Systematics and Biodiversity

ISSN: 1477-2000 (Print) 1478-0933 (Online) Journal homepage: https://www.tandfonline.com/loi/tsab20

Redescription of the western Balkan species *Xerocampylaea waldemari* and its phylogenetic relationships to other Urticicolini (Gastropoda: Hygromiidae)

Małgorzata Proćków, Michael Duda, Luise Kruckenhauser, Wim J. M. Maassen, Anton J. de Winter & Paweł Mackiewicz

To cite this article: Małgorzata Proćków, Michael Duda, Luise Kruckenhauser, Wim J. M. Maassen, Anton J. de Winter & Paweł Mackiewicz (2019) Redescription of the western Balkan species *Xerocampylaea waldemari* and its phylogenetic relationships to other Urticicolini (Gastropoda: Hygromiidae), Systematics and Biodiversity, 17:4, 367-384, DOI: 10.1080/14772000.2019.1617365

To link to this article: https://doi.org/10.1080/14772000.2019.1617365

Systematics and Biodiversity (2019), **17(4)**: 367–384

Taylor & Francis
Taylor & Francis Group

Research Article

Redescription of the western Balkan species *Xerocampylaea waldemari* and its phylogenetic relationships to other Urticicolini (Gastropoda: Hygromiidae)

MAŁGORZATA PROĆKÓW[1], MICHAEL DUDA[2], LUISE KRUCKENHAUSER[3], WIM J. M. MAASSEN[4], ANTON J. DE WINTER[4] & PAWEŁ MACKIEWICZ[5]

[1]Museum of Natural History, University of Wrocław, ul. Sienkiewicza 21, Wrocław, 50-335, Poland
[2]3rd Zoological Department, Museum of Natural History Vienna, Burgring 7, Vienna, 1010, Austria
[3]Central Research Laboratories, Museum of Natural History Vienna, Burgring 7, Vienna, 1010, Austria
[4]Naturalis Biodiversity Center, P.O. Box 9517, Leiden, 2300 RA, the Netherlands
[e]Department of Bioinformatics and Genomics, Faculty of Biotechnology, University of Wrocław, ul. F. Joliot-Curie 14a, Wrocław, 50-383, Poland

(Received 27 November 2018; accepted 25 April 2019)

The proper identification of species has significant implications for conservation and general knowledge of ecosystem variety. It is especially important for biodiversity hotspots and former Pleistocene refugia, such as the Balkans. In this paper, we evaluate the taxonomic status of the endemic Balkan species *Fruticicola waldemari* A. J. Wagner, 1912, using both recently collected material and museum specimens. Phylogenetic analyses based on two mitochondrial markers, 16S rRNA and cytochrome c oxidase subunit I, show that this species is closely related to the representatives of *Xerocampylaea*, including a conchologically similar and partly sympatric species, *X. erjaveci*. Analyses of shell and genital morphology as well as mitochondrial DNA sequences indicate that *X. waldemari* and *X. erjaveci* are separate but closely related species. These species differ in shell morphology (size, umbilicus diameter, microsculpture) as well as genital morphology. Nevertheless, a further study with more comprehensive sampling of both taxa is required to fully understand the complex pattern of genetic and morphological variation observed.

Key words: Balkans, biodiversity hotspot, endemic, mitochondrial DNA, morphology, Mollusca, phylogeny, *Trochulus*

Introduction

Land snails of the genus *Trochulus* Chemnitz, 1786 (formerly *Trichia* Hartmann, 1840), inhabit a large part of Europe and possess medium-sized (5–17 mm) shells that are far from easy to identify (Proćków, 2009). Its taxonomic history is one of continuous debates about the number of species and their classification. The *Trochulus*-like snails have been described as the members of the genus *Trochulus* only (Kerney, Cameron, & Jungbluth, 1983; Proćków, 2009), or have been separated into two (*Trochulus* and *Petasina* Beck, 1847) (Bank, 2011) or three genera (*Trochulus*, *Petasina*, and *Plicuteria* Schileyko, 1978) (Nordsieck, 1993). In a recent molecular phylogeny of the family Hygromiidae, *Trochulus* species *sensu* Kerney et al. (1983) have been split into six genera (*Trochulus*, *Edentiella*, *Plicuteria*, *Petasina*, *Xerocampylaea*, and *Noricella*) (Neiber, Razkin, & Hausdorf, 2017). Moreover, this study showed that a morphological feature traditionally thought to be typical of *Trochulus*, the presence of four dart sacs, two of which with darts, can no longer be used for the higher classification of these snails. Regardless of the generic membership of these species, most of them have limited geographic ranges. Five species are known to occur in the Balkans (Proćków, 2009), a region regarded as one of the most important hotspots for European biodiversity and one of the richest areas in terms of endemism of continental molluscs (Cuttelod, Seddon, & Neubert, 2011). Recently, several papers have presented morphological and/or genetic variation of *Trochulus* species (Duda et al., 2011, 2014; Kruckenhauser et al., 2014; Proćków, Kuźnik-Kowalska, & Mackiewicz, 2017a, 2017b; Proćków, Mackiewicz,

Correspondence to: Małgorzata Proćków. E-mail: malgorzata.prockow@uwr.edu.pl

ISSN 1477-2000 print / 1478-0933 online

https://dx.doi.org/10.1080/14772000.2019.1617365

Published online 04 Jul 2019

& Pieńkowska, 2013; Proćków, Strzała, Kuźnik-Kowalska, & Mackiewicz, 2014; Proćków, Strzała, Kuźnik-Kowalska, Proćków, & Mackiewicz, 2017), but none of them has referred to the taxa from south-eastern Europe. Clearly, this region is under collected, at least with respect to live-collected material. Thus, the phylogenetic relationships and classification of *Trochulus*-like snails in this region still remain unclear.

Two Balkan species formerly attributed to *Trochulus*, *T. waldemari* (Wagner, 1912) and *T. erjaveci* (Brusina, 1870) are the subject of our research. While *T. erjaveci* has recently been provisionally classified with the genus *Xerocampylaea* (Neiber et al., 2017), the generic affiliation of *T. waldemari* remains unanswered. Considerable uncertainty in their identification and delimitation result from the lack of detailed taxonomic studies. These species are still insufficiently known: their morphological and anatomical features are poorly documented, and molecular data are lacking. *X. waldemari* has been hardly studied since its description by Wagner (1912) (Wagner, 1914 copied the description in his other publication). Then this species was only mentioned as a part of the Bosnian malacofauna without giving localities (Jaeckel, 1954; Jaeckel, Klemm, & Meise, 1957). Their genitalia were described 73 years later since its first description (Maassen, 1985). *X. waldemari* was not included in a recent checklist of Bosnia and Herzegovina (Karaman, 2006), but has been listed as *Trochulus* (*T.*) *waldemari* in the Fauna Europea list (Bank, 2011). According to the first version of the IUCN Red List of Threatened Species, *X. waldemari* is qualified as 'Not evaluated' (Cuttelod et al., 2011), while in the online version (Fehér, 2011b) it is classified as 'Least Concern' with an estimated area of occupancy of 24 km^2. This taxon is regarded as poorly known (Fehér, 2011b) and as such taxonomic research is warranted and recommended by the IUCN (Cuttelod et al., 2011).

Xerocampylaea erjaveci is also insufficiently known. At least 10 subspecies or geographic varieties have been recognized within this species (de Winter & Maassen, 1992; Proćków, 2009). However, only few old publications addressed some aspects of this species (Bole, 1984; de Winter & Maassen, 1992; Wagner, 1914; Westerlund, 1889; Wohlberedt, 1909) and no comprehensive study of *X. erjaveci* is available.

In this paper we used recently collected material as well as specimens from the museum collections to evaluate the taxonomic status of the former *Trochulus* snails from the Balkans, currently described as *Xerocampylaea waldemari* and *X. erjaveci*. We carried out integrative molecular, morphological, and anatomical analyses of shell and soft parts of these snails.

Materials and methods

Sampling and morphological data of studied taxa

We studied material including *X. waldemari* and most of the described *X. ejaveci* subspecific taxa (i.e., *hirci*, *osoria*, *hajlensis*, *cincta*, *syrmiensis*, and *floerickei*) (Table S1, see online supplemental material, which is available from the article's Taylor & Francis Online page at https://dx.doi.org/10.1080/14772000.2019.1617365). The material included field-collected snails and museum samples from the following collections: Naturalis Biodiversity Center, Leiden, the Netherlands (ZMA); Museum für Naturkunde der Humboldt Universität, Berlin, Germany (ZMB); Croatian Natural History Museum, Zagreb, Croatia (CNHM); Museum and Institute of Zoology, Polish Academy of Science, Warszawa, Poland (IZPAN) and Museum of Natural History, Wrocław, Poland (MPW). The field-collected material is deposited in Naturhistorisches Museum Wien, Austria (NHMW, see Table S1, see supplemental material online). Fifteen adult snails from Mount Vlasič in Bosnia and Herzegovina, from which six were investigated in more detail, were regarded as putative *Xerocampylaea* sp. because of the differences in their shell surface (i.e. more distinct granulation) compared with other members of this species. Two juveniles from Foča region (Bosnia and Herzegovina) could only be ascribed to *Xerocampylaea* sp.

The same person (MP) measured in total 430 specimens from 80 sites (Fig. 1), including 351 individuals of *X. erjaveci*, 73 of *X. waldemari*, and six of *Xerocampylaea* sp., in standardized views (Proćków, 2009) from photos by using TPSdig Version 2.16 (Rohlf, 2010). Since the low systematic measurement error (with 1% error probability) does not compromise results (Duda et al., 2011), the specimens were measured once. We calculated basic statistical parameters: mean, range and standard deviation and performed the canonical discriminant analysis (CDA) on shell and genital measurements. CDA is a dimension-reduction technique, which finds linear combinations of characters that maximizes the differences between at least two data sets. We carried out the non-parametric Mann–Whitney *U* test in comparison of two data sets because variables describing them are not normally distributed. We corrected *P*-values obtained in multiple comparisons using the Benjamini and Hochberg method to control the false discovery rate. We applied Statistica 12 (Stat Soft, Inc. 1984–2014) and R package 3.5.1 (R Core Team, 2018) in the statistical analyses of the data.

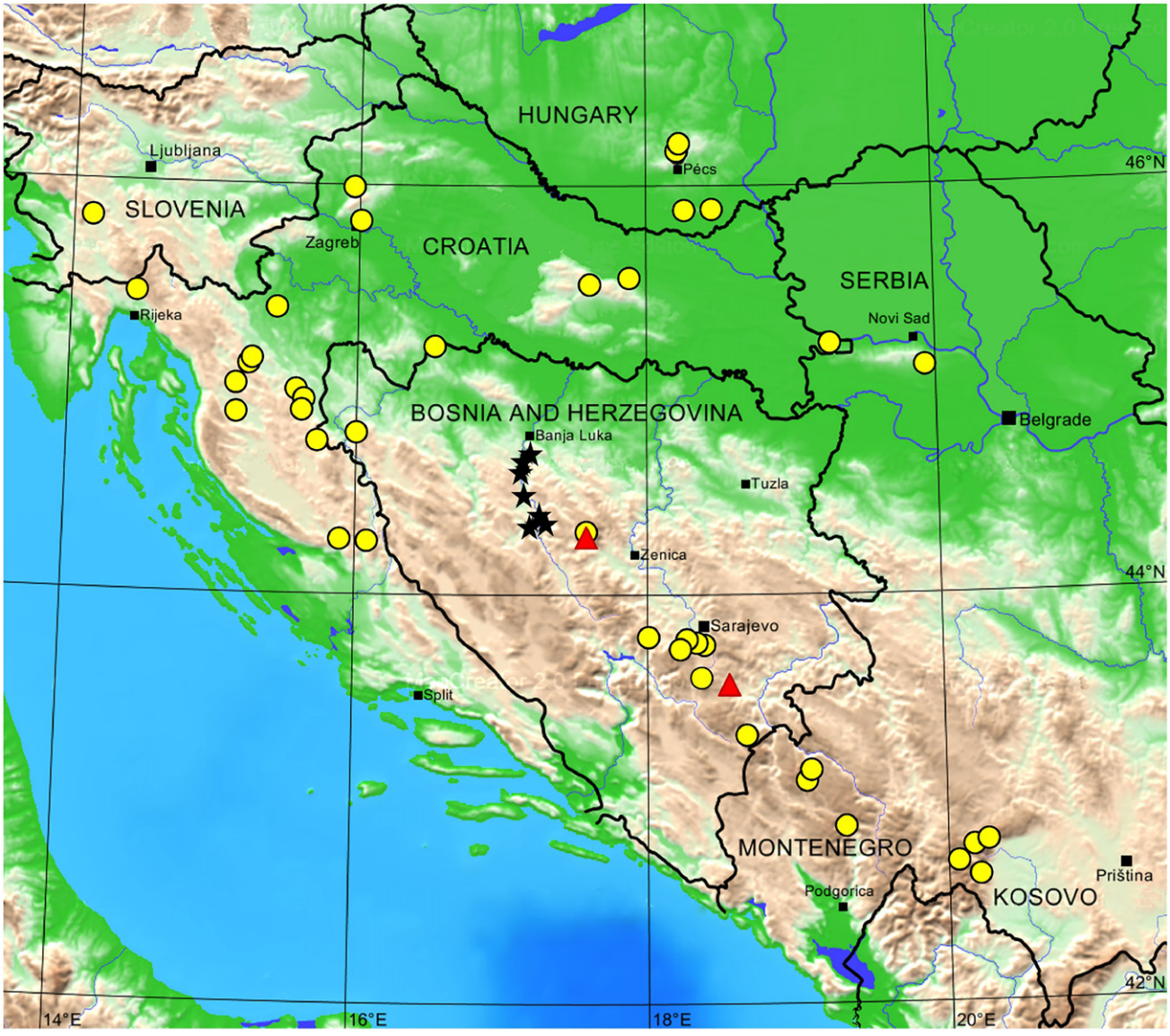


Fig. 1. Geographic locations of *X. waldemari* (stars), *X. erjaveci* (circles), and *Xerocampylaea* sp. (triangles) populations.

We dissected 38 mature snails of *X. erjaveci*, 17 of *X. waldemari*, and two of *Xerocampylaea* sp. (Table S1, see supplemental material online), and observed their external genital morphology. Additionally, we performed detailed dissections of genitalia, especially penial papilla, to record qualitative characters, e.g., the patterns of penial plicae. Altogether we dissected and analysed in detail 13 snails, including nine specimens of *X. erjaveci* (five from Barátság and one from Doczymalom both in Hungary, two from Dragos Sedlo in Bosnia and Herzegovina, one from Sarengrad in Croatia), and four *X. waldemari* (all from Vrbas, Bosnia and Herzegovina). Morphometric data were taken according to the procedures described by Proćków (2009).

Abbreviations used in the text

Shell measurements: H: shell height, W: shell width, bwH: body whorl height, h: aperture height, w: aperture width, D: shell diameter, U: umbilicus major diameter, u: umbilicus minor diameter, whl: number of whorls, H/W: height/width shell ratio, bwH/H: relative height of body whorl, U/D: relative umbilicus diameter, u/U: ratio of umbilicus minor to its major diameter, h/w: height/width aperture ratio.

Genitalia measurements: fl: flagellum, ep: epiphallus, p: penis, sd: duct of bursa copulatrix, sl: length of bursa copulatrix, sw: width of bursa copulatrix; uv: upper vagina, is/os: relative length of inner to outer dart sacs, fl/ep: flagellum/epiphallus ratio, ep/p: epiphallus/penis ratio; sd/sl: bursa copulatrix duct length/bursa copulatrix

length ratio; and sw/sl: bursa copulatrix width/length ratio.

Molecular analyses

We analysed genetically 15 specimens of *T. erjaveci* from seven distant localities as well as four adults of *X. waldemari* and two juveniles similar to *X. waldemari* from two localities (Table S1, see supplemental material online). We obtained a partial region of the mitochondrial cytochrome c oxidase subunit I (COI) gene from all individuals and larger partial regions of the mitochondrial 16S rRNA (16S) gene from 11 representatives of each clade. Primers for the COI fragment were taken from Duda et al. (2011) and primers for the 16S fragment (16Scs1, 16Scs2) from Chiba (1999). All nucleotide sequences reported in this study have been deposited in GenBank with accession numbers: MK801539–MK801559 for COI and MK811018–MK811028 for 16S rRNA.

We performed PCRs on a Master Gradient thermocycler (Eppendorf) in 25 µl with 1 µl template DNA, 0.5 unit Q5 DNA polymerase (BioLaps), 0.5 µM of each primer, and 0.2 mM of each dNTP (Roche). Each PCR comprised 35 reaction cycles with an annealing temperature of 55 °C (COI) or 50 °C (16S). Control reactions were carried out for both DNA extractions and PCR amplifications. PCR products were purified using the QIAquick PCR Purification kit (Qiagen) and analysed by direct sequencing in both directions. The sequencing was performed at Microsynth (Switzerland) using the original PCR primers.

Phylogenetic analyses

We inferred phylogenetic relationships between *Xerocampylaea* and other snails using 16S rRNA and COI gene alignments separately as well as the concatenated alignment of 16S rRNA and COI. We compared the newly obtained sequences with all homologous sequences annotated to Hygromiidae and available in the GenBank database (Table S2, see supplemental material online). We removed redundant and short sequences and selected representatives from each species to the final sets. We used three representatives of Geomitridae as outgroup. The 16S rRNA set included 159 sequences, the COI set had 198 sequences and the 16S rRNA + COI set 215 sequences. We aligned the sequence in MAFFT using a slow and accurate algorithm L-INS-i with 1,000 cycles of iterative refinement (Katoh & Standley, 2013) and inspected the resulted alignments in JalView (Waterhouse, Procter, Martin, Clamp, & Barton, 2009). The sites of the 16S rRNA alignments suitable for phylogenetic study were selected in GBlocks (Talavera & Castresana, 2007). The 16S rRNA alignment was 697 bp long, COI alignment had the length of 655 bp, while the concatenated alignment included 1259 bp.

We applied three phylogenetic approaches: the Maximum likelihood method in IQ-TREE (Nguyen, Schmidt, von Haeseler, & Minh, 2015), as well as two Bayesian analyses in MrBayes (Ronquist et al., 2012) and PhyloBayes (Lartillot & Philippe, 2004). In the case of COI set, we checked the necessity of using separate nucleotide substitution models for three codon positions, while for the concatenated alignment, we considered four potential partitions: the rRNA gene and three codon positions in COI (Table 1).

In IQ-TREE analyses (Chernomor, von Haeseler, & Minh, 2016; Kalyaanamoorthy, Minh, Wong, von Haeseler, & Jermiin, 2017), we used the substitution models as proposed according to the associated ModelFinder program (Table 1). We applied a thorough and slower nearest neighbour interchange (NNI) tree search considering all possible NNIs as well as applied Shimodara–Hasegawa-like approximate likelihood ratio test (SH-aLRT) assuming 10,000 replicates and non-parametric bootstrap with 1,000 replicates.

In MrBayes analyses, we considered partitions and associated substitution models for COI and 16S rRNA + COI alignments according to the results of PartitionFinder (Lanfear, Calcott, Ho, & Guindon, 2012) assuming BIC criterion for the model selection (Table 1). However, we applied mixed models rather than fixed ones to specify appropriate substitution models across the large parameter space (Huelsenbeck,

Table 1. Nucleotide substitution models found for studied data sets.

Data set	ModelFinder	jModelTest	PartitionFinder
16S rRNA	GTR + F+R7	TrN + I+G	NA
COI	pos. 1: TIM2 + F + R5	NA	pos. 1: GTR + I+G
	pos. 2: K3Pu + F+R3		pos. 2: K81uf + I+G
	pos. 3: TIM2 + F + R6		pos. 3: GTR + I+G
16S rRNA + COI	pos. 1: TIM2 + F + R4	NA	pos. 1: GTR + I+G
	pos. 2: TVM + F+R3		pos. 2: K81uf + I+G
	pos. 3: TIM2 + F + ASC + R6		pos. 3: GTR + I+G
	rRNA: GTR + F+R6		rRNA: GTR + I+G

Larget, & Alfaro, 2004), but the models describing heterogeneity rate across sites were adopted according to PartitionFinder. In the case of 16S rRNA set, we assumed the mixed models and the heterogeneity rate across sites described by invariant and discrete gamma models according to results of jModelTest (Darriba, Taboada, Doallo, & Posada, 2012) (Table 1).

Two independent runs starting from random trees, each using 8 (for rRNA) and 32 (for COI and rRNA + COI) Markov chains were applied. The trees were sampled every 100 generations for 10,000,000 generations in the case of single markers and 30,000,000 generations for the concatenated alignment. In the final analysis, we selected the last 1,380,000 to 9,722,000 (depending on the alignment set) trees that reached the stationary phase and convergence, i.e., when the standard deviation of split frequencies stabilized and was much below the assumed threshold 0.01.

In PhyloBayes, we applied the CAT + GTR + Γ model with the number of components, weights, and profiles inferred from the data. Two independent Markov chains were run for 100,000 generations with one tree sampled for each generation. The last 70,000–95,000 trees (depending on the alignment set) from each chain were collected to compute posterior consensus trees after reaching convergence, when the largest discrepancy observed across all bipartitions (maxdiff) was much below the recommended threshold 0.1. The gamma-distributed rate variation across the sites was approximated by five discrete rate categories in two Bayesian approaches.

Using IQ-TREE, we calculated the consensus of trees obtained in three approaches. The number of the trees supporting a given node were presented together with support values. We compared tree topologies assuming different relationships of sequences assigned to *X. waldemari* and *Xerocampylaea* sp. using Consel (Shimodaira & Hasegawa, 2001) and assuming 10,000,000 replicates. Site-wise log-likelihoods for the analysed trees were calculated in IQ-TREE under the best fitted substitution models. Competitive topologies obtained in MrBayes were compared using Bayes Factor based on the stepping-stone method estimating the mean marginal likelihood from two independent runs using eight Markov chains, 50 steps of the sampling algorithm, and 10,000,000 generations of the MCMC simulation.

Results

Morphological and anatomical analyses

Table 2 presents measurements of shell and genitalia of *X. erjaveci* and *X. waldemari*. The species are significantly distinct in size and shape of the shell (Table 2, Wilks' lambda = 0.33826, $F_{26,824} = 22.799$, $P < 0.00001$). The differences are statistically significant for 10 out of 14 features. The most discriminating features are umbilicus major (U) and minor (u) diameters as well as relative umbilicus diameter to shell diameter (U/D). They are about two times larger in *X. erjaveci* than in *X. waldemari*. However, the ranges of the shell measurements overlap and combinations of these characters are necessary to classify the samples (Fig. 2). The shells of *X. waldemari* and *X. erjaveci* are presented in Figs 3 and 4.

The shells of *X. erjaveci* are generally bigger in many dimensions and flatter than *X. waldemari*. CDA based on shell morphology can distinguish these species only by the first canonical function, which explains almost 99% of variation (Fig. 2). The samples of these species create two sets, which partially overlap. Six specimens of *Xerocampylaea* sp. are located within the set of *X. waldemari*. The height of the body whorl (bwH) and major umbilicus diameter (U) show the largest absolute values of coefficients (Table 3). CDA quite well recognized these two species, because 98.3% of *X. erjaveci* samples were correctly classified, and 84.1% of *X. waldemari*. The overall classification accuracy was 94.6%. Two specimens out of six, assigned provisionally to *Xerocampylaea* sp., were correctly classified to *X. waldemari*.

Figures 5 and 6 present the shell surface of *X. waldemari* and *X. erjaveci*. In *X. waldemari* delicate uneven growth lines cover both the upper and lower sides of the shell surface. Additionally, a granulose sculpture consisting of fine droplets can be noticed in some individuals even from the same locality (Figs 5.1–5.2). The shell surface of *X. erjaveci* is always regularly distinctly granulated (Figs 6.1–6.3), but strong growth ridges are visible only in some specimens (Figs 6.4–6.6).

The morphometric analysis of genitalia measurements revealed significant differences among *X. erjaveci* and *X. waldemari* in six out of 12 features (Table 2, Wilks' lambda = 0.44022, $F_{8,88} = 5.5790$, $P < 0.0001$). The reproductive system of *X. erjaveci* is characterized by significantly 1.3–2.3 times longer flagellum (fl), epiphallus (ep), duct of bursa copulatrix (sd) and bursa copulatrix (sl). Three out of five ratios of measurements are also statistically different between species (Table 2). However, the first two canonical functions do not clearly separate specimens of these species (Fig. 7). The whole analysis correctly classified 93.9% of *X. erjaveci* and 60% of *X. waldemari* samples. One out of two *Xerocampylaea* sp. specimens is classified to *X. waldemari*. The overall classification accuracy is 82%.

Table 2. Measurements of shell and genitalia (in mm) of *X. erjaveci* and *X. waldemari*. Statistically significant values are marked in **bold**.

	X. erjaveci			*X. waldemari*			
Features	**range**	**mean**	***SD***	**range**	**mean**	***SD***	**Corrected *P*-value**
shell							
W	8.73–16.73	12.83	1.59	6.70–13.81	10.82	1.33	**0.002**
H	5.27–11.04	7.76	1.07	5.16–9.36	7.32	0.92	**0.002**
bwH	4.54–9.27	6.53	0.85	4.18–8.01	6.19	0.79	**0.003**
h	2.67–6.55	4.25	0.68	2.97–5.53	4.14	0.56	0.280
w	4.54–9.33	6.53	0.87	3.74–7.96	6.32	0.79	0.113
D	8.36–16.80	12.79	1.62	6.70–14.04	11.03	1.37	**0.002**
U	0.73–3.64	2.13	0.53	0.33–1.44	0.96	0.24	**0.002**
u	0.55–3.09	1.78	0.44	0.22–1.29	0.85	0.21	**0.002**
whl	5.00–6.30	5.63	0.29	4.70–6.00	5.33	0.26	**0.002**
H/W	0.49–0.73	0.61	0.04	0.57–0.77	0.68	0.04	**0.002**
U/D	0.08–0.25	0.17	0.03	0.03–0.12	0.09	0.02	**0.002**
u/U	0.56–1.00	0.84	0.08	0.67–1.00	0.89	0.08	**0.002**
bwH/H	0.77–0.93	0.84	0.03	0.75–0.94	0.85	0.03	0.396
h/w	0.46–0.90	0.65	0.07	0.53–0.82	0.66	0.07	0.897
genitalia							
fl	2.13–6.35	3.28	0.86	1.44–3.98	2.45	0.84	**0.002**
ep	2.38–10.11	5.87	1.74	2.04–5.15	3.25	0.99	**0.002**
p	2.47–5.49	3.66	0.68	1.92–4.97	3.27	0.85	0.066
sd*	3.17–13.02	6.63	2.07	2.45–8.56	4.66	1.76	0.066
sl*	2.31–5.47	3.69	0.78	1.16–2.31	1.61	0.38	**0.002**
sw*	0.92–2.51	1.47	0.44	0.83–1.97	1.25	0.35	0.191
uv	0–1.63	0.23	0.49	0–3.15	0.35	0.81	0.277
is/os	0–0.87	0.44	0.22	0.17–0.52	0.32	0.11	0.066
fl/ep	0.34–0.95	0.59	0.15	0.58–1.34	0.76	0.19	**0.002**
ep/p	0.69–2.56	1.62	0.45	0.59–1.31	1.01	0.22	**0.002**
sw/sl*	0.26–0.59	0.41	0.11	0.48–1.05	0.79	0.22	**0.002**
sd/sl*	0.82–3.10	1.80	0.69	1.47–3.73	2.38	0.79	0.066

*Variables not included in CDA because they were not preserved in some specimens; for other abbreviations, see Materials and methods.

Molecular phylogenetic analyses

In order to determine the phylogenetic position of collected snail samples we compared their 16S rRNA and COI sequences with available homologues annotated to Hygromiidae. While our set included more taxa than that used recently by Neiber et al. (2017), these authors also analysed the more conserved nuclear sequences ITS2 as well as 5.8S and 28S rDNA. In our data set the trees based on the16S rRNA gene (Figs 8 and S1, see supplemental material online) and the concatenated alignments (Figs 9 and S2, see supplemental material online) were much better resolved than those for COI gene. Many tribes, recently identified by Neiber et al. (2017), can also be recognized in the inferred phylogenetic trees by at least two methods. Differences occurred in the deeper branches, which are, however, poorly supported in phylogenies obtained both by us and Neiber et al. (2017). Thus, additional molecular markers should be included to fully resolve the relationships.

Our results indicate that the collected samples, morphologically assigned to *Xerocampylaea*, are not associated either with the genus *Trochulus* nor the tribe Trochulini. Instead they were placed with a high support within the tribe Urticicolini (Figs 8 and 9). This group

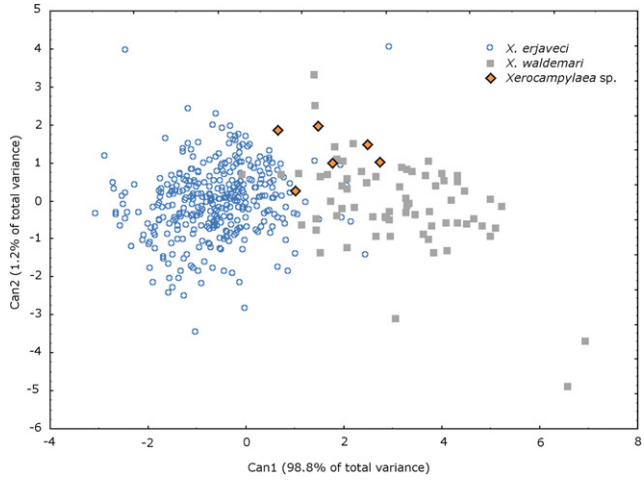


Fig. 2. CDA based on shell measurements of *X. erjaveci*, *X. waldemari*, and *Xerocampylaea* sp.

Fig. 3. Shells of *X. waldemari* from Bosnia and Herzegovina. 3.1, specimen from Jajce (YU.265). 3.2, specimen from Plivsko Jezero (YU.410). 3.3, *Xerocampylaea* sp. specimen from Mt. Vlasič (YU.494). 3.4, specimen from Vrbas gorge (Mollusca NHM 112001 AL/3377).

occurred sister to various tribes depending on the molecular markers used. Samples assigned to *X. waldemari* and *Xerocampylaea* sp. and collected from two different areas in Bosnia and Herzegovina: Banja Luka (Trwal1, 3, 6) and Foča (Trwal11, 12), created two significantly supported clades (Figs S3 and S4, see supplemental material online). Sequences from samples found in a given locality were very similar. These clades were subsequently diverged in most trees based on 16S rRNA gene and the concatenated alignments, but did not create one monophyletic group. Only in the 16S rRNA PhyloBayes tree were they clustered together (Figs S3, see supplemental material online). Samples assigned to *X. erjaveci* (Trerj) were separated with high support into two clades according to their geographic location: (1) Hungary (Trerj2-5, 10-12; Trsp19, 22-24) and Croatia (Trerj25) as well as (2) Bosnia and Herzegovina (Trerj20-22). The first clade was usually grouped with *X. zelebori* or *Semifruticicola serbica*, while the second clade was usually sister to the first one and its relatives. The samples collected in the same locality showed very small genetic difference (Figs S3 and S4, see supplemental material online).

The majority of tests verifying the monophyly of sequences assigned to *X. waldemari* (Trwal1, 3, 6) and *Xerocampylaea* sp. (Trwal11, 12) did not reject this hypothesis on the trees based on 16S rRNA and 16S rRNA + COI alignments (topology t1 in Figs S3 and S4,

Fig. 4. Shells of *X. erjaveci*. 4.1, specimen from Zagreb, Croatia (ZMA. MOLL. 403307). 4.2, specimen from Plitvica, Croatia (RMNH. MOL. 293999). 4.3, specimen from Mt. Igman, Bosnia and Herzegovina (YU.415). 4.4, specimen from Rugovska klisura, Kosovo (ZMA. MOLL. 403305).

Table 3. Canonical coefficients of discriminant analysis performed on shell measurements. The largest values for the functions are marked in **bold**.

	Standardized canonical discriminant coefficients for	
Variable	**function 1**	**function 2**
U/D	−1.736	2.826
W	0.863	0.687
U	2.951	**−2.957**
w	0.311	−0.854
h	1.477	0.938
D	−0.146	2.397
u/U	0.505	0.222
u	−1.083	−0.867
bwH/H	0.972	−0.622
whl	0.009	−0.893
H/W	2.032	0.272
bwH	**−3.444**	−0.443
h/w	−0.822	−0.487
Eigenvalue	1.889	0.023
Cum. Prop. (%)	98.78	100.00

For abbreviations, see Materials and methods.

Table S3 and S4, see supplemental material online). At least two tests showed that the alternative groupings of the monophyletic clade *X. waldemari* + *Xerocampylaea* sp. with the lineages of *Semifruticicola serbica* or other *Xerocampylaea* (topologies t2–t9 in Figs S3 and S4, Table S3 and S4, see supplemental material online) were statistically significantly worse. It means that the samples Trwal1, 3, 6, 11 and 12 have a separate evolutionary history in comparison to the other lineages.

Systematics

Redescription of *Xerocampylaea waldemari* (Wagner, 1912) (Figs 3, 5, 10–12)
Family Hygromiidae Tryon, 1866
Subfamily Trochulinae Lindholm, 1927
Tribe Urticicolini Neiber et al., 2017
Genus *Xerocampylaea* Kobelt, 1871
Fruticicola waldemari A. J. Wagner, 1912: 250

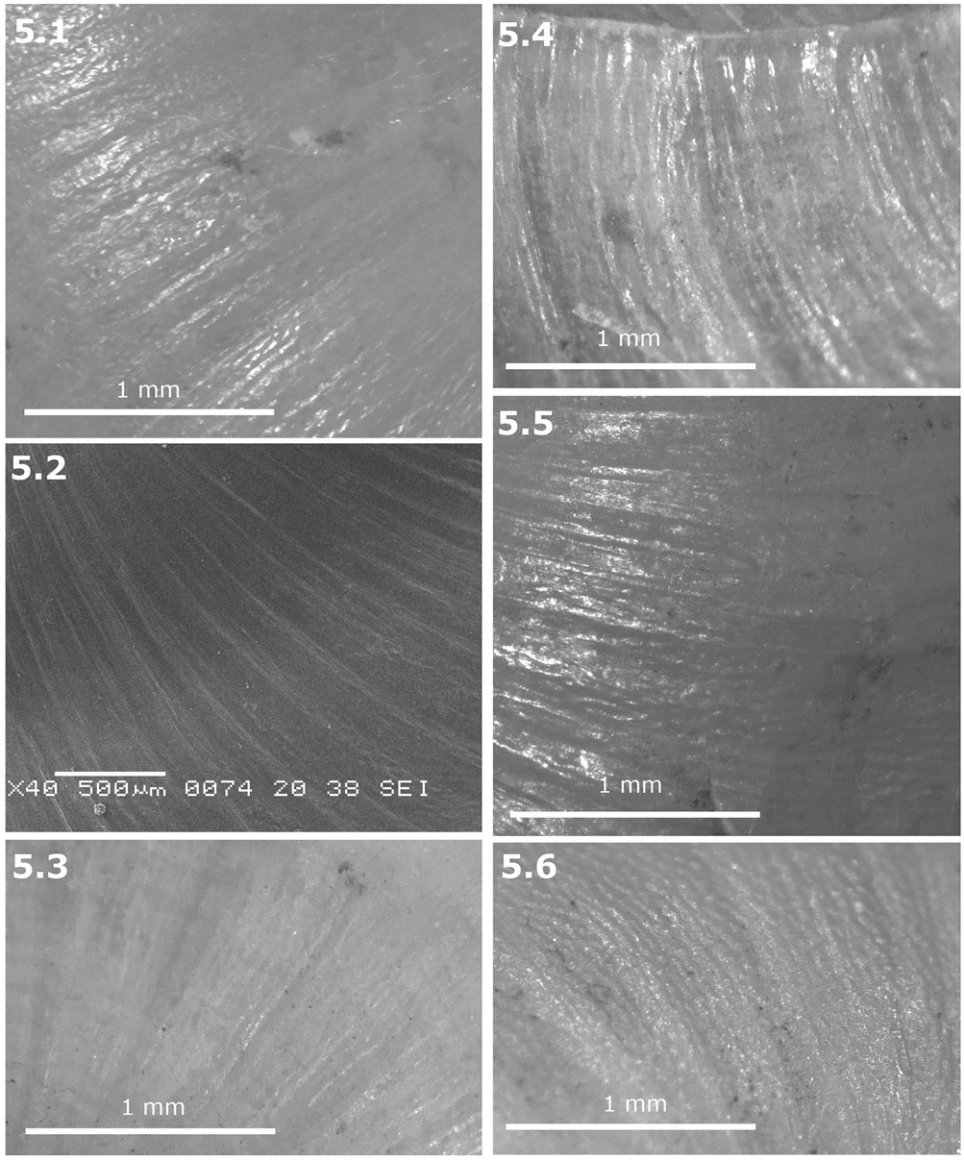


Fig. 5. Shell sculpture of *X. waldemari* from Bosnia and Herzegovina. 5.1, lower side of specimen from Jajce (YU.265). 5.2, SEM of specimen from Jajce (YU.265). 5.3, lower side of specimen from Vrbas gorge (YU.404). 5.4, upper side of specimen from Vrbas gorge (YU.571). 5.5, lower side of specimen from Plivsko Jezero (YU.410). 5.6, lower side of specimen of *Xerocampylaea* sp. from Mt. Vlasič (YU.494).

Type locality. 'Umgebung von Sarajevo, Jajce und Bočac bei Banjaluka in Bosnien'

Type material. The type material of this species cannot be located and it is assumed to have been damaged or lost because we found no original material in the collections of the two institutions that keep Wagner's material, i.e., the Naturhistorisches Museum Wien (Vienna) and Museum and Institute of Zoology, Polish Academy of Science in Warsaw, as well as in other European museums such as: Museum für Naturkunde der Humboldt Universität in Berlin (Germany), Senckenberg Naturmuseum in Frankfurt am Main (Germany), Muséum national d'Histoire naturelle in Paris (France), the Natural History Museum in London (Great Britain), and the Naturalis Biodiversity Center, formerly Rijksmuseum van Natuurlijke Historie, Leiden and Zoologisch Museum Amsterdam (The Netherlands).

Revised diagnosis. Medium-sized species possessing a conical shell with broad last whorl, almost twice as wide as penultimate whorl. Umbilicus narrow, taking up approximately 0.09 of the shell width, often partly covered by columellar aperture margin. Aperture large

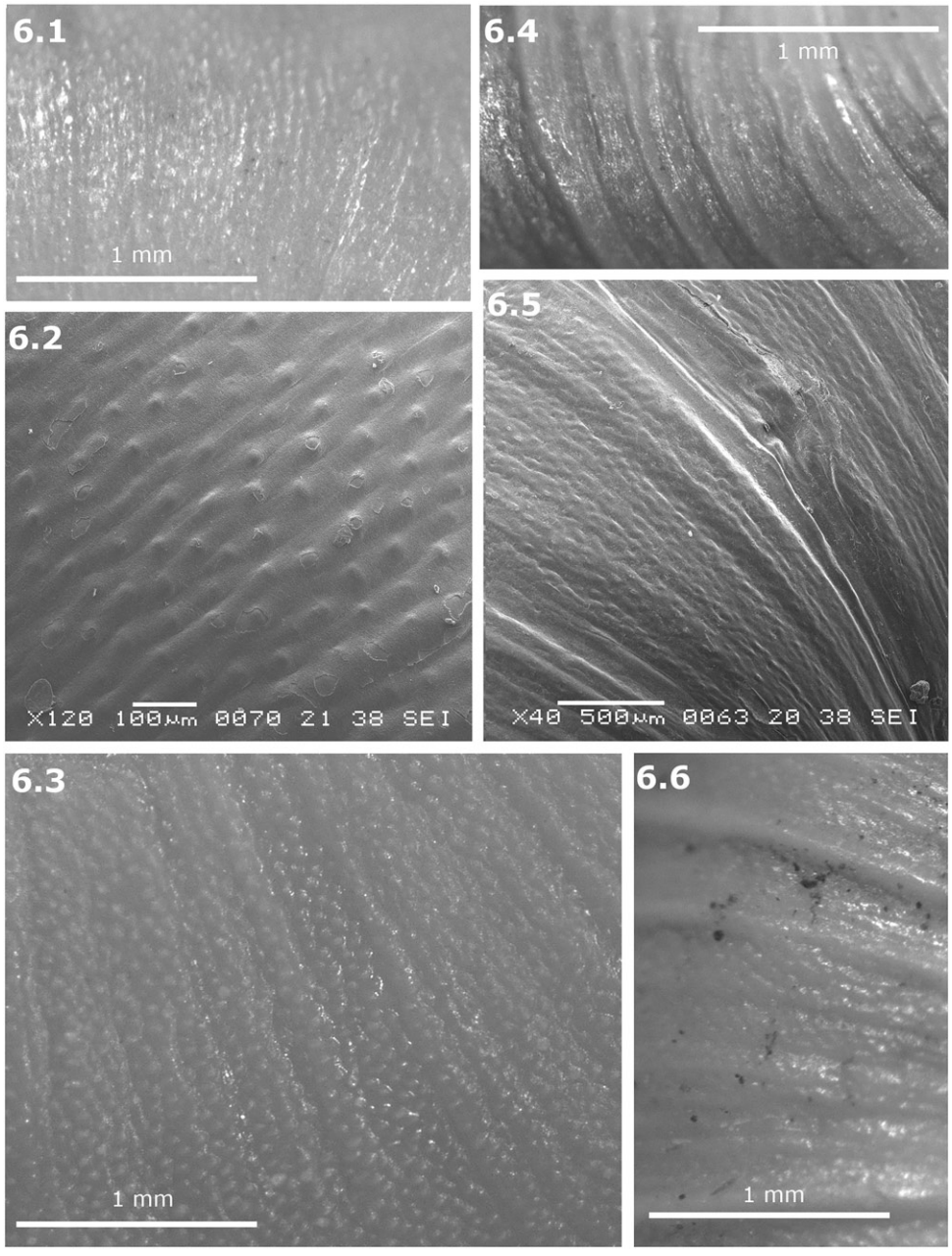


Fig. 6. Shell sculpture of *X. erjaveci*. 6.1, lower side of specimen from Zagreb, Croatia (ZMA. MOLL. 403307). 6.2, SEM of specimen from Zagreb, Croatia (ZMA. MOLL. 403307). 6.3, lower side of specimen from Mount Igman, Bosnia and Herzegovina (YU.415). 6.4, upper side of specimen from Rugovska klisura, Kosovo (ZMA. MOLL. 403305). 6.5, SEM of specimen from Durmitor Mts (YU.282). 6.6, lower side of specimen from Koprivnik Mts, Montenegro (ZMA. MOLL. 403306).

relative to the shell size, with a thin lip. Shell thin, most often pale or translucent. Shell surface with uneven, delicate growth lines, without visible scars of hairs. Four pairs of short mucous glands situated around upper vagina. Vagina long and cylindrical. Flagellum approximately as long as epiphallus or shorter. Each usually shorter than penis. Bursa copulatrix duct thick and straight. Bursa copulatrix not reaching albumen gland. In cross-section of penial papilla three intrapapillar cavities visible around seminal duct. Lower vagina with 7–8 longitudinal folds.

Shell description. Shell within the genus is medium-sized, conical, with acute apex. Whorls are regularly increasing in width. The shell surface is covered with uneven delicate growth lines. In some individuals, even

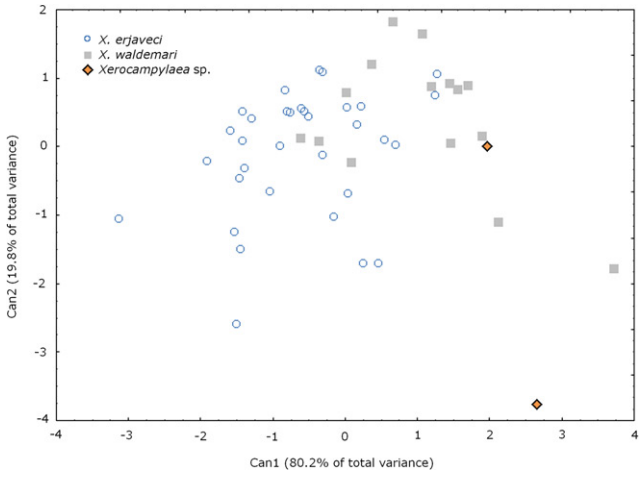


Fig. 7. CDA based on genital measurements in *X. erjaveci*, *X. waldemari*, and *Xerocampylaea* sp.

from the same locality, the surface is covered with granulose sculpture consisting of fine droplets (Fig. 5). No scars of hairs are visible. The shell is thin, most often pale or translucent. The last whorl is almost twice as wide as the penultimate whorl. It is only slightly descending towards the aperture, which is regularly semicircular, slightly wider than high, rather large relative to the shell size. The outer aperture margin is thin, light and slightly reflected. Thin, narrow, white palatal callus is developed in some individuals and populations. It is visible from the upper side of the shell (Fig. 3). Slightly broadened columella ends with a thin lip. The umbilicus is narrow, often partly covered by columellar aperture margin but always open. The shell measurements are shown in Table 2.

Genital description. Distal genitalia are presented in Fig. 10 and genital measurements are shown in Table 2. The general arrangement of the reproductive system is semidiaulic monotrematic. The penial complex consists of flagellum, epiphallus, which extends from insertion of vas deferens to penial retractor muscle, and penis ending at the atrium. The flagellum is gradually narrowing towards the tip and is of equal length as the epiphallus. Both the flagellum and the epiphallus are usually shorter than the penis, which is conical with a blunted vertical ending. Its surface is smooth for the first two-thirds of its length but with a translucent pattern caused by penial lacunas. In the third part, towards the epiphallus, holes in the penis wall appear. Cross-section of the penial papilla reveals three lacunas. Two of them are grouped laterally, the third one proximally to the crescent seminal duct (Fig. 11). All lacunas appear voluminous in the first one-third of the penial papilla but become compressed towards the epiphallus. The epiphallus shows two big and eight smaller folds in the cross-section (Fig. 11). The inner side of the penial sheath is folded from the end of the penis tip until the insert into the atrium, which is intensely folded by 20–30 longitudinal folds (Fig. 12). Four pairs of short mucous glands are situated around the upper vagina, in which 7–8 longitudinal folds are visible, and 14–16 folds appear in its cross section (Fig. 12). Bursa copulatrix duct is thick and straight. Bursa copulatrix is not reaching albumen gland.

Distribution and habitat. *Xerocampylaea waldemari* is endemic to Bosnia and Herzegovina, currently known from the Vrbas river gorge, Jajce, Krupa na Vrbasu, Mount Vlasič near Travnik (Maassen, 1985) and probably Foča area. Its distribution range needs further studies. All records of *X. waldemari* come from rocky, limestone habitats in mountainous areas.

Remarks. *Xerocampylaea waldemari* can be distinguished by a combination of characters, such as: medium size, conical shell, high spire, half-rounded aperture and narrow umbilicus. They may vary so not always sufficient to distinguish this species from its congener *X. erjaveci* and similar *Trochulus*, *Petasina*, and *Edentiella* species. These species also have similar reproductive tract structures (Proćków, 2009). Balkan species *Monachoides taraensis* and *M. kosovoensis sensu* de Winter and Maassen (1992) overlap in shell sizes with *X. waldemari*, which has smaller umbilicus diameter and completely different genitalia structure. Among other species whose geographic distribution does not overlap with that of *X. waldemari*, a Carpathian species *Plicuteria lubomirskii* (Ślósarski, 1881) is similar in shell shape but it is usually smaller, 7–10 mm in width and 5–7 mm in height (Proćków, 2009). Both species also differ in the shell surface sculpture (cf. Fig. 5 and Proćków, 2009: Fig. 9), outer appearance of genitals and genital structure (cf. Figs 10–12 and Proćków, 2009: Figs 135–139). The snails from Mount Vlasič in Bosnia and Herzegovina were assigned to *X. waldemari* by Maassen (1985). We found that their shell shape matches *X. waldemari* (Fig. 2). However, they have more conspicuous granulation on the shell surface as in *X. erjaveci* (Fig. 6), unlike other individuals of *X. waldemari* (Fig. 5). A similar situation refers to two immature specimens from Foča area (Trwal11, 12) in Bosnia and Herzegovina, whose shells showed more or less typical granulation of *X. erjaveci*, while their genitals were so poorly developed that no reliable conclusions about their assignment to species could be drawn. The only recognizable trait was the arrangement of four dart sacs, which would allow to

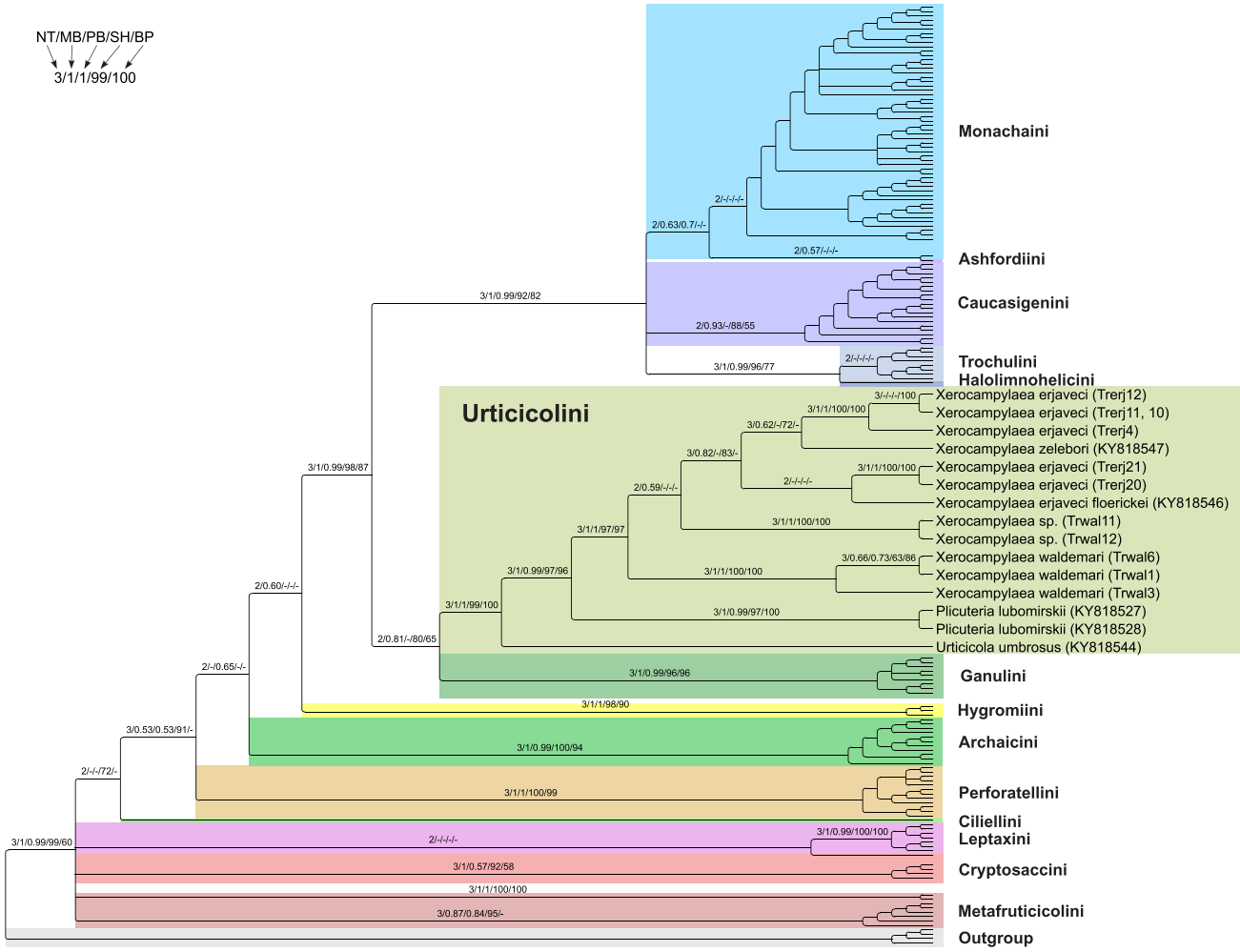


Fig. 8. The consensus of trees obtained in three approaches for the alignment of 16S rRNA. The tree is focused on relationships between analysed samples and their close relatives from tribe Urticicolini. Numbers at nodes, in the order shown, correspond to: the number of the trees that contained a given node (NT), posterior probabilities estimated in MrBayes (MB) and PhyloBayes (PB) as well as support values obtained by the approximate likelihood ratio test based on a Shimodaira–Hasegawa-like procedure (SH) and bootstrap method (BP) calculated in IQ-Tree. Values of the posterior probabilities and bootstrap percentages lower than 0.50 and 50%, respectively, were indicated by a dash '-'.

place them into the genus *Xerocampylaea*. In some phylogenetic trees these two snails grouped with other *X. waldemari* specimens (e.g., Figs S3 and S4, see supplemental material online). The most important diagnostic features differentiating *X. waldemari* and *X. erjaveci* are presented in Table S5 (see supplemental material online).

Redescription of the genitals of *Xerocampylaea erjaveci* s. str. based on the snails from Croatia and Hungary

The general arrangement of the reproductive system is semidiaulic monotrematic. The penial complex consists of flagellum, epiphallus extending from insertion of vas deferens to penial retractor muscle, and penis ending at the atrium. The flagellum is considerably shorter than the epiphallus and the penis, which is gradually narrowing towards the epiphallus. The epiphallus and the penis are approximately of equal length. The 'outer' penis has remarkable knobs at its ends, i.e., before inserting to the atrium and the epiphallus (Fig. 13). In the anterior view the penis is flattened. Viewed in the proximal direction it is club-shaped, with a rounded top, and becoming slightly slender towards the insertion of the epiphallus. Only one-quarter of the penis wall surface is smooth, the rest shows irregularly arranged longitudinal holes, connected with the penial lacunas in the inner side of the penis. The tip of the penis is flattened laterally at

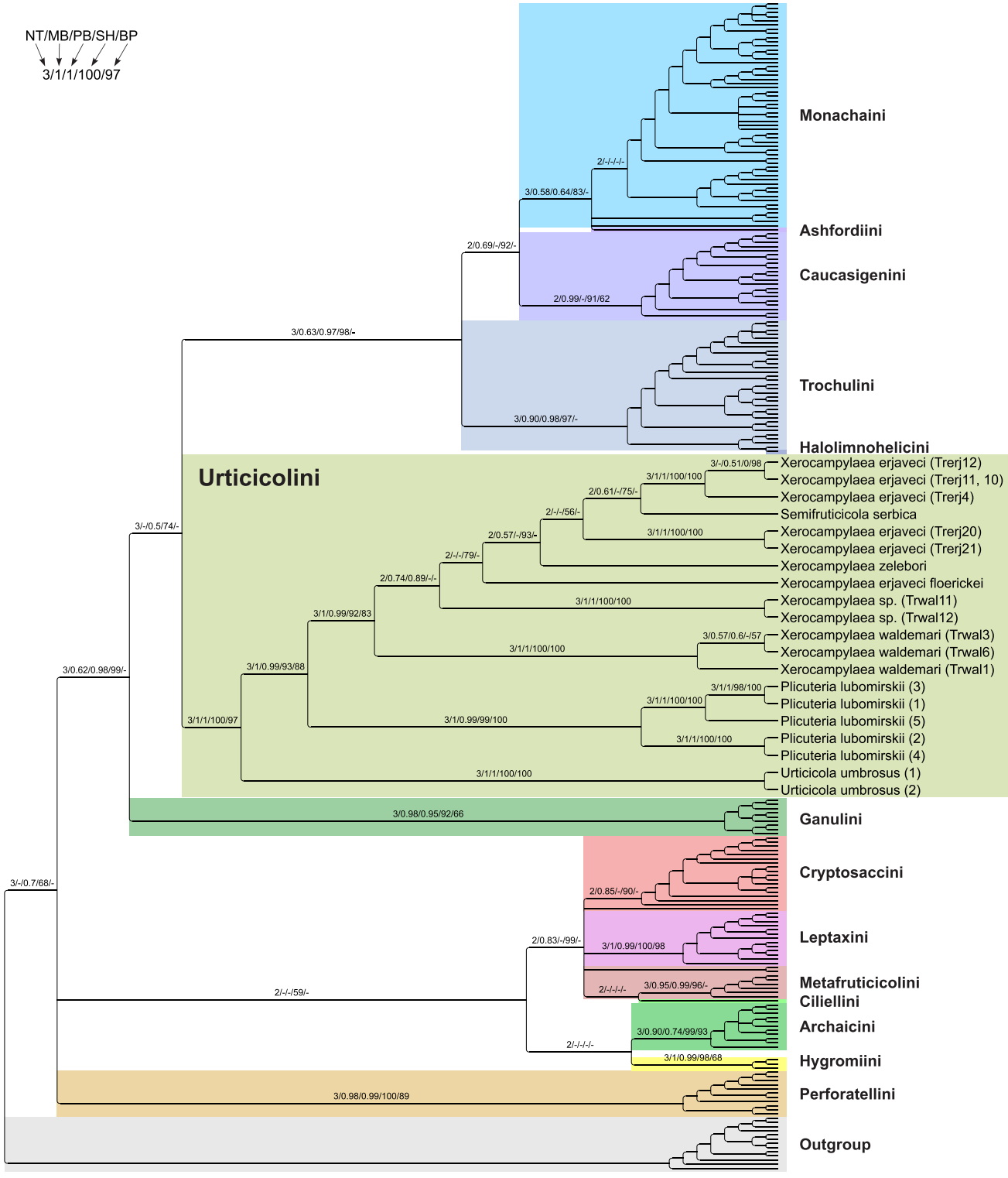
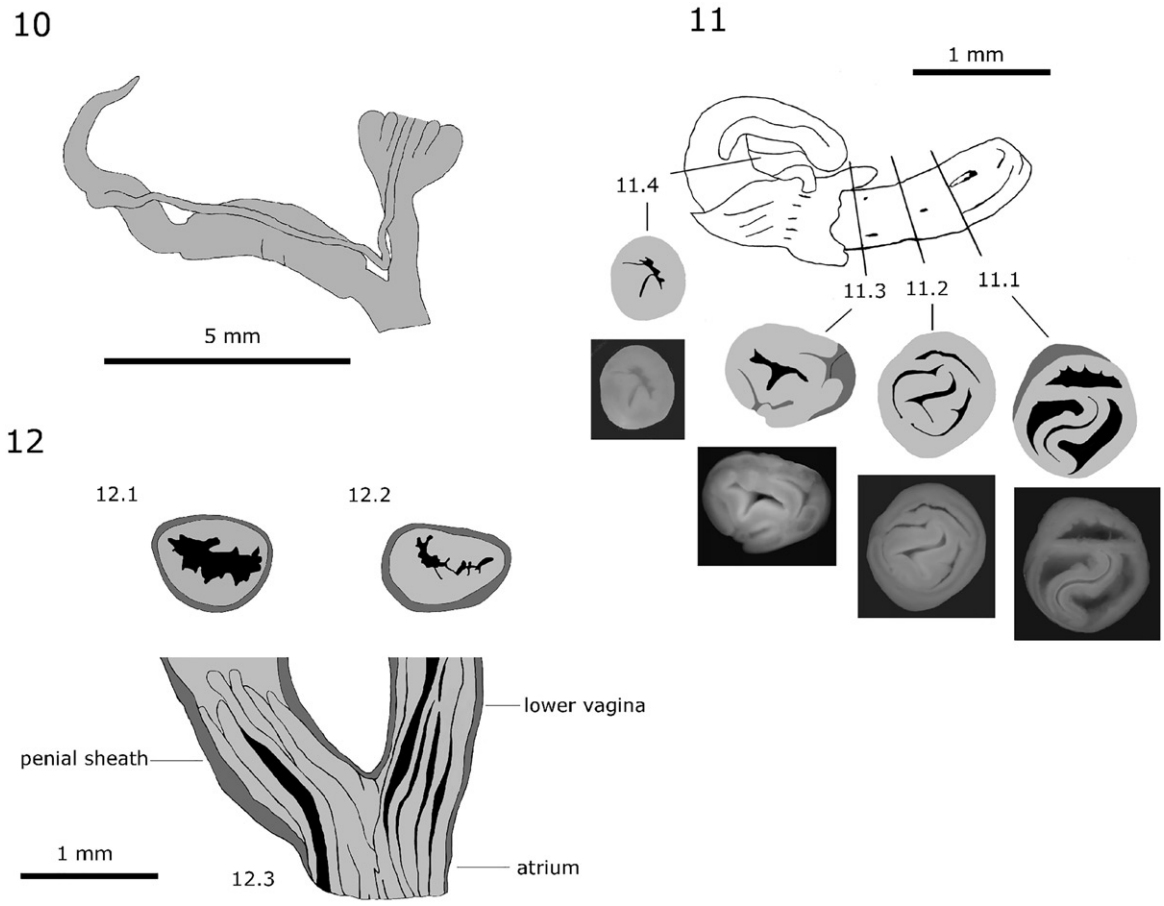


Fig. 9. The consensus of trees obtained in three approaches for the concatenated alignment of 16S rRNA + COI. The tree is focused on relationships between analysed samples and their close relatives from tribe Urticicolini. Other explanations as in Fig. 8.



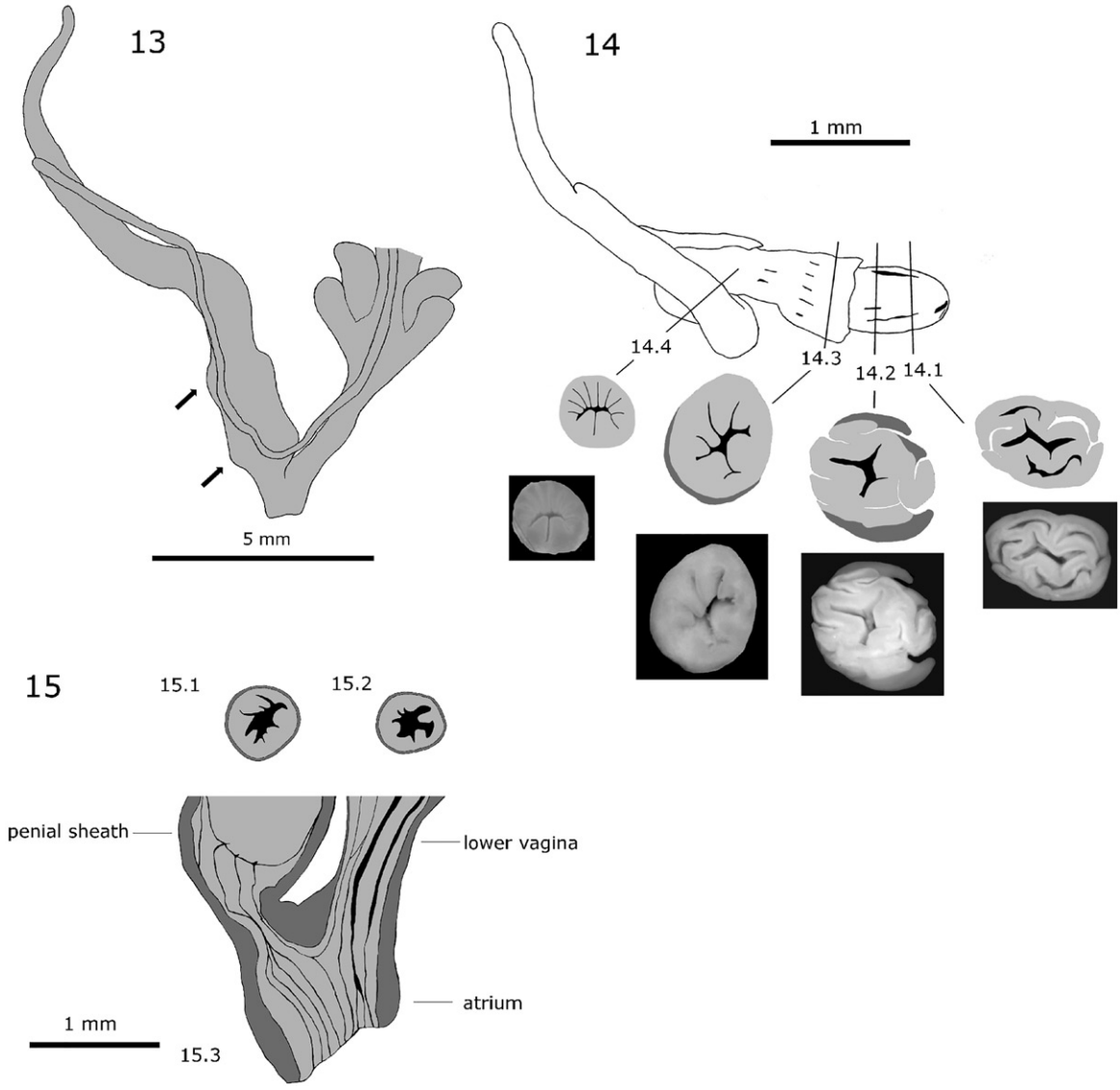
Figs 10–12. *X. waldemari*. 10. Distal genitalia. 11. Cross sections of penial papilla (11.1–11.3) and epiphallus (11.4). 12. Cross sections of penial sheath (12.1) and lower vagina (12.2); longitudinal-section of penial sheath, lower vagina, and atrium (12.3).

different levels, varying in each specimen. The cross-section of the penial papilla reveals H-shaped seminal duct. Four to five lacunas are predominately grouped around the embayments of the seminal duct. These lacunas are sometimes opened to the outer surface causing the above-mentioned longitudinal holes irregularly arranged in the papilla penis. The perforated penis wall is connected with bridges to the edges of the embankments of the seminal duct. In the last one-third towards the epiphallus, one single cavity surrounded by two bigger and five to seven smaller folds are visible. There is only one opening at the tip of the penial papilla representing the outlet of the seminal duct. The cross sections of penial papilla and epiphallus are shown in Fig. 14. Similar to *X. waldemari*, the inner side of the penial sheath is folded from the end of the penis tip until the insert into the atrium, which has 20–30 longitudinal folds (Fig. 15). Four pairs of short mucous glands are situated around the upper vagina. The vagina is cylindrical. Bursa copulatrix duct is thick and short so that bursa copulatrix does not reach the albumen gland.

Remarks. Genital traits so far apply only to the specimens of the nominate subspecies of *X. erjaveci* in Croatia and Hungary. The exact assignment of the three Bosnian individuals from Maglić (Trerj20–22) must be seen as insecure at the moment. The inner genital structure of these specimens exactly resembles those of *X. erjaveci* s. str. The only difference is that two of the four dissected specimens show an aciculate penis tip as opposed to the rounded, blunted tips of *X. erjaveci* s. str. from Hungary and Croatia.

Discussion

In the recent molecular phylogeny of Hygromiidae, Neiber et al. (2017) changed the assignment of *erjaveci* from the genus *Trochulus* and tribe Trochulini to the genus *Xerocampylaea* and a newly established tribe Urticicolini, to which also *X. zelebori*, *P. lubomirskii*, *S. serbica*, and *U. umbrosus* were classified. Our molecular phylogenies confirmed this change and also showed that



Figs 13–15. *X. erjaveci*. 13. Distal genitalia. 14. Cross sections of penial papilla (14.1–14.3) and epiphallus (14.4). 15. Cross sections of penial sheath (15.1) and lower vagina (15.2); longitudinal section of penial sheath, lower vagina, and atrium (15.3).

waldemari samples should be classified to tribe Urticicolini because they significantly grouped with the representatives of *Xerocampylaea* as well as *Semifruticicola* (Fig. 9). Since the name *Xerocampylaea* Kobelt, 1871 is older than *Semifruticicola* A. J. Wagner, 1914 and the taxonomic identity of *Semifruticicola serbica* is uncertain as it is sometimes regarded as *S. costulata serbica* subspecies (Fehér, 2011a), we prefer to assign *waldemari* to the genus *Xerocampylaea*.

Our anatomical data indicate that *X. waldemari* is a valid species distinct from *X. erjaveci*. Conversely, the shells of *X. waldemari* are not unequivocally distinguishable from *X. erjaveci* (Fig. 2). Particularly intriguing are individuals with the granulated shell surface from Mt. Vlasič (*Xerocampylaea* sp.). This feature seems to be rather characteristic of *X. erjaveci* and was not observed in many other *X. waldemari* individuals examined. We noticed clear differences in cross sections of the penial papilla between *X. waldemari* and *X. erjaveci* (Figs 11 and 14), while measurements of the reproductive organs between these species showed only weak differences (Table 2, Fig. 7). Our results are similar to the earlier findings of the *Trochulus* species, in which the genital measurements appeared to be less discriminatory (Proćków, Kuźnik-Kowalska, & Mackiewicz, 2017; Proćków et al., 2013, 2014) than anatomical traits such as the basic patterns of plicae in the penis (Duda et al., 2014).

Although only one phylogenetic tree produced the monophyly of *X. waldemari* from Banja Luka (Trwal1,

3, 6) and *Xerocampylaea* sp. from Foča (Trwal11, 12), tree topology tests showed that the monophyly is not significantly worse than other found topologies. This could suggest that the samples from Foča may also represent *X. waldemari*. In phylogenetic trees, both *X. waldemari* and *X. erjaveci* are represented by at least two quite highly diverged lineages. It means that these taxa are characterized by a quite large genetic divergence corresponding to geographic locality. On the other hand, the samples from the same locality show very low variation. The deep relationships within *Xerocampylaea* are generally not well resolved based on the mtDNA markers. Among *X. erjaveci* sequences *X. zelebori* and *S. serbica* were placed. Their taxonomic status should be verified in the comparison with more extensive sampling of this genus. Moreover, additional mitochondrial and nuclear markers are necessary to corroborate the species status of *X. waldemari* and the identity of the specimens from Foča. The placement of *X. zelebori* and *S. serbica* within *X. erjaveci* sequences breaks monophyly of the *X. erjaveci*. This topology is not consistent with clear distinction between *X. zelebori* and *X. erjaveci* in shell morphology as well as between *S. serbica* and *X. erjaveci* in genitalia morphology. This can suggest a gene flow between these taxa, incorrect species identification or high morphological variation of these snails. The remarkable morphological diversity among these taxa can likely result from phenotypic plasticity caused by local climate and/or environmental conditions, similarly to other widely distributed hygromiids, e.g., *T. hispidus* and *T. striolatus* (Proćków, Kuźnik-Kowalska, & Mackiewicz, 2017a, 2017b). Altitude was also considered as an environmental factor notably influencing substantial variation in shell morphology of Balkan hygromiids (Wagner, 1914) and was recorded in other snail families such Helicidae and Clausiliidae in the Alps and the Carpathians (Burla & Stahel, 1983; Sulikowska-Drozd, 2001, 2011).

The genetic and morphological diversity of *Xerocampylaea* is most likely much greater than presented here. This may be associated with differentiated Balkan geology consisting of limestone isolated patches, separated by regions of non-limestone, e.g., Quaternary fluvial deposits, flysch, volcanic and metamorphic bedrocks (Fehér & Szekeres, 2016). Additionally, large parts of the Balkans stayed ice-free during the last glaciations and played a role of Pleistocene refugia (Kruckeberg, 2015). These facts triggered the radiation not only in plants (Kruckeberg, 2015) but also in other immobile organisms such as land snails and slugs. The isolated island-like landscape structures in combination with long-term persistence can favour the radiation of multiple, morphologically diverse, and geographically separated forms (Gittenberger, 1991).

The Balkan region is a cradle of very rich biodiversity in terms of both flora and fauna. There are a great number of endemic species, and many are of global or European conservation importance (Kryštufek & Reed, 2004). Nevertheless, this rich part of the European fauna is poorly studied and taxonomic research is recommended by the IUCN for incorrectly described nominal or unknown cryptic taxa because this lack of knowledge often restricts effective activity in species conservation (Cuttleod et al., 2011). Our study contributes to broaden knowledge on land gastropods. The presented integrative approach including morphological, anatomical, and phylogenetic data better quantifies the evolutionary information within groups of taxa in biodiversity-rich areas than simple faunistical study (Marchese, 2015). Accordingly, increased knowledge on species evolution, ecology, and geography provides an opportunity to improve our understanding of biodiversity and change our management of the environment (Marchese, 2015).

The re-definition of *X. waldemari*, presented herein, should constitute a benchmark for further taxonomic investigations of the different populations of this species as well as very diverse congener *X. erjaveci*.

Acknowledgements

We are grateful to Christine Zorn (ZMB), Vesna Štamol (CNHM), Bram van der Bijl (Naturalis), and Dominika Mierzwa (MIZW) for the loan of snail material. We want to thank Julia Schindelar (NHMW) for technical assistance with the molecular analysis and Anita Eschner (NHMW) for processing the samples in the mollusc collection of the NHMW. We thank Christine Zorn (ZMB), Ronald Janssen (SMF), and Philippe Maestrati (MNHN), who provided information about holding no Wagner's material in the museum collections. We are also thankful to two anonymous reviewers for their helpful suggestions and comments on the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Supplemental data

Supplemental data for this article can be accessed here: https://dx.doi.org/10.1080/14772000.2019.1617365.

Funding

The scientific visits of MP (NL-TAF-4425) received support from the SYNTHESYS Project (http://www.synthesys.info). This work was partly supported by the National Science Centre, Poland (Narodowe Centrum Nauki, Polska) under Grant number 2016/21/B/NZ8/03022. Some computations were carried out at the Wrocław Center for Networking and Supercomputing under the grant no. 307 (PM).

ORCID

Małgorzata Proćków http://orcid.org/0000-0003-2240-7306

References

Bank, R. (2011). Fauna Europaea version 2.4. Retrieved from http://www.fauna-eu.org (accessed 4 October 2017).

Bole, J. (1984). Mehkužci (Mollusca: Gastropoda et Bivalvia). In G. Nonveiller (Ed.), *Fauna Durmitora 1: Posebna izdanja 18: Odjeljenje prirodnih nauka 11* (pp. 363–394). Titograd: Crnogorska Akademija Nauka i Umetnosti.

Burla, H., & Stahel, W. (1983). Altitudinal variation in *Arianta arbustorum* (Mollusca, Pulmonata) in the Swiss Alps. *Genetica*, *62*, 95–108.

Chernomor, O., von Haeseler, A., & Minh, B. Q. (2016). Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, *65*, 997–1008. doi:10.1093/sysbio/syw037

Chiba, S. (1999). Accelerated evolution of land snails *Mandarina* in the Oceanic Bonin Islands: Evidence from mitochondrial DNA sequences. *Evolution*, *53*, 460–471. doi:10.1111/j.1558-5646.1999.tb03781.x

Cuttelod, A., Seddon, M., & Neubert, E. (2011). *European red list of non-marine molluscs*. Luxembourg: Publications Office of the European Union.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772. doi:10.1038/nmeth.2109

de Winter, A. J., & Maassen, W. J. M. (1992). Two new Hygromiidae from Crna Gora and Kosovo (Gastropoda Pulmonata), with a checklist of the hygromiid species described from the Western Balkan. *Basteria*, *56*, 173–192.

Duda, M., Kruckenhauser, L., Sattmann, H., Harl, J., Jaksch, K., & Haring, E. (2014). Differentiation in the *Trochulus hispidus* complex and related taxa (Pulmonata: Hygromiidae): Morphology, ecology and their relation to phylogeography. *Journal of Molluscan Studies*, *80*, 371–387. doi:10.1093/mollus/eyu023

Duda, M., Sattmann, H., Haring, E., Bartel, D., Winkler, H., Harl, J., & Kruckenhauser, L. (2011). Genetic differentiation and shell morphology of *Trochulus oreinos* (Wagner, 1915) and *T. hispidus* (Linnaeus, 1758) (Pulmonata: Hygromiidae) in the northeastern Alps. *Journal of Molluscan Studies*, *77*, 30–40. doi:10.1093/mollus/eyq037

Fehér, Z., & Szekeres, M. (2016). Taxonomic revision of the rock-dwelling door snail genus *Montenegrina* Boettger, 1877 (Mollusca, Gastropoda, Clausiliidae). *ZooKeys*, *599*, 1–137. doi:10.3897/zookeys.599.8168

Fehér, Z. (2011a). Semifruticicola costulata. *The IUCN Red List of Threatened Species*. 2011: e.T157001A5028230. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T157001A5028230.en. (accessed 10 October 2018).

Fehér, Z. (2011b). Trochulus waldemari. *The IUCN Red List of Threatened Species* 2011: e.T156576A4966910. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T156576A4966910.en. (accessed 8 May 2017).

Gittenberger, E. (1991). What about non-adaptive radiation? *Biological Journal of the Linnean Society*, *43*, 263–272. doi:10.1111/j.1095-8312.1991.tb00598.x

Huelsenbeck, J. P., Larget, B., & Alfaro, M. E. (2004). Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. *Molecular Biology and Evolution*, *21*, 1123–1133. doi:10.1093/molbev/msh123

Jaeckel, S. (1954). Zur Systematik und Faunistik der Mollusken der nördlichen Balkaninsel. *Mitteilungen Aus Dem Museum Für Naturkunde in Berlin. Zoologisches Museum Und Institut Für Spezielle Zoologie ⟨Berlin⟩*, *30*, 54–95. doi:10.1002/mmnz.4830300106

Jaeckel, S. G., Klemm, W., & Meise, W. (1957). Die land- und süsswasser Mollusken der nördlichen Balkaninsel. *Abhandlungen Und Berichte Aus Dem Staatlichen Museum Für Tierkunde in Dresden*, *23*, 141–205.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*, 587–589. doi:10.1038/nmeth.4285

Karaman, B. (2006). Former investigations of the fauna of snails (Mollusca, Gastropoda) in Bosnia & Herzegovina. *Natura Montenegrina, Podgorica*, *5*, 55–66.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780. doi:10.1093/molbev/mst010

Kerney, M. P., Cameron, R. A. D., & Jungbluth, J. H. (1983). *Die Landschnecken Nord- und Mitteleuropas*. Hamburg und Berlin: Paul Parey.

Kruckeberg, A. (2015). *Geology and plant life: The effects of landforms and rock types on plants*. Seattle (Washington): University of Washington Press.

Kruckenhauser, L., Duda, M., Bartel, D., Sattmann, H., Harl, J., Kirchner, S., & Haring, E. (2014). Paraphyly and budding speciation in the hairy snail (Pulmonata, Hygromiidae). *Zoologica Scripta*, *43*, 273–288. doi:10.1111/zsc.12046

Kryštufek, B., & Reed, M. (2004). Patterns and process in Balkan biodiversity – An overview. In H. I. Griffiths, B. Kryštufek, & J. M. Reed (Eds.), *Balkan biodiversity: Pattern and process in the European hotspot* (pp. 1–8). Dordrecht: Kluwer Academic Publishers.

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*, 1695–1701. doi:10.1093/molbev/mss020

Lartillot, N., & Philippe, H. (2004). A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. *Molecular Biology and Evolution*, *21*, 1095–1109. doi:10.1093/molbev/msh112

Maassen, W. J. M. (1985). Nieuwe vondsten van *Trichia waldemari* A. J. Wagner, 1912. *De Kreukel*, *21*, 19–22.

Marchese, C. (2015). Biodiversity hotspots: A shortcut for a more complicated concept. *Global Ecology and Conservation*, *3*, 297–309. doi:10.1016/j.gecco.2014.12.008

Neiber, M. T., Razkin, O., & Hausdorf, B. (2017). Molecular phylogeny and biogeography of the land snail family Hygromiidae (Gastropoda: Helicoidea). *Molecular Phylogenetics and Evolution*, *111*, 169–184. doi:10.1016/j.ympev.2017.04.002

Nguyen, L. T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*, 268–274. doi:10.1093/molbev/msu300

Nordsieck, H. (1993). Das system paläarktischen Hygromiidae (Gastropoda: Stylommatophora: Helicoidea). *Archiv Für Molluskenkunde*, *122*, 1–23. doi:10.1127/arch.moll/122/1993/1

Proćków, M. (2009). The genus *Trochulus* Chemnitz, 1786 (Gastropoda: Pulmonata: Hygromiidae) a taxonomic revision. *Folia Malacologica*, *17*, 101–176. doi:10.2478/v10125-009-0013-0

Proćków, M., Kuźnik-Kowalska, E., & Mackiewicz, P. (2017a). Phenotypic plasticity can explain evolution of sympatric polymorphism in the hairy snail *Trochulus hispidus* (Linnaeus, 1758). *Current Zoology*, *63*, 389–402.

Proćków, M., Kuźnik-Kowalska, E., & Mackiewicz, P. (2017b). The influence of climate on shell variation in *Trochulus striolatus* (C. Pfeiffer, 1828) (Gastropoda: Hygromiidae) and its implications for subspecies taxonomy. *Annales Zoologici*, *67*, 199–220. doi:10.3161/00034541ANZ2017.67.2.002

Proćków, M., Mackiewicz, P., & Pieńkowska, J. R. (2013). Genetic and morphological studies of species status for poorly known endemic *Trochulus phorochaetius* (Bourguignat, 1864) (Gastropoda: Pulmonata: Hygromiidae), and its comparison with closely related taxa. *Zoological Journal of the Linnean Society*, *169*, 124–143.

Proćków, M., Strzała, T., Kuźnik-Kowalska, E., & Mackiewicz, P. (2014). Morphological similarity and molecular divergence of *Trochulus striolatus* and *X. montanus*, and their relationship to sympatric congeners (Gastropoda: Pulmonata: Hygromiidae). *Systematics and Biodiversity*, *12*, 366–384. doi:10.1080/14772000.2014.925986

Proćków, M., Strzała, T., Kuźnik-Kowalska, E., Proćków, J., & Mackiewicz, P. (2017). Ongoing speciation and gene flow between taxonomically challenging *Trochulus* species complex (Gastropoda: Hygromiidae). *Public Library of Science One*, *Plos One*, *12*, e0170460. doi:10.1371/journal.pone.0170460

R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.R-project.org/ (accessed 25 November 2018).

Rohlf, F. J. (2010). *TpsDig, program version, version 2.16*. New York: Department of Ecology and Evolution, State University of New York, Stony Brook.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. doi:10.1093/sysbio/sys029

Shimodaira, H., & Hasegawa, M. (2001). CONSEL: For assessing the confidence of phylogenetic tree selection. *Bioinformatics (Oxford, England)*, *17*, 1246–1247.

Sulikowska-Drozd, A. (2001). Shell variability in *Vestia turgida* (Rossmässler, 1836) along an altitudinal gradient. *Folia Malacologica*, *9*, 73–81.

Sulikowska-Drozd, A. (2011). Population dynamics of the Carpathian clausiliid *Vestia gulo* (E.A. Bielz 1859) (Pulmonata: Clausiliidae) under various climatic conditions. *Journal of Conchology*, *40*, 462–470.

Talavera, G., & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, *56*, 564–577. doi:10.1080/10635150701472164

Wagner, A. J. (1912). Beschreibungen neuer Land- und Süßwasserschnecken aus Südösterreich, Kroatien und Bosnien. *Verhandlungen Der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien*, *62*, 246–260.

Wagner, A. J. (1914). Beiträge zur Anatomie und Systematik der Stylommatophoren aus dem Gebiete der Monarchie und der angrenzenden Balkanländer. *Anzeiger Der Kaiserlichen Akademie Der Wissenschaften. Mathematisch-Naturwissenschaftlichen Klasse Wien*, *51*, 333–338.

Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., & Barton, G. J. (2009). Jalview Version 2–A multiple sequence alignment editor and analysis workbench. *Bioinformatics*, *25*, 189–1191.

Westerlund, C. A. (1889). *Fauna der in der paläarctischen Region (Europa, Kaukasien, Sibirien, Turan, Persien, Kurdistan, Armenien, Mesopotamien, Kleinasien, Syrien, Arabien, Egypten, Tripolis, Tunesien, Algerien und Marocco) lebenden Binnenconchylien. II. Gen. Helix*. Berlin: Friedländer.

Wohlberedt, O. (1909). Zur Fauna Montenegros und Nordalbaniens (Mollusken, Käfer, Isopoden, Chilopoden, Diplopoden). *Wissenschaftliche Mitteilungen Aus Bosnien Und Der Herzegowina*, *11*, 585–722.

Associate Editor: Barna Páll-Gergely