SARAJANA RADOMAN, 1975 (CAENOGASTROPODA: TRUNCATELLOIDEA): PREMATURE INVALIDATION OF A GENUS

SEBASTIAN HOFMAN[1], ARTUR OSIKOWSKI[2], ALEKSANDRA RYSIEWSKA[3], JOZEF GREGO[4], PETER GLÖER[5], DEJAN DMITROVIĆ[6], ANDRZEJ FALNIOWSKI[3]

[1]Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30–387 Kraków, Poland
[2]Department of Animal Anatomy, Institute of Veterinary Science, University of Agriculture in Krakow, Mickiewicza 24/28, 30–059 Kraków, Poland
[3]Department of Malacology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30–387 Kraków, Poland (e-mail: andrzej.falniowski@uj.edu.pl)
[4]Horná Mičiná 219, 97401 Banská Bystrica, Slovakia
[5]Biodiversity Research Laboratory, Schulstr. 3, D 25491 Hetlingen, Germany
[6]University of Banja Luka, Faculty of Natural Sciences and Mathematics, Department of Biology and Department of Ecology and Environment Protection, Mladena Stojanovića 2, 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Abstract Sarajana apfelbecki *(Brancsik, 1888) was assigned by Radoman to the monotypic genus, closely related to* Belgrandiella *Wagner, 1927. Later the distinctness of the genus* Sarajana *was questioned, and* S. apfelbecki *classified within the genus* Belgrandiella. *Our study on* Sarajana *from five localities in Bosnia and Herzegovina including Vrelo Bosne (type locality of the* Sarajana apfelbecki*) confirms that the morphology of the characteristic penis, as well as the female reproductive organs, with an exception of the presence of small, vestigial proximal seminal receptacle, overlooked by Radoman contradict a close relationships with* Belgrandiella. *Two molecular markers: mitochondrial cytochrome c oxidase subunit I (COI) and nuclear histone 3 (H3) were used to infer phylogeny:* Sarajana *was placed within the Hydrobiidae, Sadlerianinae, but far from* Belgrandiella, *with* Graecoarganiella *Falniowski et Szarowska, 2011 as a sister taxon.*

Key words *Female reproductive organs, penis, cytochrome c oxidase subunit I (COI), histone H3, molecular phylogeny*

INTRODUCTION

Radoman (1975) described the new genus *Sarajana* for *Frauenfeldia lacheineri* var. *apfelbecki* Brancsik, 1888, its type locality: a spring close to Vrelo Bosne, near Sarajevo, Bosnia and Herzegovina (Fig. 1). Previously these gastropods were classified as the representatives of *Belgrandiella* Wagner, 1927 (Schütt, 1959, Jaeckel, 1967), and Radoman (1975, 1983) still classified the species within the subfamily Belgrandiellinae, close to *Belgrandiella* and *Graziana* Radoman, 1975. In World Register of Marine Species – WoRMS (2018) (MolluscaBase, 2019) genus *Sarajana* is recognised as invalid, and *S. apfelbecki* assigned to the genus *Belgrandiella*, which follows Boeters, Glöer & Slavevska Stamenković (2017). Radoman (1975, 1983) listed four subspecies of *S. apfelbecki*, based on slight differences in shell characters coupled with geographic distribution. However, considering several reports on the biodiversity overestimation in the Hydrobiidae (e.g. Wilke & Falniowski, 2001; Falniowski & Beran, 2015; Osikowski, Hofman, Rysiewska, Sket, Prevorčnik & Falniowski, 2018), with the present state of the arts it seems better to treat the genus *Sarajana* as monotypic. Recently we collected a few specimens of *S. apfelbecki*, which has made possible: (1) the assignment of the collected populations to *Sarajana* based on molecular and morphological characters; (2) to assess its distinctiveness with *Belgrandiella* following the same criteria; (3) to infer their phylogenetic position within the Hydrobiidae.

Contact author : andrzej.falniowski@uj.edu.pl

MATERIAL AND METHODS

40 specimens of *Sarajana* were collected from four springs situated in mountainous middle Dinaric region in the northwestern part of Bosnia and Hercegovina, and from Vrelo Bosne spring, the type locality of the *Sarajana apfelbecki* (Fig. 1):

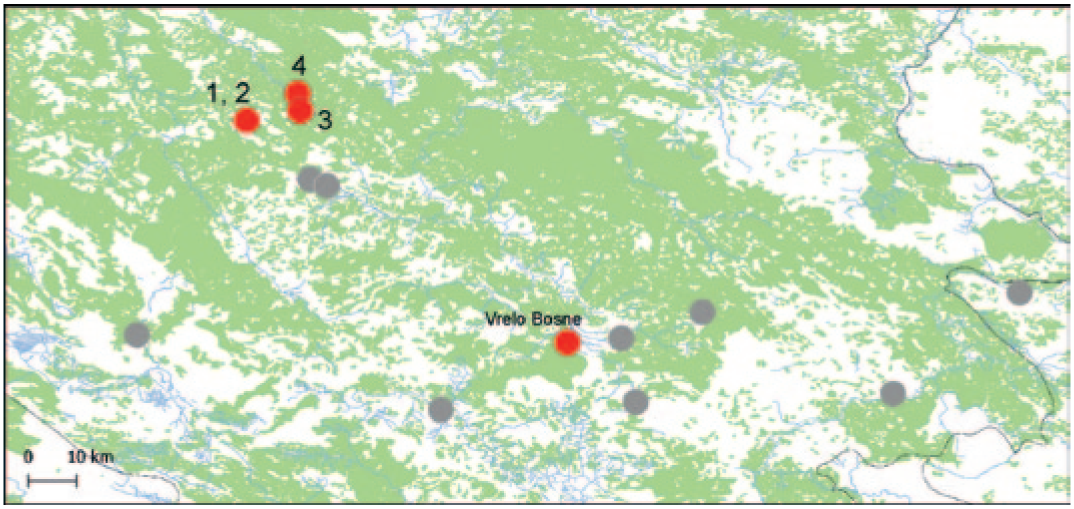

Figure 1 Map showing the localities of *Sarajana apfelbecki* listed by Radoman (1983), grey dots, and our localities, red dots

Figure 2 Photos of studied localities: a – Melina 1, b – Melina 2, c – Prisocka, d – Krusevo Brdo, e – Vrelo Bosne

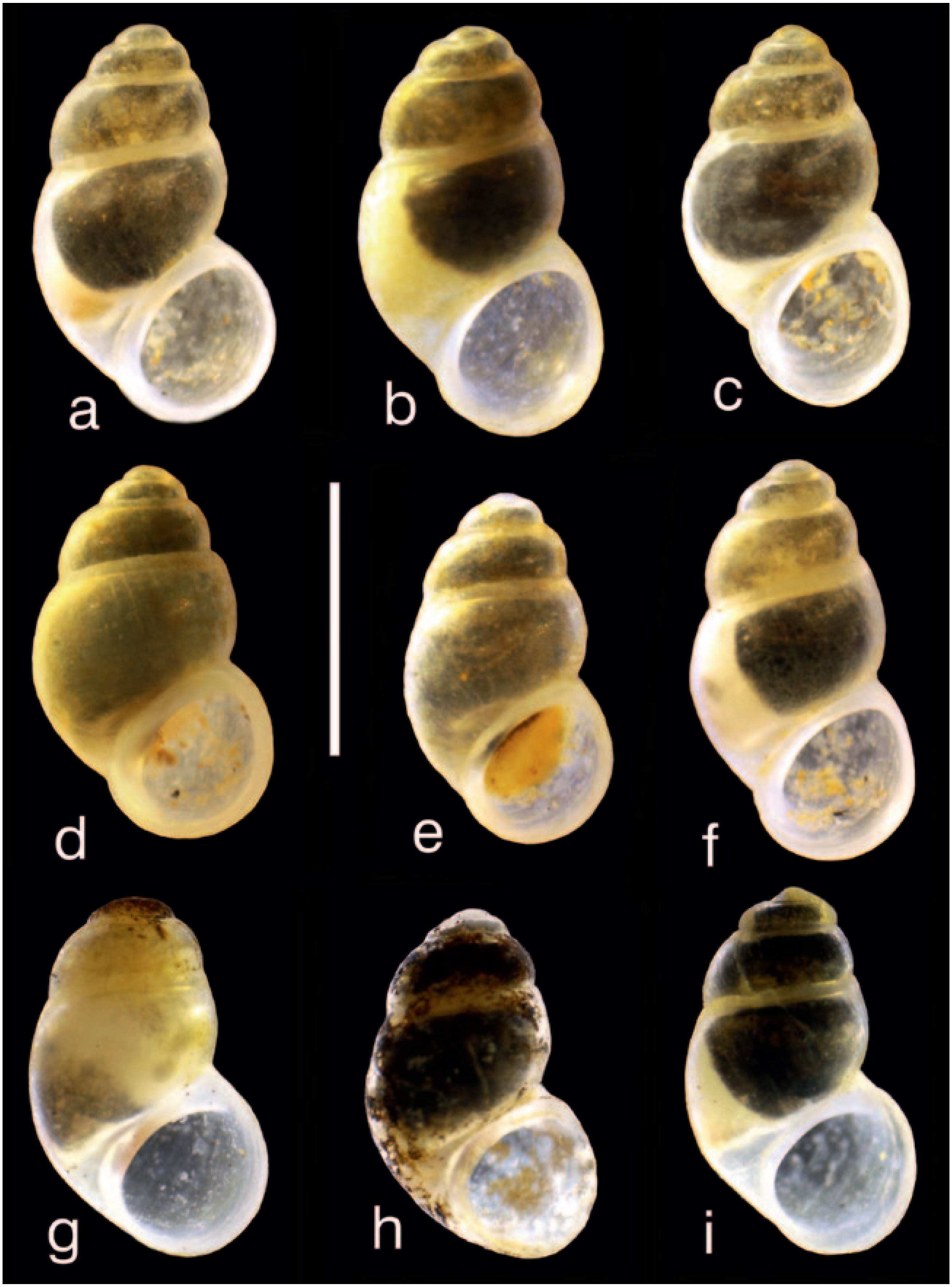

Figure 3 Shells of *Sarajana apfelbecki*: a–f – Vrelo Bosne, type locality; g–i – locality Melina 1; bar equals 500 μm

1 – Melina 1 (Fig. 2a); 44°N 23'3″ 17°E 27'50″; 597m; 18.03.2017; ten specimens; extraction number – 1I7;

2 – Melina 2 (Fig. 2b); 44°N 23'10″ 17°E 27'39″; 591m; 18.03.2017; 15 specimens; extraction number – 1I8;

3 – Prisocka (Fig. 2c); 44°N 27'19″ 17°E 35'13″; 540m; 01.07.2017; one specimen; extraction number – 1I13;

4 – Krusevo Brdo (Fig. 2d); 44°N 24'38″ 17°E 35'28″; 688m; 01.07.2017; eight specimens; extraction number – 1I14;

5 – Vrelo Bosne (Fig. 2e); 43°N 52'21″ 18°E 25'26″; six specimens, extraction numbers – 2B16, 2B17.

Springs 1 and 2 belong to Ugar river basin, while springs 3 and 4 are from upper part of Vrbanja river basin. With the exception of spring 4, which is rheocrene, all the other springs are in varying degrees adjusted for water supply, with pipes.

The shells were photographed with a CANON EOS 50D digital camera, under a NIKON SMZ18 microscope with dark field. Dissections were done under NIKON SMZ18 microscope, and photographed with dark field.

The tissue was hydrated in TE buffer (3×10 min.); then total genomic DNA was extracted with the SHERLOCK extracting kit (A&A Biotechnology), and the final product was dissolved in 20 µl TE

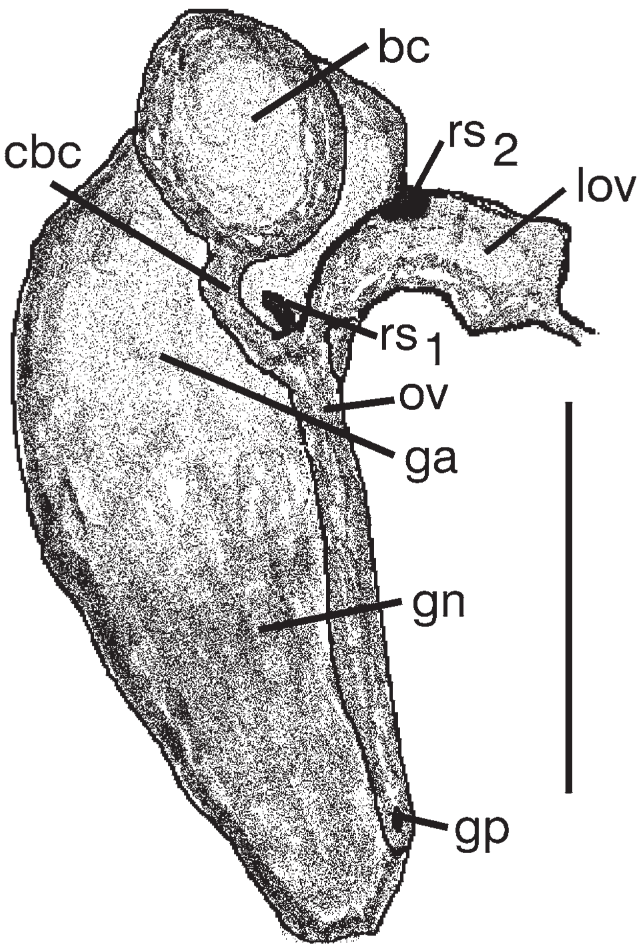

Figure 4 Renal and pallial section of the female reproductive organs of *Sarajana apfelbecki* from the locality Melina 2 (bc – bursa copulatrix, cbc – duct of bursa, ga – albuminoid gland, gn – nidamental gland, gp – gonoporus, ov – oviduct, ovl – loop of (renal) oviduct, rs_1 – distal seminal receptacle, rs_2 – proximal seminal receptacle); bar equals 500 µm

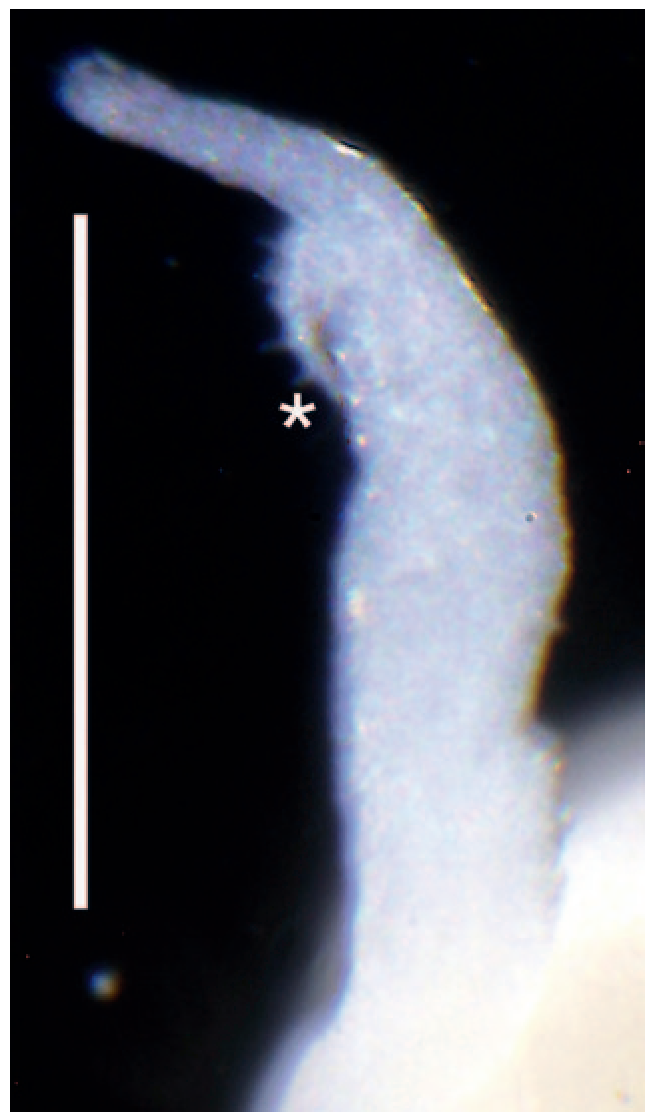

Figure 5 Penis of *Sarajana apfelbecki* from the locality Melina 2, asterisk indicates filamentous outgrowth, bar equals 200 µm

Table 1 Taxa used for phylogenetic analyses with their GenBank accession numbers and references.

Species	COI/H3 GB numbers	References
Agrafia wiktori Szarowska & Falniowski, 2011	JF906762/MG543158	Szarowska & Falniowski, 2011/ Grego *et al.*, 2017
Alzoniella finalina Giusti & Bodon, 1984	AF367650	Wilke *et al.*, 2001
Anagastina zetavalis (Radoman, 1973)	EF070616	Szarowska, 2006
Avenionia brevis berenguieri (Draparnaud, 1805)	AF367638	Wilke *et al.*, 2001
Belgrandiella cf. *kusceri* (Wagner, 1914)	KT218511/MG551366	Falniowski & Beran, 2015/ Osikowski *et al.*, 2018
Belgrandiella cf. *kuesteri* (Boeters, 1970)	MG551411	Osikowski *et al.*, 2018
Bithynia tentaculata (Linnaeus, 1758)	AF367643	Wilke *et al.*, 2001
Bythinella austriaca (von Frauenfeld, 1857)	JQ639858	Falniowski *et al.*, 2012b
Bythinella micherdzinskii Falniowski, 1980	JQ639854	Falniowski *et al.*, 2012b
Bythiospeum acicula (Hartmann, 1821)	KU341350/MK609536	Richling *et al.*, 2016/ Falniowski *et al.*, 2019
Bythiospeum alzense Boeters, 2001	KU341354	Richling *et al.*, 2016
Dalmatinella fluviatilis Radoman, 1973	KC344541	Falniowski & Szarowska, 2013
Daphniola louisi Falnowski & Szarowska, 2000	KM887915	Szarowska *et al.*, 2014a
Emmericia expansilabris Bourguignat, 1880	KC810060	Szarowska & Falniowski, 2013a
Fissuria boui Boeters, 1981	AF367654	Wilke *et al.*, 2001
Graecoarganiella parnassiana Falniowski & Szarowska, 2011	JN202352	Falniowski & Szarowska, 2011a
Graecoarganiella sp. Falniowski & Szarowska, 2011	JN202354	Falniowski & Szarowska, 2011a
Graziana alpestris (Frauenfeld, 1863)	AF367641	Wilke *et al.*, 2001
Grossuana codreanui (Grossu, 1946)	EF061919	Szarowska *et al.*, 2007
Hauffenia tellinii (Pollonera, 1898)	KY087861	Rysiewska *et al.*, 2017
Hauffenia michleri Kuščer, 1932	KY087865/KY087878	Rysiewska *et al.*, 2017
Heleobia dobrogica (Grossu & Negrea, 1989)	EU938131	Falniowski *et al.*, 2008
Heleobia maltzani (Westerlund, 1886)	KM213723/ MK609534-MK609535	Szarowska *et al.*, 2014b/ Falniowski *et al.*, 2019
Horatia klecakiana Bourguignat 1887	KJ159128	Szarowska & Falniowski, 2014a
Hydrobia acuta (Draparnaud, 1805)	AF278808	Wike *et al.*, 2000
Iglica cf. *gracilis* (Clessin, 1882)	MH720989/MH721004	Hofman *et al.*, 2018
Iglica cf. *hauffeni* (Brusina, 1886)	-/MH720995	Hofman *et al.*, 2018
Iglica cf. *forumjuliana* (Pollonera, 1887)	-/MH721006	Hofman *et al.*, 2018
Iglica hellenica Falniowski & Sarbu, 2015	KT825581/MH721007	Falniowski & Sarbu, 2015/ Hofman *et al.*, 2018
Islamia zermanica (Radoman, 1973)	KU662362/MG551320	Beran *et al.*, 2016/Grego *et al.*, 2017
Kerkia jadertina (Kuščer, 1933)	KY087868	Rysiewska *et al.*, 2017
Lithoglyphus prasinus (Küster, 1852)	JX073651	Falniowski & Szarowska, 2012
Littorina littorea (Linnaeus, 1758)	KF644330/KP113574	Layton *et al.*, 2014/ Neretina 2014, unpublished
Marstoniopsis insubrica (Küster, 1853)	AF322408	Falniowski & Wilke, 2001
Moitessieria cf. *puteana* Coutagne, 1883	AF367635/MH721012	Wilke *et al.*, 2001/Hofman *et al.*, 2018
Montenegrospeum bogici (Pešić & Glöer, 2012)	KM875510/MG880218	Falniowski *et al.*, 2014/Grego *et al.*, 2018
Paladilhiopsis bosniaca (Clessin, 1910)	-/MH721020	Hofman *et al.*, 2018
Paladilhiopsis bosnica Bole, 1970	-/MH721021	Hofman *et al.*, 2018
Paladilhiopsis grobbeni Kuscer, 1928	MH720991/-	Hofman *et al.*, 2018
Paladilhiopsis turrita (Kuščer, 1933)	MH720992/MH721015	Hofman *et al.*, 2018
Paladilhiopsis gittenbergeri (A. Reischutz & P. L. Reischutz, 2008)	MH720993/MH721025	Hofman *et al.*, 2018

Table 1 *Continued*

Species	COI/H3 GB numbers	References
Peringia ulvae (Pennant, 1777)	AF118302	Wilke & Davis, 2000
Pontobelgrandiella sp. Radoman, 1978	KU497024/MG551321	Rysiewska *et al.*, 2016/Grego *et al.*, 2017
Pseudamnicola sp. Paulucci, 1878	-/KT710579	Szarowska *et al.*, 2016
Radomaniola curta (Küster, 1853)	KC011814	Falniowski *et al.*, 2012a
Sadleriana fluminensis (Küster, 1853)	KF193067	Szarowska & Falniowski, 2013b
Sadleriana sadleriana (Frauenfeld, 1863)	MG922569	Delicado, 2018
Sadleriana robici (Clessin, 1890)	KF193076	Szarowska & Falniowski, 2013b
Salenthydrobia ferrerii Wilke, 2003	AF449213	Wilke, 2003
Tanousia zrmanjae (Brusina, 1866)	KU041812	Beran *et al.*, 2015
Ecrobia maritima (Milaschewitsch, 1916)	KJ406200/MG551322	Szarowska & Falniowski, 2014b/Grego *et al.*, 2017

buffer. The extracted DNA was stored at -80°C at the Department of Malacology of Institute of Zoology and Biomedical Research of the Jagiellonian University in Kraków (Poland). Mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear histone H3 loci were sequenced. Details of PCR conditions, primers used and sequencing are given in Szarowska *et al.* (2016).

Sequences were initially aligned in the MUSCLE (Edgar, 2004) program in MEGA 6 (Tamura, Peterson, Peterson, Stechner, Nei & Kumar, 2013) and then checked by eye in BioEdit 7.1.3.0 (Hall, 1999). Uncorrected p-distances were calculated in MEGA 6. The estimation of the proportion of invariant sites and the saturation test (Xia, 2000; Xia, Xie, Salemi, Chen & Wang, 2003) were performed using DAMBE (Xia, 2013). In a phylogenetic analysis additional sequences from GenBank were used (Table 1). The taxa were chosen to represent all the European families of the Truncatelloidea, as well as all the main lineages of the Hydrobiidae, especially the subfamily Sadlerianinae. The data were analysed using approaches based on Bayesian inference and maximum likelihood (ML). In the BI analysis, we applied the GTR model of nucleotide substitution because an over-parameterisation was apparently less critical for the BI analyses than an underparameterisation (Huelsenbeck & Rannala, 2004). For the ML analyses, the GTR model was also applied, as implemented in RaxML (Stamatakis, 2014). The Bayesian analyses (BI) were run using MrBayes v. 3.2.3 (Ronquist, Teslenko, van der Mark, Ayres, Darling, Hohna, Larget, Liu, Suchard & Huelsenbeck, 2012) with the default priors. Two simultaneous analyses were performed, each lasting 10,000,000 generations with one cold chain and three heated chains, starting from random trees and sampling trees every 1000 generations. The first 25% trees were discarded as burnin. The analyses were summarised on 50% majority-rule tree. Convergence was checked in Tracer v. 1.5 (Rambaut & Drummond, 2009). FigTree v. 1.4.4 (Rambaut, 2010) was used to visualise the trees. The ML approach was applied with RAxML v. 8.0.24 (Stamatakis, 2014). RAxML analyses were performed using the free computational resource CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010).

Results and Discussion

Morphology

The shells of *Sarajana apfelbecki* (Fig. 3a–f) resembled the ones shown and illustrated by Radoman (1975, 1983). Also the shells of *Sarajana* from the locality Melina 1 (Fig. 3g–i) and whose DNA was sequenced, resembled the ones from type locality (Vrelo Bosne). They were minute, conic (shell form after Hershler & Ponder 1998), with moderately high spire, resembling the one of *Belgrandiella*. The operculum was faded red, the mantle intensively black pigmented, the eyes big. The female reproductive organs (Fig. 4) from all the five localities, resembled the ones described and drawn by Radoman (1983): with large, nearly spherical bursa copulatrix with short and broad duct, moderately thick and rather short loop of the (renal) oviduct, and small, almost vestigial distal receptaculum seminis (rs_1). However, we

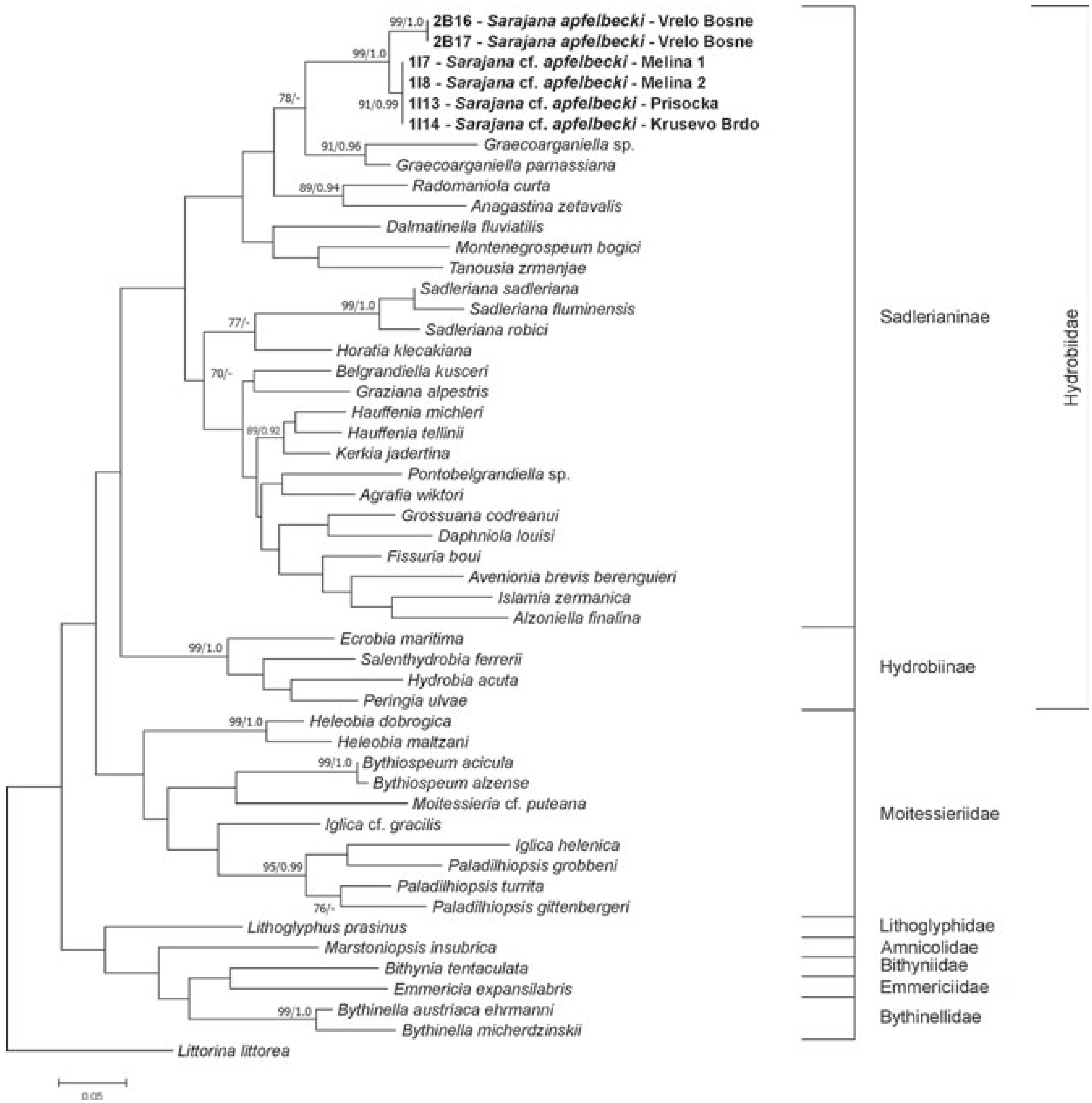

Figure 6 The maximum-likelihood tree of the COI gene. Bootstrap support and Bayesian posterior probabilities are shown

have also found, similarly vestigial, the proximal receptaculum (rs_2), overlooked by Radoman (1975, 1983) and Boeters *et al.* (2017). This structure, whose position is unexpectedly evolutionarily stabile, parallelly lost in some lineages like *Belgrandiella* (Szarowska, 2006), places *Sarajana* far from *Belgrandiella*, among the genera possessing two seminal receptacles. The penis (Fig. 5), identical at all the five localities, was similar to the one drawn by Radoman (1983), but half the size. It was characteristically long and narrow, with a filamentous outgrowth on its left side (marked with asterisk in Fig. 5). Such a penis is not characteristic for *Belgrandiella.*

Molecular relationships

In both coding loci the tests of Xia *et al*. (2003) revealed little saturation. Results from the

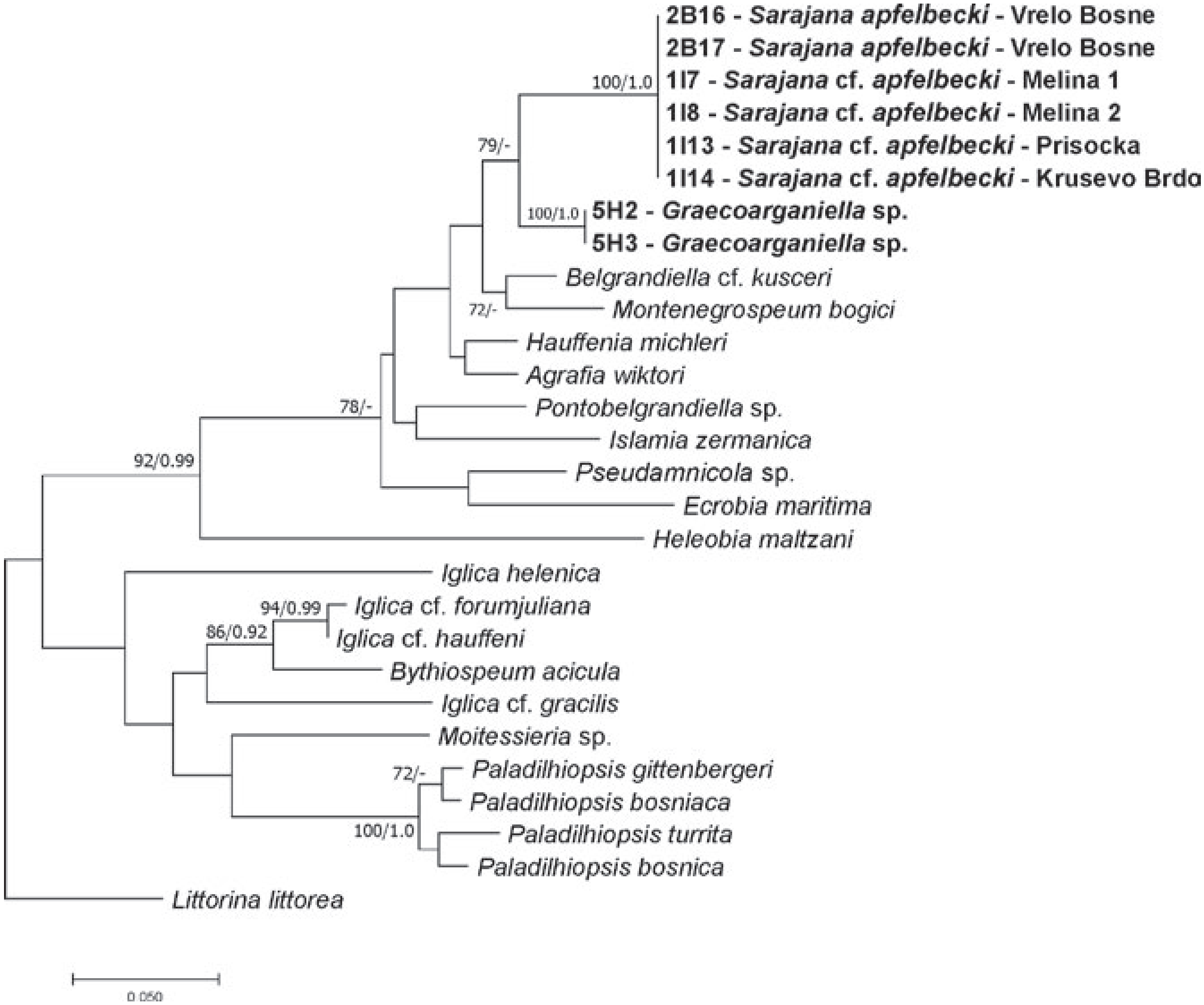

Figure 7 The maximum-likelihood tree of the H3 gene. Bootstrap support and Bayesian posterior probabilities are shown

substitution saturation analysis showed an ISS (0.72 for COI; 0.47 for H3) significantly smaller than the critical ISS value (ISSC: 0.97 for COI; 0.59 for H3), indicating that all sequences are useful in phylogenetic reconstruction

Topology of the trees obtained from BI and ML analyses were identical. In total we obtained six *Sarajana* sequences of COI (552 bp, GenBank Accession numbers MN031428–MN031433) and eight sequences of histone 3 (283 bp, GenBank Accession numbers MN031434–MN031441), six for the *Sarajana* and two for the *Graecoarganiella* sp. In the latter, for H3 we used the DNA extracts previously used by Falniowski & Szarowska, 2011a.

For both markers (Figs 6–7), sequences of *Sarajana* were closest to *Graecoarganiella*, with high bootstrap support (71 for the COI and 79 for the H3). These values were much higher for the combined tree from these two markers (96, Fig. 8). However, p-distance between these two genera was rather high: 0.137 for COI; p-distance between *Sarajana* and *Belgrandiella* was 0.176 for COI and 0.068 for H3 (compare with: Perez, Ponder, Colgan, Clark & Lydeard, 2005, Bichain, Gaubert, Samadi, Boisselier-Dubayle, 2007, Falniowski, Szarowska & Grzmil, 2007, Szarowska, Grzmil, Falniowski & Sirbu, 2007, Falniowski & Szarowska, 2011a) and 0.055 for H3. It should be noted that the

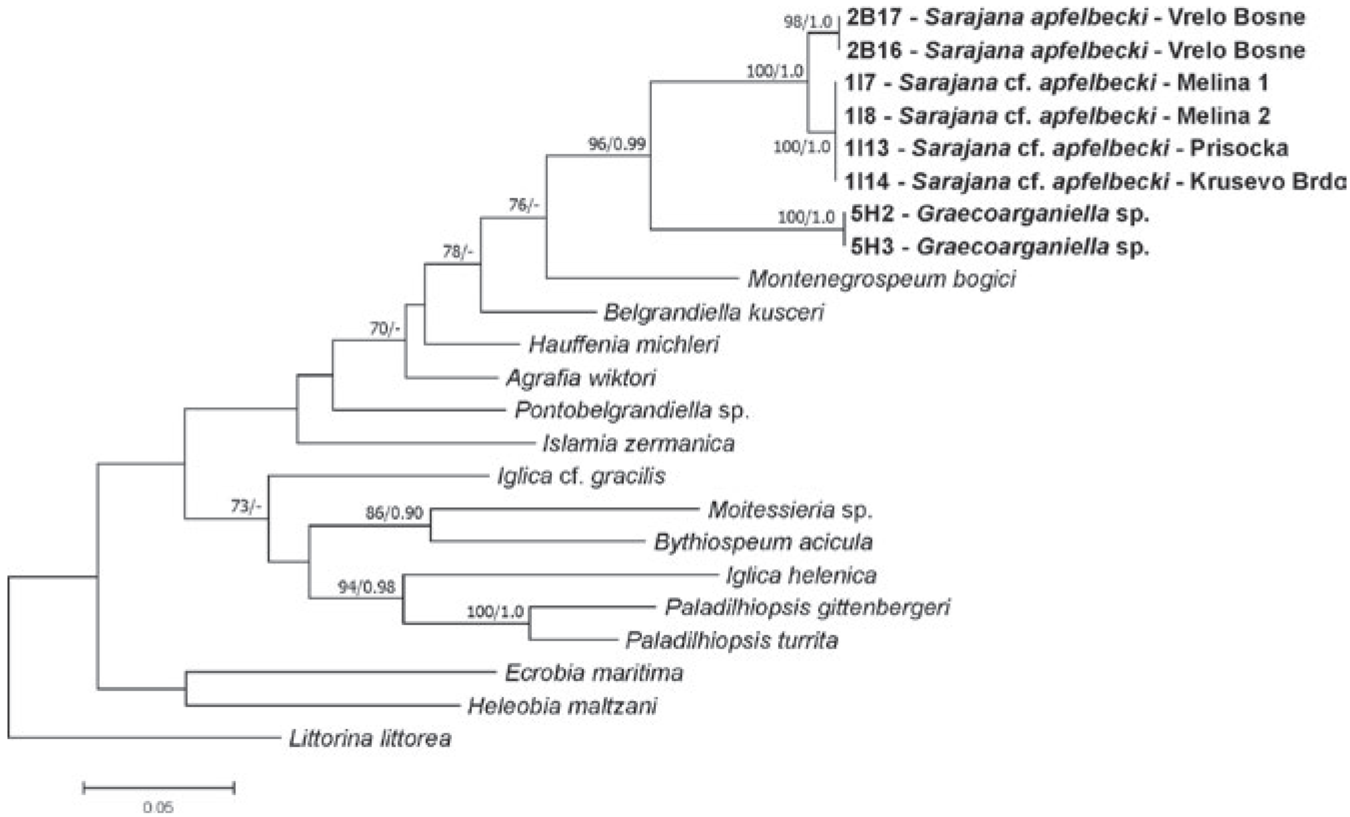

Figure 8 The maximum-likelihood tree of the COI and H3, concatenated sequences gene. Bootstrap support and Bayesian posterior probabilities are shown

morphology – including the valvatiform shell of *Graecoarganiella* Falniowski et Szarowska, 2011, lack of the ctenidium in the latter, etc. – was markedly different in the two genera. This is one more example of limited usefulness of the morphology in the phylogenetic inference in the Truncatelloidea (Szarowska & Falniowski, 2008), although the inferred sister-clade relationship is coupled with a rather high genetic distance.

In the COI tree (Fig. 6) all the deeper nodes were not supported, which is typical of this locus. In the H3 tree (Fig. 7) all the Hydrobiidae formed a well-supported (bootstrap support 78%) clade, with *Sarajana* within it. In the tree computed for the two loci (Fig. 8) there was well-supported sister-clade relationship of *Sarajana/Graecoarganiella* clade with *Montenegrospeum* Pešić & Glöer, 2013, and for these three taxa with *Belgrandiella*, thus the latter was not closely related with *Sarajana*.

As already stated above, the morphology of the gastropods studied in the present paper corresponded with the one of the genus *Sarajana*, as defined by Radoman (1975, 1983), with the exception of the proximal receptaculum which was overlooked in Radoman's description. The latter clearly distinguishes *Sarajana* from *Belgrandiella*. *Sarajana* inhabits a relatively wide range in central Bosnia, from the border with Serbia in the east, to Šuica in the west (Radoman, 1985). Our samples came from the sites close to the ones listed by Radoman (1975, 1983, 1985), around 20–25km away from the type locality of *S. apfelbecki travnicensis* Radoman, 1975 (Travnik: Fig. 1). For COI, the sequences were identical in the populations at the four localities, but not identical between them and the ones from the type locality: the genetic distance p=0.035. In the Truncatelloidea this value is within the zone of ambiguity: such values are considered as still infraspecies in some cases, but as already higher than the threshold values estimated for some genera, like *Bythinella* Moquin-Tandon, 1855 (e.g. Bichain, Boisselier-Dubayle, Bouchet & Samadi, 2007; Szarowska, Grzmil, Falniowski & Sirbu, 2007; Falniowski, Szarowska & Grzmil, 2007; Falniowski & Szarowska, 2011b). However, considering the complete lack of the polymorphism, with the same haplotype of COI in the four northern populations (from Melina 1, Melina 2, Prisocka and Krusevo Brdo), and with another single haplotype at the type locality of *S. apfelbecki* (Vrelo Bosne), suggests rather a species-level distinctness of the two *Sarajana*. The molecular phylogeny also clearly contradicts the status of this genus in the WoRMS as invalid ("unaccepted") and the assignment of *S. apfelbecki* to the genus *Belgrandiella*. *Sarajana* is clearly a distinct genus, not closely related to *Belgrandiella*.

Acknowledgments

The study was supported by a grant from the National Science Centre 2017/25/B/NZ8/01372 to Andrzej Falniowski. We thank to Frank Walther for his help. We also thank two anonymous reviewers of the former version of this paper and, especially, Anna M. Holmes for valuable comments and corrections.

References

Beran L, Hofman S & Falniowski A 2015 *Tanousia zrmanjae* (Brusina, 1866) (Caenogastropoda: Truncatelloidea: Hydrobidae): A living fossil *Folia Malacologica* **23**: 263–271.

Beran L, Osikowski A, Hofman S & Falniowski A 2016 *Islamia zermanica* (Radoman, 1973) (Caenogastropoda: Hydrobidae): morphological and molecular distinctness *Folia Malacologica* **24**: 25–30.

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

Boeters HD, Glöer P & Slavevska Stamenkovic V 2017 The *Radomaniola*/*Grossuana* group from the Balkan Peninsula, with a description of *Grossuana maceradica* n. sp. and the designation of a neotype of *Paludina hohenackeri* Küster, 1853 (Caenogastropoda: Truncatelloidea: Hydrobiidae) *Archiv für Molluskenkunde* **146**: 187–202.

Brancsik K 1888 Nachträge zur Conchylien-Fauna Bosniens. *Nachrichtablätter Deutsche malakozoologische Gesellschaft Frankfurt* **20**: 167–169.

Delicado D 2018 A rare case of stygophily in the Hydrobiidae (Gastropoda: *Sadleriana*). *Journal of Molluscan Studies* **84**: 480–485.

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

Falniowski A & Beran L 2015 *Belgrandiella* A. J. Wagner, 1928 (Caenogastropoda: Truncatelloidea: Hydrobiidae): how many endemics? *Folia Malacologica* **23**: 187–191.

Falniowski A, Pešič V & Glöer P 2014 *Montenegrospeum* Pešič et Glöer, **2013**: a representative of Moitessieriidae? *Folia Malacologica* **22**: 263–268.

Falniowski A, Prevorčnik S, Delić T, Alther R, Altermatt F & Hofman S 2019 Monophyly of the Moitessieriidae Bourguignat, 1863 (Caenogastropoda: Truncatelloidea) *Folia Malacologica* **27**: 61–70.

Falniowski A & Sarbu S 2015 Two new Truncatelloidea species from Melissotrypa Cave in Greece (Caenogastropoda) *ZooKeys* **530**: 1–14.

Falniowski A & Szarowska M 2011a A new genus and new species of valvatiform hydrobiid (Rissooidea; Caenogastropoda) from Greece *Molluscan Research* **31**: 189–199.

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

Falniowski A & Szarowska M 2012 Species distinctness of *Lithoglyphus prasinus* (Küster, 1852) (Rissooidea: Caenogastropoda) *Folia Malacologica* **20**: 99–104.

Falniowski A & Szarowska M 2013 Phylogenetic relationships of *Dalmatinella fluviatilis* Radoman, 1973 (Caenogastropoda: Rissooidea) *Folia Malacologica* **21**: 1–7.

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

Falniowski A, Szarowska M, Glöer P, Pešić V, Georgiev D, Horsak M & Sirbu I 2012b Radiation in *Bythinella* (Mollusca: Gastropoda: Rissooidea) in the Balkans *Folia Malacologica* **20**: 1–9.

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, Sirbu I, Hillebrand A & Baciu M 2008 *Heleobia dobrogica* (Grossu & Negrea, 1989)(Gastropoda: Rissooidea: Cochliopidae) and the estimated time of its isolation in a continental analogue of hydrothermal vents *Mollusan Research* **28**: 165–170.

Falniowski A & Wilke T 2001 The genus *Marstoniopsis* (Rissooidea: Gastropoda): intra- and intergeneric phylogenetic relationships *Journal of Molluscan Studies* **67**: 483–488.

Grego J, Gloer P, Rysiewska A, Hofman S & Falniowski A 2018 A new *Montenegrospeum* species from south Croatia (Mollusca: Gastropoda: Hydrobiidae) *Folia Malacologica* **26**: 25–34.

Grego J, Hofman S, Mumladze L & Falniowski A 2017 *Agrafia* Szarowska et Falniowski, 2011 (Caenogastropoda: Hydrobiidae) in the Caucasus *Folia Malacologica* **25**: 237–247.

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 Contributions to Zoology* **600**: 1–55.

Hofman S, Rysiewska A, Osikowski A, Grego J, Sket B, Prevorcnik S, Zagmajster M & Falniowski A 2018 Phylogenetic relationships of the Balkan Moitessieriidae (Caenogastropoda: Truncatelloidea) *Zootaxa* **4486**: 311–339.

Huelsenbeck JP & Rannala B 2004 Frequents properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models *Systematic Biology* **53**: 904–913.

Jaeckel SGA 1967 Gastropoda. In: Illies J. (ed.) *Limnofauna Europaea*. Gustav Fischer, Stuttgart, pp 89–104.

Layton KK, Martel AL & Hebert PD 2014 Patterns of DNA barcode variation in Canadian marine molluscs *PLoS ONE* **9**, E95003.

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

MolluscaBase 2019 MolluscaBase. *Sarajana apfelbecki* (Brancsik, 1888). Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=1003135 on 2019-05-14

Osikowski A, Hofman S, Rysiewska A, Sket B, Prevorčnik S & Falniowski A 2018 A case of biodiversity overestimation in the Balkan *Belgrandiella* A. J. Wagner, 1927 (Caenogastropoda: Hydrobiidae): molecular divergence not paralleled by high morphological variation *Journal of Natural History* **52**: 323–344.

Perez KE, Ponder WF, Colgan DJ, Clark SA, Lydeard C 2005 Molecular phylogeny and biogeography of spring-associated hydrobiid snails of the Great Artesian Basin, Australia. *Molecular Phylogenetica and Evolution* **34**: 545–556.

Radoman P 1975 Specijacija u ookviru roda *Belgrandiella* i njemu srodnih rodova na Balkanskom poluostrvu. *Glasnik Prirogdnjackog Museja, Beograd, series B* **30**: 29–69, plates 1–4 [In Serbian; English summary.]

Radoman P 1983 *Hydrobioidea a superfamily of Prosobranchia (Gastropoda). I Systematics.* Monographs of Serbian Academy of Sciences and Arts Beograd **547**, Department of Sciences **57**: 1–256.

Radoman P 1985 *Hydrobioidea, a superfamily of Prosobranchia (Gastropoda). II. Origin, zoogeography, evolution in the Balkans and Asia Minor*. Faculty of Science – Department of Biology Monographs, 1, Institute of Zoology Beograd **1**: 1–173.

Rambaut A 2010 FigTree v1.3.1. <http://tree.bio.ed.ac.uk/software/figtree>

Rambaut A & Drummond AJ 2009 Tracer v1.5. <http://beast.bio.ed.ac.uk/Tracer>.

Richling I, Malkowsky Y, Kuhn Y, Niederhöfer H–J & Boeters HD 2016 A vanishing hotspot – impact of molecular insights on the diversity of Central European *Bythiospeum* Bourguignat, 1882 (Mollusca: Gastropoda: Truncatelloidea) *Organisms Diversity & Evolution* **17**: 67–85.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA & Huelsenbeck JP 2012 Mr.Bayes 3.**2**: efficient Bayesian phylogenetic inference and model choice across a large model space *Systematic Biology* **61**: 539–542.

Rysiewska A, Georgiev D, Osikowski A, Hofman S & Falniowski A 2016 *Pontobelgrandiella* Radoman, 1973 (Caenogastropoda: Hydrobiidae): A recent invader of subterranean waters? *Journal of Conchology* **42**: 193–203.

Rysiewska A, Prevorčnik S, Osikowski A, Hofman S, Beran L & Falniowski A 2017 Phylogenetic relationships in *Kerkia* and introgression between *Hauffenia* and *Kerkia* (Caenogastropoda: Hydrobiidae) *Journal of Zoological Systematics and Evolutionary Research* **55**: 106–117.

Schütt H 1959 Zur Höhlenschnecken aus Montenegro. *Archiv für Molluskenkunde* **88**: 185–190.

Stamatakis A 2014 RAxML Version **8**: a tool for phylogenetic analysis and post-analysis of large phylogenies *Bioinformatics* **30**: 1312–1313.

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

Szarowska M & Falniowski A 2008 There is no philosopher's stone: *coup de grace* for the morphology-based systematics in the rissooidean gastropods? *5th Congress of the European Malacological Societies*, *Ponta Delgada*: 28.

Szarowska M & Falniowski A 2011 An unusual, flagellum-bearing hydrobiid snail (Gastropoda: Rissooidea: Hydrobiidae) from Greece, with descriptions of a new genus and a new species *Journal of Natural History* **45**: 2231–2246.

Szarowska M & Falniowski A 2013a Phylogenetic relationships of the Emmericiidae (Caenogastropoda: Rissooidea) *Folia Malacologica* **21**: 67–72.

Szarowska M & Falniowski A 2013b Species distinctness of *Sadleriana robici* (Clessin, 1890) (Gastropoda: Rissooidea) *Folia Malacologica* **21**: 127–133.

Szarowska M & Falniowski A 2014a *Horatia* Bourguignat, **1887**: is this genus really phylogenetically very close to *Radomaniola* Szarowska, 2006 (Caenogastropoda: Truncatelloidea)? *Folia Malacologica* **22**: 31–39.

Szarowska M & Falniowski A 2014b *Ventrosia maritima* (Milaschewitsh, 1916) and *V. ventrosa* (Montagu, 1803) in Greece: molecular data as a source of information about species ranges within the Hydrobiinae (Caenogastropoda: Truncatelloidea) *Folia Malacologica* **22**: 61–67.

Szarowska M, Grzmil P, Falniowski A & Sirbu I 2007 *Grossuana codreanui* (Grossu, 1946) and the phylogenetic relationships of the East Balkan genus *Grossuana* (Radoman, 1973) (Gastropoda: Rissooidea) *Hydrobiologia* **579**: 379–391.

Szarowska M, Hofman S, Osikowski A & Falniowski A 2014a *Daphniola* Radoman, 1973 (Caenogastropoda: Truncatelloidea) at east Aegean islands *Folia Malacologica* **22**: 269–275.

Szarowska M, Hofman S, Osikowski A & Falniowski A 2014b *Heleobia maltzani* (Westerlund, 1886) (Caenogastropoda: Truncatelloidea: Cochliopidae) from Crete and species-level diversity of *Heleobia* Stimpson, 1865 in Europe *Journal of Natural History* **48**: 2487–2500.

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

Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S 2013 MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method *Molecular Biology and Evolution* **28**: 2731–2739.

Wagner A 1927 Studien zur Molluskenfauna der Balkanhalbinsel mit besonderer Berücksichtigung Bulgariens und Thraziens, nebst monographicher Bearbeitung einzelner Gruppen. *Annales Muzei Zoologici Polonici Historia Naturalis, Warszawa*. **6**: 263–399.

Wilke T 2003 *Salenthydrobia* gen. nov. (Rissooidea: Hydrobiidae): a potential relict of the Messinian Salinity Crisis Zoological *Journal of the Linnean Society* **137**: 319–336.

Wilke T & Davis GM 2000 Infraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissoacea: Gastropoda): Do their different life histories affect biogeographic patterns and gene flow? *Biological Journal of the Linnean Society* **70**: 89–105.

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.

Wilke T & Falniowski A 2001 The genus *Adriohydrobia* (Hydrobiidae: Gastropoda): polytypic species or polymorphic populations? *Journal of Zoological Systematics and Evolutionary Research* **39**: 227–234.

Wilke T, Rolán E & Davis Gm 2000 The mudsnail genus *Hydrobia* s.s. in the northern Atlantic and western Mediterranean: a phylogenetic hypothesis *Marine Biology* **137**: 827–833.

World Register Of Marine Species 2018 www.marinespecies.org/aphia.php?p= taxdetails&id=1003135.

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.