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PHYLOGENETIC POSITION OF *TEFENNIA* SCHÜTT ET YILDIRIM, 2003 (CAENOGASTROPODA: RISSOOIDEA)

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ABSTRACT: The phylogenetic position of *Tefennia tefennica* Schutt et Yildirim, 2003, an endemic snail species from southwestern Turkey, was inferred with maximum likelihood analyses of DNA sequences of mitochondrial cytochrome oxidase subunit I and nuclear 18S rRNA. *Tefennia* belongs to the Hydrobiidae, Sadlerianinae; its sister clade comprises *Grossuana* Radoman, 1973, *Trichonia* Radoman, 1973 and *Daphniola* Radoman, 1973. Shell, radula and soft parts of *T. tefennica* are presented.

KEY WORDS: Rissooidea, endemism, COI, 18S rRNA, radula, anatomy, phylogeny, secondary loss of receptacle

INTRODUCTION

The Hydrobiidae of Turkey are represented by the subfamilies Hydrobiinae Troschel, 1857 (2 genera); Pyrgorientalinae Radoman, 1973 (2 genera) and Sadlerianinae Radoman, 1973 (8 genera) (RADOMAN 1983, SZAROWSKA 2006, YILDIRIM et al. 2006). The monotypic genus *Tefennia* Schütt et Yildirim, 2003 is known from only one locality in the Burdur Province, SW. Turkey (YILDIRIM et al. 2006). In the original description it is included in the Hydrobiidae (SCHÜTT & YILDIRIM 2003), and listed among the Orientalininae by YILDIRIM et al. (2006). The type species, *Tefennia tefennica* Schütt et Yildirim, 2003, has minute dimensions and a peculiar anatomy of female genitalia, with a bursa copulatrix in an anterior position and only one rudimentary seminal receptacle (rs_2). The aim of this paper was to establish the phylogenetic position of this interesting genus using partial sequences of the mitochondrial COI and nuclear 18S rRNA genes of *T. tefennica*. Additionally, the shell, radula and soft-part anatomy of this species were studied. The protoconch and radula, not described earlier, were examined using SEM.

MATERIAL AND METHODS

Material: Başpinar spring, Tefenni, Burdur, Turkey, leg. D. C. ÇAĞLAN

The snails were fixed with 80% ethanol. The shells were cleaned in an ultrasonic cleaner and photographed with a CANON EOS 50D digital camera. Three adult males and three females were dissected, using a NIKON SMZ-U stereomicroscope. The female genitalia (pallial oviduct) were examined using a MOTIC light microscope. The protoconchs and radulae were examined using a JEOL JSM-5410 scanning electron microscope, applying the techniques described by FALNIOWSKI (1990).

DNA was extracted from foot tissue of each snail. The tissue was hydrated in TE buffer (10 mM TRIS-HCl pH 8.0, 1 mM EDTA) (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'-TAA ACTT CAGGGTGACCAAAAAATYA-3') (WILKE & DAVIS 2000) for the cytochrome oxidase subunit I (COI) mitochondrial gene; SWAM18SF1 (5'-GAATGGCTCA TTAAATCAGTCGAGGTTCCTTAGATGATCCAAAT C-3') and SWAM18SR1 (5'-ATCCTCGTTAAAGGG TTTAAAGTGTACTCATTCCAATTACGGAGC-3') for the 18S ribosomal RNA 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 the purified PCR products were 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. All the sequences were deposited in GenBank (Table 1).

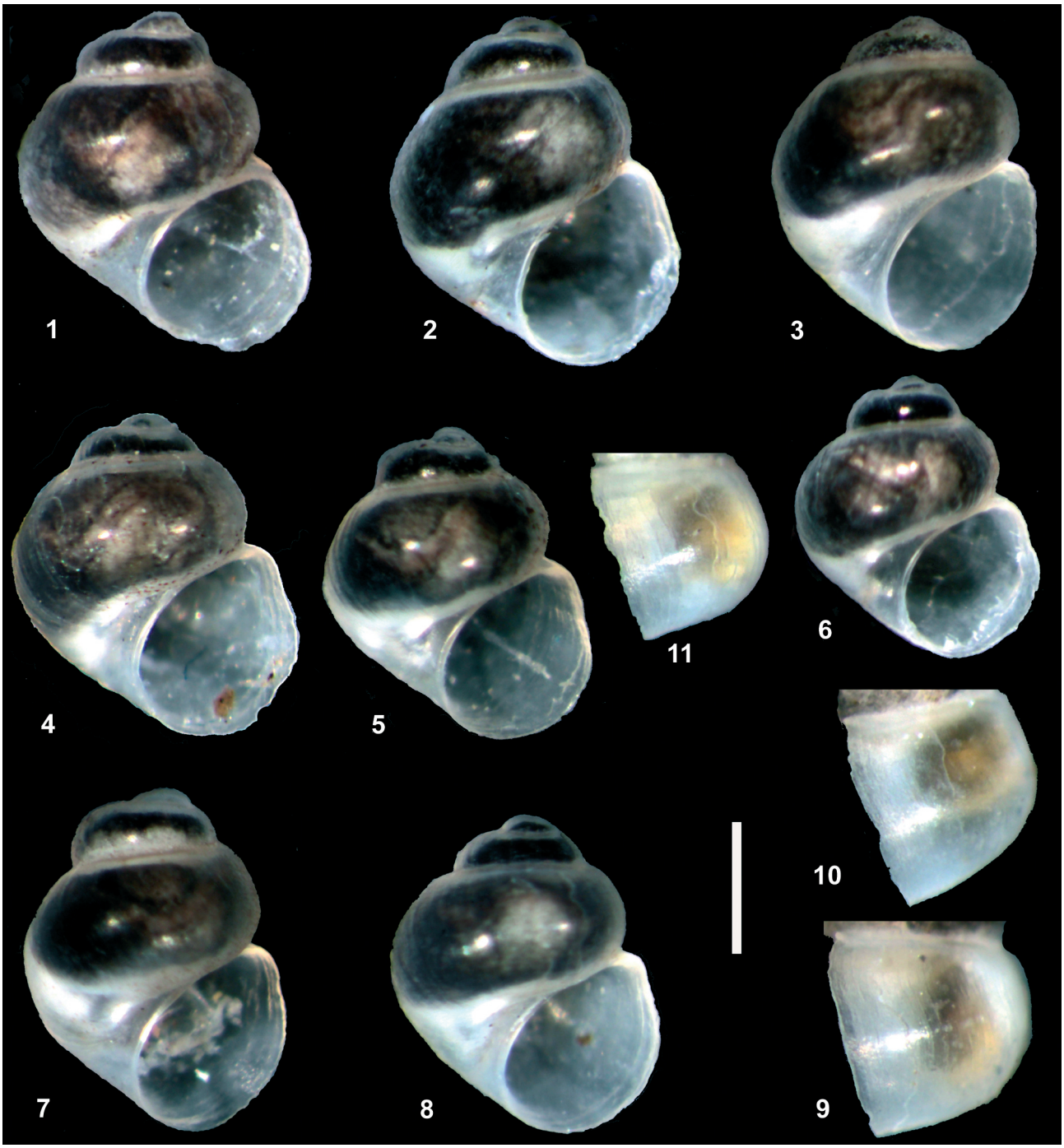
The COI sequences were aligned by eye using BioEdit 5.0.0 (HALL 1999) and edited with MACCLADE 4.05 (MADDISON & MADDISON 2002). For 18S, an initial alignment was performed using CLUSTALX 1.82 (THOMPSON et al. 1997) and edited

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)
Bythinella austriaca (Frauenfeld, 1857)	AF212917	FJ545132	FALNIOWSKI et al. (2009)
Bythiospeum sp.	AF367664	AF367634	WILKE et al. (2001)
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 (2011)
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) 1	AF367661	AF367631	WILKE et al. (2001)
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)
Tefennia tefennica Schütt et Yildirim, 2003	JX982799	JX982802	present study
	JX982800	JX982803	present study
	JX982801	JX982804	present study
Trichonia kephalovrissonia Radoman, 1973	EF070630	EF070619	SZAROWSKA (2006)
Ventrosia ventrosa (Montagu, 1803)	AF367681	AF118335	WILKE & DAVIS (2000)

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. Initially, we performed phylogeny reconstruction for 18S and COI data separately, using the maximum likelihood (ML) technique.

For each ML analysis, we used the best fit model of sequence evolution found by Modeltest v3.06 (POSADA & CRANDALL 1998, POSADA 2003). The best model for each dataset was chosen using the Akaike Informa-



Figs 1–11. Shells of *Tefennia tefennica*; bar represents 500 µm

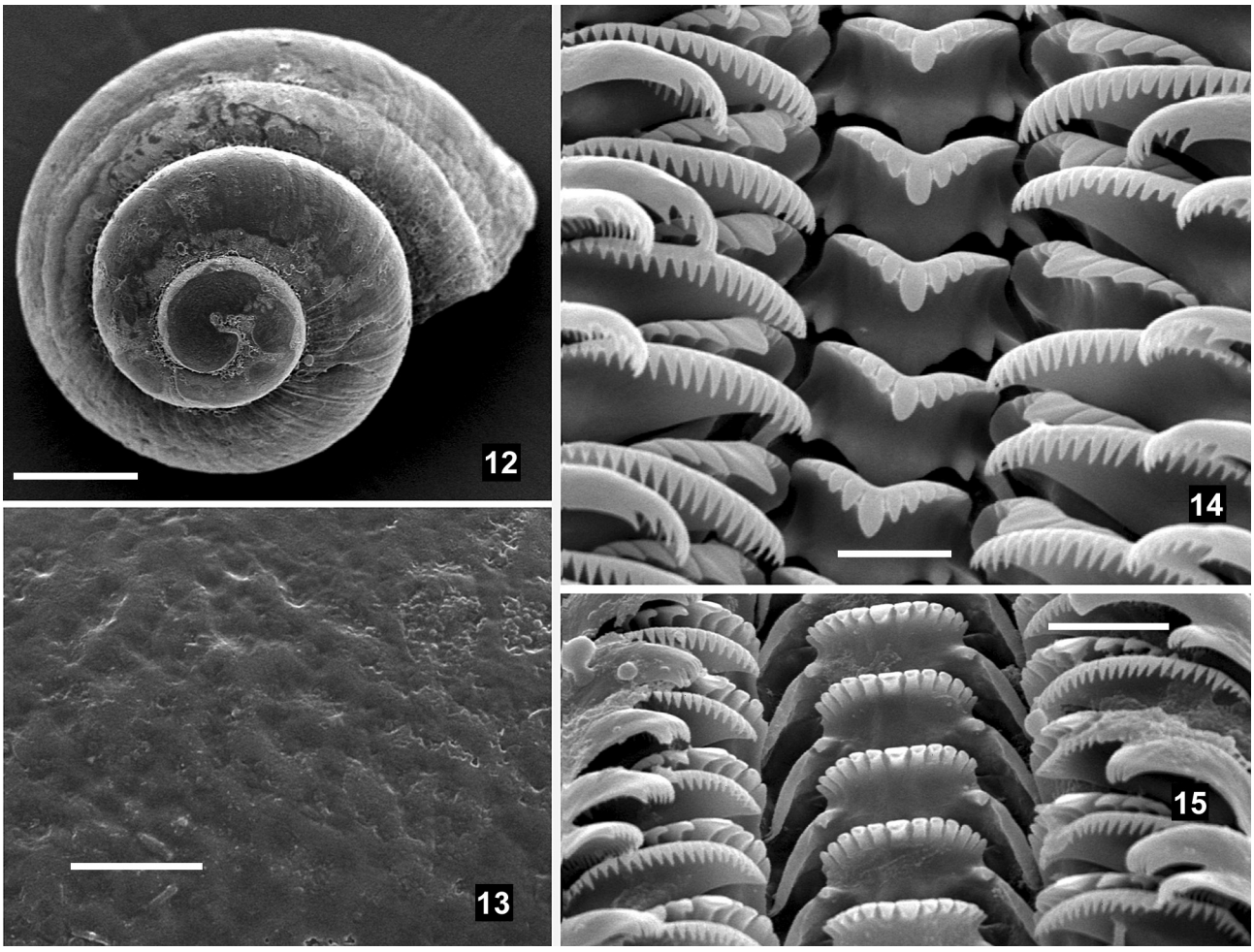
tion Criterion (AKAIKE 1974). We performed ML analyses in PAUP*4.0b10 (SWOFFORD 2002) and used a heuristic search strategy with stepwise addition of taxa, 10 random-sequence addition replicates, and tree-bisection-reconnection (TBR) branch swapping (SWOFFORD et al. 1996). Nodal support was estimated using the bootstrap (BS) approach (FELSENSTEIN 1985). Bootstrap values for ML trees were calculated using 1,000 bootstrap replicates, the "fast" heuristic search algorithm, and the same model parameters as for each ML analysis. Next, the partition homogeneity test (FARRIS et al. 1995) was performed (1,000 replicates) with PAUP*, to check whether the two genes could be analysed together. Due to its results ($p>0.7253$), the maximum likelihood heuristic search was then run for the combined molecular data.

In the phylogeny reconstruction, we used GenBank sequences from 27 rissooid taxa (Table 1). Seven of these, used as an outgroup, represented the main non-hydrobiid lineages within the Rissooidea (WILKE et al. 2001); another 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).

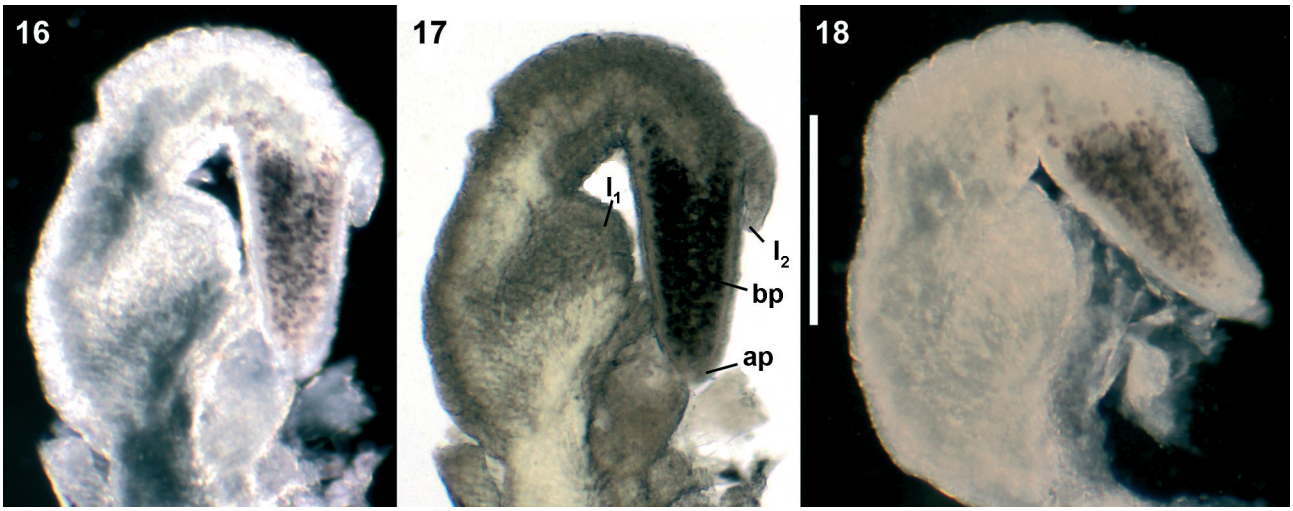
RESULTS AND DISCUSSION

The shell of *T. tefennica* (Figs 1–11) corresponds to the description of the species and drawings by SCHÜTT & YILDIRIM (2003: fig. 1). Protoconch surface malleated (Figs 12–13). Head and body pigmentation like in SCHÜTT & YILDIRIM (2003: fig. 1). Ctenidium present, number of ctenidial lamellae 8–11. Osphradium crescent-shaped. Radula (Figs 14–15) taenioglossate, central tooth formula:

$$\frac{6-1-6}{1-1}$$



Figs 12–15. SEM pictures of shell and radula of *Tefennia tefennica*: 12 – shell, 13 – protoconch surface, 14–15 – radula; bars represent 250 μm, 10 μm, 5 μm and 20 μm, respectively



Figs 16–18. Penis of *Tefennia tefennica* (ap – apical papilla, bp – black pigment, l1, l2 – lobes); bar represents 100 µm

Lateral tooth formula: 4–14. All cusps on central and lateral teeth comparatively stout and massive. Intestine course S-shaped. Stomach without caecum.

Penis (Figs 16–18) apically pigmented black, with apical papilla and 2 lobes: one bigger in basal part and one smaller at 1/3 length distal. Female genitalia (Fig. 19) correspond to the description and drawings by SCHÜTT & YILDIRIM (2003: fig. 1). Proximal seminal receptacle (rs_2) rudimentary and inconspicuous, yet present.

Three sequences of COI and three of 18S (Table 1) were analysed for *T. tefennica*. For the COI sequences the Akaike Information Criterion (AIC) with ModelTest found model GTR+I+Γ, with base frequencies: A=0.3356, C=0.1493, G=0.1434, T=0.3716; substitution rate matrix: [A-C]=0.8707, [A-G]= 8.1241, [A-T]=0.3577, [C-G]=2.7037, [C-T]=13.1246, [G-T]=1.0000, proportion of invariable sites: (I)=0.6074, and Γ distribution with the shape parameter =0.3721. For the combined data set the Akaike Information Criterion (AIC) with ModelTest found model GTR+I+Γ, with base frequencies: A=0.3356, C=0.1493, G=0.1434, T=0.3716; substitution rate matrix: [A-C]=0.8707, [A-G]=8.1241, [A-T]=0.3577, [C-G]=2.7037, [C-T]=13.1246, [G-T]=1.0000, proportion of invariable sites: (I)=0.6074, and Γ distribution with the shape parameter =0.3721.

In the COI analysis (Fig. 20) *Tefennia* was resolved within the Hydrobiidae Troschel, 1857, subfamily Sadlerianinae Radoman, 1977 (after SZAROWSKA 2006). Its sister clade consisted of *Grossuana* Radoman, 1973, *Trichonia* Radoman, 1973, and *Daphniola* Radoman, 1973. The bootstrap support for this placement was 57% (Fig. 20). In an ML tree computed for all molecular data (COI and 18S) the sister group of *Tefennia* was the same as in Fig. 20 but its support was higher (68%: Fig. 21). On the other hand, the relationships between this clade (*Tefennia*, *Daphniola*, *Grossuana* and *Trichonia*) and the other genera of the Sadlerianinae in Fig. 21 were somewhat different from the corresponding relationships shown in Fig. 20. However, in both trees (Figs 20–21) the clade was placed within the Sadlerianinae. It has to be noted, however, that low values of supports may only suggest the pattern presented.

The molecularly-inferred phylogenetic relationships of *Tefennia* suggest that the loss of the distal receptacle (rs_1) in this genus is secondary. Within the genera included in the phylogenetic analysis in the

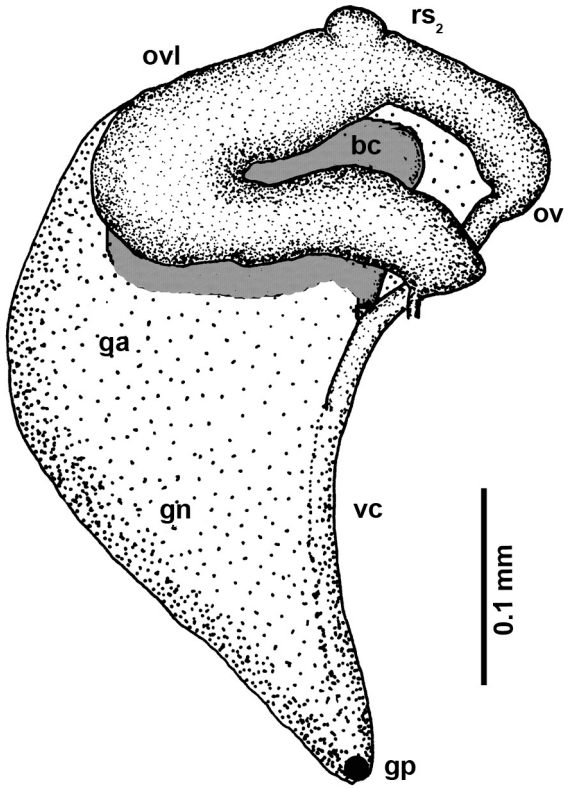


Fig. 19. Renal and pallial section of female reproductive organs of *Tefennia tefennica* (bc – bursa copulatrix, ga – albuminoid gland, gn – nidamental gland, gp – gonoporus, ov – oviduct, ovl – loop of the (renal) oviduct, rs_2 – seminal receptacle, vc – ventral channel)

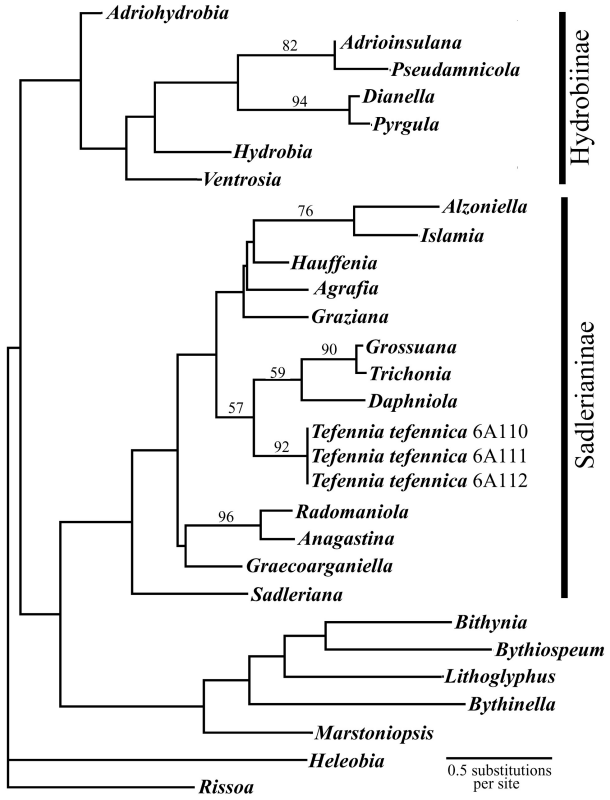


Fig. 20. ML phylogram based on COI, bootstrap supports given where >50

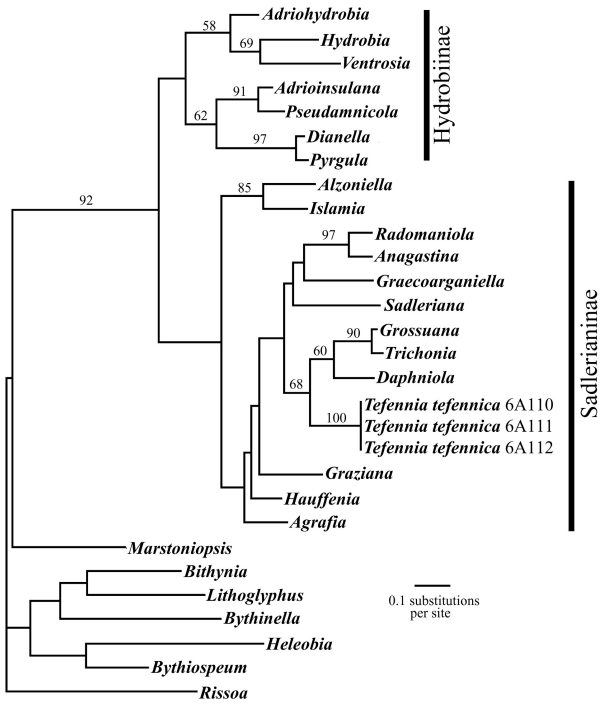


Fig. 21. ML phylogram based on COI and 18S, bootstrap supports given where >50

present study (Figs 20–21) a parallel loss of the distal seminal receptacle concerns *Hauffenia* (BODON et al. 2001). There are other valvatiform hydrobiids that have lost their rs_1 (*Lyhnidia*, *Pseudohoratia*, *Strugia*, *Zaumia*: BODON et al. 2001) but without molecular data the phylogenetic relationships of these genera are not clear.

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REFERENCES

AKAIKE H. 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19: 716–723. doi: 10.1109/TAC.1974.1100705

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.

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

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. doi: 10.1080/00222930903359636

FALNIOWSKI A., WILKE T. 2001. The genus *Marstoniopsis* (Gastropoda: Rissooidea): intra- and intergeneric phylogenetic relationships. J. Moll. Stud. 67: 483–488. doi: 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. doi: 10.1111/j.1096-0031.1994.tb00181.x

FELSENSTEIN J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. doi: 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.

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.

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

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, pp. 205–247.

POSADA D. 2003. Selecting models of evolution. In: SALEMI M., VANDAMME A.-M. (eds). The phylogenetic handbook. A practical approach to DNA and protein phylogeny. Cambridge University Press, Cambridge, pp. 256–282.

POSADA D., CRANDALL K. A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818. doi: 10.1093/bioinformatics/14.9.817

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.

SCHÜTT H., YILDIRIM M. Z. 2003. A new freshwater snail from Turkey, resembling the genus *Lyhnidia* Hadžišče 1956. Arch. Moll. 132: 97–103.

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

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, 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. doi: 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. doi: 10.1007/s10750-006-0530-4

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. doi: 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. doi: 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. doi: 10.1635/0097-3157(2001)151[0001:MSOHMG]2.0.CO;2

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

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. Phyl. Evol. 26: 1–7. doi: 10.1016/S1055-7903(02)00326-3

YILDIRIM M. Z., KOCA S. B., KEBAPÇI Ü. 2006. Supplement to the Prosobranchia (Mollusca: Gastropoda) fauna of fresh and brackish waters of Turkey. Turk. J. Zool. 30: 197–204.

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