

PLAGIGEYERIA MONTENEGRINA BOLE, 1961 (CAENOGASTROPODA: TRUNCATELLOIDEA: MOITESSIERIIDAE): MORPHOLOGY AND MOLECULES IN THE SPECIES AND GENUS TAXONOMY

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Abstract The shell habitus, teleoconch and protoconch SEM studied surface, radula, female reproductive organs and penis are described and illustrated for *Plagigeyeria montenegrina* Bole, 1961 from its type locality: Obodska Pećina Cave near Rijeka Crnojevića (Montenegro). The DNA sequences of the mitochondrial cytochrome oxidase subunit I (COI), nuclear histone H3 and ribosomal 18S and 28S genes have been used to infer phylogenetic position of *P. montenegrina*, which should belong to the genus *Paladilhiopsis* Pavlović, 1913. The anatomical and shell data strongly suggest that the genus *Plagigeyeria* Tomlin, 1930, as well as *Saxurinator* Schütt, 1960 are not distinct, but their status remains open, since type species have not been examined. The discovery of the shells of *Plagigeyeria* spp. at three interstitial habitats contradicts the common believe in isolation of stygobiotic inhabitants of caves and springs, supposed to be the main factor causing rapid speciation at (nearly) each locality. This, and the wide variability in the morphology, not only of the shell, but also of the anatomy, questions the species distinction of many nominal species described in this group.

Key words Interstitial fauna, Mollusca, phylogeography, stygobiotic fauna

INTRODUCTION

Wagner (1914) described the genus *Geyeria*, with its type species *Geyeria plagiostoma* Wagner, 1914, from springs in Bosnia (Vrelo Bosne, spring of Bosna River in Ilidža near Sarajevo). However, the name was already preoccupied by the genera of the Lepidoptera, Cephalopoda and Brachiopoda (Kabat & Hershler, 1993). Thus, Tomlin (1930) established the replacement name *Plagigeyeria* Tomlin, 1930. To our knowledge, neither soft part morphology nor anatomy of *P. plagiostoma* have been studied, only empty shells have been found (Bole, 1970; Radoman, 1973).

Bole (1970) described and figured the radula, penis, and female reproductive organs of *Plagigeyeria montenegrina* Bole, 1961, from the type locality: Obodska Pećina Cave near Rijeka Crnojevića (Montenegro). The same organs were presented for *P. montenegrina* by Radoman (1973), but, according to his opinion (Radoman 1983), the anatomy he presented for that species was

identical with the one figured by Bole (1970) for *Saxurinator sketi* Bole, 1961).

According to Schütt (1960) the genus *Saxurinator* Schütt, 1960 is characterized solely by its shell: “the shell minute and thin-walled, slim turreted with the wide apex, 5–6 whorls, massive, flat; the mouth elliptic, with its long axis about 45° diagonal to the columella, trumpet-shaped-broadened, fused with the body whorl; the sculpture fine, regular and tight as the radial growth lines; the umbilicus open to scratched. The difference from the genus *Paladilhiopsis* is marked in the broader apex, more flat whorls, slate mouth and finer sculpture”. The type species of the genus *Saxurinator* is *Paladilhiopsis buresi* Wagner, 1927, described from Temnata Dupka Cave near Lakatnik town, Stara Planina Mts. in Bulgaria (Wagner, 1927), and still known only as dead shells (Georgiev & Hubenov, 2013).

Schütt (1960) described the geographic distribution of *Saxurinator*, as vicariant to the one of more northern *Paladilhiopsis*. Similarly as in the case of the Greek “Hydrobiidae” (Schütt, 1980),

whose species' geographic ranges were always distinct (and, sometimes, in one "genus" combining the representatives of two distant families). As pointed out by Radoman (1985), Szarowska (2006) and Falniowski & Szarowska (2011) such a geographic approach to the hydrobioid taxonomy cannot be justified.

The genus *Paladilhiopsis* Pavlovic, 1913 with its type species *Paladilhia robiciana* Clessin, 1882, has been expanded to contain also the genera *Costellina* Kuščer, 1933, and possibly *Lanzaia* Brusina, 1906 (Hofman *et al.*, 2018). The latter assignment is provisional, since only a juvenile specimen of *Lanzaia bosnica* Bole, 1970 was sequenced (Hofman *et al.*, 2018). Thus, the DNA sequences of *Turbo elephantosus* Megerle von Mühlfeld, 1824, the type species of *Lanzaia*, are necessary to confirm that *Paladilhiopsis* and *Lanzaia* are congeners. If so, the ICZN rule of priority would result in considering *Paladilhiopsis* a junior synonym of *Lanzaia*. For now, we assume the genus name *Paladilhiopsis* as valid.

In September of 2019 we collected live specimens of *Plagigeyeria montenegrina* at its type locality, Obodska Pećina. The aim of the present paper is to check the morphology of this species, and to resolve its phylogenetic placement with the use of molecular (DNA) markers.

MATERIAL AND METHODS

Living snails, attached to the rock and stones, were hand-collected at the spring in the cave Obodska Pećina (Cave), near Rijeka (River) Crnojevića, Cetinje, Montenegro (Tab. 1, Figs 1–2). The spring was located inside the cave, approx. 900m from the entrance.

The Bou–Rouch method (Bou & Rouch, 1967) was additionally used to sample interstitial fauna of the Rijeka Crnojevića (Fig. 3), Rijeka Ribnica and spring near Miločani, at a depth of about 50cm below the bottom (Tab. 1, Fig. 1). The

tube was inserted in the gravel five times, and 20 litres were pumped each time and samples sieved through a 500 µm sieve. The latter technique resulted only in empty shells of *P. montenegrina* at the Rijeka Ribnica and in the spring near Miločani. The snails were fixed in 80% analytically pure ethanol, which was replaced twice, and then later sorted, if fixed with the sediment. Next, the snails were put in fresh 80% analytically pure ethanol and kept in -20°C temperature in a freezer.

The shells were photographed with a Canon EOS 50D digital camera, under a Nikon SMZ18 microscope. The dissections were done under a Nikon SMZ18 microscope with dark field, equipped with Nikon DS-5 digital camera, whose captured images were used to draw anatomical structures with a graphic tablet.

The shells were cleaned with an ultrasonic cleaner, the radulae were extracted with Clorox, applying the techniques described by Falniowski (1990), and examined and photographed using a HITACHI S-4700 scanning electron microscope.

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

Mitochondrial cytochrome oxidase subunit I (COI), and nuclear 18S ribosomal RNA (18S), 28S ribosomal RNA (28S) and histone 3 (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 7 (Kumar *et al.*, 2016) and then checked in BIOEDIT 7.1.3.0 (Hall, 1999). Uncorrected p-distances were

Table 1 Geographic coordinates of *Plagigeyeria montenegrina* sampling sites. See also the map (Fig. 1).

Id	Site	Coordinates
1	Miločani, M19-05	42°49'59.5"N 18°54'22.2"E
2	Rijeka Ribnica, M19-09	42°26'14.2"N 19°17'52.0"E
3	Rijeka Crnojevića M10	42°21'18.6"N 19°01'10.3"E
4	Obodska Pećina, M19-04	42°21'07.0"N 19°00'17.0"E



Figure 1 Localizations of sampling sites. For geographic coordinates see Table 1.

calculated in MEGA 7. The estimation of the proportion of invariant sites and the saturation test (Xia, 2000; Xia *et al.*, 2003) were performed using DAMBE (Xia, 2013). In the phylogenetic analysis additional sequences from GenBank were used as reference (Tab. 2). The data were analysed using approaches based on Bayesian Inference (BI) and Maximum Likelihood (ML). We applied the GTR model whose parameters were estimated by RaxML (Stamatakis, 2014). The Bayesian analyses were run using MrBayes v. 3.2.3 (Ronquist *et al.*, 2012) with defaults of most priors. Two simultaneous analyses were performed, each with 10,000,000 generations, with one cold chain and three heated chains, starting from random trees and sampling the trees every

1,000 generations. The first 25% of the trees were discarded as burn-in. The analyses were summarised as a 50% majority-rule tree. The Maximum Likelihood analysis was conducted in RAxML v. 8.2.12 (Stamatakis, 2014) using the 'RAxML-HPC v.8 on XSEDE (8.2.12) tool via the CIPRES Science Gateway (Miller *et al.*, 2010).

RESULTS

The six shells of *Plagigeyeria montenegrina* from Obodska Pećina Cave (Fig. 4A–F) were similar to those photographed by Radoman (1983), and show wide variability, mostly of the spire and mouth, more or less protruded. The empty *Plagigeyeria* shells from distinct localities of the

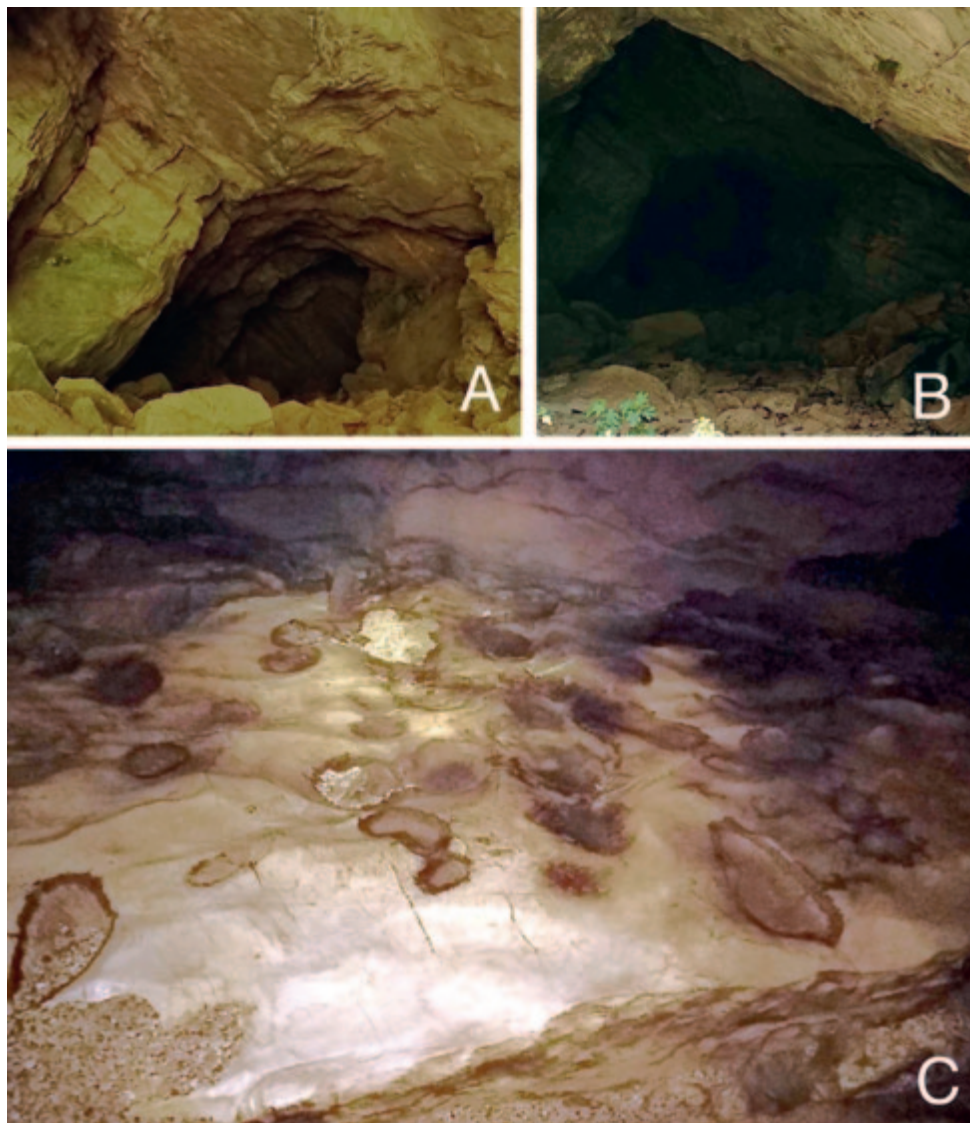


Figure 2 Obodska Pečina Cave (sampling site 4): A–B – the entrance to the cave; C – the spring inside the cave.

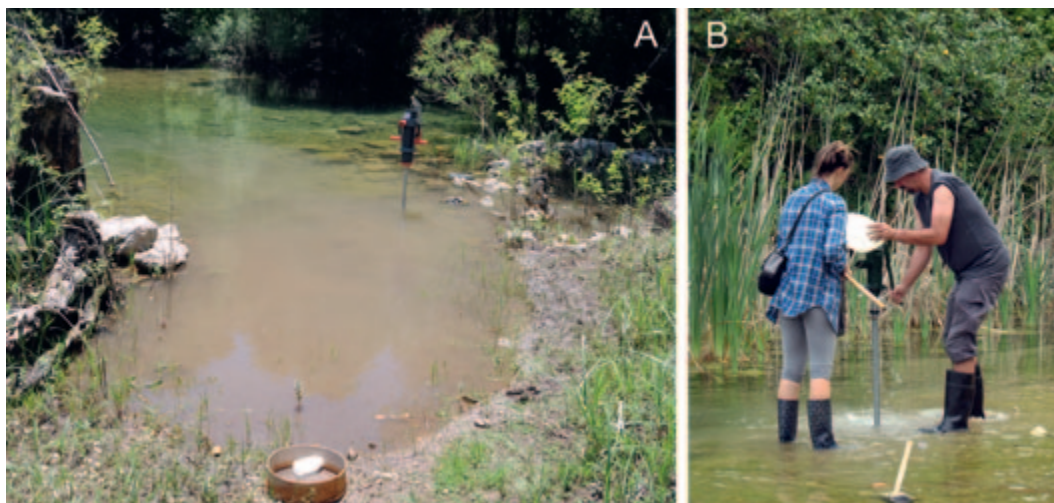


Figure 3 Rijeka Crnojevića: A – sampling site 3 (see Fig. 1); B – sampling interstitial fauna using the Bou–Rouch method.

Table 2 Reference sequences used in phylogenetic analyses.

Species	COI	H3	18S	28S	References
<i>Agrafia wiktoria</i> Szarowska & Falniowski, 2011	JF906762	MG543158	JF906761	MW449165	Szarowska & Falniowski, 2011; Grego <i>et al.</i> , 2017, present
<i>Alzoniella finalina</i> Giusti & Bodon, 1984	AF367650	-	-	-	Wilke <i>et al.</i> , 2001, present
<i>Anagastina zetavalis</i> (Radoman, 1973)	EF070616	-	-	-	Szarowska, 2006
<i>Avenionia brevis berenguieri</i> (Draparnaud, 1805)	AF367638	-	-	-	Wilke <i>et al.</i> , 2001
<i>Belgrandiella zermanica</i> Radoman, 1973	KT218511	MG551366	MW449179	MW449166	Falniowski & Beran, 2015; Osikowski <i>et al.</i> , 2018, present
<i>Bithynia tentaculata</i> (Linnaeus, 1758)	AF367643	-	-	-	Wilke <i>et al.</i> , 2001
<i>Bythinella austriaca</i> (von Frauenfeld, 1857)	JQ639858	-	-	-	Falniowski <i>et al.</i> , 2012b
<i>Bythinella micherdzinskii</i> Falniowski, 1980	JQ639854	-	-	-	Falniowski <i>et al.</i> , 2012b
<i>Bythiospeum acicula</i> (Hartmann, 1821)	KU341350	MK609534	MK629727	MH725863	Richling <i>et al.</i> , 2016; Falniowski <i>et al.</i> , 2019; Hofman <i>et al.</i> , 2018
<i>Bythiospeum alzense</i> Boeters, 2001	KU341355	-	-	-	Richling <i>et al.</i> , 2016
<i>Daphniola louisi</i> Falniowski & Szarowska, 2000	KM887915	-	-	-	Szarowska <i>et al.</i> , 2014b
<i>Dalmatinella fluviatilis</i> Radoman, 1973	KC344541	-	-	-	Falniowski & Szarowska, 2013
<i>Emmericia expansilabris</i> Bourguignat, 1880	KC810060	-	-	-	Szarowska & Falniowski, 2013a
<i>Fissuria boui</i> Boeters, 1981	AF367654	-	-	-	Wilke <i>et al.</i> , 2001
<i>Ecrobia maritima</i> (Milaschewitsch, 1916)	KX355835	MG551322	MW449180	MW449167	Osikowski <i>et al.</i> , 2016; Grego <i>et al.</i> , 2017, present
<i>Gnaecoarganiella parnassiana</i> Falniowski & Szarowska, 2011	JN202352	-	-	-	Falniowski & Szarowska, 2011
<i>Graziana alpestris</i> (Frauenfeld, 1863)	AF367641	-	-	-	Wilke <i>et al.</i> , 2001
<i>Grossuana codreamui</i> (Grossu, 1946)	EF061919	-	-	-	Szarowska <i>et al.</i> , 2007
<i>Hauffenia michtleri</i> (Kuščer, 1932)	KT236156	KY087878	MW449181	MW449168	Falniowski & Szarowska, 2015; Rysiewska <i>et al.</i> , 2017, present
<i>Hauffenia tellinii</i> (Pollonera, 1898)	KY087861	-	-	-	Rysiewska <i>et al.</i> , 2017
<i>Heleobia dalmatica</i> (Radoman, 1974)	AFI29321	-	-	-	Hershler <i>et al.</i> , 1999
<i>Heleobia dobrogica</i> (Grossu & Negrea, 1989)	EU938131	-	-	-	Falniowski <i>et al.</i> , 2008
<i>Heleobia maltzani</i> (Westerlund, 1886)	KM213723	MK629762	MW449182	MW449169	Szarowska <i>et al.</i> , 2014a; Falniowski <i>et al.</i> , 2019, present
<i>Horatia klecakiana</i> Bourguignat 1887	KJ159128	-	-	-	Szarowska & Falniowski, 2014

Table 2 Continued

Species	COI	H3	18S	28S	References
<i>Hydrobia acuta</i> (Draparnaud, 1805)	AF278808	-	-	-	Wilke et al., 2000a
<i>Iglica</i> cf. <i>forunjuliana</i> (Pollonera, 1887)	-	MH721006	MH725853	MH725885	Hofman et al., 2018
<i>Iglica</i> cf. <i>gracilis</i> (Clessin, 1882)	MH720985-89	MH721002	MH725844	MH725883	Hofman et al., 2018
<i>Iglica</i> cf. <i>hauffeni</i> (Brusina, 1886)	-	MH720995	MH725842	MH725874	Hofman et al., 2018
<i>Iglica hellenica</i> Falniowski & Sarbu, 2015	KT825581	MH721007	MH725854	MH725886	Falniowski & Sarbu, 2015; Hofman et al., 2018
<i>Islamia zermanica</i> (Radoman, 1973)	KU662362	MG551320	MW449183	MW449170	Beran et al., 2016; Grego et al., 2017, present
<i>Lanzaiopsis savinica</i> Bole, 1989	MN272428-29	-	-	-	Prevorčnik et al., 2019
<i>Littorina littorea</i> (Linnaeus, 1758)	KF644330	KP113574	DQ093437	DQ279985	Layton et al., 2014; Neretina, 2014, unpublished
<i>Lithoglyphus prasinus</i> (Küster, 1852)	JX073651	-	-	-	Falniowski & Szarowska, 2012
<i>Marstoniopsis insubrica</i> (Küster, 1853)	AF322408	-	-	-	Falniowski & Wilke, 2001
<i>Moitessieria</i> cf. <i>puteana</i> Coutagne, 1883	AF367635	MH721012	MH725859	MH725891	Wilke et al., 2001; Hofman et al., 2018
<i>Montenegrospeum bogici</i> (Pešić & Glöer, 2012)	KM875510	MG880218	MW449184	MW449171	Falniowski et al., 2014; Grego et al., 2018, present
<i>Paladilhiopsis</i> cf. <i>absoloni</i> (A. J. Wagner, 1914)	-	MH721021	MH725868	MH725900	Hofman et al., 2018
<i>Paladilhiopsis bihensis</i> (Glöer & Grego, 2015)	-	MH721015	MH725862	MH725894	Hofman et al., 2018
<i>Paladilhiopsis bosniaca</i> (Clessin, 1910)	-	MH721019	MH725866	MH725897	Hofman et al., 2018
<i>Paladilhiopsis bosnica</i> Bole, 1970	-	MH721020	MH725867	MH725899	Hofman et al., 2018
<i>Paladilhiopsis gittenbergeri</i> (A. Reischutz & P. L. Reischutz, 2008)	MH720993	MH721024	MH725872	MH725901	Hofman et al., 2018
<i>Paladilhiopsis grobbeni</i> Kušcer, 1928	MH720990-91	MH721014	MH725861	MH725892	Hofman et al., 2018
<i>Paladilhiopsis maroskoi</i> (Glöer & Grego, 2015)	-	MH721017	MH725864	MH725896	Hofman et al., 2018
<i>Paladilhiopsis turrita</i> (Kušcer, 1933)	MH720992	MH721016	MH725863	MH725895	Hofman et al., 2018
<i>Peringia ulvae</i> (Pennant, 1777)	AF118302	-	-	-	Wilke & Davis, 2000
<i>Plaggyeria montenegrina</i> Bole, 1961	MW452318-22	MW452604-08	MW449174-78	MW449160-64	present paper
<i>Pontobelgrandiella</i> Radoman, 1978; sp. n.	KU497024	MG551321	MW449186	MW449173	Rysiewska et al., 2016; Grego et al., 2017, present
<i>Pseudamnicola</i> Paulucci, 1878; sp. n.	-	KT710579	MW449185	MW449172	Szarowska et al., 2016, present
<i>Radomantiola curta</i> (Küster, 1853)	KC011814	-	-	-	Falniowski et al., 2012a
<i>Sadleriana fluminensis</i> (Küster, 1853)	KF193067	-	-	-	Szarowska & Falniowski, 2013b
<i>Salenthydrobia ferrerii</i> Wilke, 2003	AF449213	-	-	-	Wilke, 2003
<i>Tanousia zrnjanje</i> (Brusina, 1866)	KU041812	-	-	-	Beran et al., 2015

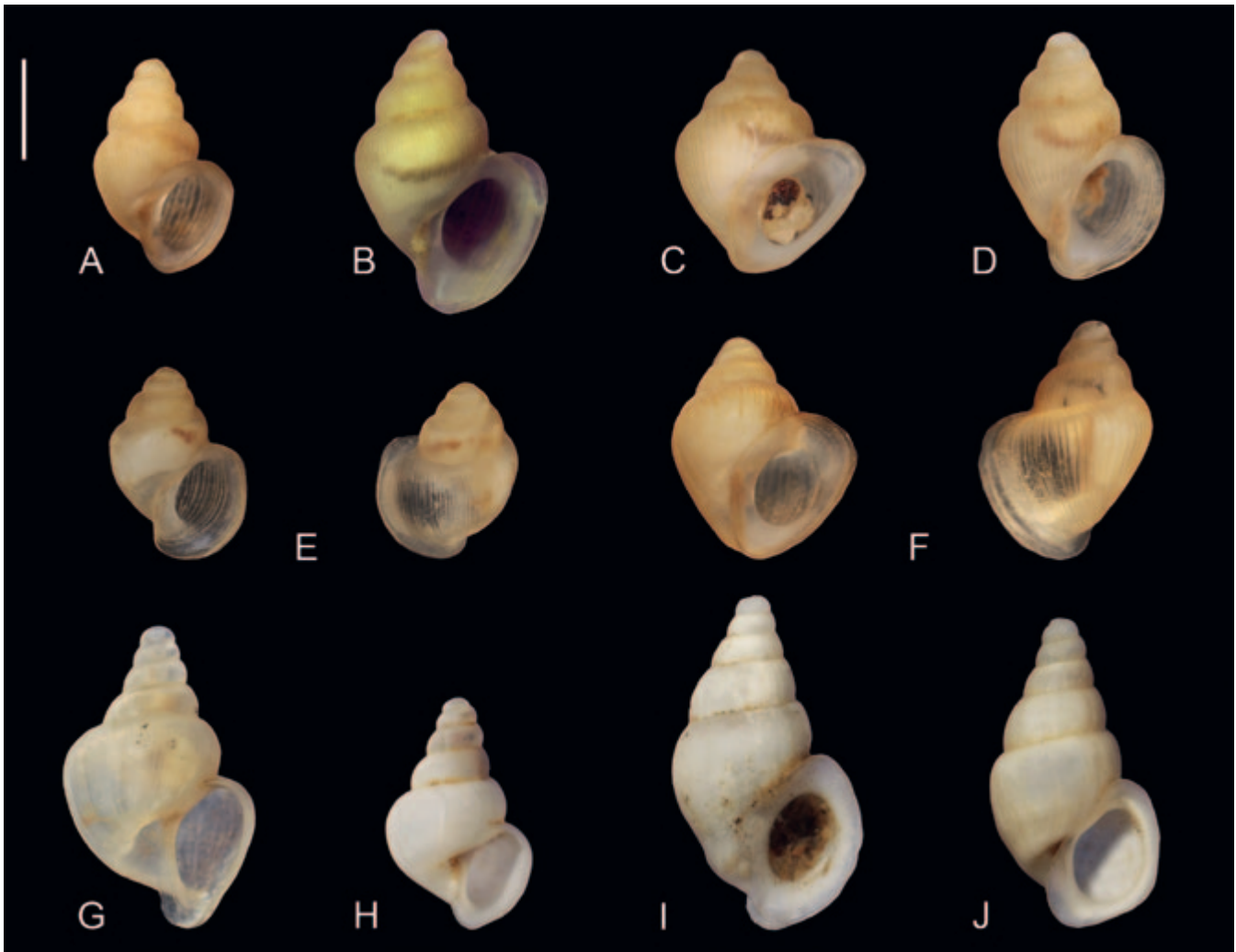


Figure 4 Shells of sequenced specimens of *Plagigeyeria montenegrina*: A–F from Obodska Pećina Cave, (sampling site 4, extraction numbers: A – 2G28, B – 2G29, C – 2G64, D – 2G65, E – 2G96, F – 2G97) and empty shells: G–H from Rijeka Ribnica, M19-09 (sampling site 2, Fig. 1) and I–J from Miločani, M19-05 (sampling site 1, Fig. 1); bar equals 1.0mm.

Skadar Lake drainage basin have mostly different shell morphology and a much higher spire (Fig. 4G–H), coarser and more distant ribbing, different body whorl shape and different protoconch sculpture. The specimen from Miločani (Fig. 4I–J), resembles *P. plagiostoma* with not completely developed mouth, and relatively delicate teleoconch sculpture. The teleoconch sculpture of *P. montenegrina* (Fig. 5A–G) was composed of rather strong radial ribs (growth lines), and much more delicate spiral riblets. The protoconch surface (Fig. 5H) is smooth.

The radula (Fig. 6A–B) with the central tooth (Fig. 6B) described by the formula:

$$\begin{array}{c} (5) \ 4-1-4 \ (5) \\ \quad \quad 1-1 \end{array}$$

On both sides of the four fully developed but short cusps on the tooth plate there are slightly developed, almost rudimentary cusps; the basal cusps very weakly developed (Fig. 6B). The lateral tooth given by the formula 4–1–5, inner marginal with about 15, and outer with about 18 rather blunt cusps.

The renal and pallial section of the female reproductive organs (Fig. 7A) with the pallial accessory gland complex somewhat shortened, and a very big bursa copulatrix. The nidamental gland folded. The loop of (renal) oviduct slightly broadened, a big, sac-shaped distal receptaculum seminis (at position of rs_1 of Radoman, 1983) with a long duct. The duct of bursa copulatrix starts at some distance from the distal termination of the

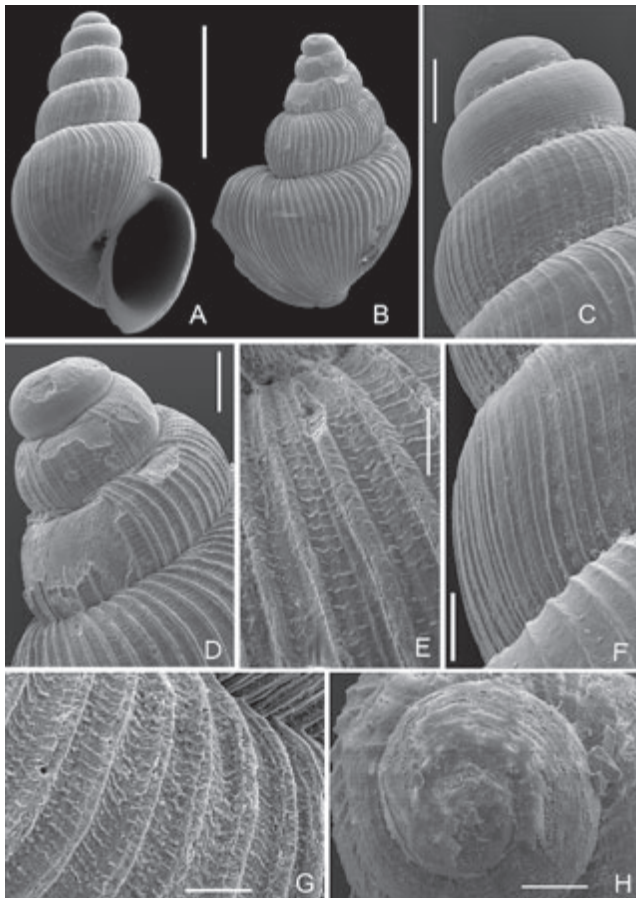


Figure 5 SEM photographs of the shells of *Plagigeyeria montenegrina*: A – M19-05, pumped from interstitial habitat; B–H – Obodska Pečina Cave: B – whole shell, C–D – spire, E–G – teleoconch sculpture, H – protoconch; bars equal: A–B – 1mm, C–D – 150 μ m, E, G – 50 μ m, F, H – 100 μ m.

bursa. The penis (Fig. 7B) simple, without any outgrowth, long and narrow, the vas deferens visible inside.

The maximum likelihood tree based on the cytochrome oxidase subunit I (COI) (Fig. 8) and all the four studied loci (Fig. 9) placed *Plagigeyeria montenegrina* within the genus *Paladilhiopsis* (bootstrap support 94%, Bayesian probability 0.99), with the sister species *P. turrita* (Kuscer, 1933). All the three studied nuclear loci (18S, 28S and H3) placed *Plagigeyeria montenegrina* in the Moitessieriidae, in the genus *Paladilhiopsis*, with *Paladilhiopsis turrita* and *P. bosnica* as a sister clade.

DISCUSSION AND TAXONOMIC CONCLUSIONS

Plagigeyeria clearly belongs to the Moitessieriidae Bourguignat, 1863, the family distinct from the Hydrobiidae Troschel, 1857. The monophyly of the Moitessieriidae has recently been confirmed (Falniowski *et al.*, 2019).

The shells of *P. montenegrina* from its type locality present a rather wide range of variability. The specimen presented in Fig. 4C, with a short spire and the mouth long axis about 45° to the columella (which resembles *Saxurinator*) is molecularly (COI) slightly different from the other *P. montenegrina* (Fig. 8), but the molecular difference is typical of intrapopulation polymorphism and similarly shaped specimen in Fig. 4F is identical molecularly with the one presented in

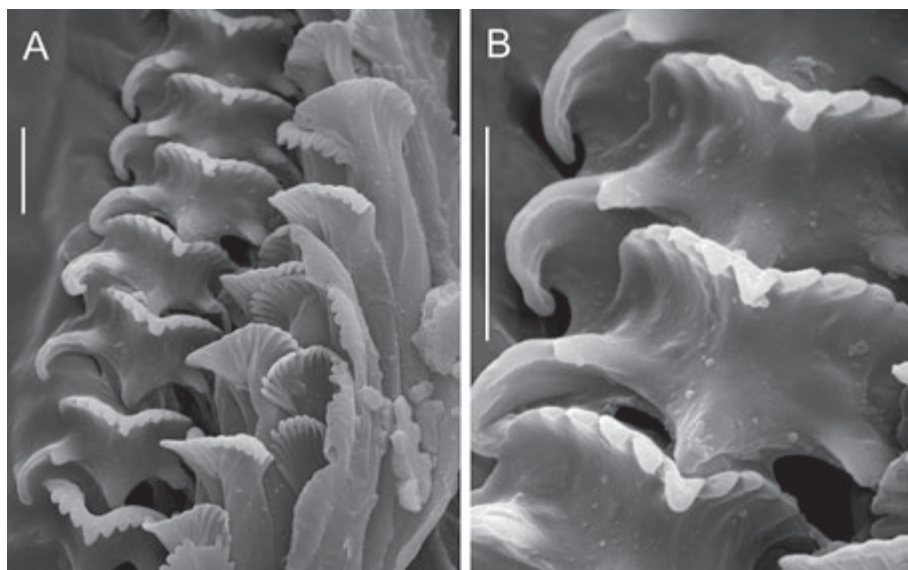


Figure 6 Radula of *Plagigeyeria montenegrina* from Obodska Pečina Cave: A – half of the row, B – central tooth; bars equal 5 μ m.

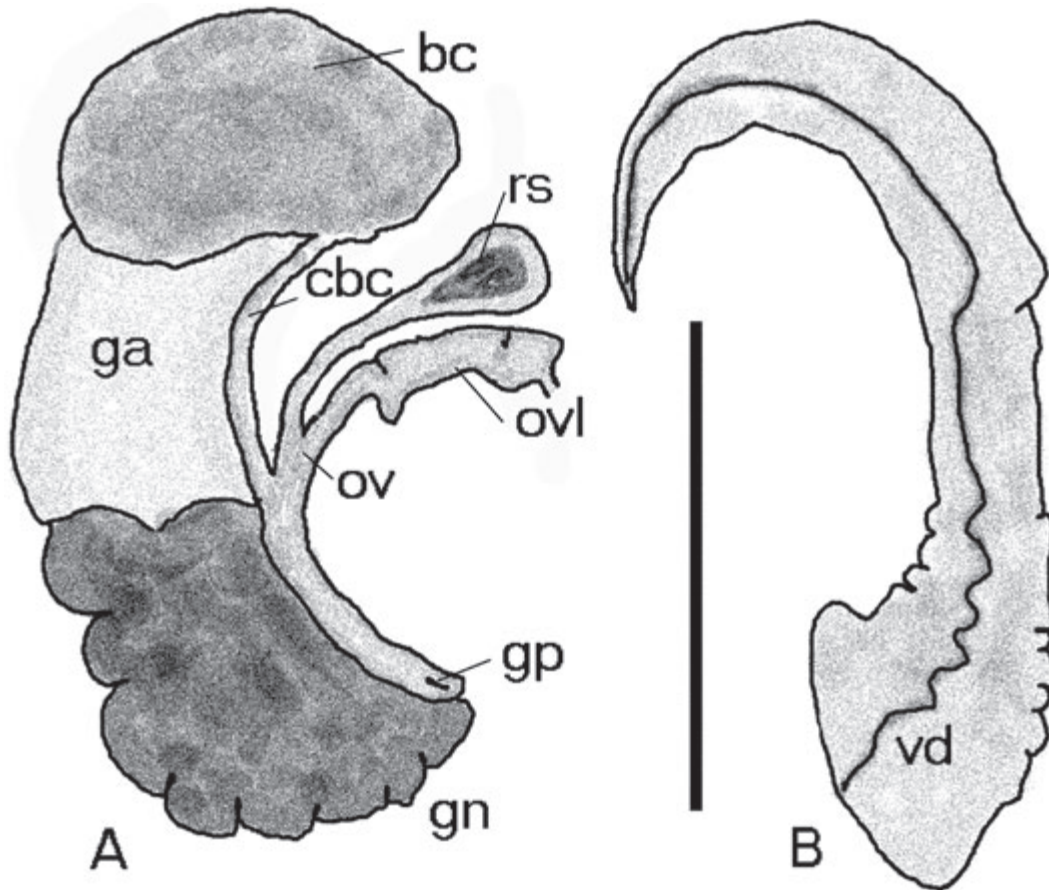


Figure 7 Reproductive organs of *Plagigeyeria montenegrina* from Obodska Pećina Cave: A – renal and pallial section of the female reproductive organs (**bc** – bursa copulatrix, **cbc** – duct of bursa copulatrix, **ga** – albuminoid gland, **gn** – nidamental gland, **gp** – gonoporus, **ov** – oviduct, **ovl** – loop of renal oviduct, **rs** – receptaculum seminis); B – penis (**vd** – vas deferens); bar equals 0.5mm.

Fig. 4E. The presented variability from one locality questions the validity of the dozens of species descriptions in *Plagigeyeria*, based on only empty and even not necessarily complete shells (e.g. Schütt, 1960, 1972; Glöer & Pešić, 2014; Grego *et al.*, 2017, 2019).

The spiral riblets of *Plagigeyeria* are typical of the Moitessieriidae (e.g. Hofman *et al.*, 2018). They are rather weak compared with some other moitessieriid species. The typical spiral sculpture of the Moitessieriidae is most prominent in *Plagigeyeria turrita*, formerly classified into a monotypic genus *Costellina* Kuser, 1933, but molecularly belonging to *Paladilhiopsis* (Hofman *et al.*, 2018). Thus, the very weak teleoconch sculpture of *Saxurinator* described by Schütt (1960) is the other extreme state of this character. The radula of *Saxurinator* corresponds with the description of Bole (1970).

The female reproductive organs, and the penis, in general resemble the ones of *Plagigeyeria*

montenegrina described and drawn by Bole (1970) and Radoman (1983). The only difference concerns the size and shape of the bursa copulatrix, and the position of its outlet into the duct of the bursa. According to the drawings of Bole (1970) this distinguishes *P. montenegrina* from *Saxurinator sketi*, but Radoman (1983) described the female reproductive organs of *Plagigeyeria montenegrina* from its type locality as identical with the ones drawn by Bole (1970) for *Saxurinator sketi*. In our specimens the states of these characters were just intermediate between the ones presented by Bole (1970) for *Plagigeyeria* and *Saxurinator*. It has to be stressed, that all the characters of the female reproductive organs show wide ranges of variability (Falniowski, 1987, 1990, 1992, 2018; Falniowski & Szarowska, 2011; Szarowska, 2006), overlooked for the long time (e. g. Radoman, 1976, 1983; Boeters, 1979, 1998). For the reasons listed by Szarowska (2006) and Falniowski (2018) such differences in the morphology of the bursa

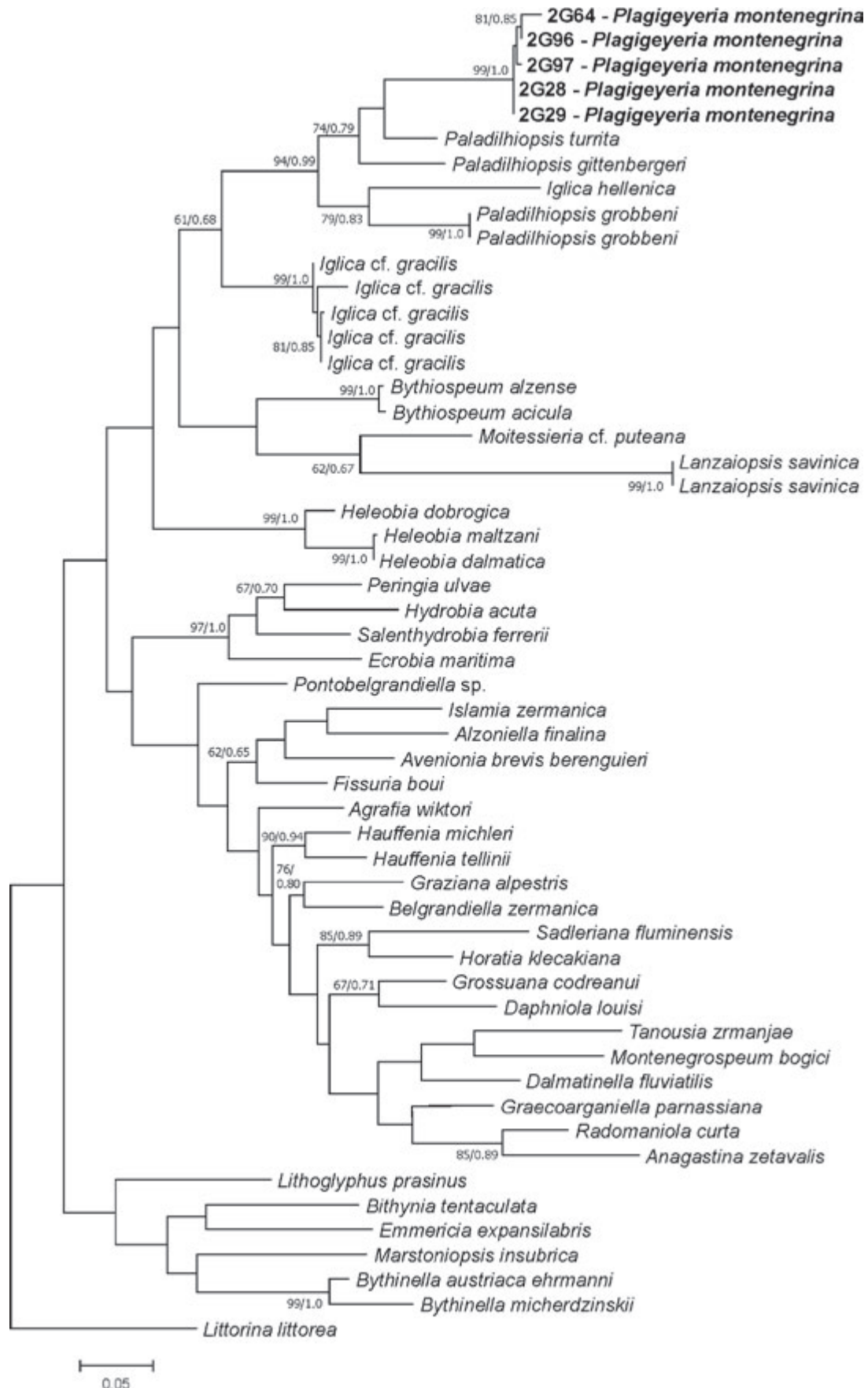


Figure 8 Maximum Likelihood tree computed for COI. Bootstrap supports and Bayesian probabilities are given.

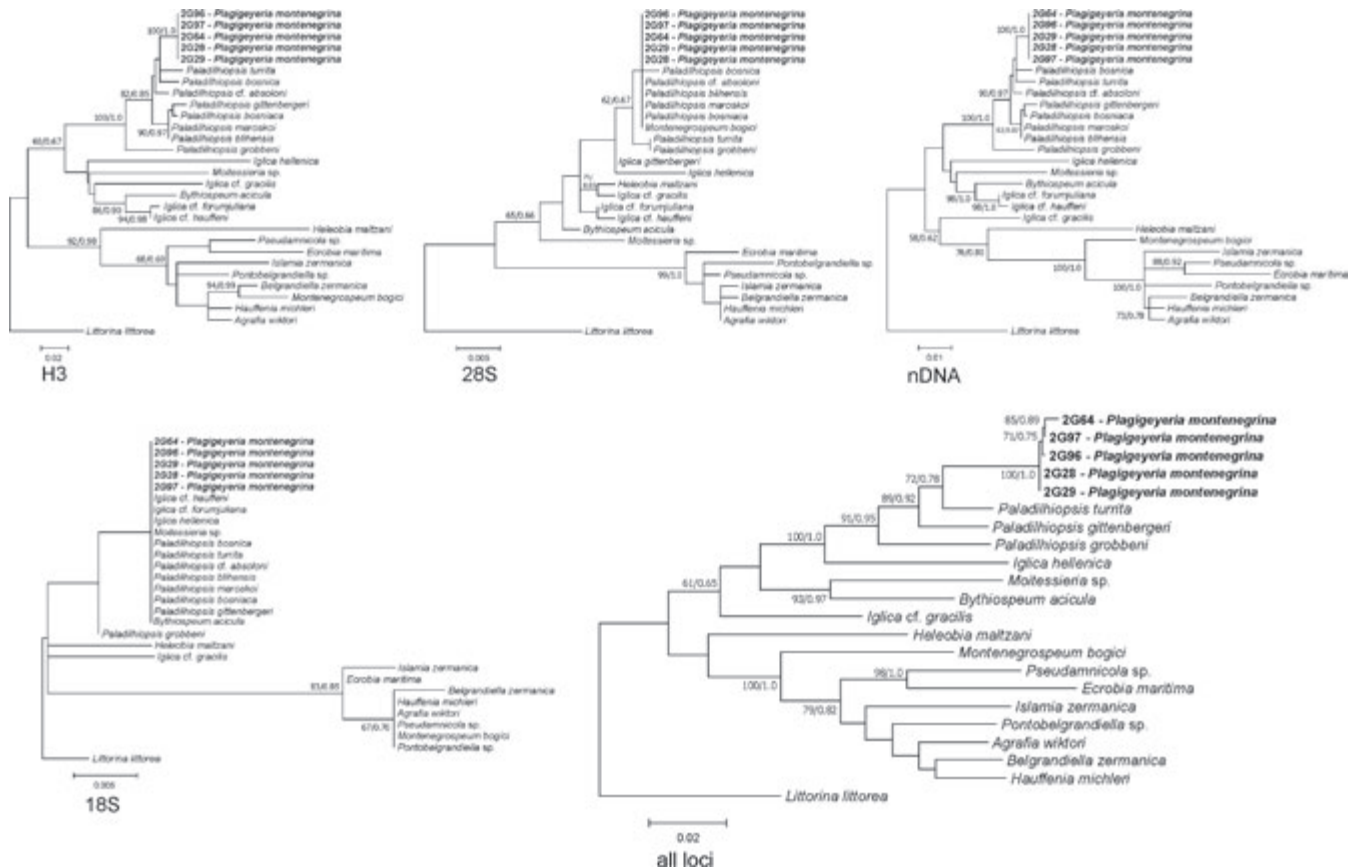


Figure 9 Maximum Likelihood trees inferred from 18S, 28S, H3, all nuclear loci (nDNA) as well as from concatenated COI and nuclear sequences. Bootstrap supports and Bayesian probabilities are given.

in the Truncatelloidea are not sufficient even for distinction of a species.

The differential diagnosis in the description of the genus *Saxurinator* given by Schütt (1960) lists characters often not sufficient to distinguish a truncatelloid species, to say nothing on genus, and clearly does not support the distinction of the *Saxurinator*. For any natural taxon there should be always at least one synapomorphy defining it. There is no such synapomorphy to be found in *Saxurinator*. The genera *Plagigeyeria* and *Saxurinator* are most probably not distinct, but, following the ICZN rules, they are still valid since their type species have not been examined. For now, there is enough evidence that “*Plagigeyeria*” *montenegrina* belongs to the genus *Paladilhopsia*.

Anatomical characters, especially of the reproductive system, became a standard in species-level taxonomy in hydrobioids. The anatomy of the female reproductive system as well as the morphology of the penis were considered in species descriptions and diagnoses (see Falniowski,

2018 for the references). On the other hand, some authors presented opinion that between the closely related representatives of the same genus differences in anatomy are not necessarily present, or even would not be expected (e. g. Radoman, 1976, 1983). The ‘lock-and-key’ mechanism, known mostly in arthropods, is hardly expected in molluscs, whose copulatory organs lack any sclerotized structures, and are more variable than it is usually noted (Falniowski, 2018). Thus, neither variable shell characters, nor slight differences in the anatomy, are sufficient to delimit a species in the hydrobioid (truncatelloid) snails. Molecular characters – DNA sequences – are helpful, but should be interpreted with appropriate care, since they reconstruct the phylogeny of a gene which should not necessarily be the same as the phylogeny of an organism (e. g. Avise, 2000).

Species delimitation in the stygobiont hydrobioid snails, however, suffers not only because of deficit of available characters, but on the commonly accepted ideas about the strict isolation

of each subterranean habitat promoting rapid speciation, as well. The latter could hardly be demonstrated. As concerns the supposed isolation: at first, the subterranean habitats of a various kind are not as rare as is usually supposed (e.g. Culver & Pipan, 2009, 2014). And, especially in stygobiotic inhabitants of subterranean habitats, one can expect the interstitial habitats as their ways of expansion. Our findings of the empty shells of *Plagigeyeria* at three interstitial localities of the Skadar Lake basin, situated along a distance of about 50km, suggest the interstitial habitats as a possible cross connection between “isolated” caves and springs.

The isolation of the stygobiotic molluscs is apparently not complete, as the single karst conduits would suggest. More extensive knowledge of the complicated karst hydrology of the Dinarides would be needed for better understanding of the potential subterranean ways of expansion thus gene flow, especially during the intermittent and seasonal fluctuations of karstwater among specific karst conduits, with alternating directions of the subterranean water flow (Grego, 2020). Typical phenomena demonstrating the complicated web of Dinaride karst water circulation are called ‘estavellas’, the function of a hole alternates seasonally either as a karst spring or a swallow hole. Additionally, the alluvial beds and spring deposit with their interstitial habitats could act as a migration corridor for many stygobiotic gastropods, especially under the large karst basins called Polje (“field”) (Grego, 2020). All the facts listed above should necessarily be considered in any further concepts of a stygobiont gastropod species.

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