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A NEW SPECIES OF *BYTHINELLA* (TRUNCATELLOIDEA: BYTHINELLIDAE) FROM BOSNIA AND HERZEGOVINA

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Abstract *A new species of* Bythinella *from Vrelo Vrioštice spring in Bosnia and Herzegovina is described. The shell, protoconch, radula, female reproductive organs and penis are illustrated and described. Cytochrome oxidase c subunit I (COI) placed the new species as the sister taxon of* B. serborientalis *Radoman, 1978. Shell biometry (PCA) and COI (genetic distance p=0.072) clearly confirm the distinctness of the newly described species.*

Key words *shell, radula, female reproductive organs, penis, cytochrome oxidase subunit I (COI), molecular phylogeny*

Introduction

Bythinella Moquin-Tandon, 1856, with its type species *Bulimus viridis* Poiret, 1801, belongs to the family Bythinellidae Locard, 1893, whose family-level distinctness was proved morphologically (Szarowska, 2006) and molecularly (Wilke *et al*., 2001). *Bythinella* is represented by more than 250 presently accepted nominal species (WoRMS, 2021) and are minute snails (2–3mm high shell), inhabiting mostly European springs. Their range spans from southern Poland to the southernmost parts of Europe (Giusti & Pezzoli, 1980; Falniowski, 1987; Boeters, 1998; Glöer, 2002), and from Pyrenees to East Europe, although former records from Caucasus were erroneous (Vinarski & Kantor, 2016; Chertoprud *et al*., 2023). Wide variability and ecophenotypic plasticity of the shell (Falniowski, 1987; Falniowski & Szarowska, 2011; Falniowski, 2018), coupled with assumptions of complete isolation of the populations inhabiting particular springs (e.g., Boeters, 1982), was rather unjustified resulted in chaos in species-level systematics. Soft parts' morphology and anatomy did not help much because of the morphostatic (as defined by Davis, 1992) evolution in *Bythinella* (Falniowski & Szarowska, 2011; Wilke *et al*., 2010). In September 2022 in Bosnia and Herzegovina we found a *Bythinella* which cannot be matched with any known species and whose description and molecular relationships we present in this study.

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Material and methods

On September 2nd, 2022 about fifty specimens of *Bythinella* were collected in Vrelo Vrioštice (43º14'15.82'' N, 17º29'7.84'' E) in Bosnia and Herzegovina (Fig. 1 and 2) by Andrzej Falniowski. The snails were fixed in 80% solution of analytically pure ethanol, which was replaced two times. Next, the snails were put in fresh 80% analytically pure ethanol and kept at -20°C temperature in a freezer. *Bythinella serborientalis* Radoman, 1978 was collected from stream in the cave in Village Potpeće near, Sevojno, Serbia (43º47'51.70'' N, 19º56'8.90'' E).

The shells were photographed with a Canon EOS 50D digital camera, under a Nikon SMZ18 microscope. The dissections were done under a Nikon SMZ18 microscope with dark field, equipped with Nikon DS-5 digital camera, whose captured images were used to draw female reproductive organs with a graphic tablet. Protoconchs were cleaned using an ultrasonic cleaner, mounted and examined applying the techniques described by Falniowski (1990), the radulae were extracted with Clorox, applying the techniques described by Falniowski (1990). Protoconchs and radulae were photographed using a HITACHI S-4700 scanning electron microscope. Penis and tubular ducts were photographed under

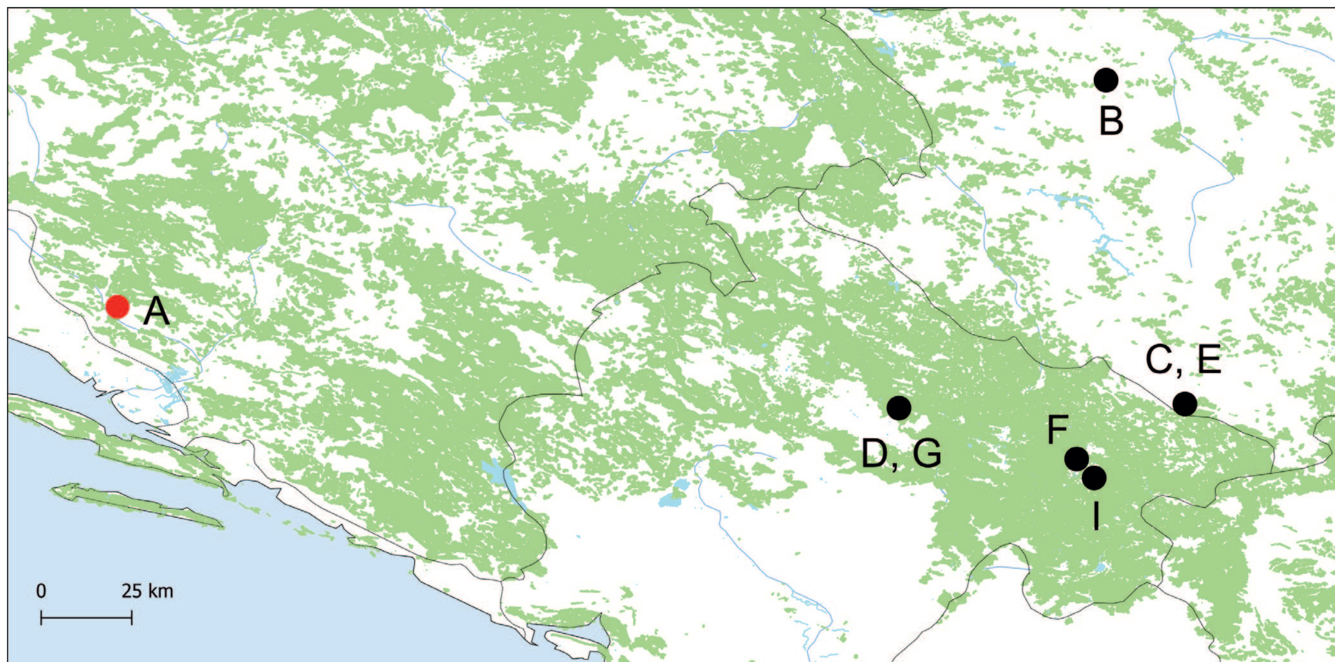

Figure 1 Type locality of *Bythinella marianramosae* (red) and localities of the phylogenetically close mOTUs (black), symbols as in Fig. 9.

Figure 2 Type locality of *Bythinella marianramosae* Vrelo Vrioštice.

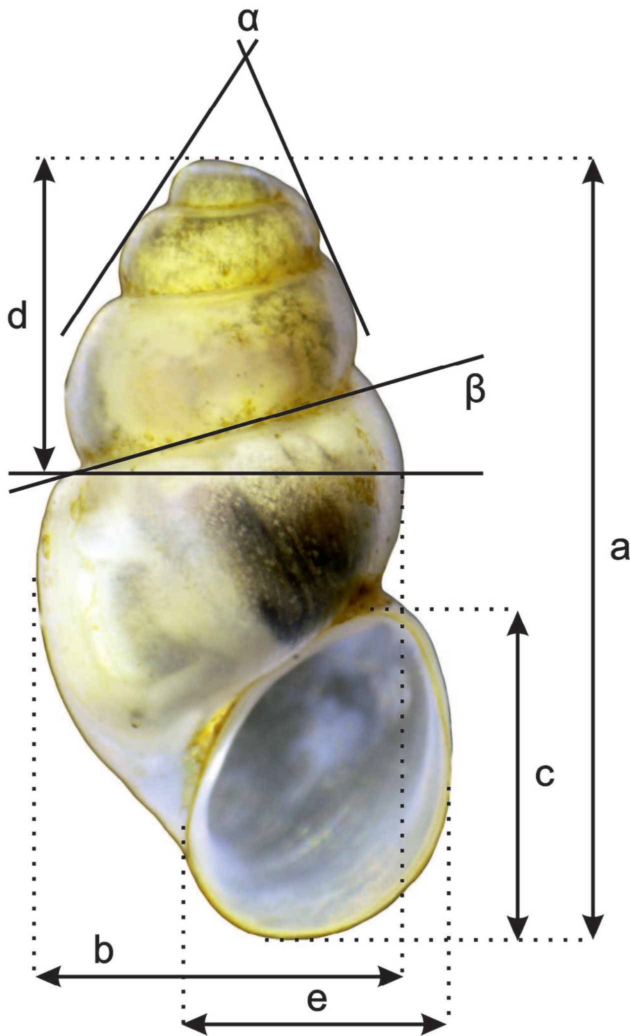

Figure 3 Measurements of the shell: a – shell height, b – body whorl breadth, c – aperture height, d – spire height, e – aperture breadth, α – apex angle, β – angle between body whorl suture and horizontal surface; measurements values given in Table 1.

Motic B3 Professional microscope with dark field.

Seven morphometric parameters of the shell (Szarowska, 2006; Falniowski *et al.*, 2007, 2012a) were measured (Fig. 3) by one person using a Nikon DS-5 digital camera and ImageJ image analysis software (Rueden *et al.*, 2017). The linear measurements were then logarithmically transformed; for angular measurements the arcsine transformation was applied. Principal component analysis (PCA), based on the matrix of correlation, was computed, applying a descriptive, non-stochastic approach. The original observations were projected into PC space, to show relationships between the specimens, without any classification given *a priori* (Falniowski, 2003; Rohlf, 1998). The transformations and PCA calculations were made by the ClustVis 2.0 (https://biit.cs.ut.ee/clustvis/) (Metsalu & Jaak, 2015). Shell character states after Hershler & Ponder (1998).

Specimens for molecular analysis were fixed in 80% ethanol. DNA was extracted from whole specimens; tissues were hydrated in TE buffer (3×10 min); then total genomic DNA was extracted with the Sherlock extraction kit (A&A Biotechnology), and the final product was dissolved in 20 µl of tris-EDTA (TE) buffer. The extracted DNA was stored at −80°C at the Department of Malacology, Institute of Zoology and Biomedical Research, Jagiellonian University in Kraków (Poland).

Mitochondrial cytochrome c oxidase subunit I (COI) locus was sequenced. Details of PCR conditions, primers used, and sequencing were given in Szarowska *et al.* (2016). Sequences were initially aligned in the MUSCLE (Edgar, 2004) program in MEGA 7 (Kumar *et al.*, 2016) and then checked in Bioedit 7.1.3.0 (Hall, 1999). Uncorrected p-distances were calculated in MEGA 7. The estimation of the proportion of invariant sites and the saturation test for entire data sets (Xia, 2000; Xia *et al.*, 2003) were performed using DAMBE (Xia, 2013). In the phylogenetic analysis, additional sequences from GenBank were used as reference (Benke *et al.*, 2011; Falniowski *et al.*, 2012b). The sequences were selected based on an alignment containing all sequences from GenBank and all belong to one clearly distinct evolutionary lineage. *Bythinella cretensis* Schütt, 1980 was used as an outgroup, as the closest relative of the clade to which the newly described species belongs. The data were analysed using approaches based on Bayesian inference (BI) and Maximum Likelihood (ML). For RAxML analysis, the jModelTest2 via the CIPRES Science Gateway (Miller *et al.*, 2010) was used to find the best-fitting model for each gene. The model TPM3uf+I was used. The ML analysis was conducted in RAxML-NG v. 0.8.0 (Kozlow *et al.*, 2019) via web service available at https://raxml-ng.vital-it.ch/, with 10 random and 10 parsimony starting trees. In the BI analysis, the K81+I model of nucleotide substitution were applied in tree reconstruction. Model was selected using MrModelTest 2.4 (Nylander, 2004). The analyses were run using MRBAYES v. 3.2.7a (Ronquist *et al.*, 2012) with defaults of most priors. Two simultaneous analyses were performed, each with 10,000,000 generations, with

one cold chain and three heated chains, starting from random trees and sampling the trees every 1,000 generations. The first 25% of the trees were discarded as burn-in. The analyses were summarised as a 50% majority-rule tree. Convergence was checked in TRACER v.1.7.1 (Rambaut *et al.*, 2018), in all cases Effective Sample Size exceeded 200. FigTree v. 1.4.4 (Rambaut, 2010) was used to visualize the trees. Three species delimitation methods were performed: Poisson Tree Processes (PTP) (Zhang *et al*., 2013), Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al*., 2011) and Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al*., 2021). The PTP approach was run using the web server https://species.h-its.org/ptp/, with 100,000 MCMC generations, 100 thinning and 0.1 burn-in. We used the RAXML output phylogenetic tree. The ABGD and ASAP approaches used the web servers (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) and (https://bioinfo.mnhn.fr/abi/public/asap/) respectively, with the default parameters. The Fastachar application (Merckelbach & Borges, 2020) and DeSignate (Hütter *et al*., 2020) were used to distinguish particular species from another species, based on COI sequences, by determining the Molecular Diagnostic Characters (MDCs). Two types of characters were accepted: at binary positions (the character state in the query group is different from the uniform character state in the reference group) and at asymmetric positions (the character state in the query group is different from the non-uniform character state in the reference group).

SYSTEMATIC

Family Bythinellidae Locard, 1893
Genus *Bythinella* Moquin-Tandon, 1856
Bythinella marianramosae Falniowski, Jaszczyńska & Hofman sp. n.

Zoobank number: lsid:zoobank.org:pub:8C629D91-4EA4-4739-8519-10E9DEF57E89
GenBank sequence numbers: OR373063-OR373064

Holotype Ethanol-fixed specimen (Fig. 4) collected on 2nd of September 2022 by Andrzej Falniowski at Vrelo Vrioštice (43º14'15.82''N, 17º29'7.84''E) in Bosnia and Herzegovina (Fig. 1–2), Museum of Natural History of the University of Wrocław, Poland, voucher number MNHW-1510.

Paratypes 30 ethanol-fixed specimens, collected at Vrelo Vrioštice (43º14'15.82''N, 17º29'7.84''E) in Bosnia and Herzegovina (Fig. 1–2), collection of the Department of Malacology, Jagiellonian

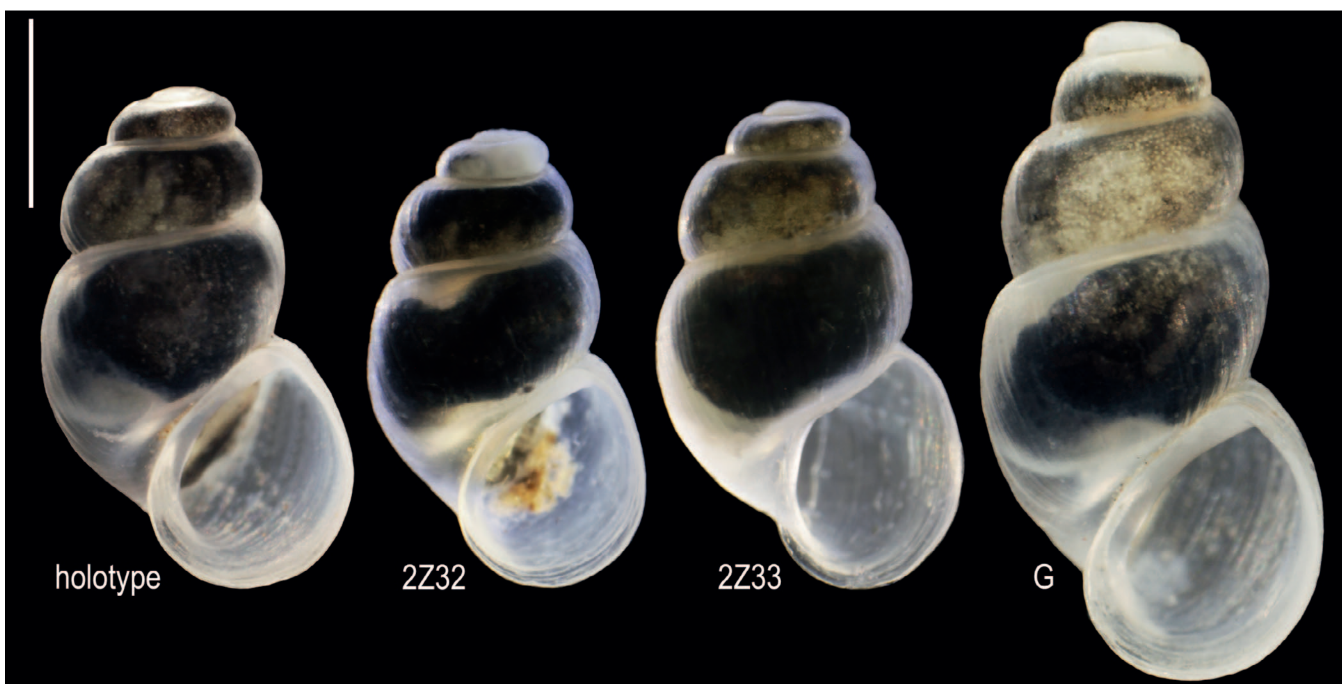

Figure 4 Shells of *Bythinella marianramosae* from the type locality: holotype, two sequenced specimens (numbers as in Fig. 7) and giant specimen (G); bar equals 1mm.

University, Krakow, Poland, voucher number ZMUJ2721, two paratypes destroyed for DNA extraction.

Description Shell (Fig. 4) ovate-conical, broad, with relatively low spire, up to 3.10mm high (3.63 in a giant specimen), with about 4.5 whorls, spire height approximately 26% of shell height, and 51% of body whorl width. Teleoconch whorls moderately convex, evenly rounded, growing regularly in diameter. Aperture broadly ovoid, outer lip simple, parietal lip complete, umbilicus slit-like. Teleoconch glossy, with delicate growth lines, periostracum whitish or white. Operculum smooth on its inner and outer surface. Protoconch (Fig. 6a) broad, no sharp border between proto- and teleoconch, its microsculpture (Fig. 6b) with regular net of depressions, formed by the covered pores. Operculum (Fig. 6c) typical of *Bythinella*, without any outgrowth.

Measurements Shell parameters for the holotype and a series of paratypes, as well as the shells of *B. serborientalis*, are given in Table 1. Principal

Table 1 Shell measurements: a – shell height; b – body whorl breadth; c – aperture height; d – spire height; e – aperture breadth; α – apex angle measured between the lines tangential to the spire; β – angle between the body whorl suture and the line perpendicular to the columella; M – mean; SD – standard deviation; Min – minimum value; Max – maximum value.

	a	b	c	d	e	α	β
Bythinella marianramosae; n=14							
Holotype	2.79	1.36	1.31	0.92	1.13	105	15
2Z32	2.53	1.30	1.20	0.80	1.03	105	13
2Z33	2.71	1.40	1.32	1.02	0.94	102	19
1	2.99	1.49	1.36	0.97	1.15	99	14
2	3.10	1.51	1.49	1.11	1.13	99	15
3	2.85	1.41	1.36	0.89	1.14	100	14
4	2.94	1.51	1.39	0.93	1.17	106	15
5	2.91	1.43	1.36	0.95	1.18	106	15
6	2.94	1.46	1.36	1.04	1.16	99	14
7	2.97	1.40	1.41	1.05	1.13	102	16
8	2.98	1.43	1.40	1.07	1.11	98	14
9	2.79	1.43	1.40	0.90	1.02	104	16
10	2.90	1.46	1.44	1.04	1.14	106	19
G	3.63	1.57	1.60	1.33	1.44	102	21
M	2.93	1.44	1.39	1.00	1.13	102.36	15.71
SD	0.245	0.068	0.091	0.127	0.111	2.977	2.335
Min	2.53	1.30	1.20	0.80	0.94	98	13
Max	3.63	1.57	1.60	1.33	1.44	106	21
Bythinella serborientalis; n=10							
1J13	2.72	1.28	1.10	1.13	0.92	100	15
1J14	2.68	1.29	1.11	1.04	0.99	103	16
1	2.67	1.32	1.18	0.97	0.98	103	23
2	2.57	1.20	1.16	0.92	0.87	100	22
3	2.56	1.27	1.13	0.99	0.92	101	22
4	2.46	1.21	1.13	0.90	0.96	102	19
5	2.55	1.26	1.10	0.94	0.95	100	18
6	2.59	1.29	1.17	0.93	1.02	103	24
7	2.56	1.24	1.15	0.99	0.99	102	22
8	2.43	1.18	1.12	0.86	0.99	103	20
M	2.58	1.25	1.14	0.97	0.96	101.70	20.10
SD	0.092	0.045	0.029	0.077	0.045	1.337	3.035
Min	2.43	1.18	1.10	0.86	0.87	100	15
Max	2.72	1.32	1.18	1.13	1.02	103	24

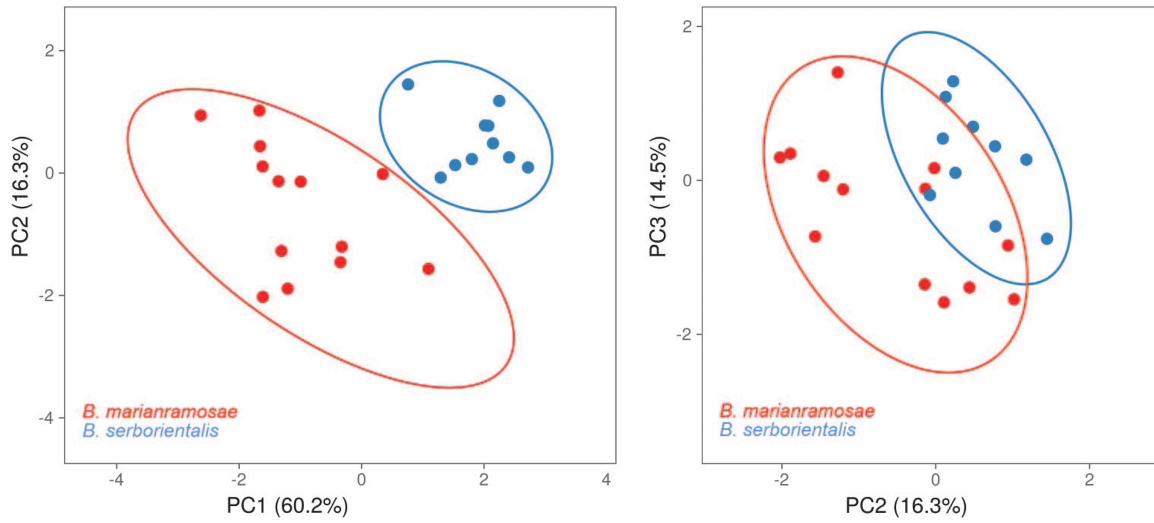

Figure 5 Principal component analysis (PCA) on the shells of *Bythinella marianramosae* (red dots) and *B. serborientalis* (blue dots).

Figure 6 Protoconch, operculum and radulae of *Bythinella marianramosae*: A–B – protoconch (bar equals: A – 100µm, B – 20µm), C – operculum (bar equals 500µm), D–E – radulae (bar equals 50µm).

component analysis (Fig. 5) shows a wide variability of the shells in *B. marianramosae* and slightly less wide in *B. serborientalis*, not overlapping for PC1 (representing mostly size differences), and partly overlapping in PC2 and PC3.

Radula (Fig. 6d–e) with central tooth with rather blunt and massive cusps following formula:

$$\frac{4-1-4}{(3)1\text{–}1(3)} \quad \text{or} \quad \frac{5-1-5}{(3)1\text{–}1(3)}$$

Lateral tooth with 3(4) – 1–4 blunt cusps, the biggest one broad and rounded. Inner marginal tooth with 19–21 long and sharp cusps, outer marginal tooth with only seven relatively big cusps on the terminal edge.

Soft parts morphology and anatomy Head and mantle pigmented black. Female reproductive organs (Fig. 7) with U-shaped moderately big bursa copulatrix, short but broad loop of oviduct and spherical and big receptaculum seminist. Penis (Fig. 8) with flagellum and the arm containing vas deferens of similar length, rather broad, tubular gland massive.

Differential diagnosis Shell ovate-conical, broader than in *B. serborientalis* and with relatively low spire, much lower than in *B. serborientalis*, female reproductive organs with U-shaped bursa copulatrix, smaller than in *B. serborientalis*, and receptaculum seminis spherical and big, bigger than in *B. serborientalis*, and loop of renal oviduct broader than in that species, penis with flagellum and the arm containing vas deferens of similar length, rather broad. The most characteristic are broad and bulky shell and big spherical receptaculum seminis. The Molecular Diagnostic Characters: binary: 18 (A), 84 (T), 108 (C), 117 (C), 123 (G), 156 (G), 216 (G), 222 (G), 234 (C), 255 (A), 267 (G), 315 (T), 432 (G), 435 (G), 447 (A); asymmetric: 264 (T), 291 (A).

Derivation of name Species name to honour the memory of Dr Marian A. Ramos, a splendid malacologist deeply devoted to the study of the Truncatelloidea, and a friend of the first author.

Distribution and habitat Known only from the type locality, narrow stream outflows from the spring Vrelo Vrioštice, which forms a small, shallow pool on the limestone rubble, with some algae and macrophytes (Fig. 2). There are no snails in this pool, but in its outlet, in a form of narrow stream, numerous *Bythinella* inhabit roots of macrophytes and grass on its banks.

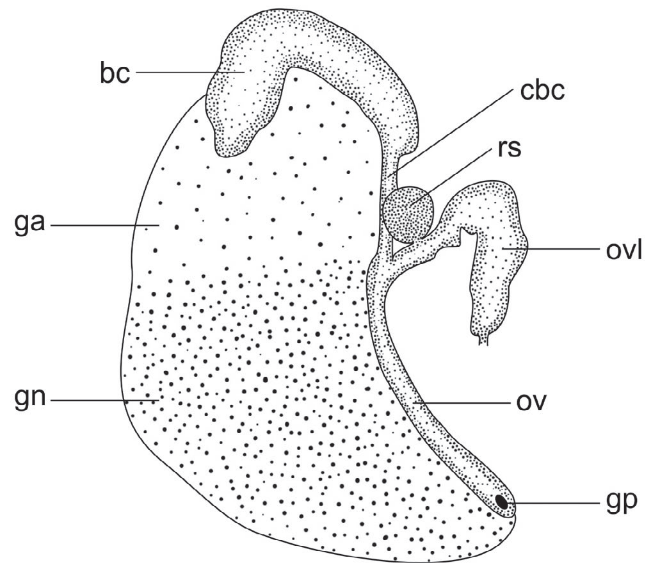

Figure 7 Female reproductive organs of *Bythinella marianramosae* (bc – bursa copulatrix, cbc – duct of bursa copulatrix, ga – albuminoid gland, gn – nidamental gland, gp – gonoporus, ov – oviduct, ovl – loop of (renal) oviduct, rs – receptaculum seminis.

MOLECULAR DISTINCTNESS AND RELATIONSHIPS OF *BYTHINELLA MARIANRAMOSAE*

We obtained four new COI sequences of *Bythinella* (457 bp, GenBank accession numbers OR373063-OR373064 of *B. marianramosae* and OR391928-OR391929 of *B. serborientalis*). The test for the substitution saturation analysis showed an ISS (0.71) significantly smaller than the critical ISS value (0.94), indicating that sequences are not saturated and thus useful in phylogenetic reconstruction. The topologies of the resulting phylograms were identical in both the ML and BI phylogram analyses; thus, we present the phylogram computed with RAxML (Fig. 9). Sequences of *B. marianramosae* (mOTU A) formed a clade with *B. serborientalis* (mOTU B) as a sister species (p-distance 0.072, Table 2), although bootstrap support is insignificant. Both mOTUs are well supported. These sequences together with 13 other formed one lineage distinct from all the other species of *Bythinella* (Jaszczyńska, in press). According to the three different delimitation methods, this clade was divided into nine mOTUs with p-distances 0.009–0.09 (Table 2).

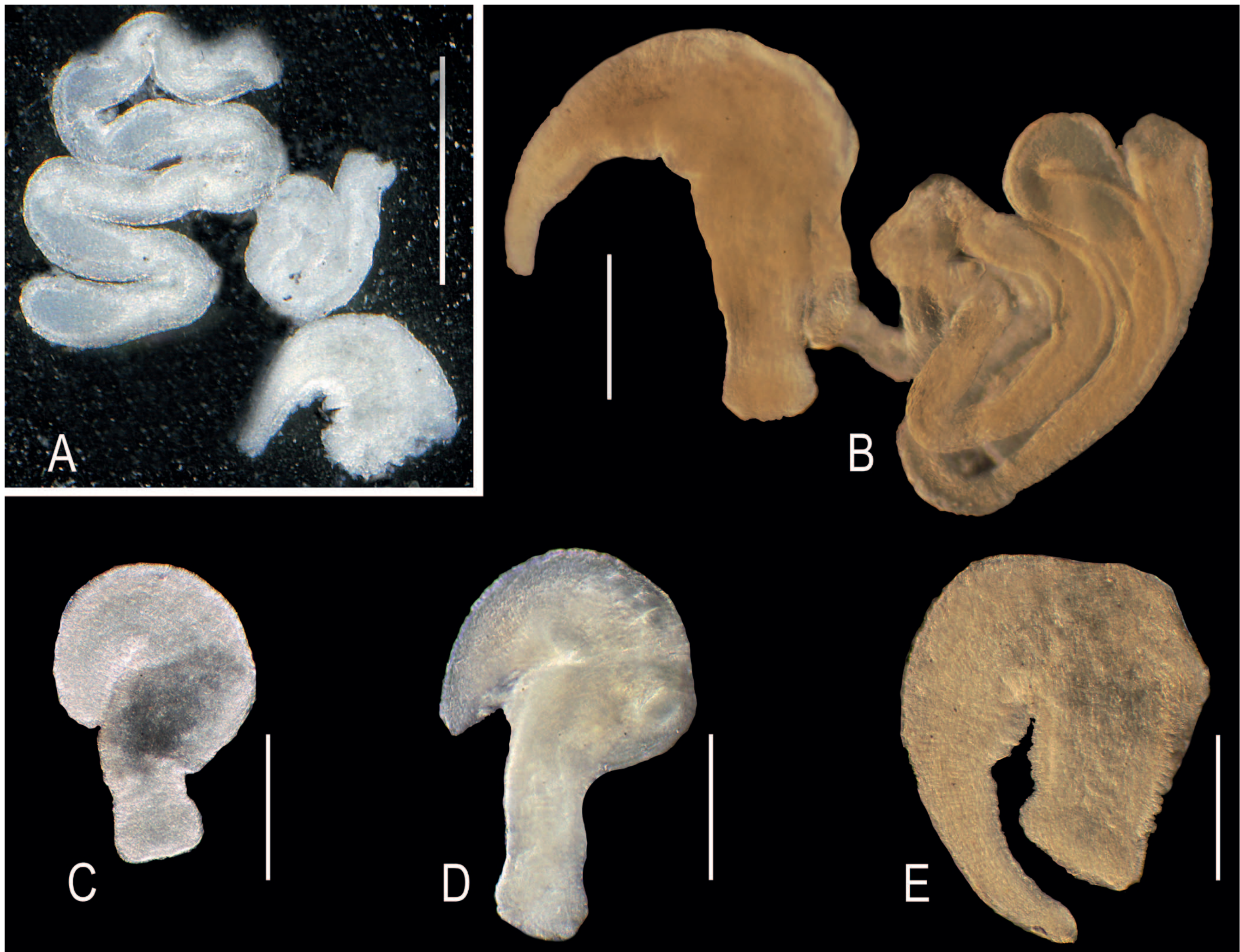

Figure 8 Penis of *Bythinella marianramosae*, A and B with tubular gland; bar equals 200µm.

Discussion

As already noted in the Introduction, the shell is of little use in the taxonomy of *Bythinella*. Thus, the variability overlap between the species is not surprising. The PCA analysis still shows some distinctness of the shell. The dense net of pores is characteristic of *Bythinella* (Falniowski, 1990), but in *B. marianramosae* they were covered by the outermost layer of the periostracum. The number of the cusps on the outer marginal tooth is lower than in the other *Bythinella* (e.g. Falniowski, 1990). As summarized by Falniowski (2018) the anatomical characters usefulness in species distinction is also limited. In the presented case, however, two sister species: *B. marianramosae* and *B. serborientalis* differ evidently in the female reproductive organs, especially the receptaculum seminis. Species status of the *B. marianramosae* is clearly confirmed by the molecular data. It formed a distinct lineage that was significantly more genetically different than the level used for interspecies variation for *Bythinella* (Bichain *et al.*, 2007a, b; Falniowski & Szarowska, 2011). All delimitation methods confirmed its separateness. The differentiation between mOTUs within the clade to which *B. marianramosae* belongs, also confirmed this phenomenon. The p-distance between this species and its sister group belongs to the larger distances within this group. The giant specimen was most probably infected by the larval trematodes – such parasitic gigantism is common in the Truncatelloidea (Falniowski, 1987).

Acknowledgments

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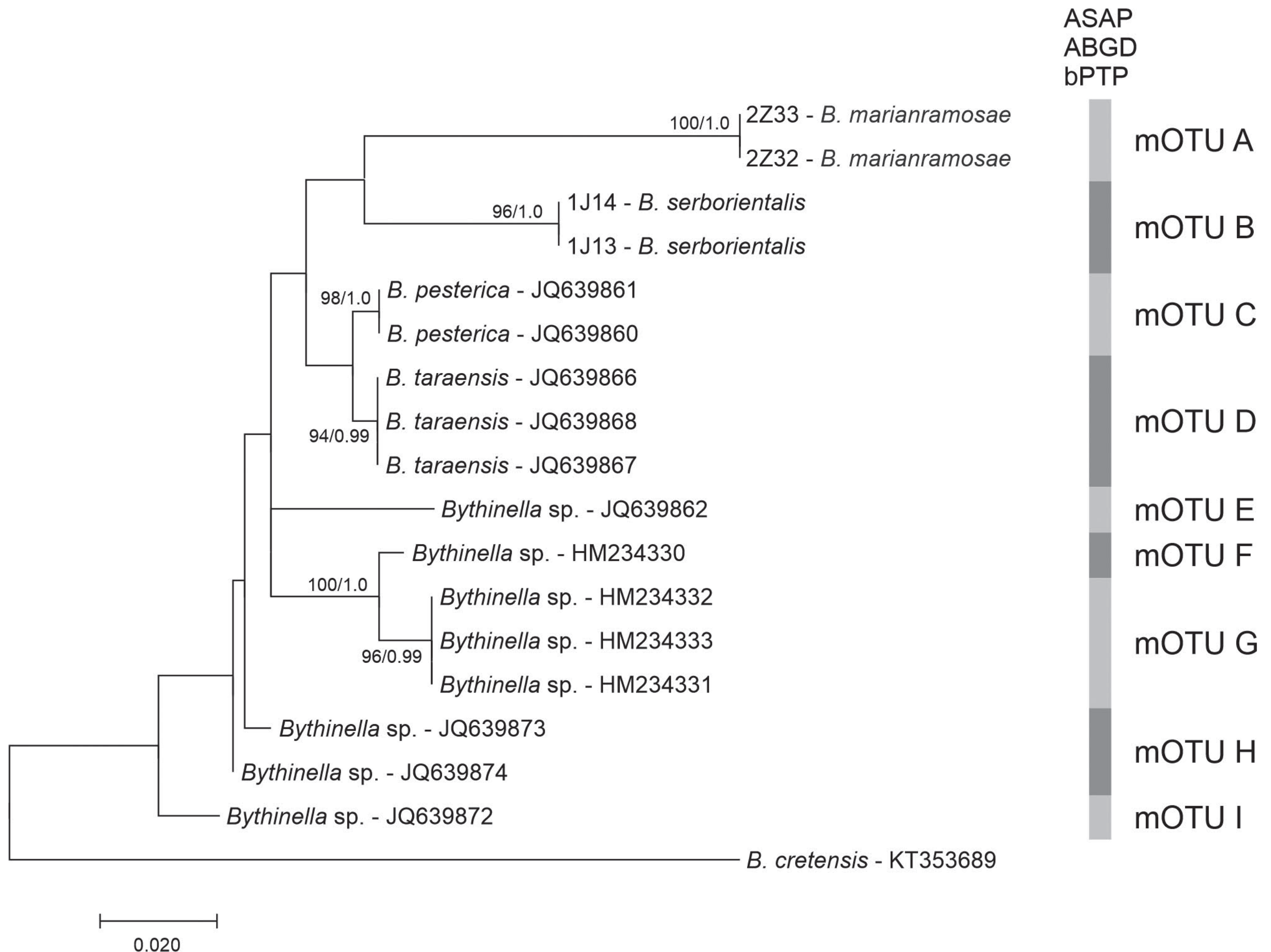

Figure 9 Maximum likelihood tree computed for cytochrome c oxidase subunit I (COI), bootstrap supports/ Bayesian probabilities given if bootstrap>70%; results of delimitation analysis are also shown. taxon names with GenBank numbers.

Table 2 p-distances for COI between (below diagonal) and within (diagonal, bold, italic) mOUTs, see Fig. 9.

	A	B	C	D	E	F	G	H	I
A	***0.000***								
B	0.072	***0.000***							
C	0.061	0.046	***0.000***						
D	0.055	0.046	0.009	***0.000***					
E	0.081	0.055	0.033	0.039	–				
F	0.085	0.057	0.039	0.039	0.048	–			
G	0.090	0.061	0.039	0.044	0.046	0.013	***0.000***		
H	0.064	0.049	0.025	0.025	0.032	0.027	0.032	***0.007***	
I	0.083	0.068	0.044	0.044	0.050	0.044	0.048	0.022	–

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References

Benke M, Brändle M, Albrecht C & Wilke T 2011 Patterns of freshwater biodiversity in Europe: lessons from the spring snail genus *Bythinella Journal of Biogeography* **38**: 2021–2032.

Bichain JM, Boisselier-Dubayle MC, Bouchet P & Samadi S 2007a Species delimitation in the genus *Bythinella* (Mollusca: Caenogastropoda: Rissooidea): A first attempt combining molecular and morphometrical data *Malacologia* **49**: 293–311.

Bichain JM, Gaubert P, Samadi S & Boisselier-Dubayle MC 2007b A gleam in the dark: Phylogenetic species delimitation in the confusing spring-snail genus *Bythinella* Moquin-Tandon, 1856 (Gastropoda: Rissooidea: Amnicolidae) *Molecular Phylogenetic and Evolution* **45**: 927– 941.

Boeters H D 1982 Species concept of prosobranch freshwater molluscs in western Europe, 2 Malacologia **22**(1–2): 499–504.

Boeters H D 1998 Mollusca: Gastropoda: Superfamilie Rissooidea in: *Süsswasserfauna von Mitteleuropa* Begründet von A. Brauer **5/1–2** (Schwoerbel J. Zwick P. eds) Gustav Fischer Verlag Jena – Lübeck – Ulm.

Chertoprud E, Grego J, Mumladze L, Hofman S, Palatov D, Osikowski A, Jaszczynska A & Falniowski A 2023 Revealing the Stygobiotic and Crenobiotic Molluscan Diversity in the Caucasus: Part IV – Crenobiotic Belgrandiellinae Radoman, 1983 (Mollusca, Hydrobiidae) from Georgia *Diversity-Basel* **15**(3): 450.

Davis G M 1992 Evolution of prosobranch snails transmitting Asian *Schistosoma*: coevolution with *Schistosoma*: a review *Progress in Clinical Parasitology* **3**: 145–204.

Edgar RC 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput *Nucleic Acids Research* **32**: 1792–1797.

Falniowski A 1987 Hydrobioidea of Poland (Prosobranchia: Gastropoda) *Folia Malacologica* **1**: 11–122.

Falniowski A 1990 Anatomical characters and SEM structure of radula and shell in the species-level taxonomy of freshwater prosobranchs (Mollusca: Gastropoda: Prosobranchia): a comparative usefulness study *Folia Malacologica* **4**: 53**–**142+78 tab. phot.

Falniowski A 2003 *Metody numeryczne w taksonomii [Numerical techniques in taxonomy]* Wydawnictwo Uniwersytetu Jagiellońskiego Kraków. 233 pp.

Falniowski A 2018 Species Distinction and Speciation in Hydrobioid Gastropods (Mollusca: Caenogastropoda: Truncatelloidea) *Archives of Zoological Studies* **1**: 003.

Falniowski A & Szarowska M 2011 Radiation and phylogeography in a spring snail *Bythinella* (Mollusca: Gastropoda: Rissooidea) in continental Greece *Annales Zoologici Fennici* **48**: 67–90.

Falniowski A, Szarowska M & Grzmil P 2007 *Daphniola* Radoman, 1973 (Gastropoda: Hydrobiidae): shell biometry, mtDNA, and the Pliocene flooding *Journal of Natural History* **41**: 2301–2311.

Falniowski A, Szarowska M, Glöer P & Pesic V 2012a Molecules vs morphology in the taxonomy of the *Radomaniola/Grossuana* group of Balkan Rissooidea (Mollusca: Caenogastropoda) *Journal of Conchology* **41**: 19–36.

Falniowski A, Szarowska M, Glöer P, Pešić V, Georgiev D, Horsák M & Sirbu I 2012b Radiation in *Bythinella* Moquin-Tandon, 1856 (Mollusca: Gastropoda: Rissooidea) in the Balkans *Folia Malacologica* **20**: 1–10.

Giusti F & Pezzoli E 1980 Gasteropodi, 2 (Gastropoda: Prosobranchia: Hydrobioidea, Pyrguloidea) *Consiglio Nazionale delle Ricerche AQ/1/47, Guide per il Riconoscimento delle Specie Animali delle Acque Interne Italiane* **8**: 1–67.

Glöer P 2002 *Mollusca I. Süsswassergastropoden Nord und Mitteleuropas. Bestimmungsschlüssel, Lebensweise, Verbreitung* 2 neubearbeitete Auflage in: Die Tierwelt Deutschlands **73**, ConchBooks Hackenheim. 327 pp.

Hall TA 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT *Nucleic Acids Symposium Series* **41**: 95–98.

Hershler R & Ponder WF 1998 A review of morphological characters of hydrobioid snails *Smithsonian Contribution to Zoology* **600**: 1–55.

Hütter T, Ganser MH, Kocher M, Halkic M, Agatha S & Augsten N 2020 DeSignate: detecting signature characters in gene sequence alignments for taxon diagnoses BMC Bioinformatics **21**: 151.

Kozlov AM, Darriba D, Flouri T, Morel B & Stamatakis A 2019 RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference *Bioinformatics* **35**: 4453–4455. doi:10.1093/bioinformatics/btz305

Kumar S, Stecher G & Tamura K 2016 MEGA**7**: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets *Molecular Biology and Evolution* **33**: 1870–1874.

Locard A 1893 Conchyliologie française. [I]. Les coquilles des eaux douces et saumâtres de France. Description des familles, genres et espèces 1–327 Paris (J.-B. Baillière). https://www.biodiversitylibrary.org/page/12977684

Merckelbach L & Borges L 2020 Make every species count: FastaChar software for rapid determination of molecular diagnostic characters to describe species *Molecular Ecology Resources* **20**: 1761–1768.

Metsalu T & Jaak V 2015 Clustvis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap *Nucleic Acids Research* **43**: 566–570.

Miller MA, Pfeiffer W & Schwartz T 2010 Creating the CIPRES Science Gateway for inference of large phylogenetic trees in*:* Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov, New Orleans, LA: 1–8.

Moquin-Tandon A 1855–1856 Histoire naturelle des mollusques terrestres et fluviatiles de France, Tome premier (1): i-viii, 1–14+Atlas: 1–16, pl. 1–9 (12 April 1855); (2): 145–256+Atlas: 17–32, pl. 10–18 (4 May 1855); (3): 257–416+Atlas: 33–48, pl. 19–27 (1 August 1855). Tome second (4): i-iii, 1–176+Atlas: 49–64, pl. 28–36 (10 September 1855); (5): 177–368+Atlas: 65–80, pl. 37–45 (2 January 1856); (6): 369–646+Atlas: i-iii, 81–92, pl. 46–54 (9 April 1856). Paris (J.-B. Baillière). https://biodiversitylibrary.org/page/32068638

Nylander JAA 2004 MrModeltest v.2. *Program distributed by the author* Uppsala: Evolutionary Biology Centre, Uppsala University.

Poiret J L M 1801 Coquilles fluviatiles et terrestres observées dans le Département de l'Aisne et aux environs de Paris: prodrome xi +119 pp. http://www.biodiversitylibrary.org/item/81286

Puillandre N, Brouillet S & Achaz G 2021 ASAP: assemble species by automatic partitioning *Molecular Ecology Resourses* **21**: 609–620.

Puillandre N, Lambert A, Brouillet S & Achaz G 2011 ABGD, Automatic Barcode Gap Discovery for primary species delimitation *Molecular Ecology* **21**: 1864–1877.

Radoman P 1973 New classification of fresh and brackish water Prosobranchia from the Balkans and Asia Minor *Posebna Izdanja Prirodnjacki Musej u Beogradu* **32**: 1–30.

Radoman P 1978 Neue Vertreter der Gruppe Hydrobioidea von der Balkanhalbinsel *Archiv für Molluskenkunde* 109 (1–3): 27–43.

Rambaut A 2010 *FigTree v1.3.1* Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/

Rambaut A, Drummond AJ, Xie D, Baele G & Suchard MA 2018 Posterior summarisation in Bayesian phylogenetics using Tracer 1.7 *Systematic Biology* **67**: 901–9.

Rohlf FJ 1998 *NTSYSpc, numerical taxonomy and multivariate analysis system*. *Version 2.0*. [Computer software and manual] Exeter Software Setauket NY.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, HohnA S, Larget B, Liu L, Suchard MA & Huelsenbeck JP 2012 Efficient Bayesian phylogenetic inference and model choice across a large model space *Systematic Biology* **61**: 539–542.

Rueden CT, Schindelin J, Hiner MC, Dezonia BE, Walter AE, Arena ET & Eliceiri KW 2017 ImageJ**2**: ImageJ for the next generation of scientific image data BMC *Bioinformatic*s **18**: 529.

Schütt H 1980. Zur Kenntnis griechischer Hydrobiiden. *Archüv für Molluskenkunde* **110**: 115–149.

Szarowska M 2006 Molecular phylogeny, systematics and morphological character evolution in the Balkan Rissooidea (Caenogastropoda) *Folia Malacologica* **14**: 99–168.

Szarowska M, Osikowski A, Hofman S & Falniowski A 2016 *Pseudamnicola* Paulucci, 1878 (Caenogastropoda: Truncatelloidea) from the Aegean Islands: a long or short story? *Organism Diversity and Evolution* **16**: 121–139.

Vinarski MV & Kantor YI 2016 Analytical Catalogue of Fresh and Brackish Water Molluscs of Russia and Adjacent Countries Tovarishchestvo Nauchnyckh Izdanii KMK Moscow.

Wilke T, Benke M, Brändle M, Albrecht C & Bichain J M 2010 The Neglected Side of the Coin: Non-adaptive Radiations in Spring Snails (*Bythinella* spp.). In: M Glaubrecht (ed) *Evolution in Action* Springer-Verlag Berlin Heidelberg: 551–578.

Wilke T, Davis GM, Falniowski A, Giusti F, Bodon M & Szarowska M 2001 Molecular systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships *Proceedings of the Academy of Natural Sciences of Philadelphia* **151**: 1–21.

WoRms Editorial Board 2023 World Register of Marine Species. Available from https://www.marinespecies.org at VLIZ. Accessed 2023-04-04.

Xia X 2000 *Data analysis in molecular biology and evolution* Kluwer Academic Publishers Boston, Dordrecht & London.

Xia X 2013 DAMBE: A comprehensive software package for data analysis in molecular biology and evolution *Molecular Biology and Evolution* **30**: 1720–1728.

Xia X, Xie Z, Salemi M, Chen L & Wang Y 2003 An index of substitution saturation and its application *Molecular Phylogenetics and Evolution* **26**: 1–7.

Zhang J, Kapli P, Pavlidis P & Stamatakis A 2013 A general species delimitation method with applications to phylogenetic placements *Bioinformatics* **29**: 2869–2876.