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A CONTRIBUTION TO THE PHYLOGENY OF ALBANIAN *AGATHYLLA* (GASTROPODA, CLAUSILIIDAE): INSIGHTS USING MORPHOLOGICAL DATA AND THREE MITOCHONDRIAL MARKERS

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ABSTRACT

In the present study, we revised Albanian *Agathylla*, combining the traditional shell morphological approach with a phylogenetic reconstruction based on three mitochondrial DNA markers (COI, COII and 16S rRNA). From the faunistic perspective, Albania was an almost completely unexplored country until the end of the 1990s. Following recent intense field sampling activity in Albania, the number of known *Agathylla* populations has increased, providing an opportunity to investigate how additional material can contribute to the resolution of the taxonomy of the Albanian *Agathylla* species. Newly discovered populations indicate greater morphological variability than previously recorded and highlight the presence of various other character state combinations. Based on these findings, we argue that only two *Agathylla* taxa occur in the region, namely *A. neutra* and *A. biloba*. Due to apparent morphological differences, they are still treated as distinct species, but high intraspecific morphological variability makes it unjustifiable to delimit subspecies. The molecular phylogeny indicates a close relationship between *A. neutra* and *A. biloba*, but could neither confirm nor refute their reciprocal monophyly, so their possible conspecificity requires further evaluation.

INTRODUCTION

Taxon delimitation is a crucial issue in biodiversity research because accurate taxon boundaries are of fundamental importance in several fields of supraindividual biology. At present, advanced molecular-based approaches (Hebert *et al*., 2003; Pons *et al*., 2006; Craft *et al*., 2010; Esselstyn *et al*., 2012; Puillandre *et al*., 2012a, b), especially within the framework of integrative taxonomy, are gaining ground. Integrative taxonomy (the delimitation of species in a single framework combining different facets of species identity including morphology, ecology, biogeography and genetic data; Padial *et al*., 2010; Puillandre *et al*., 2012a, b), provides a comprehensive toolkit to define taxon boundaries.

However, for a long time taxonomy was based almost exclusively on morphological characters and it is still a fact that the vast majority of currently acknowledged taxa have only been described in the traditional way. In addition, relatively little attention has been given to the question of how these taxon delimitations are affected by the intensity of study of a certain group. This is of special importance in the case of groups that are difficult to locate (e.g. because of cryptic behaviour, or inaccessible habitat) or distributed in less explored regions (Criscione & Köhler, 2013).

Rock-dwelling door snails (Clausiliidae), including *Agathylla* species, occurring in Albania are such a case for several reasons. Firstly, their systematics is exclusively based on a typological approach. Secondly, they are predominantly found on isolated limestone outcrops, a habitat type that is spatially scattered across wide areas of relatively inaccessible terrain. Thirdly, because from a faunistic perspective Albania is the most unexplored region in Europe, since the country was not really accessible until the 1990s.

Whereas some clausiliid genera occurring in the Balkans have been used in numerous biogeographical, ecological and evolutionary studies (Schilthuizen & Lombaerts, 1994; Schilthuizen & Lombaerts, 1995; Schilthuizen *et al*., 2004; Uit De Weerd & Gittenberger, 2004; Uit De Weerd, Piel & Gittenberger, 2004; Uit De Weerd & Gittenberger, 2005; Uit De Weerd, Schneider & Gittenberger, 2005; Uit De Weerd *et al*., 2006; Douris *et al*., 2007; Páll-Gergely, Kornilios & Giokas, 2012; Fehér *et al.*, 2013), *Agathylla* Adams & Adams, 1855 remains poorly studied. Uit de Weerd & Gittenberger (2013) have recently found that *Agathylla* exhibits affinities with Apennine genera like *Muticaria*, *Leucostigma* and *Lampedusa*, but little is known about phylogenetic relationships within the genus.

Agathylla is distributed along the western Adriatic coast from Dalmatia (Croatia) to Epirus (northwestern Greece). Available distribution data are very scarce, most of the *Agathylla* taxa being known from only a few locations (Wagner, 1922; Nordsieck, 1972; Zilch, 1977; Karaman, 2006). Until recently, this was also the case with the two species *A. biloba* and *A. neutra* that are distributed mainly in Albania.

Agathylla neutra, a small and smooth-shelled clausiliid species, was first described by Westerlund (1898) as *Clausilia (Delima) neutra*, from the Tzoumerka mountains (northern Greece). That population has not been collected again for a long time and, due to the short and uninformative description and lack of available type material in well-known collections, this species has long been forgotten. The name was reintroduced more than a century later by Nordsieck (2001) following the discovery of some topotypical material in the collections of the Naturmuseum Senckenberg in Frankfurt am Main and the Naturhistorisches Museum Wien (NHMW). In the meantime, other authors, being unaware of *A. neutra*, described some related taxa. In 1914, Wagner described two taxa from northern Albania, a ribbed one as *Alopia (Agathylla) biloba* and a smooth-shelled one as its subspecies, *Alopia (Agathylla) biloba merditana* (Sturany & Wagner, 1915). In 1996, a smooth-shelled population was found in Çorovodë (southern Albania), resulting in the elevation of *A. merditana* to species level and to the description of the Çorovodë population as its subspecies, *A. merditana scraparana* (Nordsieck, 1996). The same taxon was reported to occur in two further locations (Leskovik and Loutra) near the Albanian–Greek border (Gittenberger, 1998). When Nordsieck (2001) reintroduced *A. neutra*, it was considered as the senior synonym of *A. merditana scraparana*.

As regards *A. biloba*, Wagner's syntype material originated from two localities, "Skutari" [=Shkodër] and "Vaudenjs am Drin bei Skutari" [=Vau i Dejës]. Brandt (1961) subsequently described a subspecies, *A. biloba dabovici*, from "Fluß Shen". Later on, an *A. biloba* population found in the Fangul Pass (central Albania) was described as *A. biloba martae* (Erőss, Fehér & Szekeres, 1999). The most recent systematic concept of this group (Nordsieck, 2007) is based on knowledge of these populations. According to this concept, there are four valid taxa within two distinct species, namely *A. biloba biloba*, *A. biloba martae*, *A. neutra neutra* and *A. neutra merditana*, while *dabovici* and *scraparana* are considered synonyms of *A. b. biloba* and *A. n. neutra*, respectively.

During the past two decades, several collecting trips have taken place in Albania in an effort to rectify the gap in knowledge resulting from the fact that the country was not accessible for faunistic studies for a long time (Dhora & Welter-Schultes, 1996; Fehér *et al.*, 2004; Fehér & Erőss, 2009; Murányi, Kontschán & Fehér, 2011). As a result of this systematic field sampling, the number of known *A. neutra* and *A. biloba* populations has multiplied and their known distributional range has significantly altered, thus rendering them the most abundant and widely distributed species of the genus. This provided an opportunity to investigate how an increase in the number of sampled populations per species has affected the taxonomic delimitation of these two Albanian taxa.

In the present study, we revised the two species using the traditional shell-morphological approach to reveal the level of intraspecific phenotypic variability, considering the recently discovered populations. In addition, we performed a phylogenetic reconstruction based on three mitochondrial DNA markers (COI, COII and 16S rRNA) to test the subspecific division of the species. Representative populations from 9 of the 12 known *Agathylla* species (14 of the 24 known taxa) were included in the molecular analysis to infer the relationship of the two focal species as well as their position within the whole genus.

MATERIAL AND METHODS

Origin of specimens

The majority of the examined material was collected recently and is deposited in the Hungarian Natural History Museum (HNHM). In case of taxa with well-defined type localities, we attempted to sample topotypical populations as well. Additionally, we have investigated the material of the collections of the NHMW. Sampling localities of recently collected material are listed as precisely as possible; geographic coordinates, if available, are given in the format in which they were recorded (Systematic descriptions and Table 1). Information from the labels of old museum specimens is given in double quotation marks and, if necessary, explanations are provided within square brackets. For brevity, names of the frequently encountered collectors are abbreviated as follows: Z.E. = Zoltán Péter Erőss, Z.F. = Zoltán Fehér, A.H. = András Hunyadi, T.H. = Tamás Huszár, J.K. = Jenő Kontschán, K.K. = Kornél Kovács, D.M. = Dávid Murányi.

Morphological descriptions

We compared the newly collected material with the type lots and/or the topotypic material as well as with the descriptions in the literature (Westerlund, 1898; Sturany & Wagner, 1915; Brandt, 1961; Nordsieck, 1996; Gittenberger, 1998; Nordsieck, 2001). We gave special attention to morphological features such as shell size, width/height (W/H) ratio, sculpture of the upper whorls and the cervical part, palatal callus, cervical swelling, subsutural swelling, structures of the lunella, the upper palatal fold and the basal fold, shape and protrusion of the aperture, that are considered important taxonomic features of the group.

DNA extraction, amplification and sequence determination

Nine *Agathylla* species (29 populations) and two outgroup species (*Siciliaria pantocratoris margaritifera* and *Herilla illyrica oribates*) were included in the molecular analyses. The specimens were preserved either in absolute or 70% ethanol. Total genomic DNA was isolated from the foot muscle of one adult individual from each population (Table 1; Figure 1). To overcome problems of polymerase chain reaction (PCR) inhibition by mucopolysaccharides, DNA was extracted using the hexadecyltrimethyl-ammonium bromide (CTAB 2×) protocol of Winnepenninckx *et al.* (1993) as described by Parmakelis *et al.* (2003). Three mtDNA markers, the cytochrome oxidase subunit I (COI), the cytochrome oxidase subunit II (COII) and the 16S ribosomal RNA (16S rRNA) were used. For the amplification of the COI fragment, the universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) worked with only a few samples and, therefore, for the majority of the samples the C1-J-1718 (mt6) and C1-N-2191 (nancy) primers (Simon *et al.*, 1994) were used instead. For the amplification of COII, we used the primers reported by Hugall *et al.* (2002). The targeted fragments of COI and COII were 708 and 580 bp, respectively. In the case of 16S rRNA, two different sets of primers were used. The first was 16SAR LRN-12887 and 16SBR LR-J13398 (Simon *et al.*, 1994), which targeted a 511-bp fragment, and the second 16Scs1 and 16Sma2 (Chiba, 1999), which amplified a 950-bp fragment. The first set was used only in one sample. Each PCR was performed in a 50-ul volume, where 1-2 ul of template DNA was mixed with 0.2 mM dNTPs, 0.4 mM of each primer and 1 unit of Taq polymerase. The concentration of $MgCl_2$ was 3.5 mM. Thermocycling was performed in either a MyCycler (Biorad) or a TProfessional (Biometra) thermocycler. The cycle programmes for all three genes comprised an initial denaturation

Table 1. Geographical origins of specimens used in the molecular analyses and accession numbers of generated sequences. In accordance with the conclusions of the present study, subspecific assignments are not indicated for samples of *Agathylla biloba* and *A. neutra*.

Sample code	HNHM voucher	Taxon name	Country of origin and locality	Latitude/longitude	Accession numbers of sequences (16S/COI/COII)
B1	86066	*A. biloba* (topotype)	Albania, Shkodër, castle hill	42° 02.9′N; 19° 30.2′E	KF601259/KC756069/KC756097
B2	86065	*A. biloba* (topotype)	Albania, Vau i Dejës	42° 00.95′N; 19° 37.31′E	KF601262/KC756072/KC756100
B3	97076	*A. biloba*	Albania, Shënmarki	42° 00.88′N; 19° 37.71′E	KF601263/KC756073/KC756101
B4	85893	*A. biloba* (topotype *of 'martae'*)	Albania, Qafa e Fangul, 9 km E of Ibë	41° 14.09′N; 19° 58.63′E	KF601264/n.a./KC756102
B5	95491	*A. biloba*	Albania, 1.5 km S of Petrësh	41° 06.18′N; 20° 00.39′E	KF601260/KC756070/KC756098
B6	95492	*A. biloba*	Albania, Qafa e Gllavës	40° 30.17′N; 19° 59.07′E	KF601261/KC756071/KC756099
B7	97102	*A biloba* (transitional morph)	Albania, Koman, c. 1.5 km upstream from dam	42° 06.99′N; 19° 50.24′E	KF601278/KC756088/KC756117
N1	86067	*A. neutra*	Albania, 4 km SE of Çorovodë to Zogas	40° 29.68′N; 20° 16.23′E	KF601276/KC756086/KC756115
N2	95037	*A. neutra* (topotype of *'scraparana'*)	Albania, 4.8 km NE of Çorovodë	40° 31.25′N; 20° 15.11′E	KF601277/KC756087/KC756116
N3	95960	*A. neutra*	Albania, 11 km W of the Ulëz-junction	41° 41.51′N; 19° 49.91′E	KF601279/KC756089/KC756118
N4	97072	*A. neutra*	Albania, 3 km N of Miliskë	42° 13.21′N; 20° 05.86′E	KF601280/KC756090/KC756119
N5	97504	*A. neutra*	Albania, Nicaj-Shosh	42° 15.08′N; 19° 44.87′E	KF601275/KC756085/KC756114
A	84742	*A. abrupta abrupta*	Croatia, 2 km E of Pijavičino	42° 55.4′N; 17° 24.4′E	KF601258/KC756068/KC756096
E1	84822	*A. exarata exarata*	Bosnia–Hercegovina, 3 km E of D. Gnojnice	43° 16′N; 17° 53′E	KF601265/KC756074/KC756103
E2	84823	*A. exarata exarata*	Bosnia–Hercegovina, Počitelj	43° 08′N; 17° 44′E	KF601266/KC756075/KC756104
E3	84829	*A. exarata exarata*	Croatia, 8 km NW of Klek	42° 58.3′N; 17° 32.1′E	KF601267/KC756076/KC756105
E4	84830	*A. exarata exarata*	Croatia, Lovorje	42° 58.8′N; 17° 32.5′E	KF601268/KC756077/KC756106
E5	97067	*A. exarata exarata*	Croatia, Baćinsko Jezero, S lakeshore	43° 04.16′N; 17° 25.20′E	KF601269/KC756078/KC756107
F1	84754	*A. (Agathyllina) formosa formosa*	Croatia, Komolac	42° 40.1′N; 18° 08.1′E	n.a./KC756079/KC756108
G	97071	*A. goldi goldi*	Montenegro, 8 km above Kotor to Njeguši	42° 24.15′N; 18° 46.50′E	KF601270/KC756080/KC756109
Gh	97068	*A. goldi herminiana*	Montenegro, 16 km above Kotor to Njeguši	42° 23.96′N; 18° 46.96′E	KF601271/KC756081/KC756110
L1	84751	*A. (Agathyllina) lamellosa lamellosa*	Croatia, Čavtat	42° 35.0′N; 18° 13.0′E	KF601272/KC756082/KC756111
L2	97075	*A. (Agathyllina) lamellosa lamellosa*	Montenegro, Čanj	42° 07.4′N; 19° 03.9′E	KF601273/KC756083/KC756112
St	84788	*A. (Agathyllina) strigillata strigillata*	Croatia, Ston	42° 52.42′N; 17° 41.74′E	KF601285/KC756095/KC756124
Sl	84748	*A. (Agathyllina) strigillata latestriata*	Croatia, 2 km E of Pijavičino	42° 55.4′N; 17° 24.4′E	KF601274/KC756084/KC756113
Su1	83431	*A. sulcosa sulcosa*	Croatia, Veli Zaton	42° 41.4′N; 18° 02.2′E	KF601281/KC756091/KC756120
Su2	97070	*A. sulcosa sulcosa*	Croatia, Slano	42° 47.27′N; 17° 53.59′E	KF601283/KC756093/KC756122
Su3	97069	*A. sulcosa sulcosa*	Croatia, 1 km SE of Trsteno	42° 42.29′N; 17° 59.40′E	KF601282/KC756092/KC756121
Sa	83441	*A. sulcosa acicula*	Croatia, Komolac	42° 40.1′N; 18° 08.1′E	KF601284/KC756094/KC756123
	93265	*Herilla illyrica oribates*	Albania, Qafa e Pejes, N of Okol	42° 26.63′N; 19° 46.26′E	KF601287/n.a./KF601289
	98839	*Siciliaria pantocratoris margaritifera*	Albania, 1 km E of Progonat	40° 12.61′N; 19° 57.68′E	KF601259/KC756069/KC756097

step at 95°C for 3 min, followed by 40 cycles of 15 s at 95°C, 1 min at 45°C, 1.5 min at 72°C and a final 10-min extension at 72°C. Automated sequencing of both strands of each mtDNA product was performed in a PE-ABI3740 automated sequencer (using Big-Dye terminator chemistry). The primers in the sequencing reactions were the same as in the PCR amplifications.

Except for the samples indicated in Table 1, all three mtDNA gene segments were successfully amplified from each sample (Table 1). All sequences produced for this study have been deposited in GenBank (Table 1).

Sequence alignment and genetic data analysis

The sequence length of the 16S rRNA fragments was 743–967 bp (most above 800 bp), of COI sequences 329–700 bp (most above 500 bp) and of COII 266–551 bp (most above 500 bp). Length variation was due to low-quality reads at the beginning and at the end of the sequenced fragments. Sequences were edited using CodonCode Aligner v. 2.06 (Genecodes Corporation). Their authenticity and homology with the targeted genes were evaluated with a BLAST search in the NCBI genetic database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Each mtDNA dataset was aligned with CodonCode Aligner using the Clustal algorithm. The alignment of the 16S rRNA was visually inspected and edited.

The mean overall genetic distances between individual sequences was calculated using MEGA5 (Tamura *et al.*, 2011) and implementing the Kimura 2-parameter (K-2p) model (Kimura, 1980) of nucleotide substitution. This distance measure was estimated for the concatenated dataset and each mtDNA gene separately.

Phylogenetic analyses

Prior to the phylogenetic analyses, the datasets were investigated for substitution saturation. This was achieved by plotting the transitions and transversions of each pairwise comparison (estimated by DAMBE; Xia, 2013) *vs* the respective genetic distance. A regression analysis was performed to evaluate whether substitutions increase linearly with distance. Phylogenetic analyses were performed on the concatenated dataset of all three mtDNA genes, using maximum parsimony, Bayesian inference (BI) and maximum likelihood (ML) algorithms. BI was performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003) and a partition of the dataset according to mtDNA locus was enforced. For the selection of the appropriate substitution models fitting the three partitions, Modeltest v. 3.7 (Posada & Crandall, 1998) and the Akaike Information Criterion (Akaike, 1974) were used. Model parameter values were treated as unknown and were estimated during the MrBayes run. The separate partitions were treated as 'unlinked', with separate model parameter estimates for each one. The number of generations was set to 2×10^6 and two independent runs were performed simultaneously, with four chains in each run. The average standard deviation of split frequencies was used to determine the stationarity point of likelihoods (see MrBayes v. 3.1.2 manual), according to which stationarity was achieved well before 0.25×10^6 generations. A tree was sampled every 100th generation and, consequently, the summaries of the BI analysis relied on 4×10^4 samples (sum of four runs). From each run 15,001 samples were used, while 5,000 were discarded as burn-in. From the remaining 30,002 trees (sum of two runs), a 50% majority-rule consensus tree was constructed. Support of the nodes was assessed with the posterior probabilities (PP) of reconstructed clades. ML analysis was performed on the concatenated dataset using GARLI v. 2.0 (Zwickl, 2006). We performed heuristic phylogenetic searches using the same partition scheme and models that were implemented in MrBayes. GARLI calculates the maximum likelihood of a topology using a genetic algorithm (Lewis, 1998) to evaluate more efficiently alternative topologies. The most likely ML tree topology was inferred from 10 independent runs starting from random trees. All other parameters of GARLI were set to their default values. The separate partitions were treated as 'unlinked', and the model parameters were estimated separately for each. The independent analyses were considered to have converged when the likelihood values were less than one likelihood unit different. The ML tree with the highest likelihood score was considered as the best. The parameters estimated for the best tree were fixed in a bootstrap (BS) analysis of 200 pseudoreplicates. Based on the trees of the BS analyses, a 50% majority-rule consensus tree was created using SumTrees (http://www.nescent.org/wg_garli/Advanced_topics#Using_SumTrees). The support values at each node on the consensus tree were depicted on the best tree found by GARLI.

RESULTS

Systematic descriptions

Several characters were found to show considerable inter- and intrapopulation variability. Due to the high phenotypic variability found, a large portion of the populations was difficult to assign to any of the known subspecies. Therefore, only the parent species are described here. Regarding the alleged difference between the two species in altitudinal preference (Nordsieck 2001), *Agathylla neutra* seems to occur in higher altitudes on average, but new records revealed a significant overlap in the altitudinal ranges. *Agathylla biloba* populations were collected as high as 900 m asl (Gllavë Pass), whereas *A. neutra* populations were found at altitudes as low as 100 m asl (Mat Gorge) (Fig. 1).

***Agathylla neutra* (Westerlund, 1898)**

(Figs 2, 3A–F, 4J–L)

Clausilia (Delima) neutra Westerlund 1898: 170 (M. Pindus, Tsoumerka).

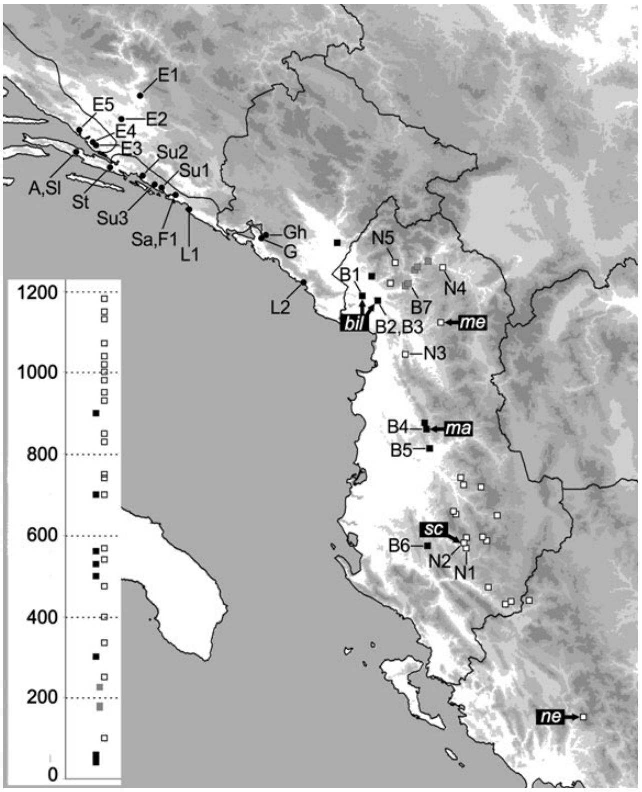

Figure 1. Distribution of the genus *Agathylla* and locations of the studied material. White squares, *A. neutra*; black squares, *A. biloba*; grey squares, populations with transitional features. Sequenced samples are indicated by the same abbreviations as in Table 1. Dots indicate populations of other *Agathylla* species, which have been included in the molecular analyses. Arrows indicate type localities of taxa that belong to the *A. neutra/biloba* group: bil, *biloba* (Shkodër and Vau i Dejës); me, *merditana* (Merdita Mts); ma, *martae* (Fangul Pass); sc, *scraparana* (Çorovodë); ne, *neutra* (Tzoumerka Mts). Note that the type locality of *dabovici* is undefined (see text). Inset illustrates the vertical distribution of the two species except for the *A. neutra* population on the Faqekuq peak region (2340 m). Note that the doubtful *A. biloba* record in Virpazar is not indicated (see text).

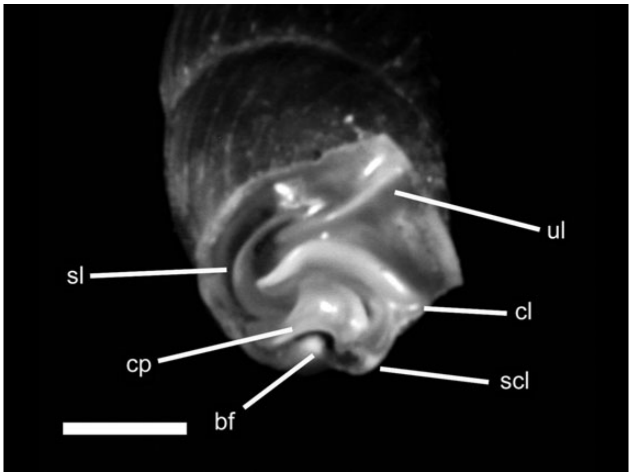

Figure 2. *In situ* position of the bilobate clausilial plate in *Agathylla neutra* (Fierzë–Tetaj, HNHM 97073). Palatal part of the aperture and the ultimate whorl are partly removed. Note the notch, which fits around the basal fold. Abbreviations: ul, upper lamella; cl, collumellar lamella; scl, subcollumellar lamella; bf, basal fold; cp, clausilial plate; sl, spiral lamella. Scale bar = 1 mm.

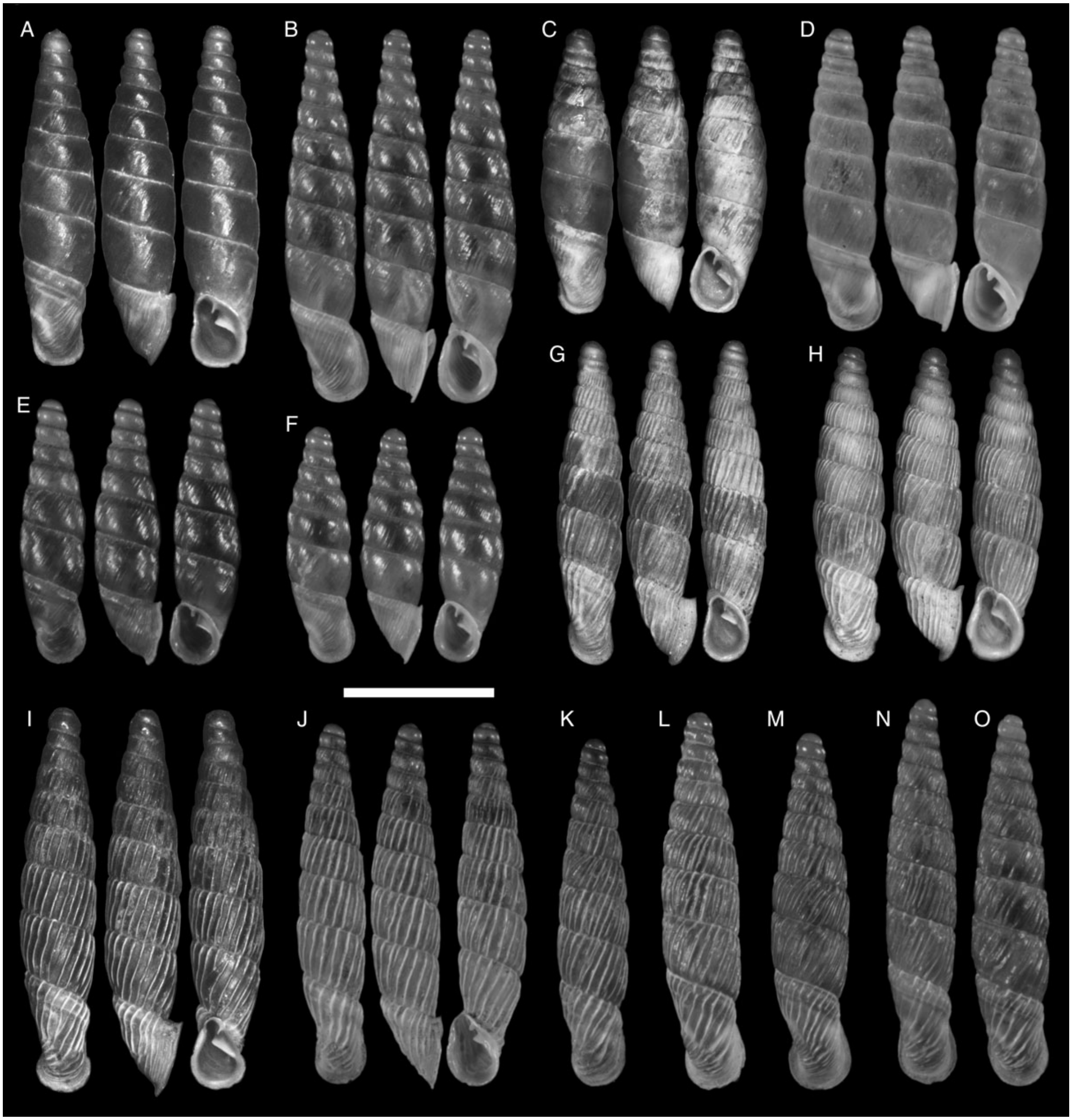

Figure 3. A. Syntype of '*Alopia (Agathylla) biloba merditana*' (NHMW 43930). **B.** *Agathylla neutra*, Zogas (HNHM 86067). **C.** Topotypical specimen of *A. neutra*, Tzoumerka Mts (NHMW 102878). **D.** *A. neutra*, Turbehovë (HNHM 97490). **E.** *A. neutra*, Mat gorge (HNHM 95106). **F.** *A. neutra*, NE of Çuka Partizan, 1130 m asl. (HNHM 97497). **G.** Syntype of '*Alopia (Agathylla) biloba*', Skutari Festungmauern (NHMW 43345). **H.** Syntype of '*Alopia (Agathylla) biloba*', Vaudenjs (NHMW 43347). **I.** Paratype of '*Agathylla biloba dabovici*', "Am Fluße Shen" (NHMW 79000/K/6003). **J.** Holotype of '*Agathylla biloba martae*', Fangul Pass (HNHM 70830). **K.** Paratype of '*Agathylla biloba martae*', Fangul Pass (HNHM 70831). **L, M.** *A. biloba*, Gllavë Pass (HNHM 95492). **N, O.** *A. biloba*, Petrësh (HNHM 95491). Scale bar = 5 mm.

Agathylla neutra—Nordsieck, 2001: 25, fig. 1.
Agathylla neutra neutra—Fehér & Erőss, 2009: 30.
Alopia (Agathylla) biloba merditana Wagner '1914' in Sturany & Wagner, 1915: 52, pl. 15, fig. 85 (Fandi bei Oroshi in der Merdita).
Agathylla biloba merditana—Nordsieck, 1972: 5. Zilch, 1977, 125, pl. 6, fig. 6 (syntype).
Agathylla merditana merditana—Nordsieck, 1996: 7.
Agathylla neutra merditana—Fehér & Erőss, 2009: 30.
Agathylla merditana scraparana Nordsieck, 1996: 8, pl. 2, fig. 1a, b (holotype; Mali Gradecit, 2–5 km E Çorovodë). Dhora & Welter-Schultes, 1996: 129, pl. 9, figs 91–93 (paratypes). Gittenberger, 1998: 187, figs 1, 2–3 (clausilium), 4 (genital system).

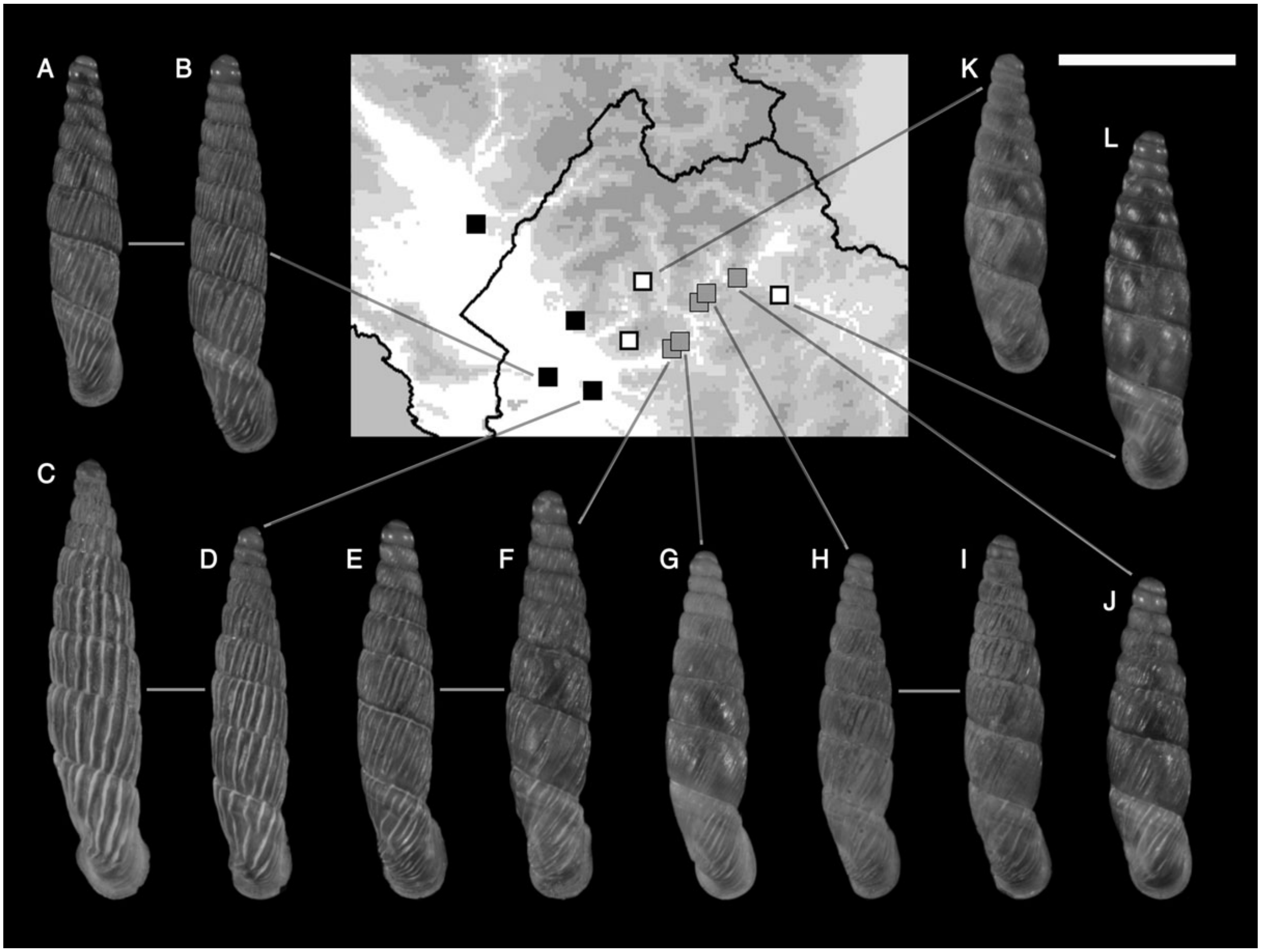

Figure 4. *Agathylla* in northern Albania. **A, D.** Topotypical specimens of *A. biloba*. **A, B.** Shkodër (HNHM 86066). **C, D.** Vau i Dejës (HNHM 86064). **E-J.** Specimens from populations in the Drin Valley area with transitional features. **E, F.** *A. biloba,* Koman (HNHM 97501). **G.** *A. biloba,* 1.5 km upstream from Koman (HNHM 97102). **H, I.** *A. biloba,* 18 km upstream from Koman (HNHM 97500). **J.** *A. neutra,* Fierzë–Tetaj (HNHM 97073). **K.** *A. neutra*, Nicaj-Shosh (HNHM 97504). **L.** *A. neutra*, Miliskë (HNHM 97072). Scale bar = 5 mm.

Type material examined: “Fandi bei Oroshi Mirdita Albanien”, 1908, leg. Winneguth (NHMW 43930/3, 79000/K/2) [*merditana* syntypes]. *Other material examined*: GREECE: Epirus, Tzoumerka Mts, Neraida, Sattel im N, c. 1600 m asl., 18.08.1990, leg. H. Sattmann (NHMW 102878). ALBANIA: Ersekë district, 6 km SW of Leskovik along the Leskovik–Përmet road, beneath the Maja e Melesinit, N40°09.66′ E20°34.12′, 07.07.1996, leg. Z.E. & Z.F. (HNHM 84755); Ersekë district, Leskovik, beneath Maja e Melesinit, 930 m asl., N40°09.06′ E20°35.69′, 03.07.2003, leg. Z.E., Z.F., J.K. & D.M. (HNHM 97508); Gramsh district, Tërvol, Pr. i Holtit gorge, 250 m asl., N40°55.56′ E20°13.39′, 26.08.2006, leg. Z.F., A.H., T.H. & D.M. (HNHM 97493); Gramsh district, Vallamarë Mts, Kukur, 890 m asl., N40°51.99′ E20°22.64′, 19.05.2011, leg. Z. Barina & D. Pifkó (HNHM 97489); Gramsh district, Vinë, 700 m asl., N40°52.98′ E20°14.66′, 04.07.2003, leg. Z.E., Z.F., J.K. & D.M. (HNHM 95960); Korçë district, 25 km W of Maliq, along the road to Gramsh, at the Gjinikos junction, gorge of Lumi i Devollit, 750 m asl., N40°41.52′ E20°30.01′, 04.07.2003, leg. Z.E., Z.F., J.K. & D.M. (HNHM 97507); Mat district, in the gorge of Lumi i Matit, Burrel–Milot road, 11 km W of Ulëz junction, 100 m asl., N41°41.51′ E19°49.91′, 08.10.2004, leg. Z.F., J.K. & D.M. (HNHM 95106); same locality, 27.06.2003, leg. Z.E., Z.F., J.K. & D.M. (HNHM 93266); Përmet district, Benjë, gorge of Lumi i Lengaricës, 335 m asl., N40°14.68′ E20°26.26′, 18.08.2007, leg. Z.F. & L. Tamás (HNHM 97498); Pukë district, 3 km N of Miliskë, 567 m asl., N42°13.22′ E20°05.69′, 07.10.2005, leg. T. Deli, Z.E., Z.F. & D.M. (HNHM 97072); Shkodër district, Toplanë, 20.5 km upstream from Koman, gorge on the right bank of Koman Lake, 180 m asl., N42°14.03′ E19°52.45′, 18.06.2012, leg. Z.F., T. Kovács & D.M. (HNHM 98844); Shkodër district, Prokletije Mts, Nicaj-Shosh, (on the Kir–Bregu i Lumit road, 4 km E of the mountain pass), 980 m asl., N42°15.08′ E19°44.87′, 23.05.2010, leg. Z.F., D.M. & Zs. Ujvári (HNHM 97504); Shkodër district, W of Shllak (18 km from the Mes bridge), 1020 m asl., N42°07.84′ E19°42.64′, 16.04.2006, leg. Z.E., Z.F., A.H. & D.M. (HNHM 97491); Skrapar district, 4 km SE of Çorovodë towards Zogas, by the right side of the Lumi i Osumit gorge, 400 m asl., N40°29.68′ E20°16.23′, 12.04.2001, leg. Z.E., Z.F. & K.K. (HNHM 86067); Skrapar district, 4.5 km NE of Turbehovë, lower part of Pr.i Krishovës gorge, 1040 m asl., N40°33.54′ E20°23.40′, 23.08.2006, leg. Z.F., A.H., T.H. & D.M. (HNHM 97490); Skrapar district, c. 15 km NE of Çorovodë, at the Gradec junction, 1000 m asl., N40°32.61′ E20°16.31′, 22.08.2006, leg. Z.F., A.H., T.H. & D.M. (HNHM 97505); Skrapar district, c. 4.8 km NE of Çorovodë, Pr. i Çorovodës gorge, 475 m asl., N40°31.19′ E20°15.37′, 10.10.2004, leg. Z.F., J.K. & D.M. (HNHM 95037); same locality, 540 m asl., N40°31.34′ E20°15.44′, 22.08.2006, leg. Z.F., A.H., T.H. & D.M. (HNHM 97499); Skrapar district, Maja e Faqekuqit, peak region, 2340 m asl., N40°31.95′ E20°25.54′, 21.08.2006, leg. Z.F., A.H., T.H. & D.M. (HNHM 97503); Skrapar district, Ostrovicë Mts, S of Faqekuq, upper part of Pr. i Krishovës gorge, 1071 m asl., 07.07.2005, leg. Z. Barina, D. Pifkó & D. Schmidt (HNHM 95323); Skrapar district, Qafa e Dëvris, NE of Radesh, along the Çorovodë–Zaloshnje road, E side of the gorge, 1150 m asl., N40°33.33′ E20°16.73′, 22.08.2006, leg. Z.F., A.H., T.H. & D.M. (HNHM 97495); same locality, 830 m asl., 08.08.2004, leg. Z.F. (HNHM 95022); same locality, W side of the gorge, 1180 m asl., N40°33.23′ E20°16.30′, 08.08.2004, leg. Z.F. (HNHM 95023); Skrapar district, Tomor Mts, c. 1.5 km W of Terovë, in a dry gorge, 850 m asl., N40°42.76′ E20°11.03′, 24.08.2006, leg. Z.F., A.H., T.H. & D.M. (HNHM 97496); Skrapar district, Tomor Mts, c. 4 km NW of Terovë, NE of Çuka Partizan, 1130 m asl., N40°43.27′ E20°10.32′, 24.08.2006, leg. Z.F., A.H., T.H. & D.M. (HNHM 97497); Tropojë district, 3 km W of the Lumi i Valbonës mouth on the Fierzë–Tetaj road, 226 m asl., N42°15.78′ E19°59.40′, 07.10.2005, leg. T. Deli, Z.E., Z.F. & D.M. (HNHM 97073).

Diagnosis: Clausilial plate bilobed, i.e. bears a notch towards the outer end that fits around basal fold. This structure distinguishes *A. neutra* from other *Agathylla* species except for *A. biloba*, *A. goldi* and *A. abrupta*. Unlike *A. goldi* and *A. abrupta*, the basal fold is more or less well visible in frontal view. Unlike *A. biloba*, it has a visible cervical swelling and (almost) smooth body whorls.

Description: Shell slender, fusiform, *H* 8–12 mm, *W* 2–2.5 mm, *W*/*H* ratio 0.20–0.24. Sculpture almost smooth on body whorls, regular and dense riblets on cervical part. Shell colour horn-brown, growth lines and riblets of same colour. Basal keel distinct to weak. Cervical swelling parallel to apertural lip varies from prominent to weak but visible. Aperture round, oval or pear-shaped, moderately (sometimes barely) protruding. Apertural lip reflected. Subcollumellar lamella visible in frontal or at least in oblique view. Principal fold long, posterior upper palatal fold short. Lunella dorsal, its upper part usually (but not always) weak or missing, its lower part connected to strong basal fold, which is visible in frontal view. Anterior upper palatal fold varies from whitish palatal callus to strong denticle (in latter case, it can be connected with posterior upper palatal fold). Clausilial plate bilobed (i.e. a notch towards outer end, which fits around basal fold; Fig. 2).

Intraspecific variability: Types of *neutra* are not known (Nordsieck, 2001) but, based on topotypical material (Nordsieck, 2001: fig. 1 and Sattmann & Reischütz, 1994: NHMW 102878) this morph can be characterized by a less slender shell (*W*/*H* ratio 0.23), rounded aperture that barely protrudes, relatively thick peristome and a clear (sometimes very strong) cervical swelling. We have found very similar populations in some southern Albanian locations (Dëvris Pass, Turbehovë, Faqekuq). There is another morph, which can be characterized by a more slender shell (*W*/*H* ratio 0.21), clearly protruding oval or pear-shaped aperture (depressed on the upper part of its palatal region) and weaker cervical swelling. Its anterior upper palatal fold is visible as a palatal callus, but does not form a prominent denticle. The shell height varies widely (from 8 mm in Terovë to 12 mm in Zogas). This morph was found in numerous locations in southern Albania (Vinë, Gjinikos, Terovë and Zogas) and the syntype of *merditana* (Oroshi in Mirditë Mts) and the topotypical *scraparana* population (Çorovodë Gorge) correspond to this morph.

Regarding the northern Albanian populations, the one in the Mat Gorge comprises small, slender, reddish-brown specimens with clearly protruding oval or pear-shaped aperture, visible cervical swelling, but weak to absent palatal callus. Based on the shape of the shell and aperture, populations from Miliskë, Nicaj-Shosh and Fierzë resemble typical *neutra*, but both their cervical swelling and palatal callus are absent or hardly visible. Their sculpture is also different; there can be regular riblets or conspicuous ribs on the cervical part of the last whorl (Miliskë), and the upper whorls can have regular riblets (Nicaj-Shosh) or even conspicuous ribs (Fierzë).

Remarks: Making taxonomic assignments somewhat complicated is the fact that Nordsieck (2001, 2007) synonymized *scraparana* and *neutra*, while he kept *merditana* separate. This is in contrast with our findings, i.e. that *scraparana* resembles *merditana* more than *neutra*. Distinguishing features, which were believed to be important by Nordsieck (1996)—i.e. the basal fold of *scraparana* is longer and situated nearer to the subcollumellar lamella, and the clausilium of *scraparana* is more clearly visible in frontal apertural view—show such intra- and interpopulation variability that any distinction between *merditana* and *scraparana* is unjustified. Though *scraparana* and *merditana* are therefore apparently synonyms, their relation to *neutra* is more complicated. The typical *neutra* and the typical *scraparana* morphs are generally easy to distinguish, but they do not show clear geographical separation. Moreover, in at least one population (on the western slope of the Dëvris Pass) typical *neutra*, typical *scraparana* and transitional specimens were all found together. This suggests that they should not be treated as distinct taxa. Morphological variability among the northern Albanian populations also supports the view that *A. neutra* should be considered as a single morphologically heterogeneous taxon.

Agathylla biloba **(Wagner, 1914)**

(Figs 3G–O, 4A–I)

Alopia (Agathylla) biloba Wagner ‘1914’ in Sturany & Wagner, 1915: 51, pl. 15, fig. 84 a–c (Skutari Festungmauern; Vaudenjs am Drin bei Skutari).

Agathylla biloba biloba—Nordsieck, 1972: 5. Zilch, 1977: 125, pl. 6, fig. 5 (syntype). Fehér & Erőss, 2009: 30.

Agathylla biloba dabovici Brandt, 1961: 15, pl. 2, fig. 14 (holotype; am Fluß Shen in Nord-Albanien). Nordsieck, 1972: 5. Zilch, 1977: 125. Fehér & Erőss, 2009: 30.

Agathylla biloba martae Erőss & Szekeres in Erőss *et al.*, 1999: 446, fig. 1 (holotype; Mangull Pass SE of Tiranë, 7 km from Ibë along the road to Kllojkë). Fehér & Erőss, 2009: 30.

Type material examined: “Skutari Festungmauern” [=Shkodër, castle], 28.04.1905, leg. Sturany (NHMW 43345, 43346, 79000/K/10192) [*biloba* syntypes]; “Vaudenjs” [=Vau i Dejës], leg. Sturany (NHMW 43347) [*biloba* syntypes]; “Am Fluße Shen”, leg. Dabović (NHMW 79000/K/6003) [*dabovici* paratypes]; Tiranë district, Qafa e Fangul, 9 km E of Ibë towards Kllojkë, over the N side of the gorge of the Pr. i Murdharit, 700 m asl., N41°14.09′ E19°58.63′ (HNHM 70830, 70831/5) [*martae* holotype and paratypes].

Other material examined: MONTENEGRO: “Hügel Šipčanik b. Tuzi Podgorica Montenegro”, leg. Dabović (NHMW 79000/K/6002, 75000/E/21596); Virpazar, leg. Dabović (NHMW 75000/E/48291, 75000/E/49068). ALBANIA: “Felsen oberhalb Deraja am Fuss des Maranaj”, 1937, leg. Dabović (NHMW 75000/E/21528); “Vaudenjs am Drinfluss auf Felsen”, leg. Dabović (NHMW 75000/E/21529, 79000/K/6001); “Skutari, Festungmauern”, 1937, leg. Dabović 1937 (NHMW 75000/E/21608, 79000/K/54267, 79000/K/5958, 79000/K/6000, 79000/K/4633); “Skutari”, ex. coll. Wagner (NHMW 10042); Shkodër, SE side of the castle hill, 40 m asl., N42°02.87′ E19°30.21′, 07.04.2001, leg. Z.E., Z.F. & K.K.

(HNHM 86066); Tiranë district, NW of Ibë, in the gorge of Lumi i Erzenit, 300 m asl., N41°15.50′ E19°57.86′, 11.04.2001, leg. Z.E., Z.F. & K.K. (HNHM 97506); Shkodër district, Vau i Dejës (20 km SE of Shkodër) left side of the dam, 55 m asl., N42°00.95′ E19°37.31′, 02.07.1996, leg. Z.E. & Z.F. (HNHM 84752); same locality, 08.04.2001, leg. Z.E., Z.F. & K.K. (HNHM 86065); same locality, right side of the dam, 08.04.2001, leg. Z.E., Z.F. & K.K. (HNHM 86064); Shodër district, Shënmarki, c. 1.2 km NE of Mjedë, N42°00.88′ E19°37.71′, 04.2007, leg. Z. Barina & D. Pifkó (HNHM 97076); Berat district, Qafa e Gllavës, along the Berat–Këlcyrë road, 900 m asl., N40°30.17′ E19°59.07′, 13.04.2001, leg. Z.E., Z.F. & K.K. (HNHM 95492); Tiranë district, Qafa e Fangul, 9 km E of Ibë towards Kllojkë, over N side of gorge of Pr. i Murdharit, 700 m asl., N41°14.09′ E19°58.63′, 11.04.2001, leg. Z.E., Z.F. & K.K. (HNHM 85893); Tiranë district, Skuterrë, 1 km along footpath to Pashkashesh, 530 m asl., N41°13.67′ E19°58.88′, 29.06.2003, leg. Z.E., Z.F., J.K. & D.M. (HNHM 96831); same locality, 2 km along footpath to Pashkashesh, over gorge of Pr. i Murdharit, 560 m asl., N41°13.81′ E19°58.89′, 29.06.2003, leg. Z.E., Z.F., J.K. & D.M. (HNHM 93268); Elbasan district, 1.5 km S of Petrësh, along Tiranë–Elbasan main road, 500 m asl., N41°06.24′ E20°00.44′, 14.04.2001, leg. Z.E., Z.F. & K.K. (HNHM 95491); same locality, 22.06.2012, leg. Z.F., T. Kovács & D.M. (HNHM 98842); Shkodër district, Koman, Liqeni i Komanit, right bank, ferry harbour, 180 m asl., N42°06.52′ E19°49.58′, 14.04.2006, leg. Z.E., Z.F., A.H. & D.M. (HNHM 97501); same locality, 19.06.2012, leg. Z.F., T. Kovács & D.M. (HNHM 98845); Shkodër district, c. 1.5 km upstream from Koman dam, Liqeni i Komanit, right bank, 180 m asl., N42°07.30′ E19°49.88′, 14.04.2006, leg. Z.E., Z.F., A.H. & D.M. (HNHM 97494); same date and locality, left bank, 180 m asl., N42°06.99′ E19°50.24′ (HNHM 97102); Shkodër district, c. 15.5 km upstream from Koman dam, Liqeni i Komanit, right bank, 180 m asl., N42°12.64′ E19°53.68′, 15.04.2006, leg. Z.E., Z.F., A.H. & D.M. (HNHM 97492); Pukë district, c. 18 km upstream from Koman dam, Mertur Stream on left side of Liqeni i Komanit, 170 m asl., N42°13.61′ E19°54.30′, 15.04.2006, leg. Z.E., Z.F., A.H. & D.M. (HNHM 97500); same locality, 18.06.2012, leg. Z.F., T. Kovács & D.M. (HNHM 98843).

Diagnosis: Bilobed clausilial plate distinguishes this species from other *Agathylla* species except for *A. neutra*, *A. goldi* and *A. abrupta*. Can be distinguished from *A. goldi* and *A. abrupta* by its basal fold, which is clear in frontal view. Can be distinguished from *A. neutra* by the absence of cervical swelling and the presence of ribs on all parts of the shell.

Description: Shell slender to very slender, fusiform, *H* 10–14 mm, *W* 2.1–2.6 mm, *W*/*H* ratio 0.19–0.21. Shell colour horn-brown, but whitish ribs give overall greyish-white appearance. Body whorls with strong, widely spaced ribs. Cervical part regularly or irregularly ribbed; ribs as strong or even stronger than on body whorls. Basal keel clear or strong. No cervical swelling behind peristome. Aperture oval or pear-shaped, clearly protruding. Apertural lip broadly reflected. No palatal callus inside aperture. Subcollumellar lamella visible in frontal or at least in oblique view. Principal fold long; posterior upper palatal fold short. Lunella dorsal, upper part usually weak or absent, lower part connected to a strong basal fold, which is visible in frontal view.

Remarks: For some reason, Nordsieck (1972, 1996) claimed that the type locality of *dabovici* ("Fluß Shen") is identical with one of the type localities of *biloba* ("Vaudenjs" [Vau i Dejës]). Investigation of other lots in NHMW, also collected by Petar Dabović, showed that "Fluss Shen" and "Vaudenjs" are two different sites. Assuming that the coordinates provided by Dabović (N42° 2′, E37° 13′ (sic!)) should read N42° 2′ ?", E19° 37′ 13″, then the *dabovici* type locality is somewhere north of Vau i Dejës. Therefore, Vau i Dejës should still be treated as the type locality of *biloba*.

In NHMW there are two lots from Virpazar (Montenegro), collected by Dabović. In spite of several attempts by Z.E. and Z.F., as well as by P. Subai (personal communication), to find *A. biloba* in the Virpazar area, this record could not be verified.

Intraspecific variability: The specimens from Shkodër (type locality of *biloba*) are smaller (*H* 10–12 mm) and more finely ribbed. Fangul Pass specimens (type locality of *martae*) are taller (*H* 11.5–13.5 mm), more robust and more strongly ribbed (irregularly on the cervix). The Vau i Dejës population (another type locality of *biloba*) exhibits high intrapopulation variability; all transitional forms between typical Shkodër-like and typical Fangul-like specimens can be found there. In the close vicinity of the type locality of *martae* (Erzen gorge and Skuterrë), we have found small, typical Shkodër-like specimens, whereas the average shell height was found to be the largest in Petrësh (also central Albania). In Brandt's (1961) view, the larger size, the stronger ribbing and the shape of the aperture are the main features distinguishing between *dabovici* and the nominate form. However, paratypes of *dabovici* (NHMW 79000/K/6003) fall within the variability of the material originating from the two *biloba* type localities. Taking this into consideration, we see no reason to treat either *martae* or *dabovici* as distinct taxa, but as synonyms of *biloba*. Ribs of the Petrësh population are somewhat lower and look papillate, as not the entire ribs but only their upper part beneath the suture is white. This morphological feature clearly differentiates the Petrësh population from the others; however, considering the phenotypic heterogeneity of this species, it seems premature to attempt discrimination at the subspecific level at the present time.

The Drin Valley area between Koman and Fierzë is worth a special note (Fig. 4). The population near Koman has a typical *biloba* shell shape although with somewhat lower ribs. East of Koman there are populations with less slender shells and lower (sometimes just wrinkle-like) ribs, which are as widely spaced as in the typical form.

mtDNA sequence divergence and molecular phylogeny

The aligned, concatenated dataset consisted of 2327 bp. Of these, 1128 sites were variable and 952 parsimony-informative. In the concatenated dataset, and regarding only *Agathylla* sequences, the mean overall sequence divergence (K-2p) was 0.247 (24.7%). For the individual loci, this value was 0.248 for 16S rDNA, 0.193 for COI and 0.303 for COII. The same analysis performed on the 12 sequences of the *A. neutra*/*A. biloba* clade gave 0.150, 0.137, 0.142 and 0.189, respectively.

Figure 5 shows the 50% majority-rule consensus from the BI analysis. The topology of the ML tree was identical to this and is therefore not presented, but both BS support values of the major nodes from the ML analysis and PP values from the BI analysis are shown. Based on this tree there are several strongly supported clades. The phylogram supports the monophyly of the subgenus *A. (Agathyllina)* (including *A. lamellosa*, *A. formosa* and *A. strigillata*), which is sister to the rest of the genus, that is *Agathylla* s. s. Within the latter group, there are three distinct clades comprising *A. abrupta* + *A. goldi*, *A. exarata* + *A. sulcosa* and *A. biloba* + *A. neutra*, respectively. The clades *A. abrupta*/*A. goldi* and *A. biloba*/*A. neutra* are not sister groups, suggesting that the bilobed clausilium plate is a homoplastic trait. Within the *A. exarata*/*A. sulcosa* clade, *A. sulcosa* is paraphyletic. As regards the *A. neutra*/*A. biloba* clade, *A. biloba* sequences form a monophyletic subclade. Though *A. neutra* appears as paraphyletic, due to its low BS support our analysis is unable to confirm or refute the two species' reciprocal monophyly.

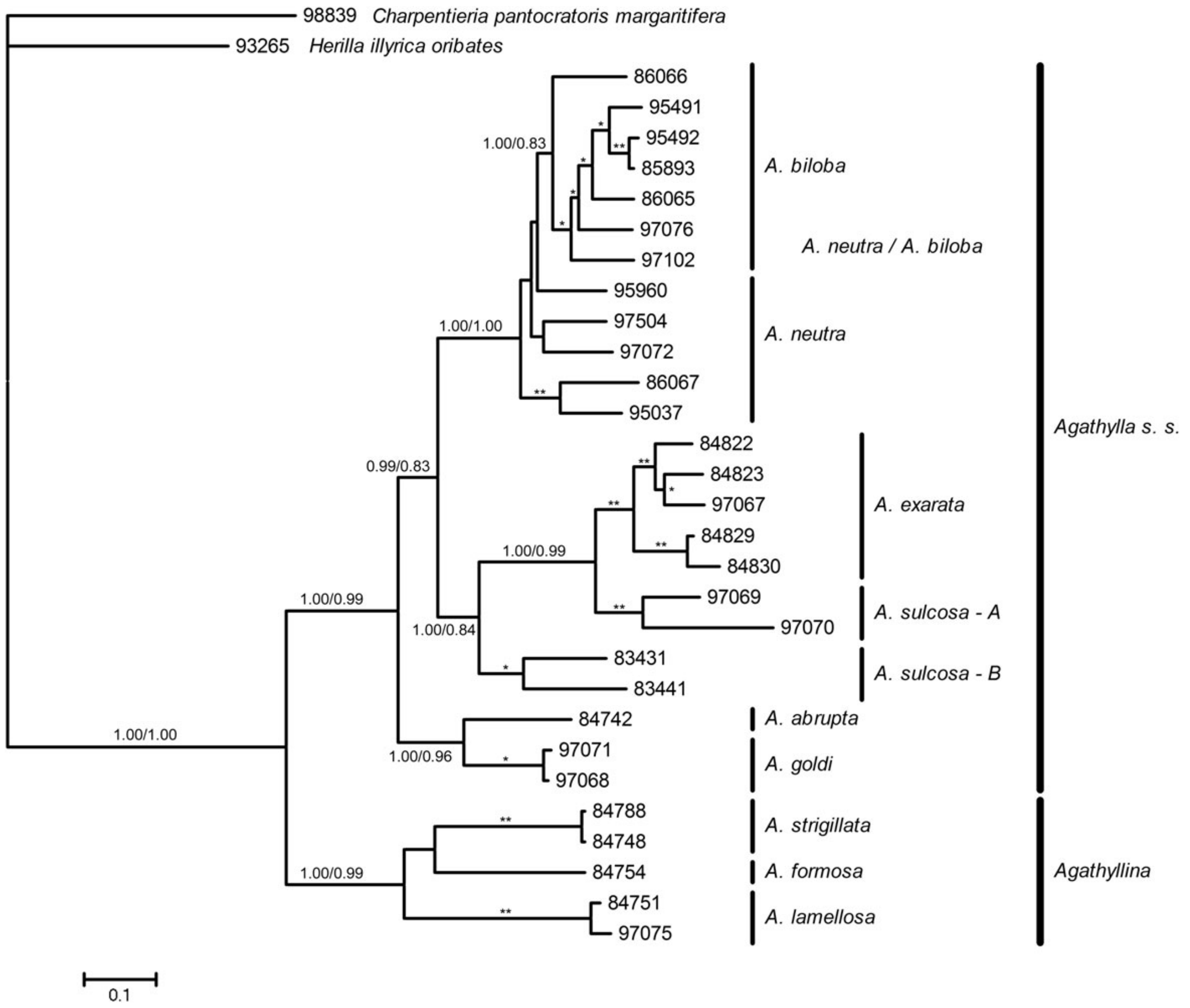

Figure 5. Bayesian tree showing phylogenetic relationships within *Agathylla*, based on concatenated COI, COII and 16S rRNA sequences. This is a 50% majority-rule consensus tree; numbers over branches are Bayesian PP and BS support values from the maximum-likelihood analysis (PP/BS). Only values above 0.75 (or 75%) are shown. At shallow phylogenetic levels the following symbols are used: **BI ≥ 0.99 and ML ≥ 0.95, *BI ≥ 0.75 and ML ≥ 0.75. Scale bar indicates the expected number of substitutions per site. For voucher specimens see Table 1. Note that *A. neutra* is inferred to be paraphyletic in this analysis.

DISCUSSION

Among the rock-dwelling gastropods in the Mediterranean area, and specifically among the subfamily Alopiinae, a significant portion of the taxa are known—at least at the time of their description—only from one or a few localities. In this group, taxonomic delimitations at the lower taxonomic levels (subspecies or species) are traditionally based on the combination of a few shell morphological characters. This applies also to *Agathylla* and specifically to the two Albanian *Agathylla* species, where the current taxonomy (Nordsieck, 1996; Nordsieck, 2007; Bank, 2012) has been based on only a few (four *A. biloba* and four *A. neutra*) known populations. Based on eight known populations, Nordsieck (1996) distinguished two subspecies within each species. However, in the newly discovered populations we have found wider boundaries of quantitative morphological features (size, shape, ribbing and colour of the shell, as well as the structure of the lunella and the cervical swelling) as well as new character state combinations. In the light of this new information, the current taxonomic scheme seems untenable. A possible way to resolve such an issue is to delineate further subspecies, but in the present case such a large number of new character state combinations were found that further splitting seems to be out of the question. Rather, we propose that there are only two taxa, which are each morphologically variable, and we see no reason to delimit further subspecies or even to maintain the subspecies already described within the two species.

A puzzling issue is the presence of morphologically transitional populations in the Drin Valley, for which more than one possible hypothesis can be proposed. One could argue that the transition from the unribbed morph to the ribbed one along the Fierzë–Vau i Dejës transect is due to adaptation along an environmental cline. However, the distribution of ribbed and unribbed populations throughout the whole range is too random (Fig. 1) to support this scenario. If we accept the view of

Gittenberger (1991) that speciation and/or phenotypic diversification of rock-dwelling gastropods is driven primarily by nonadaptive processes, there are still two possibilities. Transitional populations could be explained either as a transition in the contact zone between two conspecific morphotypes or, alternatively, as hybrids between two distinct species. A better understanding of the origin of these transitional populations would help in the resolution of the taxonomic confusion of the *A. biloba*/*A. neutra* group. Therefore, we consider the Drin Valley an interesting target for future research. Dense geographical sampling, applying population-genetic approaches in order to test the extent of gene flow, and applying niche modelling to test adaptive processes, will facilitate progress towards a taxonomy that adequately captures the evolutionary history of these species.

Though some changes might occur in the phylogenetic tree if the missing taxa are added, this study has supported an important aspect of the morphology-based division of the genus, namely the reciprocal monophyly of the two subgenera, *Agathyllina* and *Agathylla* s.s. Regarding the focal species of this study, mitochondrial markers have demonstrated that they are closely related and together form a monophyletic clade. Due to low BS values, our analysis could neither confirm nor refute the reciprocal monophyly of the two species. Even if the paraphyly of *A. neutra* had been supported by higher BS values, this would not necessarily have challenged the current system, because incomplete lineage sorting is as reasonable explanation for this phenomenon as the conspecific status of *A. biloba* and *A. neutra*. Considering that the level of mtDNA sequence divergence between land snails has been reported to be in the range of 2 to 34.5% (Parmakelis, Kotsakiozi & Rand, 2013), the level of sequence divergence recorded between *A. biloba* and *A. neutra* (13.7–18.9%, depending on the gene fragment) falls well within this range. However, land snails are also characterized by very high intraspecific mitochondrial sequence divergence that in some cases reaches up to 13.8% (Parmakelis *et al*., 2013) or even 24.2% (Scheel & Hausdorf, 2012). Consequently, delimiting land-snail species based on mtDNA sequence divergence is not straightforward. Our point is that despite indications in the molecular phylogeny and sequence divergence of the focal species, we consider that for the time being we should rely primarily on morphological features. Distinction of subspecies does not seem justified but, considering the conspicuous shell morphological differences between *A. biloba* and *A. neutra*, it seems reasonable to retain them as distinct species for the present. At the same time, we intend to seek further morphological and molecular evidence to investigate their status as distinct species. To this end, we propose a more comprehensive phylogenetic study using multiple populations per species and involving nuclear molecular markers as well.

To summarize, in the light of the recently collected material, the present classification of the Albanian *Agathylla* seems untenable. The high number of subspecific taxa relative to the number of known populations was probably an artefact of the limited number of populations investigated. New populations have revealed that *A. biloba* and *A. neutra* are too variable morphologically to justify their further taxonomic subdivision. This is similar to the findings for some *Albinaria* species after a thorough investigation of the Cretan fauna (Welter-Schultes, 2010). The most important conclusion to be drawn from this analysis is that exhaustive population sampling is indispensable for proper taxonomic assessments and that taxonomic revisions of poorly explored Mediterranean rock-dwelling gastropods will be necessary after comprehensive sampling has been performed.

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