

# A new *Fridericia* species (Clitellata, Enchytraeidae) and the enchytraeid fauna of the Őrség National Park (Hungary)

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**Abstract.** The enchytraeid fauna of the Őrség National Park (Western Hungary), hitherto unknown, was investigated in this study. 14 enchytraeid genera including 47 species and one other annelid worm (*Hrabeiella periglandulata*) were identified. One enchytraeid species was found to be new to science and is described in this paper as *Fridericia zicsii* sp. nov. The new species is distinguishable based on both morphological characters and molecular data (mitochondrial cytochrome c oxidase subunit I, nuclear histone 3 genes and nuclear ribosomal ITS region sequences) from similar species. The enchytraeid fauna of Őrség NP indicated well the subalpine nature of this area. The most species-rich site was the hay meadow (32 species) and interestingly, the species number in the *Sphagnum* bog of Szóce was unusually high (19 species).

**Keywords.** *Fridericia*, new species, Enchytraeidae, fauna, Őrség National Park

## INTRODUCTION

An intensive investigation of the Hungarian enchytraeid fauna was launched in 2001 with support from the Hungarian Scientific Research Fund (OTKA), and so far resulted in publication of three comprehensive works on the enchytraeid fauna of Hungary. First, the enchytraeid fauna of the Northern Hungarian Mountains (Bükk, Mátra, Zemplén, Börzsöny Mts.) was investigated resulting in recording of 77 species belonging to 14 genera including five species new to science (*Mari-onina sexdiverticulata* Dózsa-Farkas, 2002, *Achaeta unibulba* Graefe, Dózsa-Farkas & Christensen, 2005, *Fridericia eiseni* Dózsa-Farkas, 2005, *F. schmelzi* Cech & Dózsa-Farkas, 2005 and *F. crassiductata* Dózsa-Farkas & Cech, 2006 (Dózsa-Farkas 2002, Dózsa-Farkas 2005, Cech & Dózsa-Farkas 2005, Graefe, Dózsa-Farkas & Christensen 2005, Dózsa-Farkas & Cech 2006, Dózsa-Farkas 2007). Between 2005–2009 the fauna of the Vértes Mts. (belonging to the Transdanubian Mountains) was investigated. From this

area 41 species and one subspecies distributed in 11 genera were identified including a new species and also a new subspecies (*Fridericia mahunkai* and *Fridericia gamotheca hungarica*) (Dózsa-Farkas 2013). As third region, the exploration of the Danube–Dráva National Park was carried out in 2011–2014 and a total of 14 enchytraeid genera including 49 species and two other annelid worms were identified of which four species were new to science (*Fridericia connatiformis*, *F. phaeostriata*, *F. longiducta*, and *Cernovitoviella buekkhathi*) (Dózsa-Farkas *et al.* 2015).

Within the last project in 2014 and 2015, the Őrség National Park was investigated and the faunistic results of this study and description of a new species *Fridericia zicsii* sp. nov. are herewith presented. The morphological studies were supplemented with molecular-taxonomic analyses targeting the nuclear ribosomal ITS region, the mitochondrial cytochrome c oxidase subunit I (COI) gene and the nuclear histone 3 (H3) gene.

## MATERIAL AND METHODS

**Study area.** The Órség National Park is situated in Western Hungary (46° 51'–55°N, 16° 06'–24'E). The yearly average precipitation is 700–800 mm, the mean annual temperature is 9.0–9.5 °C. The elevation range of the area is approximately 250–350 m above sea level, and the landscape is divided into hills and valleys (Dövényi 2010). The bedrock consists of alluvial gravel and loess, the most frequent soil types are pseudogleyic and lessivaged brown forest soils, which are nutrient poor. The pH of the soil is acidic, ranging from 4.0 to 4.8 (mean 4.3) (Juhász *et al.* 2011). Forests cover an area of ca. 350 km<sup>2</sup>, which represents 80% of the Órség NP. Stands are dominated by *Fagus sylvatica*, *Quercus petraea*, *Q. robur*, *Carpinus betulus*, *Pinus sylvestris* and *Picea abies*. Mixed stands with great compositional diversity are frequent, but some are dominated by a single tree species. The most frequent non-dominant tree species are *Betula pendula*, *Populus tremula*, *Castanea sativa*, *Prunus avium*, *Tilia* spp., and *Acer* spp. Most of the original forests of the region were cut in the middle ages and in the regrown secondary forest the proportion of pioneer tree species (such as *Pinus sylvestris* and *Betula pendula*) and the cover of acidofrequent herbs, bryophytes and lichens increased (Gyöngyössi 2008, Tímár *et al.* 2002).

**Collection sites.** In total, 16 macro- and microhabitats were sampled at 9 localities (Appendix 1).

**Morphological methods.** The animals were extracted from the soil by the wet funnel method (O'Connor 1962). Worms were first studied and measured alive, and subsequently preserved in 70% ethanol. Later, a part of the adult *F. zicsii* specimens was stained with borax-carmin, then passed through an ethanol (70% to absolute) dehydration series, mounted temporarily in clove oil, and later mounted in Euparal in a slide between two coverslips. The important morphological structures were recorded *in vivo*, drawn, and photographed using an Axio Imager.A2 microscope with DIC (differential interference contrast) il-

lumination and an AxioCam MRc 5 (Zeiss) digital camera with Axiovision software. The whole-mounted specimens were reinvestigated and also photographed. Holotype and paratypes of the new species are deposited in the collection of the Department of Systematic Zoology and Ecology, Eötvös Loránd University (Budapest, Hungary).

**Methods of molecular analysis.** From the individuals subjected to molecular taxonomic analysis, genomic DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen) following the instructions given by the manufacturer. The mitochondrial cytochrome c oxidase subunit I (CO1) gene, the nuclear histon 3 (H3) gene and the nuclear ribosomal ITS region were amplified using the primers HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') (Folmer *et al.* 1994), H3a-F (5'-ATG GCT CGT ACC AAG CAG ACV GC-3') and H3a-R (5'-ATA TCC TTR GGC ATR ATR GTG AC-3') (Colgan *et al.* 1998), and ETTS1 (5'-TGC TTA AGT TCA GCG GGT-3') and ETTS2 (5'-TAA CAA GGT TTC CGT AGG TGA A-3') (Kane & Rollinson 1994), respectively. PCRs were performed applying the parameters given by Dózsa-Farkas & Felföldi (2015). Purification and sequencing of PCR products were carried out by LGC Genomics GmbH (Berlin, Germany). Removal of primer sequences and manual correction of automatic base calling on chromatograms were performed using the Chromas software v. 1.45 (Technelysium). Phylogenetic analyses (which included the search for the best-fit models) were conducted with the MEGA 6.0 software (Tamura *et al.* 2013). Sequences determined in this study were deposited in GenBank under the following accession numbers: KU586612-KU586627 (ITS), KU586582-KU586595 (CO1) and KU586596-KU586611 (H3).

## RESULTS

### Results of morphological analysis

In total 47 species were recorded belonging to 14 enchytraeid genera, moreover a terrestrial po-

lychaete, *Hrabeiella periglandulata*, was also collected (Appendix 2). All species represent new records for the Órség National Park. The status of one *Fridericia* species (*Fridericia* sp. 1) has not been ascertained yet. This probably also represents a new species for science, but further investigations are needed to clarify its status. A list of species recorded in individual samples, representing microhabitats sampled at the individual sites, is given in Appendix 2.

## DESCRIPTION OF THE NEW SPECIES

### *Fridericia zicsii* sp. nov.

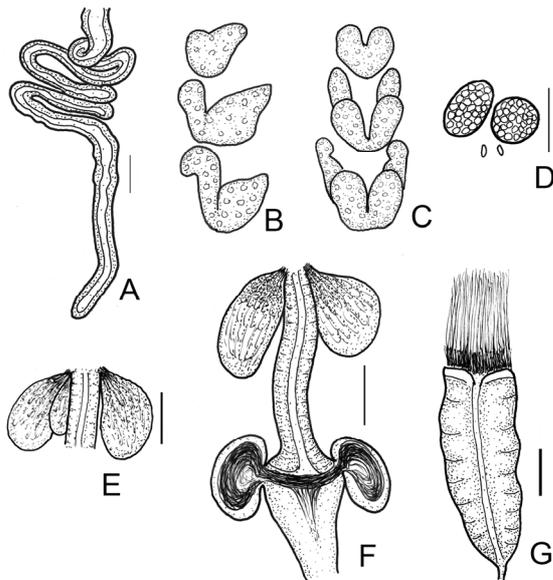
(Figures 1–5)

*Material examined. Holotype.* F.25 slide No. 2078, adult, stained whole mounted specimen. *Type locality.* Órség, Gödörházi rétek (specially protected area) on the edge of alder carr with *Hemerocallis lilio-asphodelus*. 46°44.782N 16°21.227E, 229 m a.s.l., leg. K. Dózsa-Farkas, J. Farkas, Z. Tóth & F. Hoc, 31.03.2014 (site 3b in Table 1). *Paratypes* (in total, 30 specimens). P.108.1.1–6 slide No. 1007–1009, 1066–1067, 2077. Six specimens from type locality, leg. K. Dózsa-Farkas, J. Farkas, Z. Tóth & F. Hoc, 31.03.2014. P.108.2.1–3 slide No. 2059–2060, 2076. Three specimens from Gödörházi rétek, hay meadow with *Salix rosmarinifolia*, 46°44.931N 16°21.177E, 229 m a.s.l., leg. K. Dózsa-Farkas, J. Farkas, Z. Tóth & F. Hoc, 31.03.2014. P.108.3.1–4 slide No. 2103–2106. Four specimens from Gödörházi rétek, hay meadow with *Salix rosmarinifolia*, 46°44.931N 16°21.177E, 229 m a.s.l., leg. Z. Tóth, 09.11.2014. P.108.4.1. Four specimens in ethanol from type locality, leg. K. Dózsa-Farkas, J. Farkas, Z. Tóth & F. Hoc, 31.03.2014. P.108.4.2. Ten specimens in ethanol Órség, Gödörházi rétek, hay meadow with *Salix rosmarinifolia*, 46°44.931N 16°21.177E, 229 m a.s.l., leg. Z. Tóth, 09.11.2014. P.108.4.3. Three specimens in ethanol from Órség, Gödörházi rétek, hay meadow with *Salix rosmarinifolia*, 46°44.931N 16°21.177E, 229 m a.s.l., leg. Z. Tóth, 26.10.2015.

*Further material examined.* 16 specimens from both localities (sites 3b and 4 in Table 1).

*Diagnosis.* The new species can be recognized by the following combination of characters: (1) large size (14–20 mm long and about 400 wide *in vivo*), but the cuticle thin (maximum 1.5  $\mu\text{m}$ ), segments 48–65; (2) maximum 4–5 chaetae per bundle; (3) clitellum girdle-shaped, gland cells arranged in transverse, dense rows, gland cells absent between the bursal slits but area glareaosa developed instead; (4) five preclitellar pairs of nephridia; (5) dorsal blood vessel originating in XVII–XX, blood light pink; (6) coelomo-mucocytes with hyaline globulose matrix, lenticytes scarce; (7) chylus cells in XII–XIV (2 segments); (8) seminal vesicle large; (9) subneural glands absent; (10) sperm funnel cylindrical, about the same length or 2/3 as long as body diameter; (11) spermathecae with short ectal duct, with 2 (3) very large ectal glands, the spherical ampulla with two oval or bean-shaped, large diverticula (diameter 60–90  $\mu\text{m}$  *in vivo*), separate openings into oesophagus.

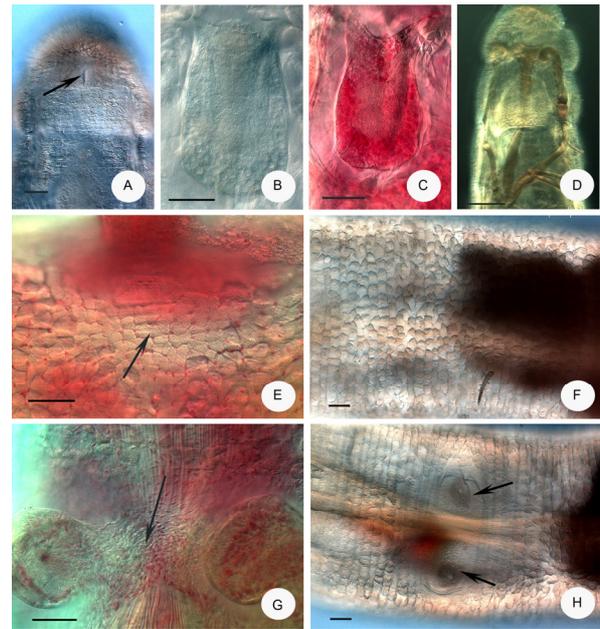
*Description.* Large species, holotype 17.5 mm long, 420  $\mu\text{m}$  wide at VIII and 550  $\mu\text{m}$  at clitellum *in vivo*, 11.8 mm long, 490  $\mu\text{m}$  wide at VIII and 570  $\mu\text{m}$  at clitellum (fixed), segments 59. Body length of paratypes 14–20 mm, width 350–450  $\mu\text{m}$  at VIII and 370–580  $\mu\text{m}$  at clitellum *in vivo*, length of fixed specimens 8–13 mm, width 360–500  $\mu\text{m}$  at VIII and 450–590  $\mu\text{m}$  at clitellum, segments 48–65. Chaetal formula: 3,4–4,3,(1),2 : 3,4,5–4,3,2. The chaetae within a bundle arranged in pairs with the outer being longer and thicker than the inner (40–50  $\times$  5  $\mu\text{m}$  vs. 45–35  $\times$  4  $\mu\text{m}$ , in preclitellar ventral bundles), the length of the chaetae in the lateral bundles somewhat longer (54 and 40  $\mu\text{m}$ , respectively). Chaetal lengths about the same in postclitellar segments. From about XXV–XXXII, only two chaetae per bundle, these 60–70  $\mu\text{m}$  long in terminal segments. Head pore a longitudinal slit at 0/I (Fig. 2A). Dorsal vessel from XVII–XX, blood light pink (Fig. 2D). Epidermal gland cells inconspicuous. Clitellum in XII–1/3XIII, girdle-shaped, hyalocytes and granulocytes arranged in dense rows dorsally (Fig. 2F), sometimes irregularly, around the male copulatory organs only granulocytes (Fig. 2E), but between the bursal slits the glandular cells absent (Fig. 2H)



**Figure 1.** *Fridericia zicsii* sp. nov. A = oesophageal appendage, B = pharyngeal glands in lateral view (schematic), C = pharyngeal glands in dorsal view (schematic), D = coelomocytes, E = three ectal glands of spermatheca, F = spermatheca, G = sperm funnel; scale bars = 50  $\mu$ m (except G = 100  $\mu$ m).

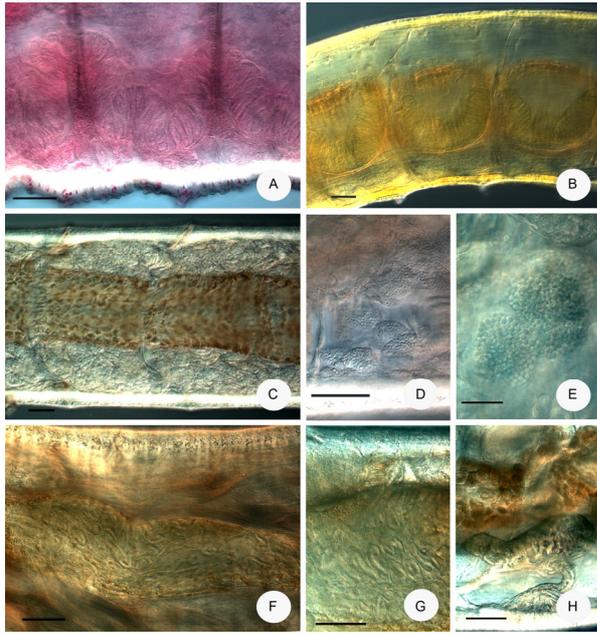
while here an area glareosa well developed (Fig. 2G). Body wall about 25–40  $\mu$ m thick, cuticula thin, 1–1.5  $\mu$ m *in vivo*.

Brain (Fig. 2B, C) about 200  $\mu$ m long and 1.8 times longer than wide *in vivo*, anterior and posterior margin with slight convexity. Oesophageal appendages (Fig. 1A) long, coiled, unbranched, type b (rarely two short branches at the end). All pairs of pharyngeal glands united dorsally and with ventral lobes in V and VI (Fig. 1B, C). Chloragocytes from V, 23–26  $\mu$ m long, brown *in vivo*. Midgut pars tumida in XXX–XLIV (in 7–10 segments) [but in two specimens in XXV–XXXI (8 segments)] the height of cells 63–65  $\mu$ m (Fig. 3A–B). Five pairs of preclitellar nephridia from 6/7 to 10/11; length ratio anteseptale : post-septale about 1 : 1.5, medial origin of efferent duct (Fig. 3H). Coelomo-mucocytes with characteristic hyaline globulose matrix (Fig. 1D, 3C–E), but in aggregations dark grey, length mostly 30–45  $\mu$ m, lenticytes scarce, 7–10  $\mu$ m long, *in vivo*. Chylus cells (Fig. 3F–G) between XII–XIV, occupying 2 segments. Seminal vesicle large (X–XI). Sperm funnels cylindrical (Figs. 1G, 4A–B), 320–500  $\mu$ m long *in vivo*, 2.5–4 times longer than



**Figure 2.** Micrograph of *Fridericia zicsii* sp. nov. A = head-pore (marked with arrow), B–C = brain, D = dorsal blood vessel anteriorly (blood pink), E = clitellar gland cells near to the male copulatory organ, laterally only granulocytes (marked with arrow), F = clitellar gland cells dorsally (hyalocytes and granulocytes in dense transverse rows), G = between the two bursal slits the glands absent, but area glareosa well developed (marked with arrow), H = clitellum in ventral view (the two male copulatory organs are marked with arrows). A, B, D, F, H *in vivo*, C, E, G fixed, stained; scale bars = 50  $\mu$ m.

wide, but in fixed specimens the length of funnels 230–330  $\mu$ m, and about 1.5–3 times longer than wide. Collar slightly narrower than funnel body. Length of spermatozoa 190–250  $\mu$ m, heads 60–80  $\mu$ m, *in vivo*. Diameter of sperm ducts about 10  $\mu$ m, *in vivo*. Male copulatory organs large (Fig. 4C–D), 250–300  $\mu$ m long, 140–160  $\mu$ m wide and 110–130  $\mu$ m high, *in vivo* (200–285, 120–160 and 95–130  $\mu$ m, fixed, respectively), the modiolus and area glareosa well developed, and covering the medial surface of the bursa (Fig. 4C). The bursal slits are T-shaped (Fig. 4E). No subneural glands. Ectal ducts of spermathecae short, length 180–260  $\mu$ m and width 26–30  $\mu$ m (130–170  $\mu$ m long and 25–28  $\mu$ m wide, fixed), the canal of duct 5–6  $\mu$ m wide *in vivo*. Two very large brownish glands at the ectal openings of the spermathecal ducts (but in size different, 80–160  $\mu$ m long *in vivo* and fixed alike). Rarely, there is a third smaller gland

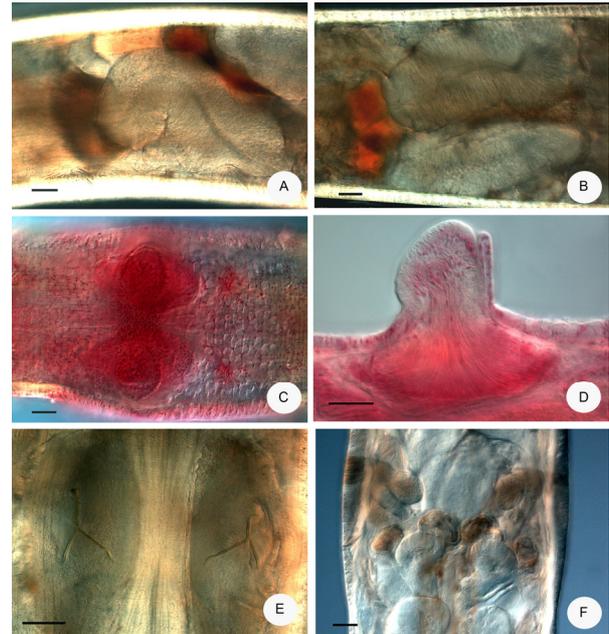


**Figure 3.** Micrograph of *Fridericia zicsii* sp. nov. A = pars tumida in XXVI-XXVII (fixed and stained), B = pars tumida in XXXVI-XXXVIII (fixed but not stained), C-E coelomocytes, F-G = chylus cells in XIII-XIV, H = second anteclellular nephridium. C-H *in vivo*; scale bars = 50 µm (except E = 20 µm).

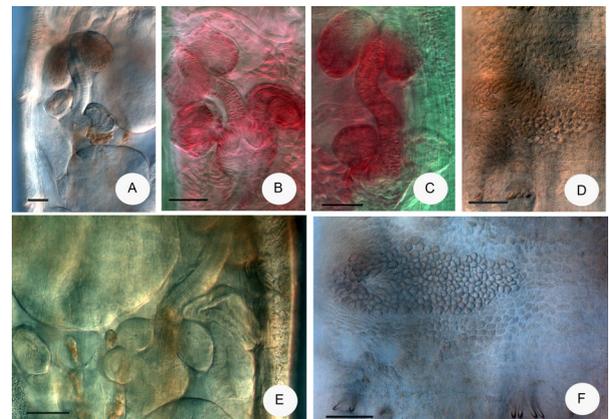
(Fig. 1E-F, 4F, 5A-C). The glands consist of a complex of many gland cells, which open one by one to the surface (Fig. 5F). Ampullae onion-shaped, diameter 75–85 µm *in vivo* (fixed 50–80 µm), with two sessile oval, bean-shaped or rounded thin-walled diverticula about 70–90 µm long and 60–70 µm wide when fixed, mostly filled with sperm (Fig. 1F, 4A-C). In one case, three diverticula were present in one of the two spermathecae (Fig. 5E). The ectal duct projecting into the lumina of ampullae, ental bulbs 40–50 µm wide, fixed (Figs. 5B). Distal and proximal parts of ampullae mostly not set off by a constriction, separate openings into oesophagus. One to four mature eggs at a time.

**Distribution and habitat.** Only in the specially protected area Gödörházi rétek of Órség National Park: on the edge of alder carr with *Hemerocallis lilio-asphodelus* and a hay meadow with *Salix rosmarinifolia*.

**Etymology.** Named in honour of my late colleague, the excellent earthworm taxonomist, Prof. Dr. András Zicsi.



**Figure 4.** Micrograph of *Fridericia zicsii* sp. nov. A-B = sperm funnels, C = male copulatory organs and the clitellar region ventrally, D = bursa everted, E = bursal slits, F = the two spermathecae. A, B, E, F *in vivo*, C-D stained; scale bars = 50 µm.



**Figure 5.** Micrograph of *Fridericia zicsii* sp. nov. A-C = spermathecae with two diverticula and large ectal glands, E = spermatheca exceptionally with three diverticula, D, F = gland openings of the complex spermathecal ectal glands. A, D, E, F *in vivo*, B-C fixed, stained; scale bars = 50 µm.

**Remarks.** Among the previously described *Fridericia* species with oval or bean-shaped spermathecal diverticula and separate openings into oesophagus, five species (*F. strenua* Rota, 1995; *F. aurita* Issel, 1905; *F. auritoides* Schmelz, 2003; *F.*

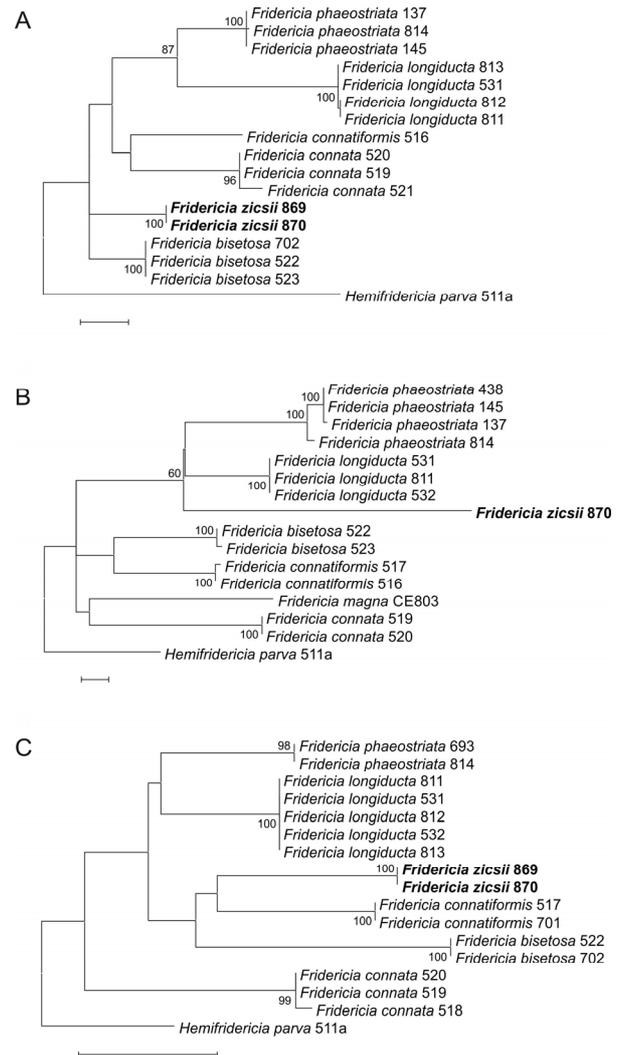
*longiducta* Dózsa-Farkas, 2015; and *F. phaeostriata* Dózsa-Farkas, 2015) are similar to the new species (Issel 1905, Schmelz 2003, Rota 1995, Dózsa-Farkas *et al.* 2015). The main differences that distinguish the new species from all the above-mentioned ones are the blood colour (in the new species light pink, in the others colourless) and the very large spermathecal ectal glands. Another species, *F. magna* Friend, 1899, has red blood and similar spermathecae with two large ectal glands. However, *F. zicsii* sp. nov. can be easily distinguished from *F. magna* by the following characters: 1) smaller size (14–20 mm long and 48–64 segments vs. 30–50 mm and 70–96 segments); 2) more chaetae: mostly 4–5 in preclitellar bundles both ventrally and laterally (2 or 3, only very rarely 4 in *F. magna*); 3) oesophageal appendages type b (type c in *F. magna*); 4) coelomo-mucocytes with a characteristic texture, the nucleoli are not visible (nucleoli conspicuous in *F. magna*); 5) seminal vesicle large (in *F. magna* absent). For descriptions of *F. magna* see Friend (1899) and Schmelz (2003).

### Results of molecular analysis

Results of molecular analyses are shown in Fig. 6. In total, 16, 14 and 16 sequences were determined from various *Fridericia* specimens in the case of ITS, CO1 and H3, respectively. In addition to two *F. zicsii* sp. nov. individuals, specimens of five other, morphologically similar species (*F. phaeostriata*, *F. longiducta*, *F. connatifformis*, *F. bisetosa* and *F. connata*, all collected from woodland habitats in Hungary; Appendix 3) were also sequenced. The results of molecular analyses confirmed our morphological results, since the sequences of the three studied taxonomic markers for *F. zicsii* sp. nov. were clearly separated from those of all similar *Fridericia* species.

### DISCUSSION

The enchytraeid fauna (47 species of 14 genera) of this area is quite diverse, and consists mostly of species typical to the Hungarian or wider



**Figure 6.** Maximum likelihood (ML) phylogenetic trees of selected *Fridericia* species based on the ITS region (A), CO1 (B) and H3 genes (C). Bootstrap values greater than 50 are shown at the nodes. Sequences from the new species described here appear in bold. A = ML tree of the ITS region based on 425 nucleotide positions using the K2+G nucleotide substitution model. B = ML tree of the CO1 gene based on 405 nucleotide positions using the TN93+G+I substitution model. C = ML tree of the H3 gene based on 175 nucleotide positions using the K2+G substitution model. Scale bars = 0.05 substitutions per nucleotide position. Sequences from the new species are highlighted with bold letters.

Central European fauna (Schmelz & Collado 2010). In terms of species numbers recorded in the investigated sites, the hay meadow of Gödörházi rétek (site 5 in Appendix 1) showed with 32 species the highest value. The species compo-

sition of this site reflects best the mountain or subalpine character of the area. Moreover only here (and at the neighbouring site 4, the edge of an alder carr), specimens of *F. zicsii* sp. nov. were found. This new species was clearly distinguishable from other *Fridericia* species based on morphological characters and molecular taxonomical analyses.

Of all other studied Hungarian mountain ranges, the fauna of Őrség NP is most similar to the fauna of Zemplén Mts. (Dózsa-Farkas 2007). *Mesenchytraeus armatus*, *Mesenchytraeus glandulosus* and *Marionina simillima* are North European or subalpine fauna elements. Comparing the two *Sphagnum* mires studied here (sites 8 and 9a) with the four other *Sphagnum* mires in the north-eastern part of Hungary investigated earlier (Dózsa-Farkas 1990, 1991), it is worth noting that the enchytraeid fauna of the mire in Farkasfa (site 8) was poor in species (4 species) while the mire in Szőce (site 9) with its 20 recorded species widely differed from these. Interestingly, this species-rich sampling site was located in the middle of the mire (site 9a), while at its edge (sites 9b and c) only seven species were recorded. We expected an opposite trend. The reason for this can be the higher pH value measured (6.8–6.9) in the mire in Szőce, the site was not as nutrient-poor as in case of the other mires (Pócs *et al.* 1958) and furthermore, besides *Sphagnum* spp. other moss species were also present.

*Chamaedrillus* (= *Cognettia*) *chalupskyi* is a new species for the Hungarian fauna. It should be noted that almost all *Cognettia* species were recently revised and relegated to the genus *Chamaedrillus* Friend, 1913 (Martinsson *et al.* 2015), but this nomenclatural act was questioned and a case has been submitted to the International Commission on Zoological Nomenclature (Schmelz *et al.* 2015), therefore both genus names appear throughout this article.

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