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HORATIA BOURGUIGNAT, 1887: IS THIS GENUS REALLY PHYLOGENETICALLY VERY CLOSE TO *RADOMANIOLA* SZAROWSKA, 2006 (CAENOGASTROPODA: TRUNCATELLOIDEA)?

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ABSTRACT: *Horatia* Bourguignat, 1887 was the first genus established for hydrobiid snails with valvatoid shell, and numerous valvatoid-shelled hydrobioids were classified as *Horatia*. The genus was the type one for some tribe/family-rank taxa. Thus it is one of the "crucial" hydrobiid genera. *Horatia* seems to inhabit only Croatia and Macedonia, and its type species: *H. klecakiana* Bourguignat, 1887, inhabits the springs in the Cetina River Valley. In the present paper the shell, operculum, soft part pigmentation, protoconch SEM microsculpture, female reproductive organs, and penis of *H. klecakiana* from the spring Studenci, N of Kučiće, in the valley of the Cetina River, Croatia, are described, to confirm the identity of the studied specimens with this species. Mitochondrial cytochrome oxidase subunit I (COI) and nuclear 18S ribosomal RNA gene sequences are used to infer phylogenetic relationships of *Horatia*, especially with *Radomaniola* and the sequence of *Horatia* from GenBank. The results suggest close relationships of the genus with *Sadleriana*, not with *Radomaniola*.

KEY WORDS: Truncatelloidea, COI, 18S rRNA, protoconch, anatomy, molecular phylogeny

INTRODUCTION

BOURGUIGNAT (1887) described a new genus *Horatia*, with its type species *H. klecakiana* Bourguignat, 1887, from "sorgente près de Ribaric, dans la vallée de Cetina" in Croatia. RADOMAN (1983) identified this type locality with the Vrijovac spring in the source area of the Cetina River. *Horatia* was the first nominal genus described for the European hydrobiids with valvatiform shell (BODON et al. 2001). Thus, it is one of the "crucial" hydrobiid genera. For example, TAYLOR (1966) established Horatiini as tribe in Hydrobiidae, within the subfamily Cochliopinae, and BOLE (1993) established a distinct family Horatiidae. RADOMAN (1973) included *Horatia* in Orientalinidae. KABAT & HERSHLER (1993) presented a review of understanding of this genus in the literature. RADOMAN (1983) listed three species of *Horatia* from the former Yugoslavia: *H. klecakiana*, *H. novoselensis* Radoman, 1966, and *H. macedonica* (Kuščer, 1936). Species of *Horatia* were reviewed by SCHÜTT (1961), BOETERS (1974, 1998) and BOLE (1993). According to ANGELOV (1967) *Horatia* is known from Bulgaria as well, but this seems doubtful.

In fact, there is a number of valvatiform hydrobiid snails, often minute in size, and with hardly known anatomy, many of them assigned to *Horatia*. *Horatia sturmi* (Rosenhauer, 1856) from Spain (= *Boetersiella sturmi*: ARCONADA & RAMOS 2001) could be an example. Another "*Horatia*" described from Greece, *H. hadei* (GITTENBERGER 1982) belongs to the genus *Daphniola* Radoman, 1973 (FALNIOWSKI & SZAROWSKA 2011a). Anyway, the identity and the phylogenetic position of the "real" *Horatia* remain enigmatic.

WILKE et al. (2001), applying molecular data, inferred phylogenetic position of *Horatia*, very close to *Radomaniola*, and the same was confirmed in recent

study (WILKE et al. 2013). However, this placement raises doubts. The single sequence in GenBank, used several times, also in our studies (e.g. SZAROWSKA 2006, FALNIOWSKI & SZAROWSKA 2011b, SZAROWSKA & FALNIOWSKI 2011), always with the same placing of *Horatia* very close to *Radomaniola* Szarowska, 2006, was intriguing. As co-authors of the study of WILKE et al. (2001), introducing this sequence, and thus co-authors of this possible error, we felt obliged to check the morphology of *Horatia klecakiana* from the Cetina Valley, to confirm that it belongs to this species, then to check if its cytochrome oxidase subunit I (COI) sequence might represent the same species as the sequence of *Horatia* in GenBank, and, finally, to infer its phylogenetic relationships using molecular data.

MATERIAL AND METHODS

About twenty specimens of *Horatia klecakiana* were collected, using a sieve (0.5 mm mesh diameter), from the spring Studenci, N of Kučiće, in the valley of the Cetina River, Croatia, 43°26'41.3"N, 16°48'25.5"E, 45 m a. s. l., on the 21st of June 2011.

Snails were washed twice in 80% ethanol and left to stand in it for around 12 hours. Then the ethanol was changed twice more within 24 hours and finally, after a few days, the 80% solution was replaced with a 96% one, in which the samples were stored at –20°C. The shells were photographed with a CANON EOS 50D digital camera. Five adult males and five females were dissected, using a NIKON SMZ-U stereoscope microscope. The penis and female genitalia (pallial oviduct) were examined using a MOTIC light microscope. The protoconch (after ultrasonic cleaning) was examined using a JEOL JSM-5410 scanning electron microscope, applying the techniques described by FALNIOWSKI (1990).

DNA was extracted from foot tissue of seven specimens not presented in the photographs. 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 buffer. The PCR reaction was performed with the following primers: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') (FOLMER et al. 1994) and COR722b (5'-TAAACTTCAGGGTGACCAAAAAATYA-3') (WILKE & DAVIS 2000) for the cytochrome oxidase subunit I (COI) mitochondrial gene and SWAM18SF1 (5'-GAATGGCTCATTAAATCAGTCGAGGTTCCTTAGATGATCCAAATC-3'), and SWAM18SR1 (5'-ATCCTCGTTAAAGGGTTTAAAGTGTACTCATTCCAATTACGG AGC-3') for the 18S rRNA gene (PALUMBI 1996). The PCR conditions were as follows: COI – initial denaturation step of 4 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and a final extension of 4 min at 72°C; 18S – initial denaturation step of 4 min at 94°C, followed by 40 cycles of 45 s at 94°C, 45 s at 51°C, 2 min at 72°C and, after all cycles were completed, an additional elongation step of 4 min at 72°C was performed. The total volume of each PCR reaction mixture was 50 μl. To check the quality of the PCR products 10 μl of the PCR product was run on 1% agarose gel. The PCR products were purified using Clean-Up columns (A&A Biotechnology) and were then amplified in both directions (HILLIS et al. 1996) using BigDye Terminator v3.1 (Applied Biosystems), following the manufacturer's protocol and with the primers described above. The sequencing reaction products were purified using ExTerminator Columns (A&A Biotechnology); DNA sequences then underwent electrophoresis on an ABI Prism sequencer. The sequences were deposited in GenBank (Table 1).

Five sequences of COI of *Radomaniola* from the GenBank (WILKE et al. 2001, FALNIOWSKI et al. 2012) were used, together with the one of *Horatia* from GenBank, and of our *Horatia* were used to calculate pairwise p-distances with MEGA5.10 (TAMURA et al. 2011). MEGA was also used to infer maximum likelihood (ML) tree, with the same methodology as described below.

In the phylogeny reconstruction, we used sequences from 29 rissooid taxa from GenBank (Table 1). Seven of them, used as an outgroup, represented the main non-hydrobiid lineages within the Rissooidea (WILKE et al. 2001); the other seven taxa represented the Hydrobiinae (including "Pyrgulinae": SZAROWSKA et al. 2005). The remaining taxa were chosen to represent all the main lineages within the European Sadlerianinae (SZAROWSKA 2006).

The COI sequences were aligned by eye using BioEdit 5.0.0 (HALL 1999) and edited with MACCLADE 4.05 (MADDISON & MADDISON 2002). For 18S, an initial alignment was performed using CLUSTALX 1.82 (THOMPSON et al. 1997) and edited with MACCLADE. Mutational saturation for the COI dataset was examined by plotting the numbers of transitions and transversions for all the codon positions together, and for the 3rd position separately, against the percentage sequence divergence, using DAMBE 5.2.9 (XIA 2000). We also used DAMBE 5.2.9 to perform the saturation test (XIA et al. 2003). It revealed a significant degree of saturation in the third position of the sequences. In rissooids, COI approaches saturation with about 18.6% or 120 nucleotide differences (DAVIS et al. 1998), which seems to happen after approximately 10 million years. However, to avoid a substantial loss of information in the case of closely related species, this position was not excluded from the dataset and it was used for the analysis. In fact, the analysis conducted on 2nd and 3rd position only resulted in similar deep

Table 1. Taxa used for phylogenetic analyses, with their GenBank Accession Numbers and references

Species	18S GB#	COI GB#	References
Adriohydrobia gagatinella (Küster, 1852)	AF367657	AF317881	Wilke & Falniowski (2001)
Adrioinsulana conovula (Frauenfeld, 1863)	AF367656	AF367628	Wilke et al. (2001)
Agrafia wiktori Szarowska et Falniowski, 2011	JF906758	JF906762	Szarowska & Falniowski (2011)
Alzoniella finalina Giusti et Bodon, 1984	AF367686	AF367650	Wilke et al. (2001)
Anagastina zetavalis (Radoman, 1973)	EF070622	EF070616	Szarowska (2006)
Bithynia tentaculata (Linnaeus, 1758)	AF367675	AF367643	Wilke et al. (2001)
Boleana umbilicata (Kuščer, 1932)	JX982797	JX982795	Falniowski & Szarowska (2012)
Bythinella austriaca (Frauenfeld, 1857)	AF212917	FJ545132	Falniowski et al. (2009)
Bythiospeum sp.	AF367664	AF367634	Wilke et al. (2001)
Dalmatinella fluviatilis Radoman, 1973	KC344539	KC344541	Falniowski & Szarowska (2013)
Daphniola graeca Radoman, 1973	EF070624	EF070618	Szarowska (2006)
Dianella thiesseana (Kobelt, 1878)	AY676125	AY676127	Szarowska et al. (2005)
Graecoarganiella parnassiana Falniowski et Szarowska, 2011	JN202341	JN202348	Falniowski & Szarowska (2011b)
Graziana alpestris (Frauenfeld, 1863)	AF367673	AF367641	Wilke et al. (2001)
Grossuana codreanui (Grossu, 1946)	EF061916	EF061919	Szarowska et al. (2007)
Hauffenia tellinii (Pollonera, 1898)	AF367672	AF367640	Wilke et al. (2001)
Heleobia dalmatica (Radoman, 1974)	AF367661	AF367631	Wilke et al. (2001)
Horatia klecakiana Bourguignat, 1887	KJ159127	KJ159128	present study
"Horatia" sturmi (Rosenhauer, 1856)	AF212912	AF213345	Wilke et al. (2000)
Hydrobia acuta (Draparnaud, 1805)	AF367680	AF278808	Wilke & Davis (2000)
Islamia piristoma Bodon et Cianfanelli, 2001	AF367671	AF367639	Wilke et al. (2001)
Lithoglyphus naticoides (C. Pfeiffer, 1828)	AF367674	AF367642	Wilke et al. (2001)
Marstoniopsis insubrica (Küster, 1853)	AF367676	AY027813	Falniowski & Wilke (2001)
Pseudamnicola lucensis (Issel, 1866)	AF367687	AF367651	Wilke et al. (2001)
Pyrgula annulata (Linnaeus, 1767)	AY676124	AY341258	Szarowska et al. (2005)
Radomaniola callosa (Paulucci, 1881)	AF367685	AF367649	Wilke et al. (2001)
Rissoa labiosa (Montagu, 1803)	AY676126	AY676128	Szarowska et al. (2005)
Sadleriana fluminensis (Küster, 1853)	AF367683	AY273996	Wilke et al. (2001)
Trichonia kephalovrissonia Radoman, 1973	EF070630	EF070619	Szarowska (2006)
Ventrosia ventrosa (Montagu, 1803)	AF367681	AF118335	Wilke & Davis (2000)

phylogeny, but with several polytomies within more terminal nodes.

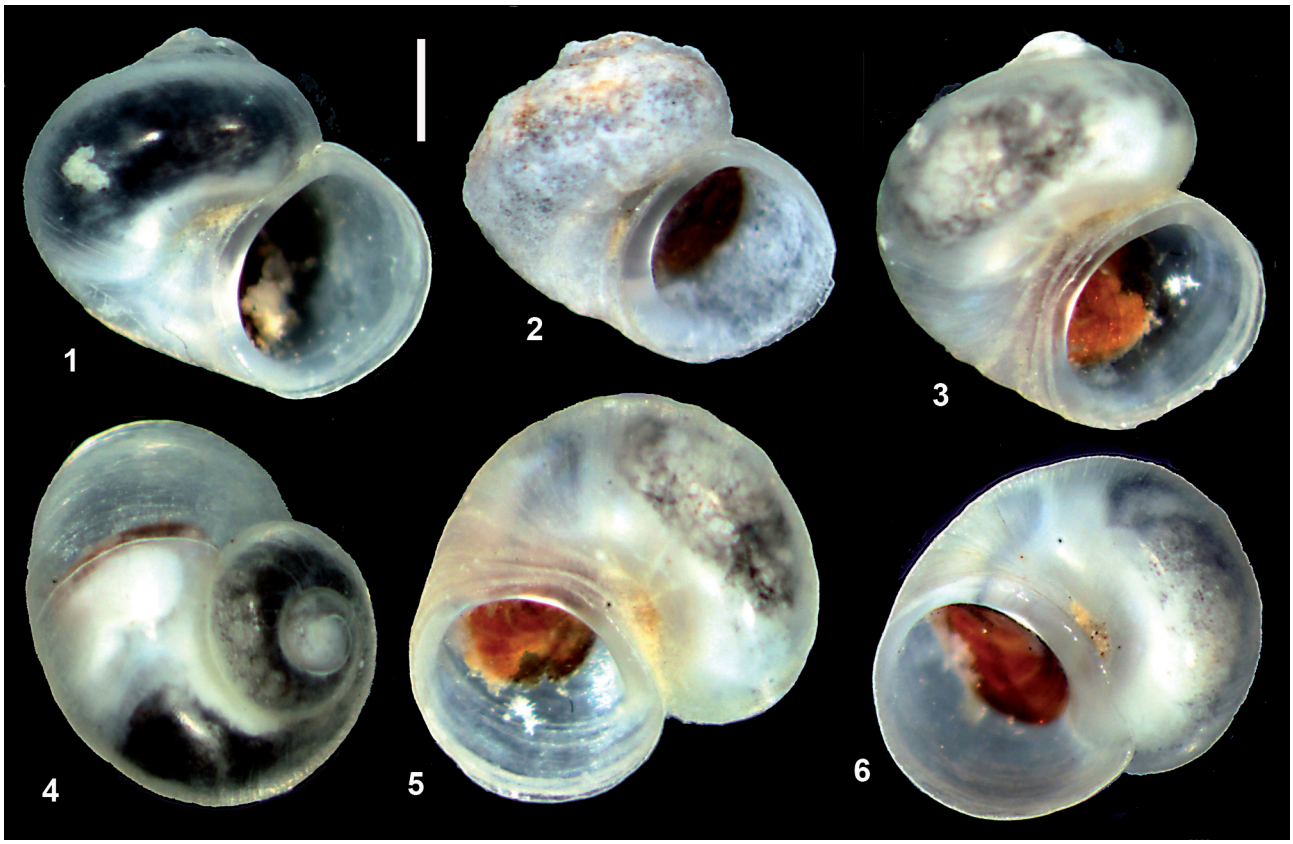
Initially, we performed phylogeny reconstruction for 18S and COI data separately, using the maximum likelihood (ML) technique. Next, the partition homogeneity test (Farris et al. 1995) was performed (1,000 replicates) with PAUP*4.0b10 (Swofford 2002), to check whether the two genes could be analysed together. Since $p>0.783$, the maximum likelihood heuristic search was then run for the combined molecular data. Following the recommendations of Posada & Buckley (2004) and Sober (2002), the best model for each dataset was chosen using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC); both chose the same model. We performed ML analyses in PAUP* and used a heuristic search strategy with stepwise addition of taxa, 10 random-sequence addition replicates, and tree-bisection-reconnection (TBR) branch swapping (Swofford et al. 1996), and with MEGA5.10 (Tamura et al. 2011). Nodal support was estimated using the bootstrap (BS) approach (Felsenstein 1985). Bootstrap values for ML trees were calculated using 10,000 bootstrap replicates, with MEGA5.10 and the same model parameters as for ML analysis.

RESULTS

Shell (Figs 1–6) minute, valvatiform, with rapidly growing whorls, continuous peristome and slit-like umbilicus, whitish and translucent; operculum orange.

Mantle intensively pigmented black (Figs 1, 4), or its pigmentation is less intensive (Figs 3, 5, 6), there is nearly no pigment on the head, eyes are present.

Protoconch (Fig. 7) formed by about 1¼ whorl, its sculpture regular and characteristic (Figs 8–9).



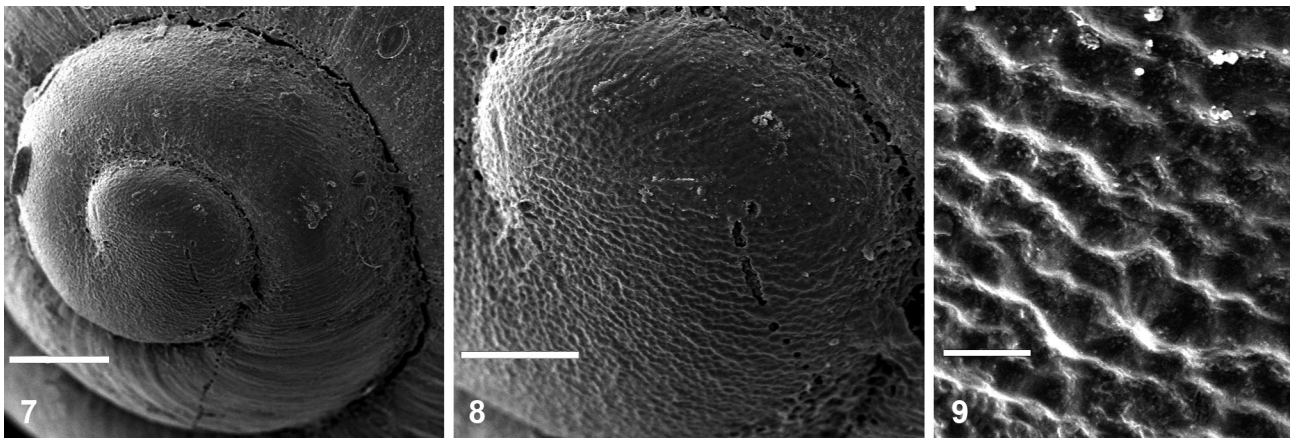
Figs 1–6. Shells of *Horatia klecakiana*, scale bar equals 0.5 mm

Female reproductive organs (Fig. 10) with two moderately big receptacula, rs_1 somewhat bigger than rs_2. Bursa copulatrix big, pear-shaped, with long duct. Loop of "renal" oviduct massive. Penis (Fig. 11) with sharp tip and a bi-lobed outgrowth on its left side, vas deferens visible inside, running nearly straight.

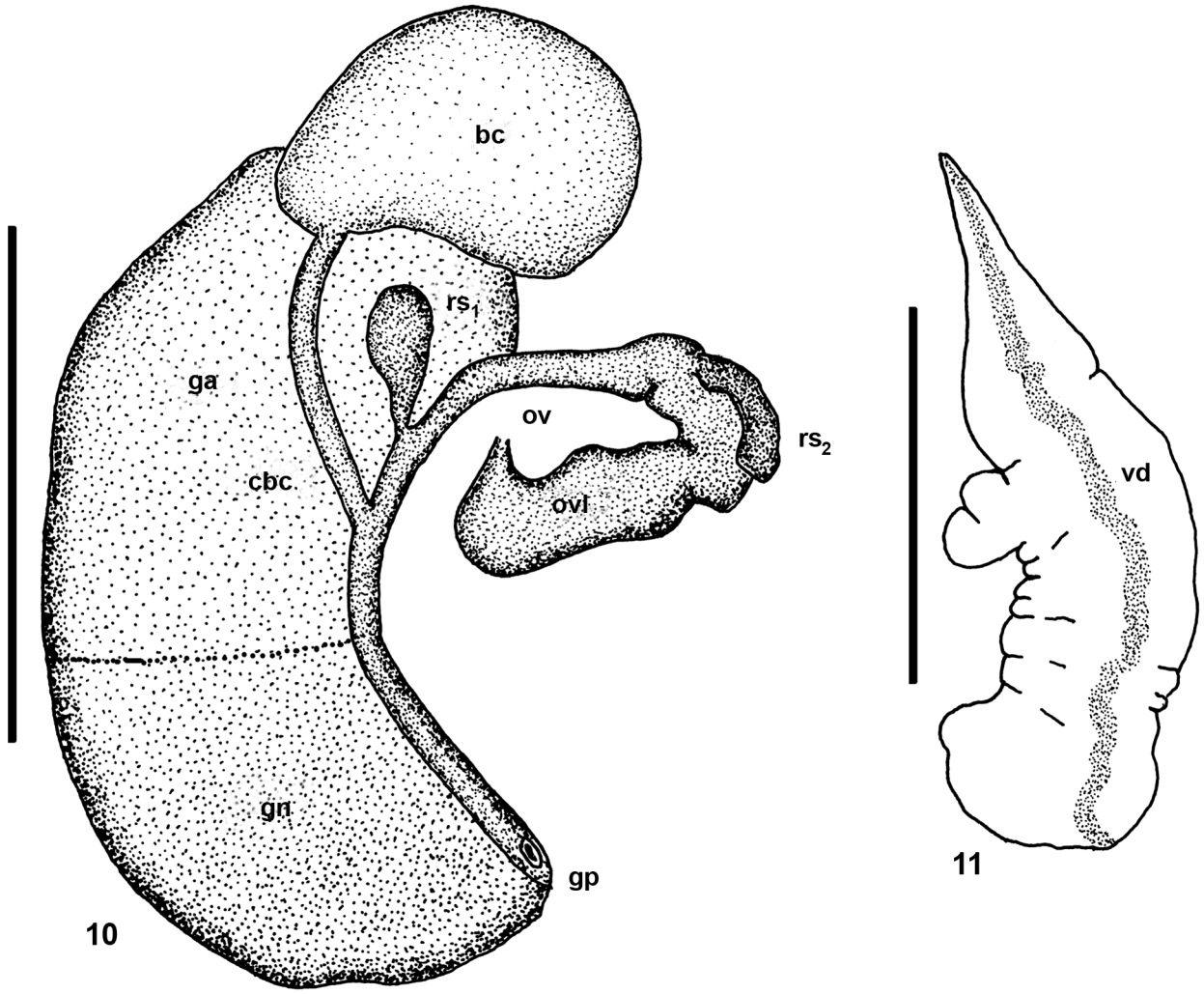
Seven sequences of cytochrome oxidase subunit I (COI) were identical – there was no intrapopulation variability. There was also no variability between 18S sequences.

Pairwise p-distances between COI sequences of our *Horatia*, five species of *Radomaniola*, and the sequence of *Horatia* from GenBank (Table 2) were characteristic of the species level between all the species of *Radomaniola* and between all of them and *Horatia* from GenBank, but more than twice higher between all six species and our *Horatia*. In the inferred ML tree (with the model of Tamura 3-parameter +Γ) *Horatia* from the GenBank clustered within the *Radomaniola* group (Fig. 12), and the bootstrap support for the clade including all the species but our *Horatia* was as high as 81%.

For the combined data set the Bayesian Information Criterion (BIC) and corrected Akaike Information Criterion (AICc) with MEGA5 found



Figs 7–9. Protoconch of *Horatia klecakiana*, scale bars equal 100 μm, 50 μm and 5 μm, respectively



Figs 10–11. *Horatia klecakiana*: 10 – female reproductive organs (bc – bursa copulatrix, cbc – duct of bursa copulatrix, ga – albuminoid gland, gn – nidamental gland, gp – gonoporus, ov – oviduct, ovl – loop of oviduct, rs_1, rs_2 – receptaculum seminis 1 and 2, respectively); 11 – penis (vd – vas deferens); scale bars equal 0.5 mm

model TN93 (Tamura-Nei 1993 – NEI & KUMAR 2000) + I + Γ, with base frequencies: A = 0.259, C = 0.209, G = 0.222, T = 0.310; substitution rate matrix: [A-C] = 0.031, [C-A] = 0.038, [A-G] = 0.134, [G-A] = 0.157, [A-T] = 0.045, [T-A] = 0.038, [C-G] = 0.033, [G-C] = 0.031, [C-T] = 0.248, [T-C] = 0.167, [G-T] = 0.045, [T-G] = 0.033; proportion of invariable sites: (I) = 0.63, Γ distribution with the shape parameter = 0.68, and transition/transversion bias R = 2.37.

The inferred phylogram (Fig. 13) obviously does not confirm that *Boetersiella sturmi*, previously known as *Horatia* (and the only *"Horatia"* apart from *H. klecakiana* whose sequence could be found in GenBank), belongs to the genus *Horatia*: its closest taxa are *Alzoniella* Giusti et Bodon, 1984, and

Table 2. Pairwise p-distances between COI sequences of five species of *Radomaniola*, *"Horatia klecakiana"* from GenBank, and our *H. klecakiana*. GenBank accession numbers after WILKE et al. (2001), FALNIOWSKI et al. (2012) and present study

	1	2	3	4	5	6
1. AF367649 *Radomaniola callosa*						
2. KC011803 *Radomaniola montana*	0.0453					
3. KC011809 *Radomaniola curta anagastica*	0.0489	0.0399				
4. KC011810 *Radomaniola curta curta*	0.0471	0.0453	0.0054			
5. KC011814 *Radomaniola curta narentana*	0.0435	0.0380	0.0362	0.0417		
6. AF367637 *"Horatia klecakiana"*	0.0507	0.0634	0.0562	0.0507	0.0634	
7. KJ159128 *Horatia klecakiana*	0.1721	0.1558	0.1522	0.1522	0.1649	0.1612

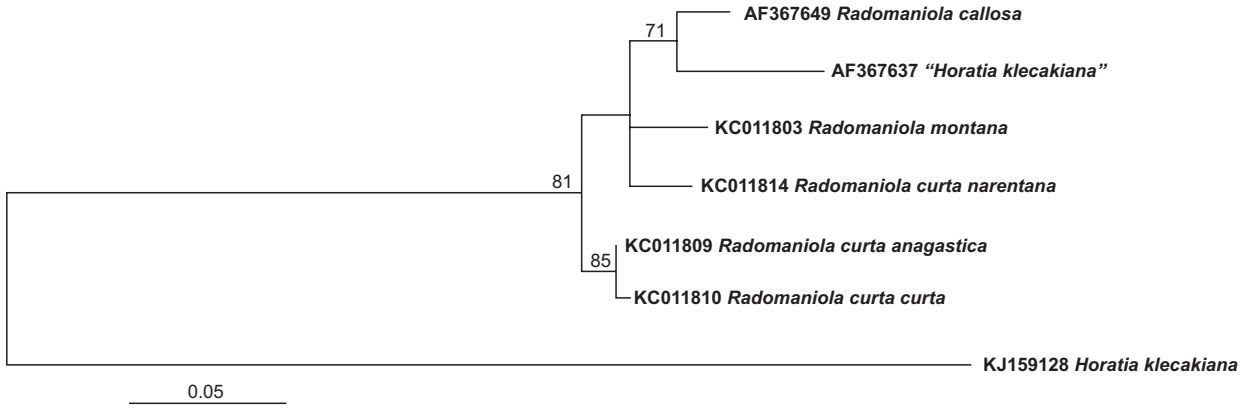


Fig. 12. Maximum likelihood tree of COI sequences for five species of *Radomaniola*, "*Horatia klecakiana*" from GenBank, and our *H. klecakiana*. GenBank accession numbers after WILKE et al. (2001), FALNIOWSKI et al. (2012) and present study

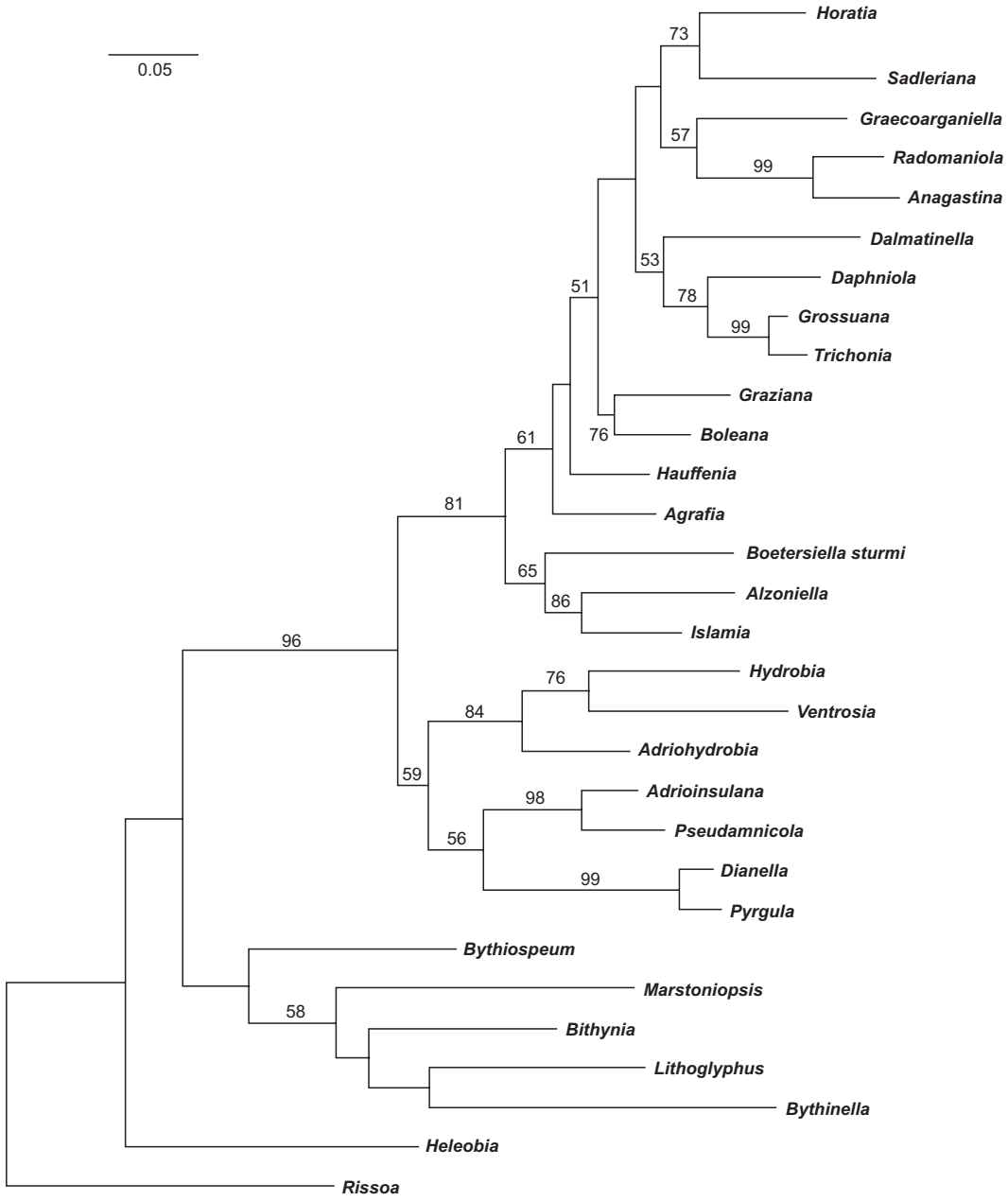


Fig. 13. Maximum likelihood tree of the two concatenated sequences (18S and COI), bootstrap supports (10,000 replicates) given if >50%

Islamia Radoman, 1973. *Horatia* belongs to the family Hydrobiidae (bootstrap support 96%), and to the subfamily Sadlerianinae Szarowska, 2006 (bootstrap support 81%), certainly not to Cochliopidae (represented by *Heleobia* Stimpson, 1865). *Horatia* belongs to the same clade with *Sadleriana* (bootstrap support 73%). *Radomaniola* forms a distinct clade with *Anagastina* Radoman, 1973, and *Graecoarganiella* Falniowski et Szarowska, 2011 (although the bootstrap support is low: 57%). The bootstrap support of the clade common to our *Horatia* and *Radomaniola* is as low as 29%.

DISCUSSION

The shells and opercula resemble the ones presented and described for *H. klecakiana* by SCHÜTT (1961), BOETERS (1974), RADOMAN (1983), BOLE (1993) and BODON et al. (2001). There is a protoconch of *H. klecakiana* presented by BODON et al. (2001); however, nothing but some diatoms can be seen on it.

Both the female reproductive organs and the penis resemble the ones drawn and described by BOETERS (1974), RADOMAN (1983), BOLE (1993) and BODON et al. (2001). RADOMAN (1983) presented a wide range of variability of the penis in *H. klecakiana*. All the morphological data confirm the assignment of our specimens to the type species of the genus *Horatia*. This, coupled with as many as seven specimens sequenced, all of them identical, strengthens our results.

Molecular data confirm that *H. klecakiana* belongs to the Hydrobiidae, not Cochliopidae as suggested by TAYLOR (1966), and it belongs to Sadlerianinae, as proposed by SZAROWSKA (2006). Both p-distances' values and ML tree for *Radomaniola*, our *Horatia*, and *Horatia* from GenBank clearly show that the latter belongs to *Radomaniola*. We can only speculate about the reasons for this mistake. In our phylogeny the sister taxon of *H. klecakiana* is *Sadleriana*, certainly not *Radomaniola*. In this case molecularly based systematics resembles the morphology-based one of RADOMAN (1973, 1983).

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REFERENCES

ANGELOV A. 1967. *Horatia (Hauffenia) lucidulus* n. sp., ein neuer Vertreter der Mollusken Fauna Bulgariens. Arch. Moll. 96: 145–148.

ARCONADA B., RAMOS M. A. 2001. New data on Hydrobiidae systematics: two new genera from the Iberian Peninsula. J. Nat. Hist. 35: 949–984. http://dx.doi.org/10.1080/002229301300323884

BODON M., MANGANELLI G., GIUSTI F. 2001. A survey of the European valvatiform hydrobiid genera, with special reference to *Hauffenia* Pollonera, 1898 (Gastropoda: Hydrobiidae). Malacologia 43: 103–215.

BOETERS H. D. 1974. *Horatia* Bourguignat, *Plagigeyeria* Tomlin und *Litthabitella* Boeters (Prosobranchia). Arch. Moll. 104: 85–92.

BOETERS H. D. 1998. Mollusca: Gastropoda: Superfamilie Rissooidea. Süsswasserfauna von Mitteleuropa. Begründet von A. Brauer, 5/1–2, Gustav Fischer Verlag, Jena–Lübeck–Ulm.

BOLE J. 1993. Podzemelijski polzi is druzine Horatiidae (Gastropoda, Prosobranchia) v Sloveniji in njihov taksonomski polozaj. Razprave, Slov. Akad. Znan. Umetn. Razr. Prirodos. Medic. Vede Odd. Prirodos. Vede 34: 3–11.

BOURGUIGNAT J.-R. 1887. Étude sur les noms génériques des petites paludinidées a opercule spirescent suivie de la description du nouveau genre *Horatia*. V. Tremblay, Paris. http://dx.doi.org/10.5962/bhl.title.10453

DAVIS G. M., WILKE T., SPOLSKY C., QIU C.-P., QIU D.-C., XIA M.-Y., ZHANG Y., ROSENBERG G. 1998. Cytochrome oxidase I-based phylogenetic relationships among the Pomatiopsidae, Hydrobiidae, Rissoidae and Truncatelidae (Gastropoda: Caenogastropoda: Rissoacea). Malacologia 40: 251–266.

FALNIOWSKI A. 1990. Anatomical characters and SEM structure of radula and shell in the species-level taxonomy of freshwater prosobranchs (Mollusca: Gastropoda: Prosobranchia): a comparative usefulness study. Folia Malacol. 4: 53–142.

FALNIOWSKI A., SZAROWSKA M. 2011a. Genus *Daphniola* Radoman, 1973 (Caenogastropoda: Hydrobiidae) in the Peloponnese, Greece. Folia Malacol. 19: 131–137. http://dx.doi.org/10.2478/v10125-011-0020-9

FALNIOWSKI A., SZAROWSKA M. 2011b. A new genus and new species of valvatiform hydrobiid (Rissooidea; Caenogastropoda) from Greece. Moll. Res. 31: 189–199.

FALNIOWSKI A., SZAROWSKA M. 2012. Phylogenetic position of *Boleana umbilicata* (Kuščer, 1932) (Caenogastropoda: Rissooidea). Folia Malacol. 20: 265–270. http://dx.doi.org/10.2478/v10125-012-0022-2

FALNIOWSKI A., SZAROWSKA M. 2013. Phylogenetic relationships of *Dalmatinella fluviatilis* Radoman, 1973 (Caenogastropoda: Rissooidea). Folia Malacol. 21: 1–7. http://dx.doi.org/10.12657/folmal.021.001

FALNIOWSKI A., SZAROWSKA M., GLÖER P., PEŠIĆ V. 2012. Molecules vs morphology in the taxonomy of *Radomaniola/Grossuana* group of the Balkan Rissooidea (Mollusca, Caenogastropoda). J. Conch. 41: 19–36.

FALNIOWSKI A., SZAROWSKA M., SIRBU I. 2009. *Bythinella* Moquin-Tandon, 1856 (Gastropoda: Rissooidea: Bythinellidae) in Romania: species richness in a glacial refugium. J. Nat. Hist. 43: 2955–2973. http://dx.doi.org/10.1080/00222930903359636

FALNIOWSKI A., WILKE T. 2001. The genus *Marstoniopsis* (Gastropoda: Rissooidea): intra- and intergeneric phylogenetic relationships. J. Mollus. Stud. 67: 483–488. http://dx.doi.org/10.1093/mollus/67.4.483

FARRIS J. S., KÄLLERSJÖ M., KLUGE A. G., BULT C. 1995. Testing significance of incongruence. Cladistics 10: 315–319. http://dx.doi.org/10.1111/j.1096-0031.1994.tb00181.x

FELSENSTEIN J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. http://dx.doi.org/10.2307/2408678

FOLMER O., BLACK M., HOEH W., LUTZ R. A., VRIJENHOEK R. C. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

GITTENBERGER E. 1982. Eine neue *Horatia* Art aus Griechenland (Prosobranchia: Hydrobiidae). Basteria 46: 68.

HALL T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.

HILLIS D. M., MABLE B. K., LARSON A., DAVIS S. K., ZIMMER E. A. 1996. Nucleic acids IV: sequencing and cloning. In: HILLIS D. M., MORITZ C., MABLE B. K. (eds). Molecular systematics. 2 ed. Sinauer Associates Inc., Sunderland, Massachusetts, pp. 321–381.

KABAT A. R., HERSHLER R. 1993. The prosobranch snail family Hydrobiidae (Gastropoda: Rissooidea): review of classification and supraspecific taxa. Smithson. Contrib. Zool. 547: 1–94. http://dx.doi.org/10.5479/si.00810282.547

MADDISON D. R., MADDISON W. P. 2002. MACCLADE. Ver. 4.05. [Computer software and manual]. Sinauer Associates Inc., Sunderland, Massachusetts.

NEI M., KUMAR S. 2000. Molecular evolution and phylogenetics. Oxford University press, Oxford, UK–New York.

PALUMBI S. R. 1996. Nucleic acids II: the polymerase chain reaction. In: HILLIS D. M., MORITZ C., MABLE B. K. (eds). Molecular Systematics. 2 ed. Sinauer Associates Inc., Sunderland, Massachusetts, pp. 205–247.

POSADA D., BUCKLEY T. R. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over Likelihood Ratio Tests. Syst. Biol. 53: 793–808. http://dx.doi.org/10.1080/10635150490522304

RADOMAN P. 1973. New classification of fresh and brackish water Prosobranchia from the Balkans and Asia Minor. Posebna Izdanja, Prirodn. Mus. Beograd 32: 1–30.

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

SOBER E. 2002. Instrumentalism, parsimony, and the Akaike framework. Philos. Sci. 69: 112–123. http://dx.doi.org/10.1086/341839

SCHÜTT 1961. Das Genus *Horatia* Bourguignat. Arch. Moll. 90: 145–152.

SWOFFORD D. L. 2002. PAUP*–Phylogenetic analysis using parsimony (* and other methods). Ver. 4. [Computer software and manual]. Sinauer Associates Inc., Sunderland, Massachusetts.

SWOFFORD D. L., OLSEN G. J., WADDELL P. J., HILLIS D. M. 1996. Phylogenetic Inference. In: HILLIS D. M., MORITZ C., MABLE B. K. (eds), Molecular systematics. 2 ed. Sinauer Associates Inc., Sunderland, Massachusetts, pp. 407–514.

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

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. J. Nat. Hist. 45: 2231–2246. http://dx.doi.org/10.1080/00222933.2011.591067

SZAROWSKA M., FALNIOWSKI A., RIEDEL F., WILKE T. 2005. Phylogenetic relationships of the subfamily Pyrgulinae (Gastropoda: Caenogastropoda: Hydrobiidae) with emphasis on the genus *Dianella* Gude, 1913. Zootaxa 891: 1–32.

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. http://dx.doi.org/10.1007/s10750-006-0530-4

TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M., KUMAR S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method. Mol. Biol. Evol. 28: 2731–2739. http://dx.doi.org/10.1093/molbev/msr121

TAYLOR D. W. 1966. A remarkable snail fauna from Coahuila, México. Veliger 9: 152–228.

THOMPSON J. D., GIBSON T. J., PLEWNIAK F., JEANMOUGIN F., HIGGINS D. G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24: 4876–4882. http://dx.doi.org/10.1093/nar/25.24.4876

WILKE T., DAVIS G. M. 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? Biol. J. Linn. Soc. 70: 89–105. http://dx.doi.org/10.1111/j.1095-8312.2000.tb00202.x

WILKE T., DAVIS G. M., FALNIOWSKI A., GIUSTI F., BODON M., SZAROWSKA M. 2001. Molecular systematics of Hydrobiidae (Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. Proc. Acad. Nat. Sci. Philadelphia 151: 1–21. http://dx.doi.org/10.1635/0097-3157(2001)151[0001:MSOHMG]2.0.CO;2

WILKE T., DAVIS G. M., GONG X., LIU H. X. 2000. *Erhaia* (Gastropoda: Rissooidea): phylogenetic relationships and the question of *Paragonimus* coevolution in Asia. Am. J. Trop. Med. Hyg. 62: 453–459.

WILKE T., FALNIOWSKI A. 2001. The genus *Adriohydrobia* (Hydrobiidae: Gastropoda): polytypic species or polymorphic populations? J. Zool. Sys. Evol. Res. 39: 227–234. http://dx.doi.org/10.1046/j.1439-0469.2001.00171.x

WILKE T., HAASE M., HERSHLER R., LIU H. P., MISOF B., PONDER W. 2013. Pushing short DNA fragments to the limit: Phylogenetic relationships of "hydrobioid" gastropods (Caenogastropoda: Rissooidea). Mol. Phylogenet. Evol. 66: 715–736. http://dx.doi.org/10.1016/j.ympev.2012.10.025

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

XIA X., XIE Z., SALEMI M., CHEN L., WANG Y. 2003. An index of substitution saturation and its application. Mol. Phylogenet. Evol. 26: 1–7. http://dx.doi.org/10.1016/S1055-7903(02)00326-3

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