (on the average ca. 1.2 mm). The radula ribbon differs by its greater length,

**Table 4** Investigated sympatric *Iberozospeum* species living in Spanish caves

| Cave | Species 1 | Species 2 |
|---|---|---|
| Herrería | NMBE 557253 *praetermissum* (purple clade) | NMBE 557136 *percostulatum* (green clade) |
| Irutxin | NMBE 557238 *biscaiense* (grey clade) | NMBE 557221 *vasconicum* (light blue clade) |
| Las Paules | No. 162 and 163 Weigand 2013 *zaldivarae* (grey clade) | NMBE 557178 and NMBE 557244 *vasconicum* (light blue clade) |
| Otxas | NMBE 557240 *biscaiense* (grey clade) | NMBE 557223 *vasconicum* (pink clade) |
| Valdemora | NMBE 557246 *praetermissum* (purple clade) | NMBE 557247 *gittenbergeri* (green clade) |

regular broadness below the adhesive zone and its perfectly straight base. Radular teeth are smaller and more numerous per transverse row. The basal plates are more compact and shorter than the long, narrow versions in *Zospeum* species. At the microstructural level, it differs by localized, dense, rough, overlapping wedge-like scales of crystallographic structure on the surface of the columellar lamellae. These sites are located only at the points of contact with the columellar muscle. It also appears to differ by the correlating, vascularized humps of columellar muscle to the corresponding zones of microstructural texture on the lamellae.

**Etymology** The name *Iberozospeum* derives from combining the region of origin of the type species, the Iberian Peninsula, and the generic name of *Zospeum* (Bourguignat, 1856).

Included taxa: all hitherto known species from the Iberian Peninsula:

*Iberozospeum schaufussi* (von Frauenfeld, 1862).
*Iberozospeum bellesi* (Gittenberger, 1973).
*Iberozospeum biscaiense* (Gómez & Prieto, 1983).
*Iberozospeum vasconicum* (Prieto et al., 2015 in Jochum et al., 2015a).
*Iberozospeum zaldivarae* (Prieto et al., 2015 in Jochum et al., 2015a).
*Iberozospeum percostulatum* (Alonso et al., 2018).
*Iberozospeum praetermissum* (Jochum et al., 2019).
*Iberozospeum gittenbergeri* (Jochum et al., 2019).

## *Iberozospeum costulatum* Prieto and Jochum, n. sp.

**Type locality** Mina del Pedreo (Bizkaia: Arcentales; 43.26800 -3.21402, 440 m).

**Holotype** A shell of 1.43 mm [MNCN 15.05/200128, ex. ZUPV 1952]; 22.02.2014, C. Prieto, A. Calvo, P. Jiménez leg.

**Paratypes** Mina del Pedreo [type locality]; 22.02.2014, C. Prieto, A. Calvo, P. Jiménez leg. [ZUPV 1952: 2 shells + 2 juvenile shells] 31.08.2014, C. Prieto, A. Calvo leg. [ZUPV 2578: 15 shells + 3 juvenile shells, ZUPV 2583: 1 shell] [MNCN 15.05/200129: 5 shells]. Cueva de Valdebeci (Bizkaia: Sopuerta: Beci; 43.24516 -3.17316, 188 m); 20.10.2015, A. Calvo leg. [ZUPV 3078: 6 shells] [NMBE 557231: 1 specimen, sequenced; Fig. 11c]. Cueva de Cuvias Negras (Cantabria: Soba: Asón; 43.25132 -3.60688, 250 m); 12.04.2017, C. Prieto, S. Quiñonero, A. Alonso, J. Ruiz-Cobo leg. [ZUPV 4714: 38 shells + 2 specimens] [NMBE 557227: 1 specimen, sequenced; Fig. 11b; NMBE 568196: 5 shells] [SMF 349,424: 5 shells] [MHNG-MOLL-0137391: 5 shells] [MNCN 15.05/200130: 10 shells] [CAA·w/o nº: 19 shells] [CSQS·w/o nº: 25 shells].

**Other material** Cueva del Cesáreo (Cantabria: Liérganes: Extremera; 43.32034 -3.72279, 258 m); 21.03.2016, S. Quiñonero, J. Ruiz-Cobo, A. Alonso leg. [ZUPV 3807: 3 shells] [CAA·w/o nº: 1 shell] [NMBE 559626: 2 specimens, sequenced; Fig. 11a]. Cueva de Asunción (Cantabria: Ramales de la Victoria: Guardamino; 43.25837 -3.44820, 180 m); 21.03.2016, S. Quiñonero, J. Ruiz-Cobo leg. [ZUPV 3808: 4 shells]. Cueva del Comellante (Cantabria: Ruesga; 43.31111 -3.60806, 170 m); 30.03.2015, S. Quiñonero, J. Ruiz-Cobo leg [ZUPV 3804: 1 shell] [CSQS·w/o nº:3 shells]. Cueva de Covallarco (Cantabria: San Roque de Riomiera: Merilla; 43.25654 -3.73412, 402 m); 18.06.2016, CP, J. Fernández leg. [ZUPV 3974: 1 shell]. Cueva de Cullalvera (Cantabria: Ramales de la Victoria; 43.25577 -3.45808, 95 m); 19.09.2014, C. Prieto, A. Calvo leg. [ZUPV 2604: 1 shell]. Torca de El Porrón (Cantabria: Ruesga: Porracolina; 43.25111 -3.66356, 920 m); 09.09.2016, M. Gutiérrez leg. [ZUPV 4180: 1 shell]. Cueva de La Puntida (Cantabria: Miera: Ajanedo; 43.25883 -3.71042, 500 m); 12.10.2015, C. Prieto, A. Calvo leg. [ZUPV 3032: 90 shells] [CSQS·w/o nº: 10 shells]. Fuente de La Revilla (Cantabria: Voto: San Miguel de Aras; 43.31972 -3.52036, 55 m); 30.03.2015, S. Quiñonero leg. [ZUPV 3806: 3 shells]. Cueva de Las Cascajosas (Cantabria: San Roque de Riomiera; 43.25457


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◂**Fig. 17** *Iberozospeum costulatum* n. sp. (**a**–**e**) holotype, 1.43 mm height [MNCN 15.05/200128, ex. ZUPV 1952], Mina del Pedreo (Bizkaia: Arcentales; 43.26800 -3.21402, 440 m). (**f**–**g**) paratype shells from the type locality [ZUPV 1952, F: juvenile shell, G: shell of 1.51 mm height. (**h**–**i**) no paratype shell of 1.48 mm height [ZUPV 3807] from Cueva del Cesáreo (Cantabria: Liérganes: Extremera; 43.32034 -3.72279, 258 m). (**j**–**k**) paratype shells from Cueva de Cuvias Negras (Cantabria: Soba: Asón; 43.25132 -3.60688, 250 m) [CSQS·w/o nº]. (**l**) no paratype shell from Cueva del Cesáreo [CSQS·w/o nº]. Photos a–i by Carlos Prieto; j–l by Sergio Quiñonero and Álvaro Alonso

-3.71924, 328 m); 09.03.2018, C. Prieto, J. Ruiz-Cobo leg. [ZUPV 5154: 2 shells]. Cueva del Molino de la Peña (Cantabria: Rasines; 43.29101 -3.36840, 180 m); 04.08.2013, S. Quiñonero, J. Ruiz-Cobo leg. [ZUPV 3802: 2 shells, broken]. Sima PO-153 (Cantabria: San Roque de Riomiera: Porracolina; 43.22984 -3.69050, 585 m); 08.10.2016, M. Gutiérrez leg. [ZUPV 4184: 4 shells]; 12.11.2016, C. Prieto, M. Gutiérrez, J. A. Noriega leg. [ZUPV 4203: 20 shells]. Sima PO-27 (Cantabria: Miera; 43.24695 -3.67023, 910 m); 07.05.2016, M. Gutiérrez leg. [ZUPV 3822: 8 shells]. Cueva de San Juan de Socueva (Cantabria: Arredondo: Socueva; 43.26671 -3.61339, 430 m); 12.04.2017, C. Prieto, S. Quiñonero, A. Alonso, J. Ruiz-Cobo leg. [ZUPV 4722: 12 shells].

**Etymology** The specific epithet derives from the well-defined ribbing of the last whorls of the shell.

**Diagnosis** A medium-sized (average, 1.35 mm, $n=52$) and wide (average, 0.76 mm, $n=52$) *Iberozospeum* species with convex whorls bearing well-defined ribs, oblique, reniform aperture with broad straight-edged parietal callus and a strong columellar lamella clearly seen inside the aperture.

Description (Fig. 17). Shell of medium size (1.24–1.55 mm, $n=52$, holotype, 1.43 mm), ovate conical. Spire formed by five (4.6–5.9, n = 52) rather convex whorls separated by a deep suture. Protoconch (nucleus, 0.175 mm wide) and apical whorls smooth (Fig. 17f) with teleoconch bearing regularly spaced (5–6/0.5 mm; 27 in the body whorl, n = 52), well-defined ribs, extending from one whorl to the next; ribs of the hind peristome region are more densely aligned and thinner. Body whorl large (holotype, 60% of the shell height), with upper convexity, ascending behind the aperture. Umbilicus closed, with ribs reaching the umbilical cavity. Aperture reniform, obliquely transverse (holotype, 47% of the shell height), attached to the body whorl by a thickly callused, broad, straight-edged parietal callus. Peristome wide and reflexed; lateral and palatal side regularly arched; columellar side almost straight and vertical. Inner lamella singular, large, oblique, (Fig. 17g) located above the parieto-columellar junction; lamella visible in oblique apertural view (Fig. 17c). Shell surface with micro sculpture of irregular spiral striae.

**Geographical distribution** *Iberozospeum costulatum* n. sp. inhabits caves throughout western Bizkaia and the eastern part of Cantabria (Fig. 18). In Bizkaia, *I. costulatum* is known from two caves located 4.2 km apart: Mina del Pedreo, where it was first discovered, and Cueva de Valdebeci (holotype, sequenced population). Both caves belong to the same limestone formation (Alén-Lujar) cut by the Barbadun river. The remaining localities are in Cantabria. They are separated from the Bizkaian caves by a distance of 20 km, with Cueva Cullalvera and Cueva de Asunción being the nearest. All Cantabrian localities are located in the limestone massifs belonging to the Miera and Asón river basins with Cueva del Cesáreo (sequenced, Fig. 11a) constituting the most north-western cave separated from this central block. We remark that *I. costulatum* appears to be sympatric with *I. biscaiense* in the Montes de Triano massif west of Bilbao (Prieto & Calvo 2013; unpublished records) and that the east-westward geographic span recorded for *I. costulatum* likely represents the true geographic distribution of this species.

**Variability** Ribbing is the most conspicuous feature on the shell of *I. costulatum*. Although the shells from the same cave show similar rib strength, spacing and length, a notable spectrum of variability is present in different cave populations. Shells deriving from populations from the Miera upper basin, such as La Puntida, PO-153 or Cuvias Negras (Fig. 17j–k), show poorly developed ribs and a weak columellar lamella, while individuals from Fuente de La Revilla bear strong and irregular ribs. On the other hand, shells from Asunción have well separated ribs (5/0.5 mm). Although obscured by wide intra-populational variation, shell size also varies between populations. Shells from Cuvias Negras are the smallest and those from Valdebeci are the largest. The inner columellar lamella also varies somewhat in form in shells from Cueva de Cesáreo, whereby in addition to the columellar lamella, a small basal lamella can be seen from the opening in oblique view (Fig. 17h–i).

***Remarks*** The conspicuous ribbing differentiates *I. costulatum* from all other iberozospeid taxa except *I. percostulatum*. The shell of *I. percostulatum* is higher (1.34–1.80 mm, average 1.55 mm) and narrower (1.32–1.65 mm, average 1.51 mm, $n=42$) and lacks inner lamellae (Alonso et al., 2018). Additionally, their geographic ranges are 80 km apart.

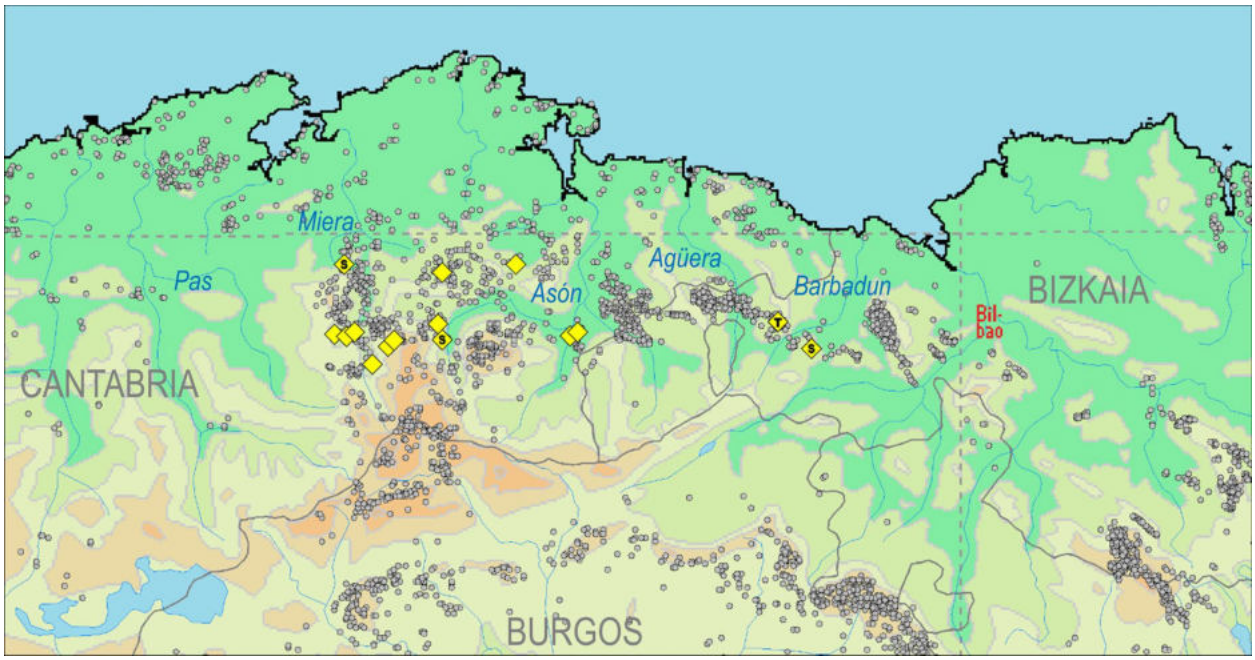


**Fig. 18** Geographical distribution of *Iberozospeum costulatum* n. sp. The type locality and sequenced populations are indicated by T and S, respectively

## Conclusion

In this integrative study, we investigated 57 populations of cavernicolous zospeid snails using genetic and morphological data. Our study revealed a separate radiation for species inhabiting northern Spanish caves for which the new genus, *Iberozospeum*, has been defined. *Iberozospeum* populations show on-going speciation in situ within isolated cave systems of northern Spain. Our investigations corroborated the proposed ancestral area reconstruction of Weigand et al. (2013) indicating that the "Cantabrian Mountains + Alps" or "Cantabrian Mountains + Dinaric Alps" was the ancestral area of the *Iberozospeum* radiation. Coherent mitochondrial and nuclear sequence patterns as well as common morphological traits were observed in most of the clades. Three new species were molecularly revealed and one, *Iberozospeum costulatum*, is described using additional morphological data.

Our comparative histological investigations revealed the huge size of the albumen gland for the first time in a sexually mature member in the female phase within the subterranean Carychiidae. We found significant morphological and structural differences in both the columellar muscle and the radulae of Dinaride *Zospeum* and *Iberozospeum* species. SEM investigations of the radular ribbon reveal that dentition form, size and alignment, consistency in ribbon shape, length, broadness and the straight-edged base are systematically significant in *Iberozospeum* species and that it morphologically differentiates these species from those of the Eastern Alpine and Dinaride *Zospeum* taxa.

Our investigations are ongoing. Future collection efforts and phylogeographic investigations will do well to reveal deeper patterns of phylogenetic relatedness and evolutionary processes in *Iberozospeum* populations.

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**Data availability** All data is published in the manuscript. Sequences are deposited in Genbank.

**Code availability** Software and programs are cited in the manuscript.

## Declarations

**Ethics approval** Not applicable.

**Consent for publication** We give permission for the following paper to be published in Organisms Diversity & Evolution.

**Conflict of interest** Not applicable.



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# On the verge of extinction – revision of a highly endangered Swiss alpine snail with description of a new genus, *Raeticella* gen. nov. (Gastropoda, Eupulmonata, Hygromiidae)

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## Abstract

The phylogenetic status of the alpine land snail *Fruticicola biconica* has remained questionable since it was described by Eder in 1917. Considered a microendemic species from mountain tops in Central Switzerland, the shell is specially adapted for life under stones. Herein, we show via molecular and anatomical investigations that *F. biconica* neither belongs to the land snail genus *Trochulus*, nor to any other genus within Trochulini, but rather warrants placement within the newly established genus *Raeticella* Kneubühler, Baggenstos & Neubert, 2022. Phylogenetic analyses reveal that *R. biconica* is clearly separated from *Trochulus*. These findings are supported by morphological investigations of the shell and genitalia.

## Keywords

Endemism, integrative taxonomy, LGM, mountains, nunataks, phylogeny, Switzerland, *Trochulus*

## Introduction

Discovered in the Bannalp, Nidwalden and known from only a few localities in the Central Swiss Alps, *Fruticicola biconica* was described by the Swiss zoologist Leo Eder in 1917.

Later, *F. biconica*, known as the Nidwaldner hairy snail, was moved to the widely used genus *Trichia* W. Hartmann, 1840 and circulated throughout the European literature under this designation (e.g., Kerney et al. 1983). The generic name, *Trichia*, was subsequently replaced by *Trochulus* Chemnitz, 1786 due to homonymy with *Trichia* De Haan, 1839 (Crustacea, Xanthidae).

Previous studies (Pfenninger et al. 2005; Dépraz et al. 2009; Duda et al. 2014; Kruckenhauser et al. 2014; Proćków et al. 2021) included *T. biconicus* individuals in their genetic analyses of *Trochulus* species. Pfenninger et al. (2005) and Dépraz et al. (2009) used the same sequence of *T. biconicus* collected at the type locality at Bannalp. This sequence clustered within the so far known *Trochulus* species and some newly identified lineages, which were not further described (fig. 2 in Pfenninger et al. 2005; fig. 1 in Dépraz et al. 2009). Most likely, Pfenninger et al. (2005) and Dépraz et al. (2009) used misidentified specimens in their phylogenetic studies, or some samples were mixed. Since these authors did not publish images of the investigated specimens, an unequivocal identification is not possible. Duda et al. (2014) and Kruckenhauser et al. (2014) found that *T. biconicus*, "*T. oreinos oreinos*" (A.J. Wagner, 1915), and "*T. oreinos scheerpeltzi*" (Mikula, 1957) form basal lineages in comparison to specimens of *Trochulus* s. str. The latter two taxa were elevated from subspecies to species level (Bamberger et al. 2020) and are today known to belong to the newly described genus *Noricella* (Neiber et al. 2017). Proćków et al. (2021) found the same phylogenetic pattern as Duda et al. (2014) and Kruckenhauser et al. (2014) and questioned the affiliation of *biconicus* to *Trochulus*. Already Turner et al. (1998) had disputed the phylogenetic position of *T. biconicus*. Until today, the phylogenetic position of *T. biconicus* within the Trochulini remained unclear. Hence, an integrative taxonomic approach is applied in this study to investigate the phylogenetic affiliation of *T. biconicus*.

## Materials and methods

### Specimens investigated

Living individuals of *T. biconicus* were collected in September 2020 at 11 sites of the known distribution area in Central Switzerland (see Fig. 1 for detailed sampling localities). *Trochulus biconicus* is classified as Vulnerable by Swiss law (Federal Office of Environment) and is protected. It is also considered Endangered by the IUCN (https://www.iucnredlist.org/species/22107/9360310). Collecting permits were obtained from the cantonal administrations of Nidwalden, Obwalden, and Uri. At each site, 3–5 snails were collected from large populations (>20 individuals)

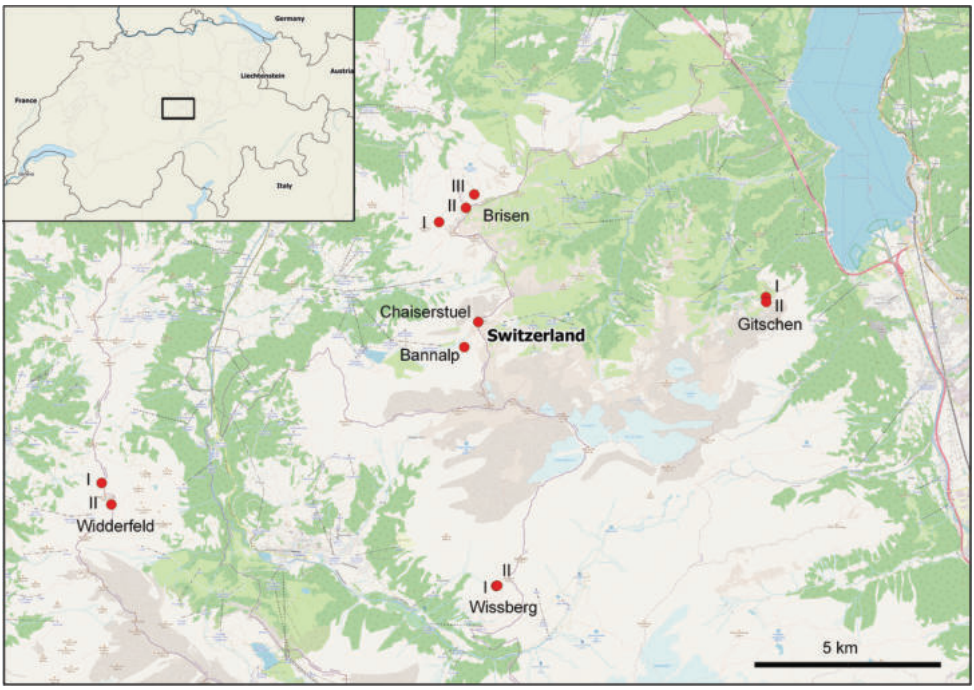


**Figure 1.** Sampling locations of the investigated individuals of *T. biconicus.*

from under rocks on stony outcrops. The individual snails were preserved in 80% ethanol to keep the body tissue soft for proper anatomical investigations and DNA extraction. In Table 1, sampling localities and GenBank accession numbers are listed for all sequenced specimens of *T. biconicus*, *Trochulus* spp., and *Edentiella edentula*. Usually, two specimens of *T. biconicus* per population were sequenced. Those not destroyed in the extraction process are deposited at the NMBE as voucher material. The map was produced with QGIS (2016, v. 2.18.13) using the Natural Earth data set.

## Acronyms of collections

**NMBE** Natural History Museum Bern, Switzerland;
**MNHW** Museum of Natural History Wrocław, University of Wrocław, Poland.

## Shell morphology and anatomical study of the genitalia

One animal was selected from each population for investigations of the shell morphology and the genital organs. The dissection of the genitalia was performed under a Leica MZ12 stereomicroscope using thin tweezers. The genital organs were removed from the body, spread on a wax-lined bowl and properly pinned with small needles. The

total length of the situs was measured using Mitutoyo callipers. Proportions between different parts of the genitalia were estimated using the total situs length as a reference. Additionally, the inner structures of the penis and the penial papilla were investigated. Pictures of the situs and the shells were taken with a Leica M205 microscope camera using an image-processing program (Leica LAS X v. 3.6.0.20104, Switzerland). The shells were imaged in frontal, lateral, apical, and ventral position. Shell height and shell width were measured using the callipers to assess perpendicularity with the shell axis.

**Table 1.** Sequenced *T. biconicus* specimens from Central Switzerland. Asterisk (*) marks the type localities of the species studied. Additionally, *Edentiella edentula* (Draparnaud, 1805) and some species of *Trochulus* were sequenced and included for phylogenetic analyses.

| Voucher-No. | Species | Locality | Coordinates | Altitude [m] | GenBank accession number COI | GenBank accession number 16S | GenBank accession number ITS2 |
|---|---|---|---|---|---|---|---|
| NMBE 567164 | *T. biconicus* | Bannalp Schonegg* | 46.87°N, 8.46°E | 2232 | MW435154 | MW433778 | MW433799 |
| NMBE 567165 | *T. biconicus* | Bannalp Schonegg* | 46.87°N, 8.46°E | 2232 | MW435155 | MW433779 | MW433800 |
| NMBE 567167 | *T. biconicus* | Chaiserstuel | 46.87°N, 8.46°E | 2263 | MW435156 | MW433780 | MW433801 |
| NMBE 567168 | *T. biconicus* | Chaiserstuel | 46.87°N, 8.46°E | 2263 | MW435157 | MW433781 | MW433802 |
| NMBE 567149 | *T. biconicus* | Wissberg I | 46.81°N, 8.47°E | 2335 | MW435158 | MW433782 | MW433803 |
| NMBE 567150 | *T. biconicus* | Wissberg I | 46.81°N, 8.47°E | 2335 | MW435159 | MW433783 | MW433804 |
| NMBE 567152 | *T. biconicus* | Wissberg II | 46.81°N, 8.47°E | 2355 | MW435160 | MW433784 | MW433805 |
| NMBE 567153 | *T. biconicus* | Wissberg II | 46.81°N, 8.47°E | 2355 | MW435161 | MW433785 | MW433806 |
| NMBE 567155 | *T. biconicus* | Widderfeld I | 46.83°N, 8.33°E | 2120 | MW435162 | MW433786 | MW433807 |
| NMBE 567156 | *T. biconicus* | Widderfeld I | 46.83°N, 8.33°E | 2120 | MW435163 | MW433787 | MW433808 |
| NMBE 567159 | *T. biconicus* | Widderfeld II | 46.83°N, 8.33°E | 2290 | MW435164 | MW433788 | MW433809 |
| NMBE 567161 | *T. biconicus* | Brisen I | 46.90°N, 8.45°E | 2045 | MW435165 | MW433789 | MW433810 |
| NMBE 567137 | *T. biconicus* | Brisen I | 46.90°N, 8.45°E | 2045 | MW435166 | MW433790 | MW433811 |
| NMBE 567139 | *T. biconicus* | Brisen II | 46.90°N, 8.46°E | 2130 | MW435167 | MW433791 | MW433812 |
| NMBE 567140 | *T. biconicus* | Brisen II | 46.90°N, 8.46°E | 2130 | MW435168 | MW433792 | MW433813 |
| NMBE 567142 | *T. biconicus* | Brisen III | 46.90°N, 8.46°E | 2090 | MW435169 | MW433793 | MW433814 |
| NMBE 567143 | *T. biconicus* | Brisen III | 46.90°N, 8.46°E | 2090 | MW435170 | MW433794 | MW433815 |
| NMBE 567145 | *T. biconicus* | Gitschen I | 46.88°N, 8.57°E | 1890 | MW435171 | MW433795 | MW433816 |
| NMBE 567146 | *T. biconicus* | Gitschen I | 46.88°N, 8.57°E | 1890 | MW435172 | MW433796 | MW433817 |
| NMBE 567148 | *T. biconicus* | Gitschen II | 46.88°N, 8.57°E | 1970 | MW435173 | MW433797 | MW433818 |
| NMBE 567162 | *T. biconicus* | Gitschen II | 46.88°N, 8.57°E | 1970 | MW435174 | MW433798 | MW433819 |
| NMBE 568100 | *T. hispidus* | Sweden, prov. Uppland, Uppsala, Linnaeus Garden* | 59.8619°N, 17.6342°E | | ON477947 | – | – |
| NMBE 568103 | *T. hispidus* | Sweden, Östergötland, Vist | 58.3294°N, 15.729°E | 70 | ON477948 | ON479908 | ON479901 |
| NMBE 564609 | *Trochulus* sp. | Bullet, Le Chasseron | 46.8517°N, 6.5377°E | 1606 | ON477944 | ON479905 | ON479898 |
| NMBE 564607 | *Trochulus* sp. | Mervelier, Scheltental | 47.336°N, 7.5153°E | 615 | ON477943 | ON479904 | ON479897 |
| NMBE 543063 | *Trochulus* sp. | St-Cergue, Route de Cuvaloup | 46.4487°N, 6.123°E | 1208 | ON477941 | ON479902 | ON479895 |
| NMBE 564601 | *Trochulus* sp. | Zernez | 46.6998°N, 10.0943°E | 1473 | ON477942 | ON479903 | ON479896 |
| NMBE 568094 | *Trochulus* sp. | Lac du Mont d'Orge | 46.2321°N, 7.333°E | 624 | ON477946 | ON479907 | ON479900 |
| NMBE 565821 | *T. alpicola* | Bannalp Schonegg* | 46.8706°N, 8.2491°E | 2234 | ON477945 | ON479906 | ON479899 |
| MNHW_S_15_29_101 | *T. villosus* | Montagne De Cernier | 47.0763°N, 6.8888°E | 1385 | MW440985 | MW447773 | MW440678 |
| MNHW_S_15_29_02 | *T. clandestinus* | Montagne De Cernier | 47.0763°N, 6.8888°E | 1385 | MW440983 | MW447772 | MW440676 |
| MNHW_S_15_27_12 | *Trochulus* sp. | Gorges de Court | 47.2553°N, 7.3439°E | 608 | MW440984 | MW621002 | MW440677 |
| MNHW_S_15_21_02 | *T. caelatus* | Gorges de Moutier* | 47.2856°N, 7.3819°E | 477 | MW440982 | MW621001 | MW440675 |
| MNHW_S_Er_50 | *E. edentula* | Erschwil | 47.3673°N, 7.555°E | 459 | MW440986 | MW621003 | MW440679 |
| MNHW_S_Er_51 | *E. edentula* | Erschwil | 47.3673°N, 7.555°E | 459 | MW440987 | MW621004 | MW440680 |

## Abbreviations used in the anatomical descriptions and figures

| | | | |
|---|---|---|---|
| **AG** | albumen gland; | **Pe** | penis; |
| **BC** | bursa copulatrix; | **PP** | penial papilla; |
| **DS** | dart sacs; | **sh** | shell height; |
| **Ep** | epiphallus; | **sw** | shell width; |
| **Fl** | flagellum; | **Va** | vagina; |
| **HD** | hermaphroditic duct; | **VD** | vas deferens. |
| **MG** | mucous glands; | | |

## DNA extraction, PCR amplification and sequence determination

For total DNA extraction of the specimens, the Qiagen Blood and Tissue Kit (Qiagen; Hilden, Germany) was used in combination with a QIAcube extraction robot. Circa 0.5 $cm^3$ of tissue was cut and placed in a mixture of 180 µl ATL buffer and 20 µl Proteinase K. It was then incubated for ca. 4 hours at 56 °C in a heater (Labnet, Vortemp 56, witec AG, Littau, Switzerland). For subsequent DNA extraction, the QIAcube extraction robot was used with the Protocol 430 (DNeasy Blood Tissue and Rodent tails Standard). In this study, two mitochondrial markers (COI and 16S) and one nuclear marker (5.8S rRNA+ITS2) were investigated. PCR mixtures consisted of 12.5 µl GoTaq G2 HotStart Green Master Mix (Promega M7423), 4.5 µl $ddH_2O$, 2 µl forward and reverse primer each, and 4 µl DNA template. The primer pairs implemented for the PCR are listed in Table 2. The following PCR cycles were used: for COI, 2 min at 94 °C, followed by 40 cycles of 1 min at 95 °C, 1 min at 47 °C and 1 min at 72 °C and finally, 5 min at 72 °C; for 16S, 3 min at 96 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C, and finally, 1 min at 72 °C; and for 5.8S rRNA+ITS2, 3 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C, and finally, 5 min at 72 °C (SensoQuest Tabcyclet and Techne TC-512, witec AG, Littau, Switzerland). The purification and sequencing of the PCR product was performed by LGC (LGC Genomics Berlin, Germany).

## Phylogenetic analyses

The phylogenetic analyses were conducted using sequences obtained from GenBank and from this study, which were included as outgroup: *Ichnusotricha berninii* Giusti & Manganelli, 1987, *Plicuteria lubomirskii* (Ślósarski, 1881), *Petasina unidentata* (Draparnaud, 1805), *Noricella oreinos* (A.J. Wagner, 1915), *Noricella scheerpeltzi* (Mikula, 1957) (GenBank numbers and sampling localities published by Neiber et al. 2017), *Edentiella edentula* (Draparnaud, 1805), and several ingroup specimens of *Trochulus* (Table 1). These species were selected to identify the phylogenetic position of *T. biconicus*.

For sequence processing and editing, the software package Geneious v. 9.1.8 (Biomatters Ltd) was used. Topologies were estimated using two different phylogenetic methods: Bayesian Inference (BI) and Maximum Likelihood (ML). Bayesian Inference was performed using Mr. Bayes v. 3.2.6 x64 (Huelsenbeck and Ronquist 2001;

**Table 2.** Primer pairs used for PCR.

| Gene | Primer | Sequence | Sequence length (bp) | Reference |
|---|---|---|---|---|
| COI | LCO1490 | 5′-GGTCAACAAATCATAAAGATATTGG-3′ | 655 | Folmer et al. 1994 |
| | HCO2198 | 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ | | |
| 16S | 16S cs1 | 5′-AAACATACCTTTTGCATAATGG-3′ | 440 | Chiba 1999 |
| | 16S cs2 | 5′-AGAAACTGACCTGGCTTACG-3′ | | |
| ITS2 | ITS2 LSU1 | 5′-GCTTGCGGAGAATTAATGTGAA-3′ | 900 | Wade and Mordan 2000 |
| | ITS2 LSU3 | 5′-GGTACCTTGTTCGCTATCGGA-3′ | | |

Ronquist and Huelsenbeck 2003; Altekar et al. 2004) via the HPC cluster from the University of Bern (http://www.id.unibe.ch/hpc). Evolutionary models for each subset were set to mixed models. The Monte Carlo Markov Chain (MCMC) parameter was set as follows: starting with four chains and four separate runs for 20 million generations with a tree sampling frequency of 1000 and a burn in of 25%. RAxML plug-in for Geneious (Stamatakis 2014) was implemented for computing ML inference, using Geneious' plug-in with rapid bootstrapping setting, the search for the best scoring ML tree and 1000 bootstrapping replicates. The model, GTR CAT I was implemented.

## Results

### Phylogenetic analyses

The BI analysis of the concatenated data set (Fig. 2) shows two major clades within the tribe Trochulini. These two clades are separated with full support. One clade contains representative specimens of *Edentiella* and *Noricella* which form a polytomy. The second major clade within Trochulini contains representatives of *Petasina*, *Trochulus*, and the investigated *T. biconicus* specimens. *Trochulus biconicus* is the sister lineage to the selected *Trochulus* specimens. This node has full posterior probability support. *Trochulus hispidus* from the type locality in Sweden clusters together with a second specimen from Sweden and forms the sister group to two Swiss *Trochulus* specimens from Zernez and Lac du Mont d'Orge. The resolution within the *T. biconicus* clade is moderate because the investigated individuals differ in only few nucleotides in all three investigated markers.

The ML analysis of the concatenated data set (Fig. 3) shows a similar topology as that of the BI analysis. The difference in the ML and the BI tree is the relationship of *Edentiella* and *Noricella*. In the ML tree, *E. edentula* clusters together with *N. scheerpeltzi.* This node has low support value (bootstrap support of 51 in Fig. 3), whereas in the BI analysis (Fig. 2), *Edentiella* and *Noricella* show a polytomy. In both analyses, *T. biconicus* forms the sister lineage to the selected *Trochulus* species. This node has full ML support. The support values within the *Trochulus* clade are moderate to high.

The *p*-distance, which shows the number of base differences per site from between sequences (Kumar et al. 2018) for the COI was calculated using MEGA


**Figure 2.** Bayesian Inference (BI) tree based on the concatenated data set of COI, 16S, and 5.8S rRNA+ITS2. Numbers represent Bayesian posterior probabilities.

v. 10.1.8 (https://www.megasoftware.net/). The *p*-distance for *T. biconicus* and the remaining investigated *Trochulus* species ranges from 0.153–0.189, for *T. biconicus* and *E. edentula* from 0.183–0.189, for *T. biconicus* and *Noricella* species from 0.128–0.166, for *T. biconicus* and *P. unidentata* from 0.171–0.176, for *T. biconicus* and *I. berninii* from 0.142–0.147 and for *T. biconicus* and *P. lubomirksi* from 0.177–0.188 (see Suppl. material 1). The genetic investigations in this study clearly show that *T. biconicus* is neither a member of the *Trochulus* clade nor does it belong to another known genus in the Trochulini. It thus, warrants designation in a separate new genus.

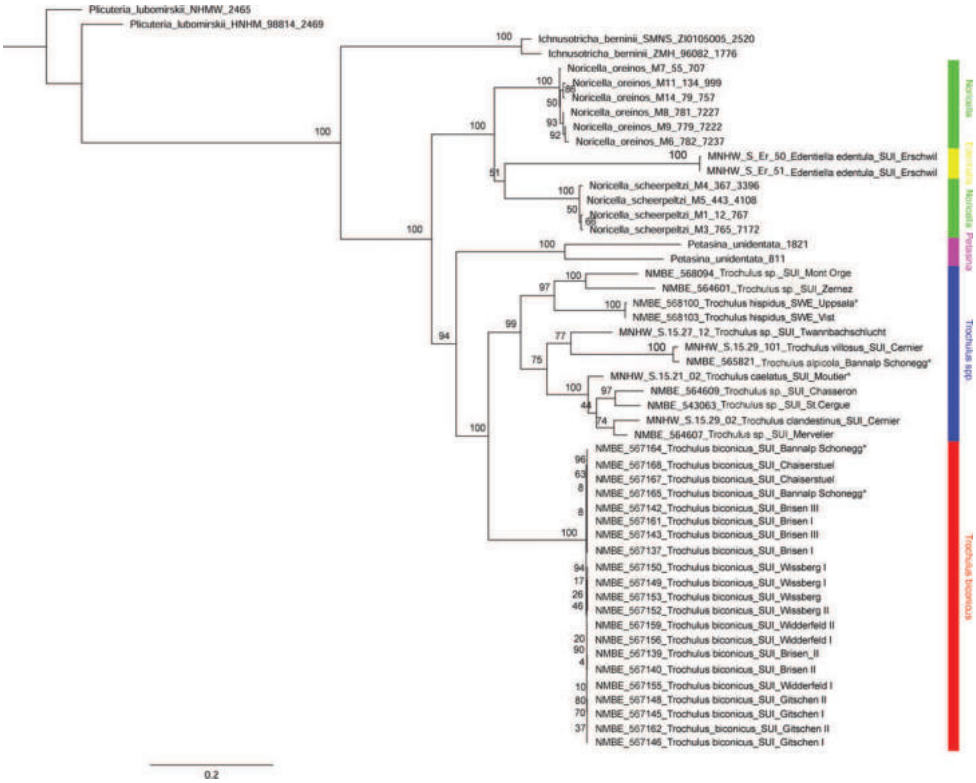


**Figure 3.** Maximum Likelihood (RAxML) tree based on the concatenated data set of COI, 16S, and 5.8S rRNA+ITS2. Numbers represent bootstrap support values from the ML analysis.

## Shell morphology

The shell of *T. biconicus* is flattened, tightly coiled, and beige to brownish. The mean shell width of the investigated individuals ($N$ = 13) is 5.63 mm (range: 5.3–6.1 mm; SD = 0.23 mm) with mean shell height reaching 2.67 mm (range: 2.34–2.9 mm; SD = 0.17 mm) (Table 3). The shell bears 5.5–6 whorls which increase only slightly in width towards the perimeter. The umbilicus is entirely open and wide. The crescent-shaped aperture contains a white, poorly developed lip. Neither juveniles nor adults show hairs on the shell (Figs 4–10).

## Morphology of the genitalia

The genitalia are characterised by four stylophores, symmetrically placed in two pairs on both sides of the vagina (see fig. 11 in Proćków 2009). The inner dart sacs are somewhat longer and slenderer than the outer sacs. The outer stylophores contain the love darts (see also Proćków 2009). The mucous glands consist of four simple and thin tubes branching off the free oviduct directly above the dart sacs. The vagina is a rather long tube, which is

almost smooth inside or shows some faint elongate tissue folds that connect to the atrium (not shown in the figures). The bursa copulatrix branches off from the free oviduct above the dart sacs and the mucous glands and is terminated by an elongated vesicle.

The penis is fusiform and contains a club-shaped penial papilla which points into the lumen of the penial chamber. The epiphallus is as long as the penis; the penis retractor muscle inserts at the transition zone between epiphallus and penis. The flagellum is about 1.5× the length of the penis and epiphallus each. The epiphallial lumen contains longitudinal tissue ridges (e.g., Fig. 4C). The penial chamber is characterised by smooth walls. The penial papilla contains a lateral subapical pore. The cross section of the penial papilla (Figs 4D, 5D) reveals a central duct surrounded by small folds.

The anatomy of the genitalia of *T. clandestinus* differs from *T. biconicus* by having eight long, thin mucous glands (Fig. 11). The inner dart sacs of the investigated *T. clandestinus* are slightly longer in length than the outer dart sacs. The flagellum has about the same length as the bulbous penis, and the epiphallus is slightly longer than the penis. The cross section of the penial papilla differs in *T. clandestinus* by having several tissue layers around the main tube of the penial papilla (Fig. 11D).

**Table 3.** Morphological analysis: measurements of the shell and genital organs of *T. biconicus* and *T. clandestinus*. Additionally, some collected dry shells from Bannalp Schonegg (NMBE 567170) and Chaiserstuel (NMBE 567171) were included in the analysis. Asterisk (*) marks the type locality of *T. biconicus*. Umbilicus minor diameter is measured according to Proćków (2009). All measurements are in mm.

| Voucher No. | Species | Locality | Coordinates | Altitude [m] | shell width | shell height | umbilicus minor diameter | penis length | epiphallus length | flagellum length | Figure number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NMBE 567151 | *T. biconicus* | Wissberg I | 46.81°N, 8.47°E | 2335 | 5.56 | 2.55 | 0.8 | 1.81 | 2.01 | 5.98 | Fig. 4 |
| NMBE 567160 | *T. biconicus* | Widderfeld II | 46.83°N, 8.33°E | 2290 | 5.73 | 2.59 | 0.88 | 2.79 | 3.42 | 8.13 | Fig. 5 |
| NMBE 567138 | *T. biconicus* | Brisen I | 46.90°N, 8.45°E | 2045 | 5.61 | 2.34 | 0.73 | 2.86 | 3.06 | 7.25 | Fig. 6 |
| NMBE 567163 | *T. biconicus* | Gitschen II | 46.88°N, 8.57°E | 1970 | 5.67 | 2.87 | 0.84 | 2.23 | 3.67 | 6.38 | Fig. 7 |
| NMBE 567166 | *T. biconicus* | Bannalp Schonegg* | 46.87°N, 8.46°E | 2232 | 5.75 | 2.76 | 0.96 | 1.84 | 1.98 | 4.26 | Fig. 8 |
| NMBE 567169 | *T. biconicus* | Chaiserstuel | 46.87°N, 8.46°E | 2263 | 5.3 | 2.46 | 0.99 | 2.66 | 3.21 | 4.1 | Fig. 9 |
| NMBE 567170_1 | *T. biconicus* | Bannalp Schonegg* | 46.87°N, 8.46°E | 2232 | 5.7 | 2.82 | 1.19 | – | – | – | Fig. 10A |
| NMBE 567170_2 | *T. biconicus* | Bannalp Schonegg* | 46.87°N, 8.46°E | 2232 | 6.03 | 2.73 | 1.08 | – | – | – | Fig. 10B |
| NMBE 567170_3 | *T. biconicus* | Bannalp Schonegg* | 46.87°N, 8.46°E | 2232 | 5.39 | 2.54 | 0.99 | – | – | – | Fig. 10C |
| NMBE 567170_4 | *T. biconicus* | Bannalp Schonegg* | 46.87°N, 8.46°E | 2232 | 6.1 | 2.9 | 1.07 | – | – | – | Fig. 10D |
| NMBE 567171_1 | *T. biconicus* | Chaiserstuel | 46.87°N, 8.46°E | 2263 | 5.46 | 2.76 | 1.08 | – | – | – | Fig. 10E |
| NMBE 567171_2 | *T. biconicus* | Chaiserstuel | 46.87°N, 8.46°E | 2263 | 5.41 | 2.61 | 0.79 | – | – | – | Fig. 10F |
| NMBE 567171_3 | *T. biconicus* | Chaiserstuel | 46.87°N, 8.46°E | 2263 | 5.46 | 2.83 | 0.89 | – | – | – | Fig. 10G |
| NMBE 571318 | *T. clandestinus* | Bern, Bümpliz | 46.9435°N, 7.3922°E | 540 | 9.64 | 5.57 | 1.29 | 4.24 | 5.79 | 4.69 | Fig. 11 |

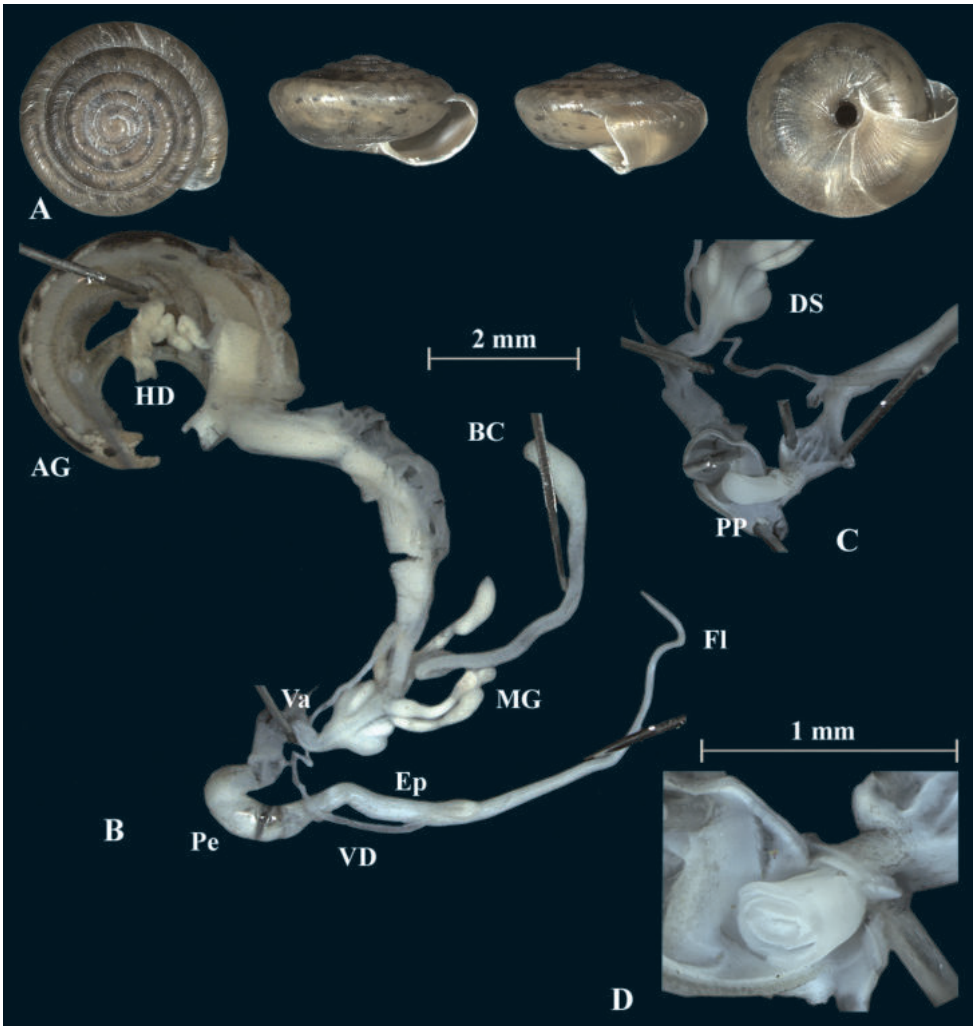


**Figure 4.** *Trochulus biconicus* (NMBE 567151) collected from Wissberg I **A** shell, sw = 5.56 mm, sh = 2.55 mm **B** situs **C** penis (Pe) with penial papilla (PP) **D** cross section of the penial papilla. Shell × 5.

## Taxonomic and systematic implications

The fully supported split between *T. biconicus* and currently known *Trochulus* species (Figs 2, 3) warrants description of a new genus, *Raeticella* gen. nov., based on *Fruticicola biconica*.

**Genus *Raeticella* gen. nov.**
http://zoobank.org/D7620E37-3AA3-45D2-BB3C-B55114AF36F2

**Type species.** *Fruticicola biconica* Eder, 1917.

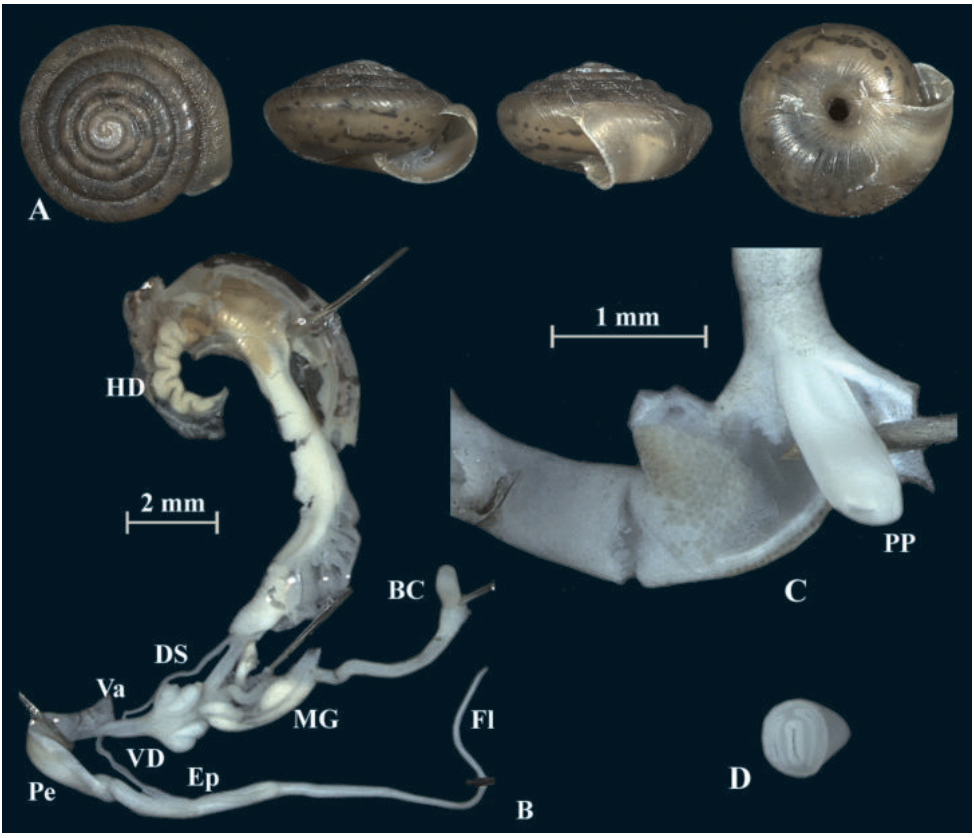


**Figure 5.** *Trochulus biconicus* (NMBE 567160) collected from Widderfeld II **A** shell, sw = 5.73 mm, sh = 2.59 mm **B** situs **C** penis (Pe) with penial papilla (PP) **D** cross section of the penial papilla. Shell × 5.

## Genus *Trochulus* Chemnitz, 1786

*Trochulus biconicus* (Eder, 1917)

**Diagnosis.** Shell flattened and thin-walled, translucent, compressed in the direction of the axis; no trichome formation; whorls 5.5–6, gradually increasing so that the body whorl is only about twice as wide as the first whorl; the aperture is oblique, narrow, crescent-shaped; lip sharp, whitish and slightly reflexed; the four mucous glands are long, thick and pointed; penis and epiphallus are about the same length; the flagellum is barely separated from the epiphallus.

**Differential diagnosis.** *Raeticella* gen. nov. differs from *Trochulus* by having a flat, biconical shell, devoid of any periostracal hairs, even in juveniles, and in having only four instead of occasionally six or eight (see Duda et al. 2014) mucous glands. It differs from *Noricella* by lacking a basal tooth, being devoid of any periostracal hairs, the absence of coarse ripples and the absence of an additional fold and bulge in the penial papilla, which occurs in *N. oreinos* (Duda et al. 2014).

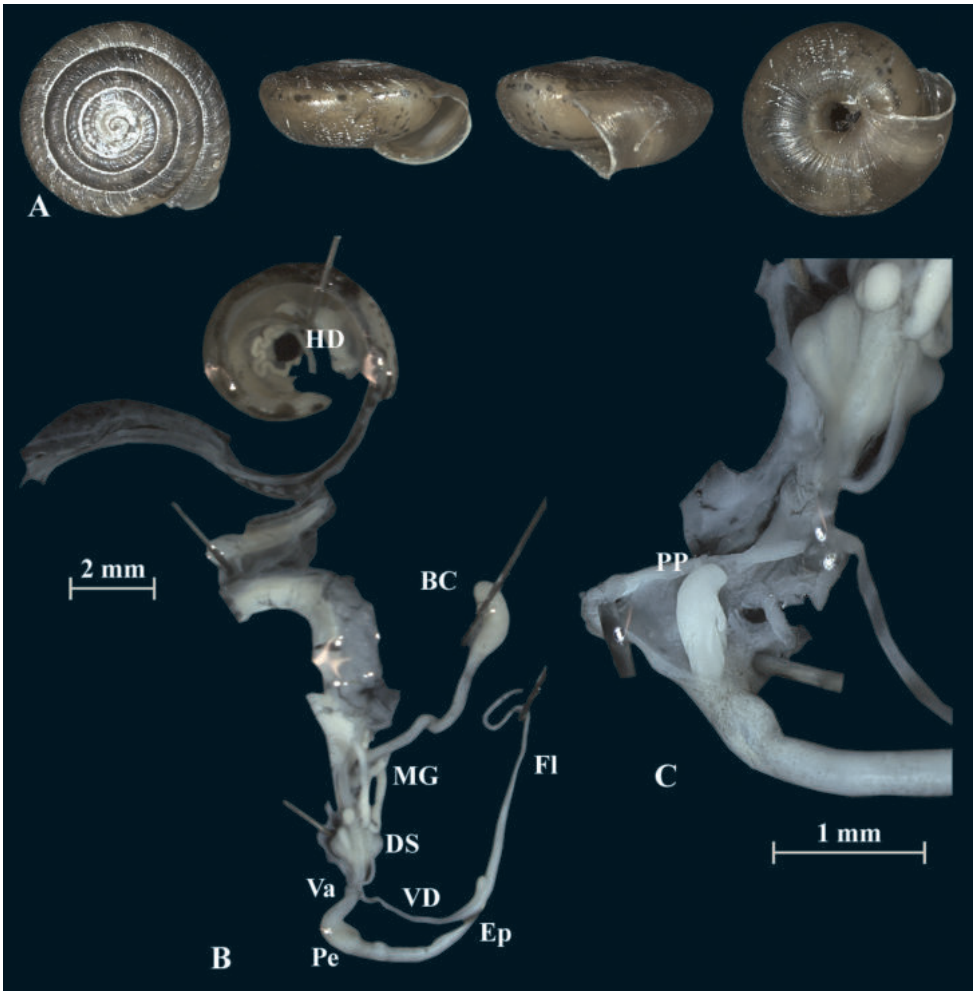


**Figure 6.** *Trochulus biconicus* (NMBE 567138) collected from Brisen I **A** shell, sw = 5.61 mm, sh = 2.34 mm **B** situs **C** penis (Pe) with penial papilla (PP). Shell × 5.

**Etymology.** The name is derived from the Roman province of Raetia, which comprised within its larger expansion, the area of what is now known as eastern and central Switzerland. It also refers to the generic name, *Noricella*, which is another recently detected spin-off from *Trochulus* and whose name derives in part from the eastern border province of Raetia (Noricum – now Austria and Slovenia).

## Discussion

Neiber et al. (2017) clarified the phylogenetic positions of some species within the Trochulini by establishing the new genus *Noricella* Neiber, Razkin & Hausdorf, 2017. In their study it was proven that *N. oreinos* and *N. scheerpeltzi* differed from the closest

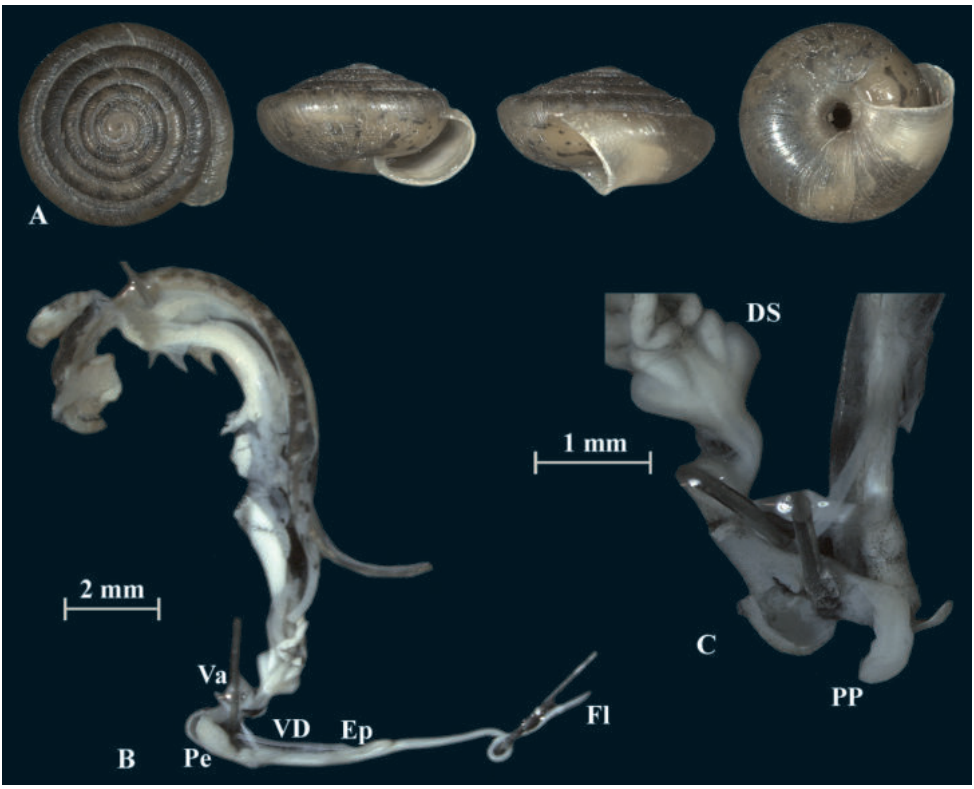


**Figure 7.** *Trochulus biconicus* (NMBE 567163) collected from Gitschen II **A** shell, sw = 5.67 mm, sh = 2.87 mm **B** situs **C** penis (Pe) with penial papilla (PP). Shell × 5.

related genus *Edentiella* Poliński, 1929 in some apomorphic nucleotide substitutions and by morphological characters. *Edentiella* contains at least one longitudinal septum separating an additional lacuna in the penial papilla which is lacking in *N. oreinos*, in most *Trochulus* species, and in *Petasina* (Neiber et al. 2017). These authors also included some representatives of *Trochulus* but did not have specimens of *R. biconica* available. Turner et al. (1998) had already considered *R. biconica* to be only distantly related to *Trochulus* s. str. because of 1) the lack of periostracal hair even in juveniles, 2) a very long flagellum, and 3) only four instead of six or eight mucous glands. Hence, Turner (1991) suggested to move *R. biconica* into a subgenus of *Trochulus*. The questionable position of *biconicus* in *Trochulus* was recently re-addressed by Proćków et al. (2021). In our analysis, the calculated *p*-distance of *R. biconica* and the investigated *Trochulus* specimens comprises the highest values. The *p*-distance of *R. biconica* and *Noricella* species is lower than for *Trochulus*, which means that *Raeticella* is genetically closer, based on COI, to *Noricella* than to *Trochulus*. Even *Ichnusotricha*, which belongs to the tribe of Ganulini is genetically more similar to *Raeticella* than *Trochulus* is to *Raeticella*.

The shell morphology of *R. biconica* differs from all known *Trochulus* species by having a flat shell with a low spire. The last whorl is bluntly keeled. Adults are always hairless (Proćków 2009). In this regard, it is most like the shells of the two *Noricella*

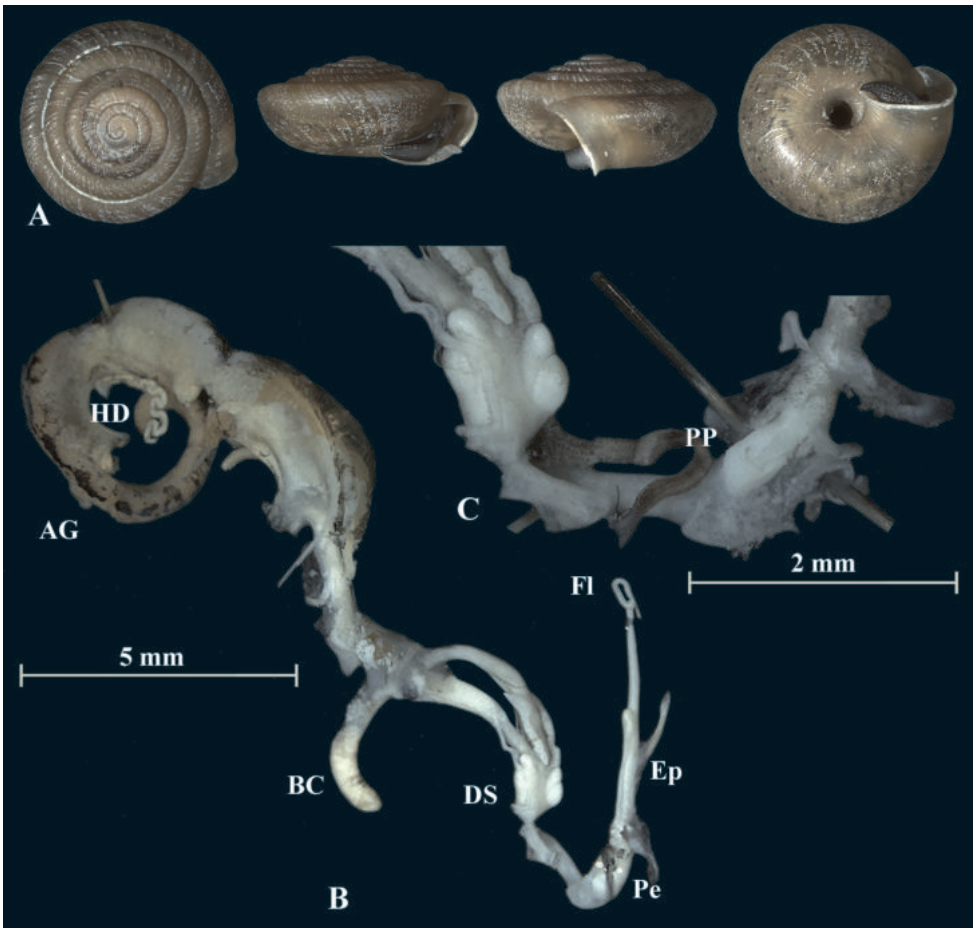


**Figure 8.** *Trochulus biconicus* (NMBE 567166) collected from Bannalp Schonegg **A** shell, sw = 5.75 mm, sh = 2.76 mm **B** situs **C** penis (Pe) with penial papilla (PP). Shell × 5.

species (Duda et al. 2011, 2014), but the anatomy of the genital organs of these species is different. Both *Noricella* species have four pairs of mucous glands, compared to two pairs in *R. biconica*. *Noricella oreinos* possesses an additional fold and bulge in the penial papilla, which seems to be unique to this species (Duda et al. 2014). The section of the penial papilla in *R. biconica* shows similar internal features as in *T. caelatus* (Proćków 2009), *T. striolatus* (Proćków 2009; Duda et al. 2014; Proćków et al. 2021), and *T. suberectus* (Proćków 2009). *Raeticella biconica* does not possess periostracal hairs, neither as a juvenile nor as an adult. This, however, is considered a typical feature for *Trochulus* species (Proćków 2009).

Hewitt (2004) observed that many taxa in temperate refugial regions in Europe and North America show relatively deep DNA divergence, indicating their presence over several ice ages and suggesting a mode of speciation by repeated allopatry. On the one hand, this possibly explains the deep split between *Raeticella* and *Trochulus* and shifts

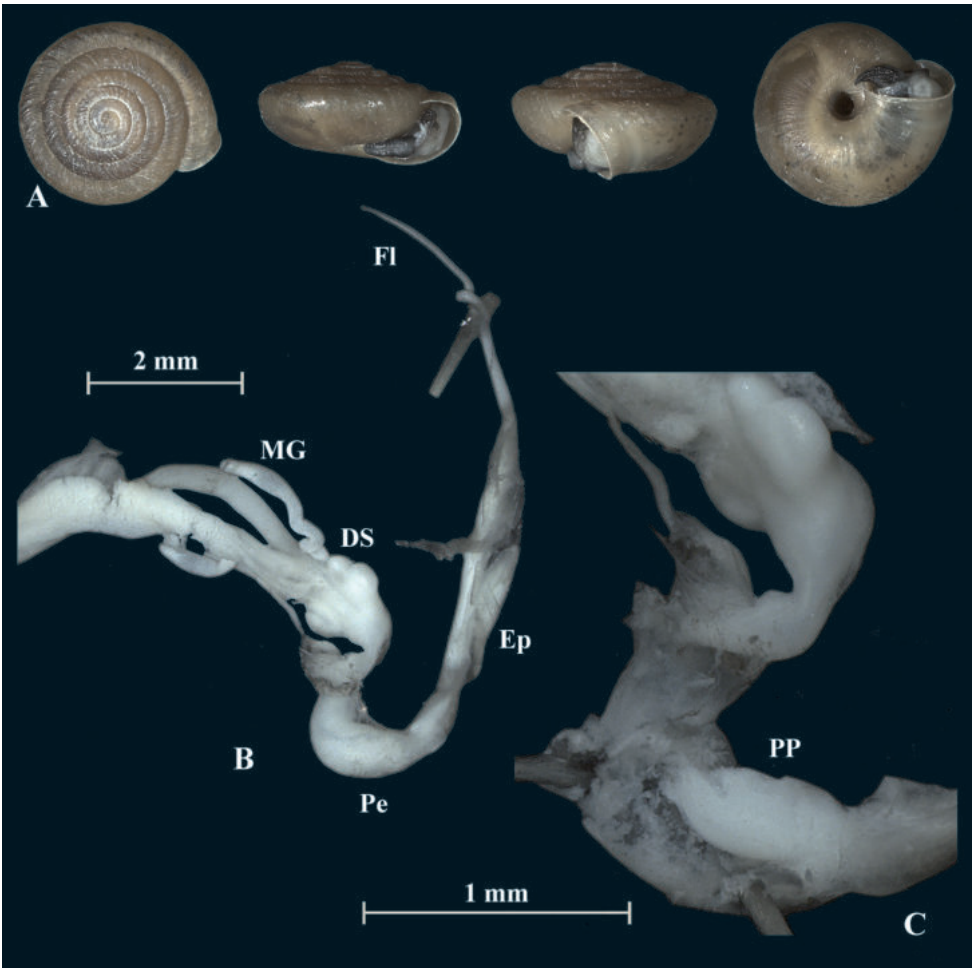


**Figure 9.** *Trochulus biconicus* (NMBE 567169) collected from Chaiserstuel **A** shell, sw = 5.30 mm, sh = 2.46 mm **B** situs **C** penis (Pe) with penial papilla (PP). Shell × 5.

the splitting event of these groups to the Pliocene. On the other hand, we observed a low genetic diversity within our analysed populations. So, this species probably underwent a bottleneck event during the Pleistocene and the Last Glacial Maximum (LGM). Some isolated populations obviously survived this icy period. The LGM lasted about 30–19 ka in the Alps. During that period, this area was covered by massive ice sheets, and the glaciers reached out to the forelands of both, the northern and southern side of the main alpine chains. However, mountain tops above more than 2000 m were not covered by ice during the LGM. The recession of the glaciers from their maximum extent started around 24 ka (see Ivy-Ochs 2015). We hypothesize that the original distribution area of *R. biconica* was much larger, but only a few individuals survived on neighbouring nunataks (glacial islands) during the LGM. A similar scenario is assumed

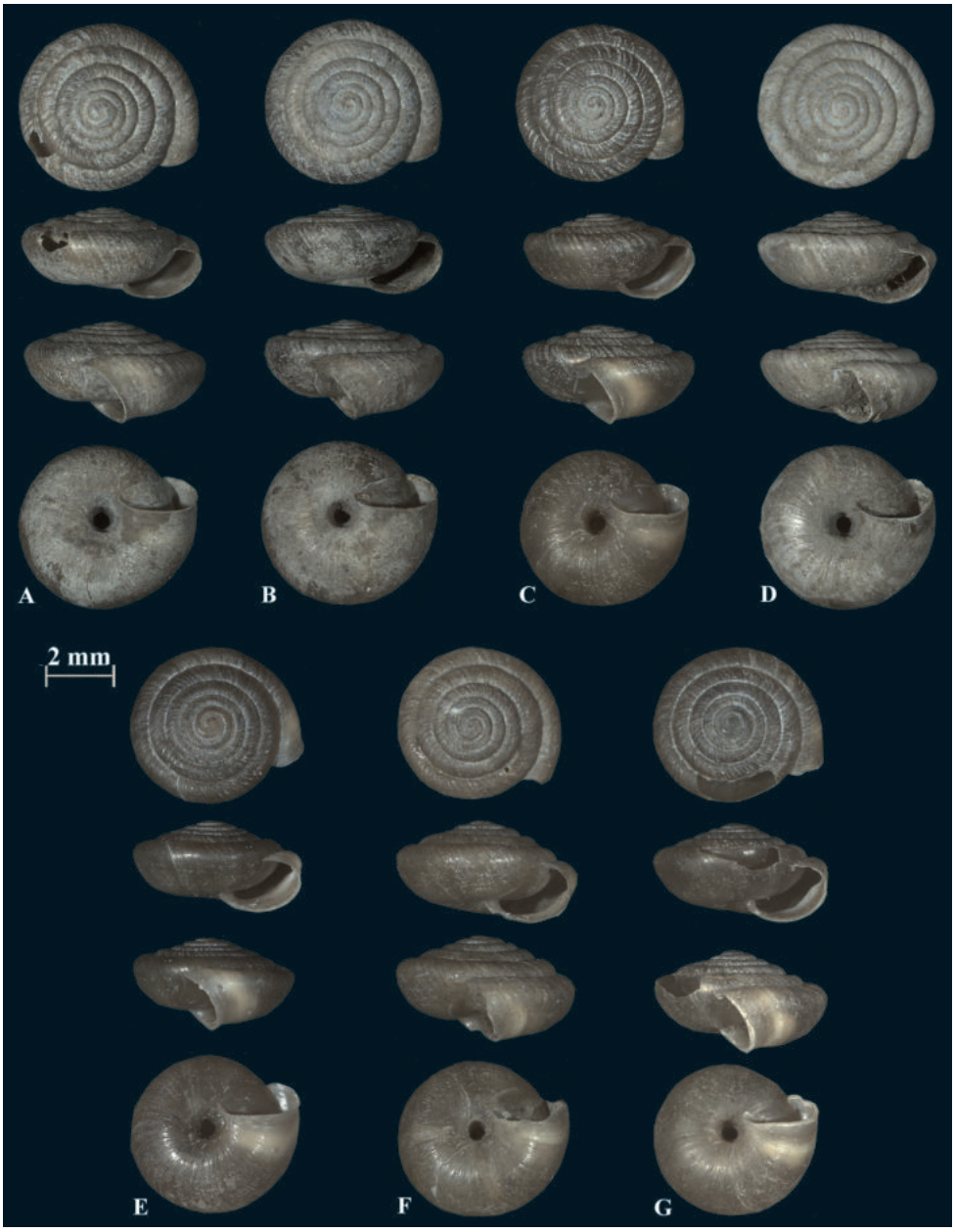


**Figure 10.** Shells of *Trochulus biconicus* from Bannalp Schonegg (**A–D**) and from Chaiserstuel (**E–G**).

for the evolution of the two *Noricella* species (Duda et al. 2011, 2014; Kruckenhauser et al. 2014). Gittenberger et al. (2004) also hypothesized the survival of *Arianta arbustorum alpicola* (A. Férussac, 1821) on nunataks. A similarly fragmented distribution pattern can be observed in the eastern alpine mollusc species *Cylindrus obtusus* (Draparnaud,

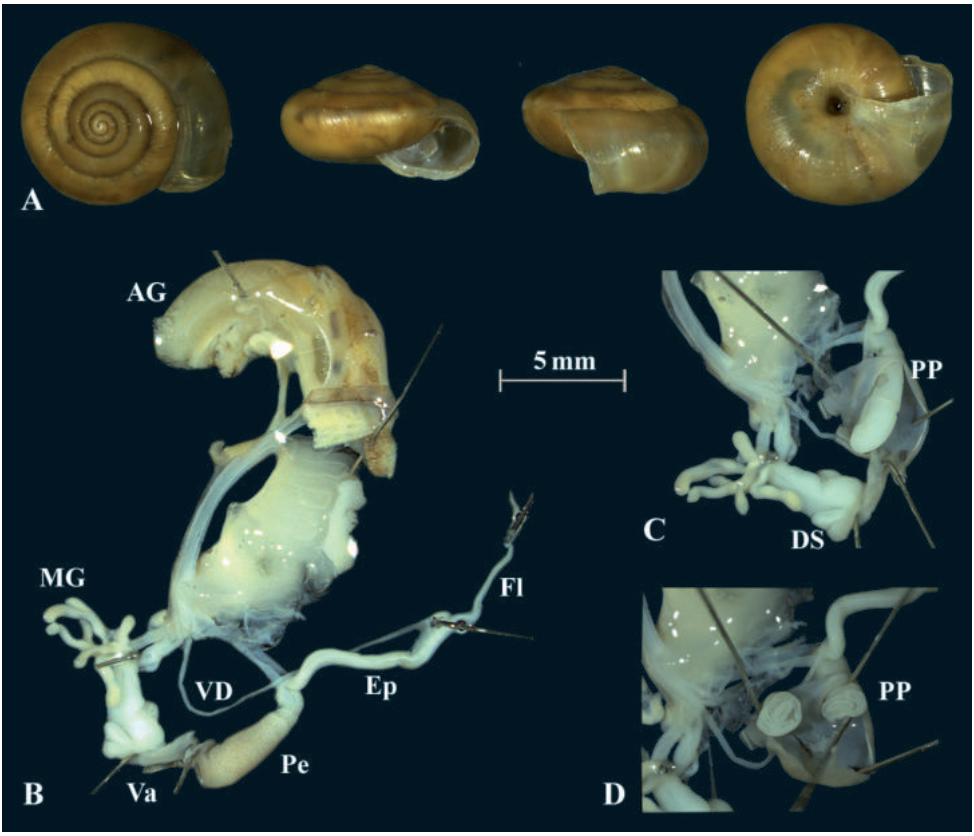


**Figure 11.** *Trochulus clandestinus* (NMBE 571318) collected from Bümpliz, Bern, Switzerland **A** shell, sw = 9.64 mm, sh = 5.57 mm **B** situs **C** penis (Pe) with penial papilla (PP) **D** cross section of the penial papilla. Shell × 3.

1805) (Schileyko 2012: 95, fig. 2). Schileyko argued that the missing fossil record for this species proves that it was formed at the end of the Würm glaciation approximately 10–12 ka ago. As a species adapted to cold environmental conditions, this species was then assumed to be forced to follow the retreating snow and ice fields, which subsequently lead to habitat fragmentation. This assumption requires an ancestor from interglacials (which is also not found in the fossil record), and has to explain the rapid transformation of an Ariantine species from a globular or even depressed shell to a turriform shell. This is most unlikely. Based on COI sequences, Cadahía et al. (2014) estimated 1.5–12 mya for the split between *Arianta* and *Cylindrus*. So, we assume that *Raeticella* gen. nov., like the monotypic genus *Cylindrus*, evolved much earlier and survived the Pleistocene by chance on nunatak mountain tops.

The current distribution pattern does not necessarily and strictly reflect the "survivor" populations. ARNAL (2018) found a limited gene flow between the "isolated" populations of *R. biconica*. This shows that dispersal is not completely impossible, but,

**Figure 12.** Typical habitat of *Raeticella biconica*. This photograph was taken on 25.05.2009 on Chaiserstuel (46.8762°N, 8.4671°E, 2263 m) by Markus Baggenstos.

due to the high-altitude adaptation of the species, it is rather limited to other, hitherto unpopulated high alpine areas. Possible vectors may be large pasturing animals like sheep and goats, but also ibex, chamois, or birds.

In alpine environments, microendemic species with a relict distribution pattern may occur, which were much more widespread in earlier times. They are now restricted to a very small area due to changes in environmental condition (Turner 1991; Cook 2008; Veron et al. 2019). The distribution area of *R. biconica* is currently known to encompass 150 isolated sites on both sides of the Engelberger valley, all situated between 1890 and 2575 m of altitude (Baggenstos 2010).

The habitat of *R. biconica* is very special, and only few other snail species are known to survive in this harsh environment (Eder 1917; Baggenstos 2010). Apart from the occurrence of limestone scree, the snails very much depend on small-scale relief. Slope edges or hilltops, ridges and summits as well as rocky heads and rocky steps are more likely to be colonised by the snail than slope hollows and slope foothills. The highest density of *R. biconica* is reached in areas with more than 50% of rocky scree (Baggenstos 2010). All these sites are covered with snow for a relatively short time in winter. With its flat shell, *R. biconica* is perfectly adapted to live under or between stones (Figs 12, 13). Flatness was interpreted as an adaptation to the cold

**Figure 13.** Close-up of *Raeticella biconica* crawling on the underside of a stone. This photograph was taken on 09.09.2009 on Chaiserstuel (46.8762°N, 8.4671°E, 2263 m) by Markus Baggenstos.

climate at the top of the mountains and may protect the animals from predators (Baur 1987). When it gets too hot, the snails retreat into the ground. The individuals are mainly active during night (Baggenstos 2010). Almost all known *R. biconica* habitats are blue grass meadows. These are alpine grasslands rich in flowers with a great diversity and a remarkably high proportion of Leguminosae. The prominent structural elements are *Sesleria caerulea* and *Carex sempervirens*. The soil cover is relatively thin, interspersed with gravel and stones and dries out quickly (Delarze et al. 2008). Wigger (2007) observed that *R. biconica* mainly feeds on decaying leaves of blue grass (*Sesleria caerulea*). The landscape of these meadows is strongly influenced by extensive pasturing and hiking tourism. Pasture animals like sheep, goats, and cows can modify the position of large stones and thus create new micro habitats for the snails. However, stronger interventions, such as the removal of stones or a climate-related transfer of the rubble-rich sites into closed meadows or woodland formations would cause the snail to disappear (Turner 1991).

This stenoecious species is prone to extinction because of climate change. Over the last 100 years temperatures have increased by about 0.12–0.20 °C per decade in the Swiss Alps and the snow seasons have shortened (Kohler et al. 2014). *Raeticella biconica* already reached the summits of the mountains in their vicinity, and there is no more alternative for avoiding unsuitable climate conditions. Considering that global warming is ongoing, *R. biconica* may well become extinct in just a few years.

## Conclusion

Long known morphological characteristics in conjunction with our genetic analyses show that *R. biconica* should be assigned to a new genus. Morphologically, the investigated individuals of *R. biconica* strongly resemble *N. oreinos* (Duda et al. 2011). But the genetic analyses of several different species from all genera within Trochulini reveal that *R. biconica* does not belong to any currently known genus. Therefore, a new monotypic genus within Trochulini is introduced.

## Acknowledgements

We are indebted to Małgorzata Proćków for providing tissue samples of *E. edentula* and some *Trochulus* specimens, to Ted von Proschwitz for providing *T. hispidus* samples from the Swedish type locality, to Adrienne Jochum for the linguistic revision, to Tom Burri for paleoecological insights, to the Swiss Federal Office of Environment (FOEN) for financial support (contract no. 110010344 / 8T30/00.5147.PZ/0006), and to the cantonal authorities of Nidwalden (Felix Omlin), Obwalden (Andreas Bacher), and Uri (Georges Eich) for providing sampling permits.

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## Supplementary material 1

**Calculated p-distances of the COI of the investigated specimens.**

Authors: Jeannette Kneubühler, Markus Baggenstos, Eike Neubert

Data type: excel file

Explanation note: Calculated p-distances of the COI of the investigated specimens.



Link: https://doi.org/10.3897/zookeys.1104.82866.suppl1

# How many species? Limits of morphological species concepts in the genus *Trochulus* (Gastropoda, Eupulmonata, Hygromiidae)

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## Abstract

In this study, the genus *Trochulus* is revised including specimens from the whole distribution area of the genus, and, if possible, from type localities. Species are redefined based on clades as result of the genetic research. Species boundaries are derived from the genetically recorded clades. This structure is used as a backbone to explore, whether these clades can be described by apomorphic traits in shell morphology and in characters of the genital organs. A total of 551 *Trochulus* specimens covering almost the complete distribution range of the genus are sequenced by using two mitochondrial (COI and 16S) and one nuclear marker (5.8S rRNA+ITS2). GenBank data and outgroup specimens are used to complement the data set, which sums up to 710 specimens treated. The analysis is performed by using Bayesian Inference, Maximum Likelihood and IQ-TREE calculation. These methods and the current taxon sampling yield a stable framework of 21 *Trochulus* clades with differing relationships depending on the method used. A landmark-based geometric morphometric

study is conducted to reveal shell morphological differences. Some lineages could be well separated morphologically, but some cryptic (?) species could not be identified even with shape-based morphometric analyses. Five tentatively new species have been identified. These species are cryptic and cannot be distinguished based on shell morphology only.

For the following taxa, neotypes are selected: *Trochulus circinnatus* (S. Studer, 1820), *Trochulus sericeus* (O. F. Müller, 1774), *Trochulus plebeius* (Draparnaud, 1805).

Lectotypes here designated: *Trochulus montanus* (S. Studer, 1820), *Trochulus clandestinus* (Hartmann, 1821), *Trochulus plebicola* (Locard, 1888), *Trochulus pascali* (Mabille, 1867).

### Keywords

Cryptic species, endemism, geometric morphometrics, integrative taxonomy, phylogeny, RAD sequencing

## Introduction

The genus *Trochulus* Chemnitz, 1786 and its species has been target of many studies in the past. One of the major research impediments in this genus was the historical burden of opinions, revisions, and descriptions. In polymorphic groups like this, authors tended to oversplit and described too many nominal taxa. The increasing diversification of interpretations about the attribution of names to a biological species finally hampered the development of robust species concepts.

In many cases, the whereabout of type specimens was (and is) unclear, descriptions were imprecise, figures insignificant, or the species was recorded from vast areas without a designation of type locality by the original author. Thus, the application of names changed several times throughout the centuries, making it impossible to follow a stringent line. Finally, the “clash of cultures” with the ignorance on both sides between the German school and the French Nouvelle École led to an increase of names, which only can be aligned by cumbersome and pertinacious labour.

Usually, these species descriptions were based on shell morphological traits. If the texts of the descriptions were very simple and short at the beginning, they became too complicated with authors like Bourguignat or Locard, and essential features were emphasised far in excess. Draparnaud knew four species of *Trochulus* (*hispidus*, *sericeus*, *plebeius,* and *villosus*) and it was to the credit of Samuel Studer who soon realised that the Swiss species of the genus could not be covered with this concept. This was the starting point for the subsequent authors of the 19th century, who recognised several new taxa within a short time, re-interpreted the species of previous authors without deeper knowledge, and then added some fossil taxa of different ages to this melange. This is not the place for a

comprehensive historical treatise on the course of the research history on this subject, but it should be noted that we have compiled > 180 names so far, with 10 of them based on fossils, which we consider describing a species of *Trochulus*.

It was Forcart (1966) who, based on Kloeti-Hauser (1920), ventured a new beginning not only for the Swiss *Trochulus* species. He rejected the abusive authorship of Draparnaud for *Helix sericeus,* attributed it to O. F. Müller and presented the species as a mere shell variation of *H. hispidus*; recognized *H. striolatus* C. Pfeiffer, 1828 and *H. concinna* Jeffreys 1830 as distinct species, included *H. plebeia* Draparnaud, 1805 as a western European species, and identified *H. montana* S. Studer, 1820 and *H. caelata* S. Studer, 1820 as distinct endemic Swiss species; and finally affiliated the fossil *H. suberecta* Clessin, 1878 with recent snails from the Engadine (Switzerland) based on an anatomical study of a misidentified *H. sericea* (Wagner, 1915: pl. 19, Fig. 165). Another progress was the inclusion of morphological character states of the genital organs. Hence, Forcart's work represented the starting point for all following "modern" workers on the group, who tried to apply Forcart's concept to the respective area they treated. Some corrections were made, such as the recognition of *H. sericea* as a separate species (Klemm 1974: 393), the summary of the main European taxa in Kerney et al. (1983), and the discussion of French taxa by Falkner et al. (2002). Other work led to the exclusion of some taxa from the genus, such as *Edentiella* and *Petasina*, as independent genera (see Nordsieck 1993). A detailed summary and appreciation of many other works can be found in Proćków (2009). However, all morphology-based approaches failed to address the diversity of *Trochulus*, and leaves for example cryptic species unrevealed. A species is called cryptic, when two or more distinct species are classified as one species based on morphological similarity (Bickford et al. 2007; Pfenninger & Schwenk 2007).

More recent studies combined molecular methods with the investigation of the morphological variation of shells and genital organs, which serves as the backbone for taxonomic decisions (Jörger & Schrödl 2013). Dépraz et al. (2009) studied some Swiss *Trochulus* specimens from a very small area in Canton Vaud finding large morphological overlap between the two lineages recorded. In their work on the genus focusing on Austria, Kruckenhauser et al. (2014) assume a widespread species in Europe, viz. *T. hispidus* (Linnaeus, 1758), which splits into nine clades. Duda et al. (2014) tried to define this and several other species morphologically by an intensive study of the shells and the morphology of the genital organs. In this context, the authors regarded *T. hispidus* as a complex of (cryptic?) species in which species delimitation according to the biological species concept of Mayr (1942) was impossible. The authors reported localities where several clades occur together. Kruckenhauser et al. (2014) showed an unresolved paraphyly, which is explained either by

budding speciation and incomplete lineage sorting, introgression into the mtDNA or the presence of cryptic species. Proćków et al. (2017a) extended the taxon sampling to some southern German and French sites. Here, too, the paraphyletic results complicated the interpretation of the results. In two follow-up papers (Proćków et al. 2017c, 2021), this phenomenon was explained by gene flow between taxa or ancestral polymorphism.

Kruckenhauser et al. (2014) point out that the resolution of the species complex is prerequisite for a stable taxonomy. We fully agree with this statement. After a careful analysis of the aforementioned papers, we seek to resolve the problem by eliminating two major problems. One of them is the size of the taxon sampling, the other refers to the species concept used. All studies published so far widely ignore the enormous radiation of the genus in Switzerland and the neighbouring Franche-Comté in France. Furthermore, we did not base our analysis on *a priori* identified specimens but took a different approach, which is fundamentally different if compared to the aforementioned works, and paraphyletic situations cannot occur. Species are redefined based on clades as result of the genetic research. Species boundaries are derived from the genetically recorded clades.

After an extensive analysis of the literature, we have compiled a list of nominal taxa in question in Europe. The taxon sampling contains, as far as possible, animals from the type localities or nearby, so that a rather reliable relation between a clade and a name could be established. This name has then been used for those animals which co-occur in one lineage.

This study includes 551 *Trochulus* specimens using two mitochondrial (COI and 16S) and one nuclear marker (5.8S rRNA+ITS2) for the genetic analysis. To cover the entire distribution range of species affiliated to this genus, additional sequences from databases of 131 specimens were added. Moreover, the variation of shell morphology in *Trochulus* is analysed using landmark and semi landmark-based geometric morphometric methods (GMM) instead of traditional, linear measurements. The advantage of using landmarks and semi landmarks is, that shape variations of morphologies are covered. Something, which is sometimes overseen or not recognized by linear measurements (Neige 1999). Additionally, GMM allow the separate analysis of size and shape (e.g., Zelditch et al. 2012; Polly & Motz 2016) without introducing artefactual patterns of covariance (Gerber 2017). To morphologically define the lineages found, a 2D landmark-based geometric morphometric analysis was carried out on photos of 195 adult *Trochulus* and *Raeticella* Kneubühler, Baggenstos & Neubert, 2022 specimens. For this, we used the frontal and ventral photo view and combined these two data sets in the analyses. Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) were run to visualize the multivariate data.

To date, there is no coherent concept to resolve the currently unsatisfactory situation. In our study we resolve some of the most urgent questions addressing the taxonomy and the nomenclature of species included in *Trochulus*.

## Material and methods

### Abbreviations

MNHN Muséum National d'Histoire Naturelle, Paris, France

NMBE Naturhistorisches Museum der Burgergemeinde Bern, Bern, Switzerland

NMSG Naturmuseum St. Gallen, St. Gallen, Switzerland

SMNS Staatliches Museum für Naturkunde Stuttgart, Stuttgart, Germany

SMF Senckenberg Museum Frankfurt, Frankfurt am Main, Germany

### Specimens investigated in the molecular study

We examined 551 *Trochulus* specimens. Additionally, 21 *Raeticella biconica* (Eder, 1917) and seven *Edentiella edentula* (Draparnaud, 1805) specimens were included in the genetic analysis (Supplementary material I). From each population, 1–3 specimens were used. The specimens are housed in the collection of the NMBE, MNHN, SMNS and NMSG or have been freshly collected in recent years, mainly by MP and JK. Our data set was supplemented with specimens from the databases GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and BOLD (https://www.bold systems.org/) in order to cover the complete distribution range of *Trochulus*. In total, we included 131 specimens from the publications of Kruckenhauser et al. (2014), Duda et al. (2017), and Proćków et al. (2021), of which the specimens of *Noricella oreinos* (A.J. Wagner, 1915) and *N. scheerpeltzi* (Mikula, 1957) served as outgroup (Bamberger et al. 2020). This sums up to a total of 710 specimens for the genetic analysis. The distribution map of the investigated *Trochulus* and *Raeticella* specimens is shown in Figure 1.

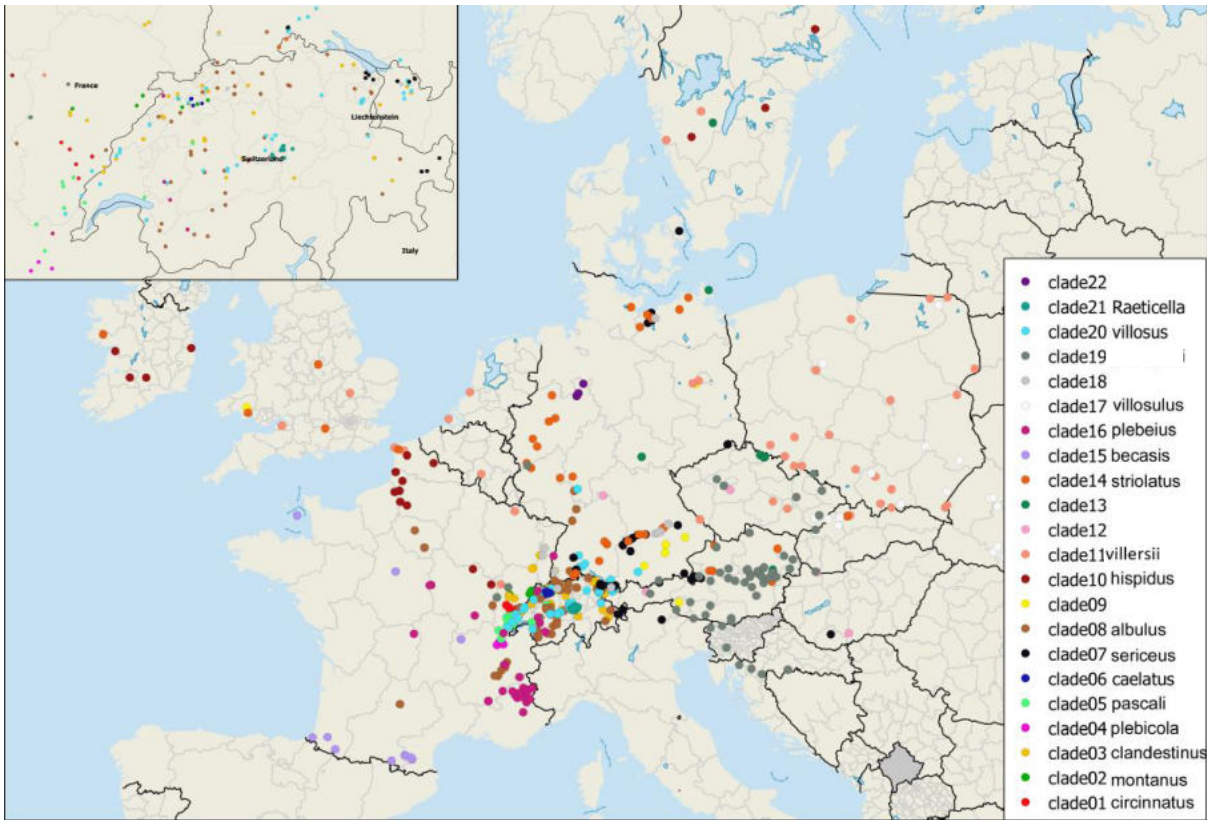



**Figure 1**: Sampling locations of *Trochulus* and *Raeticella* specimens grouped into clades and designated by coloured circles.

## DNA Extraction, PCR Amplification and Sequence Determination

Live specimens were preserved in 80% ethanol. Before DNA extraction, every specimen was imaged under sterile conditions from apical, frontal, lateral and ventral view with a Leica DFC425 microscope camera using the image-processing program (IMS Client V15Q4, Imagic, Switzerland). Three to ten sequential, stacked images were taken from top to bottom of each shell with a single, multistacked image being generated via the Leica software. For total DNA extraction of the specimens the Qiagen Blood and Tissue Kit (Qiagen; Hilden, Germany) was used in combination with a QIAcube extraction robot. Circa 0.5 $cm^3$ of tissue was cut and placed in a mix of 180 µl ATL buffer and 20 µl Proteinase K. It was then incubated for ca. 4 hours at 56 °C in a heater (Labnet, Vortemp 56, witec AG, Littau, Switzerland). For subsequent DNA extraction the QIAcube extraction robot with the Protocol 430 (DNeasy Blood Tissue and Rodent tails Standard) was used. In this study, two mitochondrial markers (COI and 16S) and one nuclear marker (5.8S rRNA+ITS2) were investigated. For some animals only COI was amplified and analysed. PCR mixtures consisted of 12.5 µl GoTaq G2 HotStart Green Master Mix (Promega M7423), 4.5 µl $ddH_2O$, 2 µl forward and reverse primer each, and 4 µl DNA template. In Table 1, the used primer pairs for the PCR are listed. For some specimens, the primers 16Scs1/cs2 were used instead, which generated a longer sequence. Following PCR cycles were used: for COI

2 min at 94 °C, followed by 40 cycles of 1 min at 95 °C, 1 min at 47 °C and 1 min at 72 °C and finally, 5 min at 72 °C; for 16S 3 min at 96 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C, and finally, 1 min at 72 °C; and for 5.8S rRNA+ITS2 3 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C, and finally, 5 min at 72 °C (SensoQuest Tabcyclet and Techne TC-512, witec AG, Littau, Switzerland). The purification and sequencing of the PCR product was performed by LGC (LGC Genomics Berlin, Germany).

**Table 1:** Used primer pairs for the PCR.

| Gene | Primer | Sequence | Sequence length (bp) | Reference |
|---|---|---|---|---|
| COI | LCO1490 | 5'-GGTCAACAAATCATAAAGATATTGG-3' | 655 | Folmer et al. (1994) |
| | HCO2198 | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' | | |
| 16S | 16S ar | 5'-CGC CTG TTT ATC AAA AAC AT-3' | 440 | Simon et al. (1994) |
| | 16S br | 5'- CCG GTC TGA ACT CTG ATC AT -3' | | |
| | 16Scs1 | 5'-AAACATACCTTTTGCATAATGG-3' | 900 | Chiba (1999) |
| | 16Scs2 | 5'-AGAAACTGACCTGGCTTACG-3' | | |
| ITS2 | LSU-1 | 5'-CTAGCTGCGAGAATTAATGTGA-3' | 920 | Wade & Mordan (2000) |
| | LSU-3 | 5'-ACTTTCCCTCACGGTACTTG-3' | | |

## Phylogenetic analyses

The software package Geneious v9.1.8 (Biomatters Ltd) was used for sequence processing and editing. Maximum Likelihood analysis (ML) was performed using RAxML v8.2.12 (Stamatakis 2014) through the HPC cluster from the University of Bern (http://www.id.unibe.ch/hpc). The rapid bootstrapping setting to compute the best scoring ML tree and 1000 bootstrapping replicates were chosen. The protein-coding gene fragments of COI was defined in three data blocks. The non-coding regions from 16S and 5.8S rRNA+ITS2 were defined as single data blocks each. Partitionfinder-2.1.1 (Lanfear et al. 2016) was applied for searching optimal evolutionary models for the partitions using the corrected Akaike Information Criterion (cAIC). The nucleotide model GTR CAT I was used. Additional Maximum Likelihood trees were calculated with the command line software IQ-TREE v2.1.3 (Nguyen et al. 2015). The model GTR+G+I was used, the ultrafast bootstrap (UFBoot; Hoang et al. 2018) and the SH-aLRT branch test (Guindon et al. 2010) with 1000 replicates were performed. For speeding up the computation, 4 CPU cores were used. One would typically start to rely on the clade if its SH-aLRT >= 80% and UFBoot >= 95%. UFBoot support values are more unbiased than normal bootstrap support. 95% UFBoot support correspond roughly to a probability of 95%, that a clade is true (Minh et al. 2013).

Bayesian Inference (BI) was performed using Mr. Bayes v3.2.6 x64 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Altekar et al. 2004) through the HPC cluster from the University of Bern. For the concatenated data set, Partitionfinder-2.1.1 was used for finding the optimal evolutionary models for each subset with the model = all function. The Monte Carlo Markov Chain (MCMC) parameter was set as follows: starting with four chains and four separate runs for 20 million generations with a tree sampling frequency of 1000 and a burn in of 25%.

## Analyses of shell morphology

For the first time, the morphology in *Trochulus* was analysed using landmark and semi landmark-based geometric morphometric methods (GMM) instead of traditional, linear measurements. Of the 572 *Trochulus* and *Raeticella* specimens examined genetically in this study, 195 specimens could be used for morphometric analyses. For the remaining specimens, there were either no photos available, the shell was broken, or the animals were juveniles. Wherever possible, photos of type material were included.

We used the frontal and ventral photo view of the *Trochulus* and *Raeticella* specimens and performed a 2D landmark-based geometric morphometric analysis. The software tpsDig2 v2.31 (Rohlf 2010) was used to obtain the landmarks. On the frontal view, 24 landmarks have been set, of which 16 are fixed landmarks and 8 are sliding semi landmarks. On the ventral view, of the 16 landmarks set, 7 are fixed landmarks and 9 are sliding semi landmarks (Fig. 2). Fixed landmarks are discrete anatomical loci (i.e., transitions of whorls or curvature of the aperture or umbilicus) which are homologous between specimens. The sliding semi landmarks are placed along the surface between two landmarks, in such a way that they best describe the curvature of the outline. Sliding the semi landmarks removes the effect of arbitrary initial spacing (Gunz & Mitteroecker 2013).

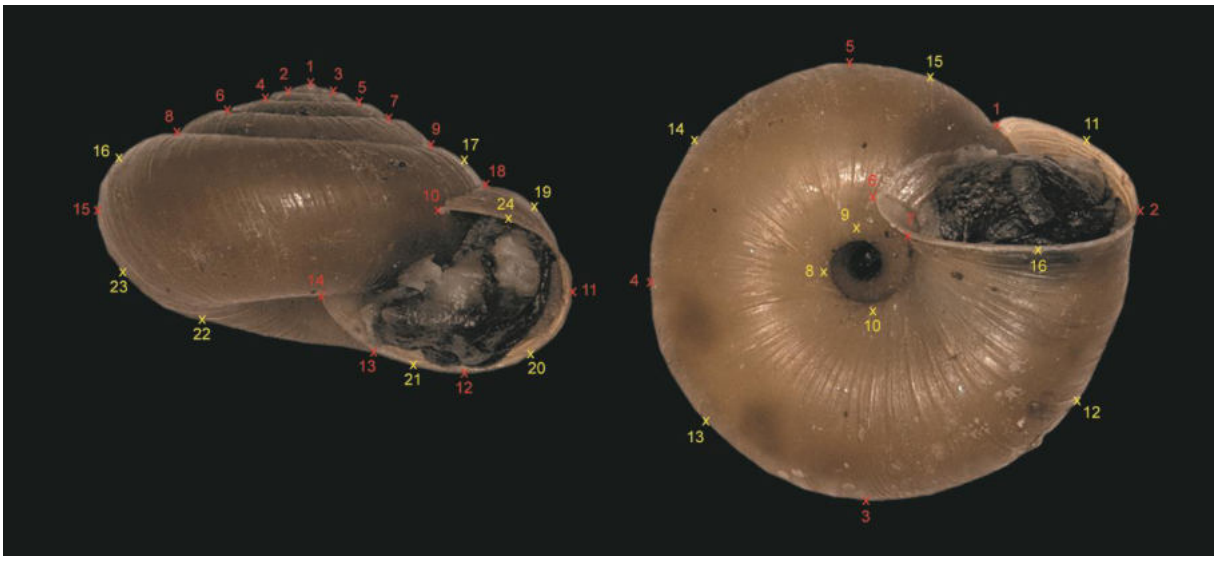


**Figure 2**: Position of landmarks on the frontal and ventral shell view. Red crosses: fixed landmarks; yellow crosses: sliding landmarks.

### Geometric morphometric analysis

For the geometric morphometric analyses, the software R v.4.1.2 (R Core Team, 2021) was used with the packages geomorph v4.0.1 (Adams et al. 2020) and Morpho v2.9 (Schlager 2017). For subsequent analyses, we used modified scripts from Bischof et al. (2021, 2022). With the R function *Morpho::procSym*, the 2D landmark coordinates underwent a full generalised Procrustes alignment (GPA). The semi landmarks were slid in a way to minimize Procrustes distance. The full Procrustes fit standardizes position, orientation and size while leaving only Procrustes shape coordinates (e.g., Bookstein 1991; Zelditch et al. 2012). The frontal and ventral data was combined with the R function *geomorph::combine.subsets* and set to unit centroid size. The centroid size (CS) is the square root of the sum of the squared distances of the landmarks from the centroid (Zelditch et al. 2012). We then ran a Principal Component Analysis (PCA) for the frontal and ventral data set each and for the combined data set and additionally, a Canonical Variate Analysis (CVA) of the frontal and ventral data set each to visualize the multivariate data.

For modelling shapes at the extreme PC-values, the R function *GeometricMorphometricsMix::reversePCA* (Fruciano 2019) was used. This function recalculates artificial Procrustes shape variables from the extreme PC-values in a morphospace. The thin plate spline (TPS) deformation grid were calculated with the R function *geomorph::plotRefToTarget.* TPS deformation grids visualize shape differences between a reference shape and a target shape based on a set of homologous coordinates (Gunz & Mitteroecker 2013). The frontal and ventral views were analysed separately, as we want to show the extreme values for both views and each clade separately. All plots were created with ggplot2.

## Results

### Phylogeny

The Bayesian Inference, Maximum Likelihood and IQ-TREE trees (Fig. 3, 4 and 5) show the concatenated data set (COI, 16S and 5.8S rRNA+ITS2) of 710 specimens. The data set contains additional sequences of Kruckenhauser et al. (2014), Duda et al. (2017) and Proćków et al. (2021). The trees were rooted by specimens of *N. oreinos* from Bamberger et al. (2020). The topologies of all three methods show the same 21 *Trochulus* clades, but relationships of these clades differ to each other between the methods. These clades follow a geographic pattern (cf. Fig. 1). In all three methods, *R. biconica* (Eder, 1917) forms the

sister clade to the remaining *Trochulus* species. These nodes have high support values in all analyses (Fig. 3, 4 and 5; see also Kneubühler et al. 2022).

In the Bayesian Inference analysis (Fig. 3), we see a large split that divides the *Trochulus* lineages into two large clades. This node has a posterior probability of 0.67. One clade consists of the lineages that lie more in the Jura mountains (referred here as the “Jurassic” clade) and the surrounding countryside, and the other consists of the lineages that are widespread in Europe. The resolution in the “Jurassic” clade is better, as more genes were examined here. The support values for the lineages within the “Jurassic” clade are moderate to high. Within the clades, some of the nodes are not supported. In the clade with the widespread lineages, only the COI was examined for most specimens which results in a polytomy with this large number of individuals.

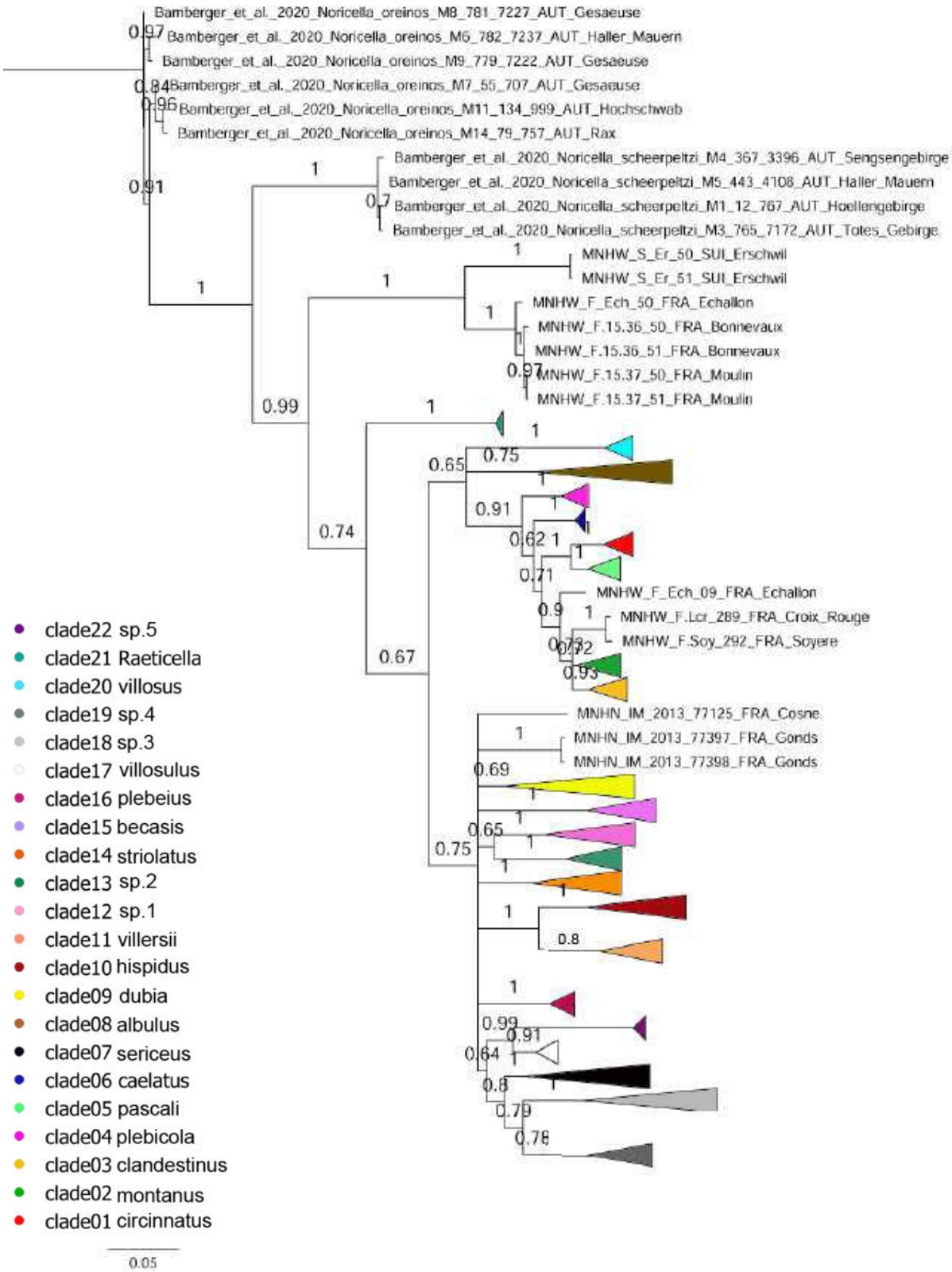



**Figure 3**: Bayesian Inference (BI) tree based on the concatenated data set of COI, 16S and 5.8S rRNA+ITS2. The analysis comprises 710 individuals of which 10 are outgroup specimens from *N. oreinos* and *N. scheerpeltzi*. Additionally, sequences of Kruckenhauser et al. (2014), Duda et al. (2017) and Proćków et al. (2021) were included. Numbers at the nodes are the posterior probabilities.

In the Maximum Likelihood analysis (Fig. 4), the node of the “Jurassic” clades and the widespread clades has a ML support of 90. In contrast to the BI analysis, where *T. villosus* and *T. albulus* form separate not related clades, they cluster together in the ML analysis. But this node is not supported with a bootstrap support of 57. However, the topology in the rest of the "Jurassic" clade is the same in the BI and ML analysis. The topology of the clades in the widespread clade differs, except for *T. hispidus* and *T. villersii* and *T.* sp. 3 and *T.* sp. 4, which form sister groups in the BI and ML analyses respectively. The node of *T. hispidus* and *T. villersii* has a bootstrap support of 94 and a posterior probability of 1 (Fig. 3). The node of *T.* sp. 3 and *T.* sp. 4 is not supported in the ML and BI analysis respectively. The resolution in the tree is better, as no polytomy occurs with ML analysis.

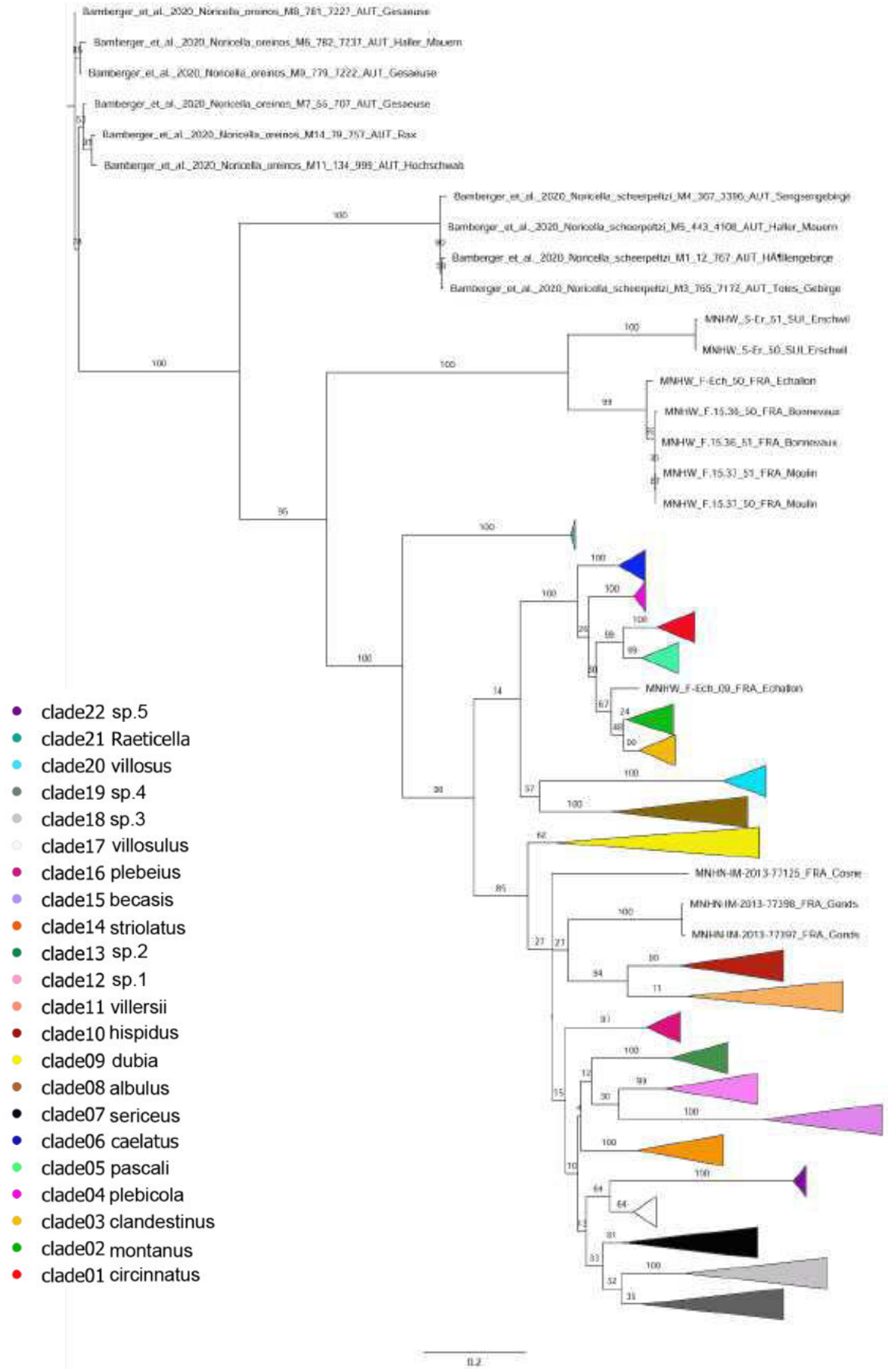
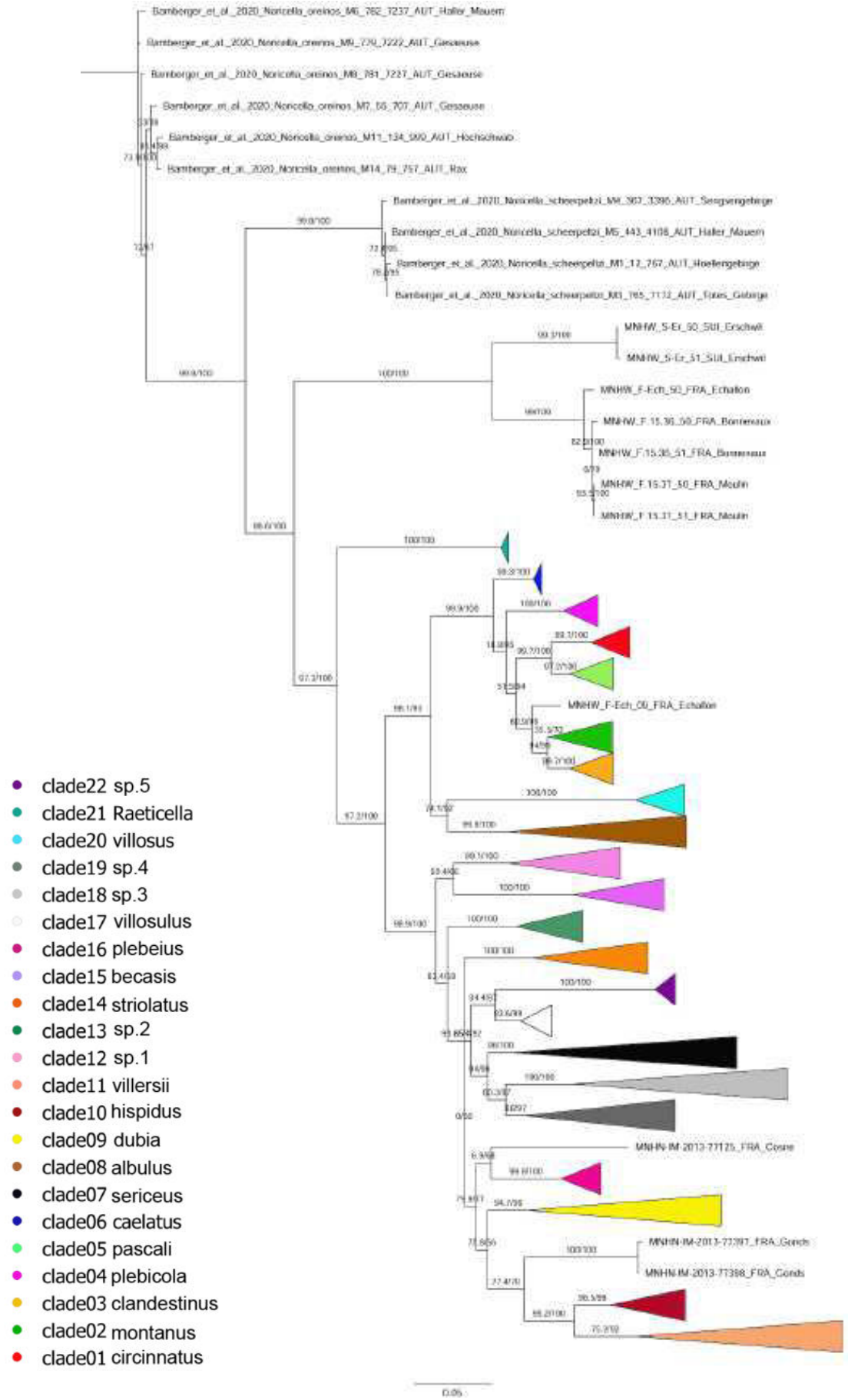

clade22 sp.5
clade21 Raeticella
clade20 villosus
clade19 sp.4
clade18 sp.3
clade17 villosulus
clade16 plebeius
clade15 becasis
clade14 striolatus
clade13 sp.2
clade12 sp.1
clade11 villersii
clade10 hispidus
clade09 dubia
clade08 albulus
clade07 sericeus
clade06 caelatus
clade05 pascali
clade04 plebicola
clade03 clandestinus
clade02 montanus
clade01 circinnatus
MNHW_F-Ech_09_FRA_Echallon
MNHN-IM-2013-77125_FRA_Cosne
MNHN-IM-2013-77398_FRA_Gends
MNHN-IM-2013-77397_FRA_Gends
0.2

**Figure 4**: Maximum Likelihood (ML) tree based on the concatenated data set of COI, 16S and 5.8S rRNA+ITS2. The analysis comprises 710 individuals of which 10 are outgroup specimens from *N. oreinos* and *N. scheerpeltzi*. Additionally, sequences of Kruckenhauser et al. (2014), Duda et al. (2017) and Proćków et al. (2021) were included. Numbers at the nodes are the bootstrap support values.

The IQ-TREE analysis (Fig. 5) shows a consensus tree with SH-aLRT (left) and ultrafast bootstrap (right) supports. The IQ-TREE analysis shows a similar topology to RAxML (Fig. 4), as the approach is also based on Maximum Likelihood (ML) trees. We see again the split in “Jurassic” clades and the widespread clades. This node is well supported with SH-aLRT value of 97.2 and UFBoot value of 100. The nodes show overall higher support values as in the BI or RAxML analyses.


clade22 sp.5
clade21 Raeticella
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clade18 sp.3
clade17 villosulus
clade16 plebeius
clade15 becasis
clade14 striolatus
clade13 sp.2
clade12 sp.1
clade11 villersii
clade10 hispidus
clade09 dubia
clade08 albulus
clade07 sericeus
clade06 caelatus
clade05 pascali
clade04 plebicola
clade03 clandestinus
clade02 montanus
clade01 circinnatus
MNHW_F-Ech_00_FRA_Echallon
MNHN-IM-2013-77125_FRA_Cosne
MNHN-IM-2013-77397_FRA_Gonds
MNHN-IM-2013-77398_FRA_Gonds
0.05

**Figure 5**: Maximum Likelihood (ML) tree based on the concatenated data set of COI, 16S and 5.8S rRNA+ITS2. The analysis comprises 710 individuals of which 10 are outgroup specimens from *N. oreinos* and *N. scheerpeltzi*. Additionally, sequences of Kruckenhauser et al. (2014), Duda et al. (2017) and Proćków et al. (2021) were included. Numbers at the nodes are the SH-aLRT values (left) and UFBoot support values (right).

## Evolutionary distances

For the analysis of the sequence distances of the COI, the software MEGA v10.1.8 (https://www.megasoftware.net/) was used. The sequences were assigned to groups according to their affiliation to a clade, following the numbering of the clades. The outgroup genera *Raeticella*, *Edentiella* and *Noricella* were included. In Table 2 the number of base differences per site from averaging over all sequence pairs within each group are shown (Kumar et al. 2018). This analysis involved 665 nucleotide sequences and 655 positions in the final data set. The highest value is found in *T.* sp. 3, which means that within this clade the greatest differences are to be found with regard to the different bases per site. *Trochulus* sp. 3 consists of many genetically different animals with a large geographical distribution. *Trochulus caelatus* has the lowest value in this analysis. However, this clade also contains only six sequences with little genetic differences.

In Table 3, the number of base differences per site from averaging over all sequence pairs between groups are shown (Kumar et al. 2018). This analysis involved 665 nucleotide sequences and 655 positions in the final data set. The "Jurassic" clades comprise the lowest values in this analysis. The highest values result from the comparison of *Edentiella* with *Trochulus* species. The second highest values result from the comparison of *T. becasis* and *T. villosus* with the "Jurassic" clades.

**Table 2**: The number of base differences per site from averaging over all sequence pairs within each species (group).

| Species (group) | d |
|---|---|
| *T. circinnatus* | 0.026 |
| *T. montanus* | 0.0165 |
| *T. clandestinus* | 0.014 |
| *T. plebicola* | 0.0256 |
| *T. pascali* | 0.0104 |
| *T. caelatus* | 0.0018 |
| *T. sericeus* | 0.0851 |
| *T. albulus* | 0.0881 |
| *T. dubia* | 0.0139 |
| *T. hispidus* | 0.0318 |
| *T. villersii* | 0.0112 |

| | |
|---|---|
| *T.* sp. 1 | 0.0613 |
| *T.* sp. 2 | 0.0384 |
| *T. striolatus* | 0.042 |
| *T. becasis* | 0.0495 |
| *T. plebeius* | 0.0047 |
| *T. villosulus* | 0.0242 |
| *T.* sp. 3 | 0.1062 |
| *T.* sp. 4 | 0.0342 |
| *T. villosus* | 0.0183 |
| *T.* sp. 5 | 0.0122 |
| *Edentiella* | 0.0031 |
| *Noricella* | 0.0744 |
| *Raeticella* | 0.0029 |

**Table 3**: The number of base differences per site from averaging over all sequence pairs between species. Abbreviations used: cir = *circinnatus*, mon = *montanus*, cla = *clandestinus*, ple = *plebeius*, pas = *pascali*, cae = *caelatus*, ser = *sericeus*, alb = *albulus*, dub = *dubia*, his = *hispidus*, vii = *villersii*, str = *striolatus*, bec = *becasis*, viu = *villosulus*, vil = *villosus*, Ede = *Edentiella*, Nor = *Noricella*, Rae = *Raeticella*

| | cir | mon | cla | ple | pas | cae | ser | alb | dub | his | vii | T. sp.1 | T. sp.2 | str | bec | ple | viu | T. sp.3 | T. sp.4 | vil | T. sp.5 | Ede | Nor | Rae |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cir | | | | | | | | | | | | | | | | | | | | | | | | |
| mon | 0.0915 | | | | | | | | | | | | | | | | | | | | | | | |
| cla | 0.0917 | 0.0364 | | | | | | | | | | | | | | | | | | | | | | |
| ple | 0.0944 | 0.0834 | 0.0775 | | | | | | | | | | | | | | | | | | | | | |
| pas | 0.0700 | 0.0914 | 0.0863 | 0.1023 | | | | | | | | | | | | | | | | | | | | |
| cae | 0.0969 | 0.0837 | 0.0799 | 0.0811 | 0.0946 | | | | | | | | | | | | | | | | | | | |
| ser | 0.1728 | 0.1714 | 0.1651 | 0.1754 | 0.1766 | 0.1719 | | | | | | | | | | | | | | | | | | |
| alb | 0.1526 | 0.1416 | 0.1397 | 0.1523 | 0.1558 | 0.1426 | 0.1713 | | | | | | | | | | | | | | | | | |
| dub | 0.1490 | 0.1462 | 0.1419 | 0.1568 | 0.1513 | 0.1532 | 0.1447 | 0.1544 | | | | | | | | | | | | | | | | |
| his | 0.1613 | 0.1628 | 0.1604 | 0.1671 | 0.1637 | 0.1546 | 0.1570 | 0.1566 | 0.1283 | | | | | | | | | | | | | | | |
| vii | 0.1565 | 0.1665 | 0.1604 | 0.1440 | 0.1556 | 0.1469 | 0.1715 | 0.1606 | 0.1452 | 0.1395 | | | | | | | | | | | | | | |
| T. sp.1 | 0.1578 | 0.1619 | 0.1691 | 0.1744 | 0.1650 | 0.1640 | 0.1550 | 0.1718 | 0.1486 | 0.1550 | 0.1600 | | | | | | | | | | | | | |
| T. sp.2 | 0.1664 | 0.1642 | 0.1604 | 0.1673 | 0.1686 | 0.1554 | 0.1460 | 0.1730 | 0.1430 | 0.1404 | 0.1560 | 0.1402 | | | | | | | | | | | | |
| str | 0.1519 | 0.1561 | 0.1545 | 0.1643 | 0.1583 | 0.1549 | 0.1422 | 0.1639 | 0.1344 | 0.1365 | 0.1419 | 0.1289 | 0.1305 | | | | | | | | | | | |
| bec | 0.1792 | 0.1866 | 0.1853 | 0.1894 | 0.1834 | 0.1752 | 0.1598 | 0.1719 | 0.1556 | 0.1396 | 0.1615 | 0.1499 | 0.1441 | 0.1466 | | | | | | | | | | |
| ple | 0.1601 | 0.1585 | 0.1543 | 0.1669 | 0.1716 | 0.1628 | 0.1405 | 0.1600 | 0.1339 | 0.1307 | 0.1285 | 0.1421 | 0.1275 | 0.1104 | 0.1354 | | | | | | | | | |
| viu | 0.1490 | 0.1471 | 0.1498 | 0.1524 | 0.1548 | 0.1514 | 0.1234 | 0.1524 | 0.1242 | 0.1248 | 0.1441 | 0.1226 | 0.1145 | 0.1016 | 0.1438 | 0.1012 | | | | | | | | |
| T. sp.3 | 0.1740 | 0.1683 | 0.1680 | 0.1800 | 0.1754 | 0.1691 | 0.1602 | 0.1706 | 0.1629 | 0.1624 | 0.1814 | 0.1725 | 0.1657 | 0.1555 | 0.1740 | 0.1525 | 0.1442 | | | | | | | |
| T. sp.4 | 0.1610 | 0.1454 | 0.1453 | 0.1706 | 0.1528 | 0.1561 | 0.1421 | 0.1589 | 0.1502 | 0.1363 | 0.1501 | 0.1504 | 0.1453 | 0.1346 | 0.1552 | 0.1244 | 0.1279 | 0.1478 | | | | | | |
| vil | 0.1742 | 0.1773 | 0.1709 | 0.1821 | 0.1801 | 0.1667 | 0.1731 | 0.1706 | 0.1648 | 0.1556 | 0.1556 | 0.1633 | 0.1427 | 0.1521 | 0.1588 | 0.1579 | 0.1569 | 0.1862 | 0.1669 | | | | | |
| T. sp.5 | 0.1742 | 0.1674 | 0.1710 | 0.1699 | 0.1777 | 0.1665 | 0.1415 | 0.1702 | 0.1450 | 0.1509 | 0.1712 | 0.1586 | 0.1522 | 0.1463 | 0.1474 | 0.1457 | 0.1030 | 0.1644 | 0.1451 | 0.1783 | | | | |
| Ede | 0.1947 | 0.1931 | 0.1883 | 0.1945 | 0.1834 | 0.1856 | 0.1972 | 0.1816 | 0.1880 | 0.1771 | 0.1672 | 0.1818 | 0.1651 | 0.1920 | 0.1786 | 0.1760 | 0.1710 | 0.1934 | 0.1718 | 0.1790 | 0.1824 | | | |
| Nor | 0.1545 | 0.1572 | 0.1507 | 0.1560 | 0.1479 | 0.1483 | 0.1665 | 0.1503 | 0.1437 | 0.1469 | 0.1481 | 0.1444 | 0.1544 | 0.1482 | 0.1599 | 0.1414 | 0.1410 | 0.1625 | 0.1508 | 0.1707 | 0.1654 | 0.1730 | | |
| Rae | 0.1676 | 0.1556 | 0.1543 | 0.1700 | 0.1687 | 0.1679 | 0.1711 | 0.1674 | 0.1578 | 0.1625 | 0.1629 | 0.1615 | 0.1726 | 0.1726 | 0.1733 | 0.1549 | 0.1487 | 0.1718 | 0.1513 | 0.1768 | 0.1664 | 0.1860 | 0.1479 | |

## Landmark analysis

The shape analysis of the data from the frontal view shows, that the first three Principal Components (PC) account for 65.8% (PC1 = 29.8%, PC2 = 23.4%, PC3 = 12.6%) of the total variation. *Raeticella biconica* is separated from all other clades along PC1. The two investigated specimens of *T. caelatus* are separated from the other clades along PC2 (Fig. 6A) even though the type specimen of *T. caelatus* (blue diamond in Fig. 6) clusters within the main point cloud of the remaining specimens. Even though most of the remaining clades cover a limited area in the morphospace, the overall amount of overlap is too large to make a clear distinction.

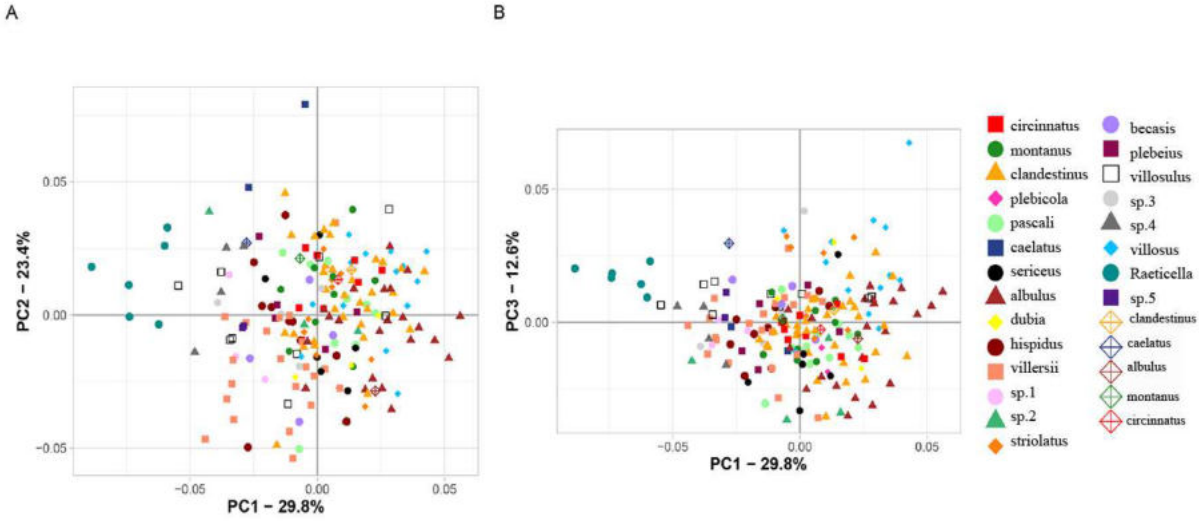



**Figure 6**: Principal Component Analysis (PCA) of the frontal view data of all investigated specimens (n = 195). (A) Principal Component 1 and 2; (B) Principal Component 1 and 3. Values at the axes account for the variation of the data. Diamonds represent type specimens of the respective species.

The shape analysis of the data from the ventral view shows, that the first three Principal Components (PC) account for 70% (PC1 = 35.6%, PC2 = 17.8%, PC3 = 16.6%) of the total variation. The analysis of the ventral data shows a large overlap of the clades (Fig. 7). The species cannot be distinguished based on ventral characteristics alone. Running the analysis with fewer clades does not enhance the separation significantly.

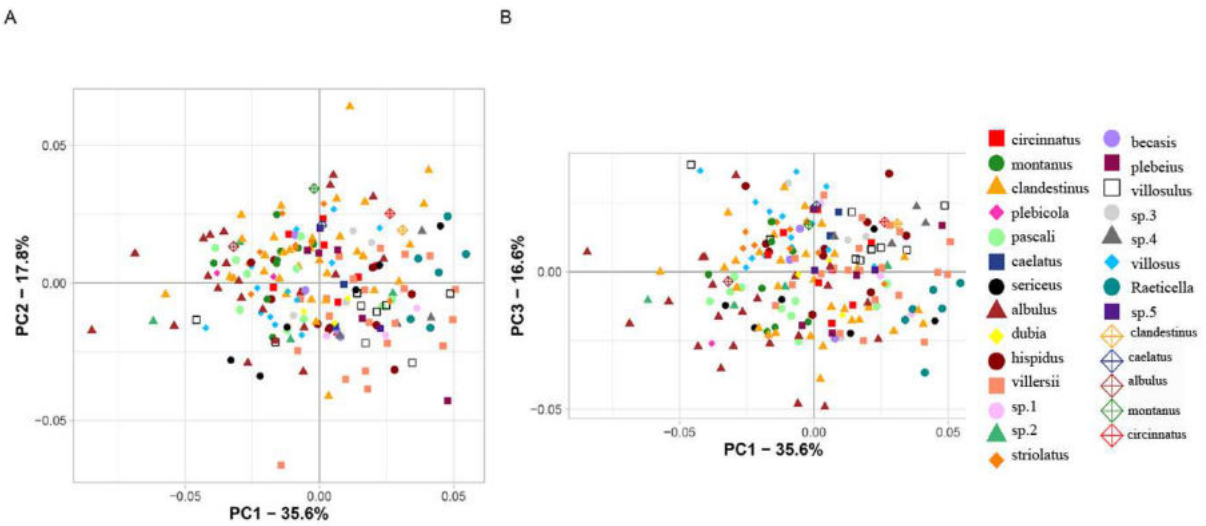



**Figure 7**: Principal Component Analysis (PCA) of the ventral view data of all investigated specimens (n = 195). (A) Principal Component 1 and 2; (B) Principal Component 1 and 3. Values at the axes account for the variation of the data. Diamonds represent type specimens of the respective species.

The shape analysis of the combined data of the frontal and ventral view shows, that the first three Principal Components (PC) account for 52.6% (PC1 = 26.8%, PC2 = 15.4%, PC3 = 10.4%) of the total variation. The PCA of PC1 and PC2 (Fig. 8A) and of PC1 and PC3 (Fig. 8B) reveals a large overlap of the clades, except for *R. biconica*, which is separated from the other clades along PC1. This shape analysis supports our hypothesis that *R. biconica* does not belong to the genus *Trochulus* Chemnitz, 1786, but is placed in a new genus (Kneubühler et al. 2022). The type specimen of *T. albulus* (brown diamond in Fig. 8) clusters well within the point cloud of *T. albulus* (Fig. 8). The type specimen of *T. clandestinus* (orange diamond in Fig. 8), *T. montanus* (green diamond) and *T. circinnatus* (red diamond) cluster close to each other, which means, they are not well distinct based on frontal and ventral shell shape. The point cloud of *T. clandestinus* spans that of *T. montanus* and *T. circinnatus*, which means that the intraspecific variability of *T. clandestinus* is greater than the interspecific variability of those species. Specimens from *T. sericeus*, *T.* sp. 1 and *T. villosulus* show great intraspecific variability.

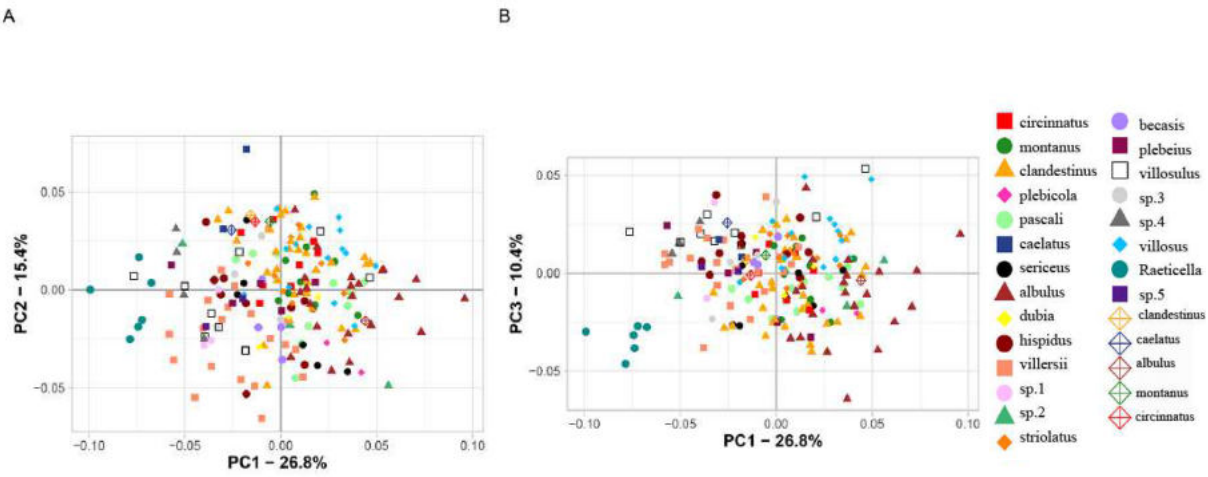



**Figure 8**: Principal Component Analysis (PCA) of the combined data from frontal and ventral view of all investigated specimens (n = 195). (A) Principal Component 1 and 2; (B) Principal Component 1 and 3. Values at the axes account for the variation of the data. Diamonds represent type specimens of the respective species.

In Figure 9, the thin plate spline (TPS) of Procrustes shape of the extreme PC values against the mean shapes of each clade for the frontal view is shown. *Trochulus circinnatus*, *T. montanus*, *T. clandestinus*, and *T. pascali* look very similar. These specimens have a rather flat shell with a large, roundish aperture. *Trochulus caelatus* (Fig. 9) is flat, where the first whorls contribute very little in terms of shell height. Specimens of *T. plebicola* and *T. albulus* have a globular shell with a relatively large aperture. *Trochulus villosus* (Fig. 9) has a flat shell with an elongated aperture. *Raeticella biconcia* (Fig. 9) has a flat, keeled shell with a lunate aperture. *Trochulus hispidus* and *T. villersii* have a rather flat shell with a relatively smaller aperture than clades with a similar shell shape (e.g., *T. circinnatus*, *T. montanus*, and *T. clandestinus*).

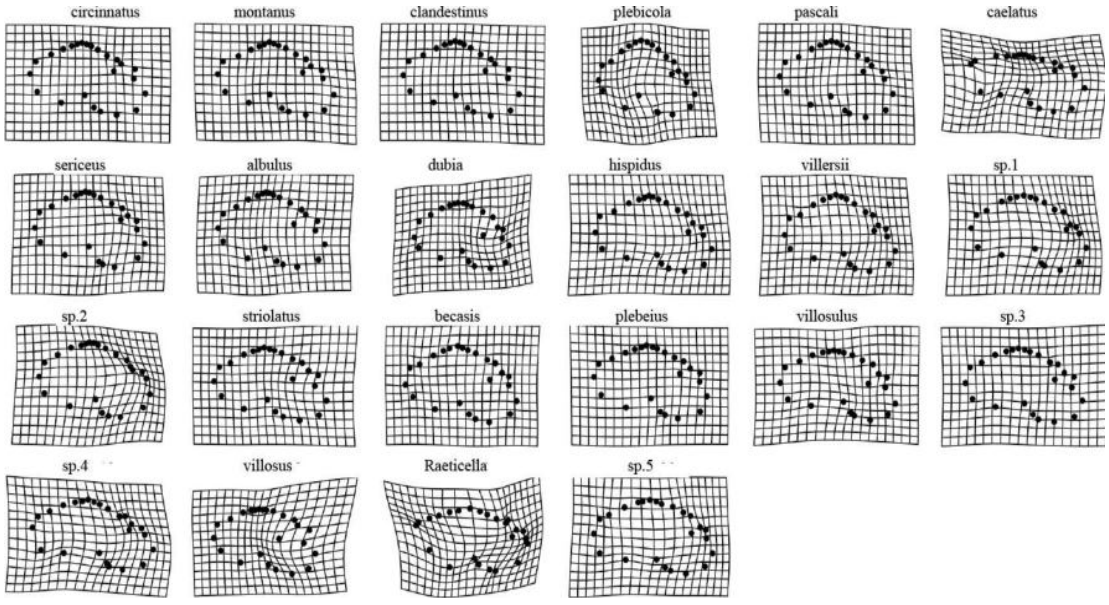


**Figure 9**: The thin plate spline (TPS) of Procrustes shape of the extreme PC values against the mean shapes of each clade for the frontal view.

Figure 10 shows the thin plate spline (TPS) of Procrustes shape of the extreme PC values against the mean shapes of each clade for the ventral view. *Trochulus plebicola* and *T. albulus* have a narrow umbilicus, slightly hidden by the aperture. The remaining clades have a similar wide, open umbilicus.

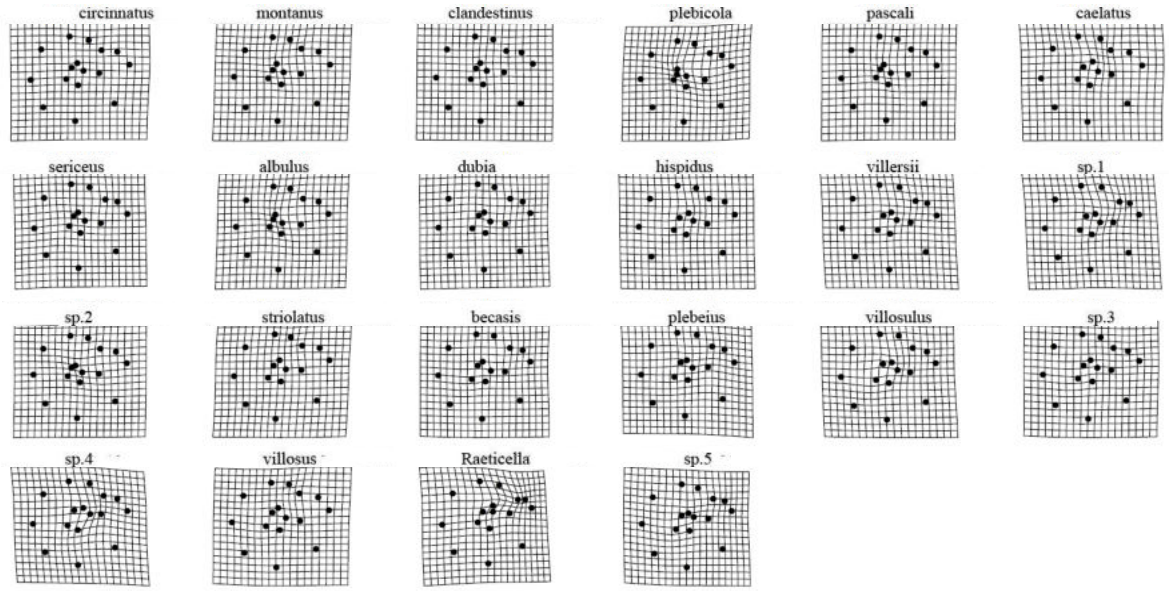



**Figure 10**: The thin plate spline (TPS) of Procrustes shape of the extreme PC values against the mean shapes of each clade for the ventral view.

Canonical Variate Analysis (CVA) is used to identify and measure the associations among two sets of variables. We calculated a CVA for the frontal (Fig. 11) and the ventral view (Fig. 12) each. *Raeticella biconica* falls out morphologically completely in the analysis. In order to better show the differences between the other species, *R. biconica* was left out of the analyses. In Figure 10, the CVA of the frontal shell view is shown. *Trochulus villosus* is well separated from the remaining species. *Trochulus albulus* and *T. villosulus* show slight tendencies towards delimitation, but nevertheless have an overlap with other species. The scatter within a clade is significantly smaller than with PCA.

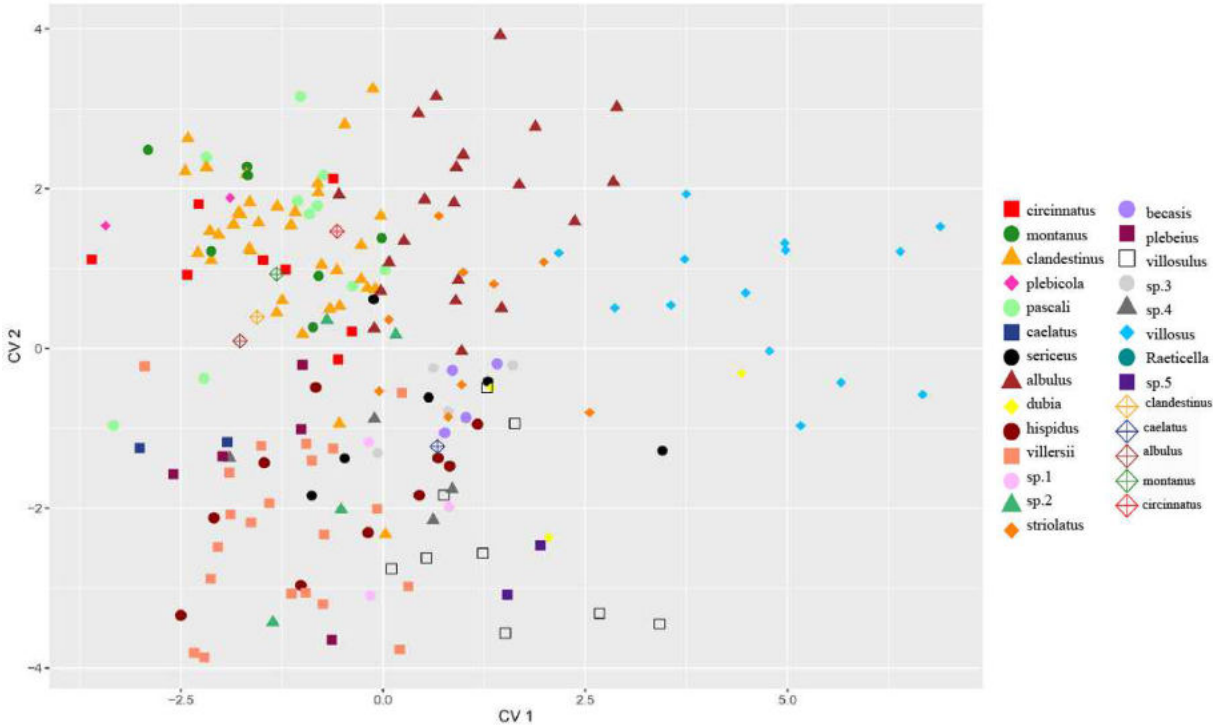



**Figure 11**: Canonical Variate Analysis (CVA) of the frontal view data of the investigated specimens without *R. biconica* (n = 189). Diamonds represent type specimens of the respective species.

The separation of the specimens based on the ventral view (Fig. 12) is less characteristic than with the frontal data. Only some specimens of *T. albulus* with the very narrow, point-like umbilicus can be separated from the other species in the analysis. The other specimens have a big overlap which indicates that they are morphologically very similar.

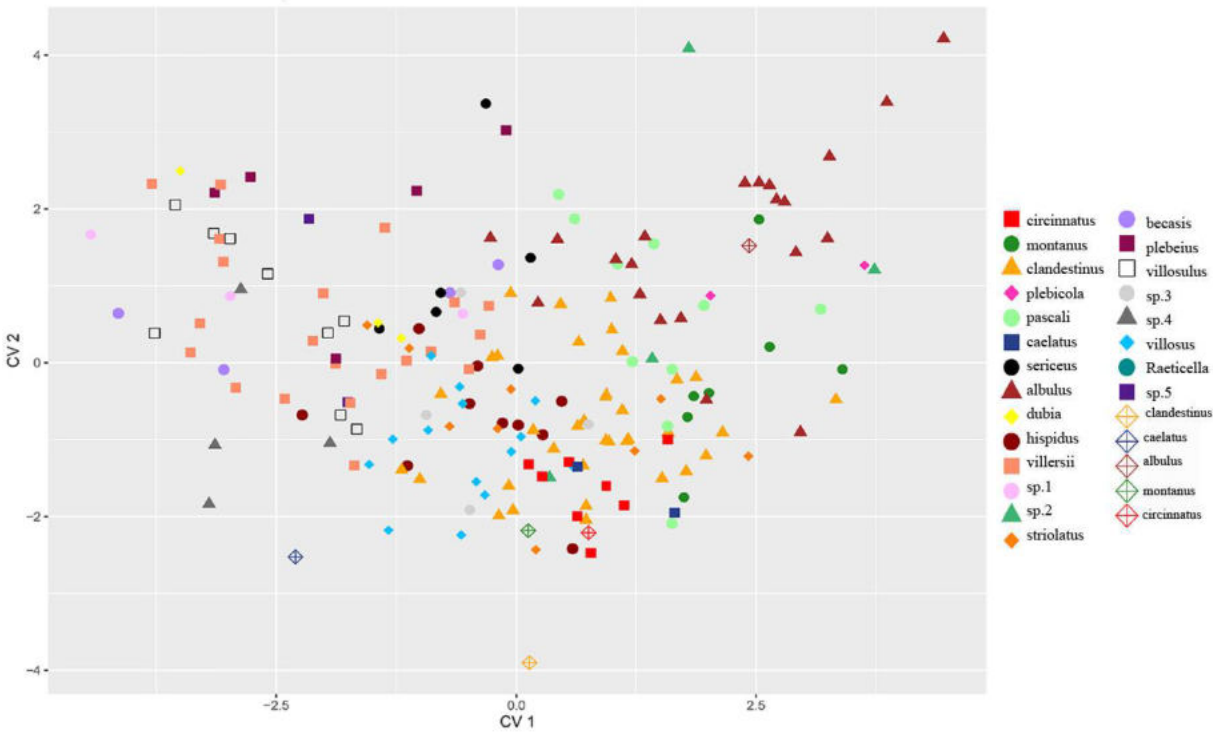


**Figure 12**: Canonical Variate Analysis (CVA) of the ventral view data of the investigated specimens without *R. biconica* (n = 189). Diamonds represent type specimens of the respective species.

## Taxonomic account

The clades are here translated into a taxonomic structure according to the number where they occur in the tree. Variation in shell shape and morphology can be estimated from our plates, which mainly show sequenced specimens (except for some dry type specimens).

## *Trochulus circinnatus* (S. Studer, 1820) [clade01] Figs 13a-f

1820 *Glischrus* (*Helix*) *circinnata* S. Studer, Naturw. Anz. schweiz. Ges. Naturw., 3 (11): 86 [or: Syst. Verz. Schweizer-Conchylien: 12] ["Auf dem Jura"].

1821 *Helix depressa* W. Hartmann, Neue Alpina: 1: 237 ["sie kommt in der Schweiz, aber selten, vor"; Remark: refers to Draparnaud, 1805, Hist. nat. moll. terr. fluv. France: 104, pl. 7 fig. 22 as *Helix hispida* var. γ; non *Helix depressa* Montagu, 1803].

Type specimens: We consider S. Studer as author of this species based on his indication of the name and differentiation from *Helix* (*Glischrus*) *montana* in the description of the latter species. *Trochulus circinnatus* was mentioned a year later by Hartmann (1821, in: Steinmüller, Neue Alpina, 1: 237, pl. 2 fig. 13) as *Helix hispida* var. *circinnata* with the note: "ich erhielt sie besonders schön aus dem Kanton Wadt und durch Herrn von Charpentier von Mont zwischen Valorbe und Lac de Joux [I received a nice specimen from the Canton Waadt and from Mr. von Charpentier from Mont between Valorbe and Lac de Joux]". Unfortunately, no type specimen could be traced in Studer's collection. The specimens from Hartmann do not originate from Studer, and no other original specimens could be traced in contemporary collections, the designation of a neotype is needed to stabilize the application of the name. We herewith designate NMBE 564609 as lectotype for *T. circinnatus*.

Description: The investigated specimens of *T. circinnatus* have a pale brown shell without any hairs. The shell is roundish-conical with an aperture that is about half of the shell width. The shell is finely striated. Shell width ranges from 9.48–11.98 mm and shell height from 5.48–7.1 mm (N = 9). All measurements can be found in the appendix (supplementary material II). The body whorl height accounts for about 80% of shell height and contains a light band. The specimens have between 5.25–6 whorls. The umbilicus is wide and open. The body is pale brown to greyish.

Distribution (Fig. 14): This species is a small range endemic for the Central Swiss and French Jura. Genetically identified populations inhabit an area roughly between Neuenburg and Lausanne in Switzerland north to Dole and Besançon in France.

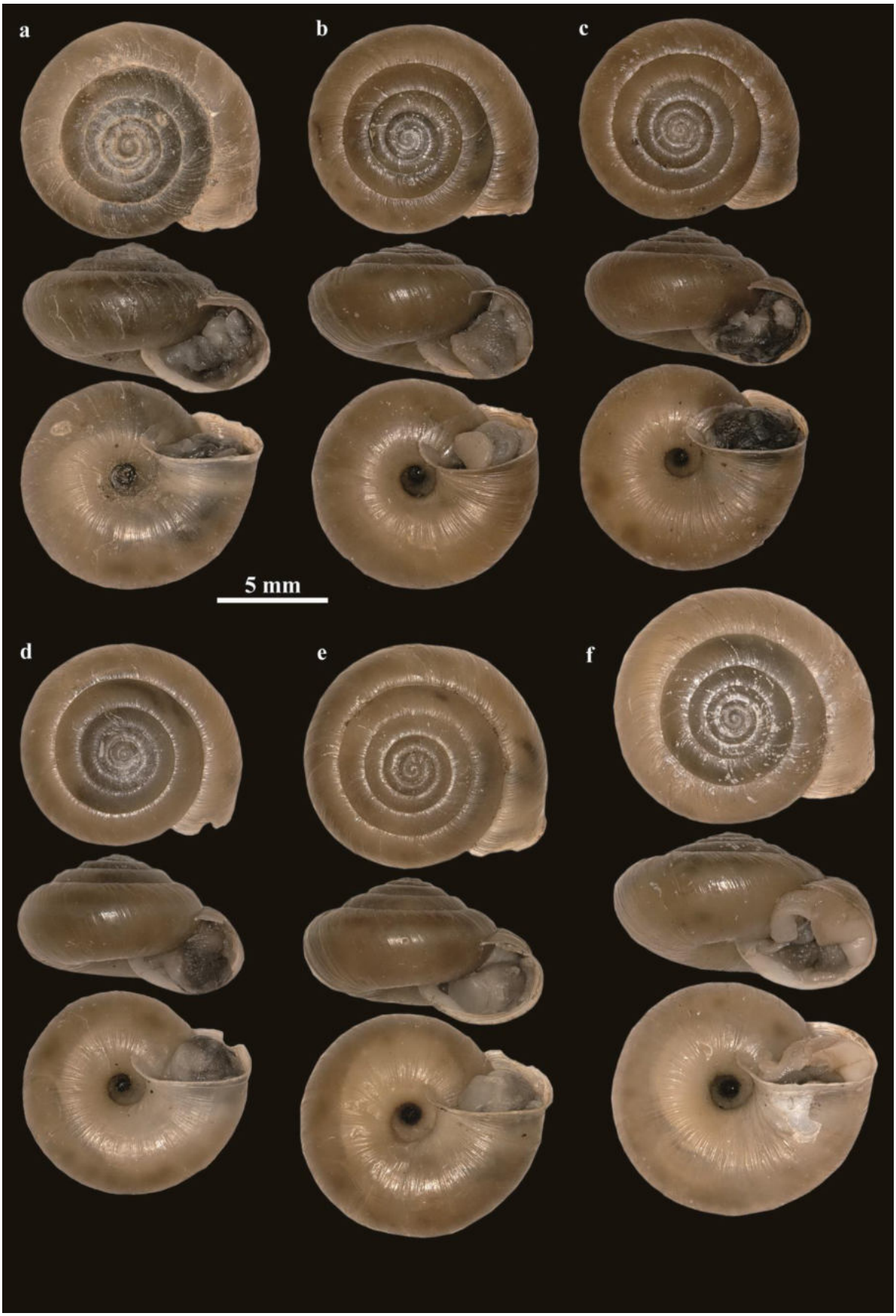

a
b
c
5 mm
d
e
f

**Figure 13**: *Trochulus circinnatus*. Clade 01; (a) MNHW_F.15.38_06; France, Chaux-des-Crotenay; (b) MNHW_F.15.35_01; France, Mouthe, Source du Doubs; (c) MNHW_F.15.36_01; France, Bonnevaux; (d) MNHW_F.15.37_06; France, Cascade du Moulin du Saut; (e) MNHW_F.15.37_05; France, Cascade du Moulin du Saut; (f) MNHW_F.15.34_01; France, La Chevry.

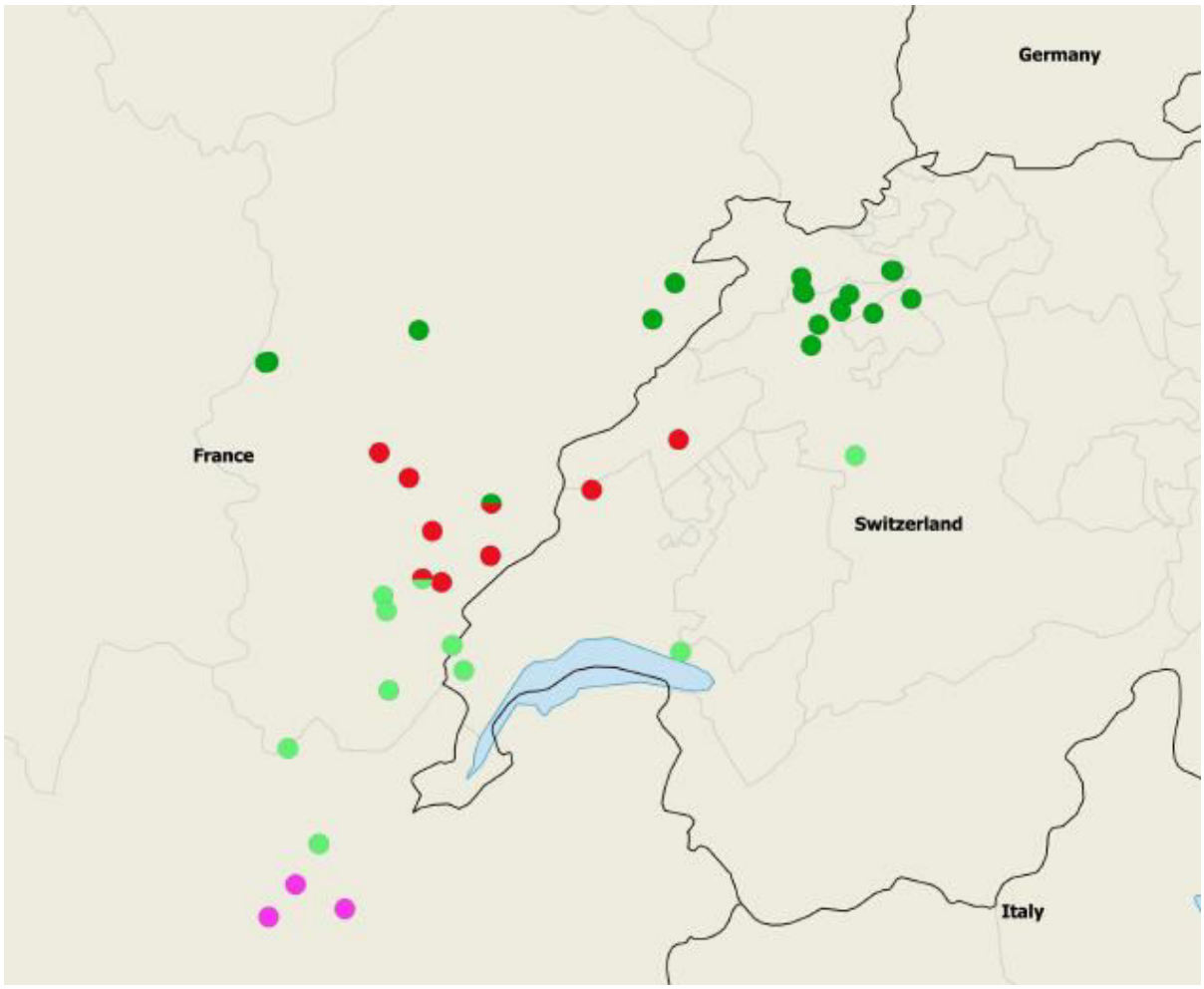



**Figure 14**: Distribution map of the investigated *T. circinnatus* (red circles), *T. montanus* (dark green circles), *T. plebicola* (pink circles), and *T. pascali* (pale green circles) specimens.

## *Trochulus montanus* (S. Studer, 1820) [clade02] Figs 15a-h

1820 *Glischrus* (*Helix*) *montana* S. Studer, Naturw. Anz. schweiz. Ges. Naturw., 3 (11): 86 [or: Syst. Verz. Schweizer-Conchylien: 12] [Auf dem Jura].

1855 *Helix glabra* Dumont, Bull. Inst. natn. genevois: 3: 355 [“du Jura”; junior homonym of *Helix glabra* Gmelin, 1790 In: Linnaeus, Systema Naturae, ed. 13, I: 3658].

Type specimens: The original *Glischrus montana* series of Studer as found by Forcart in 1957 labelled with Studer’s catalogue number “13” contained 12 shells. Without providing a reason, Forcart split the original lot “13” into the following subsets: 13, 13a, 13b, and 13c. So, the original number “13” was selected by him as lectotype for *Glischrus* [*Helix*] *montana* STUDER, 1820 containing a single specimen (NMBE 15422). Under “13a”, he separated four specimens, under “13b” three specimens, and finally under “13c” another four specimens. All

these lots constitute paralectotypes of *Glischrus montana*. Later, these lots have been revised by Wüthrich (1977) and Gosteli (1991), both unpublished.

Wüthrich recognized that the lectotype selection by Forcart was erroneous and identified specimen “13” as a juvenile *Urticicola umbrosus* (C. Pfeiffer, 1828). Number “13a” contains *T. montanus* sensu auctores, and Gosteli selected a new lectotype specimen from this series. “13b” contains another three paralectotype specimens of *T. montanus*, while “13c” is a compositum mixtum of two shells of *U. umbrosus*, a single shell of *T. montanus* and a single shell of *T. striolatus*.

We consider Forcart’s lectotype selection as invalid using ICZN 74.2. This article regulates that “If it is demonstrated that a specimen designated as a lectotype was not a syntype, it loses its status of lectotype”. The type locality of *montana* is “Auf dem Jura” – which can be either the Swiss Jura or the French part. However, in both Jura parts, *U. umbrosus* does not occur (in fact, *U. umbrosus* does not occur in the whole of France nor the whole of Switzerland). The shell that has been selected as the lectotype by Forcart can therefore not be a syntype. Hence, Gosteli’s selection is correct, the lectotype is registered under NMBE 15466 (D = 10.9 mm; Fig. 15a).

Description: *Trochulus montanus* has a pale brown, shiny shell without any hairs. The shell is roundish-conical. Shell width of the investigated specimens ranges from 7.84–12.4 mm and shell height from 5.04–6.44 mm (N = 7). All measurements can be found in the appendix (supplementary material II). The body whorl height accounts for about 80% of shell height and contains on some specimens a light band. Specimens contain 5.5–6 whorls. The umbilicus is wide and open. The body is beige to brown.

Distribution (Fig. 14): This species is genetically identified from the Swiss Jura south of Basel and inhabits the mountainous part of the Franche-Comté towards the promontory of Dijon. The southernmost record is from the French Jura between Bourg-en-Bresse and Geneva.

Habitat: We consider this species an endemic of the western Central Jura, where it lives in sympatry with *T. circinnatus*.

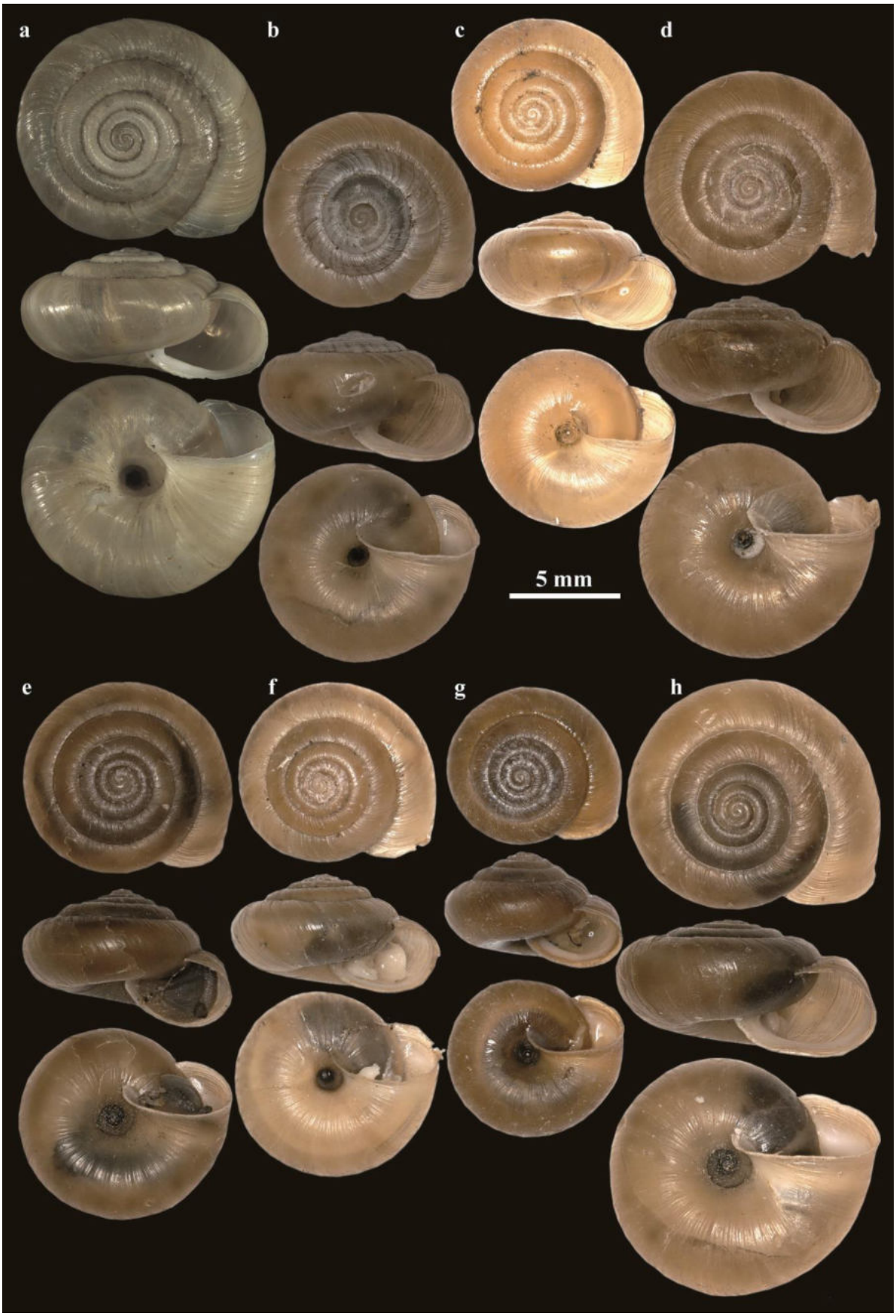

a
b
c
d
5 mm
e
f
g
h

**Figure 15**: *Trochulus montanus*. Clade02; (a) Lectotype NMBE 15466 «Auf dem Jura»; (b) MNHW_S.15.22a_10 Switzerland, Gorges du Pichoux; (c) MNHW_F.SF_283; France, Source de la Foulotière; (d) MNHW_S.15.17_01; Switzerland, Gorges de Court; (e) MNHW_S.15.20_17; Switzerland, Gorges de Court 4; (f) MNHW_F.Lcr_289; France, La croix rouge; (g) MNHW_F.LR_290; France, La Ruchotte; (h) MNHW_S.15.26_03; Switzerland, Frinvillier 2.

## *Trochulus clandestinus* (Hartmann, 1821) [clade03] Figs 16a-h

1821 *Helix corrugata* var. *clandestina* HARTMANN, in: STEINMÜLLER, Neue Alpina, 1: 236 [ich erhielt sie von Wien und fand sie in der Schweiz bey Zürich [Wien: ex BORN]].

1821 *Helix corrugata* W. Hartmann, Neue Alpina: 1: 236 [Neuwied [identified with Neuweid, Zürich, 47.32701 8.42324]; junior homonym of *Helix corrugata* Gmelin, 1789].

1874 *Helix* [*Fruticiola*] *putonii* Clessin: Jahrb. dtsch. malakozool. Ges., 1 (4): 314 [Belgium and NE France: Bussange, Vogesen].

Type specimen: Lectotype NMSG M942 (D = 10.07 mm), here designated for the specimen from Zurich.

Description: The investigated specimens of *T. clandestinus* have a shiny grey to pale brown shell without any hairs. The shell is roundish-conical. The shell is finely striated. Shell width ranges from 7.18–11.9 mm and shell height ranges from 4.08–6.96 mm (N = 36). All measurements can be found in the appendix (supplementary material II). The last whorl is less wide than in *T. circinnatus* and *T. montanus*. The specimens have 5.25–6 whorls. The umbilicus is wide and open. The body is beige.

Distribution (Fig. 17): This species occurs in France (eastern Franche-Comté and southern Alsace), Germany (southern Black Forest), and Austria (Vorarlberg). In Switzerland, it inhabits the complete northern area, but does not live in the region south of the main alpine chain.

Habitat: *Trochulus clandestinus* is found in the herb layer in damp deciduous forest, below bushes near rivers, but also in cultivated areas like gardens and parks.

Remarks: One of the historical confusions have been started by Hartmann himself, who recorded the species from Vienna and Zurich. However, according to our results, *T. clandestinus,* as used by modern authors, is restricted to the western alpine area. The record from Vienna (specimen not preserved in the Hartmann collection) is a confusion with another *Trochulus* species.

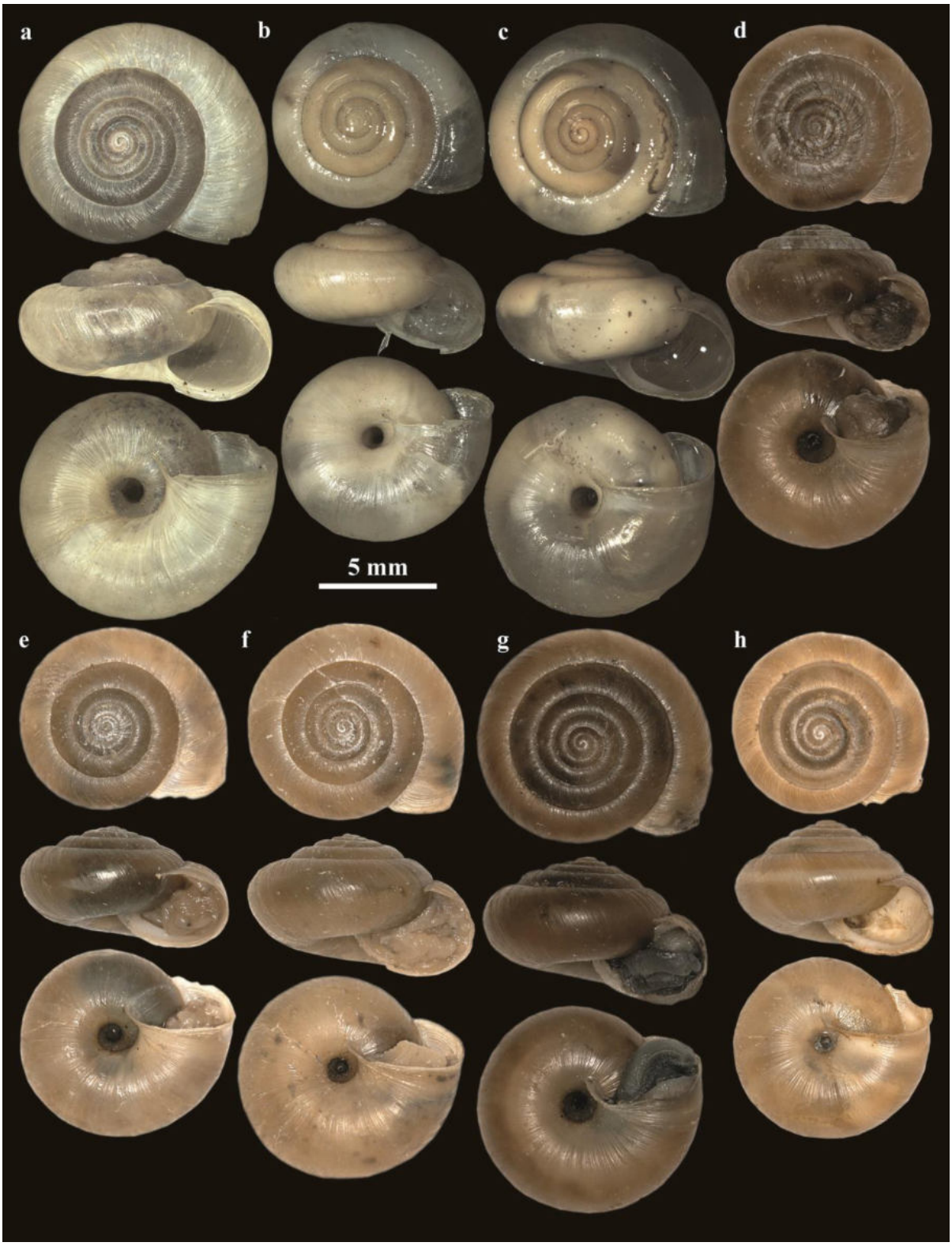



**Figure 16**: *Trochulus clandestinus*. Clade03; (a) Lectotype NMSG M942; Switzerland, Zürich; (b) NMBE 571142; Switzerland, Bergün; (c) NMBE 571235; Switzerland, Bürglen; (d) MNHW_F.15.31_02; France, Mont d'Or; (e) MNHW_A.Me_05; Austria, Mellau; (f) MNHW_F.15.14_13; France, Source de la Moselle n. Bussang; (g) MNHW_S.15.20_18; Switzerland, Gorges de Court 4; (h) MNHW_F.VM_294; France, Vallon du Moulinot.

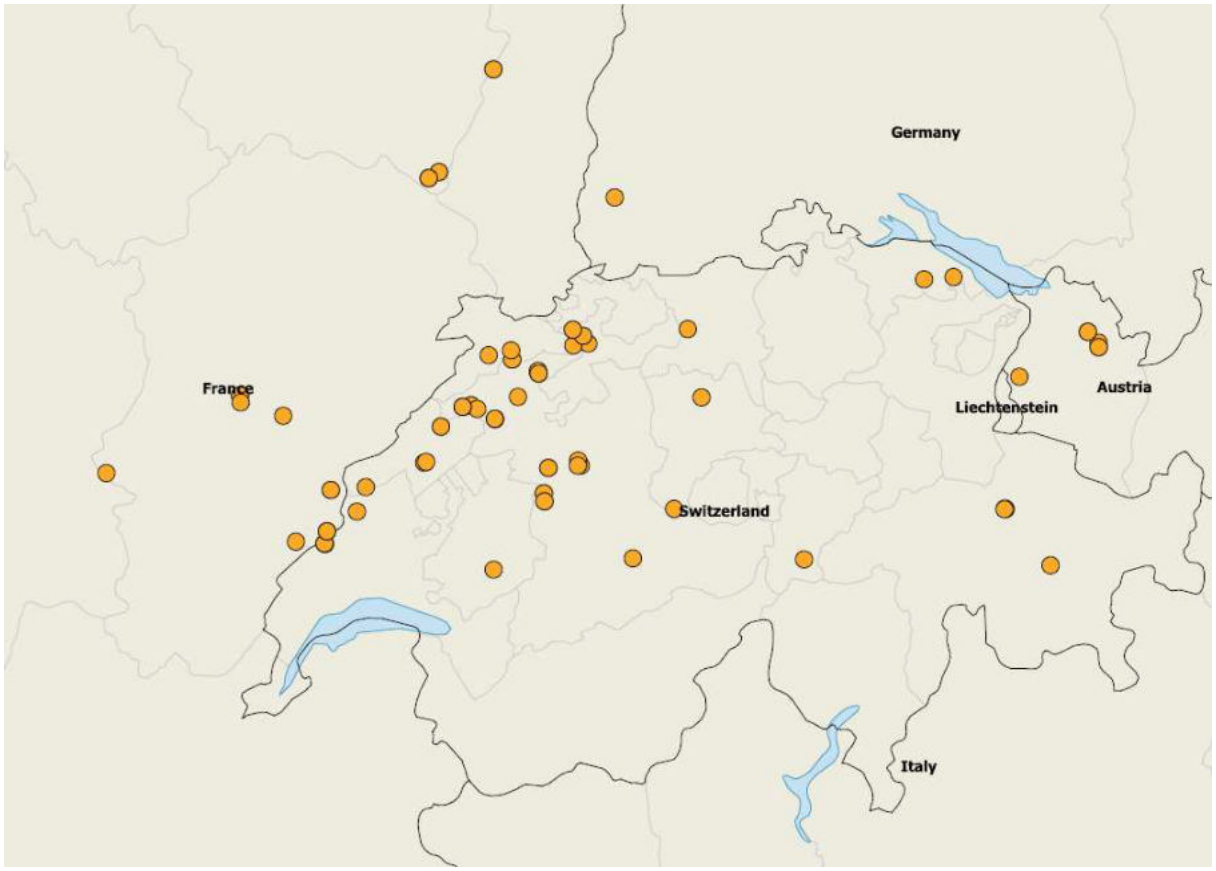



**Figure 17**: Distribution map of the investigated *T. clandestinus* specimens.

## *Trochulus plebicola* (Locard, 1888) [clade04] Figs 18a-c

1888 *Helix plebicola* Locard, Ann. Soc. Linn. Lyon, (N.S.) 34 ["1887"]: 366-368. Type locality: "Tenay, le Colombier, dans l'Ain; les alluvions du Rhône au nord de Lyon; Évian, dans la Haute-Savoie; Bief-du-Fourg, dans le Jura; l'Aube.

Type specimens: MNHN XXXXXX (D = 9.35 mm), coll. Locard. We herewith select Tenay (Ain) as type locality for *H. plebicola*. All our specimens originate from localities around Tenay in Dept. Ain and thus are considered to constitute topotypes.

*Trochulus plebicola* has a pale brown shiny shell without hairs. The shell is globular and finely striated. Shell width of the two investigated specimens are 9.19 mm and 9.89 mm and shell height 6.23 mm respectively 6.75 mm. All measurements can be found in the appendix (supplementary material II). Adult specimens have six whorls. The umbilicus is narrow and open. The body is greyish.

Distribution (Fig. 14): *Trochulus plebicola* has been recorded from three localities in a very small area in the southwestern French Jura. However, this may be a collection bias as we have no records of *Trochulus* specimens between Lyon and Aix-les-Bains.

Remark: We have selected Tenay as type locality of this species. The other places recorded by Locard are unprecise or based on confusion with similar species.

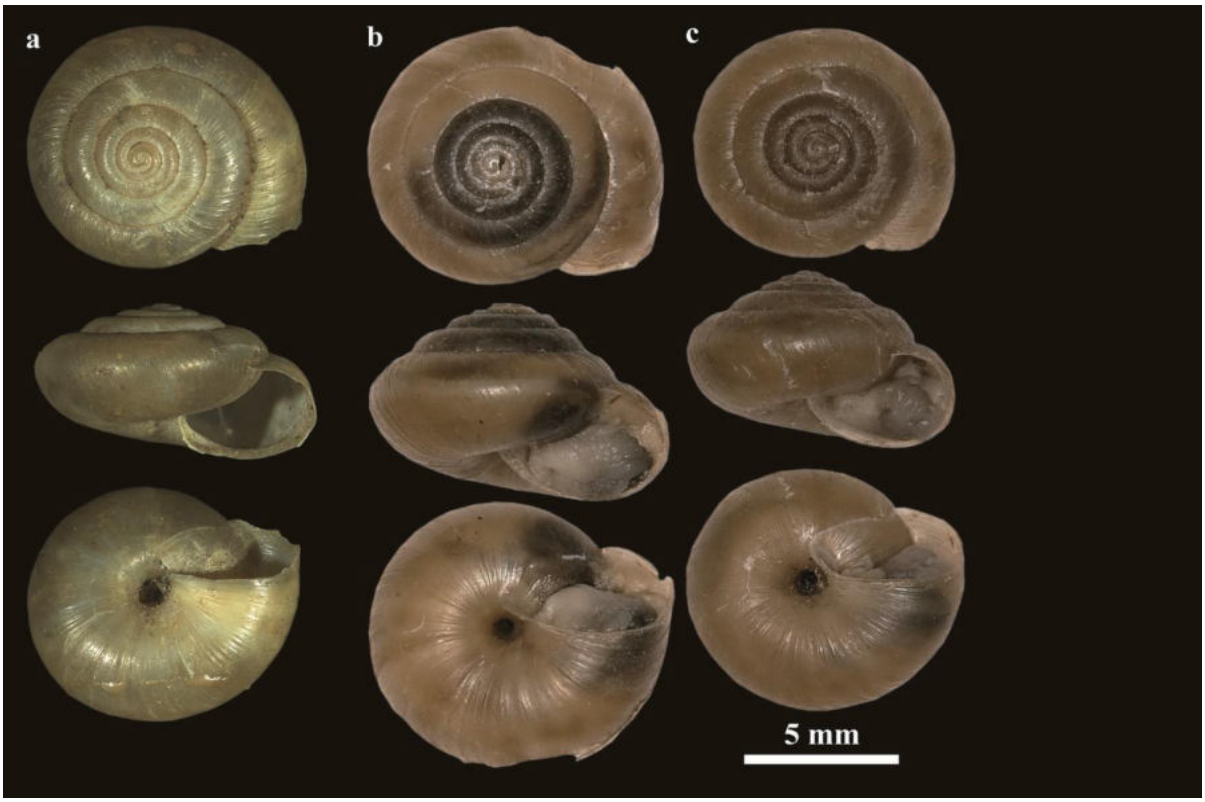



**Figure 18**: *Trochulus plebicola*. Clade04; (a) MNHN XXXXXX; France, Tenay; (b) MNHW_F.15.42a_03; France, Arandas; (c) MNHW_F.15.42a_04; France, Arandas.

## *Trochulus pascali* (Mabille, 1867) [clade05] Figs 19a-h

1867 *Helix pascali* Mabille, Arch. malac., fasc. 2: 29–30 [Cette espèce habite les lieux frais et montreux de l'est de la France; nous l'avons reçu de Saint-Amour (Jura) et de Bellegarde (Ain)].

1868 *Helix submontana* Mabille, 1868, Rev. Mag. Zool.: (2) 20 (1): 22-23 [Saint-Amour (Jura) et de Bellegarde (Ain); published as *Helix pascali* by Mabille in Arch. malac., fasc. 2: 29–30 (1867)].

1882 *Helix silanica* Bourguignat in Locard, 1882, Prodrome de malacologie française. [I]. Catalogue général des Mollusques vivants de France. Mollusques terrestres, des eaux douces et des eaux saumâtres: 314 [Lac de Silan près de Nantua].

Type specimens: Lectotype MNHN [to be completed].

Description: The specimens of *T. pascali* have a brown shell with fine strips. Some of the specimens have short, straight hairs. The shell is roundish-conical and contains 5–6 whorls. Shell width of the investigated specimens range from 7.49–10.97 mm and shell height from 4.07-7.19 mm (N = 10). All measurements can be found in the appendix (supplementary material II). The umbilicus is narrower compared to the species *T. circinnatus*, *T. montanus* and *T. clandestinus*. The body is pale grey to dark grey.

Distribution (Fig. 14): Most records of this species come from the Haute Jura in France and the neighbouring area in Switzerland. This species is also recorded from the Central Swiss area from Bern to Lausanne but is probably introduced there.

Remark: We herewith select the specimen MNHN XXXXX as lectotype. Our sampling shows that the species lives in partial sympatry with *T. montanus, T. circinnatus, T. villosus*, and *T. plebeius*.

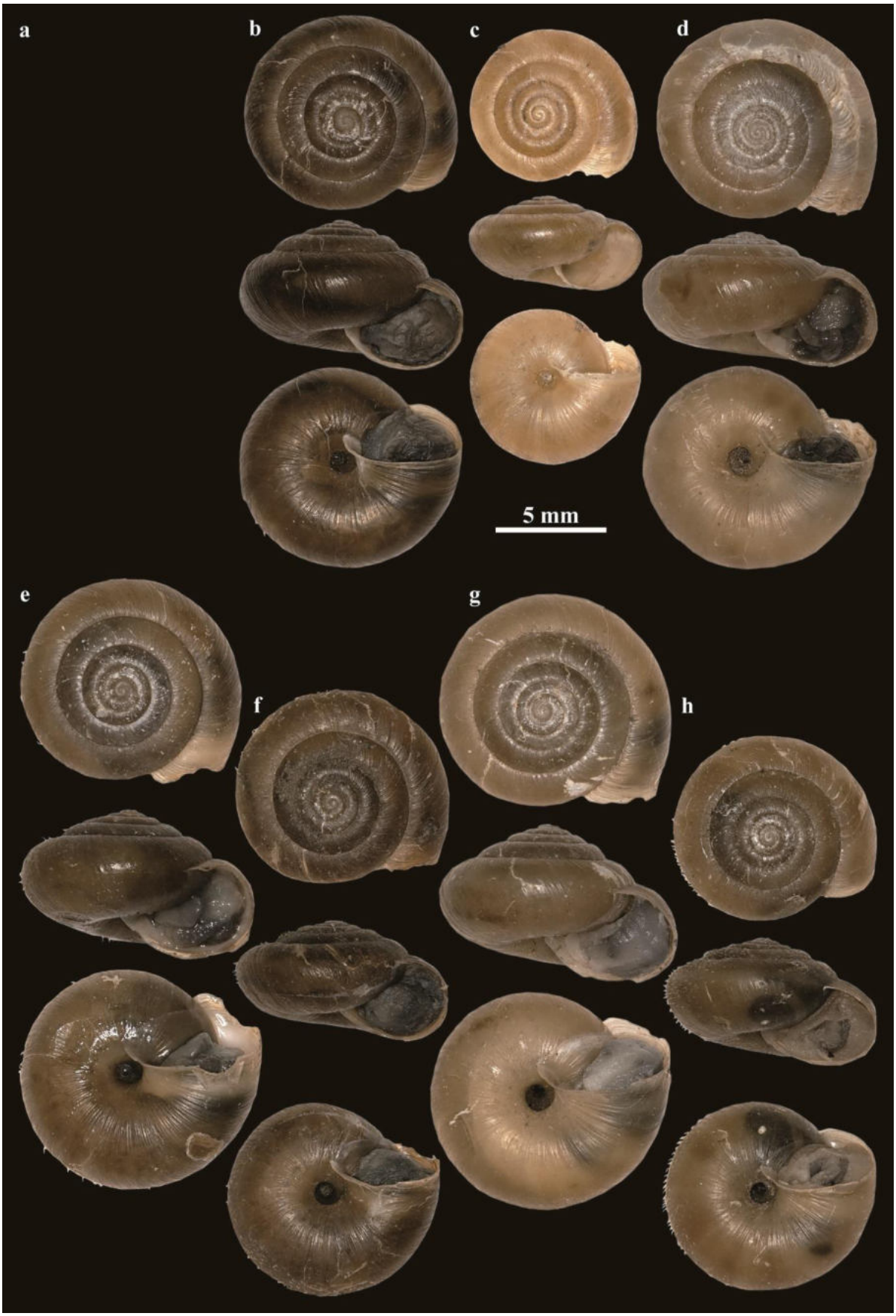

a
b
c
d
5 mm
e
f
g
h

**Figure 19**: *Trochulus pascali*. Clade05; (a) TYPE?; (b) MNHW_F.15.33_06; France, Les Rousses-d'Amont; (c) MNHW_F.Col_228; France, Col. De Vac.; (d) MNHW_F.15.38_05; France, Chaux-des-Crotenay; (e) MNHW_F.15.39_01; France, Cascades du Hérisson; (f) MNHW_F.15.47_05; France, Jalinard; (g) MNHW_F.15.39a_01; France, Cascades du Hérisson; (h) MNHW_F.15.47_04; France, Jalinard.

## *Trochulus caelatus* (S. Studer, 1820) [clade06] Figs 20a-c

1820 *Glischrus* (*Helix*) *caelata* S. Studer, 1820, Syst. Verz. Schweizer-Conchylien: 12 ["Auf dem Jura, in Wäldern, an feuchten Felsen"; Lectotype selected by Forcart (1957: 194), who also restricted the type locality based on a manuscript of Studer: "Kt. Bern, Birstal unterhalb Moûtier, an Felsen"]

1821 *Helix coelata* by Hartmann, in: Steinmüller, Neue Alpina, 1: 236, pl. 2 fig. 12 ("in den Gebirgen des Berneroberlandes und auf dem Jura") misspelling, non *Helix coelata* Vallot, 1801].

Type specimen: Lectotype NMBE 15419 (D = 8.2 mm).

*Trochulus caelatus* is characterised by its strongly flattened, discoidal shell, the wide, open umbilicus and the absence of hairs. The shell is pale brown to greyish with fine strips. Shell width of the two investigated specimens is 7.8 mm and 8.93 mm and shell width 3.67 mm respectively 4.52 mm. The diameter of the type specimen is 8.2 mm. All measurements can be found in the appendix (supplementary material II). The body whorl height accounts for about 90% of the shell height. The last whorl is clearly separated in width from the previous whorls. The body is black.

Distribution (Fig. 21): *Trochulus caelatus* is an endemic species in Switzerland only known around Moutier in the canton Jura and Solothurn.

Habitat: The species lives in shaded limestone habitats (Proćków 2009).

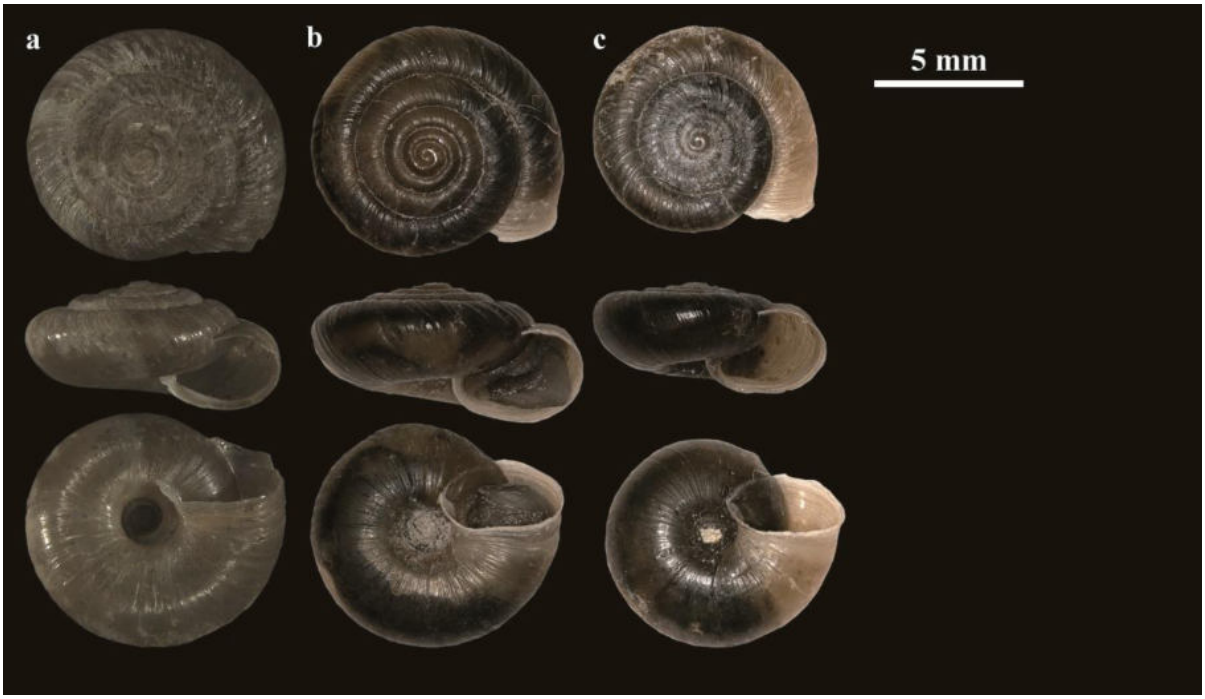


**Figure 20**: *Trochulus caelatus*. Clade06; (a) Lectotype NMBE 15419; Switzerland, «Auf dem Jura»; (b) MNHW_S.15.21_01; Switzerland, Gorges de Moutier; (c) MNHW_S.15.21_02; Switzerland, Gorges de Moutier.

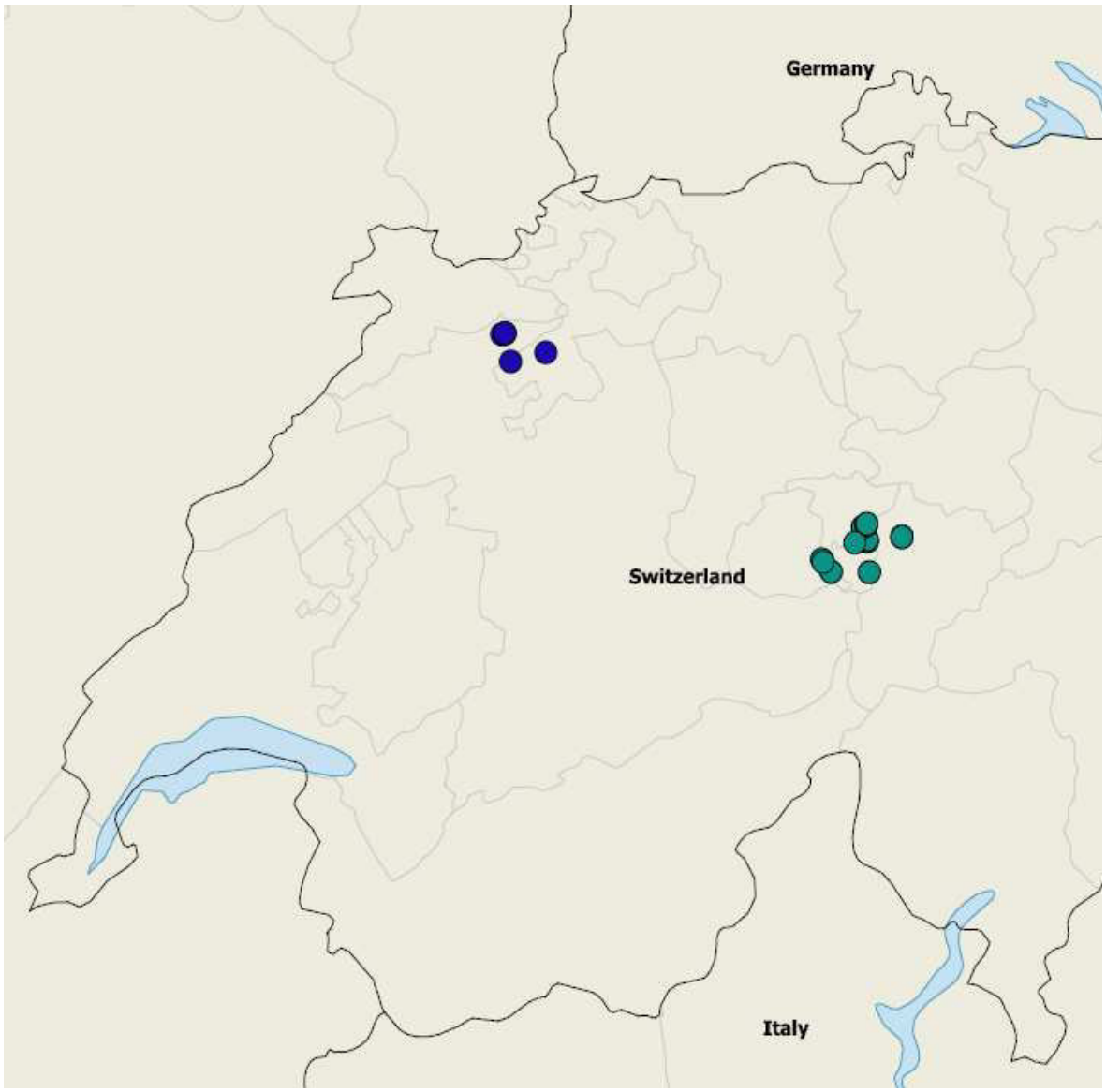



**Figure 21**: Distribution map of the investigated *T. caelatus* (dark blue circles) and *R. biconica* (turquoise circles).

## *Trochulus sericeus* (O. F. Müller, 1774) [clade07] Figs 22a-f

1774 *Helix sericea* O. F. Müller, 1774, Verm. terr. fluv.: 2: 62–63 [“agri Fridrichsdalensis, sue Daniae”].

1871 *Helix* [*Trichia*] *liberta* Westerlund, Fauna moll. terr. fluv. Sveciae Norvegiae Daniae: 1: 83, 139–140 [prov. Skåne: near Lund].

1874 *Helix* [*Fruticiola*] *septentrionalis* Clessin, Jahrb. dtsch. malakozool. Ges.: 1 (4): 311–312 [Pyrmont, (Norddeutschland), Fredricksdal (Dänemark), Bolteberga, Kinnekulle und Skane (in Schweden), Corbach, Cöln, Breidenstein, Sternberg bei Lemgo (Deutschland); described as having a rather narrow umbilicus].

Type specimens: Syntype *Helix sericea* ZMUC-GAS-670. This type specimen cannot be the original specimen of Müller because it is a juvenile shell of *Monachoides incarnatus* (O. F. Müller, 1774). In his description, Müller talks about the hairs on the shell: “Testa cornei transparentis coloris, aliquantum carinata; pelluciditas carinae major fasciam pallidam mentitur; tota resta tomento minutissimo obtegitur, hoc tamen oculo armato & certo situ, quovis pilo resplendente, tantum conspicitur“ which translates to “Shell of transparent horn colour, somewhat keeled; the transparency of the keel resembles a bright band; the rest of the [shell] is covered with a fine felt, but this is visible only with the armed eye and when the [shell] is in a fixed position, on which each hair shines brightly”. This proves that Müller’s shell was haired, so ZMUC-GAS-670 cannot be the type. Additional research in Müller’s (and Fabricius’ collection, who bought the collection of Müller) surfaced no specimens that could be selected as lectotypes (Tom Schiøtte, pers. comm. Dec. 2021).

For almost a century or longer, the name was attributed to Draparnaud as taxonomic author. However, reading his text it becomes clear that it is an almost verbatim copy of Müller’s text. Certainly, Draparnaud never intended to describe a new taxon; he also does not specify where this species should be living in France.

To stabilize the application of this widely used name, we herewith select the sequenced topotype specimen NMBE 571112 (D = 7.13 mm) as neotype of *Helix sericea* O. F. Müller, 1774.

Description: The sequenced specimens of *T. sericeus* have a brown to greyish, shiny shell. Most of the shells are covered by short, straight hairs. The shell is rather globular with a narrow umbilicus. Shell width ranges from 7.13–8.74 mm and shell height from 4.26–5.51 mm (N = 6). All measurements can be found in the appendix (supplementary material II). The body is pale grey with dark patches.

Distribution (Fig. 23): *Trochulus sericeus* is found in Denmark (Fredriksdal), at the Baltic Sea, in Saxon Switzerland, southern Germany (Black Forest and Bavaria), eastern Switzerland, Tyrol, Austria and Hungary.

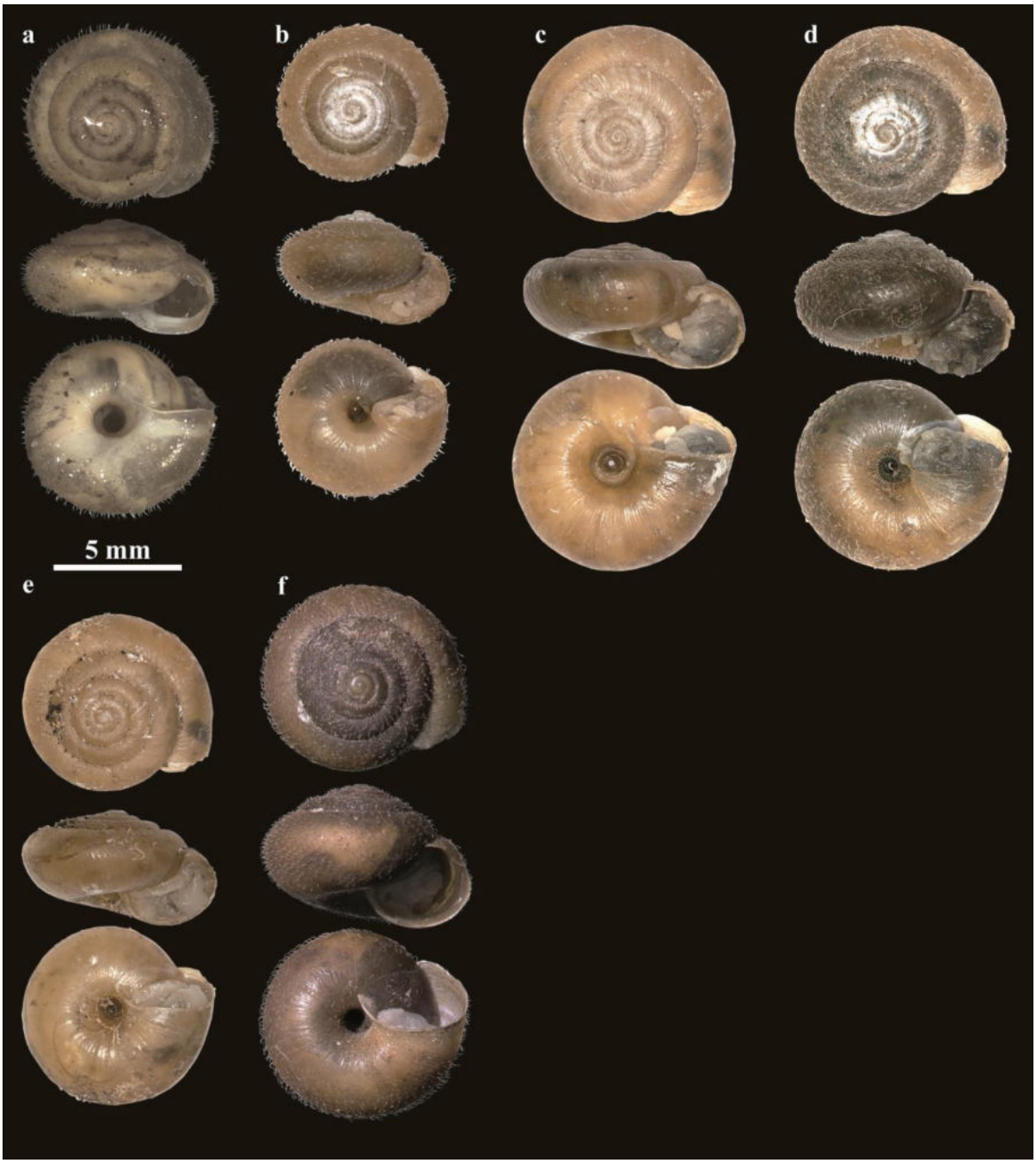



**Figure 22**: *Trochulus sericeus*. Clade07; (a) topotype NMBE 571112; Denmark, Frederiksdal, Furesø; (b) MNHW_A.Gens_201; Austria, Genabend-Sibratsgfäll; (c) MNHW_G.17.05_298; Germany, Pfatter; (d) MNHW_G.17.17_228; Germany, Thannlehen b. Berchtesgaden; (e) MNHW_G.Ott_227; Germany, Ottendorf; (f) MNHW_Re5; Germany, Reichenau.

**Figure 23**: Distribution map of the investigated *T. sericeus* specimens.

## *Trochulus albulus* (S. Studer, 1820) [clade08] Figs 24a-h

1820 *Glischrus* (*Helix*) *albula* Studer, Syst. Verz. Schweizer-Conchylien: 12 [“Ich fand es an schattigen Rainen um Bern”; Lectotype selected by FORCART (1957: 194)].

1821 *Helix charpentieri* W. Hartmann, Neue Alpina: 1: 236 [“bey St. Maurice in Wallis” (ex. Charpentier)].

1821 *Helix glabra* W. Hartmann, Neue Alpina: 1: 238 [“Ich fand sie bey St. Gallen und erhielt sie auch von Bex und aus der Gegend von Joerdon”; junior homonym of *Helix glabra* Gmelin, 1790 In: Linnaeus, Systema Naturae, ed. 13, I: 3658].

1821 *Helix similis* W. Hartmann, Neue Alpina: 1: 237 [in der ganzen Schweiz].

1842 *Trichia erecta* W. Hartmann, Erd- und Süssw.-Gaster. Schweiz: 5: 129 [“Neuwied”; “kommt in dem Jura des Kantons Waadt in der Schweiz, besonders in Val de Joux vor”; “Wien”; “England”].

1855 *Helix pratensis* Dumont, Bull. Inst. natn. genevois: 3: 355 [Sur le sommet des montagnes nues, comme au Méry …. dans la région des gazons; junior homonym of *Helix pratensis* L. Pfeiffer, Proc. Zool. Soc. London, XIII (153) Feb. 1846, 132].

1864 *Helix phorochaetia* Bourguignat, Malac. Grande-Chartreuse: 52-54, pl. 6 figs 9-14 [le long des sentiers de Saint-Bruno et de Chartreusette].

1882 *Helix vocoutiana* Bourguignat in Locard, Prodr. malac. française: 1: 76, 317 [La Salette près Corps, Isère].

2005 *Trochulus piccardi* Pfenninger & Pfenninger, Archiv für Molluskenkunde: 134 (2): 264-268, figs 2-5 [Chateau d’Oex, Hügel um die Kirche].

Type specimen: Lectotype NMBE 15461 (D = 7.3 mm); Switzerland, “Um Bern”

Description: *Trochulus albulus* is characterized by a globular shell with short, slightly curved hair. The shell of the type specimen lost its hairs, but hair scars are still visible. Shell width ranges from 6.37–8.91 mm and shell height from 3.95–5.92 mm (N = 21). All measurements can be found in the appendix (supplementary material II). The shell contains 4.75–6 whorls and a narrow umbilicus, which is slightly covered by the bent aperture. Some of the specimens have a strong callus. The body is pale grey with dark spots.

Distribution (Fig. 25): *Trochulus albulus* is found everywhere in Switzerland except in Ticino. The species is further found in Austria (Vorarlberg), south-western Germany as far as Frankfurt am Main and western France with its southern most record from the district of Aveyron.


**Figure 24**: *Trochulus albulus*. Clade08; (a) Lectotype NMBE 15461; Switzerland, “Um Bern”; (b) MNHW_G.15.05_285; Germany, Ketsch; (c) MNHW_S.15.19_11; Switzerland, Gorges de Court 3; (d) MNHW_Sau1; Austria, Sausteig; (e) NMBE 569689; Switzerland, Stansstad; (f) NMBE 569677; Switzerland, Schwarzwasser; (g) NMBE 571095; Switzerland, Grimentz; (h) MNHN-IM-2013-76922; France, Saint-Laurent-du-Pont.

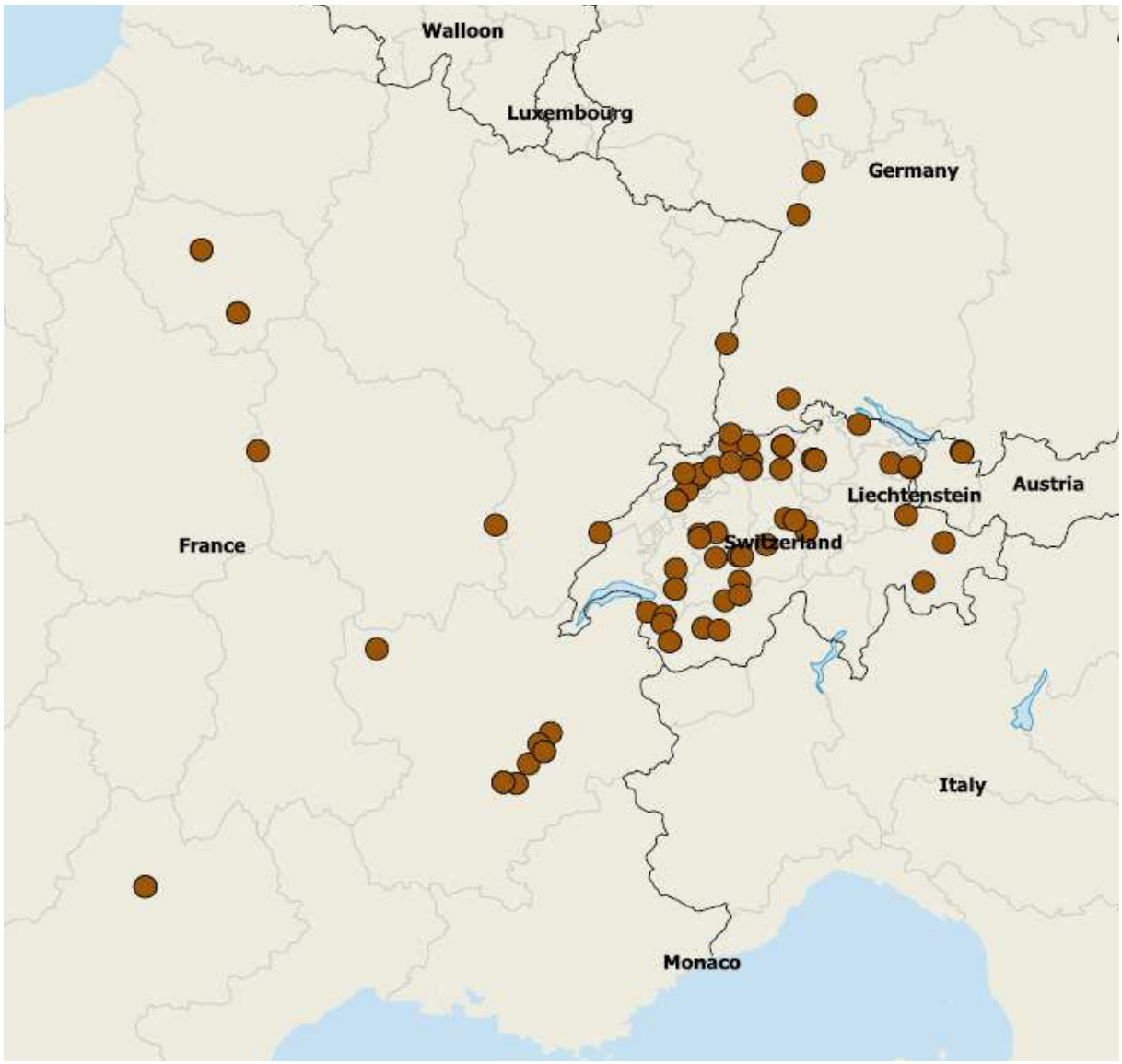



**Figure 25**: Distribution map of the investigated *T. albulus* specimens.

## *Trochulus dubia* (Clessin, 1874) [clade09] Figs 26a-c

1874 *Helix* [*Fruticiola*] *dubia* Clessin, Jahrb. dtsch. malakozool. Ges.: 1 (4): 327–328, pl. 13 fig. 1 [Sillschlucht bei Innsbruck].

1874 *Helix* [*Fruticiola*] *corneola* Clessin, Jahrb. dtsch. malakozool. Ges.: 1 (4): 326–327, pl. 13 fig. 2 [Eibsee].

1874 *Helix* [*Fruticiola*] *expansa* Clessin, Jahrb. dtsch. malakozool. Ges.: 1 (4): 326, pl. 12 fig. 5 [Dietramszell].

Type specimen: [not yet investigated].

The investigated specimens of *T. dubia* have a globular shell with short, straight hairs. The umbilicus is wide and open. The inner whorls are not clearly visible. Shell width ranges from

6.92–7.73 mm and shell height from 4.18–4.98 mm (N = 3). All measurements can be found in the appendix (supplementary material II). The body is pale grey with dark spots.

Distribution (Fig. 27): *Trochulus dubia* is found in Bavaria along the river Danube, in western Austria. Single records were made in Potsdam (Germany), Carmel (UK), and Lussat (Central France).

Remark: the denomination of *dubia* Clessin, 1874 is based on a topotypic sequence by Kruckenhauser et al. (2014) from near Innsbruck.

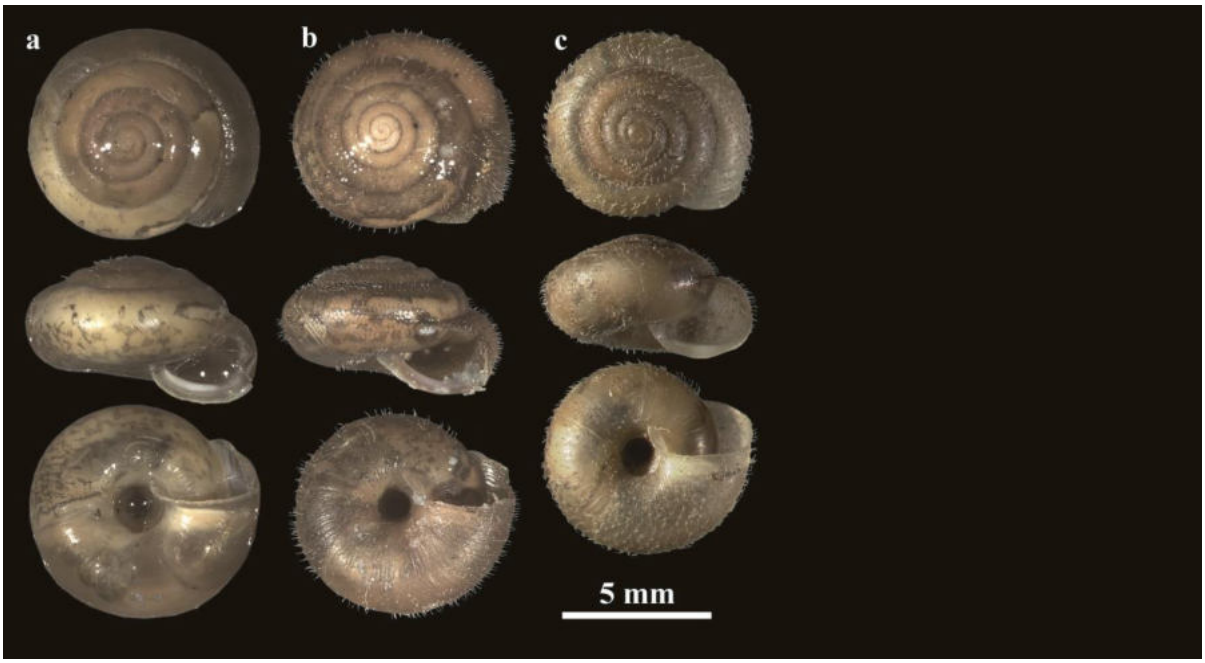



**Figure 26**: *Trochulus dubia* (Clessin, 1874). Clade09; (a) NMBE 571108; Germany, Diessen am Ammersee; (b) NMBE 571125; United Kingdom, Carmel; (c) MNHW_G-Mo5; Germany, Moosburg.

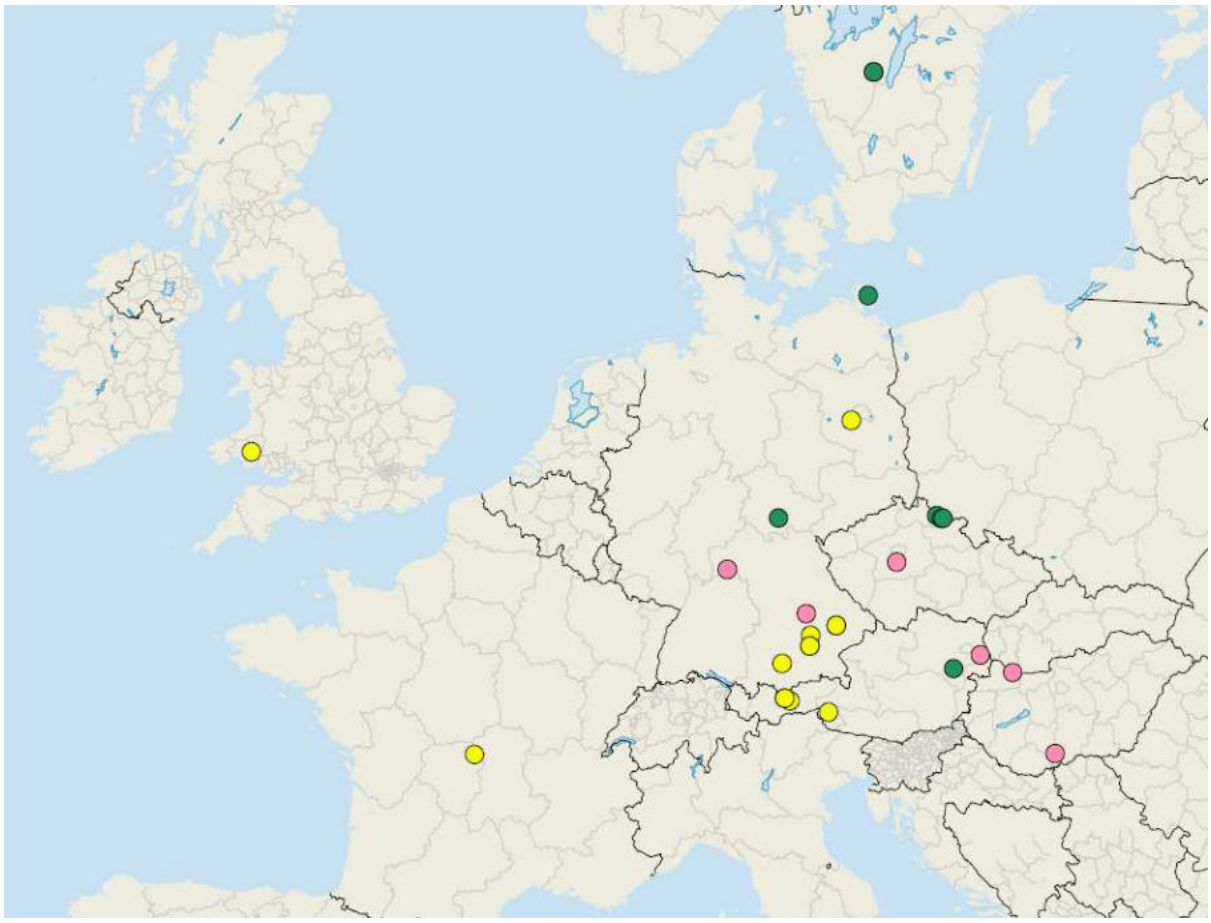


**Figure 27**: Distribution map of the investigated *T. dubia* (yellow circles), *T.* sp. 1 (light pink circles) and *T.* sp. 2 (forest green circles) specimens.

## *Trochulus hispidus* (Linnaeus, 1758) [clade10] Figs 28a-h

1758 *Helix hispida* Linnaeus, Systema Naturae: 10: 771 ["Svecia"].

1840 *Helix pallida* Picard, Bull. Soc. linn. Nord France: 1 (3): 228 [type locality not mentioned; junior homonym of *Helix pallida* E. Donovan, 1803].

1841 *Helix conoidea* J. Brown, Ann. Mag. nat. Hist.: (1) 7 (45): 428, 429, pl. 2 fig. 4 [“beds which compose the fluvio-marine deposit at Clacton, on the eastern coast of Essex”; junior homonym of *Helix conoidea* Draparnaud, 1801].

1856 *Helix* [*Zenobia*] *subrufa* Moquin-Tandon, Hist. nat. Moll. terr. fluv. France: 2 (5): 224 [type locality not mentioned; refers to Draparnaud, 1805, Hist. nat. moll. terr. fluv. France: 104 sub *Helix hispida* var. β and Picard, 1840, Bull. Soc. linn. Nord France, 1 (3): 228 sub *Helix hispida* var. minor].

1856 *Helix* [*Zenobia*] *rufescens* var. *rubens* Moquin-Tandon, Hist. nat. Moll. terr. fluv. France: 2 (5): 206, 207 [environs de Boulogne].

1862 *Helix conica* Jeffreys, Brit. Conch.: 1: 199 [“At the roots of *Rosa spinosissima* on the sand-hills near Swansea”; junior homonym of *Helix conica* Draparnaud, 1801].

1862 *Helix conica* Baudon, Mém. Soc. acad. Archéol. Sci. Arts Dépt. Oise: 5: 192 [“Prairie d’Hondainville”; junior homonym of *Helix conica* Draparnaud, 1801].

1862 *Helix decora* Baudon, Mém. Soc. acad. Archéol. Sci. Arts Dépt. Oise: 5: 191 [dans la forêt de Hez, près de La Garde; junior homonym of Helix decora, Ziegler in E. A. Rossmaessler, 1837].

1862 *Helix nana* Jeffreys, Brit. Conch.: 1: 199 [Freshwater, Isle of Wight; junior homonym of *Helix nana* Pennant, 1777].

1862 *Helix nitida* Baudon, Mém. Soc. acad. Archéol. Sci. Arts Dépt. Oise: 5: 191 [Terrain marécageux des bois de Fourneau à Mouy; junior homonym of *Helix nitida* O. F. Müller, 1774].

1862 *Helix pratensis* Baudon, Mém. Soc. acad. Archéol. Sci. Arts Dépt. Oise: 5: 192 [type locality not mentioned; junior homonym of *Helix pratensis* L. Pfeiffer, 1846].

1877 *Helix bellovacina* Mabille, Bull. Soc. zool. France: 2 (3/4): 305 [in humidis silvisque pagi Compendiaci Galliae].

Type specimens: According to Forcart (1966), the type specimens of *H. hispida* are lost. We consider our specimens from the garden of Linnaeus in Uppsala as topotypes of this taxon. In order to stabilize the application of this name, we herewith designate NMBE 568100 as neotype for *H. hispida*.

Description: *Trochulus hispidus* has a pale brown, shiny shell with short, straight hairs. The shell is roundish-conical with an aperture that is slightly less than half of the shell width. The shell contains fine strips. Shell width of the investigated specimens ranges from 6.72–8.82 mm and shell height from 3.88–5.58 mm (N = 10). All measurements are in the appendix (supplementary material II). The specimens have 5–5.5 whorls. The body pale brown with black spots.

Distribution (Fig. 29): *Trochulus hispidus* is found in southern Sweden with northernmost occurrence at the type locality in Uppsala, in Ireland, at the northern tip of France and in Burgundy near Dijon.

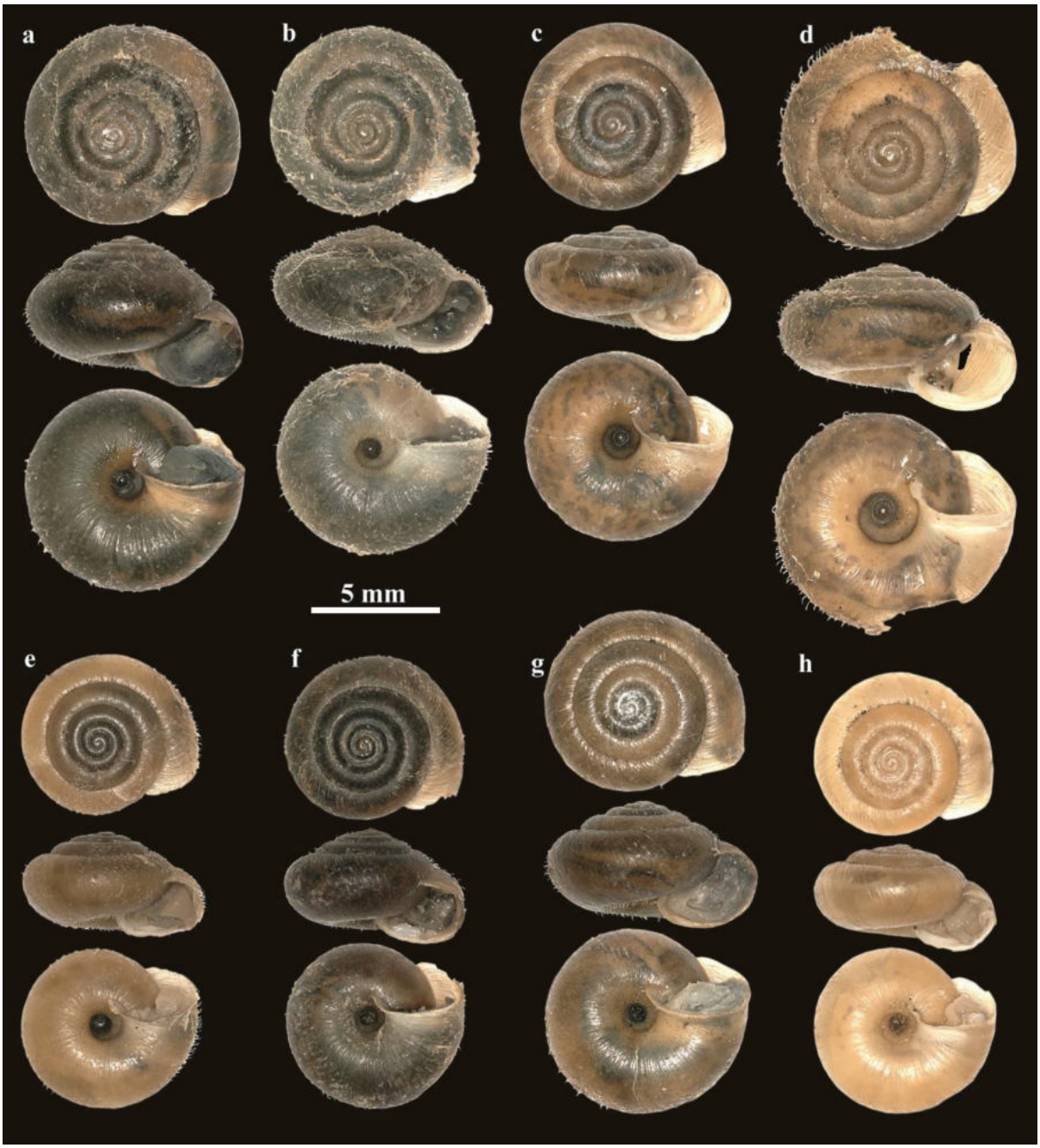



**Figure 28**: *Trochulus hispidus*. Clade10; (a) MNHW_F.18.05_277; France, Fouquenies; (b) MNHW_F.18.08_221; France, Eaucourt-Sur-Somme; (c) MNHW_I.16.10_232; Ireland, Bealaclugga; (d) MNHW_I.16.3_229; Ireland, Monastery; (e) MNHW_F.18.24_223; France, Le Mont Gournoy 2 (Forêt Indivise d'Eu); (f) MNHW_F.18.14_225; France, Larré; (g) MNHW_F.18.25_279; France, Escames; (h) MNHW_I.16.4_230; Ireland, Rock of Cashel.

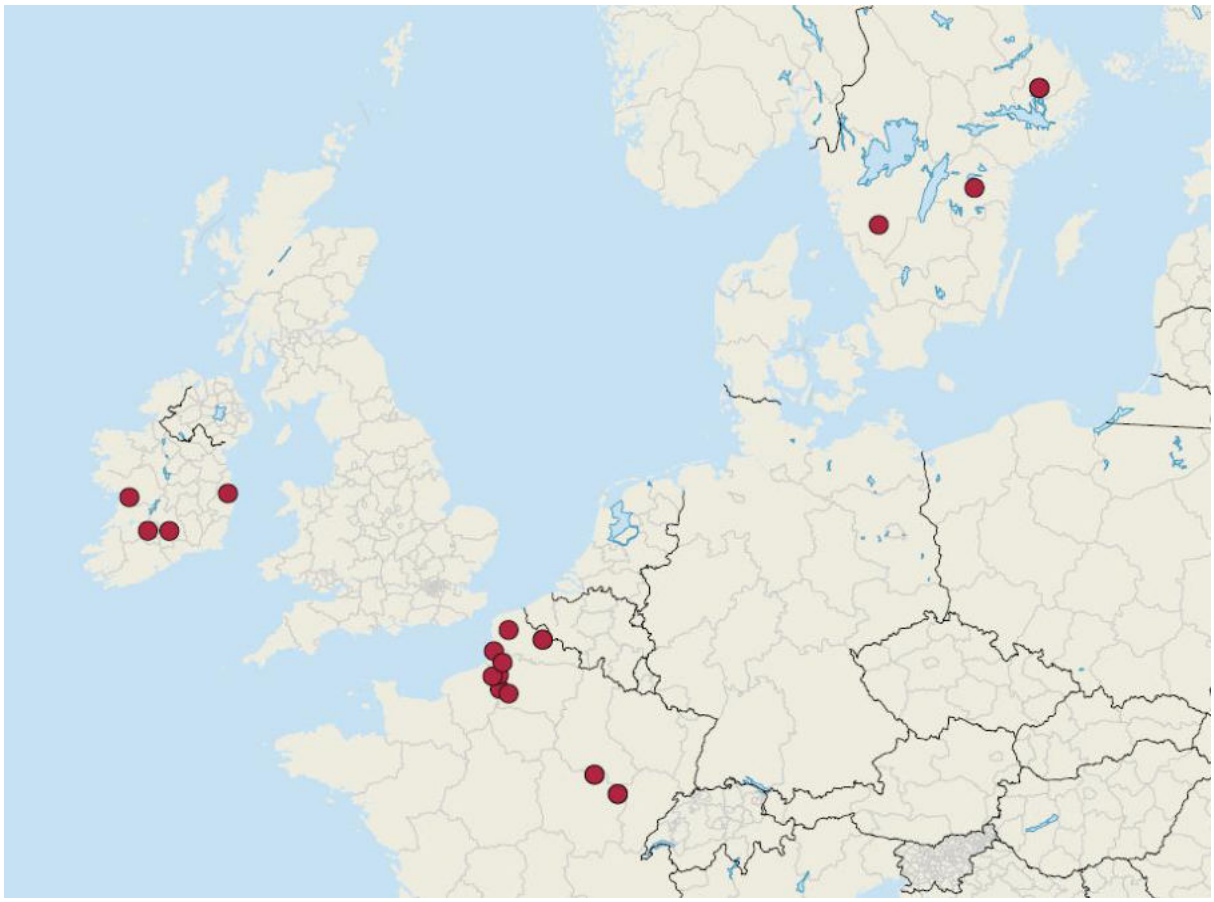


**Figure 29**: Distribution map of the investigated *T. hispidus* specimens.

## *Trochulus villersii* (De Malzine, 1867) [clade11] Figs 30a-h

1867 *Helix villersii* De Malzine, Essai fauna malac. Belgique: 74 [L'abbaye de Villers].

1868 *Helix fontainei* Colbeau, 1868, Mém. Soc. malac. Belgique: 1: 33, 77 [Papignies, dans les prairies aux bords de la Dendre].

1872 *Helix conoidea* Van den Broeck, Bull. Soc. Malac. Belgique: 6 ["1871"]: XLIV [dans un fossé à Sluys-Kill; junior homonym of *Helix conoidea* Draparnaud, 1801].

1873 *Helix rosea* Van den Broeck, Ann. Soc. Malac. Belgique: 7: LXIII-LXIV [vallée de l'Hermeton].

1888 *Helix abludens* Locard, Ann. Soc. Linn. Lyon, (N.S.): 34 ["1887"]: 334-338 ["en Angleterre de l'île de Jersey; environs de Boulogne-sur-Mer, dans le Pas-de-Calais"; type locality restricted by Proækków et al. (2021): Boulogne-sur-Mer].

Type specimens: [to be completed].

The investigated specimens of *Trochulus villersii* have a pale brown, finely striated shell. The shell is roundish and contains short, slightly curved hairs. Shell width ranges from 6.83–9.4 mm and shell height from 3.99–5.85 mm (N = 21). All measurements are in the appendix (supplementary material II). The umbilicus is wide and open and the inner whorls are clearly visible. The body is pale brown with dark spots.

Distribution (Fig. 31): *Trochulus villersii* is widely distributed species with occurrence in southern Sweden, southern England, the Netherlands, Belgium, north-eastern France, northern Germany, Czech Republic, Poland, and Slovakia.

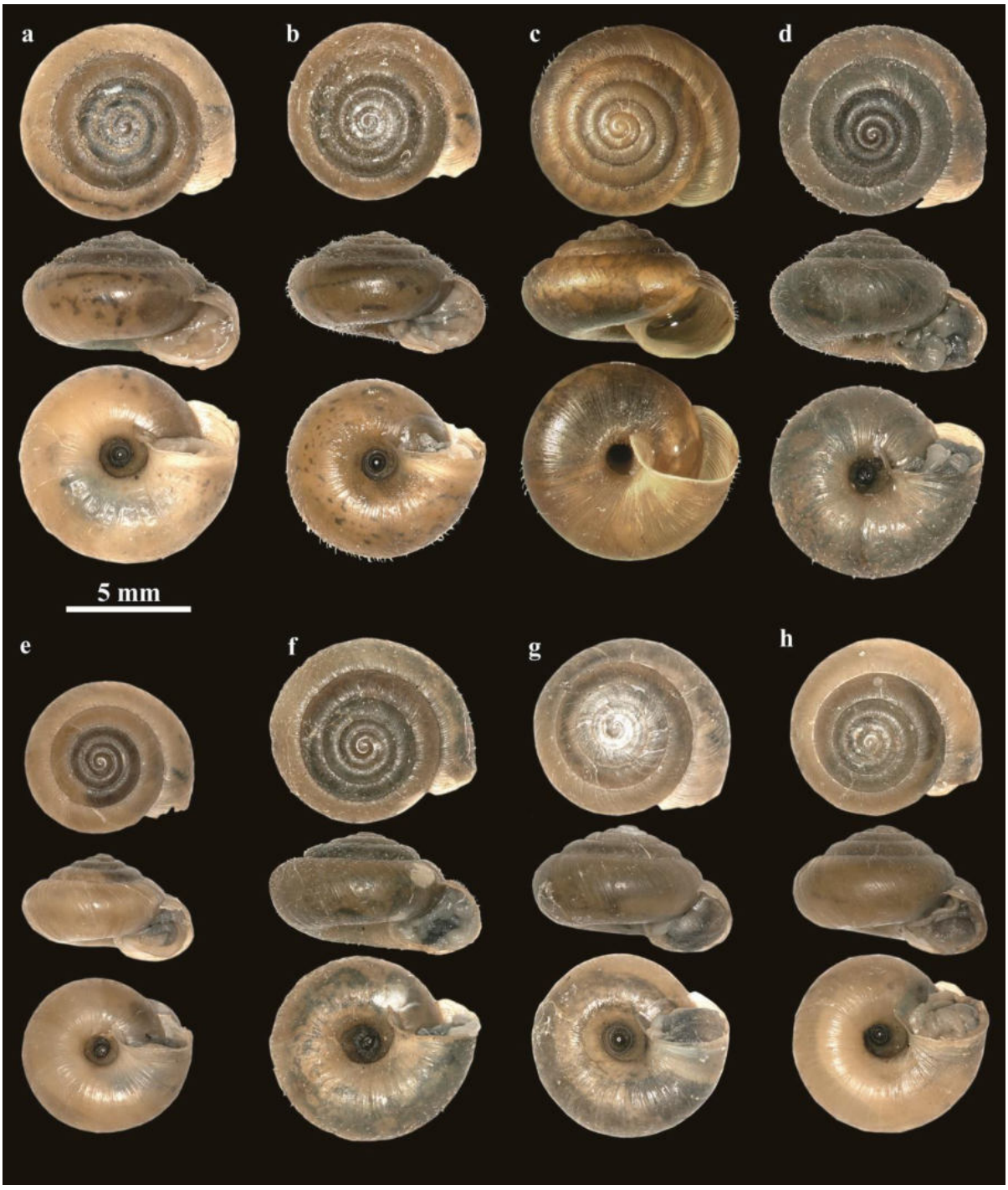



**Figure 30**: *Trochulus villersii*. Clade11; (a) MNHW_CZ.17.145_212; Czech Republic, Závist; (b) MNHW_CZ.19.43_216; Czech Republic, Debrník; (c) MNHW_UK.En-D_5; United Kingdom, Downside; (d) MNHW_NL.Ost_233; Netherlands, Oestgeest near Leiden; (e) MNHW_F.18.12_224; France, Licques; (f) MNHW_PL.19.10_250; Poland, Mątki near Sztum; (g) MNHW_PL.20.22_245; Poland, Stebnik; (h) MNHW_F.15.07_219; France, Bertrange.

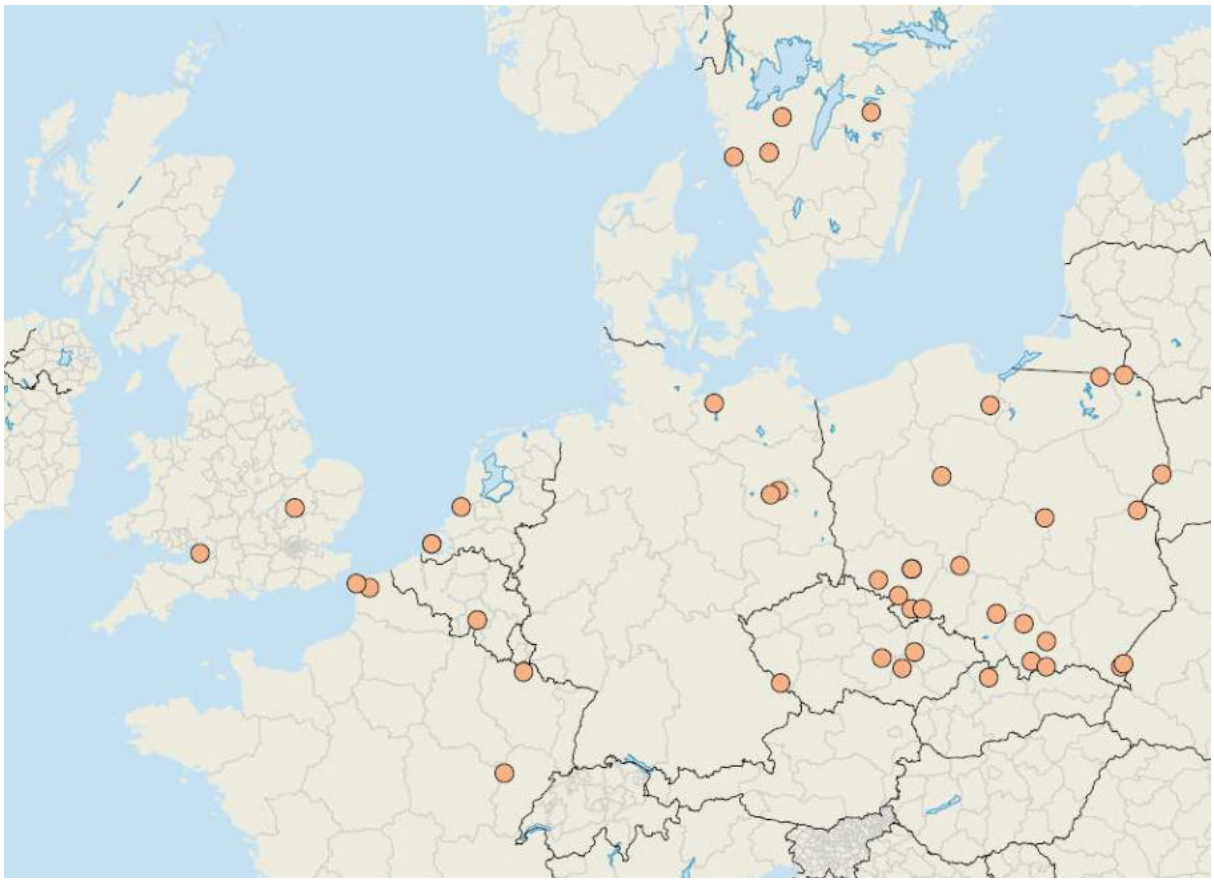


**Figure 31**: Distribution map of the investigated *T. villersii* specimens.

## *Trochulus* sp. 1 (sp. nov.?) [clade12] Figs 32a-c

Type specimen: [to be completed].

Description: The species is characterized by a pale brown, roundish-conical shell. Shell width of the investigated specimens ranges from 7.56–9.27 mm and shell height from 4.6–5.82 mm (N = 3). All measurements are in the appendix (supplementary material II). The umbilicus is wide and open. The body is beige to greyish with small dark dots.

Distribution (Fig. 27): The species is distributed in southern Germany, Czech Republic, eastern Austria, and Hungary.

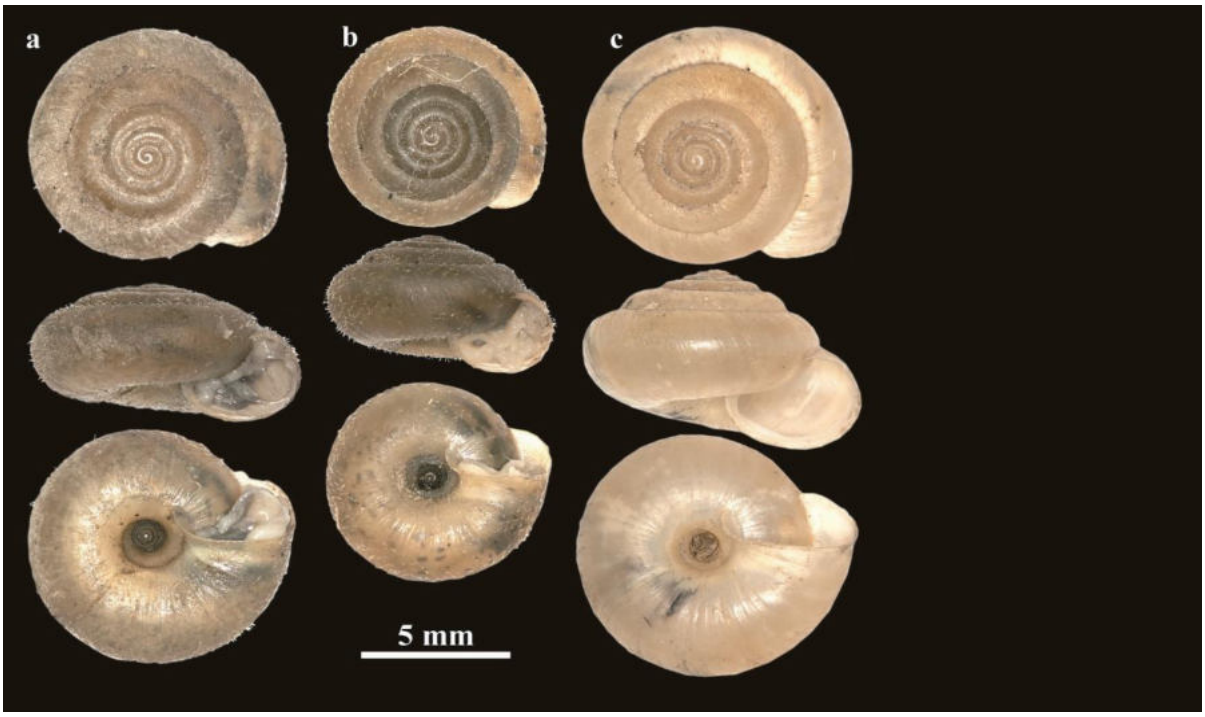



**Figure 32**: *Trochulus* sp. 1. Clade12; (a) MNHW_SK.19.62_261; Slovakia, Čičov (Čičovský les); (b) MNHW_CZ.19.38_214; Czech Republic, Štěchovice (Maják); (c) MNHW_St-14; Germany, Staubing.

## *Trochulus* sp. 2 (sp. nov.?) [clade13] Figs 33a-d

Type specimen: [to be completed].

Description: The species is characterized by a brown, roundish-conical shell. Shell width of the investigated specimens ranges from 6.87–7.3 mm and shell height from 3.65–4.92 mm (N = 4). All measurements are in the appendix (supplementary material). The umbilicus is narrow and slightly covered by the lip. The body is beige to greyish.

Distribution (Fig. 27): The species is found in southern Sweden, at the Baltic Sea and in Central Germany, in Czech Republic, and eastern Austria.

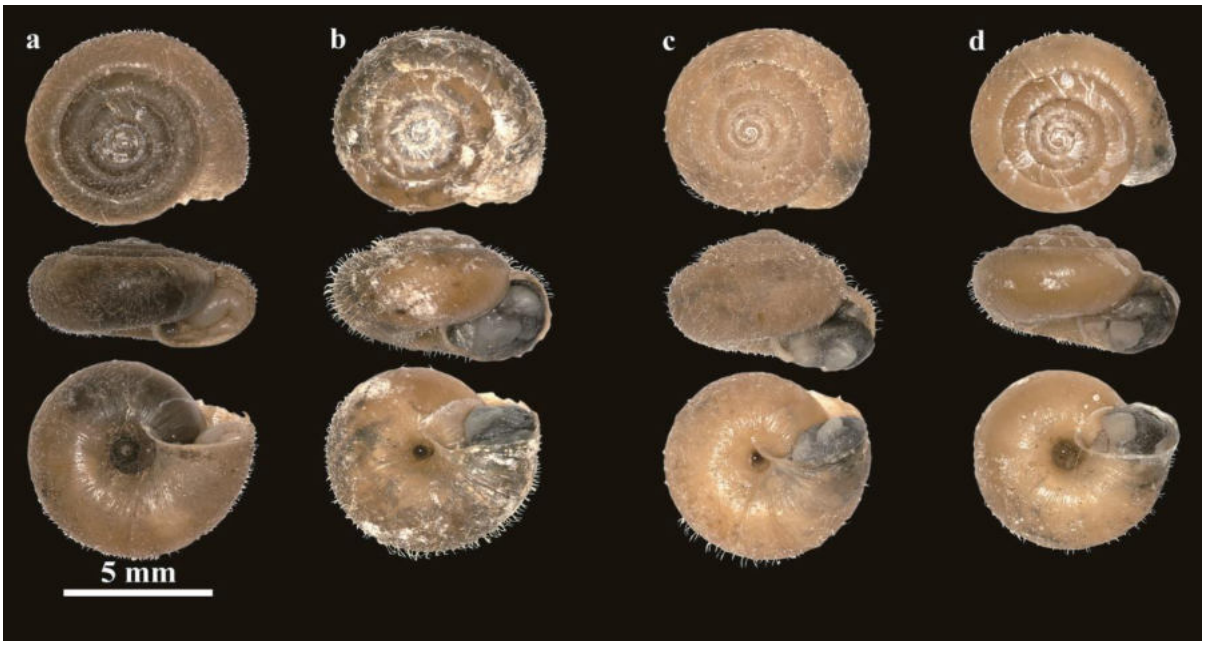



**Figure 33**: *Trochulus* sp. 2. Clade13; (a) MNHW_A.17.28_203; Austria, Durnbachtal; (b) MNHW_CZ.CD_272; Czech Republic, Černý Důl; (c) MNHW_CZ.Lan_273; Czech Republic, Lánov; (d) MNHW_CZ.Vit_274; Czech Republic, Vitkovice.

## *Trochulus striolatus* (C. Pfeiffer, 1828) [clade14] Figs 34a-f

1828 *Helix striolata* C. Pfeiffer, Naturgesch. dtsch. Land- u. Süßwass.-Moll., 3: 28, pl. VI, fig. 8 [“Bei Heidelberg in ebengelegenen Gärten” (ex Leukart)].

1830 *Helix concinna* Jeffreys, Trans. Linn. Soc. London: (1) 16 (2): 336–337 [“under stones & in dry places in the neighbourhood of Swansea .... and very plentyfully among the rejectamenta of the Avon river, near Bristol”; “Irish”; homonym of *Helix concinna* Dupuy, 1848

1841 *Helix rufina* L. Pfeiffer, Symbolae ad historiam Heliceorum: 1: 39 [Germania].

1842 *Trichia imminuata* W. Hartmann, Erd- und Süssw.-Gaster. Schweiz: 5: 130 [Stuttgart].

1842 *Helix complana* W. Hartmann, Erd- und Süssw.-Gaster. Schweiz: 5: 126, 130 [“Stuttgart”; “Neuwied”].

1871 *Helix* [*Trichia*] *gyratus* Westerlund, Fauna moll. terr. fluv. Sveciae Norvegiae Daniae: 1: 138 [prov. Skåne: near Lund].

1874 *Helix* [*Fruticiola*] *subcarinata* Clessin, Jahrb. dtsch. malakozool. Ges.: 1 (3): 181, 189, pl. 8 fig. 6 [Eberbach].

1874 *Helix* [*Fruticiola*] *danubialis* Clessin, Jahrb. dtsch. malakozool. Ges.: 1 (3): 184, pl. VIII, fig. 4 [Bayern, bei Dillingen].

1876 *Helix* [*Fruticiola*] *clessini* Weinland, Jahresh. Ver. Vaterl. Naturk. Württemberg: 32: 285 [Waldtrauf der Vöttelwiese [Wittlingen bei Bad Urach]].

1877 *Helix elaverana* Mabille, Bull. Soc. zool. France: 2 (3/4): 305 [Ivry près Paris].

1877 *Helix goosensis* Mabille, Bull. Soc. zool. France: 2 (3/4): 306 [in locis limpsis ad Firmitatem Adelahidis Galliae” [= La Ferté-Alais]].

1877 *Helix saporosa* Mabille, Bull. Soc. zool. France: 2 (3/4): 305–306 [In silvis pagi Suessoniensis Galliae].

1882 *Helix altenana* Locard, Prodr. malac. française: 79 [dep. Nord: vicinity of Lille; dep. Pas-de-Calais: Boulogne-sur-Mer; dep. Aube: Bar-sur-Seine; Replacement name for *Helix rufescens* sensu Drouët, 1855 (non Pennant); nomen nudum in Locard, 1882; nomen museorum in coll. Bourguignat Geneve].

1888 *Helix coelomphala* Locard, Ann. Soc. Linn. Lyon, (N.S.): 34 ["1887"]: 352–355 [Mt. Righi, Zurich, Lucerne and Jura neuchâtelois; Germany: Dillingen near Saarlouis, Günzburg, Dinkelsbühl; E France: dep. Savoie: environs of Annecy, Chambéry, Aix-les-Bains, Mouxy, Albertville; dep. Isère: vicinity of Genoble, Sassenage, Saint-Martin-le-Vinoux, Allevard-les-Bains, Grande-Chartreuse, Sappey, Voreppe; dep. Hautes-Alpes: Barcelonnette; dep. Rhône: environs de Lyon, alluvions de Rhône N Lyon, Brotteaux, Sathonay, Pape; dep. Ain: Laumusse, Bugey, alluvions of Suran; dep. Jura: Bief-du-Fourg, Saint-Claude, Poligny; dep. Côte-d'Or: Châtillon-sur-Seine; environs of Paris; Bionville near Metz; dep. Finistère: Brest].

1894 *Helix* (*Trichia*) *britannica* Westerlund, Nachrichtsbl. dtsch. malakozool. Ges.: 26 (9/10): 164–165 [London].

1894 *Helix rufescentella* Locard, Coq. terr. France: 128–129 [dep. Nord: Valenciennes and Lille].

1914 *Helix juvavensis* Geyer, Verh. k. k. zool.-bot. Ges. Wien: 65 (5/6): 276–278 [Salzburg, Schafberg].

Type specimens: [to be completed].

Description: *Trochulus striolatus* (clade14) is characterized by a pale brown, roundish-conical shell with coarse ribs. Adult shells are strong and have a strong white lip are They are usually without hairs. Shell width of the investigated specimens ranges from 7.99–11.90 mm and shell height from 4.54–7.3 mm (N = 9). All measurements can be found in the appendix (supplementary material II). The umbilicus is wide and open. The body is beige to greyish.

Distribution (Fig. 35): *Trochulus striolatus* is found Ireland, south England, Germany, northern Switzerland (Schaffhausen), north-east Austria, and Slovakia.

Habitat: The species prefers shaded and humid habitats, lives in forests, along roadsides or under scrubs (Proćków 2009; Proćków et al. 2014).

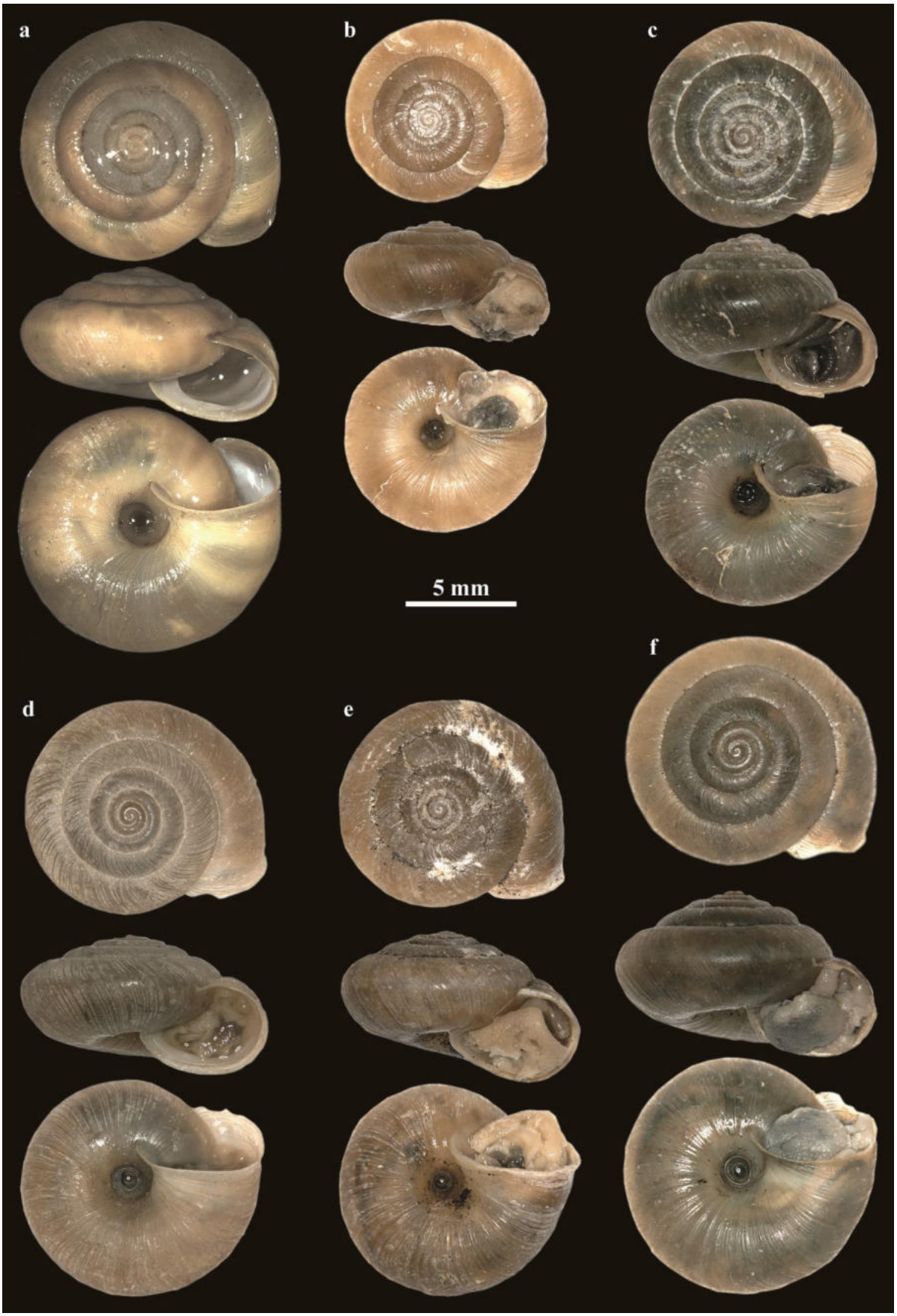

a
b
c
5 mm
d
e
f

**Figure 34**: *Trochulus striolatus*. Clade14; (a) NMBE 571160; Germany, Dorsel; (b) MNHW_A.17.12_01; Austria, Schafberg; (c) MNHW_I.16.12_01; Ireland, Maam; (d) MNHW_F.18.10_06; France, Blecquenecques n. Marquise; (e) MNHW_GB.Br_01; United Kingdom, Bramley near Basingstoke; (f) MNHW_SK.19.71_01; Slovakia, Varín.

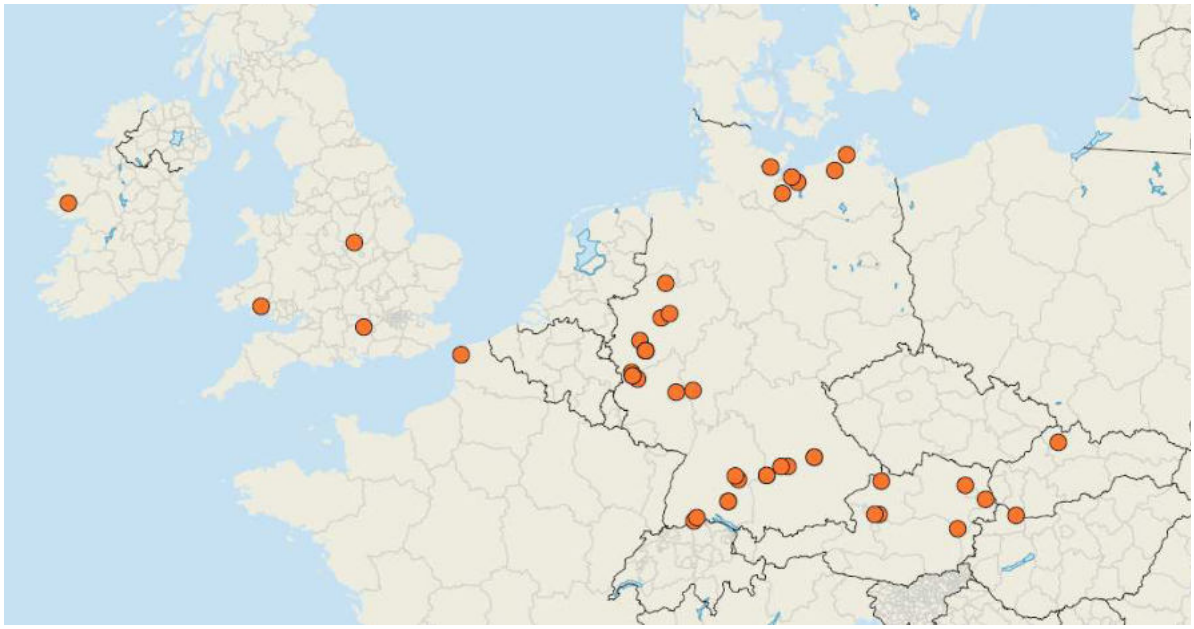


**Figure 35**: Distribution map of the investigated *T. striolatus* specimens.

## *Trochulus becasis* (Rambur, 1868) [clade15] Figs 36a-d

1868 *Helix becasis* Rambur, Journal de Conchyliologie: 16 (3): 267–268 [Ad basin montis "Canigou" dicti, Galliae].

1869 *Helix vendeana* Letourneux, Revue et Magasin de Zoologie: 21: 60 [Bois-Plat, près de Fontenay-le-Comte].

1877 *Helix steneligma* Mabille, Bull. Soc. zool. France: 305 [Pyrenees; dep. Oise: in silvis pagi Compendiaci [= probably Compiègne].

1881 *Helix deobrigana* Servain, Moll. Espagne Portugal: 59–60 [fontaine ferrugineuse de Luchon sur le chemin de l'Hospice].

1882 *Helix microgyra* Bourguignat in Locard, Prodr. malac. française: 1: 79, 319 [Val du pic du Gers, B. Pyr.].

1883 *Helix ataxiaca* Fagot, Bull. Soc. Hist. nat. Toulouse: 17: 220 [Quillau, Fanges forest].

1888 *Helix globulosa* Locard, Ann. Soc. Linn. Lyon, (N.S.): 34 ["1887"]: 337 [de l'île de Jersey].

1894 *Helix alixae* Locard, Coq. terr. France: 120 [Loudres].

1894 *Helix pictavica* Bourguignat, Coq. terr. France: 127 [Clain].

Type specimens: [to be completed].

Description: The investigated specimens of *T. becasis* have a roundish-conical shell with fine strips. The shell contains short, straight hair. The mummy in Figure 20a lost its hairs, but hair scars are still visible. Shell width ranges from 8.41–10.67 mm and shell height from 5.31–6.26 mm (N = 4). All measurements can be found in the appendix (supplementary material II). The umbilicus is wide and open. The body is beige with dark spots.

Distribution (Fig. 37): *Trochulus becasis* is found north of the Pyrenees, in Perreux in south-east France and near Orléans in Central France.

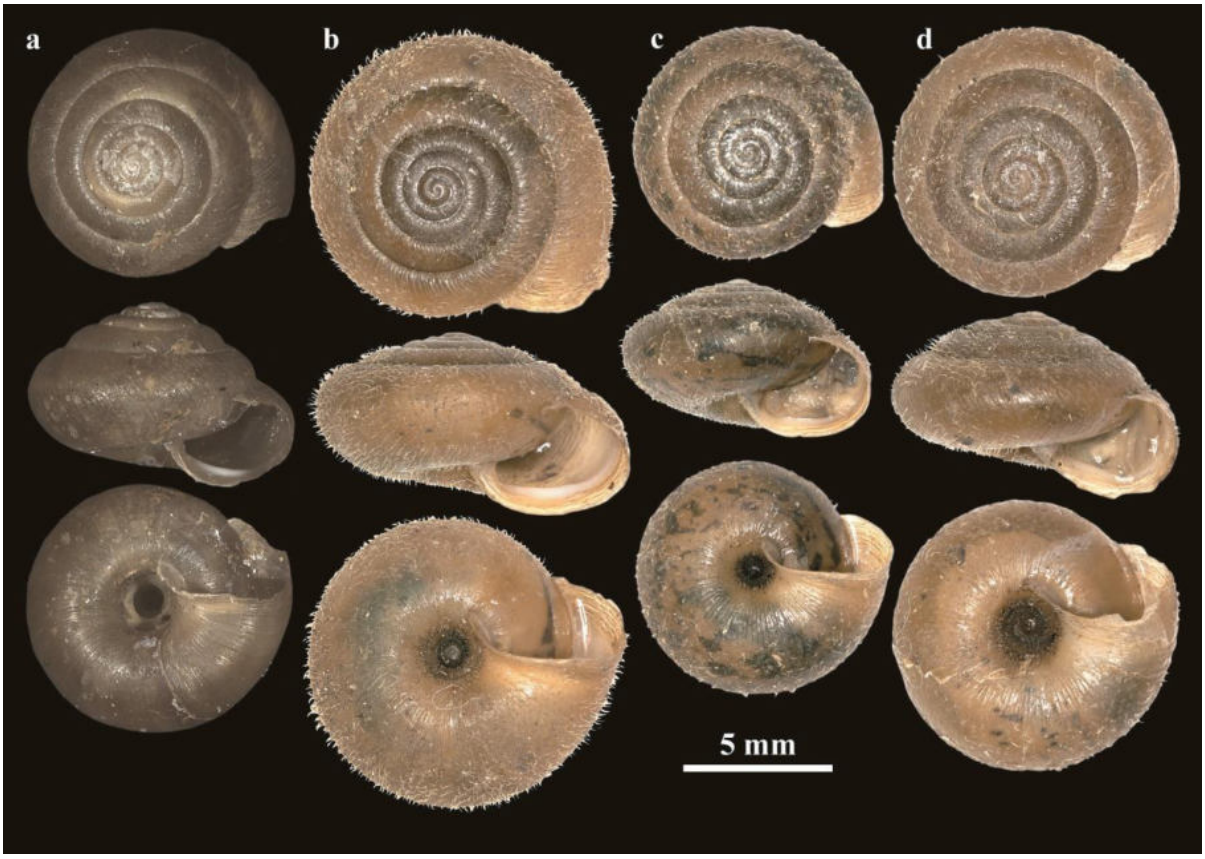



**Figure 36**: *Trochulus becasis*. Clade15; (a) NMBE 571128; United Kingdom, Jersey; (b) MNHW_F.18.37_301; France, Forêt de Beleste; (c) MNHW_F.18.35_300; France, Le Chandelier; (d) MNHW_F.18.30_299; France, Forêt Bac Estable 2.

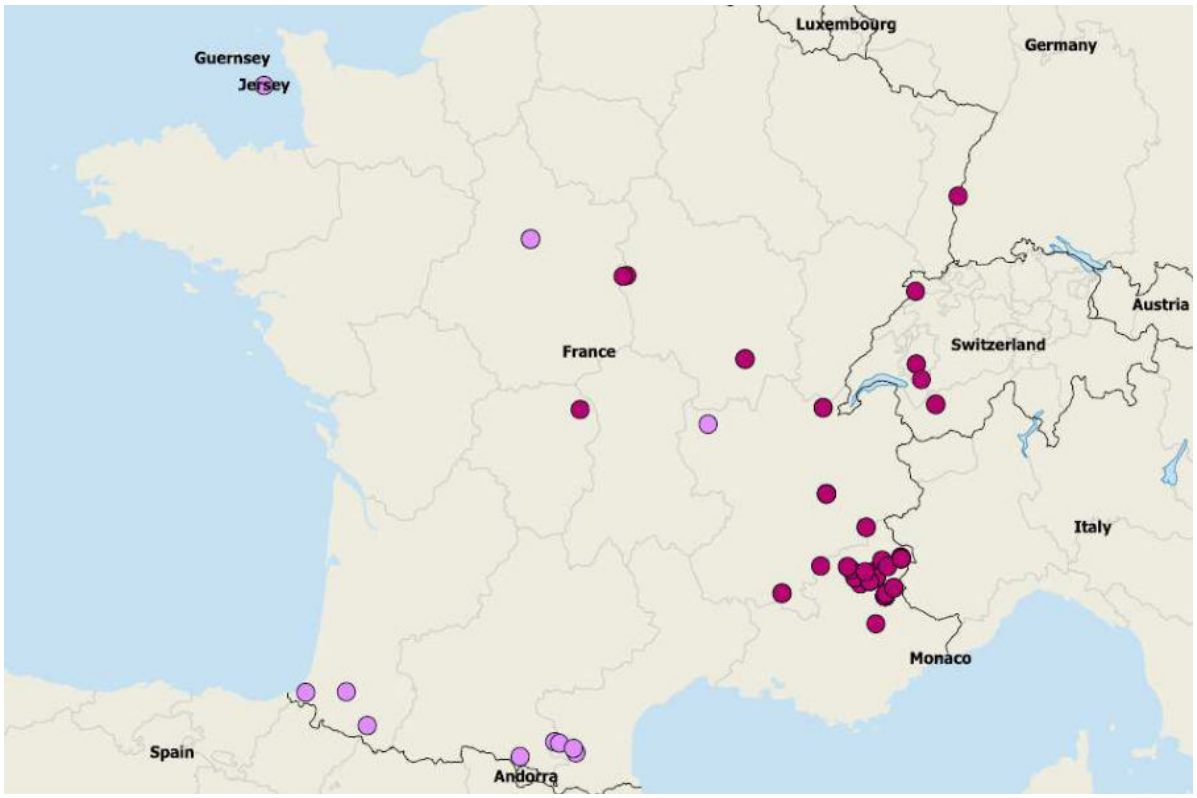



**Figure 37**: Distribution map of the investigated *T.s becasis* (lilac circles) and *T. plebeius* (bordeaux circles) specimens.

## *Trochulus plebeius* (Draparnaud, 1805) [clade16] Figs 38a-e

1805 *Helix plebeium* Draparnaud, Hist. nat. Moll. terr. fluv. France: 105, pl. 7 fig. 5 [unknown].

1820 *Glischrus* (*Helix*) *rudis* Studer, Syst. Verz. Schweizer-Conchylien: 12 [not mentioned; Remark (1): refers to *Helix plebeium* Draparnaud, 1805, pl. 7 fig. 5. Remark (2): name to be treated as a nomen novum? Remark (3): also mentioned by Hartmann, 1821, in: Steinmüller, Neue Alpina, 1: 237, 238; but he refers to Draparnaud, 1805: 104, pl. 7 fig. 21 sub *Helix hispida* var. β].

1826 *Helix prevostiana* Risso: IV: 73 [nos collines [in Dep. Alpes-Maritimes]].

1832 *Helix hispidula* De Cristofori & Jan, Mantissa: 2 [Ital. bor. [northern Italy]].

1855 *Helix hispida* Dumont, Bull. Inst. natn. genevois: 3: 355 [des Alpes; junior homonym of *Helix hispida* Linnaeus, 1758].

1855 *Helix depressula* Dumont, Bull. Inst. natn. genevois: 3: 356 [Mont-Saxonnet, sous l'Eglise, 900 m"; "Brizon, 1100 m"; "St.-Pierre-d'Entremont, 800 m"; "Grande-Chartreuse, 950 m"; "environs de Montiers, 488 m"; "Bramant, 1300 m"; "Mont-Cenis"; "Lanslevillard, de 1500 à 1700 m; junior homonym of Helix depressula E. A. Rossmaessler, Iconographie II (3 & 4) Sept. 1839, 6.].

1864 *Helix bourniana* Bourguignat, Malac. Grande-Chartreuse: 54-55, pl. 7 figs 13–17 [le long du sentier de Chartreusette].

1869 *Helix gratianopolitana* Rambur, Journal de Conchyliologie: 17: 267 [dans les environs de Grenoble].

1879 *Helix hemisphaerica* Lessona, Atti r. Accad. Sci. Torino: 15 (2): 295, pl. 7 figs 19-21 [Brossasco].

1879 *Helix ripularum* Lessona, Atti r. Accad. Sci. Torino: 15 (2): 293, 294, 296, pl. 6 figs 1-5 [Rivoli (V. della Doria Riparia)].

1879 *Helix subcoelata* Lessona, Atti r. Accad. Sci. Torino: 15 (2): 294, pl. 6 fig. 9 [Susa].

1879 *Helix subplebeia* Lessona, Atti r. Accad. Sci. Torino: 15 (2): 295, 296, pl. 6 figs 6-8 [Tornino].

1879 *Helix trochiformis* Lessona, Atti r. Accad. Sci. Torino: 15 (2): 294, 295, 296, pl. 7 figs 22-24 [Tornino; junior homonym of trochiformis Helix, Grateloup, Bull. H. N. Soc. Linn. Bordeaux, II (7) 1827].

1879 *Helix vulgaris* Lessona, Atti r. Accad. Sci. Torino: 15 (2): 292, 294, 295, pl. 6 figs 16-18 [Tornino; junior homonym of *Helix vulgaris*, E. M. da Costa, Brit. Conch. 1778, 72].

1879 *Helix globus* Lessona, Atti r. Accad. Sci. Torino: 15 (2): 296, pl. 7 figs 28-30 [Tornino].

1882 *Helix subbadiella* Bourguignat in Locard, Prodr. malac. française: 1: 74, 317 [Alpes Maritimes: Monton].

1882 *Helix vendoperanensis* Bourguignat in Locard, Prodr. malac. française: 1: 76, 317 [dep. Aube: Vendeuvre-sur Barse and Viélaines near Rosières; environs de Lyon].

1882 *Helix cularensis* Bourguignat in Locard, Prodr. malac. française: 1: 79, 319 [Sassenage près Grenoble].

1886 *Helix vagienna* Pollonera, Boll. Mus. Zool. Anat. comp. Univ. Torino: 1 (17): 3 [Purriac, Valle Stura di Cuneo tra 2100 e 2500m].

1894 *Helix barcelonnettensis* Bourguignat, Coq. terr. France: 125 [Hauteville (Ain), Albertville (Savoie), Barcelonnette (H. Alpes); photographed specimen from Barcelonnette].

1909 *Helix* [*Capillifera*] *maynardi* Caziot, Bull. Soc. zool. France: 34 (5/6): 90, fig. 21 [Mont-Mounier (2.800m); sur le plateau du Demant (2400m)].

1910 *Helix* [*Capillifera*] *orzeszkoi* Caziot, Moll. terr. fluv. Monaco: 117 [fréquente dans les alluvions du Loup].

1910 *Helix* [*Capillifera*] *subniverniaca* Caziot, Moll. terr. fluv. Monaco: 115 [Dans les prés, à l'Est du champ de courses du Var, près du bord de la mer].

Type specimens: The collection of Draparnaud is located in the Natural History Museum Vienna. Under the number NHMW 14828 a syntype of *H. plebeium* is preserved. Investigation of this specimen revealed that it is a specimen of *Urticiola umbrosa*; the original specimen of Draparnaud is not preserved. For this reason, we herewith designate NMBE 569666 (D = 7.86 mm; France, Forest-Saint-Julien; Fig. 38e) as neotype to stabilize the application of the name.

Description: The investigated specimens of *T. plebeius* have a pale brown shell with short, slightly curved hairs. The shell is finely striated. Shell width ranges from 7.51–9.01 mm and shell height from 4.32–4.99 mm (N = 5). All measurements can be found in the appendix (supplementary material II). The specimens have 5.5 whorls. The body is beige with dark spots.

Distribution (Fig. 37): *Trochulus plebeius* is found in the northern area of the province Provence-Alpes-Côte d'Azur and Rhône-Alpes in south-east France, in Central France, and in western Switzerland.


**Figure 38**: *Trochulus plebeius*. Clade16; (a) MNHW_F-Com_227; France, Chameyer; (b) MNHN-IM-2013-76890; France, Guillestre; (c) MNHW_F-Ech_02; France, Échallon; (d) NMBE 569626; France, Avonçon; (e) neotype NMBE 569666; France, Forest-Saint-Julien.

## *Trochulus villosulus* (Rossmässler, 1838) [clade17] Figs 39a-h

1838 *Helix villosula* Rossmässler, Iconographie: (1) 2 (1/2): 1 [Hungary; name attributed to Ziegler.].

1914 *Fruticicola* (*Trichia*) *elatior* Polinski, Spraw. Kom. Fizyogr.: 48: 28 [Ojców].

Type specimens: [to be completed].

Description: *Trochulus villosulus* is characterized by a flattened shell with long hairs. The umbilicus is wide and open and accounts for about 1/5 of the shell diameter. Shell width of the investigated specimens ranges from 6.87–8.92 mm and shell height from 3.96–5.37 mm (N = 9). All measurements can be found in the appendix (supplementary material II). The body is grey with few dark spots.

Distribution (Fig. 40): *Trochulus villosulus* is found in Poland, eastern Czech Republic, Slovakia and Ukraine.

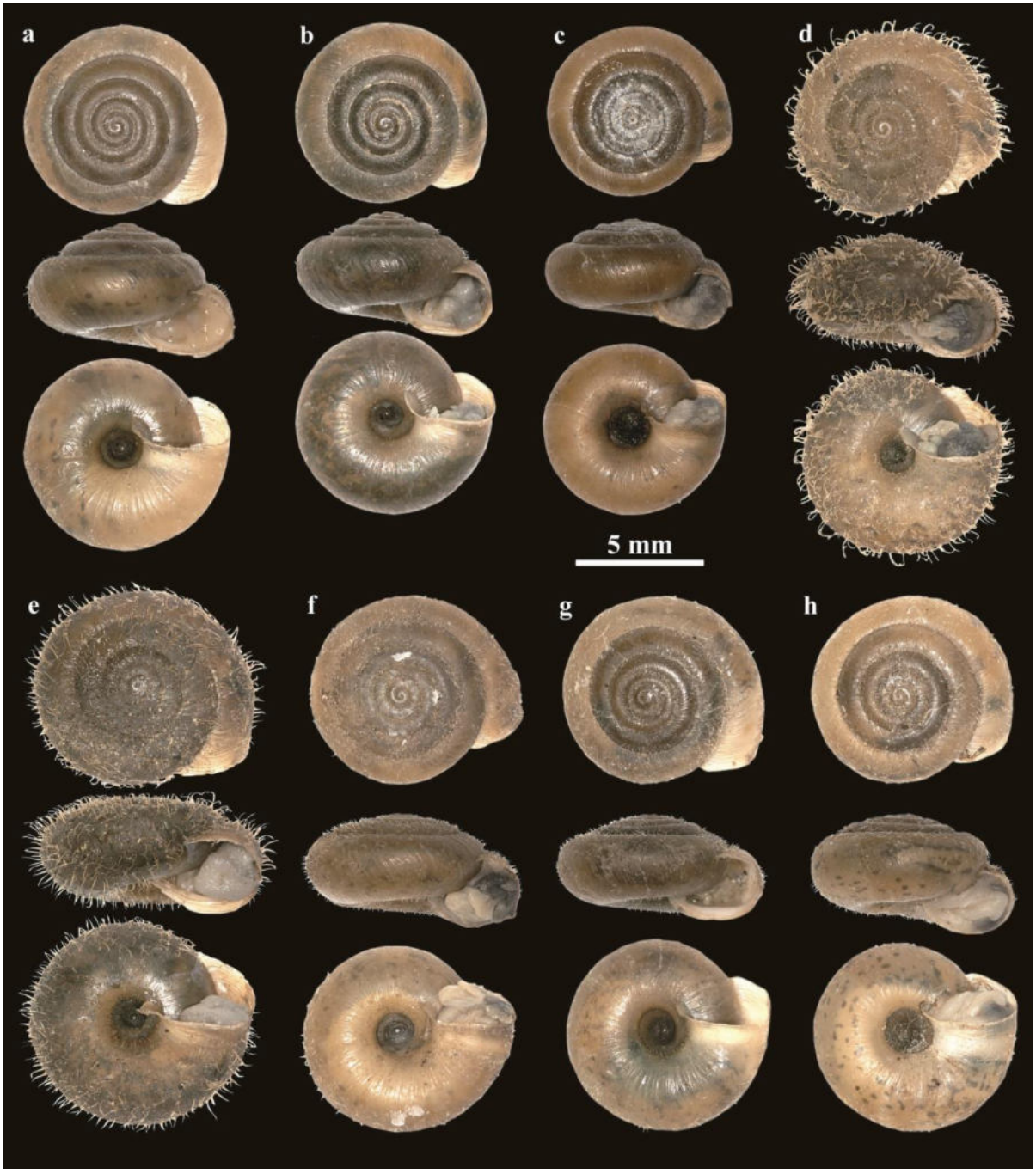


**Figure 39**: *Trochulus villosulus*. Clade17; (a) MNHW_PL.19.09_257; Poland, Barcin-Górne Wolice; (b) MNHW_PL.20.05_249; Poland, Cisowy Jar; (c) MNHW_PL.20.13_253; Poland, Gamlówka; (d) MNHW_PL.20.16_306; Poland, Wapowce; (e) MNHW_PL.20.32_307; Poland, Muszyna 2 ; (f) MNHW_UA.18.55_264; Ukraine, Bibrka; (g) MNHW_UA.18.56_265; Ukraine, Swirz; (h) MNHW_UA.18.58_267; Ukraine, Mikuliczyn.


**Figure 40**: Distribution map of the investigated *T. villosulus* specimens.

## *Trochulus* sp. 3 (sp. nov.?) [clade18] Figs 41a-d

Type specimens: [to be completed].

Description: The shell of the investigated specimens is roundish-conical. Shell width of the investigated specimens ranges from 6.5–9.6 mm and shell height from 3.73–5.99 mm (N = 4). All measurements can be found in the appendix (supplementary material II). The shell comprises of short straight hairs. The umbilicus is wide and open.

Distribution (Fig. 42): The species is found in eastern France in the Alsace, in northern Switzerland, at the Baltic Sea, in the southern Black Forest and southern Bavaria in Germany, and in Vorarlberg and along the Danube near Linz in Austria.

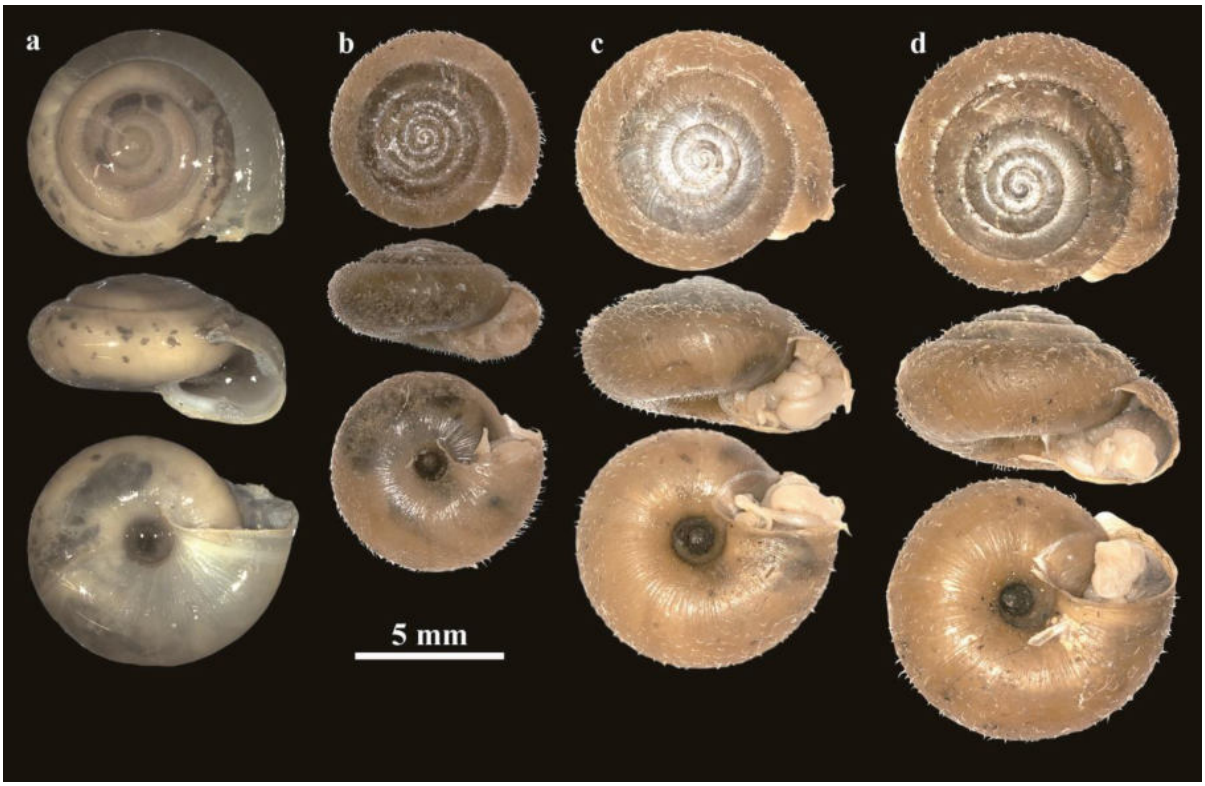



**Figure 41**: *Trochulus* sp. 3. Clade18; (a) NMBE 571152; Germany, Kelheim; (b) MNHW_A.Schw_202; Austria, Schwarzenberg-Loch; (c) MNHW_F.15.09_09; France, between Aubure & La Petite Verrerie; (d) MNHW_F.15.09_10; France, between Aubure and La Petite Verrerie.

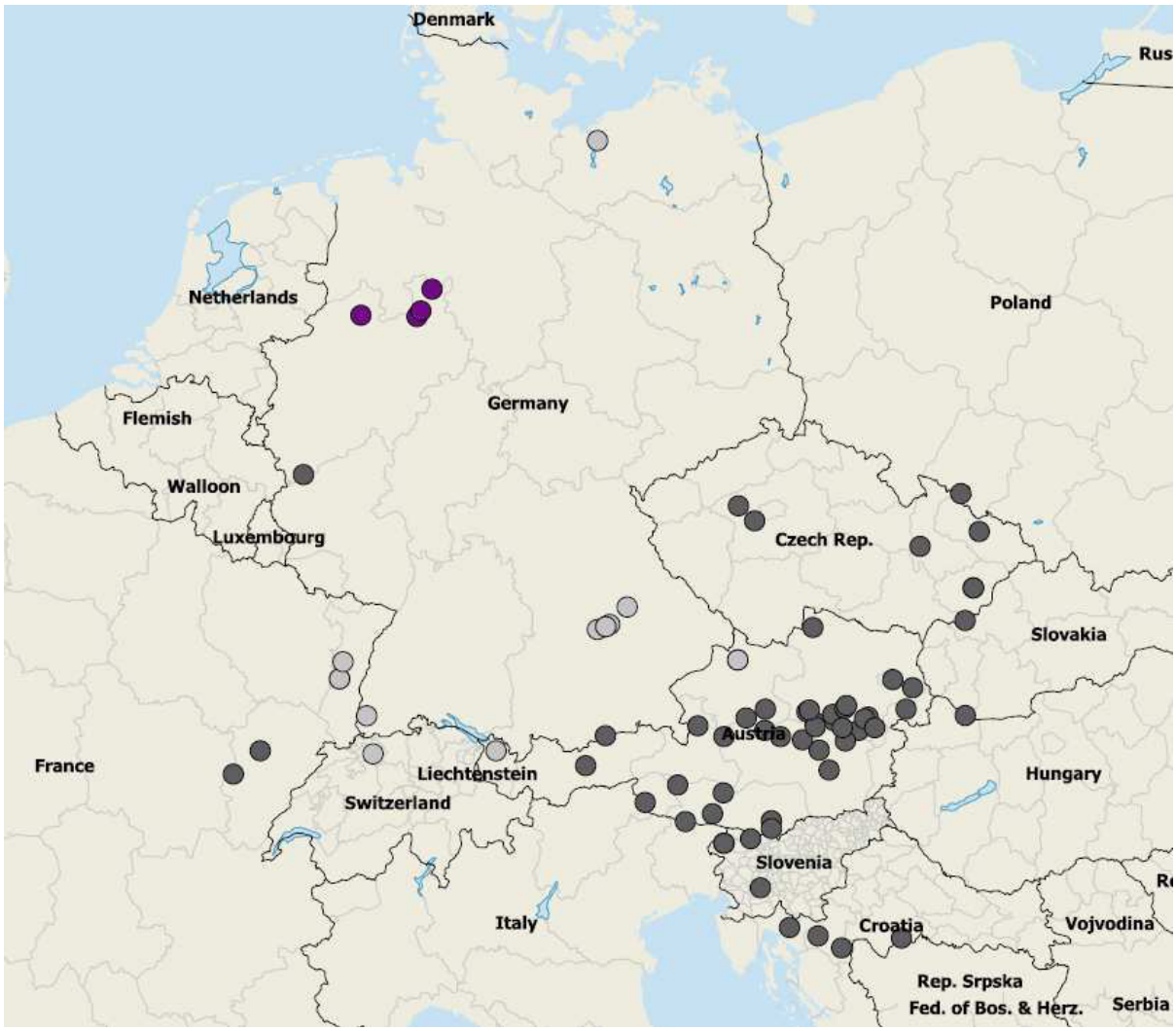



**Figure 42**: Distribution map of the investigated *Trochulus* sp. 3 (pale grey circles), *T.* sp. 4 (dark grey circles), and *T.* sp. 5 (purple circles) specimens.

## *Trochulus* sp. 4 (sp. nov.?) [clade19] Figs 43a-d

Type specimens: [to be completed].

Description: The investigated specimens have a pale brown shell with short straight hairs. The shell is flattened and the umbilicus wide and open. Shell width ranges from 7.31–9.17 mm and shell height from 3.93–5.27 mm (N = 4). All measurements can be found in the appendix (supplementary material II). The specimens have 5.5 whorls. The body is beige with black spots.

Distribution (Fig. 42): The species is found throughout Austria except Vorarlberg, in Czech Republic, western Slovakia, Slovenia, and northern Croatia.

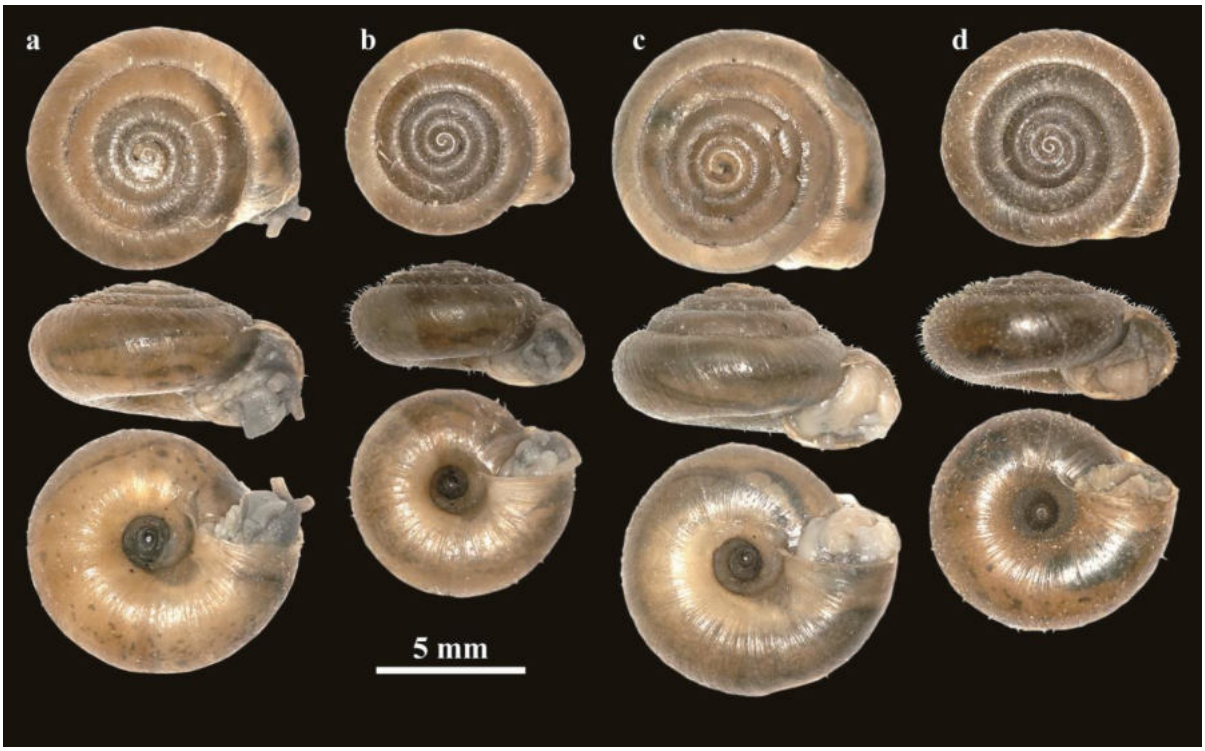



**Figure 43**: *Trochulus* sp. 4. Clade19; (a) MNHW_CZ.19.78_218; Czech Republic, Pitárné; (b) MNHW_CZ.19.35_213; Czech Republic, Srbsko 3; (c) MNHW_SK.19.63_262; Slovakia, Medveďov; (d) MNHW_SLO.17.140_260; Slovenia, Vintgar Gorge.

## *Trochulus villosus* (Draparnaud, 1805) [clade20] Figs 44a-f

1805 *Helix villosa* Draparnaud, Hist. nat. Moll. terr. fluv. France: 104-105, pl. 7 fig. 18 ["dans les montagnes de Savoie"; refers to Draparnaud, 1801, Tabl. moll. terr. fluv. France: 85 sub Helix sericea var. b].

1812 *Helix pilosa* von Alten, Systemat. Abhandl. Erd-und Flussconchyl. Augsburg: 46-47, pl. 4 fig. 7 ["Man findet sie an schattigen Orten in Waldungen, auf verschiedenen Pflanzen, wie auch auf Erlen, besonders auf Weidensträuchen, an den Lechkanälen auf dem Siebenbrunnenfeld, wie auch in der Gegend von Steßling"].

1821 *Helix detrita* W. Hartmann, Neue Alpina: 1: 235 [in bergigten Gegenden der Schweiz, Deutschland und Frankreich; Remark: see also Hartmann, 1824, in: Sturm, Deutschlands Fauna, 6 (7): 13, figs 13a-b.].

1871 *Helix* [*Fruticiola*] *rubra* Clessin, Ber. Naturh. Ver. Augsburg: 21: 93 [bei Günzburg].

1914 *Fruticicola alpicola* Eder, Archiv für Molluskenkunde: 53 (5): 228-231 [in den Churfirsten"; "im Alpsteingebirge (Hoher Kasten, Ebenalp)"; "In Unterwalden ..... von ca. 1700 m. bis 2200 m. zu beiden Seiten des Bannalppasses].

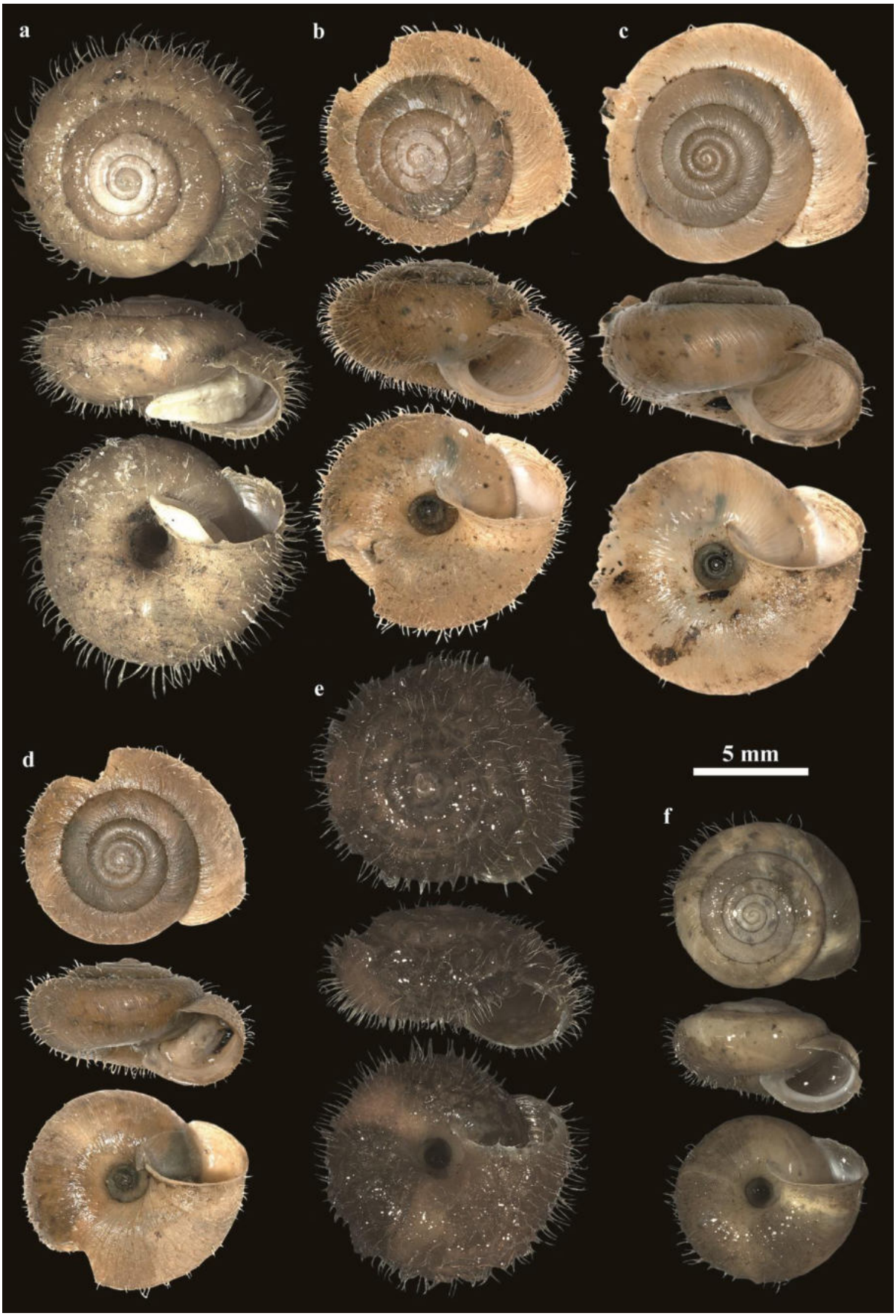
Type specimens: [not yet researched, to be found in Vienna].

Description: *Trochulus villosus* is characterized by a flat shell with very long, strong hairs. The shell is strong and contains a strong lip. Shell width of the investigated specimens ranges from 9.61–13.48 mm and shell height from 5.21–9.73 mm (N = 14). All measurements can be found in the appendix (supplementary material II). The body whorl height accounts for about 85-90% of the shell height. The body is beige with dark spots.

Distribution (Fig. 45): *Trochulus villosus* is found north of the Swiss alps, in southern Germany (Black Forest and Bavaria) and Vorarlberg in western Austria.

Habitat: The species lives in humid and shaded montane areas, in alpine pastures and in forests (Proćków 2009).

Remark: Some *T. alpicola* specimens were collected and examined near Torgon in the Valais, Beatenberg and on the mountain Säntis. There is no genetic difference in the genes studied between *T. villosus* and *T. alpicola*. The latter is most likely a smaller altitudinal form of *T. villosus*, which is why it is synonymised with *T. villosus*.


a
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5 mm

**Figure 44**: *Trochulus villosus*. Clade20; (a) NMBE 569640; Switzerland, Bürgenstock; (b) MNHW_S.15.29_100; Switzerland, Montagne De Cernier; (c) MNHW_G.15.52_109; Germany, Geisingen; (d) MNHW_A.MtK_105; Austria, Mt Kojenkopf; (e) NMBE 569639; Switzerland, Stansstad; (f) NMBE 571203; Switzerland, Säntis, Meglisalp.

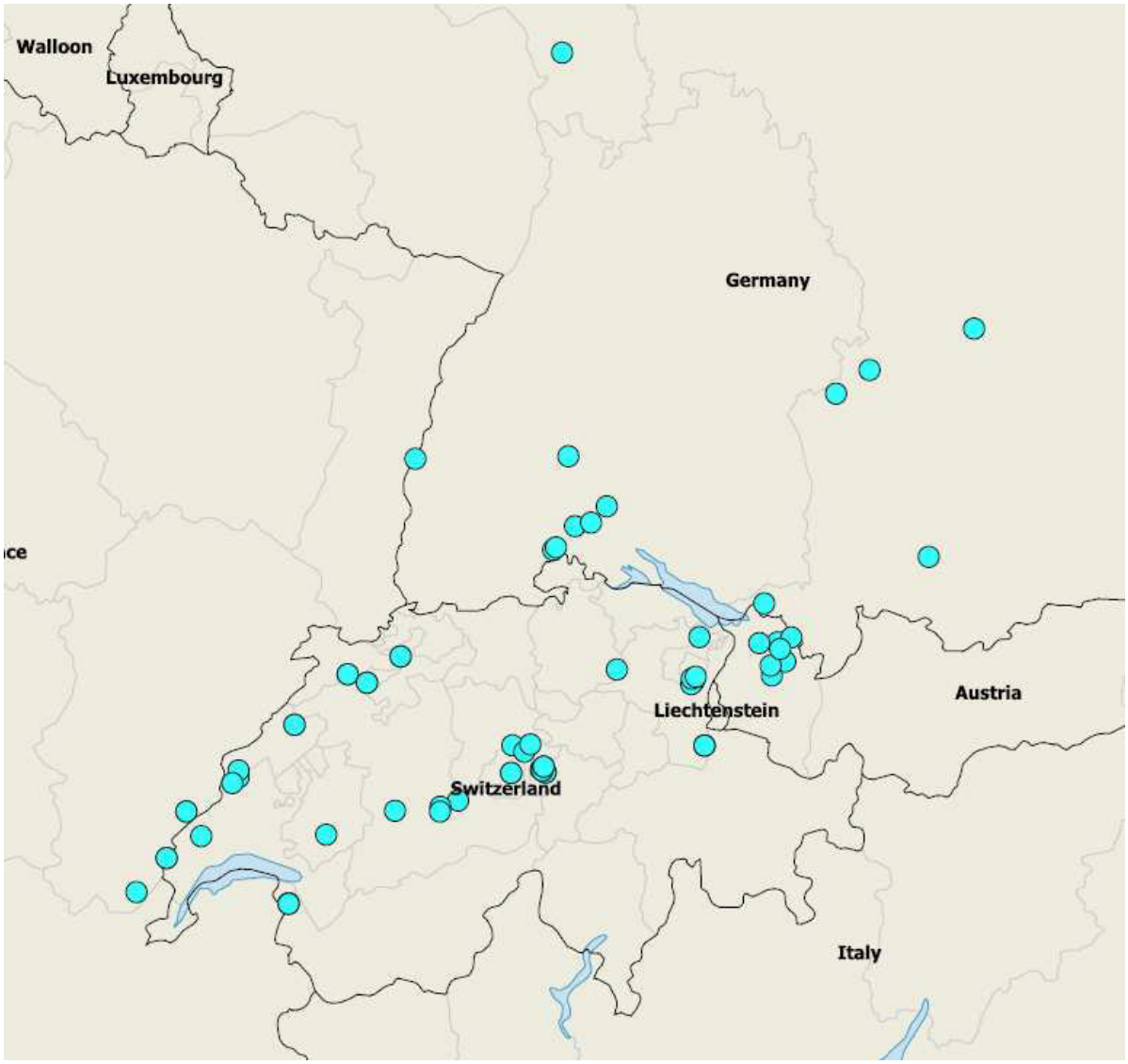


**Figure 45**: Distribution map of the investigated *Trochulus villosus* specimens.

## *Trochulus* sp. 5 (sp. nov.?) [clade22] Figs 46a-b

Type specimens: [to be completed].

Description: The investigated specimens have a roundish-conical shell which is pale brown and shiny. The shell contains short, slightly curved hairs. The aperture is lunate. The umbilicus is wide and open. The body is beige with black spots.

Distribution (Fig. 42): The species is found in a small area around Bielefeld in North Rhine-Westphalia in northern Germany.


**Figure 46**: *Trochulus* sp. 5. Clade22; (a) NMBE 571329; Germany, Kreis Herford, Enger, Belke-Steinbeck, Brandbach; (b) NMBE 571333; Germany, Kreis Minden-Lübbecke, Hille, Mindenerwald.

## Discussion

This study is the first that comprehensively examines specimens from almost the entire distribution range of the genus *Trochulus*. A total of 551 specimens was collected and studied with an integrative approach including a genetic and morphometric survey. In the phylogenetic part, three different methods were used to calculate trees (Bayesian Inference and two Maximum Likelihood methods) based on the concatenated data set of COI, 16S and 5.8S rRNA+ITS2. These studies yielded 21 stable clades, with some of them detected for the first time. The trees include the data of previous studies. The phylogenies of these studies were largely retrieved in clade structures. To facilitate their recognition, we provide a table supplying the corresponding data (Appendix, supplementary material III). Due to the lack of some markers, the resolution within the clades is poor and some nodes are not supported. During the investigation, a saturation in clade recognition could be observed, i.e. additional specimens sorted well into the clade structure, and only randomly a new clade was encountered. For this reason, we conclude that these clades represent species; however, at the current state a sound phylogenetic statement is impossible due to missing node support and presence of an unravelled polytomy in the BI analysis (Fig. 3), which is reflected in the ML analysis by a group of less related clades (Fig. 4). Also, the position of some clades varies to some extent, while their specimen composition stays stable. To overcome this

weakness of the Sanger analysis, a subset of 100 specimens representing all clades recovered so far is currently analysed by using a ddRADseq approach.

However, the basic split in the tree, which is rather well supported in both ML analyses and also present in the BI tree (not supported), separates the genus into two geographically well sorted groups. The first is the subgroup living in the Jurassic Mountains and the Franche-Comté in France and is composed of *T. circinnatus, T. montanus, T. clandestinus, T. caelatus, T. plebicola, T. pascali, T. albulus,* and *T. villosus*. The other one comprises the remaining clades from the distribution area of the genus in Europe. The Jurassic species may have retreated to refuges in unglaciated parts of the Jura Mountains during the Last Glacial Maximum (LGM; e.g., Graf et al. 2015; Claude et al. 2019). After the ice layers have retreated from the Swiss midlands, these species, except *T. clandestinus, T. albulus,* and *T. villosus*, could not spread that far (see Fig. 1). In contrast, the species of the European group such as *T. hispidus* and *T. villersii*, had greater dispersal potential and were able to occupy empty niches rather fast.

Our unbiased approach ignoring *a priori* determinations of specimens resolves the paraphyletic situation as reported by previous authors (e.g., Kruckenhauser et al. 2014; Duda et al. 2014, 2017; Proćków et al. 2013, 2017a, 2021), which may be demonstrated by the example of *T. hispidus*. This species was considered to live widespread in Europe. Restriction of the type locality for this species to Linnaeus' Garden in Uppsala recovers clade10 as *T. hispidus*. This lineage now denominates a boreal group with genetic records from Sweden, Ireland, and north-eastern France. Certainly, the British Isles are also inhabited by that species as well as we infer its presence in the western and more coastal parts of Belgium, The Netherlands, Germany and Denmark. But contrary to all previous determinations, *T. hispidus* is not recorded from Switzerland, nor from southern Germany or Austria. Thus, our concept of *T. hispidus* is radically different to the papers referenced so far, including the mainstream opinions of the last two centuries. Although the framework seems to be settled, rather large sampling gaps in western France and northern and central Germany hamper a final re-grouping of the genus. It may well be that such gaps harbour more small-range endemic lineages like *Trochulus* sp. 5 n. sp., which unexpectedly occurred in a small area in North Rhine-Westphalia, Germany. Since we unfortunately do not have any specimens from Lower Saxony, we do not know exactly how widespread this species is in the north of German. Still, there are single specimens from France that do not fit into the established clades (MNHN-IM-2013-77397 and MNHN-IM-2013-77398 in Fig. 3, 4 and 5), and probably represent uncovered lineages in an ill-sampled area. Future sampling efforts in deficit regions could further clarify the distribution of individual species and possibly reveal new cryptic taxa.

Several of our clades could relate to a name. A prerequisite for this is that ideally topotypic specimens were included in the analysis adjusting the application of a given name. We were able to identify 16 clades with validly described taxa. Given that still almost 100 names need to be evaluated under a taxonomical and nomenclatorial point of view, we refrain to describe the remaining five taxa as new species. No statistical differences in the morphology of the shells of *Trochulus* sp. 1 n. sp. and *Trochulus* sp. 2 n. sp. could be detected. *Trochulus* sp. 3 n. sp. and *Trochulus* sp. 4 n. sp. form sister lineages in the phylogenies (Figs. 3, 4, and 5). They are widespread in Europe, with *Trochulus* sp. 4 appearing to have its radiation hotspot in Austria. So far it was not possible to allocate a name for this lineage.

Nonetheless, the second aim was to investigate the presence of synapomorphic morphological traits differentiating the clades from each other; these could serve as identification features beyond the molecular methods and serve all applied field biologists. Several geometric morphometric methods were tested. Even though we have tried to capture the shape of adult shells as well as possible with landmarks, we have not succeeded in statistically separating all clades from each other. There are several cases, where genetically well supported species also form clear geographical entities, while they remain inseparable by shell morphometry (Fig. 6, 7, and 8). The shape of the shell is very similar for most species and only slight trends are discernible (Fig. 11 and 12), but no clear delineation. Of the specimens examined in the GMM, only *T. caelatus* can be morphologically determined with certainty. But here the type specimen stands out. This could be, since the shell is slightly tilted forward on the frontal picture and therefore the first whorls appear higher than they actually are. The exact orientation of the shells can have a big influence on the landmark analyses. The number of individuals also differs greatly within the clades. Most animals were examined in *T. clandestinus* (N = 36) and the fewest in *T. plebicola*, *T. caelatus* and *Trochulus* sp. 5 (N = 2). This also has an influence on the analyses because with a small number of individuals, it is difficult to estimate the intraspecific variability.

For the other species than *T. caelatus*, several combinations of characteristics are needed to identify the species morphologically. For example, *T. villosus* can be clearly identified by its size and long hairs. Since most cryptic species are not syntopic, they can be identified relatively well by their distribution or ecology.

*Trochulus* species colonize a wide range of habitats: from rocky alpine sites to lowlands, forests, near rivers, and nettle fields, to tall grasses in open areas (e.g., Duda et al. 2014; Proćków et al. 2017b). *Trochulus* species seem to be very adaptable and were able to colonize different habitats. At the moment, best practice to identify a species is to use our distribution maps reporting genetically identified clades or species, identify sympatric species

also occurring in the area, and exclude all other species. In all cases, a simple barcode can help to correctly identify the animals. This reduces the number of misidentifications.

## Outlook

Although we have tried to cover the entire distribution range of the genus *Trochulus*, we are still missing some regions that may harbour new species. We still have collection gaps in western France and in central and northern Germany. Only by conscientiously sampling these regions and examining the specimens, will we get a complete picture. Even though we have collected over 180 *Trochulus* names, we have discovered five new species which will be described in a future paper. The phylogeny will be clarified by results of an ongoing ddRADseq approach; the data were not yet available.

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# Appendix

**Supplementary material I:** Database number, country of origin, geographical data, and GenBank accession numbers for the investigated *Trochulus, Raeticella* and *Edentiella* specimens. Specimens with † where used in the landmark analysis. Specimens with * where used in the ddRADseq analysis.

| Species | Data base nr° | Country | Locality | Lat | Long | Alt | GenBank accession number COI | GenBank accession number 16S | GenBank accession number ITS2 |
|---|---|---|---|---|---|---|---|---|---|
| *T. circinnatus* | NMBE_564589 | Switzerland | Chasseron | 46.85 | 6.54 | 1606 | MW703573 | MW709376 | - |
| *T. clandestinus* | NMBE_564590 | Switzerland | Villeret, Combe Grède | 47.14 | 7.03 | 911 | MW703574 | MW709329 | - |
| *T. montanus* | NMBE_564591 | Switzerland | Moutier, Gorges de Moutier | 47.28 | 7.37 | 515 | MW703575 | MW709377 | - |
| *T. clandestinus* | NMBE_564592 | Switzerland | Villeret, Combe Grède | 47.14 | 7.03 | 911 | MW703576 | MW709330 | MW709295 |
| *T. montanus* | NMBE_564593 | Switzerland | Sornetan, Gorges du Pichoux | 47.29 | 7.23 | 643 | MW703577 | MW709378 | MW709296 |
| *T. montanus* | NMBE_564594 | Switzerland | Montoz | 47.22 | 7.27 | 1327 | MW703578 | MW709331 | MW709297 |
| *T. clandestinus* | NMBE_564595 | Switzerland | Beinwil, Hohe Winde | 47.34 | 7.58 | 1204 | MW703579 | MW709379 | MW709298 |
| *T. clandestinus* | NMBE_564596 | Switzerland | Vechigen, Boll | 46.95 | 7.54 | 570 | MW703580 | MW709380 | MW709299 |
| *T. clandestinus* * | NMBE_564597 | Switzerland | Malix | 46.81 | 9.54 | 1130 | MW703581 | MW709332 | MW709300 |
| *T. clandestinus* | NMBE_25267 | Switzerland | Andermatt, Teufelsbrücke | 46.65 | 8.59 | 1405 | MW703551 | MW709353 | MW709283 |
| *T. clandestinus* | NMBE_509933 | Switzerland | Leissigen, Meielisalp | 46.65 | 7.79 | 800 | MW703553 | MW709355 | MW709285 |
| *T. clandestinus* | NMBE_564598 | Switzerland | Bolligen, Ferenberg | 46.97 | 7.53 | 722 | - | MW709381 | MW709301 |
| *T. clandestinus* * | NMBE_564599 | Switzerland | Vechigen, Sinneringen | 46.95 | 7.53 | 570 | MW703582 | MW709382 | MW709302 |
| *T. sericeus* | NMBE_509907 | Switzerland | Zernez, Val Laschadura | 46.70 | 10.16 | 2100 | MW703552 | MW709354 | MW709284 |
| *T. striolatus* * | NMBE_564600 | Switzerland | Hallau, Lochgraben | 47.69 | 8.41 | 350 | MW703583 | MW709383 | - |
| *T. sericeus* | NMBE_564601 | Switzerland | Zernez | 46.70 | 10.09 | 1473 | ON477942 | ON479903 | ON479896 |
| *T. sericeus* | NMBE_564602 | Switzerland | Zernez, Inn | 46.70 | 10.09 | 1400 | MW703585 | MW709334 | MW709304 |
| *T. montanus* | NMBE_564604 | Switzerland | Selzach, Hasenmatt | 47.24 | 7.45 | 1444 | MW703586 | MW709335 | - |
| *T. clandestinus* | NMBE_564605 | Switzerland | St-Brais | 47.31 | 7.11 | 967 | - | MW709385 | - |
| *T. montanus* * | NMBE_564607 | Switzerland | Mervelier, Scheltental | 47.34 | 7.52 | 615 | ON477943 | ON479904 | ON479897 |
| *T. clandestinus* * | NMBE_564608 | Switzerland | Vallorbe, Grotte de l'Orbe | 46.70 | 6.35 | 760 | MW703589 | MW709336 | MW709307 |
| *T. circinnatus* * | NMBE_564609 | Switzerland | Bullet, Le Chasseron | 46.85 | 6.54 | 1606 | ON477944 | ON479905 | ON479898 |
| *T. clandestinus* | NMBE_564610 | Switzerland | Villeret, Combe Grède | 47.14 | 7.03 | 911 | MW703591 | MW709389 | MW709309 |
| *T. clandestinus* | NMBE_564606 | Switzerland | Chasseral | 47.13 | 7.06 | 1607 | MW703587 | MW709386 | MW709305 |
| *T. clandestinus* | NMBE_564567 | Switzerland | Areuse, Gorges de l' | 46.96 | 6.81 | 516 | - | MW709357 | MW709287 |
| *T. montanus* * | NMBE_564568 | Switzerland | Günsberg, Chamben | 47.27 | 7.57 | 1250 | MW703555 | MW709358 | - |
| *T. albulus* | NMBE_564569 | Switzerland | Reichenbach i. K., Kiental, Gamchi | 46.53 | 7.78 | 1650 | MW703556 | MW709359 | - |
| *T. albulus* | NMBE_564570 | Switzerland | Sigriswil, Justistal, 1100m | 46.71 | 7.77 | 1100 | - | MW709360 | MW709288 |
| *T. sericeus* | NMBE_564571 | Switzerland | Scuol, Puors | 46.81 | 10.30 | 1559 | MW703557 | MW709361 | MW709289 |
| *T. albulus* | NMBE_564572 | Switzerland | Brienz, Brienzer Rothorn | 46.79 | 8.05 | 2349 | MW703558 | MW709328 | - |
| *T. albulus* | NMBE_564573 | Switzerland | Alpnach, Pilatus, | 46.97 | 8.24 | 2128 | MW703559 | MW709362 | MW709290 |

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|---|---|---|---|---|---|---|---|---|---|
| | | | Tomlishorn | | | | | | |
| *T. villosus* | NMBE_564574 | Switzerland | Pohlern, Chrümmelwäge | 46.71 | 7.52 | 1467 | MW703560 | MW709363 | - |
| *T. villosus* | NMBE_564575 | Switzerland | Chasseron | 46.85 | 6.54 | 1606 | MW703561 | MW709364 | MW709291 |
| *T. sericeus* * | NMBE_564576 | Switzerland | Heiden | 47.44 | 9.52 | 794 | MW703562 | MW709365 | - |
| *T. plebeius* | NMBE_564577 | Switzerland | Soubey | 47.31 | 7.05 | 476 | MW703563 | MW709366 | - |
| *T.* sp.3 | NMBE_564578 | Switzerland | Langenbruck, Chilchzimmersattel | 47.36 | 7.80 | 990 | MW703564 | MW709367 | - |
| *T. sericeus* | NMBE_564579 | Switzerland | Goldach | 47.47 | 9.47 | 447 | MW703565 | MW709368 | - |
| *T. albulus* | NMBE_564580 | Switzerland | Erlenbach, Stockhorn | 46.69 | 7.54 | 2190 | MW703566 | MW709369 | - |
| *T. pascali* * | NMBE_543063 | Switzerland | St-Cergue, Route de Cuvaloup | 46.45 | 6.12 | 1208 | ON477941 | ON479902 | ON479895 |
| *T. albulus* | NMBE_564581 | Switzerland | Leukerbad, Allmei | 46.39 | 7.63 | 1600 | MW703567 | MW709370 | MW709292 |
| *T. pascali* * | NMBE_564582 | Switzerland | Chardonne, Mont Pèlerin | 46.49 | 6.83 | 1032 | - | MW709371 | MW709293 |
| *T. caelatus* * | NMBE_564584 | Switzerland | Bettlach, Wandflue | 47.23 | 7.40 | 1300 | MW703569 | MW709372 | MW709294 |
| *T. caelatus* * | NMBE_564585 | Switzerland | Oberdorf, Weissenstein | 47.25 | 7.50 | 1280 | MW703570 | MW709373 | - |
| *T. caelatus* * | NMBE_564586 | Switzerland | Moutier, Gorges de Moutier | 47.28 | 7.37 | 515 | MW703571 | MW709374 | - |
| *T. sericeus* | NMBE_564587 | Switzerland | Steinach, Obersteinach | 47.49 | 9.44 | 417 | MW703572 | MW709375 | - |
| *T. sericeus* | NMBE_24820 | Germany | Blumberg, Eichberg/Baden-Württemberg | 47.85 | 8.52 | | MW703549 | MW709351 | MW709281 |
| *T. sericeus* | NMBE_24833 | Germany | Neuschwaig/ Bayern | 48.65 | 10.68 | | MW703550 | MW709352 | MW709282 |
| *E. edentula* | MNHW_S-Er_50 | Switzerland | Erschwil | 47.37 | 7.56 | | MW440986 | MW621003 | MW440679 |
| *E. edentula* | MNHW_S-Er_51 | Switzerland | Erschwil | 47.37 | 7.56 | | MW440987 | MW621004 | MW440680 |
| *E. edentula* * | MNHW_F-Ech_50 | France | Échallon | 46.20 | 5.75 | | - | MW772316 | MW775782 |
| *E. edentula* | MNHW_F.15.36_51 | France | Bonnevaux | 46.82 | 6.21 | | - | MW772295 | MW775759 |
| *E.edentula* | MNHW_F.15.36_50 | France | Bonnevaux | 46.82 | 6.21 | | - | MW772294 | MW775758 |
| *E. edentula* | MNHW_F.15.37_50 | France | Cascade du Moulin du SAustria | 46.76 | 6.02 | | - | MW772298 | MW775760 |
| *E. edentula* | MNHW_F.15.37_51 | France | Cascade du Moulin du SAustria | 46.76 | 6.02 | | - | MW772299 | MW775761 |
| *T. albulus* | MNHW_F-ChJ_38 | France | Château de Joux | 46.87 | 6.37 | | MW774498 | - | MW775776 |
| *T. albulus* | MNHW_S.15.17_12 | Switzerland | Gorges de Court | 47.26 | 7.34 | | MW774513 | MW772329 | MW775793 |
| *T. albulus †* | MNHW_S.15.19_10 | Switzerland | Gorges de Court 3 | 47.25 | 7.34 | | - | MW772330 | - |
| *T. albulus †* | MNHW_S.15.19_11 | Switzerland | Gorges de Court 3 | 47.25 | 7.34 | | MW774518 | MW772331 | MW775797 |
| *T. albulus* | MNHW_S.15.25_04 | Switzerland | Frinvillier | 47.17 | 7.25 | | MW774538 | MW772344 | - |
| *T. albulus †* | MNHW_S.15.21_13 | Switzerland | Gorges de Moutier | 47.29 | 7.38 | | MW774524 | MW772338 | MW775803 |
| *T. albulus †* | MNHW_S.15.21_14 | Switzerland | Gorges de Moutier | 47.29 | 7.38 | | MW774525 | MW772339 | MW775804 |
| *T. plebeius †* | MNHW_F-Ech_02 | France | Échallon | 46.20 | 5.75 | | MW774499 | MW772312 | MW775779 |
| *T. plebeius* | MNHW_F-Ech_03 | France | Échallon | 46.20 | 5.75 | | MW774500 | MW772313 | MW775780 |
| *T. albulus †* | MNHW_S.15.27_12 | Switzerland | Twannbachschlucht | 47.10 | 7.14 | | MW440984 | MW621002 | MW440677 |

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|---|---|---|---|---|---|---|---|---|---|
| *T. albulus* | MNHW_S.15.27_13 | Switzerland | Twannbachschlucht | 47.10 | 7.14 | | MW774543 | MW772345 | MW775821 |
| *T. albulus* | MNHW_S.15.27_14 | Switzerland | Twannbachschlucht | 47.10 | 7.14 | | MW774544 | MW772346 | MW775822 |
| *T. albulus* | MNHW_S-Me_05 | Switzerland | Mervelier | 47.34 | 7.51 | | MW774561 | - | MW775842 |
| *T. clandestinus †* | MNHW_S.15.20_01 | Switzerland | Gorges de Court 4 | 47.25 | 7.35 | | MW774519 | MW772332 | MW775798 |
| *T. clandestinus †* | MNHW_S.15.20_02 | Switzerland | Gorges de Court 4 | 47.25 | 7.35 | | - | MW772333 | - |
| *T. montanus* | MNHW_S.15.19_08 | Switzerland | Gorges de Court 3 | 47.25 | 7.34 | | | | |
| *T. clandestinus* | MNHW_S.15.19_09 | Switzerland | Gorges de Court 3 | 47.25 | 7.34 | | MW774516 | MW776381 | MW775796 |
| *T. montanus* | MNHW_S.15.25_01 | Switzerland | Frinvillier | 47.17 | 7.25 | | MW774517 | MW776382 | - |
| *T. montanus ** | MNHW_S.15.25_03 | Switzerland | Frinvillier | 47.17 | 7.25 | | MW774536 | MW776388 | - |
| *T. clandestinus †** | MNHW_S.15.26_02 | Switzerland | Frinvillier 2 | 47.17 | 7.25 | | MW774537 | MW776389 | MW775815 |
| *T. montanus †* | MNHW_S.15.26_03 | Switzerland | Frinvillier 2 | 47.17 | 7.25 | | MW774539 | MW776390 | MW775816 |
| *T. clandestinus* | MNHW_S.15.27_04 | Switzerland | Twannbachschlucht | 47.10 | 7.14 | | MW774540 | MW776391 | MW775817 |
| *T. clandestinus* | MNHW_S.15.27_05 | Switzerland | Twannbachschlucht | 47.10 | 7.14 | | MW774541 | MW776392 | MW775818 |
| *T. clandestinus* | MNHW_S.15.27_06 | Switzerland | Twannbachschlucht | 47.10 | 7.14 | | - | MW776394 | MW775820 |
| *T. clandestinus †* | MNHW_S.15.28_06 | Switzerland | Saint-Imier/Les Pontins | 47.14 | 6.99 | | MW774545 | MW776395 | MW775823 |
| *T. villosus* | MNHW_S.15.22a_100 | Switzerland | Gorges du Pichoux | 47.29 | 7.22 | | MW774530 | - | MW775809 |
| *T. villosus* | MNHW_S.15.22a_101 | Switzerland | Gorges du Pichoux | 47.29 | 7.22 | | MW774531 | MW776384 | MW775810 |
| *T. villosus †* | MNHW_S.15.29_100 | Switzerland | Montagne De Cernier | 47.08 | 6.89 | | MW774549 | MW776396 | MW775827 |
| *T. villosus †* | MNHW_S.15.29_101 | Switzerland | Montagne De Cernier | 47.08 | 6.89 | | MW440985 | MW447773 | MW440678 |
| *T. villosus ** | MNHW_F.15.33_100 | France | Les Rousses-d'Amont | 46.51 | 6.09 | | MW774474 | MW776373 | MW775747 |
| *T. villosus* | MNHW_F.15.33_101 | France | Les Rousses-d'Amont | 46.51 | 6.09 | | MW774475 | MW772284 | MW775748 |
| *T. villosus* | MNHW_F.15.35_100 | France | Mouthe, Source du Doubs | 46.71 | 6.21 | | MW774481 | MW772290 | MW775754 |
| *T. villosus* | MNHW_F.15.35_101 | France | Mouthe, Source du Doubs | 46.71 | 6.21 | | MW774482 | MW772291 | MW775755 |
| *T. villosus* | MNHW_F.15.44_100 | France | Grand Colombier | | | | MW774495 | MW776377 | MW775772 |
| *T. villosus* | MNHW_S-Er_100 | Switzerland | Erschwil | 47.37 | 7.56 | | MW774558 | MW776398 | MW775838 |
| *T. villosus* | MNHW_S-Er_101 | Switzerland | Erschwil | 47.37 | 7.56 | | MW774559 | MW772359 | MW775839 |
| *T. villosus* | MNHW_S-Bu_100 | Switzerland | Buttes | 46.88 | 6.54 | | MW774557 | MW772356 | MW775835 |
| *T. villosus ** | MNHW_S.15.17_100 | Switzerland | Gorges de Court | 47.26 | 7.34 | | MW774514 | MW776379 | MW775794 |
| *T. villosus* | MNHW_S.15.17_101 | Switzerland | Gorges de Court | 47.26 | 7.34 | | MW774515 | MW776380 | MW775795 |
| *T. montanus ** | MNHW_S.15.23_01 | Switzerland | Gorges du Pichoux 2 | 47.29 | 7.22 | | MW774532 | MW776385 | MW775811 |
| *T. montanus* | MNHW_S.15.23_02 | Switzerland | Gorges du Pichoux 2 | 47.29 | 7.22 | | MW774533 | MW776386 | MW775812 |
| *T. montanus* | MNHW_S-Me_14 | Switzerland | Mervelier | 47.34 | 7.51 | | MW774562 | MW772362 | MW775843 |
| *T. montanus* | MNHW_S-Mell_12 | Switzerland | Mervelier 2 | 47.34 | 7.51 | | - | MW772363 | MW775844 |
| *T. montanus* | MNHW_S-Mell_13 | Switzerland | Mervelier 2 | 47.34 | 7.51 | | MW774563 | MW772364 | |
| *T. montanus* | MNHW_S-Er_05 | Switzerland | Erschwil | 47.37 | 7.56 | | - | MW772357 | MW775836 |

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|---|---|---|---|---|---|---|---|---|---|
| *T. montanus* | MNHW_S-Er_08 | Switzerland | Erschwil | 47.37 | 7.56 | | - | MW772358 | MW775837 |
| *T. caelatus †* | MNHW_S.15.21_01 | Switzerland | Gorges de Moutier | 47.29 | 7.38 | | MW774522 | MW772336 | MW775801 |
| *T. caelatus †* | MNHW_S.15.21_02 | Switzerland | Gorges de Moutier | 47.29 | 7.38 | | MW440982 | MW621001 | MW440675 |
| *T. caelatus* | MNHW_S.15.21_04 | Switzerland | Gorges de Moutier | 47.29 | 7.38 | | MW774523 | MW772337 | MW775802 |
| *T. montanus †* | MNHW_S.15.22a_10 | Switzerland | Gorges du Pichoux | 47.29 | 7.22 | | MW774528 | MW772341 | MW775807 |
| *T. albulus* | MNHW_S.15.22a_11 | Switzerland | Gorges du Pichoux | 47.29 | 7.22 | | MW774529 | MW772342 | MW775808 |
| *T. clandestinus* | MNHW_S.15.32_01 | Switzerland | Sources de l'Orbe | 46.70 | 6.34 | | MW774553 | MW772351 | MW775830 |
| *T. clandestinus* | MNHW_S.15.32_02 | Switzerland | Sources de l'Orbe | 46.70 | 6.34 | | MW774554 | MW772352 | MW775831 |
| *T. clandestinus* | MNHW_S.15.32_03 | Switzerland | Sources de l'Orbe | 46.70 | 6.34 | | - | MW772353 | MW775832 |
| *T. pascali †* | MNHW_F.15.33_04 | France | Les Rousses-d'Amont | 46.51 | 6.09 | | MW774471 | MW772283 | MW775744 |
| *T. pascali †* | MNHW_F.15.33_05 | France | Les Rousses-d'Amont | 46.51 | 6.09 | | MW774472 | MW776371 | MW775745 |
| *T. pascali †* | MNHW_F.15.33_06 | France | Les Rousses-d'Amont | 46.51 | 6.09 | | MW774473 | MW776372 | MW775746 |
| *T. pascali †* | MNHW_F.15.39_01 | France | Cascades du Hérisson | 46.62 | 5.86 | | MW774488 | MW772302 | MW775764 |
| *T. pascali †* | MNHW_F.15.39_02 | France | Cascades du Hérisson | 46.62 | 5.86 | | - | MW772303 | MW775765 |
| *T. pascali †* | MNHW_F.15.39a_01 | France | Cascades du Hérisson | 46.62 | 5.86 | | MW774489 | MW776374 | MW775766 |
| *T. plebicola* | MNHW_F.15.41_05 | France | HAustriaville-Lompnes 2 | 45.97 | 5.58 | | MW774490 | MW772304 | MW775767 |
| *T. plebicola †* | MNHW_F.15.42a_03 | France | Arandas | 45.89 | 5.49 | | MW774491 | MW772305 | MW775768 |
| *T. plebicola †* | MNHW_F.15.42a_04 | France | Arandas | 45.89 | 5.49 | | MW774492 | MW772306 | MW775769 |
| *T. plebicola ** | MNHW_F.15.43_01 | France | Virieu-Le-Petit | 45.91 | 5.74 | | MW774493 | MW776375 | MW775770 |
| *T. plebicola* | MNHW_F.15.43_02 | France | Virieu-Le-Petit | 45.91 | 5.74 | | MW774494 | MW776376 | MW775771 |
| *T. pascali †** | MNHW_F.15.47_04 | France | Jalinard | 46.06 | 5.65 | | - | MW772307 | MW775773 |
| *T. pascali †* | MNHW_F.15.47_05 | France | Jalinard | 46.06 | 5.65 | | MW774496 | MW776378 | MW775774 |
| *T. montanus* | MNHW_F-Ech_09 | France | Échallon | 46.20 | 5.75 | | MW774501 | MW772315 | MW775781 |
| *T. montanus †* | MNHW_S.15.17_01 | Switzerland | Gorges de Court | 47.26 | 7.34 | | MW774511 | MW772326 | MW775791 |
| *T. clandestinus †* | MNHW_S.15.17_02 | Switzerland | Gorges de Court | 47.26 | 7.34 | | MW774512 | MW772327 | MW775792 |
| *T. montanus †* | MNHW_S.15.20_17 | Switzerland | Gorges de Court 4 | 47.25 | 7.35 | | MW774520 | MW772334 | MW775799 |
| *T. clandestinus †* | MNHW_S.15.20_18 | Switzerland | Gorges de Court 4 | 47.25 | 7.35 | | MW774521 | MW772335 | MW775800 |
| *T. clandestinus* | MNHW_S.15.22_06 | Switzerland | Gorges du Pichoux | 47.29 | 7.22 | | MW774527 | MW776383 | MW775806 |
| *T. clandestinus* | MNHW_S.15.22_05 | Switzerland | Gorges du Pichoux | 47.29 | 7.22 | | MW774526 | MW772340 | MW775805 |
| *T. clandestinus †* | MNHW_S.15.24_01 | Switzerland | Undervelier | 47.32 | 7.22 | | MW774534 | MW776387 | MW775813 |
| *T. montanus ** | MNHW_S.15.24_07 | Switzerland | Undervelier | 47.32 | 7.22 | | MW774535 | MW772343 | MW775814 |
| *T. clandestinus †** | MNHW_S.15.28_07 | Switzerland | Saint-Imier/Les Pontins | 47.14 | 6.99 | | MW774546 | MW772347 | MW775824 |
| *T. clandestinus †* | MNHW_S.15.28_08 | Switzerland | Saint-Imier/Les Pontins | 47.14 | 6.99 | | MW774547 | - | MW775825 |
| *T. clandestinus †* | MNHW_S.15.29_01 | Switzerland | Montagne De Cernier | 47.08 | 6.89 | | MW774548 | MW772348 | MW775826 |
| *T. clandestinus †* | MNHW_S.15.29_02 | Switzerland | Montagne De Cernier | 47.08 | 6.89 | | MW440983 | MW447772 | MW440676 |

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|---|---|---|---|---|---|---|---|---|---|
| *T. clandestinus* | MNHW_S.15.30_01 | Switzerland | Gorges de L'Areuse | 46.96 | 6.82 | | MW774550 | MW772349 | MW775828 |
| *T. circinnatus †* | MNHW_S.15.30_02 | Switzerland | Gorges de L'Areuse | 46.96 | 6.82 | | MW774551 | MW772350 | MW775829 |
| *T. circinnatus ** | MNHW_S.15.30_03 | Switzerland | Gorges de L'Areuse | 46.96 | 6.82 | | MW774552 | MW776397 | - |
| *T. clandestinus* | MNHW_F.15.31_01 | France | Mont d'Or | 46.74 | 6.35 | | MW774468 | MW772280 | MW775741 |
| *T. clandestinus †* | MNHW_F.15.31_02 | France | Mont d'Or | 46.74 | 6.35 | | MW774469 | MW772281 | MW775742 |
| *T. clandestinus †* | MNHW_F.15.31_03 | France | Mont d'Or | 46.74 | 6.35 | | MW774470 | MW772282 | MW775743 |
| *T. circinnatus †* | MNHW_F.15.34_01 | France | La Chevry | 46.65 | 6.05 | | MW774476 | MW772285 | MW775749 |
| *T. circinnatus †** | MNHW_F.15.34_03 | France | La Chevry | 46.65 | 6.05 | | MW774477 | MW772286 | MW775750 |
| *T. circinnatus †* | MNHW_F.15.34_05 | France | La Chevry | 46.65 | 6.05 | | MW774478 | MW772287 | MW775751 |
| *T. circinnatus †* | MNHW_F.15.35_01 | France | Mouthe, Source du Doubs | 46.71 | 6.21 | | MW774479 | MW772288 | MW775752 |
| *T. clandestinus* | MNHW_F.15.35_02 | France | Mouthe, Source du Doubs | 46.71 | 6.21 | | MW774480 | MW772289 | MW775753 |
| *T. circinnatus †** | MNHW_F.15.36_01 | France | Bonnevaux | 46.82 | 6.21 | | MW774483 | MW772292 | MW775756 |
| *T. montanus* | MNHW_F.15.36_02 | France | Bonnevaux | 46.82 | 6.21 | | MW774484 | MW772293 | MW775757 |
| *T. circinnatus †* | MNHW_F.15.37_05 | France | Cascade du Moulin du SAustria | 46.76 | 6.02 | | - | MW772296 | - |
| *T. circinnatus †* | MNHW_F.15.37_06 | France | Cascade du Moulin du SAustria | 46.76 | 6.02 | | MW774485 | MW772297 | - |
| *T. pascali †* | MNHW_F.15.38_05 | France | Chaux-des-Crotenay | 46.65 | 5.99 | | MW774486 | MW772300 | MW775762 |
| *T. circinnatus †** | MNHW_F.15.38_06 | France | Chaux-des-Crotenay | 46.65 | 5.99 | | MW774487 | MW772301 | MW775763 |
| *T. clandestinus* | MNHW_S-Gr_10 | Switzerland | Grindel | 47.39 | 7.51 | | - | MW772360 | MW775840 |
| *T. clandestinus* | MNHW_S-Gr_11 | Switzerland | Grindel | 47.39 | 7.51 | | MW774560 | MW772361 | MW775841 |
| *T. circinnatus* | MNHW_F-Pr_17 | France | Pretin | 46.93 | 5.85 | | MW774502 | MW772317 | MW775783 |
| *T. circinnatus* | MNHW_F-Pr_18 | France | Pretin | 46.93 | 5.85 | | MW774503 | MW772318 | MW775784 |
| *T. circinnatus* | MNHW_F-An-M_06 | France | Andelot-en-Montagne | 46.88 | 5.95 | | - | MW772308 | MW775775 |
| *T. circinnatus* | MNHW_F-An-M_09 | France | Andelot-en-Montagne | 46.88 | 5.95 | | MW774497 | MW772309 | - |
| *T. clandestinus* | MNHW_S-Bu_05 | Switzerland | Buttes | 46.88 | 6.54 | | MW774556 | - | - |
| *T. clandestinus* | MNHW_F-ChJ_43 | France | Château de Joux | 46.87 | 6.37 | | - | MW772310 | MW775777 |
| *T. clandestinus* | MNHW_F-ChJ_44 | France | Château de Joux | 46.87 | 6.37 | | - | MW772311 | MW775778 |
| *T. villosus* | NMBE_565855 | Switzerland | Chrüzhütte 1746m | 46.89 | 8.44 | 1746 | MW703604 | MW709348 | MW709322 |
| *T. villosus* | NMBE_565856 | Switzerland | Chrüzhütte 1746m | 46.89 | 8.44 | 1746 | MW703605 | MW709390 | MW709323 |
| *T. albulus* | NMBE_565858 | Switzerland | Schwanden 1459m | 46.88 | 8.45 | 1460 | MW703606 | MW709391 | MW709324 |
| *T. albulus ** | NMBE_565860 | Switzerland | Schwanden 1459m | 46.88 | 8.45 | 1460 | MW703608 | MW709392 | MW709326 |
| *T. villosus* | NMBE_565862 | Switzerland | Grüenboden 2015m | 46.87 | 8.47 | 2015 | MW703609 | MW709350 | MW709327 |
| *T. villosus* | NMBE_565859 | Switzerland | Grüenboden 2015m | 46.87 | 8.47 | 2015 | MW703607 | MW709349 | MW709325 |
| *T. villosus ** | NMBE_565821 | Switzerland | Schonegg 2234m | 46.87 | 8.25 | 2234 | ON477945 | ON479906 | ON479899 |
| *T. villosus* | NMBE_565822 | Switzerland | Schonegg 2234m | 46.87 | 8.25 | 2234 | MW703593 | MW709338 | MW709311 |

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|---|---|---|---|---|---|---|---|---|---|
| *T. villosus* | NMBE_565824 | Switzerland | | | | | - | MW709339 | MW709312 |
| *T. villosus* | NMBE_565825 | Switzerland | Lang Band? 1636minsgäu bach | 46.88 | 8.44 | 1640 | MW703594 | MW709340 | MW709313 |
| *T. villosus* | NMBE_565832 | Switzerland | Lombachalp | 46.75 | 7.92 | 1600 | MW703600 | MW709344 | MW709319 |
| *T. clandestinus* | NMBE_565831 | Switzerland | Churwalden | 46.81 | 9.53 | 1120 | MW703599 | MW709343 | MW709318 |
| *T. villosus* | NMBE_565828 | Switzerland | Jura | 46.83 | 6.50 | 1120 | MW703596 | - | MW709315 |
| *T. clandestinus* | NMBE_565829 | Switzerland | Jura | 46.80 | 6.50 | 1330 | MW703597 | MW709341 | MW709316 |
| *T. villosus* | NMBE_565830 | Switzerland | Pilatus | 46.99 | 8.26 | 1308 | MW703598 | MW709342 | MW709317 |
| *T. villosus* | NMBE_565827 | Switzerland | Mont Tendre | 46.60 | 6.30 | 1630 | MW703595 | - | MW709314 |
| *T. villosus* | NMBE_565833 | Switzerland | Beatenberg | 46.73 | 7.80 | 1800 | MW703601 | MW709345 | MW709320 |
| *T. villosus* | NMBE_565834 | Switzerland | Beatenberg, 1728m | 46.70 | 7.80 | 1730 | MW703602 | MW709346 | - |
| *T. albulus* | NMBE_565836 | Switzerland | Beatenberg, 1751m | 46.70 | 7.80 | 1730 | MW703603 | MW709347 | MW709321 |
| *T. villosus* | NMSG-IM-28906 | Switzerland | Bad Ragaz/Pizol SG | 46.99 | 9.46 | | | | |
| *T. albulus* | NMSG-IM-28907 | Switzerland | Mittelsass Kurve in den Wald SG | 47.00 | 9.46 | | | | |
| *T. villosus* | NMSG-IM-28908 | Switzerland | Wiese Pardiel SG | 46.99 | 9.46 | | | | |
| *T. villosus* | NMSG-IM-28909 | Switzerland | oberhalb Pardiel SG | 46.99 | 9.46 | | | | |
| *R. biconica* | NMBE_567164 | Switzerland | Bannalp Schonegg | 46.87 | 8.47 | 2234 | MW435154 | MW433778 | MW433799 |
| *R. biconica* | NMBE_567165 | Switzerland | Bannalp Schonegg | 46.87 | 8.47 | 2234 | MW435155 | MW433779 | MW433800 |
| *R. biconica* * | NMBE_567167 | Switzerland | Chaiserstuel | 46.88 | 8.47 | 2263 | MW435156 | MW433780 | MW433801 |
| *R. biconica* | NMBE_567168 | Switzerland | Chaiserstuel | 46.88 | 8.47 | 2263 | MW435157 | MW433781 | MW433802 |
| *R. biconica* | NMBE_567149 | Switzerland | Wissberg I | 46.81 | 8.47 | 2335 | MW435158 | MW433782 | MW433803 |
| *R. biconica* * | NMBE_567150 | Switzerland | Wissberg I | 46.81 | 8.47 | 2335 | MW435159 | MW433783 | MW433804 |
| *R. biconica* | NMBE_567152 | Switzerland | Wissberg II | 46.81 | 8.36 | 2355 | MW435160 | MW433784 | MW433805 |
| *R. biconica* | NMBE_567153 | Switzerland | Wissberg II | 46.81 | 8.36 | 2355 | MW435161 | MW433785 | MW433806 |
| *R. biconica* | NMBE_567155 | Switzerland | Widderfeld I | 46.84 | 8.33 | 2120 | MW435162 | MW433786 | MW433807 |
| *R. biconica* * | NMBE_567156 | Switzerland | Widderfeld I | 46.84 | 8.33 | 2120 | MW435163 | MW433787 | MW433808 |
| *R. biconica* | NMBE_567159 | Switzerland | Widderfeld II | 46.83 | 8.33 | 2290 | MW435164 | MW433788 | MW433809 |
| *R. biconica* | NMBE_567161 | Switzerland | Brisen I | 46.90 | 8.45 | 2045 | MW435165 | MW433789 | MW433810 |
| *R. biconica* | NMBE_567137 | Switzerland | Brisen I | 46.90 | 8.45 | 2045 | MW435166 | MW433790 | MW433811 |
| *R. biconica* | NMBE_567139 | Switzerland | Brisen II | 46.90 | 8.46 | 2130 | MW435167 | MW433791 | MW433812 |
| *R. biconica* | NMBE_567140 | Switzerland | Brisen II | 46.90 | 8.46 | 2130 | MW435168 | MW433792 | MW433813 |
| *R. biconica* | NMBE_567142 | Switzerland | Brisen III | 46.91 | 8.47 | 2090 | MW435169 | MW433793 | MW433814 |
| *R. biconica* | NMBE_567143 | Switzerland | Brisen III | 46.91 | 8.47 | 2090 | MW435170 | MW433794 | MW433815 |
| *R. biconica* | NMBE_567145 | Switzerland | Gitschen I | 46.88 | 8.57 | 1890 | MW435171 | MW433795 | MW433816 |
| *R. biconica* | NMBE_567146 | Switzerland | Gitschen I | 46.88 | 8.57 | 1890 | MW435172 | MW433796 | MW433817 |
| *R. biconica* * | NMBE_567148 | Switzerland | Gitschen II | 46.88 | 8.57 | 1970 | MW435173 | MW433797 | MW433818 |
| *R. biconica* | NMBE_567162 | Switzerland | Gitschen II | 46.88 | 8.57 | 1970 | MW435174 | MW433798 | MW433819 |
| *T. villosus* | NMBE_568160 | Switzerland | Torgon, La Cheurgne | 46.31 | 6.85 | 1282 | | | |
| *T. albulus* | NMBE_568161 | Switzerland | Torgon, La Cheurgne | 46.31 | 6.85 | 1282 | | | |
| *T. villosus* | NMBE_568164 | Switzerland | Torgon, La Cheurgne | 46.31 | 6.85 | 1318 | | | |
| *T. villosus* | NMBE_568167 | Switzerland | Torgon, La Cheurgne | 46.31 | 6.85 | 1342 | | | |
| *T. hispidus* | NMBE_568099 | Sweden | prov. Uppland, Uppsala, Linnaeus Garden | 59.86 | 17.63 | | | | |
| *T. hispidus* * | NMBE_568100 | Sweden | prov. Uppland, Uppsala, Linnaeus Garden | 59.86 | 17.63 | | ON477947 | - | - |

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|---|---|---|---|---|---|---|---|---|---|
| *T. hispidus* | NMBE_568107 | Sweden | prov. Västergötland, Boras, Rya asar | 57.74 | 12.92 | | | | |
| *T. villersii ** | NMBE_568108 | Sweden | prov. Västergötland, Boras, Rya asar | 57.74 | 12.92 | | | | |
| *T. hispidus ** | NMBE_568103 | Sweden | prov. Östergötland, Vist | 58.33 | 15.73 | | ON477948 | ON479908 | ON479901 |
| *T. villersii* | NMBE_568104 | Sweden | prov. Östergötland, Vist | 58.33 | 15.73 | | | | |
| *T. villosus* | MNHW_A.EE_100 | Austria | Ebenwald-Egg | 47.43 | 9.92 | | MW774452 | MW772262 | - |
| *T. villosus †* | MNHW_A.Me_101 | Austria | Mellau | 47.35 | 9.97 | | MW774457 | MW772268 | - |
| *T. villosus* | MNHW_A.Dor_102 | Austria | Dornbündt | 47.45 | 10.01 | | MW774451 | MW772261 | - |
| *T. villosus* | MNHW_A.Bo_103 | Austria | Bödele | 47.42 | 9.81 | | MW774448 | MW772258 | MW775722 |
| *T. villosus* | MNHW_A.Da_104 | Austria | Damüls | 47.29 | 9.89 | | MW774449 | MW772259 | MW775723 |
| *T. villosus †* | MNHW_A.MtK_105 | Austria | Mt Kojenkopf | 47.33 | 9.88 | | MW774459 | MW772270 | MW775731 |
| *T. villosus †* | MNHW_A.MtB_106 | Austria | Mt Baumgarten | 47.40 | 9.94 | | MW774458 | MW772269 | MW775730 |
| *T. villosus* | MNHW_G.15.51_107 | Germany | Fützen | 47.82 | 8.51 | | MW774505 | MW772320 | MW775786 |
| *T. villosus †* | MNHW_G.15.50a_108 | Germany | Buchberg 2 | 47.83 | 8.53 | | MW774504 | MW772319 | MW775785 |
| *T. villosus †* | MNHW_G.15.52_109 | Germany | Geisingen | 47.92 | 8.65 | | MW774506 | MW772321 | MW775787 |
| *T. villosus* | MNHW_G.15.53_110 | Germany | Willingendorf | 48.21 | 8.61 | | MW774507 | MW772322 | MW775788 |
| *T. villosus* | MNHW_G.Bi_111 | Germany | Bittenbrunn | 48.74 | 11.16 | | MW774508 | MW772323 | MW775789 |
| *T. villosus* | MNHW_G.Im_112 | Germany | Immendingen | 47.93 | 8.75 | | MW774510 | MW772325 | MW775790 |
| *T. villosus ** | MNHW_G.Dil_113 | Germany | Dillingen a.d. Donau | 48.57 | 10.50 | | MW774509 | MW772324 | |
| *T. villosus* | MNHW_S.15.49_01 | Switzerland | Brisen | 46.90 | 8.45 | | - | MW772354 | MW775833 |
| *T. villosus* | MNHW_S.15.49_02 | Switzerland | Brisen | 46.90 | 8.45 | | MW774555 | MW772355 | MW775834 |
| *T. albulus* | MNHW_A.Dor_02 | Austria | Dornbündt | 47.45 | 10.01 | | - | MW772260 | - |
| *T. sericeus* | MNHW_A.Kap_03 | Austria | Kapf n. Egg | 47.42 | 9.95 | | MW774455 | MW772265 | - |
| *T. sericeus* | MNHW_A.Kap_04 | Austria | Kapf n. Egg | 47.42 | 9.95 | | MW774456 | MW772266 | MW775727 |
| *T. clandestinus †* | MNHW_A.Me_05 | Austria | Mellau | 47.35 | 9.97 | | - | MW772267 | MW775728 |
| *T. clandestinus* | MNHW_A.FA_07 | Austria | Forst-Au | 47.33 | 9.97 | | MW774453 | MW772263 | MW775725 |
| *T. clandestinus* | MNHW_A.FA_08 | Austria | Forst-Au | 47.33 | 9.97 | | MW774454 | MW772264 | MW775726 |
| *T.* sp.3 *†* | MNHW_F.15.09_09 | France | between Aubure & La Petite Verrerie | 48.21 | 7.25 | | MW774460 | MW772271 | MW775732 |
| *T.* sp.3 *†* | MNHW_F.15.09_10 | France | between Aubure & La Petite Verrerie | 48.21 | 7.25 | | MW774461 | MW772272 | MW775733 |
| *T. clandestinus †** | MNHW_F.15.11_11 | France | Tellure | 48.21 | 7.13 | | MW774462 | MW772273 | MW775734 |
| *T. clandestinus †* | MNHW_F.15.11_12 | France | Tellure | 48.21 | 7.13 | | - | MW772274 | MW775735 |
| *T. clandestinus †* | MNHW_F.15.14_13 | France | Source de la Moselle n. Bussang | 47.89 | 6.88 | | MW774463 | MW772275 | MW775736 |

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|---|---|---|---|---|---|---|---|---|---|
| *T. clandestinus †* | MNHW_F.15.14_14 | France | Source de la Moselle n. Bussang | 47.89 | 6.88 | | MW774464 | MW772276 | MW775737 |
| *T. clandestinus †* | MNHW_F.15.15_15 | France | Bussang | 47.87 | 6.83 | | MW774465 | MW772277 | MW775738 |
| *T. clandestinus †* | MNHW_F.15.15_16 | France | Bussang | 47.87 | 6.83 | | MW774466 | MW772278 | MW775739 |
| *T. clandestinus †* | MNHW_F.15.15_17 | France | Bussang | 47.87 | 6.83 | | MW774467 | MW772279 | MW775740 |
| *T. albulus* | NMBE_568077 | Switzerland | Vex | 46.19 | 7.41 | 654 | | | |
| *T. albulus* | NMBE_568079 | Switzerland | Bex, Mines de Sel | 46.28 | 7.03 | 598 | | | |
| *T. albulus †* | NMBE_568084 | Switzerland | St. Maurice, Grotte aux Fées | 46.22 | 7.00 | 455 | | | |
| *T. albulus* | NMBE_568086 | Switzerland | Martigny, Amphitheater | 46.09 | 7.07 | 497 | | | |
| *T. albulus* | NMBE_568087 | Switzerland | Martigny, Amphitheater | 46.09 | 7.07 | 497 | | | |
| *T. albulus* | NMBE_568089 | Switzerland | Vex, Römerbrücke | 46.19 | 7.41 | 670 | | | |
| *T. plebeius* | NMBE_568094 | Switzerland | Lac du Mont d'Orge | 46.23 | 7.33 | 624 | ON477946 | ON479907 | ON479900 |
| *T. plebeius* | NMBE_568095 | Switzerland | Lac du Mont d'Orge | 46.23 | 7.33 | 624 | | | |
| *T. clandestinus †* | NMBE_569597 | Switzerland | Bern-Bümpliz, Schlosspark | 46.94 | 7.39 | 540 | | | |
| *T. clandestinus †* | NMBE_569598 | Switzerland | Bern-Bümpliz, Schlosspark | 46.94 | 7.39 | 540 | | | |
| *T. albulus* | NMBE_569671 | Switzerland | Münsingen | 46.87 | 7.54 | | | | |
| *T. villosus †* | NMBE_569638 | Switzerland | Broc, Waldlichtung | 46.61 | 7.09 | 744 | | | |
| *T. plebeius* | NMBE_569672 | Switzerland | Bulle, Schlosspark | 46.62 | 7.06 | 777 | | | |
| *T. plebeius* | NMBE_569673 | Switzerland | Bulle, Schlosspark | 46.62 | 7.06 | 777 | | | |
| *T. clandestinus* | NMBE_569674 | Switzerland | Châtel-sur-Montsalvens, Staumauer | 46.61 | 7.14 | 847 | | | |
| *T. albulus* | NMBE_569675 | Switzerland | Châtel-sur-Montsalvens, Staumauer | 46.61 | 7.14 | 847 | | | |
| *T. striolatus* | NMBE_569618 | Germany | Stormsdorf, NSG Reppeliner Bachtal | 54.07 | 12.45 | | | | |
| *T. sericeus* | NMBE_569620 | Germany | Insel Poel | 54.00 | 11.38 | | | | |
| *T. striolatus* | NMBE_569641 | Germany | Gross Woltersdorf | 53.87 | 11.39 | | | | |
| *T. sericeus* | NMBE_569643 | Germany | Beidendorf | 53.82 | 11.39 | | | | |
| *T.* sp.3 * | NMBE_569645 | Germany | Greese | 53.87 | 11.54 | | | | |
| *T. striolatus* | NMBE_569647 | Germany | Tarnewitzer Bach | 53.95 | 11.22 | | | | |
| *T. sericeus* * | NMBE_569649 | Germany | Wüstenmark | 53.77 | 11.25 | | | | |
| *T. striolatus* * | NMBE_569651 | Germany | Flemendorf, Kirche | 54.33 | 12.80 | | | | |
| *T.* sp.2 * | NMBE_569655 | Germany | Rügen | 54.50 | 13.57 | | | | |
| *T. sericeus* | MNHW_Ka-3 | Germany | Kaisheim | 48.73 | 10.87 | 401 | | | |
| *T. sericeus* | MNHW_La-7 | Germany | Lauingen | 48.56 | 10.42 | 443 | | | |
| *T. sericeus* | MNHW_G-Gu9 | Germany | Günzburg, Reisensburg | 48.47 | 10.30 | 436 | | | |
| *T. sericeus* | MNHW_Gm-1 | Germany | Gremheim | 48.63 | 10.65 | 445 | | | |
| *T. villosulus* | MNHW_SK.19.54_308 | Sloveniavakia | Hlboké 2 | 49.12 | 18.12 | 522 | | | |
| *T. villosulus †* | MNHW_PL.20.45_305 | Poland | Pieskowa Skała (PN) | 50.24 | 19.78 | 435 | | | |
| *T. villosulus †* | MNHW_PL.20.32_307 | Poland | Muszyna 2 | 49.50 | 20.92 | 477 | | | |
| *T. villosulus †** | MNHW_PL.20.16_306 | Poland | Wapowce | 49.80 | 22.65 | 213 | | | |

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|---|---|---|---|---|---|---|---|---|---|
| *T. plebeius* | MNHW_S.15.48_309 | Switzerland | Chateau-D'Oex | 46.47 | 7.13 | 933 | | | |
| *T. albulus* | MNHW_S.15.48_311 | Switzerland | Chateau-D'Oex | 46.47 | 7.13 | 933 | | | |
| *T. albulus* | MNHW_S.15.48_310 | Switzerland | Chateau-D'Oex | 46.47 | 7.13 | 933 | | | |
| *T. sericeus* | MNHW_Ei6 | Germany | Eichberg | 47.85 | 8.51 | 829 | | | |
| *T. becasis* | MNHW_F.18.48_302 | France | Arties near Auzat | 42.74 | 1.48 | 984 | | | |
| *T. becasis †** | MNHW_F.18.30_299 | France | Forêt Bac Estable 2 | 42.78 | 2.26 | 1391 | | | |
| *T. becasis †* | MNHW_F.18.37_301 | France | Forêt de Beleste | 42.89 | 1.97 | 844 | | | |
| *T. becasis †* | MNHW_F.18.35_300 | France | Le Chandelier | 42.88 | 2.03 | 826 | | | |
| *T. becasis* | MNHW_F.18.52_303 | France | Saint-Martin-Lys | 42.83 | 2.23 | 333 | | | |
| *T. clandestinus †* | MNHW_F.VM_295 | France | Vallon du Moulinot | 47.18 | 5.95 | | | | |
| *T. clandestinus †* | MNHW_F.RP_297 | France | La Roche des Pins | 47.11 | 6.15 | | | | |
| *T. clandestinus †* | MNHW_F.VM_294 | France | Vallon du Moulinot | 47.18 | 5.95 | | | | |
| *T. clandestinus †* | MNHW_F.ML_296 | France | Mont de Lune | 47.15 | 5.95 | | | | |
| *T. striolatus* | MNHW_S.Sch_07 | Switzerland | Schleitheim | 47.76 | 8.50 | 508 | | | |
| *T. striolatus †* | MNHW_A.17.12_01 | Austria | Schafberg | 47.78 | 13.43 | 1767 | | | |
| *T. striolatus †* | MNHW_I.16.12_01 | Ireland | Maam | 53.51 | -9.53 | 20 | | | |
| *T. striolatus †** | MNHW_SK.19.71_01 | Slovakia | Varín | 49.20 | 18.87 | 406 | | | |
| *T. striolatus †** | MNHW_F.18.10_06 | France | Blecquenecques n. Marquise | 50.82 | 1.73 | 26 | | | |
| *T. striolatus* | MNHW_SK.19.63_01 | Slovakia | Medveďov | 47.80 | 17.65 | 115 | | | |
| *T. striolatus †* | MNHW_GB.Br_01 | United Kingdom | Bramley near Basingstoke | 51.33 | -1.06 | 62 | | | |
| *T. striolatus* | MNHW_G.Ri_02 | Germany | Riedensheim | 48.74 | 11.11 | 387 | | | |
| *T. albulus ** | MNHW_F.LaD_24 | France | Là Diat | 45.34 | 5.80 | 722 | | | |
| *T. albulus* | MNHW_F.LeP_34 | France | Le Pont du Lac | 45.43 | 5.88 | 750 | | | |
| *T. albulus* | MNHW_F.Sa_34 | France | Sassenage | 45.21 | 5.65 | 252 | | | |
| *T. hispidus †* | MNHW_F.18.25_279 | France | Escames | 49.55 | 1.81 | 134 | | | |
| *T. hispidus †* | MNHW_F.18.07_278 | France | Beaucamps-Le-Jeune | 49.82 | 1.77 | 199 | | | |
| *T.* sp.3 | MNHW_F.15.13_276 | France | Hoft Cascade du Hohwald | 48.40 | 7.30 | 676 | | | |
| *T. hispidus †* | MNHW_F.18.05_277 | France | Fouquenies | 49.46 | 2.06 | 29 | | | |
| *T.* sp.4 | MNHW_F.CC_284 | France | Combe aux Cordiers | 47.40 | 5.93 | | | | |
| *T.* sp.4 * | MNHW_F.SF_282 | France | Source de la Foulotière | 47.13 | 5.48 | | | | |
| *T. albulus †* | MNHW_F.LE_280 | France | Les Essarts | 46.93 | 5.32 | | | | |
| *T. montanus* | MNHW_F.CP_291 | France | Colline Planoise | 47.21 | 5.98 | | | | |
| *T. montanus †* | MNHW_F.LR_290 | France | La Ruchotte | 47.14 | 5.49 | | | | |
| *T. montanus* | MNHW_F.Soy_292 | France | Soyère | 47.31 | 6.81 | | | | |
| *T. villersii* | MNHW_F.BS_293 | France | Bord de Saône | 47.47 | 5.65 | | | | |
| *T. albulus* | MNHW_So4 | Germany | Sommerau | 47.81 | 8.27 | 878 | | | |
| *T. sericeus †* | MNHW_Re5 | Germany | Reischenau | 48.32 | 10.59 | 468 | | | |

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|---|---|---|---|---|---|---|---|---|---|
| *T. albulus †** | MNHW_G.15.05_285 | Germany | Ketsch | 49.37 | 8.52 | 107 | | | |
| *T. sericeus* | MNHW_G.17.19_286 | Germany | Ramsau b. Berchtesgaden | 47.61 | 12.94 | 621 | | | |
| *T. albulus* | MNHW_S.Ros_288 | Switzerland | Rosern | 47.50 | 7.68 | 531 | | | |
| *T. albulus* | MNHW_S.Fr_287 | Switzerland | Froburg | 47.38 | 7.89 | 833 | | | |
| *T. albulus †* | MNHW_RF4 | Switzerland | Ruine Farnsburg | 47.49 | 7.87 | 721 | | | |
| *T.* sp.1 *†* | MNHW_St-14 | Germany | Staubing | 48.89 | 11.81 | 355 | | | |
| *T. sericeus †* | MNHW_G.17.05_298 | Germany | Pfatter | 48.97 | 12.39 | 283 | | | |
| *T.* sp.3 | MNHW_Kl-2 | Germany | Kleinmehring | 48.76 | 11.54 | 378 | | | |
| *T.* sp.3 * | MNHW_Kle-8 | Germany | Kleinprüfening | 49.01 | 12.03 | 326 | | | |
| *T. albulus †** | MNHW_Sau1 | Austria | Sausteig | 47.44 | 10.02 | 994 | | | |
| *T. albulus* | MNHW_Sau2 | Austria | Sausteig | 47.44 | 10.02 | 994 | | | |
| *T.* sp.4 | MNHW_A.17.15_271 | Austria | Schlenken 2 | 47.68 | 13.21 | 1398 | | | |
| *T.* sp.2 *†* | MNHW_CZ.Vit_274 | Czech Republic | Vitkovice | 50.69 | 15.53 | 614 | | | |
| *T.* sp.2 *†** | MNHW_CZ.Lan_273 | Czech Republic | Lánov | 50.64 | 15.67 | 593 | | | |
| *T.* sp.2 *†** | MNHW_CZ.CD_272 | Czech Republic | Černý Důl | 50.65 | 15.71 | 667 | | | |
| *T. villersii †* | MNHW_CZ.19.51_275 | Czech Republic | Radňovice | 49.56 | 16.02 | 610 | | | |
| *T. montanus †* | MNHW_F.SF_283 | France | Source de la Foulotière | 47.13 | 5.48 | | | | |
| *T. clandestinus* | MNHW_F.LE_281 | France | Les Essarts | 46.93 | 5.32 | | | | |
| *T. montanus †* | MNHW_F.Lcr_289 | France | La croix rouge | 47.23 | 6.74 | | | | |
| *T. villersii †* | MNHW_PL.20.09_252 | Poland | Leśna Podlaska | 52.12 | 23.05 | 143 | | | |
| *T. villosulus* | MNHW_PL.19.18_254 | Poland | Jakubowice near AnnoPoland | 50.90 | 21.84 | 164 | | | |
| *T. villosulus †* | MNHW_PL.20.13_253 | Poland | Gamlówka | 50.56 | 23.14 | | | | |
| *T. villersii †** | MNHW_PL.20.07_251 | Poland | Budy Leśne n. Białowieża | 52.72 | 23.72 | 167 | | | |
| *T. villersii †* | MNHW_PL.19.10_250 | Poland | Mątki near Sztum | 53.85 | 19.00 | 62 | | | |
| *T. villersii †* | MNHW_PL.Ro_247 | Poland | Puszcza Romincka | 54.34 | 22.68 | | | | |
| *T. villersii †* | MNHW_PL.20.04_248 | Poland | Rapa | 54.31 | 22.02 | 107 | | | |
| *T. villosulus †** | MNHW_PL.20.05_249 | Poland | Cisowy Jar | 54.16 | 22.35 | 265 | | | |
| *T.* sp.4 | MNHW_HR.17.134_209 | Croatia | Lokve | 45.36 | 14.73 | 721 | | | |
| *T.* sp.4 * | MNHW_HR.17.108_206 | Croatia | Rosulje near Croatiaatska Kostajnica | 45.23 | 16.59 | 91 | | | |
| *T.* sp.4 | MNHW_HR.17.126_208 | Croatia | Ogulin (Dobra river) | 45.26 | 15.21 | 327 | | | |
| *T.* sp.4 | MNHW_HR.17.110_207 | Croatia | Slunij | 45.12 | 15.60 | 267 | | | |
| *T.* sp.4 | MNHW_CZ.17.33_210 | Czech Republic | Špraněk Brook valley | 49.67 | 16.90 | 430 | | | |
| *T.* sp.4 *†** | MNHW_CZ.19.78_218 | Czech Republic | Pitárné | 50.24 | 17.58 | 405 | | | |
| *T. villersii †* | MNHW_CZ.17.145_212 | Czech Republic | Závist | 49.38 | 16.58 | 403 | | | |
| *T.* sp.4 *†* | MNHW_CZ.19.35_213 | Czech Republic | Srbsko 3 | 49.94 | 14.15 | 280 | | | |
| *T.* sp.4 | MNHW_CZ.19.40_215 | Czech Republic | Brejl | 50.10 | 13.88 | 337 | | | |

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|---|---|---|---|---|---|---|---|---|---|
| *T.* sp.4 | MNHW_CZ.19.77_217 | Czech Republic | Skřipov | 49.83 | 17.89 | 409 | | | |
| *T.* sp.1 †* | MNHW_CZ.19.38_214 | Czech Republic | Štěchovice (Maják) | 49.85 | 14.39 | 198 | | | |
| *T. villersii †* | MNHW_CZ.19.43_216 | Czech Republic | Debrník | 49.12 | 13.24 | 725 | | | |
| *T. villersii* | MNHW_CZ.17.34_211 | Czech Republic | Březina | 49.67 | 16.92 | 402 | | | |
| *T. villersii †* | MNHW_PL.19.27_242 | Poland | Będzin | 50.35 | 19.17 | 258 | | | |
| *T. villersii* | MNHW_PL.Lo_240 | Poland | Łopuszna | 49.50 | 20.13 | 720 | | | |
| *T. villersii* | MNHW_PL.19.24_241 | Poland | Lipnica Dolna 2 | 49.86 | 20.55 | 280 | | | |
| *T. villersii ** | MNHW_UK.En-HW_19 | United Kingdom | Hayley Wood | 52.16 | -0.12 | 84 | | | |
| *T. villersii †* | MNHW_UK.En-D_5 | United Kingdom | Downside | 51.39 | -2.72 | 160 | | | |
| *T.* sp.4 | MNHW_A.17.144_205 | Austria | Oberdorf | 47.41 | 15.22 | 504 | | | |
| *T. sericeus* | MNHW_A.Dor_200 | Austria | Dornbündt | 47.45 | 10.01 | 880 | | | |
| *T. sericeus †* | MNHW_A.Gens_201 | Austria | Genabend-Sibratsgfäll | 47.44 | 10.01 | 1042 | | | |
| *T.* sp.2 †* | MNHW_A.17.28_203 | Austria | Durnbachtal | 47.85 | 16.02 | 613 | | | |
| *T.* sp.3 †* | MNHW_A.Schw_202 | Austria | Schwarzenberg-Loch 2 | 47.40 | 9.85 | 619 | | | |
| *T.* sp.4 | MNHW_A.17.21_204 | Austria | Präbichl 2 | 47.52 | 14.95 | 1235 | | | |
| *T. villersii* | MNHW_PL.19.87_238 | Poland | Chwalisław | 50.43 | 16.83 | 444 | | | |
| *T. villersii* | MNHW_PL.JW31 | Poland | Wrocław-Jarnołtów | 51.12 | 16.84 | 112 | | | |
| *T. villersii* | MNHW_PL.17.03_239 | Poland | Ratnowice near Otmuchów | 50.43 | 17.13 | 219 | | | |
| *T. villersii* | MNHW_PL.JW33 | Poland | Wrocław-Jarnołtów | 51.12 | 16.84 | 112 | | | |
| *T. villersii* | MNHW_PL.JW32 | Poland | Wrocław-Jarnołtów | 51.12 | 16.84 | 112 | | | |
| *T. villersii †* | MNHW_PL.19.82_236 | Poland | Wojcieszów Górny | 50.93 | 15.92 | 391 | | | |
| *T. villersii* | MNHW_PL.19.84_237 | Poland | Sokolec | 50.66 | 16.48 | 513 | | | |
| *T. villersii †** | MNHW_NL.19.03_234 | Netherlands | Veere 2 | 51.55 | 3.65 | 6 | | | |
| *T. villersii †* | MNHW_NL.Ost_233 | Netherlands | Oestgeest near Leiden | 52.18 | 4.46 | 1 | | | |
| *T. villersii* | MNHW_NL.03_235 | Netherlands | Veere 2 | 51.55 | 3.65 | 6 | | | |
| *T. hispidus †* | MNHW_F.18.14_225 | France | Larré | 50.68 | 2.06 | 65 | | | |
| *T. hispidus †* | MNHW_F.18.24_223 | France | Le Mont Gournoy 2 (Forêt Indivise d'Eu) | 49.80 | 1.59 | 217 | | | |
| *T. hispidus ** | MNHW_F.18.20_222 | France | Froise | 50.28 | 1.63 | 86 | | | |
| *T. pascali †* | MNHW_F.Col_228 | France | Col. De Vac. | 46.27 | 5.55 | | | | |
| *T. hispidus †* | MNHW_F.18.08_221 | France | Eaucourt-Sur-Somme | 50.06 | 1.88 | 11 | | | |
| *T. plebeius †* | MNHW_F.Com_227 | France | Chameyer | 44.62 | 6.50 | | | | |
| *T. villersii †** | MNHW_F.18.12_224 | France | Licques | 50.80 | 1.94 | 81 | | | |
| *T. villersii* | MNHW_F.18.17_226 | France | Cap Gris-Nez | 50.87 | 1.58 | 58 | | | |
| *T. villersii †* | MNHW_F.15.07_219 | France | Bertrange | 49.32 | 6.17 | 164 | | | |
| *T. hispidus †* | MNHW_I.16.8_231 | Ireland | Lough Gur | 52.52 | -8.52 | 66 | | | |

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|---|---|---|---|---|---|---|---|---|---|
| *T. hispidus †* | MNHW_I.16.4_230 | Ireland | Rock of Cashel | 52.52 | -7.89 | 116 | | | |
| *T. hispidus †* | MNHW_I.16.3_229 | Ireland | Monastery | 53.20 | -6.17 | 126 | | | |
| *T. hispidus †** | MNHW_I.16.10_232 | Ireland | Bealaclugga | 53.13 | -9.07 | 31 | | | |
| *T. plebeius* | MNHW_F.GrCht_42 | France | Grande Chartreuse | 45.37 | 5.79 | 851 | | | |
| *T. plebeius* | MNHW_F.GrCht_43 | France | Grande Chartreuse | 45.37 | 5.79 | 851 | | | |
| *T. plebeius* | MNHW_F.GrCht_41 | France | Grande Chartreuse | 45.37 | 5.79 | 851 | | | |
| *T. plebeius* | MNHW_F.GrCht_44 | France | Grande Chartreuse | 45.37 | 5.79 | 851 | | | |
| *T. villosulus* | MNHW_UA.18.57_266 | Ukraine | Podwysokie | 49.43 | 24.59 | 261 | | | |
| *T. villosulus ** | MNHW_UA.18.68_269 | Ukraine | Lazeschyna | 48.30 | 24.44 | 839 | | | |
| *T. villosulus †* | MNHW_UA.18.55_264 | Ukraine | Bibrka | 49.64 | 24.30 | 301 | | | |
| *T. villosulus †* | MNHW_UA.18.58_267 | Ukraine | Mikuliczyn | 48.43 | 24.60 | 569 | | | |
| *T. villosulus †* | MNHW_UA.18.56_265 | Ukraine | Swirz | 49.65 | 24.43 | 315 | | | |
| *T. villosulus* | MNHW_UA.18.65_268 | Ukraine | Wierchowina | 48.15 | 24.84 | 604 | | | |
| *T. sericeus †** | MNHW_G.17.17_228 | Germany | Thannlehen b. Berchtesgaden | 47.64 | 13.09 | 1065 | | | |
| *T. sericeus †** | MNHW_G.Ott_227 | Germany | Ottendorf | 50.94 | 14.29 | 271 | | | |
| *T. dubia †* | MNHW_G-Mo5 | Germany | Moosburg a.d. Isar | 48.48 | 11.93 | 376 | | | |
| *T. sericeus* | MNHW_RW2 | Germany | Ruine Waldau | 48.16 | 8.41 | 751 | | | |
| *T. sericeus* | MNHW_RW3 | Germany | Ruine Waldau | 48.16 | 8.41 | 751 | | | |
| *T. sericeus* | MNHW_RW1 | Germany | Ruine Waldau | 48.16 | 8.41 | 751 | | | |
| *T. villersii †* | MNHW_PL.19.08_256 | Poland | Ostrów | 52.69 | 17.66 | 128 | | | |
| *T. villosulus †* | MNHW_PL.19.09_257 | Poland | Barcin-Górne Wolice | 52.84 | 17.93 | 95 | | | |
| *T. villersii †* | MNHW_PL.20.01_255 | Poland | Siemianice n. Kępno | 51.18 | 18.17 | 174 | | | |
| *T. villersii* | MNHW_PL.19.15_258 | Poland | Grabce Józefowskie | 51.99 | 20.50 | 169 | | | |
| *T.* sp.1 *†** | MNHW_SK.19.62_261 | Slovakia | Čičov (Čičovský les) | 47.78 | 17.70 | 137 | | | |
| *T.* sp.4 *†** | MNHW_SK.19.63_262 | Slovakia | Medveďov | 47.80 | 17.65 | 115 | | | |
| *T. villersii ** | MNHW_SK.19.73_263 | Sloveniavakia | Belá | 49.22 | 18.96 | 588 | | | |
| *T. villersii †* | MNHW_PL.20.23_246 | Poland | Równia | 49.41 | 22.59 | 498 | | | |
| *T. villersii †* | MNHW_PL.20.22_245 | Poland | Stebnik | 49.46 | 22.66 | 419 | | | |
| *T.* sp.4 * | MNHW_SLO.17.137_259 | Slovenia | Planina | 45.82 | 14.25 | 458 | | | |
| *T.* sp.4 *†* | MNHW_SLO.17.140_260 | Slovenia | Vintgar Gorge | 46.39 | 14.08 | 585 | | | |
| *T. villersii †* | MNHW_PL.20.34_243 | Poland | Szlachtowa 2 | 49.41 | 20.54 | 564 | | | |
| *T. villersii †* | MNHW_PL.20.44_244 | Poland | Owczary | 50.17 | 19.93 | 352 | | | |
| *T. clandestinus †* | NMBE_569600 | Switzerland | Schwarzwasser, Ufer | 46.86 | 7.37 | 608 | | | |
| *T. clandestinus* | NMBE_569601 | Switzerland | Schwarzwasser, Ufer | 46.86 | 7.37 | 608 | | | |
| *T. albulus †* | NMBE_569677 | Switzerland | Schwarzwasser, Ufer | 46.86 | 7.37 | 608 | | | |
| *T. clandestinus* | NMBE_569603 | Switzerland | Schwarzwasser, Brücke | 46.84 | 7.37 | 617 | | | |
| *T. clandestinus †* | NMBE_569604 | Switzerland | Schwarzwass | 46.84 | 7.37 | 617 | | | |

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|---|---|---|---|---|---|---|---|---|---|
| | | | er, Brücke | | | | | | |
| *T. albulus* | NMBE_569679 | Switzerland | Schwarzwasser, Brücke | 46.84 | 7.37 | 617 | | | |
| *T. albulus* | NMBE_569606 | Switzerland | Zürich | 47.39 | 8.52 | 400 | | | |
| *T. albulus †* | NMBE_569607 | Switzerland | Zürich | 47.39 | 8.52 | 400 | | | |
| *T. albulus †* | NMBE_569680 | Switzerland | Brugg, Friedhof, unterhalb Austriaobahnbrücke | 47.48 | 8.21 | 349 | | | |
| *T. albulus †* | NMBE_569681 | Switzerland | Brugg, Friedhof, unterhalb Austriaobahnbrücke | 47.48 | 8.21 | 349 | | | |
| *T. clandestinus †* | NMBE_569609 | Switzerland | Aarau, Parkanlage Grossratsgebäude | 47.39 | 8.05 | 348 | | | |
| *T. albulus* | NMBE_569683 | Switzerland | Brugg, Aareufer | 47.48 | 8.21 | 330 | | | |
| *T. albulus †* | NMBE_569684 | Switzerland | Brugg, Aareufer | 47.48 | 8.21 | 330 | | | |
| *T. clandestinus †* | NMBE_569611 | Switzerland | Sursee, Surepärkli | 47.17 | 8.11 | 483 | | | |
| *T. albulus †* | NMBE_569686 | Switzerland | Hallwil, Schloss | 47.32 | 8.20 | 446 | | | |
| *T. albulus †* | NMBE_569689 | Switzerland | Stansstad, Kieswerk, Flussufer | 46.96 | 8.33 | 462 | | | |
| *T. albulus* | NMBE_569690 | Switzerland | Stansstad, Kieswerk, Flussufer | 46.96 | 8.33 | 462 | | | |
| *T. villosus †* | NMBE_569639 | Switzerland | Stansstad, Kieswerk, Flussufer | 46.96 | 8.33 | 462 | | | |
| *T. albulus †* | NMBE_569616 | Switzerland | Zürich, Beckenhof | 47.38 | 8.54 | 430 | | | |
| *T. villosus †* | NMBE_569640 | Switzerland | Stans, Bürgenberg, Bürgenstock | 46.99 | 8.37 | 900 | | | |
| *T. plebeius* | NMBE_569635 | France | Villard d'arène | 45.04 | 6.35 | | | | |
| *T. plebeius* | NMBE_569636 | France | Villard d'arène | 45.04 | 6.35 | | | | |
| *T. plebeius* | NMBE_569632 | France | Rousset | 44.48 | 6.27 | | | | |
| *T. plebeius* | NMBE_569629 | France | Avonçon | 44.54 | 6.20 | | | | |
| *T. plebeius* | NMBE_569630 | France | Avonçon | 44.54 | 6.20 | | | | |
| *T. plebeius †* | NMBE_569626 | France | Avonçon | 44.54 | 6.20 | | | | |
| *T. plebeius* | NMBE_569627 | France | Avonçon | 44.54 | 6.20 | | | | |
| *T. plebeius* | NMBE_569660 | France | Chateauroux les alpes | 44.54 | 6.49 | | | | |
| *T. plebeius* | NMBE_569661 | France | Chateauroux les alpes | 44.54 | 6.49 | | | | |
| *T. plebeius* | NMBE_569664 | France | Crots | 44.54 | 6.48 | | | | |
| *T. plebeius †* | NMBE_569666 | France | Forest-Saint-Julien | 44.62 | 6.14 | | | | |
| *T. plebeius* | NMBE_569667 | France | Forest-Saint-Julien | 44.62 | 6.14 | | | | |
| *T. plebeius* | NMBE_569668 | France | Chateauroux les alpes | 44.54 | 6.49 | | | | |
| *T. plebeius* | NMBE_569669 | France | Chateauroux les alpes | 44.54 | 6.49 | | | | |
| *T. plebeius* | NMBE_569623 | France | Savines-le-Lac | 44.50 | 6.40 | | | | |
| *T. plebeius* | NMBE_569624 | France | Savines-le-Lac | 44.50 | 6.40 | | | | |
| *T. villosus* | NMSG-IM-28911 | Switzerland | Bad Ragaz, Schammboden, Jonaweg | 47.31 | 8.91 | | | | |
| *T. albulus* | NMBE_571090 | Switzerland | Aarburg, Born-Gratweg | 47.32 | 7.89 | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| *T. albulus* | NMBE_571088 | Switzerland | Aarburg, Tschärpisstrasse | 47.32 | 7.88 | | | | |
| *T. pascali* * | NMBE_571084 | Switzerland | Könizbergwald | 46.93 | 7.39 | 630 | | | |
| *T. albulus †* | NMBE_571095 | Switzerland | Grimentz | 46.18 | 7.57 | 1620 | | | |
| *T.* sp.3 | NMBE_571097 | Germany | Badenweiler, Vogelbachtal | 47.79 | 7.70 | | | | |
| *T. albulus* | NMBE_571102 | Switzerland | Mümliswil-Ramiswil, Naturpark Thal | 47.37 | 7.69 | 1000 | | | |
| *T. striolatus* * | NMBE_571105 | Germany | Kelkheim-Eppenhain, Naturpark Taunus | 50.17 | 8.38 | 350 | | | |
| *T. striolatus †* | NMBE_571107 | Germany | Rheinland-Palatinate, Welterod | 50.13 | 7.90 | | | | |
| *T. dubia †** | NMBE_571108 | Germany | Diessen am Ammersee | 47.95 | 11.11 | 544 | | | |
| *T. villosus* | NMBE_571110 | Germany | Schongau, Heidewiese | 47.79 | 10.87 | 730 | | | |
| *T. sericeus †** | NMBE_571112 | Denmark | Hovedstaden, Fureso Kommune | 55.78 | 12.44 | 22 | | | |
| *T. dubia †** | NMBE_571125 | United Kingdom | Carmel, near Swansea | 51.83 | -4.05 | | | | |
| *T. striolatus* * | NMBE_571126 | United Kingdom | Pontlliw | 51.70 | -4.01 | | | | |
| *T. becasis †* | NMBE_571128 | United Kingdom | Jersey | 49.21 | -2.13 | | | | |
| *T. albulus* | NMBE_571130 | Switzerland | Waldstatt | 47.36 | 9.30 | 416 | | | |
| *T. albulus* | NMBE_571132 | Switzerland | Oberriet | 47.33 | 9.50 | 867 | | | |
| *T. clandestinus †* | NMBE_571134 | Switzerland | Flühli | 46.81 | 7.98 | 1283 | | | |
| *T. albulus* | NMBE_571141 | Switzerland | Davos | 46.80 | 9.84 | | | | |
| *T. clandestinus †* | NMBE_571142 | Switzerland | Bergün Dorf | 46.63 | 9.75 | | | | |
| *T. clandestinus †* | NMBE_571234 | Switzerland | Amriswil, Kronberg | 47.56 | 9.29 | | | | |
| *T. clandestinus †* | NMBE_571235 | Switzerland | Bürglen, Schloss | 47.55 | 9.15 | | | | |
| *T. striolatus* | NMBE_571137 | Germany | Dortmund | 51.49 | 7.48 | | | | |
| *T. clandestinus †* | NMBE_571145 | Switzerland | Twannbachschlucht | 47.10 | 7.14 | | | | |
| *T. villosus* | NMBE_571139 | Germany | Weil, Rheinauen | 48.20 | 7.65 | | | | |
| *T.* sp.3 *†* | NMBE_571152 | Germany | Kelheim, | 48.81 | 11.74 | | | | |
| *T. plebeius* | NMBE_571154 | Germany | Weil, Rheinauen | 48.20 | 7.65 | | | | |
| *T. striolatus* | NMBE_571156 | Germany | Unna | 51.57 | 7.71 | | | | |
| *T. striolatus †* | NMBE_571158 | Germany | Mettmann, | 51.08 | 6.86 | | | | |
| *T. striolatus †* | NMBE_571160 | Germany | Dorsel, | 50.38 | 6.80 | | | | |
| *T. albulus* | NMBE_571164 | Germany | Weil, Rheinauen | 48.20 | 7.65 | | | | |
| *T. albulus* | NMBE_571166 | Switzerland | Bettlingen, | 47.57 | 7.68 | | | | |
| *T. sericeus* | NMBE_571168 | Italia | Südtirol, Wolkenstein Gröden | 46.56 | 11.78 | | | | |
| *T.* sp.2 | NMBE_571172 | Germany | Ilmkreis, Gehren, Schlosspark | 50.65 | 11.01 | | | | |
| *T. striolatus* | NMBE_571174 | Germany | Ostholstein bei Oldenburg | 54.12 | 10.61 | | | | |
| *T.* sp.4 | NMBE_571180 | Germany | Euskirchen, Blankenheim, Burg | 50.44 | 6.65 | | | | |
| *T. villosus* | NMBE_571201 | Switzerland | Säntis | 47.26 | 9.40 | | | | |
| *T. villosus †** | NMBE_571203 | Switzerland | Säntis | 47.25 | 9.38 | | | | |
| *T. villosus †* | NMBE_571206 | Switzerland | Säntis, | 47.27 | 9.38 | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| | | | Schäfler | | | | | | |
| *T. villosus †* | NMBE_571208 | Switzerland | Säntis, Ebenalp | 47.28 | 9.41 | | | | |
| *T. striolatus* | NMBE_571328 | Germany | Nettersheim | 50.50 | 6.63 | 470 | | | |
| *T. striolatus* | NMBE_571319 | Germany | Blankenheim | 50.44 | 6.65 | 495 | | | |
| *T. striolatus* | NMBE_571325 | Germany | Mechernich | 50.44 | 6.65 | | | | |
| *T. villersii* | NMBE_571321 | Belgium | Fonds de Lesse bei Dinant | 50.23 | 4.90 | 105 | | | |
| *T. villosus* | NMBE_571305 | Germany | Scheidegg | 47.59 | 9.84 | 695 | | | |
| *T. villosus* | NMBE_571323 | Germany | Griesheim | 49.87 | 8.57 | | | | |
| *T. albulus* | NMBE_571312 | Germany | Rheinaue | 49.82 | 8.44 | | | | |
| *T. clandestinus* | NMBE_571316 | Germany | Badenweiler | 47.81 | 7.70 | | | | |
| *T. villosus †* | NMBE_571237 | Switzerland | Untereggen, Altrüti SG | 47.45 | 9.43 | 570 | | | |
| *T. albulus* | NMBE_571238 | Switzerland | Homburg, Mosraa TG | 47.64 | 8.98 | 570 | | | |
| *T. sericeus* | NMBE_571239 | Switzerland | Untereggen, Altrüti SG | 47.45 | 9.43 | 570 | | | |
| *T. villersii* | NMBE_571211 | Germany | Berlin, Wannseebad weg | 52.45 | 13.18 | 30 | | | |
| *T. dubia ** | NMBE_571212 | Germany | Potsdam, Schlaatz, Milanhorst | 52.38 | 13.09 | 34 | | | |
| *T. villersii ** | NMBE_571213 | Germany | Potsdam, Wildpark | 52.38 | 12.97 | 34 | | | |
| *T. villersii* | NMBE_571216 | Germany | Klein Woltersdorf | 53.89 | 11.41 | 10 | | | |
| *T. striolatus †* | NMBE_571218 | Germany | Dutzower See, Nordspitze | 53.67 | 10.95 | 40 | | | |
| *T. villosulus* | NMBE_571220 | Czech Republic | Moravia, Bohuslavice u Zlina | 49.16 | 17.62 | 240 | | | |
| *T. villosulus ** | NMBE_571222 | Czech Republic | Moravia, Bohuslavice u Zlina | 49.16 | 17.62 | 240 | | | |
| *T.* sp.4 | NMBE_571224 | Czech Republic | Moravia, Zadverice | 49.22 | 17.79 | 260 | | | |
| *T.* sp.4 | NMBE_571225 | Czech Republic | Moravia, Zadverice | 49.22 | 17.79 | 260 | | | |
| *T. striolatus* | MNHW_GB.19.01_01 | United Kingdom | Diseworth | 52.82 | -1.33 | | | | |
| *T.* sp.5 | MNHW_G.Gre_01 | Germany | Greven | 52.11 | 7.60 | | | | |
| *T. striolatus* | MNHW_G.Gre_02 | Germany | Greven | 52.11 | 7.60 | | | | |
| *T. striolatus* | MNHW_G.Col_01 | Germany | Köln-Westhoven/Ensen | 50.90 | 7.03 | | | | |
| *T. striolatus* | MNHW_G.Col_02 | Germany | Köln-Westhoven/Ensen | 50.90 | 7.03 | | | | |
| *T. striolatus* | MNHW_G.Col_04 | Germany | Köln-Westhoven/Ensen | 50.90 | 7.03 | | | | |
| *T. striolatus* | MNHW_G.Col_20 | Germany | Köln-Westhoven/Ensen | 50.90 | 7.03 | | | | |
| *T. striolatus* | MNHW_G.Col_21 | Germany | Köln-Westhoven/Ensen | 50.90 | 7.03 | | | | |
| *T.* sp.4 | NMBE_571229 | Czech Republic | Moravia, Vápenky | 48.86 | 17.65 | 820 | | | |
| *T. albulus* | NMBE_571231 | Switzerland | Appenzell, Rüfen | 47.34 | 9.49 | 718 | | | |
| *T.* sp.6 | MNHN-IM-2013-77397 | France | Gonds | 45.70 | -0.59 | | | | |
| *T. becasis* | MNHN-IM-2013-76972 | France | Saint-Martin-Bideren | 43.40 | -0.97 | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| *T. albulus †* | MNHN-IM-2013-76922 | France | Saint-Laurent-du-Pont | 45.35 | 5.76 | | | | |
| *T. hispidus ** | MNHN-IM-2013-76909 | France | Bèze | 47.47 | 5.27 | | | | |
| *T.* sp.6 * | MNHN-IM-2013-77398 | France | Gonds | 45.70 | -0.59 | | | | |
| *T. pascali ** | MNHN-IM-2013-76928 | France | Bonlieu | 46.58 | 5.87 | | | | |
| *T. plebeius ** | MNHN-IM-2013-76924 | France | Aubres | 44.39 | 5.17 | | | | |
| *T. hispidus* | MNHN-IM-2013-76932 | France | Neuville | 50.50 | 3.07 | | | | |
| *T. plebeius* | MNHN-IM-2013-77045 | France | Molines-en-Queyras | 44.73 | 6.84 | | | | |
| *T. albulus* | MNHN-IM-2013-77391 | France | Avon | 48.41 | 2.73 | | | | |
| *T. plebeius ** | MNHN-IM-2013-77107 | France | Château-Ville-Vieille | 44.74 | 6.84 | | | | |
| *T. albulus* | MNHN-IM-2013-77259 | France | Villard-de-Lans | 45.07 | 5.53 | | | | |
| *T. becasis ** | MNHN-IM-2013-77000 | France | Lourdios-Ichère | 43.06 | -0.68 | | | | |
| *T. becasis ** | MNHN-IM-2013-76902 | France | Ahetze | 43.39 | -1.54 | | | | |
| *T. albulus* | MNHN-IM-2013-77392 | France | Avon | 48.41 | 2.73 | | | | |
| *T. albulus ** | MNHN-IM-2013-86901 | France | Saint-Projet | 44.30 | 1.79 | | | | |
| *T. plebeius ** | MNHN-IM-2013-77379 | France | Lussat | 46.18 | 2.32 | | | | |
| *T. plebeius ** | MNHN-IM-2013-77128 | France | Myennes | 47.45 | 2.93 | | | | |
| *T. dubia ** | MNHN-IM-2013-77383 | France | Lussat | 46.18 | 2.32 | | | | |
| *T. albulus* | MNHN-IM-2013-86900 | France | Saint-Projet | 44.30 | 1.79 | | | | |
| *T. hispidus* | MNHN-IM-2013-76914 | France | Châtillon-sur-Seine | 47.86 | 4.58 | | | | |
| *T. hispidus* | MNHN-IM-2013-76911 | France | Bèze | 47.47 | 5.27 | | | | |
| *T. albulus* | MNHN-IM-2013-77261 | France | Villard-de-Lans | 45.07 | 5.53 | | | | |
| *T. pascali* | MNHN-IM-2013-76918 | France | Saint-Claude | 46.40 | 5.88 | | | | |
| *T. plebeius* | MNHN-IM-2013-76907 | France | Champcella | 44.71 | 6.57 | | | | |
| *T. plebeius* | MNHN-IM-2013-77046 | France | Molines-en-Queyras | 44.73 | 6.84 | | | | |
| *T. albulus †* | MNHN-IM-2013-77127 | France | Myennes | 47.45 | 2.93 | | | | |
| *T. becasis* | MNHN-IM-2013-77376 | France | Beaugency | 47.80 | 1.62 | | | | |
| *T. villosus* | MNHN-IM-2013-76921 | France | Septmoncel les Molunes | 46.36 | 5.90 | | | | |
| *T. pascali* | MNHN-IM-2013-76929 | France | Bonlieu | 46.58 | 5.87 | | | | |
| *T. plebeius* | MNHN-IM-2013-76904 | France | Champcella | 44.71 | 6.57 | | | | |
| *T. plebeius* | MNHN-IM-2013-76899 | France | Laye | 44.65 | 6.09 | | | | |
| *T. becasis* | MNHN-IM-2013-76896 | France | Perreux | 46.04 | 4.12 | | | | |
| *T. pascali* | MNHN-IM-2013-76919 | France | Saint-Claude | 46.40 | 5.88 | | | | |
| *T. becasis ** | MNHN-IM-2013-77378 | France | Beaugency | 47.80 | 1.62 | | | | |
| *T. hispidus* | MNHN-IM-2013-76915 | France | Châtillon-sur-Seine | 47.86 | 4.58 | | | | |
| *T. albulus* | MNHN-IM-2013-76884 | France | Paris | 48.84 | 2.36 | | | | |
| *T. albulus ** | MNHN-IM-2013-76886 | France | Paris | 48.84 | 2.36 | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| *T. plebeius* | MNHN-IM-2013-77108 | France | Château-Ville-Vieille | 44.74 | 6.84 | | | | |
| *T. plebeius* | MNHN-IM-2013-77154 | France | Réallon | 44.60 | 6.34 | | | | |
| *T. plebeius* | MNHN-IM-2013-77177 | France | Fley | 46.67 | 4.65 | | | | |
| *T. plebeius* | MNHN-IM-2013-77178 | France | Fley | 46.67 | 4.65 | | | | |
| *T. hispidus* | MNHN-IM-2013-76935 | France | Neuville | 50.50 | 3.07 | | | | |
| *T. plebeius* | MNHN-IM-2013-76925 | France | Aubres | 44.39 | 5.17 | | | | |
| *T. plebeius* | MNHN-IM-2013-76900 | France | Laye | 44.65 | 6.09 | | | | |
| *T. plebeius †* | MNHN-IM-2013-76890 | France | Guillestre | 44.65 | 6.65 | | | | |
| *T. plebeius* | MNHN-IM-2013-76891 | France | Guillestre | 44.65 | 6.65 | | | | |
| *T. albulus* | MNHN-IM-2013-76894 | France | Perreux | 46.04 | 4.12 | | | | |
| *T.* sp.5 * | NMBE_571337 | Germany | Bielefeld | 52.10 | 8.53 | 129 | | | |
| *T.* sp.5 *†* | NMBE_571329 | Germany | Herford | 52.15 | 8.60 | 75 | | | |
| *T.* sp.5 *†** | NMBE_571333 | Germany | Minden | 52.38 | 8.79 | 59 | | | |
| *T. clandestinus* | NMBE_571315 | Austria | Feldkirch, Schlattenburg | 47.24 | 9.60 | | | | |
| *T. plebeius* | NMBE_569657 | France | Freissinières | 44.74 | 6.49 | | | | |
| *T. plebeius* | NMBE_569658 | France | Freissinières | 44.74 | 6.49 | | | | |

**Supplementary material II:** Shell measurements and ratios of the genetically investigated adult specimens. Abbreviations used: W = shell width, H = shell height, bwH = body whorl height, h = aperture height, w = aperture width, D = shell diameter, U = major umbilicus width, u = minor umbilicus width, whl = number of whorls. All measurements are in millimetres.

| Data base nr° | locality | W | H | bwH | h | w | D | U | u | whl | H/W | U/D | u/U | bwH/H | h/w |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MNHW_S.15.19_10 | Gorges de Court 3 | 8.46 | 5.21 | 4.22 | 2.87 | 4.57 | 8.66 | 0.92 | 0.82 | 5.5 | 0.62 | 0.11 | 0.89 | 0.81 | 0.63 |
| MNHW_S.15.19_11 | Gorges de Court 3 | 8.33 | 5.92 | 4.71 | 3.00 | 4.62 | 8.35 | 0.90 | 1.05 | 5.6 | 0.71 | 0.11 | 1.16 | 0.79 | 0.65 |
| MNHW_S.15.21_13 | Gorges de Moutier | 7.60 | 5.10 | 4.22 | 2.92 | 4.21 | 8.16 | 1.11 | 0.96 | 5.3 | 0.67 | 0.14 | 0.86 | 0.83 | 0.69 |
| MNHW_S.15.21_14 | Gorges de Moutier | 8.47 | 5.31 | 4.43 | 3.08 | 4.75 | 8.09 | 0.97 | 0.71 | 5.2 | 0.63 | 0.12 | 0.73 | 0.83 | 0.65 |
| MNHW_F-Ech_02 | Échallon | 9.01 | 4.62 | 3.96 | 2.97 | 4.07 | 8.90 | 2.20 | 1.98 | 5.8 | 0.51 | 0.25 | 0.90 | 0.86 | 0.73 |
| MNHW_S.15.27_12 | Twannbachschlucht | 8.51 | 5.10 | 4.35 | 3.27 | 4.64 | 8.36 | 0.98 | 0.84 | 5.6 | 0.60 | 0.12 | 0.86 | 0.85 | 0.70 |
| MNHW_S.15.20_01 | Gorges de Court 4 | 10.79 | 5.94 | 5.14 | 3.53 | 5.63 | 11.51 | 2.25 | 2.10 | 6 | 0.55 | 0.20 | 0.94 | 0.86 | 0.63 |
| MNHW_S.15.20_02 | Gorges de Court 4 | 11.43 | 6.23 | 5.16 | 3.98 | 6.07 | 12.56 | 2.31 | 1.84 | 6 | 0.54 | 0.18 | 0.80 | 0.83 | 0.66 |
| MNHW_S.15.26_02 | Frinvillier 2 | 11.90 | 6.67 | 5.45 | 3.89 | 5.94 | 12.36 | 2.40 | 2.15 | 5.75 | 0.56 | 0.19 | 0.89 | 0.82 | 0.65 |
| MNHW_S.15.26_03 | Frinvillier 2 | 12.40 | 6.44 | 5.70 | 3.90 | 6.75 | 13.40 | 2.28 | 1.96 | 6 | 0.52 | 0.17 | 0.86 | 0.88 | 0.58 |
| MNHW_S.15.28_06 | Saint-Imier/Les Pontins | 11.23 | 6.96 | 5.51 | 3.80 | 5.52 | 11.70 | 2.28 | 1.69 | 6 | 0.62 | 0.19 | 0.74 | 0.79 | 0.69 |
| MNHW_S.15.29_100 | Montagne De Cernier | 12.89 | 8.28 | 7.08 | 4.51 | 7.14 | 13.26 | 2.74 | 2.33 | 5.5 | 0.64 | 0.21 | 0.85 | 0.86 | 0.63 |
| MNHW_S.15.29_101 | Montagne De Cernier | 11.42 | 7.21 | 6.06 | 4.23 | 5.89 | 11.63 | 2.18 | 1.81 | 5.9 | 0.63 | 0.19 | 0.83 | 0.84 | 0.72 |
| MNHW_S.15.21_01 | Gorges de Moutier | 8.93 | 4.52 | 3.94 | 2.87 | 4.35 | 8.29 | 1.55 | 1.46 | 5.5 | 0.51 | 0.19 | 0.94 | 0.87 | 0.66 |
| MNHW_S.15.21_02 | Gorges de Moutier | 7.80 | 3.67 | 3.35 | 2.69 | 4.06 | 8.14 | 1.48 | 1.55 | 5 | 0.47 | 0.18 | 1.05 | 0.91 | 0.66 |
| MNHW_S.15.22a_10 | Gorges du Pichoux | 10.04 | 6.04 | 5.18 | 3.79 | 5.84 | 9.27 | 0.97 | 0.84 | 5.6 | 0.60 | 0.10 | 0.87 | 0.86 | 0.65 |
| MNHW_F.15.33_04 | Les Rousses-d'Amont | 10.19 | 6.46 | 5.26 | 3.69 | 5.34 | 10.57 | 1.45 | 1.28 | 6 | 0.63 | 0.14 | 0.88 | 0.81 | 0.69 |
| MNHW_F.15.33_05 | Les Rousses-d'Amont | 10.97 | 6.83 | 5.74 | 4.08 | 6.18 | 11.90 | 1.70 | 1.58 | 5.75 | 0.62 | 0.14 | 0.93 | 0.84 | 0.66 |
| MNHW_F.15.33_06 | Les Rousses-d'Amont | 9.87 | 6.57 | 5.27 | 3.67 | 5.25 | 10.43 | 1.22 | 0.99 | 5.5 | 0.67 | 0.12 | 0.81 | 0.80 | 0.70 |
| MNHW_F.15.39_01 | Cascades du Hérisson | 10.82 | 6.84 | 5.67 | 4.10 | 6.26 | 11.62 | 1.24 | 1.07 | 5.8 | 0.63 | 0.11 | 0.86 | 0.83 | 0.66 |
| MNHW_F.15.39_02 | Cascades du Hérisson | 9.91 | 7.19 | 5.31 | 3.21 | 5.26 | 10.80 | 1.48 | 1.41 | 6.25 | 0.73 | 0.14 | 0.95 | 0.74 | 0.61 |
| MNHW_F.15.39a_01 | Cascades du Hérisson | 10.89 | 7.06 | 5.55 | 4.10 | 6.08 | 11.48 | 1.38 | 1.34 | 6 | 0.65 | 0.12 | 0.97 | 0.79 | 0.67 |
| MNHW_F.15.42a_03 | Arandas | 9.89 | 6.75 | 5.18 | 3.82 | 5.61 | 10.03 | 1.09 | 0.79 | 6 | 0.68 | 0.11 | 0.72 | 0.77 | 0.68 |
| MNHW_F.15.42a_04 | Arandas | 9.19 | 6.23 | 5.00 | 3.21 | 5.07 | 9.55 | 1.11 | 0.96 | 6 | 0.68 | 0.12 | 0.86 | 0.80 | 0.63 |
| MNHW_F.15.47_04 | Jalinard | 9.47 | 5.73 | 4.82 | 3.45 | 5.19 | 10.16 | 1.30 | 1.04 | 5.5 | 0.60 | 0.13 | 0.79 | 0.84 | 0.66 |
| MNHW_F.15.47_05 | Jalinard | 9.65 | 5.45 | 4.67 | 3.60 | 4.85 | 9.66 | 1.28 | 1.12 | 5.6 | 0.56 | 0.13 | 0.88 | 0.86 | 0.74 |
| MNHW_S.15.17_01 | Gorges de Court | 10.85 | 6.34 | 5.27 | 3.79 | 6.01 | 11.52 | 1.58 | 1.40 | 5.75 | 0.58 | 0.14 | 0.89 | 0.83 | 0.63 |
| MNHW_S.15.17_02 | Gorges de Court | 8.43 | 5.12 | 4.26 | 3.28 | 4.73 | 9.17 | 1.24 | 1.04 | 5.25 | 0.61 | 0.13 | 0.84 | 0.83 | 0.69 |
| MNHW_S.15.20_17 | Gorges de Court 4 | 9.51 | 6.20 | 4.83 | 3.05 | 4.86 | 9.99 | 1.72 | 1.47 | 5.9 | 0.65 | 0.17 | 0.86 | 0.78 | 0.63 |
| MNHW_S.15.20_18 | Gorges de Court 4 | 9.77 | 6.28 | 5.03 | 3.62 | 5.02 | 9.82 | 1.52 | 1.42 | 6 | 0.64 | 0.15 | 0.93 | 0.80 | 0.72 |
| MNHW_S.15.24_01 | Undervelier | 10.16 | 5.76 | 4.75 | 3.51 | 5.43 | 10.35 | 1.51 | 1.31 | 5.7 | 0.57 | 0.15 | 0.87 | 0.82 | 0.65 |
| MNHW_S.15.28_07 | Saint-Imier/Les Pontins | 10.02 | 6.07 | 4.84 | 3.46 | 5.33 | 10.72 | 1.60 | 1.30 | 5.6 | 0.61 | 0.15 | 0.82 | 0.80 | 0.65 |
| MNHW_S.15.28_08 | Saint-Imier/Les Pontins | 11.09 | 6.45 | 5.35 | 3.72 | 5.92 | 11.86 | 1.82 | 1.55 | 6.1 | 0.58 | 0.15 | 0.85 | 0.83 | 0.63 |

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| MNHW_S.15.29_01 | Montagne De Cernier | 11.99 | 7.14 | 5.85 | 4.43 | 6.44 | 12.82 | 2.22 | 1.75 | 6.25 | 0.60 | 0.17 | 0.79 | 0.82 | 0.69 |
| MNHW_S.15.29_02 | Montagne De Cernier | 11.54 | 5.96 | 5.03 | 3.61 | 5.97 | 11.62 | 1.79 | 1.62 | 5.5 | 0.52 | 0.15 | 0.91 | 0.85 | 0.60 |
| MNHW_S.15.30_02 | Gorges de L'Areuse | 9.48 | 5.63 | 4.73 | 3.64 | 5.22 | 10.26 | 1.64 | 1.29 | 5.6 | 0.59 | 0.16 | 0.79 | 0.84 | 0.70 |
| MNHW_F.15.31_02 | Mont d'Or | 9.05 | 5.61 | 4.53 | 3.13 | 4.60 | 9.45 | 1.72 | 1.51 | 5.75 | 0.62 | 0.18 | 0.88 | 0.81 | 0.68 |
| MNHW_F.15.31_03 | Mont d'Or | 10.22 | 5.69 | 4.92 | 3.89 | 5.46 | 10.00 | 1.67 | 1.30 | 5.5 | 0.56 | 0.17 | 0.78 | 0.87 | 0.71 |
| MNHW_F.15.34_01 | La Chevry | 11.98 | 7.10 | 6.04 | 4.73 | 6.49 | 13.08 | 2.05 | 1.66 | 6 | 0.59 | 0.16 | 0.81 | 0.85 | 0.73 |
| MNHW_F.15.34_03 | La Chevry | 10.58 | 5.48 | 4.66 | 3.43 | 5.21 | 10.85 | 2.07 | 1.71 | 5.6 | 0.52 | 0.19 | 0.83 | 0.85 | 0.66 |
| MNHW_F.15.34_05 | La Chevry | 10.35 | 5.62 | 4.63 | 3.35 | 5.25 | 9.87 | 1.94 | 1.57 | 5.25 | 0.54 | 0.20 | 0.81 | 0.82 | 0.64 |
| MNHW_F.15.35_01 | Mouthe, Source du Doubs | 10.18 | 6.10 | 5.21 | 3.88 | 5.36 | 10.56 | 1.64 | 1.45 | 6 | 0.60 | 0.16 | 0.88 | 0.85 | 0.72 |
| MNHW_F.15.36_01 | Bonnevaux | 9.97 | 6.00 | 4.78 | 3.37 | 5.27 | 10.91 | 1.79 | 1.38 | 5.5 | 0.60 | 0.16 | 0.77 | 0.80 | 0.64 |
| MNHW_F.15.37_05 | Cascade du Moulin du Saut | 10.87 | 6.58 | 5.25 | 3.65 | 5.67 | 11.46 | 2.08 | 1.75 | 6 | 0.61 | 0.18 | 0.84 | 0.80 | 0.64 |
| MNHW_F.15.37_06 | Cascade du Moulin du Saut | 10.26 | 6.27 | 5.02 | 3.39 | 5.29 | 11.00 | 1.82 | 1.50 | 5.6 | 0.61 | 0.17 | 0.82 | 0.80 | 0.64 |
| MNHW_F.15.38_05 | Chaux-des-Crotenay | 10.46 | 5.93 | 4.78 | 3.74 | 5.50 | 10.34 | 1.61 | 1.32 | 5.7 | 0.57 | 0.16 | 0.82 | 0.81 | 0.68 |
| MNHW_F.15.38_06 | Chaux-des-Crotenay | 11.03 | 6.63 | 5.40 | 3.80 | 5.79 | 10.87 | 1.91 | 1.46 | 6 | 0.60 | 0.18 | 0.76 | 0.81 | 0.66 |
| MNHW_A.Me_101 | Austria, Mellau | 12.79 | 7.56 | 6.72 | 4.61 | 7.06 | 11.78 | 2.15 | 1.93 | 5.5 | 0.59 | 0.18 | 0.90 | 0.89 | 0.65 |
| MNHW_A.MtK_105 | Austria, Mt Kojenkopf | 11.08 | 6.48 | 5.66 | 3.81 | 5.76 | 11.47 | 2.20 | 1.93 | 5.52 | 0.58 | 0.19 | 0.88 | 0.87 | 0.66 |
| MNHW_A.MtB_106 | Austria, Mt Baumgarten | 10.40 | 6.14 | 5.33 | 3.90 | 5.59 | 10.67 | 2.02 | 1.70 | 5 | 0.59 | 0.19 | 0.84 | 0.87 | 0.70 |
| MNHW_G.15.50a_108 | Germany, Buchberg 2 | 13.48 | 9.73 | 7.57 | 4.84 | 7.95 | 14.39 | 2.35 | 2.16 | 6.25 | 0.72 | 0.16 | 0.92 | 0.78 | 0.61 |
| MNHW_G.15.52_109 | Germany, Geisingen | 13.10 | 8.51 | 7.13 | 4.73 | 6.97 | 13.60 | 2.59 | 2.34 | 6 | 0.65 | 0.19 | 0.91 | 0.84 | 0.68 |
| MNHW_A.Me_05 | Austria, Mellau | 9.72 | 5.58 | 4.69 | 3.41 | 5.27 | 10.03 | 1.79 | 1.36 | 5.25 | 0.57 | 0.18 | 0.76 | 0.84 | 0.65 |
| MNHW_F.15.09_09 | France, between Aubure & La Petite Verrerie | 8.63 | 4.97 | 4.12 | 2.93 | 4.18 | 8.36 | 1.72 | 1.49 | 5.5 | 0.58 | 0.21 | 0.87 | 0.83 | 0.70 |
| MNHW_F.15.09_10 | France, between Aubure & La Petite Verrerie | 9.60 | 5.99 | 4.74 | 3.17 | 4.73 | 9.85 | 1.95 | 1.54 | 5.6 | 0.62 | 0.20 | 0.79 | 0.79 | 0.67 |
| MNHW_F.15.11_11 | France, Tellure | 10.58 | 6.02 | 4.87 | 3.39 | 5.38 | 10.50 | 1.82 | 1.56 | 5.5 | 0.57 | 0.17 | 0.85 | 0.81 | 0.63 |
| MNHW_F.15.11_12 | France, Tellure | 9.90 | 6.29 | 5.18 | 3.65 | 5.41 | 9.72 | 1.67 | 1.41 | 5.5 | 0.64 | 0.17 | 0.85 | 0.82 | 0.67 |
| MNHW_F.15.14_13 | France, Source de la Moselle n. Bussang | 9.64 | 5.99 | 5.01 | 3.63 | 5.07 | 9.64 | 1.28 | 1.12 | 5.5 | 0.62 | 0.13 | 0.88 | 0.84 | 0.72 |
| MNHW_F.15.14_14 | France, Source de la Moselle n. Bussang | 9.09 | 5.84 | 4.56 | 3.01 | 4.90 | 9.08 | 1.51 | 1.17 | 5.25 | 0.64 | 0.17 | 0.77 | 0.78 | 0.61 |
| MNHW_F.15.15_15 | France, Bussang | 10.61 | 6.57 | 5.31 | 3.88 | 5.73 | 10.69 | 1.60 | 1.34 | 6 | 0.62 | 0.15 | 0.83 | 0.81 | 0.68 |
| MNHW_F.15.15_16 | France, Bussang | 10.18 | 6.29 | 5.05 | 3.80 | 5.40 | 10.23 | 1.56 | 1.19 | 5.5 | 0.62 | 0.15 | 0.76 | 0.80 | 0.70 |
| MNHW_F.15.15_17 | France, Bussang | 9.88 | 5.80 | 4.79 | 3.36 | 5.08 | 9.79 | 1.76 | 1.43 | 5.5 | 0.59 | 0.18 | 0.82 | 0.83 | 0.66 |
| NMBE_568084 | St. Maurice, Grotte aux Fées | 6.89 | 4.10 | 4.01 | 4.67 | 3.88 | 7.09 | 0.70 | 0.61 | 5 | 0.60 | 0.10 | 0.87 | 0.98 | 1.20 |
| NMBE_569597 | Bern-Bümpliz, Schlosspark | 7.53 | 4.44 | 3.90 | 3.02 | 4.07 | 7.45 | 0.80 | 0.99 | 5.5 | 0.59 | 0.11 | 1.24 | 0.88 | 0.74 |
| NMBE_569598 | Bern-Bümpliz, Schlosspark | 7.18 | 4.08 | 3.46 | 2.76 | 3.91 | 7.03 | 1.15 | 0.92 | 5.5 | 0.57 | 0.16 | 0.80 | 0.85 | 0.71 |
| NMBE_569638 | Broc, Waldlichtung | 11.28 | 6.39 | 6.10 | 3.09 | 5.96 | 11.79 | 1.97 | 2.13 | 5.75 | 0.57 | 0.17 | 1.08 | 0.95 | 0.52 |
| MNHW_PL.20.45_305 | Poland, Pieskowa Skała (PN) | 7.75 | 4.34 | 3.82 | 2.40 | 3.49 | 7.96 | 1.65 | 1.43 | 5.2 | 0.56 | 0.21 | 0.87 | 0.88 | 0.69 |
| MNHW_PL.20.32_307 | Poland, Muszyna 2 | 8.92 | 4.66 | 4.38 | 2.81 | 4.42 | 9.08 | 1.78 | 1.68 | 5.75 | 0.52 | 0.20 | 0.94 | 0.94 | 0.64 |
| MNHW_PL.20.16_306 | Poland, Wapowce | 7.86 | 4.77 | 4.04 | 2.34 | 3.90 | 7.68 | 1.36 | 1.21 | 5 | 0.61 | 0.18 | 0.89 | 0.85 | 0.60 |
| MNHW_F.18.30_299 | France, Forêt Bac Estable 2 | 9.67 | 6.26 | 4.92 | 3.43 | 5.05 | 9.67 | 2.05 | 1.93 | 6 | 0.65 | 0.21 | 0.95 | 0.79 | 0.68 |

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| MNHW_F.18.37_301 | France, Forêt de Beleste | 10.67 | 6.13 | 5.15 | 3.54 | 5.51 | 10.79 | 2.05 | 1.77 | 6.2 | 0.57 | 0.19 | 0.86 | 0.84 | 0.64 |
| MNHW_F.18.35_300 | France, Le Chandelier | 8.41 | 5.31 | 4.36 | 3.34 | 4.51 | 8.76 | 1.39 | 1.20 | 5.5 | 0.63 | 0.16 | 0.86 | 0.82 | 0.74 |
| MNHW_F.VM_295 | France, Vallon du Moulinot | 9.46 | 5.76 | 4.63 | 3.13 | 5.02 | 9.33 | 1.17 | 1.07 | 5.75 | 0.61 | 0.13 | 0.91 | 0.81 | 0.62 |
| MNHW_F.RP_297 | France, La Roche des Pins | 8.65 | 5.27 | 4.20 | 3.12 | 4.57 | 8.64 | 0.97 | 0.86 | 6 | 0.61 | 0.11 | 0.89 | 0.80 | 0.68 |
| MNHW_F.VM_294 | France, Vallon du Moulinot | 8.17 | 5.51 | 4.28 | 2.89 | 4.59 | 8.23 | 0.93 | 0.89 | 5.7 | 0.67 | 0.11 | 0.95 | 0.78 | 0.63 |
| MNHW_F.ML_296 | France, Mont de Lune | 8.36 | 5.70 | 4.30 | 2.93 | 3.83 | 8.42 | 1.48 | 1.10 | 6 | 0.68 | 0.18 | 0.75 | 0.75 | 0.77 |
| MNHW_A.17.12_01 | Austria, Schafberg | 8.97 | 5.06 | 4.28 | 2.89 | 4.68 | 8.71 | 1.43 | 1.29 | 5.5 | 0.56 | 0.16 | 0.90 | 0.85 | 0.62 |
| MNHW_I.16.12_01 | Ireland, Maam | 10.46 | 7.30 | 5.81 | 3.79 | 5.53 | 10.44 | 1.82 | 1.64 | 6 | 0.70 | 0.17 | 0.90 | 0.80 | 0.68 |
| MNHW_SK.19.71_01 | Slovakia, Varín | 11.59 | 7.27 | 5.81 | 4.01 | 5.80 | 11.21 | 1.87 | 1.62 | 5.7 | 0.63 | 0.17 | 0.87 | 0.80 | 0.69 |
| MNHW_F.18.10_06 | France, Blecquenecques n. Marquise | 10.96 | 6.13 | 5.20 | 3.61 | 5.68 | 10.77 | 1.82 | 1.56 | 5.5 | 0.56 | 0.17 | 0.85 | 0.85 | 0.64 |
| MNHW_GB.Br_01 | Great Britain, Bramley near Basingstoke | 10.85 | 6.81 | 5.78 | 4.02 | 5.99 | 10.93 | 1.51 | 1.39 | 5.6 | 0.63 | 0.14 | 0.92 | 0.85 | 0.67 |
| MNHW_F.18.25_279 | France, Escames | 8.03 | 4.82 | 3.99 | 2.75 | 3.72 | 7.89 | 1.24 | 1.10 | 5.25 | 0.60 | 0.16 | 0.89 | 0.83 | 0.74 |
| MNHW_F.18.07_278 | France, Beaucamps-Le-Jeune | 8.05 | 5.58 | 4.46 | 2.62 | 4.06 | 8.15 | 1.23 | 1.13 | 5.7 | 0.69 | 0.15 | 0.92 | 0.80 | 0.65 |
| MNHW_F.18.05_277 | France, Fouquenies | 8.08 | 5.58 | 4.40 | 2.59 | 4.07 | 8.15 | 1.19 | 1.11 | 5.7 | 0.69 | 0.15 | 0.93 | 0.79 | 0.64 |
| MNHW_F.LE_280 | France, Les Essarts | 6.49 | 4.57 | 3.75 | 2.74 | 3.48 | 6.48 | 0.57 | 0.56 | 5 | 0.70 | 0.09 | 0.98 | 0.82 | 0.79 |
| MNHW_F.LR_290 | France, La Ruchotte | 7.84 | 5.04 | 3.98 | 2.56 | 4.28 | 7.80 | 1.20 | 1.05 | 5.6 | 0.64 | 0.15 | 0.87 | 0.79 | 0.60 |
| MNHW_Re5 | Germany, Reischenau | 8.02 | 5.38 | 4.51 | 3.08 | 4.18 | 8.02 | 0.99 | 0.99 | 5.25 | 0.67 | 0.12 | 1.00 | 0.84 | 0.74 |
| MNHW_G.15.05_285 | Germany, Ketsch | 8.91 | 5.79 | 4.62 | 2.84 | 4.74 | 8.97 | 1.35 | 1.10 | 6 | 0.65 | 0.15 | 0.81 | 0.80 | 0.60 |
| MNHW_RF4 | Switzerland, Ruine Farnsburg | 7.80 | 4.62 | 3.96 | 2.75 | 3.85 | 7.80 | 0.88 | 0.88 | 5.25 | 0.59 | 0.11 | 1.00 | 0.86 | 0.71 |
| MNHW_St-14 | Germany, Staubing | 9.27 | 5.82 | 4.55 | 3.09 | 4.55 | 9.09 | 1.64 | 1.45 | 5.8 | 0.63 | 0.18 | 0.89 | 0.78 | 0.68 |
| MNHW_G.17.05_298 | Germany, Pfatter | 8.74 | 4.96 | 4.29 | 3.24 | 4.59 | 8.89 | 1.76 | 1.51 | 5.6 | 0.57 | 0.20 | 0.86 | 0.87 | 0.71 |
| MNHW_Sau1 | Austria, Sausteig | 8.24 | 5.16 | 4.29 | 3.19 | 4.40 | 8.13 | 0.99 | 0.88 | 5.4 | 0.63 | 0.12 | 0.89 | 0.83 | 0.73 |
| MNHW_CZ.Vit_274 | Czech Republic, Vitkovice | 6.95 | 4.42 | 3.61 | 2.74 | 3.46 | 6.91 | 1.08 | 0.95 | 5.2 | 0.64 | 0.16 | 0.88 | 0.82 | 0.79 |
| MNHW_CZ.Lan_273 | Czech Republic, Lánov | 7.03 | 4.92 | 4.01 | 2.63 | 3.56 | 6.99 | 0.75 | 0.70 | 5.25 | 0.70 | 0.11 | 0.94 | 0.82 | 0.74 |
| MNHW_CZ.CD_272 | Czech Republic, Černý Důl | 6.87 | 4.29 | 3.69 | 2.63 | 3.57 | 6.79 | 0.67 | 0.50 | 5.6 | 0.62 | 0.10 | 0.75 | 0.86 | 0.74 |
| MNHW_CZ.19.51_275 | Czech Republic, Radňovice | 7.63 | 5.12 | 4.13 | 2.82 | 3.71 | 7.69 | 1.19 | 1.11 | 5.5 | 0.67 | 0.15 | 0.93 | 0.81 | 0.76 |
| MNHW_F.SF_283 | France, Source de la Foulotière | 8.78 | 5.23 | 4.41 | 3.04 | 4.61 | 8.77 | 1.10 | 0.87 | 5.5 | 0.60 | 0.12 | 0.79 | 0.84 | 0.66 |
| MNHW_F.Lcr_289 | France, La croix rouge | 9.45 | 5.34 | 4.32 | 3.31 | 5.00 | 9.29 | 1.26 | 1.12 | 5.7 | 0.57 | 0.14 | 0.89 | 0.81 | 0.66 |
| MNHW_PL.20.09_252 | Poland, Leśna Podlaska | 7.15 | 4.62 | 3.68 | 2.61 | 3.25 | 7.46 | 1.86 | 1.51 | 5.4 | 0.65 | 0.25 | 0.81 | 0.80 | 0.80 |
| MNHW_PL.20.13_253 | Poland, Gamlówka | 6.87 | 4.02 | 3.38 | 2.07 | 3.02 | 6.74 | 1.63 | 1.59 | 5.5 | 0.59 | 0.24 | 0.98 | 0.84 | 0.69 |
| MNHW_PL.20.07_251 | Poland, Budy Leśne n. Białowieża | 7.68 | 5.57 | 4.24 | 2.43 | 3.73 | 7.76 | 1.35 | 1.29 | 5.6 | 0.73 | 0.17 | 0.96 | 0.76 | 0.65 |
| MNHW_PL.19.10_250 | Poland, Mątki near Sztum | 8.49 | 4.79 | 3.81 | 2.77 | 3.69 | 8.32 | 2.06 | 1.57 | 6 | 0.56 | 0.25 | 0.76 | 0.79 | 0.75 |
| MNHW_PL.Ro_247 | Poland, Puszcza Romincka | 6.97 | 5.09 | 3.96 | 2.80 | 3.39 | 7.07 | 1.21 | 1.10 | 5.5 | 0.73 | 0.17 | 0.91 | 0.78 | 0.83 |
| MNHW_PL.20.04_248 | Poland, Rapa | 7.39 | 5.09 | 4.15 | 2.76 | 3.87 | 7.44 | 1.42 | 1.38 | 5.2 | 0.69 | 0.19 | 0.97 | 0.82 | 0.71 |
| MNHW_PL.20.05_249 | Poland, Cisowy Jar | 7.40 | 5.10 | 4.11 | 2.76 | 3.89 | 7.85 | 1.79 | 1.54 | 5.3 | 0.69 | 0.23 | 0.86 | 0.81 | 0.71 |
| MNHW_CZ.19.78_218 | Czech Republic, Pitárné | 9.03 | 4.77 | 4.04 | 3.10 | 4.24 | 9.07 | 2.32 | 1.74 | 5.4 | 0.53 | 0.26 | 0.75 | 0.85 | 0.73 |
| MNHW_CZ.17.145_212 | Czech Republic, Závist | 7.97 | 5.06 | 3.96 | 2.55 | 3.93 | 8.06 | 1.79 | 1.44 | 5.6 | 0.64 | 0.22 | 0.80 | 0.78 | 0.65 |

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| MNHW_CZ.19.35_213 | Czech Republic, Srbsko 3 | 7.31 | 3.93 | 3.29 | 2.10 | 3.28 | 7.24 | 1.73 | 1.51 | 5.5 | 0.54 | 0.24 | 0.87 | 0.84 | 0.64 |
| MNHW_CZ.19.38_214 | Czech Republic, Štěchovice (Maják) | 7.56 | 4.77 | 3.81 | 2.64 | 3.54 | 7.57 | 1.79 | 1.54 | 6.1 | 0.63 | 0.24 | 0.86 | 0.80 | 0.74 |
| MNHW_CZ.19.43_216 | Czech Republic, Debrník | 7.60 | 4.68 | 3.74 | 2.77 | 3.98 | 7.70 | 1.35 | 1.20 | 5.6 | 0.62 | 0.18 | 0.89 | 0.80 | 0.70 |
| MNHW_PL.19.27_242 | Poland, Będzin | 6.83 | 4.34 | 3.40 | 2.16 | 3.06 | 6.88 | 1.56 | 1.50 | 5.2 | 0.63 | 0.23 | 0.96 | 0.78 | 0.70 |
| MNHW_UK.En-D_5 | UK, Downside | 8.46 | 5.49 | 4.18 | 3.08 | 4.18 | 8.35 | 1.43 | 1.10 | 5.6 | 0.65 | 0.17 | 0.77 | 0.76 | 0.74 |
| MNHW_A.Gens_201 | Austria, Genabend-Sibratsgfäll | 6.84 | 4.59 | 3.74 | 2.82 | 3.48 | 6.86 | 0.88 | 0.79 | 5.2 | 0.67 | 0.13 | 0.90 | 0.81 | 0.81 |
| MNHW_A.17.28_203 | Austria, Durnbachtal | 7.30 | 3.65 | 3.06 | 2.60 | 3.40 | 7.32 | 1.51 | 1.41 | 5.5 | 0.50 | 0.21 | 0.93 | 0.84 | 0.76 |
| MNHW_A.Schw_202 | Austria, Schwarzenberg-Loch 2 | 6.50 | 3.73 | 2.96 | 2.24 | 3.21 | 6.49 | 1.05 | 1.01 | 5.6 | 0.57 | 0.16 | 0.96 | 0.79 | 0.70 |
| MNHW_PL.19.82_236 | Poland, Wojcieszów Górny | 8.00 | 5.30 | 4.11 | 2.81 | 3.73 | 7.93 | 1.36 | 1.13 | 5.7 | 0.66 | 0.17 | 0.83 | 0.77 | 0.75 |
| MNHW_NL.19.03_234 | the Netherlands, Veere 2 | 7.63 | 3.99 | 3.45 | 2.72 | 3.88 | 7.55 | 1.32 | 1.01 | 5.1 | 0.52 | 0.17 | 0.77 | 0.87 | 0.70 |
| MNHW_NL.Ost_233 | the Netherlands, Oestgeest near Leiden | 8.28 | 5.33 | 4.14 | 3.00 | 3.99 | 8.46 | 1.46 | 1.20 | 5.5 | 0.64 | 0.17 | 0.82 | 0.78 | 0.75 |
| MNHW_F.18.14_225 | France, Larré | 6.72 | 4.26 | 3.43 | 2.23 | 3.34 | 6.60 | 1.14 | 0.97 | 5.1 | 0.63 | 0.17 | 0.85 | 0.80 | 0.67 |
| MNHW_F.18.24_223 | France, Le Mont Gournoy 2 (Forêt Indivise d'Eu) | 6.93 | 4.05 | 3.29 | 2.11 | 3.38 | 6.89 | 1.26 | 1.02 | 5.2 | 0.58 | 0.18 | 0.81 | 0.81 | 0.63 |
| MNHW_F.Col_228 | France, Col. De Vac. | 7.49 | 4.07 | 3.45 | 2.70 | 3.85 | 7.40 | 0.90 | 0.85 | 5 | 0.54 | 0.12 | 0.95 | 0.85 | 0.70 |
| MNHW_F.18.08_221 | France, Eaucourt-Sur-Somme | 7.95 | 4.67 | 3.83 | 2.61 | 3.41 | 7.87 | 1.52 | 1.10 | 5 | 0.59 | 0.19 | 0.73 | 0.82 | 0.77 |
| MNHW_F.Com_227 | Chameyer | 7.85 | 4.99 | 4.09 | 3.07 | 3.80 | 7.91 | 1.52 | 1.30 | 5.5 | 0.64 | 0.19 | 0.86 | 0.82 | 0.81 |
| MNHW_F.18.12_224 | France, Licques | 6.85 | 4.25 | 3.35 | 2.13 | 3.22 | 6.82 | 1.38 | 1.16 | 5 | 0.62 | 0.20 | 0.85 | 0.79 | 0.66 |
| MNHW_F.15.07_219 | France, Bertrange | 7.36 | 4.99 | 3.88 | 2.35 | 3.38 | 7.49 | 1.71 | 1.32 | 5.3 | 0.68 | 0.23 | 0.77 | 0.78 | 0.70 |
| MNHW_I.16.8_231 | Ireland, Lough Gur | 7.55 | 3.88 | 3.35 | 2.46 | 3.54 | 7.56 | 1.62 | 1.35 | 5 | 0.51 | 0.21 | 0.84 | 0.86 | 0.69 |
| MNHW_I.16.4_230 | Ireland, Rock of Cashel | 6.85 | 3.99 | 3.31 | 2.16 | 3.18 | 6.84 | 1.32 | 1.14 | 5.7 | 0.58 | 0.19 | 0.86 | 0.83 | 0.68 |
| MNHW_I.16.3_229 | Ireland, Monastery | 8.82 | 5.46 | 4.54 | 2.82 | 4.01 | 9.00 | 2.21 | 1.85 | 5.7 | 0.62 | 0.25 | 0.84 | 0.83 | 0.70 |
| MNHW_I.16.10_232 | Ireland, Bealaclugga | 7.75 | 4.31 | 3.61 | 2.26 | 3.82 | 7.83 | 1.42 | 1.29 | 5.2 | 0.56 | 0.18 | 0.91 | 0.84 | 0.59 |
| MNHW_UA.18.55_264 | Ukraine, Bibrka | 8.16 | 4.32 | 3.61 | 2.52 | 3.49 | 8.13 | 2.05 | 1.57 | 6 | 0.53 | 0.25 | 0.76 | 0.84 | 0.72 |
| MNHW_UA.18.58_267 | Ukraine, Mikuliczyn | 7.60 | 4.44 | 3.78 | 2.32 | 3.50 | 7.57 | 1.80 | 1.69 | 5.5 | 0.58 | 0.24 | 0.94 | 0.85 | 0.66 |
| MNHW_UA.18.56_265 | Ukraine, Swirz | 7.71 | 3.96 | 3.26 | 2.35 | 3.54 | 7.63 | 1.82 | 1.65 | 5.6 | 0.51 | 0.24 | 0.91 | 0.83 | 0.66 |
| MNHW_G.17.17_228 | Germany, Thannlehen b. Berchtesgaden | 8.34 | 5.51 | 4.50 | 3.24 | 3.97 | 8.31 | 1.27 | 1.02 | 5.6 | 0.66 | 0.15 | 0.80 | 0.82 | 0.82 |
| MNHW_G.Ott_227 | Germany, Ottendorf | 7.24 | 4.26 | 3.59 | 2.79 | 3.66 | 7.13 | 1.11 | 1.11 | 5 | 0.59 | 0.16 | 1.00 | 0.84 | 0.76 |
| MNHW_G-Mo5 | Germany, Moosburg a.d. Isar | 6.92 | 4.18 | 3.52 | 2.42 | 3.41 | 6.70 | 1.32 | 1.21 | 5.2 | 0.60 | 0.20 | 0.92 | 0.84 | 0.71 |
| MNHW_PL.19.08_256 | Poland, Ostrów | 7.41 | 4.74 | 3.85 | 2.74 | 3.51 | 7.45 | 1.63 | 1.35 | 5.3 | 0.64 | 0.22 | 0.83 | 0.81 | 0.78 |
| MNHW_PL.19.09_257 | Poland, Barcin-Górne Wolice | 8.26 | 5.37 | 4.18 | 2.57 | 4.05 | 8.30 | 1.70 | 1.57 | 6.1 | 0.65 | 0.20 | 0.93 | 0.78 | 0.64 |
| MNHW_PL.20.01_255 | Poland, Siemianice n. Kępno | 9.40 | 5.64 | 4.54 | 3.31 | 4.55 | 9.33 | 1.71 | 1.66 | 6 | 0.60 | 0.18 | 0.97 | 0.80 | 0.73 |
| MNHW_SK.19.62_261 | Slovakia, Čičov (Čičovský les) | 8.82 | 4.60 | 4.02 | 2.78 | 3.90 | 8.71 | 2.00 | 1.63 | 5.6 | 0.52 | 0.23 | 0.82 | 0.87 | 0.71 |
| MNHW_SK.19.63_262 | Slovakia, Medveďov | 9.17 | 5.27 | 3.82 | 2.60 | 4.15 | 8.87 | 2.25 | 1.77 | 5.6 | 0.57 | 0.25 | 0.78 | 0.72 | 0.63 |
| MNHW_PL.20.23_246 | Poland, Równia | 7.94 | 5.00 | 3.63 | 2.46 | 3.58 | 7.90 | 1.85 | 1.63 | 6.1 | 0.63 | 0.23 | 0.88 | 0.73 | 0.69 |
| MNHW_PL.20.22_245 | Poland, Stebnik | 7.66 | 4.83 | 3.61 | 2.32 | 3.44 | 7.58 | 1.65 | 1.46 | 5.6 | 0.63 | 0.22 | 0.88 | 0.75 | 0.68 |
| MNHW_SLO.17.140_260 | Slovenia, Vintgar Gorge | 8.50 | 4.29 | 3.62 | 2.95 | 3.95 | 8.30 | 2.11 | 1.80 | 5.6 | 0.50 | 0.25 | 0.85 | 0.84 | 0.75 |
| MNHW_PL.20.34_243 | Poland, Szlachtowa 2 | 8.61 | 5.85 | 4.29 | 2.59 | 3.77 | 8.80 | 1.74 | 1.53 | 6.2 | 0.68 | 0.20 | 0.88 | 0.73 | 0.69 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MNHW_PL.20.44_244 | Poland, Owczary | 8.43 | 4.95 | 3.91 | 2.61 | 3.84 | 8.41 | 1.91 | 1.74 | 5.5 | 0.59 | 0.23 | 0.91 | 0.79 | 0.68 |
| NMBE_569600 | Schwarzwasser, Ufer | 10.03 | 5.69 | 5.17 | 3.62 | 5.17 | 10.14 | 1.59 | 1.79 | 5.75 | 0.57 | 0.16 | 1.13 | 0.91 | 0.70 |
| NMBE_569677 | Schwarzwasser, Ufer | 7.68 | 4.39 | 4.27 | 2.62 | 4.10 | 7.35 | 0.80 | 0.57 | 5 | 0.57 | 0.11 | 0.71 | 0.97 | 0.64 |
| NMBE_569604 | Schwarzwasser, Brücke | 9.52 | 5.67 | 4.76 | 3.22 | 5.23 | 9.74 | 1.13 | 1.45 | 5.5 | 0.60 | 0.12 | 1.28 | 0.84 | 0.62 |
| NMBE_569607 | Zürich | 7.44 | 3.95 | 3.65 | 2.80 | 3.98 | 7.28 | 0.80 | 1.11 | 4.75 | 0.53 | 0.11 | 1.39 | 0.92 | 0.70 |
| NMBE_569680 | Brugg, Friedhof, unterhalb Autobahnbrücke | 7.53 | 4.68 | 4.28 | 2.95 | 4.08 | 7.39 | 0.68 | 0.70 | 5 | 0.62 | 0.09 | 1.03 | 0.91 | 0.72 |
| NMBE_569681 | Brugg, Friedhof, unterhalb Autobahnbrücke | 8.17 | 4.73 | 4.29 | 2.77 | 4.67 | 8.00 | 0.74 | 0.58 | 5 | 0.58 | 0.09 | 0.78 | 0.91 | 0.59 |
| NMBE_569609 | Aarau, Parkanlage Grossratsgebäude | 9.13 | 5.84 | 5.01 | 3.28 | 4.96 | 9.39 | 1.35 | 1.49 | 5.5 | 0.64 | 0.14 | 1.10 | 0.86 | 0.66 |
| NMBE_569684 | Brugg, Aareufer | 6.65 | 4.32 | 3.86 | 2.46 | 3.53 | 6.83 | 0.85 | 0.68 | 5 | 0.65 | 0.12 | 0.80 | 0.89 | 0.70 |
| NMBE_569611 | Sursee, Surepärkli | 9.65 | 5.77 | 4.96 | 3.45 | 5.30 | 9.76 | 1.36 | 1.48 | 5.5 | 0.60 | 0.14 | 1.09 | 0.86 | 0.65 |
| NMBE_569686 | Hallwil, Schloss | 6.37 | 4.10 | 3.74 | 2.00 | 3.55 | 6.56 | 0.64 | 0.72 | 5 | 0.64 | 0.10 | 1.13 | 0.91 | 0.56 |
| NMBE_569689 | Stansstad, Kieswerk, Flussufer | 8.85 | 5.80 | 5.29 | 3.34 | 5.19 | 8.63 | 0.75 | 0.80 | 5.25 | 0.66 | 0.09 | 1.07 | 0.91 | 0.64 |
| NMBE_569639 | Stansstad, Kieswerk, Flussufer | 12.71 | 7.26 | 6.50 | 3.81 | 5.75 | 12.83 | 2.20 | 2.54 | 5.75 | 0.57 | 0.17 | 1.15 | 0.90 | 0.66 |
| NMBE_569616 | Zürich, Beckenhof | 7.28 | 4.37 | 3.68 | 2.42 | 3.82 | 7.39 | 0.82 | 0.92 | 5 | 0.60 | 0.11 | 1.12 | 0.84 | 0.63 |
| NMBE_569640 | Stans, Bürgenberg, Bürgenstock | 12.86 | 7.41 | 6.67 | 3.80 | 6.67 | 13.09 | 2.43 | 2.30 | 5.5 | 0.58 | 0.19 | 0.95 | 0.90 | 0.57 |
| NMBE_569626 | Avonçon | 7.51 | 4.32 | 4.08 | 3.06 | 4.05 | 7.56 | 1.02 | 1.07 | 5.5 | 0.58 | 0.13 | 1.05 | 0.94 | 0.76 |
| NMBE_569666 | Forest-Saint-Julien | 7.86 | 4.66 | 4.05 | 2.65 | 4.20 | 8.05 | 1.06 | 1.22 | 5.5 | 0.59 | 0.13 | 1.15 | 0.87 | 0.63 |
| NMBE_571095 | Grimentz | 6.37 | 4.16 | 3.57 | 2.26 | 3.41 | 6.38 | 0.77 | 0.68 | 4.75 | 0.65 | 0.12 | 0.88 | 0.86 | 0.66 |
| NMBE_571107 | Rheinland-Palatinate, Welterod | 8.25 | 4.54 | 4.21 | 2.83 | 3.90 | 8.60 | 1.42 | 1.64 | 5.5 | 0.55 | 0.17 | 1.15 | 0.93 | 0.73 |
| NMBE_571108 | Diessen am Ammersee | 7.28 | 4.72 | 3.92 | 2.28 | 3.28 | 7.39 | 1.43 | 1.53 | 5.25 | 0.65 | 0.19 | 1.07 | 0.83 | 0.70 |
| NMBE_571112 | DK, Hovedstaden, Fureso Kommune | 7.13 | 4.34 | 3.77 | 2.45 | 3.59 | 7.31 | 1.29 | 1.67 | 5.5 | 0.61 | 0.18 | 1.29 | 0.87 | 0.68 |
| NMBE_571125 | Carmel, near Swansea | 7.73 | 4.98 | 4.27 | 2.40 | 4.03 | 8.09 | 1.27 | 1.40 | 5.5 | 0.64 | 0.16 | 1.10 | 0.86 | 0.60 |
| NMBE_571128 | Jersey | 8.69 | 6.12 | 4.51 | 2.84 | 4.26 | 8.91 | 1.82 | 1.84 | 6 | 0.70 | 0.20 | 1.01 | 0.74 | 0.67 |
| NMBE_571134 | Flühli | 9.86 | 5.33 | 3.21 | 4.63 | 5.47 | 9.84 | 1.52 | 1.73 | 5.25 | 0.54 | 0.15 | 1.14 | 0.60 | 0.85 |
| NMBE_571142 | Bergün Dorf | 9.64 | 5.73 | 4.95 | 3.29 | 5.03 | 9.94 | 1.00 | 1.57 | 5.5 | 0.59 | 0.10 | 1.57 | 0.86 | 0.65 |
| NMBE_571234 | Amriswil, Kronberg | 9.77 | 5.89 | 5.09 | 3.73 | 5.49 | 9.88 | 1.59 | 1.57 | 5.5 | 0.60 | 0.16 | 0.99 | 0.86 | 0.68 |
| NMBE_571235 | Bürglen, Schloss | 10.07 | 6.29 | 5.17 | 3.74 | 5.46 | 9.88 | 1.30 | 1.34 | 5.5 | 0.62 | 0.13 | 1.03 | 0.82 | 0.68 |
| NMBE_571145 | Twann, Twannbachschlucht | 11.25 | 6.12 | 5.22 | 4.26 | 5.69 | 11.59 | 1.70 | 2.01 | 5.5 | 0.54 | 0.15 | 1.18 | 0.85 | 0.75 |
| NMBE_571152 | Bayern, Kelheim, Neustadt, Donaubrücke | 8.92 | 4.99 | 4.28 | 3.89 | 4.56 | 9.35 | 1.35 | 1.57 | 5.5 | 0.56 | 0.14 | 1.16 | 0.86 | 0.85 |
| NMBE_571158 | NRW, Mettmann, Monheim, Baumberg, Auwald | 11.77 | 7.34 | 6.11 | 3.97 | 6.79 | 12.41 | 2.14 | 2.19 | 6 | 0.62 | 0.17 | 1.02 | 0.83 | 0.58 |
| NMBE_571160 | Dorsel, Dorseler Mühle | 11.90 | 7.10 | 5.69 | 3.70 | 5.95 | 12.51 | 2.22 | 2.45 | 6.5 | 0.60 | 0.18 | 1.10 | 0.80 | 0.62 |
| NMBE_571203 | Säntis, Meglisalp | 9.61 | 5.21 | 5.06 | 3.23 | 5.61 | 9.94 | 1.51 | 1.86 | 5.75 | 0.54 | 0.15 | 1.23 | 0.97 | 0.58 |
| NMBE_571206 | Säntis, Schäfler | 11.17 | 6.32 | 5.40 | 3.67 | 5.80 | 11.61 | 2.44 | 2.68 | 6 | 0.57 | 0.21 | 1.10 | 0.85 | 0.63 |
| NMBE_571208 | Säntis, Ebenalp | 11.41 | 6.73 | 5.83 | 3.83 | 5.98 | 11.91 | 1.78 | 2.44 | 6 | 0.59 | 0.15 | 1.37 | 0.87 | 0.64 |
| NMBE_571237 | Untereggen, Altrüti SG | 12.56 | 6.76 | 6.25 | 4.05 | 5.82 | 12.78 | 2.7 | 2.2 | 5.5 | 0.54 | 0.21 | 0.81 | 0.92 | 0.70 |
| NMBE_571218 | Dutzower See, Nordspitze | 7.99 | 4.56 | 3.70 | 2.53 | 4.17 | 8.29 | 1.55 | 1.34 | 6 | 0.57 | 0.19 | 0.86 | 0.81 | 0.61 |
| MNHN-IM-2013-76922 | Saint-Laurent-du-Pont | 8.31 | 5.24 | 4.52 | 3.44 | 4.81 | 8.17 | 0.68 | 0.75 | 5 | 0.63 | 0.08 | 1.10 | 0.86 | 0.72 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MNHN-IM-2013-77127 | Myennes | 7.35 | 4.43 | 3.66 | 2.71 | 3.73 | 7.20 | 1.38 | 1.11 | 4.75 | 0.60 | 0.19 | 0.80 | 0.83 | 0.73 |
| MNHN-IM-2013-76890 | Guillestre | 7.19 | 4.22 | 3.37 | 2.39 | 3.38 | 6.97 | 1.39 | 1.34 | 5.25 | 0.59 | 0.20 | 0.96 | 0.80 | 0.71 |
| NMBE_571329 | NRW, Kreis Herford, Enger, Belke-Steinbeck, Brandbach | 8.64 | 4.68 | 3.80 | 2.52 | 4.09 | 8.55 | 1.64 | 1.47 | 5.75 | 0.54 | 0.19 | 0.90 | 0.81 | 0.62 |
| NMBE_571333 | NRW, Kreis Minden-Lübbecke, Hille, Mindenerwald | 7.54 | 4.45 | 3.55 | 2.64 | 3.60 | 7.67 | 1.71 | 1.44 | 5.75 | 0.59 | 0.22 | 0.84 | 0.80 | 0.73 |

**Supplementary material III:** Species ID, accession numbers from GenBank and BOLD, and sampling localities from sequences from external publications used in the genetic analysis.

| ID | Locality | Country | Lat | Long | Species in this study | Clade/species in original publication | COI accession no. | 16S accession no. | ITS2 accession no. | Publication |
|---|---|---|---|---|---|---|---|---|---|---|
| 4287_452 | Vätra Götalands Län | Sweden | 57.68 | 11.95 | *villersii* | 1 | KJ151495 | KJ151606 | | Kruckenhauser et al. 2014 |
| 4293_455 | Vätra Götalands Län | Sweden | 58.26 | 13.28 | *villersii* | 1 | KJ151499 | KJ151608 | KJ151747 | Kruckenhauser et al. 2014 |
| 103_33 | Nationalpark Donauau | Austria | 48.11 | 16.78 | sp. 4 | 2A | KJ151296 | KJ151551 | KJ151720 | Kruckenhauser et al. 2014 |
| 104_104 | Dürrenstein | Austria | 47.84 | 15.02 | sp. 4 | 2A | HQ204466.1 | HQ204521.1 | | Kruckenhauser et al. 2014 |
| 109_102 | Hallstatt | Austria | 47.56 | 13.64 | sp. 4 | 2A | HQ204464.1 | HQ204516.1 | | Kruckenhauser et al. 2014 |
| 140_24 | Gesäuse | Austria | 47.56 | 14.58 | sp. 4 | 2A | HQ204446.1 | HQ204520.1 | KJ151721 | Kruckenhauser et al. 2014 |
| 683_5 | Semmering | Austria | 47.63 | 15.88 | sp. 4 | 2A | HQ204476.1 | HQ204529.1 | KJ151723 | Kruckenhauser et al. 2014 |
| 688_50 | Hohe Wand | Austria | 47.78 | 16.04 | sp. 4 | 2A | HQ204473.1 | HQ204530.1 | | Kruckenhauser et al. 2014 |
| 726_94 | westliches Mittelgebirge | Austria | 47.23 | 11.34 | sp. 4 | 2A | KJ151322 | KJ151562 | | Kruckenhauser et al. 2014 |

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|---|---|---|---|---|---|---|---|---|---|---|
| 736_130 | Bruck an der Mur | Austria | 47.72 | 15.16 | sp. 4 | 2A | HQ204483.1 | HQ204526.1 | KJ151728 | Kruckenhauser et al. 2014 |
| 763_64 | Gailtaler Alpen | Austria | 46.68 | 13.45 | sp. 4 | 2A | KJ151333 | KJ151568 | | Kruckenhauser et al. 2014 |
| 769_66 | Gurktaler Alpen | Austria | 46.92 | 13.63 | sp. 4 | 2A | KJ151337 | KJ151572 | | Kruckenhauser et al. 2014 |
| 852_159 | Waldviertel | Austria | 48.78 | 15.12 | sp. 4 | 2A | KJ151343 | KJ151578 | KJ151733 | Kruckenhauser et al. 2014 |
| 926_140 | Grazer Bergland | Austria | 47.18 | 15.39 | sp. 4 | 2A | HQ204450.1 | HQ204504.1 | | Kruckenhauser et al. 2014 |
| 1294_168 | Schneeberg | Austria | 47.76 | 15.98 | sp. 4 | 2A | HQ204478.1 | | | Kruckenhauser et al. 2014 |
| 1331_167 | Bucklige Welt | Austria | 47.66 | 16.15 | sp. 4 | 2A | HQ204496.1 | | | Kruckenhauser et al. 2014 |
| 1335_93 | Achensee | Austria | 47.57 | 11.67 | sp. 4 | 2A | KJ151355 | | | Kruckenhauser et al. 2014 |
| 1346_60 | Sattnitz | Austria | 46.6 | 14.43 | sp. 4 | 2A | KJ151362 | | | Kruckenhauser et al. 2014 |
| 1472_223 | Julische Alpen | Slovenia | 46.34 | 13.64 | sp. 4 | 2A | KJ151376 | | | Kruckenhauser et al. 2014 |
| 1480_204 | Defereggen-Gebirge | Austria | 46.81 | 12.33 | sp. 4 | 2A | KJ151383 | | | Kruckenhauser et al. 2014 |
| 1832_208 | Fischbacher Alpen | Austria | 47.51 | 15.66 | sp. 4 | 2A | HQ204436.1 | HQ204515.1 | | Kruckenhauser et al. 2014 |
| 1834_231 | Donauinsel | Austria | 48.2 | 16.45 | sp. 4 | 2A | KJ151393 | | | Kruckenhauser et al. 2014 |
| 2000_237 | Warscheneck | Austria | 47.63 | 14.32 | sp. 4 | 2A | HQ204499.1 | | | Kruckenhauser et al. 2014 |
| 2879_286 | Pannonische Tiefebene | Austria | 47.87 | 16.67 | sp. 4 | 2A | KJ151413 | | | Kruckenhauser et |

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|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | al. 2014 |
| 2984_306 | Oberes Mürztal | Austria | 47.74 | 15.47 | sp. 4 | 2A | HQ204482.1 | | | Kruckenhauser et al. 2014 |
| 2994_311 | Mariazell | Austria | 47.81 | 15.45 | sp. 4 | 2A | HQ204469.1 | | | Kruckenhauser et al. 2014 |
| 3004_313 | Schwarzatal/Steinapiesting | Austria | 47.88 | 15.65 | sp. 4 | 2A | HQ204490.1 | | | Kruckenhauser et al. 2014 |
| 3133_317 | Gutensteineralpen | Austria | 47.91 | 15.68 | sp. 4 | 2A | HQ204493.1 | HQ204531.1 | | Kruckenhauser et al. 2014 |
| 3137_318 | Hochschwab | Austria | 47.67 | 15.16 | sp. 4 | 2A | HQ204470.1 | | | Kruckenhauser et al. 2014 |
| 3203_339 | Mürztal | Austria | 47.66 | 15.61 | sp. 4 | 2A | KJ151434 | | | Kruckenhauser et al. 2014 |
| 3307_341 | Lunz | Austria | 47.86 | 15.06 | sp. 4 | 2A | KJ151437 | | | Kruckenhauser et al. 2014 |
| 3575_386 | Sengsengebirge | Austria | 47.87 | 14.33 | sp. 4 | 2A | KJ151447 | | | Kruckenhauser et al. 2014 |
| 3907_385 | Totes Gebirge | Austria | 47.77 | 14.01 | sp. 4 | 2A | KJ151449 | | | Kruckenhauser et al. 2014 |
| 4263_446 | Glocknergebiet | Austria | 47.01 | 12.87 | sp. 4 | 2A | KJ151489 | | | Kruckenhauser et al. 2014 |
| 1455_200 | Karnische Alpen | Italy | 46.59 | 13 | sp. 4 | 2B | KJ151369 | KJ151589 | KJ151740 | Kruckenhauser et al. 2014 |
| 1471_223 | Julische Alpen | Slovenia | 46.34 | 13.64 | sp. 4 | 2B | KJ151375 | KJ151590 | | Kruckenhauser et al. 2014 |
| 4145_402 | Karawanken | Austria | 46.51 | 14.43 | sp. 4 | 2B | KJ151469 | | | Kruckenhauser et al. 2014 |
| 1341_86 | Achensee | Austria | 47.52 | 11.69 | *sericeus* | 3A | KJ151358 | KJ151585 | KJ151737 | Kruckenhauser et al. 2014 |

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|---|---|---|---|---|---|---|---|---|---|---|
| 1475_217 | Salzkammergut | Austria | 47.63 | 13.27 | *sericeus* | 3A | KJ151379 | KJ151591 | KJ151741 | Kruckenhauser et al. 2014 |
| 2899_291 | Mecsek Gebirge | Hungary | 46.17 | 18.23 | *sericeus* | 3A | KJ151422 | KJ151597 | KJ151753 - KJ151755 | Kruckenhauser et al. 2014 |
| 4155_407 | Berchtesgadener_Land | Germany | 47.68 | 13.01 | *sericeus* | 3A | KJ151472 | KJ151600 | KJ151746 | Kruckenhauser et al. 2014 |
| 4165_412 | Berchtesgadener_Land | Germany | 47.75 | 12.64 | *sericeus* | 3A | KJ151475 | KJ151601 | KJ151756, KJ151757 | Kruckenhauser et al. 2014 |
| 6233_549 | Inntal | Austria | 47.28 | 11.18 | *sericeus* | 3A | KJ151518 | | | Kruckenhauser et al. 2014 |
| 849_545 | Waldviertel | Austria | 48.78 | 15.13 | *sericeus* | 3B | KJ151340 | KJ151575 | KJ151732 | Kruckenhauser et al. 2014 |
| 5540_476 | Donautal (Sauwald) | Austria | 48.42 | 13.87 | *sericeus* | 4 | KJ151506 | KJ151611 | KJ151763 - KJ151764 | Kruckenhauser et al. 2014 |
| 5538_476 | Donautal (Sauwald) | Austria | 48.42 | 13.87 | sp. 3 | 5 | KJ151504 | KJ151609 | KJ151748 | Kruckenhauser et al. 2014 |
| 101_33 | Nationalpark Donauauen | Austria | 48.11 | 16.78 | sp. 1 | 6A | KJ151294 | KJ151549 | KJ151719 | Kruckenhauser et al. 2014 |
| 2889_296 | Duna-Dráva Nationalpark | Hungary | 46.2 | 18.92 | sp. 1 | 6A | KJ151419 | KJ151596 | | Kruckenhauser et al. 2014 |
| 5588_482 | Taubertal | Germany | 49.71 | 9.55 | sp. 1 | 6B | KJ151508 | KJ151613 | KJ151749 | Kruckenhauser et al. 2014 |
| 6234_549 | Inntal | Austria | 47.28 | 11.18 | sp. 1 | 6B | KJ151519 | | | Kruckenhauser et al. 2014 |
| 4291_453 | Vätra Götalands Län | Sweden | 58.03 | 13.73 | sp. 2 | 7 | KJ151497 | KJ151607 | | Kruckenhauser et al. 2014 |
| 6242_560 | Frutigen-Niedersimmental | Switzerland | 46.43 | 7.78 | *albulus* | 8A | KJ151525 | KJ151614 | KJ151750 | Kruckenhauser et al. 2014 |
| 6249_555 | Karlsruhe | Germany | 49.08 | 8.37 | *albulus* | 8A | KJ151529 | KJ151615 | | Kruckenhauser et |

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|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | al. 2014 |
| 2079_248 | Graubünden | Switzerland | 46.52 | 9.63 | *albulus* | 8B | KJ151407 | KJ151593 | | Kruckenhauser et al. 2014 |
| 725_94 | westliches Mittelgebirge | Austria | 47.23 | 11.34 | *dubia* | 9 | KJ151321 | KJ151561 | KJ151726 | Kruckenhauser et al. 2014 |
| 1478_207 | Defereggen-Gebirge | Austria | 47.01 | 12.44 | *dubia* | 9 | KJ151381 | | | Kruckenhauser et al. 2014 |
| 6229_550 | Inntal | Austria | 47.28 | 11.19 | *dubia* | 9 | KJ151515 | | | Kruckenhauser et al. 2014 |
| 6359_559 | Ost-Obwalden | Switzerland | 46.87 | 8.43 | *R. biconica* | *biconicus* | KJ151532 | KJ151617 | KJ151752 | Kruckenhauser et al. 2014 |
| 1381_199 | Graubünden | Switzerland | 46.81 | 9.53 | *clandestinus* | *clandestinus* | KJ151365 | KJ151586 | KJ151738 | Kruckenhauser et al. 2014 |
| 4044_413 | Schwaben | Germany | 48.47 | 10.29 | *sericeus* | *coelomphala* | KJ151459 | KJ151598 | KJ151743 | Kruckenhauser et al. 2014 |
| 5592_483 | Bayrisches Donautal | Germany | 48.97 | 12.39 | *sericeus* | *coelomphala* | KJ151511 | | | Kruckenhauser et al. 2014 |
| 248_33 | Nationalpark Donauau | Germany | 48.11 | 16.78 | *striolatus* | *striolatus* | KJ151301 | KJ151555 | KJ151722 | Kruckenhauser et al. 2014 |
| 251_122 | Höllengebirge | Austria | 47.82 | 13.6 | *striolatus* | *striolatus* | KJ151304 | KJ151557 | | Kruckenhauser et al. 2014 |
| 687_71 | Wechsel | Austria | 47.54 | 15.98 | *striolatus* | *striolatus* | KJ151315 | KJ151560 | KJ151724 | Kruckenhauser et al. 2014 |
| 1342_142 | Stockerauer Au | Austria | 48.38 | 16.2 | *striolatus* | *striolatus* | KJ151359 | | | Kruckenhauser et al. 2014 |
| 2077_249 | Baden Württemberg | Germany | 48.49 | 9.69 | *striolatus* | *striolatus* | KJ151405 | KJ151592 | | Kruckenhauser et al. 2014 |
| 4001_414 | Schwäbische_Alb | Germany | 48.56 | 9.6 | *striolatus* | *striolatus* | KJ151450 | | | Kruckenhauser et al. 2014 |

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|---|---|---|---|---|---|---|---|---|---|---|
| 5528_469 | Donautal (Sauwald) | Austria | 48.46 | 13.79 | *striolatus* | *striolatus* | KJ151501 | | | Kruckenhauser et al. 2014 |
| 1289_193 | Žilinský kraj | Slovakia | 48.96 | 18.52 | *villosulus* | *villosulus* | KJ151350 | KJ151581 | KJ151735 | Kruckenhauser et al. 2014 |
| 4053_428 | Žilina | Slovakia | 49.17 | 18.58 | *villosulus* | *villosulus* | KJ151462 | | | Kruckenhauser et al. 2014 |
| 4192_413 | Schwaben | Germany | 48.47 | 10.29 | *villosus* | *villosus* | KJ151481 | KJ151603 | | Kruckenhauser et al. 2014 |
| 4203_417 | Schwäbische_Alb | Germany | 48 | 8.85 | *villosus* | *villosus* | KJ151485 | KJ151605 | KJ151760 - KJ151762 | Kruckenhauser et al. 2014 |
| Hu_3 | Hundersingen | Germany | 48.0708 | 9.396 | *striolatus* | *striolatus* | MT754820 | MT755541 | MT755442 | Prockow et al. 2020 |
| Hu_13 | Hundersingen | Germany | 48.0708 | 9.396 | *striolatus* | *striolatus* | MT754819 | MT755540 | | Prockow et al. 2020 |
| Dil_11 | Dillingen a/d Donau | Germany | 48.5684 | 10.4999 | *striolatus* | *striolatus* | MT754803 | MT755524 | MT755444 | Prockow et al. 2020 |
| Dil_15 | Dillingen a/d Donau | Germany | 48.5684 | 10.4999 | *striolatus* | *striolatus* | MT754804 | MT755525 | MT755445 | Prockow et al. 2020 |
| Bi_5 | Bittenbrunn | Germany | 48.7394 | 11.1619 | *sericeus* | *coelomphala* | MT754796 | MT755517 | MT755401 | Prockow et al. 2020 |
| Bi_6 | Bittenbrunn | Germany | 48.7394 | 11.1619 | *sericeus* | *coelomphala* | MT754797 | MT755518 | MT755432 | Prockow et al. 2020 |
| Wa_5 | Wackerstein | Germany | 48.7888 | 11.6756 | sp. 3 | sp. | MT754855 | MT755576 | MT755422 | Prockow et al. 2020 |
| Wa_10 | Wackerstein | Germany | 48.7888 | 11.6756 | sp. 3 | sp. | MT754854 | MT755575 | MT755423 | Prockow et al. 2020 |
| Kel_4 | Kehlheim | Germany | 48.9151 | 11.8661 | *striolatus* | *striolatus* | MT754823 | MT755544 | MT755411 | Prockow et al. 2020 |
| Kel_6 | Kehlheim | Germany | 48.9151 | 11.8661 | *striolatus* | *striolatus* | MT754824 | MT755545 | MT755447 | Prockow et al. 2020 |
| Zu_1 | Zulling | Germany | 48.6668 | 12.6645 | *dubia* | *hispidus* | MT754856 | MT755577 | MT755428 | Prockow et al. 2020 |
| Zu_2 | Zulling | Germany | 48.6668 | 12.6645 | *dubia* | *hispidus* | MT754857 | MT755578 | MT755429 | Prockow et al. 2020 |
| EP_4 | Erding-Pretzen | Germany | 48.2804 | 11.9075 | *dubia* | *hispidus* | MT754808 | MT755529 | MT755404 | Prockow et al. 2020 |
| EP_5 | Erding-Pretzen | Germany | 48.2804 | 11.9075 | *dubia* | *hispidus* | MT754809 | MT755530 | MT755405 | Prockow et al. 2020 |
| Bo_3 | Bödele | Austria | 47.4235 | 9.8077 | *sericeus* | *hispidus* | MT754799 | MT755520 | MT755395 | Prockow et al. 2020 |
| Bo_4 | Bödele | Austria | 47.4235 | 9.8077 | *sericeus* | *hispidus* | MT754800 | MT755521 | MT755396 | Prockow et al. 2020 |
| Le_21 | Lechsend | Germany | 48.7384 | 10.9124 | *striolatus* | *striolatus* | MT754838 | MT755559 | MT755453 | Prockow et al. 2020 |
| Le_2 | Lechsend | Germany | 48.7384 | 10.9124 | *sericeus* | *coelomphala* | MT754837 | MT755558 | MT755414 | Prockow et al. 2020 |

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|---|---|---|---|---|---|---|---|---|---|---|
| Gm_2 | Gremheim | Germany | 48.5846 | 10.5393 | *sericeus* | *coelomphala* | MT754817 | MT755536 | MT755410 | Prockow et al. 2020 |
| ALNHM363-17 | Bezau | Austria | 47.3804 | 9.9208 | *clandestinus* | *clandestinus* | ALNHM362-17.COI-5P | ALNHM362-17.16S | | Duda et al. 2017 |
| ALNHM362-17 | Bezau | Austria | 47.3804 | 9.9208 | *clandestinus* | *clandestinus* | ALNHM363-17.COI-5P | ALNHM363-17.16S | | Duda et al. 2017 |
| MNHN-IM-2009-20119 | Chateau Garnier | France | 44.082 | 6.488 | *plebeius* | | MEMOL037-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20195 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL211-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20196 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL398-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20197 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL404-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20198 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL373-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20199 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL368-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20200 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL178-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20202 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL253-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20204 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL162-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20205 | Les Blancs | France | 44.357 | 6.618 | *plebeius* | | MEMOL175-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20236 | Molines en Queyras | France | 44.73 | 6.844 | *plebeius* | | MEMOL379-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20237 | Molines en Queyras | France | 44.73 | 6.844 | *plebeius* | | MEMOL374-11 | | | publ. in BOLD by Gargominy |

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|---|---|---|---|---|---|---|---|---|---|---|
| MNHN-IM-2009-20239 | Molines en Queyras | France | 44.73 | 6.844 | *plebeius* | | MEMOL366-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20240 | Molines en Queyras | France | 44.73 | 6.844 | *plebeius* | | MEMOL324-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20276 | Les la Croix Haute | France | 44.657 | 5.711 | *plebeius* | | MEMOL400-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20506 | St. Hugues | France | 45.299 | 5.81 | *albulus* | *phorochaetia* | MEMOL318-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-20507 | St. Hugues | France | 45.299 | 5.81 | *albulus* | *phorochaetia* | MEMOL540-12 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-21154 | Barcelonnette | France | 44.382 | 6.631 | *plebeius* | | MEMOL208-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-21155 | Barcelonnette | France | 44.382 | 6.631 | *plebeius* | | MEMOL210-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-21156 | Barcelonnette | France | 44.382 | 6.631 | *plebeius* | | MEMOL215-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-21157 | Barcelonnette | France | 44.382 | 6.631 | *plebeius* | | MEMOL181-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-21158 | Barcelonnette | France | 44.382 | 6.631 | *plebeius* | | MEMOL271-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-21160 | Jausiers | France | 44.442 | 6.742 | *plebeius* | | MEMOL258-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-21161 | Jausiers | France | 44.442 | 6.742 | *plebeius* | | MEMOL242-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2009-21162 | Jausiers | France | 44.442 | 6.742 | *plebeius* | | MEMOL248-11 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2013-76655 | Coranche | France | 45.0737 | 5.3984 | *albulus* | *phorochaetia* | LPRCM336-21 | | | publ. in BOLD by Gargominy |
| MNHN-IM-2013- | Coranche | France | 45.0737 | 5.3984 | *albulus* | *phorochaetia* | LPRCM337- | | | publ. in BOLD by |

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|---|---|---|---|---|---|---|---|---|---|---|
| 76656 | | | | | | | 21 | | | Gargominy |
| MNHN-IM-2013-76657 | Coranche | France | 45.0737 | 5.3984 | *albulus* | *phorochaetia* | LPRCM338-21 | | | publ. in BOLD by Gargominy |
| M1_12_767 | Heumadgupf | Austria | 47.81 | 13.71 | *N. scheerpeltzi* | *N. scheerpeltzi* | HQ204394 | | KJ151731 | Bamberger et al. 2020 |
| M3_765_7172 | Hochmölbing | Austria | 47.62 | 14.16 | *N. scheerpeltzi* | *N. scheerpeltzi* | MN193608 | | MN299115 | Bamberger et al. 2020 |
| M4_367_3396 | Haltersitz | Austria | 47.79 | 14.32 | *N. scheerpeltzi* | *N. scheerpeltzi* | HQ204392 | | KJ151742 | Bamberger et al. 2020 |
| M5_443_4108 | Westgrat | Austria | 47.65 | 14.38 | *N. scheerpeltzi* | *N. scheerpeltzi* | MN193721 | | MN299218 | Bamberger et al. 2020 |
| M6_782_7237 | Natterriegel | Austria | 47.64 | 14.48 | *N. oreinos* | *N. oreinos* | MN193670 | | MN299147 | Bamberger et al. 2020 |
| M7_55_707 | Admonter Kalbling | Austria | 47.55 | 14.52 | *N. oreinos* | *N. oreinos* | HQ204396 | | KJ151725 | Bamberger et al. 2020 |
| M8_781_7227 | Grosser Buchstein | Austria | 47.61 | 14.59 | *N. oreinos* | *N. oreinos* | MN193660 | | MN299128 | Bamberger et al. 2020 |
| M9_779_7222 | Hesshütte | Austria | 47.56 | 14.64 | *N. oreinos* | *N. oreinos* | MN193657 | | MN299121 | Bamberger et al. 2020 |
| M11_134_999 | Schiestlhaus | Austria | 47.62 | 15.14 | *N. oreinos* | *N. oreinos* | HQ204422 | | KJ151734 | Bamberger et al. 2020 |
| M14_79_757 | Bismarcksteig | Austria | 47.69 | 15.7 | *N. oreinos* | *N. oreinos* | HQ204409 | | KJ151729 | Bamberger et al. 2020 |

## Chapter 4

In several side projects, I generated COI, 16S, and H3 sequences for the micro gastropod species of *Zospeum* and *Carychium*. Since these specimens are very small (< 2 mm), a new DNA extraction protocol was developed to generate enough DNA for the PCR in the first place. Still, the Alpine-Dinaric radiation of *Zospeum* is little explored. Due to the lack of historic type specimens, insufficient original description, and the lack of freshly collected specimens from western Balkan caves, the only known southern species, *Z. troglobalcanicum,* has remained an unknown entity. Based on recent collection efforts and my sequencing and phylogenetic analyses of new individuals, new species within the Dinaric genus *Zospeum* will be described soon.

For the ongoing revision and the worldwide phylogeny of the ellobiid genus *Carychium*, I provided the genetic data and assisted in the performance and interpretation of phylogenetic analyses. The phylogeny serves as the backbone for the taxonomic revision of the genus and reveals several new clades, which will be described as new species.

## Discussion

Cryptic species have been revealed in various organism groups in recent years (e.g., Hebert *et al.* 2004; Funk *et al*. 2011; Weigand et al. 2013; Cruse *et al*. 2022). We are increasingly realising that we only see the tip of the iceberg in terms of species numbers in the World. So far, 1.2 million species have been described worldwide, but estimates suggest that up to 8.7 million eukaryotic species exist (Mora *et al.* 2011). We are still a long way from capturing all this biodiversity on Earth.

Cryptic species are a man-made concept not known by Nature. Species differ in ways that may be beyond our visual or auditory comprehension. But species have a way of distinguishing themselves and, for example, of finding the “right” partner for mating and avoiding the “wrong” ones which could lead to sterile offspring. We can only speak of cryptic species until we have fully investigated the animals using an integrative approach. In the identification of separate species, greater emphasis should be placed on the re-evaluation of morphological traits in the future. Here, the genetic findings serve as a guideline to identify possible, previously hidden convergences, which in previous work were wrongly interpreted as synapomorphies and thus, lead to paraphyly in the phylogenetic trees. The concept of integrative taxonomy follows this idea (e.g., DeSalle *et al*. 2005; Bickford *et al*. 2007; Padial *et al.* 2010; Horsáková *et al.* 2022).

Cryptic speciation is exemplified in Chapter 1 with the Spanish *Iberozospeum* radiation. The species are difficult to distinguish from shell morphology alone and show a high intraspecific variability. Among the specimens we studied, only some species such as *I. biscaiense* (Gómez

& Prieto, 1983), *I. percostulatum* (Á. Alonso, Prieto, Quiñonero-Salgado & Rolán, 2018), and *I. costulatum* Prieto & Jochum, 2022 can be well distinguished morphologically. In congeners, there is a morphological transition. An extensive sampling of northern Spanish caves and genetic methods have brought clarity now to the Spanish radiation. The investigation of a concatenated dataset with the four markers COI, 16S, H3 and ITS2, revealed a deep genetic split between the Spanish and the Alpine-Dinaric radiation. We found nine clades in the Spanish radiation, but the support values were not significant due to some missing markers for some specimens. With a reduced dataset containing only specimens with complete marker sets, we found the same nine clades, but the support values were significantly higher. This shows that the phylogeny covering all specimens available is reliable. We found three distinct clades within the hitherto existing species *I. vasconicum*. These clades are also consistent with the geographical distribution of the investigated individuals. The morphological discrimination from the eastern Alpine and Dinaric radiation of *Zospeum* could be performed with the help of histological analysis of the upper visceral complex and scanning electron microscopic (SEM) analyses of radulae. The composition and form of radular teeth is influenced by mineral composition of the environment, the diet, and other factors (Luchtel *et al.* 1997). Our observations that the columellar muscle becomes detached from the body wall from its origin on the columella are in sync with the trends in Ellobiidae (Barker 2001). These findings in particular show how important it is to carry out integrative studies and use several character states for species identification.

Chapter 2 and 3 of my doctoral thesis deal with the hairy snail genus *Trochulus*. A revision of the genus by Proćków (2009) was dominated by shell and genital anatomical analyses and raised more questions than clarity. Research in the *Trochulus* complex has been going on for years, but only small areas of the distribution range have been collected and studied (e.g., Dépraz *et al.* 2009; Duda *et al.* 2014; Kruckenhauser *et al.* 2014; Proćków *et al.* 2014, 2017a, 2021). Therefore, the general overview was missing for a thorough revision of the genus. With our extensive sampling of about 570 specimens from almost the entire distribution range of the genus, we were now able to genetically identify different clades for the first time. It turned out that many morphologically described species consist of several clades that are stable across different phylogenetic analysis methods. Some species are restricted to the Jura mountains and neighbouring areas, which could indicate, that the species retreated to refugia in the unglaciated part of the Jura mountains during the Last Glacial Maximum (LGM; e.g., Graf *et al.* 2015; Claude *et al.* 2019). These species could not spread very far after the retreat of the ice. In contrast, other species that are widespread in Europe, such as *T. hispidus*, probably had a greater potential for dispersal and could occupy different niches. The endemic species, *T. biconicus*, which we separated from the genus *Trochulus* due to its genetics, shell morphology and genital anatomical peculiarities and placed it in the monotypic genus

*Raeticella*, has presumably survived the LGM on nunataks (see Chapter 2). Here, the species lives in a barren landscape under stones and feeds on blue grass (*Sesleria caerulea*). *Trochulus* species, on the other hand, inhabit a wide range of habitats: from rocky alpine sites, lowlands, most often adjoining rivers, forests and nettle patches, or on tall grasses in open areas (e.g., Duda *et al.* 2014; Proćków *et al*. 2017b). *Trochulus* species seem to have great adaptability and have been able to colonise diverse habitats. Locally, genetically differentiated species have evolved, but we have so far missed them because of their morphological similarity. However, the shells have not changed much and only a few species can be identified by their shells. With our integrative approach, we have now uncovered some of these cryptic species.

In Chapter 4, I give some insights to our ongoing research on micro gastropods. Because the species of *Zospeum* and *Carychium* are so small and difficult to find, they remain either unknown or are often ignored due to the tedious effort required to find them. Many species descriptions are still based on shell morphology and anatomical characteristics (Bank & Gittenberger 1985). With the help of an integrative taxonomic approach, incorporating multiple lines of evidence, the species, *Z. kolbae* sp. n. for example, was genetically flagged in my analyses and is currently being processed in another work. This case constitutes yet another species that brings us closer to the real number of species in the World. Moreover, the global phylogeny with the inclusion of topotypic specimens brings valuable insights towards clarifying the systematics of *Carychium*. Within the species *C. minimum*, for example, we have detected various lineages that will soon be described as separate species.

In various land snail groups, I am endeavouring to reveal the diversity within cryptic species using integrative taxonomy. Only with the help of integrative studies can we manage to identify the World's biodiversity.

## Outlook

In my studies these past few years, I was able to corroborate some species' concepts within certain land snail groups, leading to description of new species, but also to the synonymising of validly described taxa. This procedure requires the use of a significant number of animals from the whole known ecological and biogeographic range and the investigation of as many as possible different character states (Horsáková *et al.* 2022). All the aforementioned examples show that we cannot, in most cases, interpret so many species using only shell morphology. For future studies, it is essential to also focus on other character states than morphology and genetic data. Seldomly used traits such as mating signals, behavioural traits, bioacoustics data and biogeography may be promising. The chemical communication strategies in molluscs should also be studied more closely. Ballard *et al.* (2021) have shown

that the animals communicate with each other by means of proteins and pheromones in the trail mucus. This could be an approach for “cryptic” *Trochulus* species to identify species-specific differences. For smaller gastropod species such as *Iberozospeum*, *Zospeum* and *Carychium*, CT scans and histological analyses have proven successful. Since histological investigations are very tedious and time consuming beyond the few years of a doctoral thesis (A. Jochum, pers. comm. 2022) and CT scans constitute expensive methods, they are rarely used to the level we need. Still, their use would further enhance our knowledge of these miniscule snails especially if extended to several individuals per species to better identify morphological differences between species. It would also be interesting to examine the hairs of *Trochulus* with high-resolution SEM. The different lengths and shapes of the hairs can be determined well with a binocular microscope, but species-specific differences may be found microscopically. Microscopic examination of the love darts and spermatophores may also reveal species-specific differences. Although we have tried to cover the entire distribution range of the traditionally recognized taxonomic units, we are still missing some regions that may harbour “cryptic” species. For *Trochulus*, we still have collection gaps in western France and in central and northern Germany. Only by conscientiously sampling these regions and examining the specimens, will we get a complete picture. At least we were able to provide a deeper insight into the taxonomy of different land snail groups. In summary, some biological aspects should be added to the integrative taxonomy method. These include the fields described above, whose potential in the recognition of species, including “cryptic” species, has not yet been exploited.

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