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.

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 & 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 Orientalinae 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 (rs₂). 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 protoconchs and radula, not described earlier, were examined using SEM.

MATERIAL AND METHODS

Material: Başpinar spring, Tefenni, Burdur, Turkey, leg. D. 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’-GGTCAACAATCATAAGATATTGG-3’)
(FOLMER et al. 1994) and COR722b (5'-TAAACTTCAGGGTGACCAAAAAATYA-3') (WILKE & DAVIES 2000) for the cytochrome oxidase subunit I (COI) mitochondrial gene; SWAM18SF1 (5'-ATCCTCGTTAAAGGGTTAAAGTCTACTGCAATCC-3') and SWAM18SR1 (5'-ATCCTCGTTAAAGGGTTAAAGTGTACTCATTCCAATTACGGAGC-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) |
| Adrioinosula conovula (Frauenfeld, 1863) | AF367656 | AF367628 | WILKE et al. (2001) |
| Agrafia wiktori Szarowska et Falniowski, 2011 | JF906758 | JF906762 | SZAROWSKA & FALNIOWSKI (2011) |
| Alizoniella finalina Giusti et Bodon, 1984 | AF367686 | AF367650 | WILKE et al. (2001) |
| Anagastina zetavalis (Radoman, 1973) | EF070622 | EF070616 | SZAROWSKA (2006) |
| Bathybia tentaculata (Linnæus, 1758) | AF367675 | AF367643 | WILKE et al. (2001) |
| Bythinella austriaca (Frauenfeld, 1857) | AF212917 | EF070624 | SZAROWSKA (2006) |
| Bythiopeum sp. | AF367664 | AF367634 | WILKE et al. (2001) |
| Daphniola graeca Radoman, 1973 | EF070624 | EF070618 | SZAROWSKA (2006) |
| Diasellina thiesseana (Kobelt, 1878) | AF367612 | AF367612 | SZAROWSKA et al. (2005) |
| Graecoarganiella pannassiana (Falniowski et Szarowska, 2011) | JN202341 | JN202348 | FALNIOWSKI & SZAROWSKA (2011) |
| Graziana alestris (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) |
| Hydrobia acuta (Draparnaud, 1805) | AF367680 | AF278808 | WILKE & DAVIS (2000) |
| Islamia piristoma Bodon et Cianfanelli, 2001 | AF367671 | AF367639 | WILKE et al. (2001) |
| Lithogyphus natricoides (C. Pfeiffer, 1828) | AF367674 | AF367642 | WILKE et al. (2001) |
| Marstoniopsis insulica (Küster, 1853) | AF367676 | AF278813 | FALNIOWSKI & WILKE (2001) |
| Pseudamnicola lucensis (Issel, 1866) | AF367687 | AF367651 | WILKE et al. (2001) |
| Pyrgula annulata (Linnæus, 1767) | AF367672 | AF367640 | WILKE et al. (2001) |
| Radomaniola callosa (Paulucci, 1881) | AF367685 | AF367649 | WILKE et al. (2001) |
| Rissoa labiosa (Montagu, 1803) | AF367672 | AF367640 | WILKE et al. (2001) |
| Sodleriara fluminensis (Küster, 1853) | AF367683 | AF278808 | WILKE & DAVIS (2000) |
| Tefenna tefenna Schütz et Yildirim, 2003 | JX982799 | JX982802 | 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 Tefennisia 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. Oosphra-

![Image](image_url)

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
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 (rs₂) 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 (rs₁) in this genus is secondary. Within the genera included in the phylogenetic analysis in the

![Fig. 16–18. Penis of Tefennia tefennica (ap – apical papilla, bp – black pigment, l₁, l₂ – lobes); bar represents 100 µm](image)

![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, rs₂ – seminal receptacle, vc – ventral channel)](image)
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 rs1 (*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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