The role of Anatolia in the origin of the Caucasus biodiversity hotspot illustrated by land snails in the genus *Oxychilus*

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Abstract

Several taxa that are distributed in the Caucasus and/or the adjacent Pontic Mountains also have representatives in the East Mediterranean region. These disjunctions could have been caused by long-distance dispersal or be the result of extinctions in Central Anatolia caused by the aridification of the Anatolian Plateau during the Pliocene. We studied the *Longiphallus*–*Hiramia* group of *Oxychilus* as an example showing such distribution patterns. Phylogenetic analyses of the *Oxychilus* species previously classified in *Longiphallus*, *Hiramia* and related subgenera resulted in a new delimitation of these taxa and the recognition of *Anatoloxychilus* Neiber, Walther & Hausdorf n. subgen. as an additional clade. Based on phylogenetic and population genetic analyses, *O. reticulatus* from Mingrelia is revalidated and the populations from the Pontic Mountains previously identified with *O. mingrelicus koutaisanus* are recognised as a distinct species. Three species pairs of the *Longiphallus*–*Hiramia* group with deep splits predating the aridification of the Anatolian Plateau during the Pliocene show disjunctions between the Caucasus/Pontic region and the Mediterranean. The majority of taxa with such a distribution pattern probably had more continuous distributions before the aridification started. The relationships between the *Hiramia* species from the Caucasus, the Pontic Mountains and the East Mediterranean highlight the importance of the Anatolian land as a source area for the colonisation of the Caucasus region. The dating of the divergences of the Caucasian *Hiramia* species in the middle to late Miocene indicated that they colonised the Caucasus when it was still an island in the Paratethys Sea and that their divergence was triggered by the orogenesis of the Greater Caucasus. A common pattern within the Caucasus region, also found in *Hiramia*, is the separation of taxa in the northwestern Greater Caucasus from taxa inhabiting the southern slopes of the central Greater Caucasus.

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Introduction

The Caucasus region is among the most important global biodiversity hotspots and harbours a rich endemic fauna and flora (Myers et al., 2000; Zazanashvili et al., 2004). The first islands in the Caucasus region formed in the Paratethys in the Oligocene and fused after considerable uplift with Asia anterior towards the end of the Miocene (Popov et al., 2004; Vincent et al., 2016). Little is known about the origin of the Caucasian biota and its radiation within the hotspot (Tarkhnishvili et al., 2000; Tarkhnishvili, 2014; Neiber and Hausdorf, 2015; Koch et al., 2016; Neiber et al., 2016, 2017, 2018; Walther et al., 2016; Hausdorf et al., 2018; Hrivniak et al., 2020; Murtshkhvaladze et al., 2020) because the distributions and phylogeny are well documented only for few taxa that radiated within the Caucasus region. However, the importance of the Caucasian region as a glacial refugium where Neogene relict species survived has previously been appreciated (Pokryszko et al., 2011; Tarkhnishvili et al., 2012;
Nakhutsrishvili, 2013; Tarkhnishvili, 2014; Walther et al., 2014). Several taxa that are distributed in the Caucasus region and/or the adjacent Pontic Mountains also have disjunct representatives in the Eastern Mediterranean region (e.g. Nordsieck, 1994), another global biodiversity hotspot (Myers et al., 2000). Such distribution patterns may be relics of formerly continuous distributions, which were disrupted by the extinction of populations on the species-poor Anatolian Plateau. Alternatively, they could also have been caused by long-distance dispersal.

We studied the relationships between Caucasus/Pontic and Mediterranean taxa and the described disjunct representatives in the Eastern Mediterranean and Macaronesian Islands and the adjacent Pontic Mountains and the described disjunct Mediterranean taxa and the described disjunct representatives in the Eastern Mediterranean and Macaronesian Islands. Such distribution patterns may be relics of formerly continuous distributions, which were disrupted by the extinction of populations on the species-poor Anatolian Plateau. Alternatively, they could also have been caused by long-distance dispersal.

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We selected the subgenus *Longiphallus* Riedel, 1958 of *Oxychilus* (Oxychilidae Hesse, 1927 (1879)) as delimited by Riedel (1966, 1980, 1989) and related taxa as a model group (Fig. 1). *Oxychilids* are hygro- or mesophilic (Kenney and Cameron, 1979) and their relationships and distribution patterns may reflect the colonisation of the Caucasus region and the subsequent fragmentation of forests. Endemic *Longiphallus* species are present in the north-western region of the Greater Caucasus, the southern slopes of the Greater Caucasus, the Talysh and Alborz mountains south of the Caspian Sea and several regions around the Black Sea, and one species even occurs on Crete in the Eastern Mediterranean (Riedel, 1966, 1980, 1989). There are no cladistic analyses of the relationships of *Longiphallus* within *Oxychilus* based on morphological characters, but there are some hypotheses derived from comparisons of the genitalia. Riedel (1966) suggested that *Hiramia* Riedel, 1962 from the Eastern Mediterranean and Middle East and *Cellariopsis* Wagner, 1914 from the Carpathian Mountains are derived from *Longiphallus*, which is closely related to the *Ortizius* Forcart, 1957 group including several taxa from the Macaronesian Islands (Atlasxychilus Riedel, 1964, Drouetia Gude, 1911, Radiulus Wollaston, 1878), as well as *Calloretinella* Haas, 1934 from Cyprus (see also Riedel, 1991). Riedel (1983) doubted even the distinctness of *Hiramia* and *Longiphallus* because of the similarity of *O. (Hiramia) cyprius* (Pfeiffer, 1847) and *O. delius* (Bourguignat, 1857), classified by him in *Longiphallus*. However, he retained them as different subgenera of *Oxychilus* (Riedel, 1995). Furthermore, Riedel (1980, 1990) stated that

Helicophana Westerlund, 1886 from Crete is anatomically close to *Longiphallus*. Finally, Colville and Riedel (1998) supposed that *Araboxychilus* Riedel, 1977 from the south-western part of the Arabian Peninsula is related to *Ortizius* and *Longiphallus*. In their review of the *Oxychilus* taxonomy, Giusti and Manganelli (1999) suggested to combine the ill-defined subgenera *Calloretinella*, *Helicophana*, *Longiphallus*, *Ortizius*, the Macaronesian taxa and several additional taxa in *Oxychilus* (*Oxychilus*), whereas they tentatively synonymised *Hiramia* with *Oxychilus* (*Schistophallus*) Wagner, 1914 and kept *Oxychilus* (*Cellariopsis*) as a distinct subgenus.

We analysed the phylogenetic relationships of the *Longiphallus* species and the possibly related subgenera *Araboxychilus*, *Calloretinella*, *Cellariopsis*, *Helicophana*, *Hiramia*, and *Ortizius* using mitochondrial and nuclear sequences to reconstruct their biogeographic history. We examined the species delimitation, phylogeographic patterns, and population structure of the Caucasian *Longiphallus* species in more detail based on mitochondrial sequences and multilocus AFLP markers.

**Material and methods**

**Sampling**

Most of the material for this study was collected between 2011 and 2014 in Georgia, Armenia, Azerbaijan, the Russian Caucasus, the Crimean Peninsula and north-eastern Turkey (see Table S1 for details). Newly collected samples of *O. delius*, *O. filicum* (Krynicki, 1836), *O. mingrelicus mingrelicus* (Mousson, 1863), *O. mingrelicus koutaisanus* (Mousson, 1863) (note that the name *mingrelicus* Mousson, 1863 does not accept priority over *koutaisanus* Mousson, 1863 following ICZN, 1999 Art. 24.1.), *O. oschienicus* (Boettiger, 1888) and *O. reticulatus* (Boettger, 1881) are housed in the collection of the Zoological Museum Hamburg, Germany (ZMH). Specimens were determined using Riedel (1958, 1966, 1989, 1993, 1995) and by comparison with type material in the collections of the Senckenberg Forschungsmuseum, Frankfurt, Germany (SMF). Additionally, (in part older) samples of *O. camaelius* (Bourguignat, 1852), *O. cypris*, *O. debellii* (Wagner, 1914), *O. navronooustakistis* (Haas, 1934), *O. spatiosus* (Lindholm, 1922) (= *O. costarius* Riedel, 1989, see Walther and Neiber, 2018), *O. secernendus* (Retowski, 1889), *O. paphlagonicus* Riedel, 1993,*O. sabaeus* (Martens, 1889), *O. aegopinoides* (Maltzan, 1883) and *O. superflua* (Pfeiffer, 1849) were used. Tissue samples of the foot of newly collected specimens were stored in 96% ethanol or in 100% isopropanol at −20 °C for molecular genetic analyses. *Oxychilus* cellarius (Müller, 1774), *O. drapparnaudi* (Beck, 1837), *O. diphanellus* (Krynicki, 1836), *O. navariicus* (Bourguignat, 1870) and *O. allarius* (Miller, 1822) were included as outgroup taxa. Data on sampling sites, voucher numbers and the classification of each specimen used in this study are compiled in Table S1.

**DNA extraction, amplification, sequencing and sequence assembly**

Total genomic DNA was extracted following a slightly modified version of the protocol of Sokolov (2000) as detailed by Scheel and Hausdorf (2012). Parts of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) and the 16S rRNA (*16S*) genes as well as the
nuclear ribosomal gene cluster including parts of the 5.8S rRNA (5.8S) gene, the complete internal transcribed spacer (ITS2) and parts of the 28S rRNA (28S) gene of a representative subsample (Table S1) were amplified by polymerase chain reaction (PCR) to test the subgeneric affiliation (subgenus data set) of species using the primer pairs LCO1490 plus HCO2198 (Folmer et al., 1994), 16Scs1 plus 16Scs2 (Chiba, 1999) and LSU1 plus LSU3 (Wade and Mor- dan, 2000), respectively (for primer sequences, see Table S2). For species delimitation and phylogeographic analyses, a part of cox1 was sequenced from additional specimens (Table S1). PCR amplifications were carried out as detailed in Neiber et al. (2017). Both strands of the amplified products were sequenced at Macrogen Europe Laboratory (Amsterdam, The Netherlands). ChromasPro 1.7.1 (Technelysium, Tewantin, Australia) was used to assemble forward and reverse sequence reads. Sequences were deposited in GenBank (see Table S1 for GenBank accession numbers).

**Sequence alignment and analyses of DNA sequences**

Sequences were aligned with Maft (Katoh and Standley, 2013) using the Q-INS-i iterative refinement algorithm and otherwise default settings. PartitionFinder 2.1.1 (Lanfear et al., 2012) was used to select the best evolutionary model for the Bayesian inference (BI) and maximum likelihood (ML) analyses of the phylogenetic relationships, conducting exhaustive searches, with separate estimation of branch lengths for each partition and with the Bayesian information criterion to select among models. The models were limited to those
available in MrBayes 3.2.6 (Ronquist et al., 2012). Models were selected for mitochondrial and nuclear data sets separately. The mitochondrial sequences of the subgenus data set were initially divided into four partitions corresponding to 1st, 2nd and 3rd codon positions of cox1 as well as 16S. The nuclear sequences were not further subdivided into partitions. The extended cox1 data set was initially subdivided according to codon position.

The BI analysis was performed using MrBayes. Metropolis-coupled Monte Carlo Markov chain (MCMC) searches were run with four chains in two separate runs with 50,000,000 generations with default priors, trees sampled every 1,000 generations under default heating using the evolutionary models and data partitions as suggested by the PartitionFinder analyses. The first 50,000 generations of each run were discarded as burn-in. Diagnostics obtained from the MrBayes output were used to assess stationarity and convergence.

The ML analyses were performed using GARLI (Zwickl, 2006) with evolutionary models and data partitions as suggested by the PartitionFinder analyses and otherwise default settings. Support values were calculated by bootstrapping with 1,000 replications. For comparison of support values, an additional bootstrap analysis (1,000 non-parametric bootstrap replications) was conducted using IQ-TREE (Chernomor et al., 2016; Minh et al., 2020) using the same partitions and evolutionary models as in the analysis with GARLI.

Heuristic maximum parsimony (MP) searches were conducted with PAUP* 4.0b10 (Swofford, 2002) with unordered characters, 100 random sequence addition replicates, tree bisection reconnection (TBR) branch swapping, and gaps treated as missing data. Support for internal branches was assessed in PAUP* by bootstrapping with 1,000 replications, using full heuristic searches with 10 random addition sequence replicates, TBR branch swapping, and one tree held at each step during stepwise addition. For comparison of support values, an additional bootstrap analysis (1,000 non-parametric bootstrap replications) was conducted using TNT (Goloboff et al., 2008) with 10 random addition sequence replicates, TBR branch swapping, and one tree held at each step during stepwise addition.

Bootstrap support (BS) values from the ML and MP analyses as well as posterior probabilities (PP) from the BI analyses were mapped on the respective BI 50% majority-rule consensus trees with SumTrees 3.3.1, which is part of the DendroPy 3.8.0 package ( Sukumaran and Holder, 2010). PP ≥ 0.95 and BS ≥ 70 were interpreted as support for branches.

Median-joining networks (Bandelt et al., 1999) were constructed with PopART (Leigh and Bryant, 2015) to illustrate relationships among cox1 haplotypes.

**Molecular dating and ancestral area estimation**

Divergence times were estimated using the Bayesian algorithm implemented in BEAST 2.4.1 (Bouckaert et al., 2014) assuming an uncorrelated relaxed log-normal molecular clock and the birth–death model as tree prior. Calibration was based on a previous estimation of the split of Limacidae Batseh, 1789 and Oxychilidae (66 Ma, 95 highest posterior density (HPD) interval: ca 59–75 Ma) obtained from a phylogenomic analysis of Panpulmonata by Teasdale (2017) that was calibrated using six heterobranch gastropod fossils. This was done because of the scarcity or absence of fossil material assignable with certainty to Limacoidea Batseh, 1789 or Oxychilidae from deposits prior to the Miocene (Nordsieck, 2014; Harzhauser and Neubauer, 2021). A log-normal distribution with parameters \( M = 66.0 \) and \( s = 0.066 \) in real space was set as the prior for the calibration node. Additional sequences of oxychilids as well as sequences of representatives of the families Limacidae, Boettgerillidae Wiktor & Lakharev, 1979, Agriolimacidae Wagner, 1935 and Parmacellidae Fischer, 1856 (1855) from the study of Neiber et al. (2020) were added to the subgenus data set (Table S1) for the molecular dating analysis. Sequences were aligned with MAFFT as detailed above. Because some ITS2 sequences were very divergent, ambiguously aligned positions were excluded from the nuclear sequences with Gblocks (Castresana, 2000) as implemented on the Gblocks server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using the following settings: (i) allow for smaller final blocks, (ii) allow gap positions within the final blocks and (iii) allow less strict flanking positions. Data partitions and evolutionary models were selected with PartitionFinder as detailed above.

The BEAST analysis was run for 100,000,000 generations with a sampling frequency set to 10,000. Tracer 1.7.1 (Rambaut et al., 2018) was used to check whether effective sample sizes for the relevant parameters were above 200. Ten per cent of the generations were discarded as burn-in, and a maximum clade credibility tree with median node heights was calculated with TreeAnnotator 2.4.1, which is part of the BEAST 2.4.1 distribution.

Historical distributions patterns were evaluated using ancestral range estimation with the R 3.5.1 (R Core Team, 2018) package BioGeoBEARS (Matzke, 2013). The following discrete areas were considered: (i) Caucasian region, (ii) western part of the Arabian Peninsula, (iii) coastal regions of the Levant (excluding Turkey), (iv) Cyprus, (v) southern Turkey, (vi) northern Turkey, (vii) Crete, (viii) Caspian region of Iran and Azerbaijan and (ix) Europe. Human-mediated introductions of O. delius in the Caucasian region were not considered in the analyses. The maximum number of areas allowed was set to five. Two models were implemented in BioGeoBEARS: (i) the dispersal–extinction–cladogenesis (DEC) model (Ree et al., 2005; Ree and Smith, 2008) and (ii) the DEC model allowing for founder-event speciation (+J) (Matzke, 2014). A likelihood ratio test (LRT) was conducted to select between the two models.

**AFLP data**

AFLP data were generated based on the protocol by Vos et al. (1995) with slight modifications as detailed in Scheel and Hausdorf (2012) for 137 specimens (Tables S1 and S3). Preselective PCRs were carried out with one selective base on each primer (Sauer and Hausdorf, 2012, Table S2) and selective PCRs were carried out with two additional selective bases on fluorescence-labelled primers and three additional selective bases on the unlabelled primers (Sauer and Hausdorf, 2010, Table S2). Six primer combinations in total were run for selective PCRs. For used adapters and primers, see Table S2. Three differently labelled PCR products were pooled (2 µl of each PCR product) and 1 µl of the pooled sample was combined with 9.85 µl Hi Dye Formamid (Thermo Fisher Scientific) and 0.15 µl of GS-500 ROX size standard (Thermo Fisher Scientific). The samples were denatured at 95 °C for 8 min and electropheretically separated using the pop4 polymer (Thermo Fisher Scientific) on an ABI PRISM 3730 (Applied Biosystems, Waltham, MA, USA) capillary sequencer.

Peak Scanner 1.0 (Applied Biosystems) was used to detect and calculate the size of AFLP bands. Parameters were left at their default values except for allowing a light peak smoothing. The RawGeno 2.0.1 (Arrigo et al., 2009) package for R 3.5.1 was used for automated binning and scoring of AFLP bands, with the scoring range set to 50–400 base pairs (bp), the minimum intensity set to 100 relative fluorescence units (rfu), the bin width set to 1–1.5 bp and all other parameters left at their default values. To assess reproducibility, reactions were repeated for \( n = 13 \) randomly chosen individuals. The replicate reproducibility rate was then calculated as the mean percentage of matching character states between replicates of the same individual according to Pompeano et al. (2005). For AFLP data, see Table S3.

A neighbour-net phylogenetic network (Bryant and Moulton, 2004) using Jaccard distances based on the AFLP data was constructed with SplitsTree4 4.15.1 (Huson and Bryant, 2006). The
number of genetically homogeneous clusters and the amount of admixture among individuals was estimated with Structure 2.3.4 (Pritchard et al., 2000; Falush et al., 2007). An admixture analysis allowing for correlated allele frequencies was conducted based on the AFLP data (including only species represented by \( n \geq 5 \) specimens). A total of 20 runs with 80,000 iterations after a burn-in period of 20,000 iterations for \( K = 1 \) to \( K = 15 \) were carried out. The resulting estimates of the posterior probabilities \( \Pr(K) \) of the data for each \( K \) and each run were used to compute the ad hoc quantity \( \Delta K \) proposed by Evanno et al. (2005) with Structure Harvester (Earl and vonHoldt, 2012). For relevant values of \( K \) (i.e. those corresponding to species taxonomy or those that showed peaks for \( \Delta K \)), a Structure run each with 800,000 iterations after a burn-in period of 200,000 and otherwise identical settings was carried out, and the results of these runs were displayed as bar plots using Structure Plot 2.0 (Ramosasy et al., 2014).

Results

Phylogeny based on concatenated mitochondrial and nuclear DNA sequences

The final alignment had a length of 2583 base pairs (bp) (\( \text{cox1}: 655 \) bp, 16S: 917 bp, 5.8S + ITS2 + 28S: 1012 bp; Data S1). The selection of a suitable partitioning strategy and evolutionary models with PartitionFinder suggested to combine 1st and 2nd codon positions of \( \text{cox1} \) (GTR + I + G model), the 3rd codon positions of \( \text{cox1} \) and the 16S sequences (HKY + I + G model) and the 5.8S + ITS2 + 28S sequences (K80 + I + G) into separate partitions.

The species from the Greater and Lesser Caucasus assigned to \( \text{Longiphallus} \) by Riedel (1966, 1980), \( O. \) mingrelicus, \( O. \) reticulatus and \( O. \) oschtenicus, did not cluster with the type species of \( \text{Longiphallus} \), \( O. \) filicum, but formed a strongly supported clade together with the Pontic \( O. \) delius and the species previously assigned to \( \text{Hiramia} \), \( O. \) cyprius, \( O. \) camelinus and \( O. \) paphlagonicus, in all phylogenetic analyses (Fig. 2). In the following, we will use the name \( \text{Hiramia} \) for this clade. Although previously assigned to \( \text{Longiphallus} \), \( O. \) delius proved to be the sister species of \( O. \) cyprius. Additionally, a specimen identified as \( O. \) mingrelicus \( \text{koutaisanus} \) from Turkey (following Riedel, 1989) was also part of this clade but did not cluster with \( O. \) mingrelicus. This specimen represents an as yet undescribed species (referred to hereafter as \( O. \) cf. \( O. \) mingrelicus \( \text{koutaisanus} \); Fig. 2). \( O. \) oxychilus \( \text{deubeli} \) from the Carpathian region, the type species of the monotypic subgenus \( \text{Cellariopsis} \), formed the sister group of \( \text{Hiramia} \) in all phylogenetic analyses (Fig. 2).

\( O. \) oxychilus \( \text{filicum} \), the type species of \( \text{Longiphallus} \), from the Talysh in Azerbaijan and the adjacent Alborz Mountains in Iran formed a maximally supported clade with \( O. \) \( \text{superflius} \) from Crete, and the representatives of the monotypic subgenera \( \text{Araboxychilus} \) from the south-western Arabian Peninsula, \( \text{Calloretinella} \) from Cyprus and \( \text{Helicophana} \) from Crete in all phylogenetic analyses (Fig. 2). Within this clade, \( O. \) \( \text{filicum} \) clustered with \( O. \) \( \text{superflius} \) from Crete, which Riedel (1980) also included in \( \text{Longiphallus} \), albeit with low statistical support (Fig. 2).

Neither of the two species from the Pontic Mountains in north-eastern Turkey, i.e., \( O. \) \( \text{secernendus} \) and \( O. \) \( \text{spatiosus} \) (= \( O. \) \( \text{costatus} \)), which were also classified in \( \text{Longiphallus} \) by Riedel (1966, 1980, 1989), clustered with \( \text{Hiramia} \) nor with the \( \text{Longiphallus} \) clade but formed a maximally supported clade of their own. The relationships of this clade were, however, not resolved (Fig. 2). We propose the name \( \text{Anatoloxychilus} \) Neiber, Walther & Hausdorf n. subgen. (type species: \( \text{Patula spatiosa} \) Lindholm, 1922) for this clade. Whereas the monotypic subgenera \( \text{Cellariopsis} \), \( \text{Araboxychilus} \), \( \text{Calloretinella} \) and \( \text{Helicophana} \) are characterised by morphological autapomorphies, there are no morphological characters known that characterise the subgenera \( \text{Hiramia} \), \( \text{Longiphallus} \) and \( \text{Anatoloxychilus} \) n. subgen. as delimited based on the molecular phylogeny.

To fulfil the requirements of the International Code for Zoological Nomenclature (ICZN, 1999) we provide the following diagnosis based on molecular characters: Oxychilinae associated by the apomorphic nucleotide substitutions (based on Data S1 and the tree from Fig. 2 in File S1): 905: A \( \rightarrow \) T, 913: A \( \rightarrow \) T, 1334: A T, 1389: A \( \rightarrow \) T, 1540: C \( \rightarrow \) T, 1725: G \( \rightarrow \) T, 1726: G \( \rightarrow \) T, 1727: A \( \rightarrow \) T, 1981: A \( \rightarrow \) G, 1982: G \( \rightarrow \) T, 2006: C \( \rightarrow \) G, 2195: A \( \rightarrow \) C; only positions considered with data for all taxa and only changes with a consistency index of 1.00 given (ZooBank registration: http://zoobank.org/NomenclaturalActs/1d4c93f6-dcbd-4cf0-a9a6-00805f747dc0). The proposed name is a composite word (masculine) combined from Anatolia and the generic name \( \text{Oxychilus} \).

Finally, the subgenus \( \text{Ortizius} \), represented by \( O. \) \( \text{aliarius} \) and \( O. \) \( \text{navarricus} \), grouped together with \( \text{Oxychilus} \) s. str., represented by \( O. \) \( \text{cellarius} \) and \( O. \) \( \text{draparnaudi} \), and \( O. \) \( \text{diaphanellus} \), the type species of the monotypic \( \text{Tauroxychilus} \) from the Crimean Peninsula, albeit with low statistical support (Fig. 2).

Molecular dating

The final alignment had a length of 2928 bp (\( \text{cox1}: 655 \) bp, 16S: 1012 bp, 5.8S + ITS2 + 28S: 1261 bp (635 bp excluded)) (Data S2). The selection of a suitable partitioning strategy and evolutionary models with PartitionFinder suggested to combine 1st and 2nd codon positions of \( \text{cox1} \) into one partition (GTR + I + G model) and to keep the 3rd codon positions of \( \text{cox1} \) (GTR + G model), the 16S sequences (GTR + I + G model) and the alignable positions of K1t to K15 were carried out. The resulting estimates of the posterior probabilities \( \Pr(K) \) of the data for each \( K \) and each run were used to compute the ad hoc quantity \( \Delta K \) proposed by Evanno et al. (2005) with Structure Harvester (Earl and vonHoldt, 2012). For relevant values of \( K \) (i.e. those corresponding to species taxonomy or those that showed peaks for \( \Delta K \)), a Structure run each with 800,000 iterations after a burn-in period of 200,000 and otherwise identical settings was carried out, and the results of these runs were displayed as bar plots using Structure Plot 2.0 (Ramosasy et al., 2014).
the 5.8S + ITS2 + 28S sequences (GTR + I + G) as separate partitions.

The tree was calibrated by the divergence of Oxychilidae and Limacoidea in the early Paleogene, ca. 66 Ma, as suggested by the analysis of Teasdale (2017). It was estimated that the splitting of the crown group of Oxychilidae started 45.2 Ma (95% HPD: 36.0–55.0 Ma) in the middle Eocene with the divergence of Nastiinae Riedel, 1989 (Fig. 3). The divergence between Daudebardiinae Kobelt, 1906 and Selenochlamydinae + Oxychilinae followed at 42.0 Ma (95% HPD: 33.2–50.6 Ma) and Selenochlamydinae Likharev and Wiktor, 1980 separated from Oxychilinae 37.1 Ma (95% HPD: 29.4–45.4 Ma) in the late Eocene (Fig. 3). The onset of the radiation of Oxychilinae was dated at 28.6 Ma (95% HPD: 22.6–35.2 Ma) in the Oligocene (Fig. 3).

Within Oxychilinae, the strongly supported clade including the subgenera Hiramia, Cellariopsis, Araboxychilus, Calloretinella, Helicophana and Longiphallus as delimited here originated 22.9 Ma (95% HPD: 18.0–28.4 Ma) (Figs 2 and 3). Hiramia + Cellariopsis diverged from the remaining groups 21.4 Ma (95% HPD: 16.8–26.4 Ma) in the early Miocene (Fig. 3). The split of Hiramia and Cellariopsis was dated at 18.3 Ma (95% HPD: 14.0–22.7 Ma; Fig. 3), with an estimated age of the onset of diversification of Hiramia of 15.8 Ma (95% HPD: 12.1–19.5 Ma) in the middle Miocene (Fig. 3). The Caucasian Hiramia species diverged in the middle or late Miocene (Fig. 3). The onset of diversification of the clade including Longiphallus, Calloretinella, Helicophana and Araboxychilus was dated at 7.2 Ma (95% HPD: 5.1–9.5 Ma) in the late Miocene (Fig. 3).

**Ancestral area estimation**

The LRT ($D = 1.62, df = 1, P = 0.0001$) comparing the DEC (In likelihood = −63.42) and DEC + J (In likelihood = −55.84) models favoured the DEC + J model over the DEC model. The DEC model suggested an origin of the included Oxychilus species in a
Fig. 3. Maximum clade credibility tree of Limacoidei obtained from the molecular dating analysis with BEAST. Numbers at nodes represent node ages in Ma and the bars at nodes represent 95% highest posterior density intervals for node ages. The node used for calibration is marked by a star. Black dots at nodes indicate posterior probabilities ≥0.95. Q, Quaternary.
region comprising Europe and northern Anatolia (Fig. 4). The origin of the clade including the subgenera Helicophana, Calloretinella, Longiphallus and Araboxychilus was inferred to be in Crete (Fig. 4). Hiramia, as here delimited was inferred to have originated in northern Turkey, with a dispersal event to the Caucasus region of the clade comprising O. mingrelicus, O. oschtenicus and O. reticulatus (Fig. 4). The ancestor of the clade comprising O. delius and O. cyprius expanded into the East Mediterranean region and O. camelinus originated also by a long-distance dispersal to that region (Fig. 4).

Phylogeography of Caucasian Hiramia

The alignment of the coxl fragment had a length of 655 bp (Data S3). The PartitionFinder analysis suggested a single partition (HKY + I + G model). In the tree based on coxl sequences alone, all (sub-)species belonging to Hiramia were monophyletic and most were strongly supported (Fig. 5; for details, see also Fig. S1). The 77 specimens of O. mingrelicus koutaisanus from Georgia formed a strongly supported clade in all analyses. The O. mingrelicus koutaisanus clade formed the sister group of a clade including specimens identified morphologically as O. mingrelicus mingrelicus from the north-western Greater Caucasus and specimens from northern Armenia that are morphologically more similar to O. mingrelicus koutaisanus (Fig. 5 and Fig. S1). In the median-joining network (Fig. 6), these two clades were also separated (see also Table S1 for information on haplotypes). Geographic groupings were less evident within the O. mingrelicus koutaisanus clade. Populations from the southern slopes of the Greater Caucasus, the Colchis region and the Lesser Caucasus in Georgia generally included haplotypes from distant parts of the median-joining network (Fig. 6).

In line with the analyses based on concatenated mitochondrial and nuclear sequences (Fig. 2), the BI analysis joined a strongly supported clade including a total of 31 O. oschtenicus specimens with O. mingrelicus, albeit with low support (Fig. 5 and Fig. S1). Within the O. oschtenicus clade, specimens from the southern slopes of the Greater Caucasus (Sochi region) formed a strongly supported clade as the sister group of a likewise strongly supported clade including specimens from the northern slopes of the Greater Caucasus and a specimen from a population between Dagomys and Solokhaul south of the main chain of the Greater Caucasus (Figs 5 and 6, Fig. S2 and Table S1). There is a high haplotype diversity at the southern slopes of the Greater Caucasus, contrasted by a low diversity at the northern slopes (Figs 5 and 7).

Specimens identified morphologically as O. reticulatus (11 specimens) formed a strongly supported clade with unresolved relationships (Fig. 5). The 15 specimens of O. cf. mingrelicus koutaisanus from the Turkish regions Trabzon, Rize and Giresun were placed together in a clade that was widely separated from the Caucasian O. mingrelicus and showed little geographic structuring (Figs 5 and 7; Fig. S1).

Relationships among Hiramia species based on nuclear AFLP data

The AFLP data set included 2061 loci obtained from six selective PCRs of a total 137 individuals (excluding 13 replicates; Table S3). The replicate reproducibility rate according to Pompanon et al. (2005) was calculated as 94.8%.

The grouping of species and subspecies belonging to the Hiramia clade (Fig. 2) in the neighbour-net network based on the nuclear AFLP data (Fig. 8) was consistent with the grouping of specimens based on the mitochondrial coxl sequences (Fig. 5). The species O. cyprius, O. delius, O. camelinus and O. cf. mingrelicus koutaisanus, from the Mediterranean and Pontic regions clustered together. Oxychilus reticulatus and O. oschtenicus were clearly separated. The O. oschtenicus cluster (28 individuals) was subdivided into subclusters from the southern and the northern slopes of the Greater Caucasus with a specimen from a population between Dagomys and Solokhaul in an intermediate position (Fig. 8, Table S1). These results are similar to those obtained from the analyses of the coxl sequences (Figs 5 and 7, Fig. S1).

The widespread O. mingrelicus was strongly structured with O. mingrelicus mingrelicus (five individuals) in an intermediate position between O. mingrelicus koutaisanus (71 individuals) and O. oschtenicus (Fig. 8). Specimens of O. mingrelicus koutaisanus from the Colchis Lowlands, the southern slopes of the Georgian part of the Greater Caucasus and the Lesser Caucasus and the northern slopes of the Greater Caucasus formed subclusters, with specimens generally clustering according to populations within these subclusters (Fig. 8, Table S1). The only exception is a specimen from the vicinity of Chrebalo (DNA voucher 3577), which did not cluster with other specimens from the same population (Fig. 8, Table S1).

Admixture among Hiramia species based on AFLP data

The results of the admixture analysis for K = 5 clusters (Fig. 9, Fig. S1) grouped specimens according to species, i.e., delimited O. mingrelicus, O. oschtenicus, O. reticulatus, O. delius and O. cf. mingrelicus koutaisanus as distinct groups. This solution suggested
Fig. 4. Ancestral area estimation under the DEC + J model for species included in Oxychilinae based on the dated phylogeny shown in Fig. 3. Regions: (A) Caucasus region, (B) western part of the Arabian Peninsula, (C) coastal regions of the Levant (excl. Turkey), (D) Cyprus, (E) southern Turkey, (F) northern Turkey, (G) Crete, (H) Caspian region of Iran and Azerbaijan and (I) Europe. Squares at tips and nodes refer to areas or combination of areas representing (ancestral) ranges of species.
Fig. 5. Bayesian 50% majority-rule consensus tree of *Oxychilus* (*Hirania*) taxa (in colour; see legend) and related taxa based on *coxl* sequences. Stars at nodes indicate support in all phylogenetic analyses (posterior probabilities $\geq 0.95$ or bootstrap values $\geq 70$). For details on node support and specimen vouchers, see Fig. S1.
Fig. 6. Median-joining network and distribution of cox1 haplotypes of *Oxychilus mingrelicus mingrelicus* (triangles) and *O. mingrelicus koutaisanus* (stars). Circles in the median-joining network are sized according to the number of observed haplotypes. Black dots represent hypothetical haplotypes and ticks on branches indicate the number of mutations separating haplotypes. For information on populations and haplotypes, see Table S1.
Fig. 7. Median-joining network and distribution of cox1 haplotypes of *Oxychilus deilus* (circles), *O. oschtenicus* (triangles), *O. reticulatus* (diamonds) and *O. cf. mingrelicus koutaisanus* (crosses). Circles in the median-joining networks are sized according to the number of observed haplotypes. Black dots represent hypothetical haplotypes and ticks on branches indicate the number of mutations separating haplotypes. For information on populations and haplotypes, see Table S1.
admixture especially between *O. mingrelicus* mingrelicus and the geographically overlapping *O. oschtenicus* but also between *O. cf. mingrelicus koutaisanus* from the Pontic Mountains and *O. mingrelicus* (Fig. 9, Fig. S1).

In the Structure solution for $K = 8$ (Figs 9 and 10, Fig. S1) suggested on the basis of the ad hoc quantity $\Delta K$, *O. oschtenicus, O. reticulatus, O. deilus* and *O. cf. mingrelicus koutaisanus* were retained as separate clusters but the *O. mingrelicus* cluster was split into four clusters (Figs 9 and 10). One cluster corresponded to specimens from the *O. mingrelicus mingrelicus* clade and strongly admixed specimens from the botanical garden in Tbilisi (Figs 9 and 10, Fig. S1). A second cluster was formed by individuals from the Colchis Lowlands and a third included specimens from the Laba catchment at the northern slopes of the Greater Caucasus (Figs 9 and 10, Fig. S1). The largest cluster included specimens from the Lesser Caucasus and the southern slopes of the Greater Caucasus in Georgia (Figs 9 and 10, Fig. S1).
Fig. 9. Distribution of sampled Oxychilus (Hirania) populations and results of admixture analyses with Structure. (a) Distribution of sampled populations of O. mingrelicus mingrelicus, O. mingrelicus koutaisanus, O. oschtenicus, O. reticulatus, O. cf. mingrelicus koutaisanus and O. deilus. (b) Result of admixture analysis for $K = 5$ (number of species). (c) Result of admixture analysis for $K = 8$ (peak for $\Delta K$). Bars in the plots (b-c) represent ancestry of specimens in different ancestral populations. See Fig. S2 and Table S1 for information on vouchers.
Admixture between population groups within *O. mingrelicus* was often high, especially between geographically adjacent clusters. Several individuals from the Colchis Lowlands showed a high proportion of ancestry in the neighbouring cluster from the Lesser Caucasus and the southern slopes of the Greater Caucasus in Georgia (Fig. 10). Similarly, several specimens from the *O. mingrelicus koutaisanus* clade from the northern slopes of the Greater Caucasus showed admixture with specimens from the geographically neighbouring cluster corresponding to *O. mingrelicus mingrelicus* (Fig. 10). More surprisingly, some individuals from the southern slopes of the Greater Caucasus in Georgia showed admixture with the cluster corresponding to *O. mingrelicus mingrelicus* (Fig. 10). Specimens from the botanical garden in Tbilisi are mainly composed of shares of the cluster from the Colchis Lowlands and *O. mingrelicus mingrelicus*.

**Discussion**

*Systematics of the Longiphallus–Hiramia group of Oxychilus*

The phylogenetic analyses of *Longiphallus* in the sense of Riedel (1966, 1980, 1989) and related taxa revealed several surprising insights, both with regard to systematics and to its biogeographical implications. *Longiphallus* in the sense of Riedel (1966, 1980, 1989) proved to be polyphyletic and has to be split into three groups. *Longiphallus* in the strict sense includes only the type species, *O. filicum* from the Talysh Mountains in Azerbaijan and the adjacent Alborz Mountains in Iran and, perhaps, *O. superflus* from Crete (Fig. 2). In all phylogenetic analyses, these species formed a maximally supported clade with an also strongly supported clade including the representatives of the
monotypic subgenera *Helicophana* from Crete, *Calloretinella* from Cyprus and *Araboxychilus* from the south-western Arabian Peninsula (Fig. 2).

The Caucasian and Pontic species previously included in *Longiphallus* are not related to this clade but to the *Hiramia* species from the Levant and can be classified in this subgenus. *Oxychilus deilitus* and *O. cypricus*, whose similarity led Riedel (1983) to doubt the distinctness of *Hiramia* and *Longiphallus*, are in fact sister species that both belong to *Hiramia*. *Hiramia* is the sister group of *Cellariopsis* from the Carpathian Mountains. *Oxychilus spatiosus* and *O. secernendus* from the eastern Pontic Mountains form a third clade of species previously included in *Longiphallus*, *Anatoloxchilus* Neiber, Walther & Hausdorf n. subgen., which is neither closely related to *Longiphallus* nor to *Hiramia*. *Hiramia* is not related to *Schistophallus*, with which it was tentatively synonymised by Giusti and Manganelli (1999). As previously shown, *Schistophallus* does not belong to Oxychilinae but to Nastiinae (Neiber et al., 2020).

Within *Hiramia* an endemic Caucasian group separated from the more widespread circum-Pontic and Eastern Mediterranean group (Fig. 2). Unfortunately, both groups are not statistically supported. The lack of long internodes in the network based on the AFLP data indicates that the divergence of the *Hiramia* species was probably a rapid radiation (Fig. 7), explaining the lack of statistical support for the clades in the tree (Fig. 2). The Caucasus group includes the widespread *O. mingrelicus* and *O. oschtenicus* from the north-western Caucasus, which were already recognised as distinct species by Riedel (1966). In addition, *O. reticulatus* from Mingrelia turned out to represent a distinct lineage, which is supported by the phylogenetic analyses of the sequence data (Figs 2 and 3), as well as the network (Fig. 6) and the Structure analyses of the AFLP data (Fig. 8). Riedel (1966) considered *O. reticulatus* a form of *O. mingrelicus*, although he had not seen the holotype, the only specimen known at that time. *Oxychilus reticulatus* differs from *O. mingrelicus* in the distinct microsculpture both on the apical and the umbilical side of the shell.

In the optimal Structure solution based on the AFLP data (with *K* = 8), *O. mingrelicus* was split into four population groups, in the Laba catchment on the northern slope of the Greater Caucasus, in the Colchis Lowland, in western Georgia and a disjunct cluster found in the north-western Greater Caucasus and in Armenia (Figs 8 and 9). All four population clusters are connected by notable admixture. The cluster in the north-western Greater Caucasus and Armenia corresponds to one of the two basal sister groups within *O. mingrelicus* in the phylogenetic analyses (Figs 2 and 3). This group includes populations from the north-western Greater Caucasus, which were separated as a distinct subspecies, *O. mingrelicus mingrelicus*, by Riedel (1966) based on a pronounced microsculpture on the apical side of the shell from the mainly Georgian *O. mingrelicus koutaisanus*. However, this group also includes—besides the populations from the north-western Greater Caucasus—populations from Armenia, which do not differ morphologically from the Georgian *O. mingrelicus koutaisanus* and were classified as such by Akramowski (1976). Based on the genetic data, we classify them as *O. m. mingrelicus*.

A final taxonomic surprise was the finding that the populations from the eastern Pontic Mountains in the Turkish provinces Giresun, Trabzon and Rize, which Riedel (1989, 1995) identified as *O. mingrelicus koutaisanus*, represent a distinct species, supported by the phylogenetic analyses of the sequence data (Figs 2 and 3), as well as the network (Fig. 7) and the Structure analyses of the AFLP data (Fig. 8). The populations of this species are also geographically separated from *O. mingrelicus* by a distribution gap in the Turkish province Artvin. This new species will be described elsewhere.

**Caucasian–Pontic–Mediterranean disjunctions and their possible causes**

The phylogenetic and biogeographic analyses of the *Longiphallus–Hiramia* group (Figs 1, 2, and 4) revealed two or three Caucasian/Pontic–Mediterranean disjunctions. The separation of *O. (Longiphallus) filicum* from the Talysh and Alborz mountains from the endemic “*Longiphallus*” species in the Greater and Lesser Caucasus, which actually belong to *Hiramia* (Fig. 2), was not the result of a fragmentation of a continuous forest refuge in the Caucasus region by the aridification in the eastern part of the Greater Caucasus. Rather, *O. (Longiphallus) filicum* is probably the sister species of *O. (Longiphallus) superflus* from Crete. These species belong to a group of taxa with a relictual distribution in the Eastern Mediterranean and south-western Arabia, namely *Helicophana* from Crete, *Calloretinella* from Cyprus, and *Araboxychilus* from south-western Arabia (Fig. 1). The biogeographic analysis implies an origin of *O. (Longiphallus) filicum* by a long-distance dispersal from Crete approximately 5 mya (Fig. 4).

A second Pontic–Mediterranean disjunction concerns the sister species *O. (Hiramia) deilitus* in the Pontic region and *O. (Hiramia) cypricus*, which extends from Cyprus and southern Anatolia across the Aegean Islands to the mainland of Greece. Both species are often found synanthropically and the extent of their native ranges is difficult to assess. The biogeographic analysis suggests that their ancestral species may have expanded its range from the Pontic region through western Anatolia to the eastern Mediterranean (Fig. 4).
Oxychilus (Hiramia) paphlagonicus from the Pontic Mountains and O. (Hiramia) camelinus from the Levant may represent a third disjunct species pair (Fig. 4). However, the monophyly of this pair needs further corroboration. In any case, the occurrence of O. (Hiramia) camelinus in the Levant can be explained by another long-distance dispersal from the Pontic region.

There are also several other land snail groups that show similar Caucasian/Pontic–Mediterranean disjunctions like Pilorchula Germain, 1912 and Pagodulina Clessin, 1876 (Hausdorf, 1996), Leiostyla Lowe, 1852 (Hausdorf, 1990; Subai, 1993), Gigantomilax Boettger, 1883 (Likharev and Wiktor, 1980; Heller, 2009), Dobatia Nordsieck, 1973 (Nordsieck, 1994; Schütt, 2010), Serrulina serrulata (Pfeiffer, 1847) (Nordsieck, 1994; Schütt, 2010), Elia Adams & Adams, 1855 (in the strict sense; Nordsieck, 1994; Schütt, 2010), Galeata Boettger, 1877 (Nordsieck, 1994; Schütt, 2010), or Armenica laevicollis (Charpentier, 1852) (Nordsieck, 1994; Schütt, 2010). Some of these disjunctions may be the result of long-distance dispersal. However, the large number of cases indicates that it is more likely that the majority of the disjunct ranges are relicts of previously more continuous distributions across Anatolia that became disjunct by the successive aridification of the Anatolian Plateau that intensified in the late Pliocene following the uplift of the Anatolian Plateau and the subsequent uplift of the Pontic and Taurus mountains during the late Miocene to Pliocene (Kayseri-Özer, 2017; Huang et al., 2019; Meijers et al., 2020). This hypothesis is difficult to test because the splits between the disjunct pairs were associated with or may even predate the colonisation events that may have occurred at different times in the different taxa. However, the splits are expected to predate the vicariance event, the aridification of the Anatolian Plateau in the late Pliocene. This is the case for the disjunctions in the Longiphallus–Hiramia group (Fig. 4).

Colonisation and diversification patterns of the Caucasian fauna

There are three possible explanations for the disjunct ranges of O. m. mingrelicus. It might be hypothesised that one of the two distribution areas is the result of human introduction. Arguments for this hypothesis are that several other Hiramia species (O. camelinus, O. cyprius, O. delius) were widely distributed by humans and that a population from orchards in Ashgabat in Turkmenistan, described as Hyalinia emigrata Lindholm, 1922, has been inferred also to be an introduction of O. m. mingrelicus (Likharev and Rammelmeier, 1952). However, Riedel (1966) suggested that H. emigrata might actually be O. filicum. More importantly, O. m. mingrelicus is found in the north-western Greater Caucasus, as well as in Armenia in natural habitats and there is no ecological or historical evidence that either of these two occurrences can be explained by introduction. The second possible explanation for the disjunction is a natural long-distance dispersal event, perhaps by birds, which have repeatedly been discussed as probable vectors in the literature (e.g., Dörge et al., 1999; Gittenberger et al., 2006). The final explanation is that the occurrences of O. m. mingrelicus in the north-western Caucasus and in Armenia are relics of a formerly continuous distribution. An argument for this hypothesis is the occurrence of genetic shares of O. m. mingrelicus in several populations of O. mingrelicus koutaisanum in Racha-Lechkhumi and Kvemo Svaneti, which are currently not in contact with O. m. mingrelicus. In contrast, the admixed population in the botanical garden in Tbilisi is probably the result of a mixing of introduced populations of O. m. mingrelicus and O. mingrelicus koutaisanum.

Although Riedel (1966) supposed that O. mingrelicus might occur in the Laba catchment, he has not seen specimens from that area. He expected that this region might have been colonised by O. m. mingrelicus from the west. However, the analyses of the specimens that we found in the Laba catchment showed that they belong to O. mingrelicus koutaisanum and, thus, suggest that the species has colonised this area from the south-east by crossing the main ridge of the Greater Caucasus. The geographical pattern of small-scale outposts on the northern slopes of the Greater Caucasus that are isolated from a main distribution range in Central Georgia is also found in other land snail species, e.g. Imerezia lederi (Boettger, 1881), Peristoma boettgeri (Clessin, 1883) or Pilorchula trifilaris trifilaris (Mousson, 1856).

The occurrence of the Hiramia species O. paphlagonicus and a new species (O. cf. mingrelicus koutaisanum) in the Pontic Mountains and of O. camelinus and O. cyprius in the Eastern Mediterranean region indicate that Hiramia colonised the Greater Caucasian Island (see Popov et al., 2004) from the south, which is supported by our ancestral area estimation (Fig. 4). The presence of the widespread O. delius on Crimea is not an argument against this hypothesis because O. delius occurs also in Turkey and is the sister species of the Eastern Mediterranean O. cyprius. The divergence of the Caucasian Hiramia species in the middle to late Miocene (Fig. 3) in the Greater Caucasus during this time period support reconstructions that suggest that there was already a considerable uplift of the Greater Caucasus during the Oligocene and Miocene (Popov et al., 2004; Vincent et al., 2016) rather than models that assume that the major phase of orogenesis of the Greater Caucasus started only in the Pliocene (Avdeev and Niemi, 2011; Forte et al., 2014). Similar
divergence patterns were also reconstructed for *Caucasotachaea* Boettger, 1909 subspecies (Neiber et al., 2016), mayflies of the subgenus *Epeorus* (*Caucasiron*) Kluge, 1997 (Hrviňak et al., 2020) and endemic species of the genus *Darevskia* Arribas, 1997 (Murtskhvaladze et al., 2020).

The overlapping ranges of the Caucasian species *O. oschtenicus*, *O. mingrelicus* and *O. reticulatus* (Fig. 9) indicate that their origin cannot be explained by vicariance as a result of the fragmentation of a formerly continuous forest refuge alone. Instead, lineage diversification and distribution might be related to a colonisation-extinction scenario and the north-western Greater Caucasus was probably colonised repeatedly. First, *O. oschtenicus* diverged from *m. mingrelicus*. The north-western Greater Caucasus was then colonised again by *O. mingrelicus*. Finally, *O. mingrelicus* split into *m. mingrelicus koutaisanus* and *O. m. mingrelicus*. The latter was probably originally widespread from Armenia to the north-western Greater Caucasus but was later replaced by *O. mingrelicus koutaisanus* in western Georgia, which subsequently also spread across the main ridge of the Greater Caucasus into the Laba catchment at the northern slope of the Grater Caucasus.

The separation of *O. oschtenicus* in the north-western Greater Caucasus and especially the split of *O. mingrelicus* into *m. mingrelicus* and *O. mingrelicus koutaisanus* resembles the pattern observed in *Caucasotachaea atrolobiata* (Krynicki, 1833), i.e., a split into the north-western *C. a. atrolobiata* and the south-eastern *C. atrolobiata calligera* (Dubois de Mont–pè reux, 1840), which also occurred around the Miocene–Pliocene boundary (Neiber et al., 2016). Similar patterns also occur in other snail groups so that there are groups of species restricted to the north-western Caucasus and to western Georgia. The geographical separation of the Northwest Caucasian species was already recognised by Likharev and Rammelmeier (1952) and Riedel (1966) who referred to the area occupied by these species as the “Kuban-Abkhasian subdistrict”.

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Conflict of interest

None declared.

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**Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig. S1.** Bayesian 50% majority-rule consensus tree of Oxychilus (Hiramia) taxa (in colour) and related taxa based on the cox1 sequences.

**Fig. S2.** Distribution of sampled Oxychilus (Hiramia) populations and results of admixture analyses with structure.

**Table S1.** Museum registration numbers, population numbers, haplotype numbers, GenBank accession numbers, availability of AFLP data and locality data for the specimens used in the molecular phylogenetic analyses.

**Table S2.** Primers used for the PCR amplifications.

**Table S3.** AFLP data for the Oxychilus (Hiramia) specimens used in this study.

**Data S1.** Partial sequences of the cytochrome c oxidase subunit 1 (cox1) and 16S rRNA (16S) genes, as well as partial sequences of the 5.8S rRNA (5.8S) gene, the complete internal transcribed spacer (ITS2) and partial sequences of the 28S rRNA (28S) gene of Oxychilidae in nexus format.

**Data S2.** Partial sequences of the cytochrome c oxidase subunit 1 (cox1) and 16S rRNA (16S) genes, as well as partial sequences of the 5.8S rRNA (5.8S) gene, the complete internal transcribed spacer (ITS2) and partial sequences of the 28S rRNA (28S) gene of Oxychilidae in nexus format.

**Data S3.** Partial cytochrome c oxidase subunit 1 (cox1) sequences of Oxychilidae in nexus format.

**File S1.** Bayesian 50% majority-rule consensus tree of Oxychilus species based on the analysis of concate- nated cox1, 16S, 5.8S, ITS2 and 28S sequences (Data S1).