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THE ENCHYTRAEID FAUNA (ENCHYTRAEIDAE, CLITELLATA) OF THE RAX MOUNTAIN (AUSTRIA) WITH DESCRIPTION OF TWO NEW SPECIES AND COMPARISON OF *FRIDERICIA DISCIFERA* HEALY, 1975 AND *F. ALPICA* SP. N.

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The enchytraeid fauna of the Rax mountain range (Austria) was investigated. Samples were taken from five different habitats: under *Pinus mugo*, under *Picea abies*, under *Larix decidua*, from a mixed forest (*P. abies* with *Betula pendula*) and from an alpine meadow. Forty one enchytraeid species belonging to 12 genera were found and additionally the terrestrial polychaete *Hrabeiella periglandulata*. In addition to the very frequent species, *Fridericia discifera* Healy, 1975, another species with very similar spermathecae but differing in some traits was also observed. The characters which could be used to discriminate this new species, *F. alpica* sp. n. from *F. discifera* are discussed in this paper. Another species collected from the study site, *F. raxiensis* sp. n. was also new to science and is described based on both morphological and molecular taxonomic methods.

Keywords: enchytraeid fauna, new *Fridericia* species, molecular analysis, Rax Mountain.

INTRODUCTION

The enchytraeid fauna of the Austrian Alps is still little known. Schmidegg (1938) reported 14 species from Northern Tirol. However, the true identity of several species given in that study is uncertain, as was pointed out by Schmelz (2003). Nurminen (1977) reported 32 enchytraeid species from Grossglockner. Later, Bauer (1993, 1996*a, b*, 1998 and Bauer *et al.* 1994) reported 21 species from spruce forests, subalpine meadows and pasture in Central Alps, in the Northern Limestone Alps and Eastern Austria. Subsequently, for frost survival experiments, she studied the enchytraeid fauna and the abundance of enchytraeids on the Rax plateau in a subalpine meadow, where she found 12 species (Bauer 2002*a, b*).

In 2012, we started an investigation focusing on the enchytraeid fauna of the Kőszeg Mountains (Günzer Mountains) of Hungary and Austria. To compare these subalpine areas and the Rax mountain range, soil samples were taken between 2012–2016 from the upper terminal of the Rax cable car and from the western side of the mountain. Besides, some soil samples were collected in 2008 and sent to us by Roswitha Bauer. In this paper, faunistic

results and the description of two new species, *Fridericia raxiensis* sp. n. and *F. alpica* sp. n. are presented, furthermore a detailed comparison between two similar species, *F. discifera* and *F. alpica* sp. n., is also given. The morphological studies were supplemented with molecular taxonomical analyses targeting the nuclear ribosomal ITS region, the mitochondrial cytochrome c oxidase subunit I (CO1) gene and the nuclear histone 3 (H3) gene.

MATERIAL AND METHODS

Study area – Rax Mountain, Lower Austria. Soil samples were taken from the upper terminal of the Rax cable car and from various habitats at the western side of the mountain, the bedrock is calcareous, pH 5–5.5 (Bauer 2002*a*). Leg. K. Dózsa-Farkas, J. Farkas & Z. Tóth (years 2012–2016), and R. Bauer (year 2008):

- subalpine meadows (4 samples): 47°43.172N, 15°45.218E, 1613 m a.s.l.; 47°43.001N, 15°45.403E, 1621 m a.s.l., (15.05.2012), 47°43.088N, 15°45.365E 1584 m a.s.l. (22.05.2014); 47° 41.138N, 15° 42.542E, 1331 m. a.s.l. (13.05.2016).
- under *Pinus mugo* (7 samples): 47°71.666N, 15°77.305E, 1613 m a.s.l (10.06.2008). 47°43.233N, 15°45.164E, 1612 m a.s.l.; 47°43.125N, 15°45.277E, 1619 m a.s.l; 47°43.036N, 15°46.024E, 1620m a.s.l (15.05.20012); 47°42.590N, 15°45.413E 1622 m a.s.l.; 47°43.067N; 15°46.073E 1630 m a.s.l. (22.05.2014); 47°43.007N, 15°45.401E, 1613 m a.s.l. (15.05.2015).
- under *Picea abies* (5 samples): 47°43.008N, 15°46.319E, 1563 m a.s.l. (15.05.2012); 47°43.012N, 15°46.317E 1560 m a.s.l. (22.05.2014, 15.05.2015); 47°41.055N, 15°42.561E, 1308 m a.s.l.; 47°40.537N, 15°43.006E, 1260 m a.s.l. (13.05.2016).
- under *Larix decidua* (1 sample): 47°41.094N, 15°42.560E, 1314 m a.s.l. (13.05.2016).
- from mixed forest (*Picea abies* + *Betula pendula*) (1 sample): 47°40.538N, 15°43.140E, 1116 m a.s.l. (13.05.2016).

Morphological methods – About 15 x 15 x 10 cm soil sample has been dug (no quantitative sampling). 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 specimens was stained with borax-carmine then passed through an ethanol (70% to absolute) dehydration series, mounted temporarily in clove oil, and mounted in Euparal 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) illumination and an AxioCam MRc 5 (Zeiss) digital camera with Axiovision software. The whole-mounted specimens were reinvestigated and also photographed. Selected materials were catalogued with collection numbers for the holotypes ("F"), paratypes ("P") and with slide numbers, and were deposited in the collection of the Department of Systematic Zoology and Ecology, Eötvös Loránd University (Budapest, Hungary).

Methods of molecular analysis – Genomic DNA was extracted from the individuals with the DNeasy Blood & Tissue Kit (Qiagen) following the instructions given by the manufacturer. Three different regions were amplified with PCR: the mitochondrial cytochrome c oxidase subunit I (CO1) gene, the nuclear histone 3 (H3) gene and the nuclear ribosomal ITS region 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). If amplification failed in the case of the H3 gene, two additional primer sets (designed by AllGenetics, A Coruña, and used here with permission of ECT Oekotoxikologie GmbH, Flörsheim) were applied: H3a-new-F (5′-TGG CTC GTA CCA AGC AGA CSG-3′) with H3a-new-R (5′-ATG ATG GTG ACG CKY TTG GC-3′), and H3Frid-M13F (5′-GTA AAA CGA CGG CCA GTT ACC AAG CAG ACG GCH CGY-3′) with H3Frid-M13tR (5′-GCG GAT AAC AAT TTC ACA CAG GGG CGT GAA TBG CRC ACA GGT-3′) as described in Dózsa-Farkas *et al.* (2015). Purification and sequencing of PCR products were performed by LGC Genomics GmbH (Berlin, Germany). Removal of primer sequences and manual correction of automatic base calling on chromatograms were carried out 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 accession numbers KX985868- KX985875 (ITS), KX985876-KX985884, MG921590 (CO1) and KX985885-KX985896 (H3).

RESULTS

Forty one enchytraeid species were recorded, representing 12 genera, and additionally one terrestrial polychaeta, *Hrabeiella periglandulata* (Table 1). Two *Fridericia* species are considered new to science, namely *F. alpica* sp. n. and *F. raxiensis* sp. n., and described below. Two other *Fridericia* species, be-

Table 1. List of recorded species and their distribution in the investigated habitats of the Rax Mountain (Austria)

	meadow	*Pinus mugo* stands	*Picea abies* stands	*Larix decidua* stand	mixed forest
Achaeta danica?		+	+		
Buchholzia appendiculata		+	+		
B. fallax *			+		
B. simplex *	+	+	+		+
Euenchytraeus clarae		+	+		
Chamaedrilus cognettii		+			
Ch. chlorophilus *		+	+		
Enchytraeus buchholzi s.l. *		+	+		+
E. norvegicus	+	+	+		
E. bulbosus				+	+
Enchytraeus sp.					+
Enchytronia christenseni		+			

Table 1 (continued)

	meadow	*Pinus mugo* stands	*Picea abies* stands	*Larix decidua* stand	mixed forest
En. parva		+	+		
En. baloghi			+		
***Fridericia alpica* sp.n.**	+	+	+		
F. aurita s.l.1 *		+	+	+	+
F. aurita s.l.2				+	
F. benti		+			
F. bisetosa		+		+	
F. connata	+	+	+		
F. discifera		+	+		+
F. galba			+		+
F. miraflores			+		
F. paroniana		+			
F. perrieri					+
***F. raxiensis* sp.n.**	+	+			
F. semisetosa					+
F. tubulosa	+			+	
F. waldenstroemi					+
Fridericia sp.					+
Hemifridericia parva	+	+			
Henlea glandulifera		+	+		
H. heleotropha	+				
H. perpusilla *	+	+		+	+
H. nasuta		+	+		
Marionina argentea s.l. *		+			
M. communis	+				
Mesenchytraeus armatus		+			
Me. glandulosus	+				
Me. pelicensis		+			
Oconnorella cambrensis		+			
Total number of enchytraeid species: 41	11	26	18	6	12
Hrabeiella periglandulata		+	+		

* species found previously by Bauer (2002*a*, *b*) too

longing to the *F. aurita* species complex are listed as *F. aurita* sensu lato 1 and 2, while an additional *Fridericia* species represents probably another new species (*F.* sp.). Moreover, one undescribed *Enchytraeus* species was found but its identity requires further studies. The list of recorded species and their distribution in the investigated habitats are given in Table 1. Seven enchytraeid species were found on the Rax Mt. previously by Bauer (2002*a, b*) too (marked with * in Table 1). In Table 1, *Chamaedrilus chlorophilus* (after Martinsson *et al.* 2015) is surely identical with *Cognettia sphagnetorum* found by Bauer. Two species recorded by her, *F. ratzeli* and *Stercutus niveus*, were not found in our study. The possible reason for the absence of *S. niveus*, is that this species occurs at the surface only in autumn and winter, from April or May until July or August, without feeding, they retreat to the deeper layers of the soil and aggregate into smaller groups (Dózsa-Farkas 1973), and we collected the soil only in summer so this species did not came to the samples, contrary Bauer collected her worms also in September and December.

DESCRIPTIONS OF THE NEW SPECIES

Fridericia alpica sp. n.
(Figs 1–2)

Type material – Holotype. F. 26. slide No. 2136, Rax Mountain, close to the Rax cable car terminal, under *Pinus mugo* 47°43.233N, 15°45.164E, 1612 m a.s.l., 15.05.2012.

Paratypes. In total 13 specimens. P.110.1.1. slide No. 2137 at the type locality. 15.05.2012; P.110.1.2–110.1.3, slides No. 2120, 2156, two specimens on Rax Mountain at the type locality, under *Pinus mugo,* 47°43.038N, 15°46.024E, 1623 m a.s.l. and 47°43.007N, 15°45.401E, 1613 m a.s.l., 22.05.2014; P.110.1.4-109.1.6 slides No. 2129, 2132–2133, three specimens, on Rax Mountain, near to type locality under *Picea abies* 47°43.012N, 15°46.317E, 1560 m a.s.l., 15.05.2015; P.110.2.1–109.2.2 slide No. 2118–2119 two specimens in Kőszeg Mts (Günzer Mts), near to Reichnitz, Austria, mixed forest (*Pinus silvestris, Picea abies, Quercus petraea, Fagus sylvatica*), 47°19.453N, 16°25.400E, 589 m a.s.l., 21.05.2014; P110.3.1–109.3.5 slides No. 2121–2123, 2157, 2159, five specimens in Kőszeg Mts near to Steirer Houses, Hungary, *Quercus petraea* woodland with some *Fagus sylvatica* 47°22.411N, 16°30.016E 521 m a.s.l.

Etymology – Named after the region where this species is an inhabitant of the eastern margin of Alps.

Diagnosis – The new species can be recognized by the following combination of characters: (1) medium size (7–16 mm *in vivo*), segments 38–52; (2) maximum 5–6 chaetae per bundle; (3) clitellum girdle-shaped: hyalocytes and granulocytes arranged in transverse rows; (4) five preclitellar pairs of nephridia; (5) coelomo-mucocytes numerous, a-type (according to Möller 1971), lenticytes 7–12 μm; (6) chylus cells mostly in XVI–XVII; (7) bursal slit T or Y-shaped; (8) seminal vesicle large; (9) subneural glands in XIII–XV; (10)

sperm funnel cylindrical, approximately half as long as body diameter, collar narrower as funnel body, sperm 150–220 μm long, sperm heads 60–75 μm long (fixed); (11) spermatheca separate entally, with two sessile, sphaerical diverticula and a large ectal gland.

Description – Holotype *in vivo* 6.9 mm long, 350 μm wide at VIII and 380 μm at the clitellum (fixed), 51 segments. Body length of the paratypes 7–16 mm, width 300–400 μm at VIII and 340–400 μm at the clitellum (*in vivo*). Length of fixed specimens 4.7–11 mm,

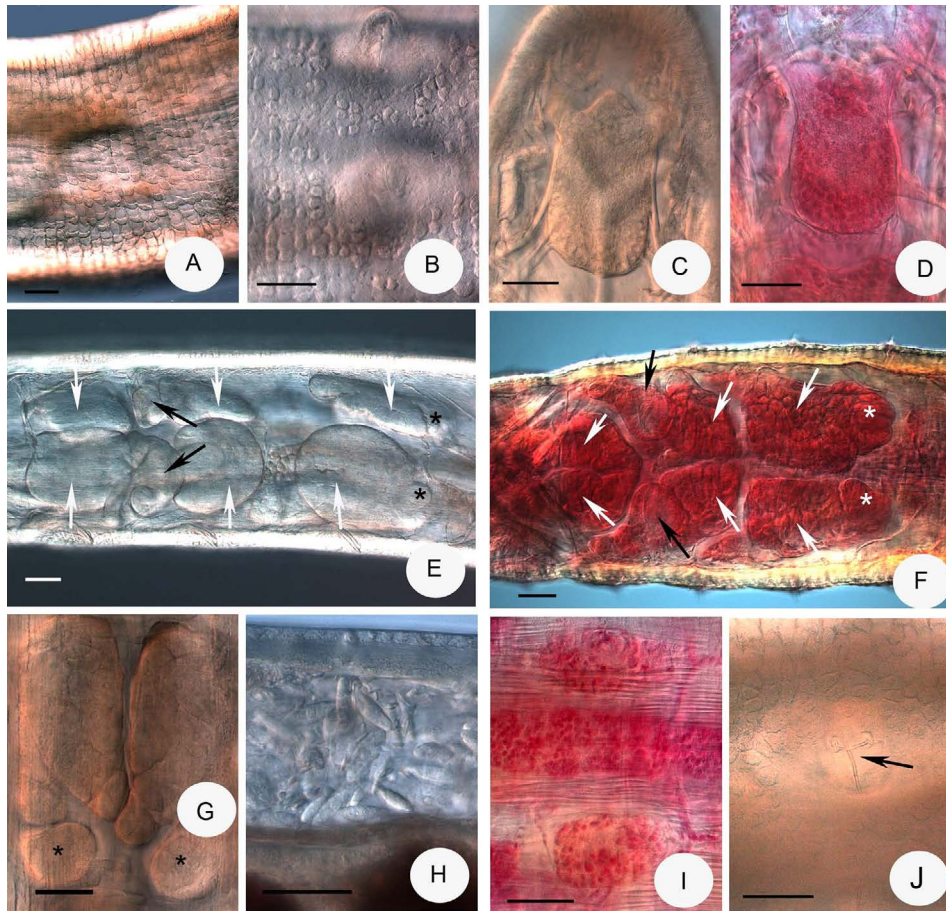

Fig. 1. Micrograph of *Fridericia alpica* sp. n. A = clitellar glands dorsally, B = clitellar glands ventrally, C–D = brain, E–F = pharyngeal glands (marked with white arrows, * = ventral projections, spermathecae marked with black arrows, E = dorso-lateral view, F = dorsal view), G = third pairs of pharyngeal glands, ventral view (* = ventral projections), H = coelomocytes, I = male copulatory organs, J = bursal slit (marked with arrow). (A–C, E, G–H, J *in vivo*, D, F, I fixed, stained; scale bars = 50 μm)

width 270–380 μm at VIII and 310–430 μm at the clitellum. Segments 38–52. Chaetal formula: 2,(1),3,4,5 – 5,4,3,2 : (1,2),3,4,5,6 – 6,5,4,3,2(1). As in other *Fridericia* species, chaetae in bundles arranged in pairs with the outer pair being longer and thicker than the inner pairs: 58–70 by 5–6 μm against 43–60 by 4–5 μm and 25–40 by 3 μm (preclitellar bundles), at body-end only 2 (1) chaetae per bundle, length about 65–75 by 5–6 μm. Head pore at 0/I. Dorsal pores from VII. Epidermal gland cells weakly developed. Clitellum in XII–1/2XIII, girdle-shaped, hyalocytes and granulocytes arranged in transverse rows dorsally (Fig. 1A), glands ventrally mostly weakly developed only (Fig. 1B). Thickness of body wall about 30–60 μm, cuticle about 1–1.5 μm *in vivo*. Brain egg-shaped, about 140–160 μm long, 2 times longer than wide *in vivo* (Fig. 1C) and 120–140 μm and 1.5 times longer than wide in the fixed specimens (Fig. 1D).

Oesophageal appendages, long, without branches. Pharyngeal glands in 4/5 united dorsally, with small ventral lobes, in 5/6 united dorsally or free with medium large ventral lobes, in 6/7 free dorsally with large ventral lobes and ventral projections (Figs. 1E–G). Chloragocytes from V, brown *in vivo*. Dorsal vessel from XVI–XXI, blood colourless. Midgut pars tumida in XXVII–XXXIII occupying 5–6 segments (Fig. 2A). Five pairs of preclitellar nephridia from 6/7 to 10/11, length ratio anteseptale : postseptale 1:1.5–2, midventral origin of efferent duct. Coelomo-mucocytes numerous, a-type (according to Möller 1971) (length 29–42 μm *in vivo*, (10–20 μm fixed), lenticytes, 6–10 μm long (Fig. 1H). Chylus cells between XVI–XVII, occupying 2 segments. Seminal vesicle large. Sperm funnels cylindrical (Figs 2C, D), about 150–280 μm long and 2–3 times as long as wide (*in vivo*). Funnel length in fixed specimens 140–180 μm. Collar not narrower than funnel body. Spermatozoa about 200–300 μm long, heads 70–150 μm *in vivo*, in fixed specimens 150–220 μm and 60–75 μm, respectively. Diameter of sperm ducts 9–10 μm (*in vivo*). Male copulatory organs (Fig. 1I) small 100–150 μm long, 55–75 μm wide and 45–55 μm high (*in vivo*), (80–120, 50–70 and 40–45 μm in fixed specimens, respectively). Bursal slits T- or Y-shaped (Fig. 1J). Subneural glands in XIII–XV, the first are the largest (Fig. 2B). Spermathecae (Figs 2E–F): one 45–50 μm long (*in vivo*) stalked ectal gland at the orifice (Fig. 2F), 35–57 μm, fixed. Ectal ducts about 150–250 μm long and 18–20 μm wide, ectally slightly widening (20–30 μm wide), projecting into ampullae, ental bulbs about 40–60 μm wide, canals not widened. Each ampulla with two rounded, mostly sessile diverticula, located on opposite sides at the ampulla. The diameter of the diverticula 45–60 μm, ampullae 60–80 μm wide, proximal part of ampulla considerably set off from distal part by a constriction, 45–50 μm long (fixed); separate openings into oesophagus. 2–3 mature eggs at a time.

Distribution and habitat – Austria: Rax Mountain in the habitats of mountain meadow and *Pinus mugo* and *Picea abies* stands, further Kőszeg Mts (Günser Mts) in mixed forest. Hungary: Kőszeg Mts near to Steirer Hauses, in *Quercus petraea* woodland with some *Fagus sylvatica.* The species lives presumably in the Alps and their foothills in the Western geographic region of Hungary.

Remarks – The new species can be well distinguished from all *Fridericia* species having two sessile spermathecal divericula and non-fused ampullae (see the Tables 3–4 in Dózsa-Farkas 2009 and Dózsa-Farkas *et al.* 2015), except *F. discifera*. The differential diagnosis from this species is given below.

Comparison of *F. alpica* sp. n. and *Fridericia discifera* Healy, 1975 – The two species are very similar, so for their proper discrimination we have combined morphological and molecular methods. Previously Schmelz (2003) also

indicated that some specimens found in the Alps and identified as *F. discifera* could belong to a yet undescribed *Fridericia* species. We found that the two above-mentioned species occur together both in the Rax Mt. and also in the Kőszeg Mts in Hungary, moreover *F. alpica* sp. n. was found in Hungary and also Austria (where this mountain range is called Günser Mountains).

We made a comparison on the basis of the descriptions of *F. discifera* by Healy (1975) and Schmelz (1999) as well as based on specimens collected in this study. Many traits of the two species are very similar, the morphological comparison of the similar characters are given in Table 2. Dissimilar charac-

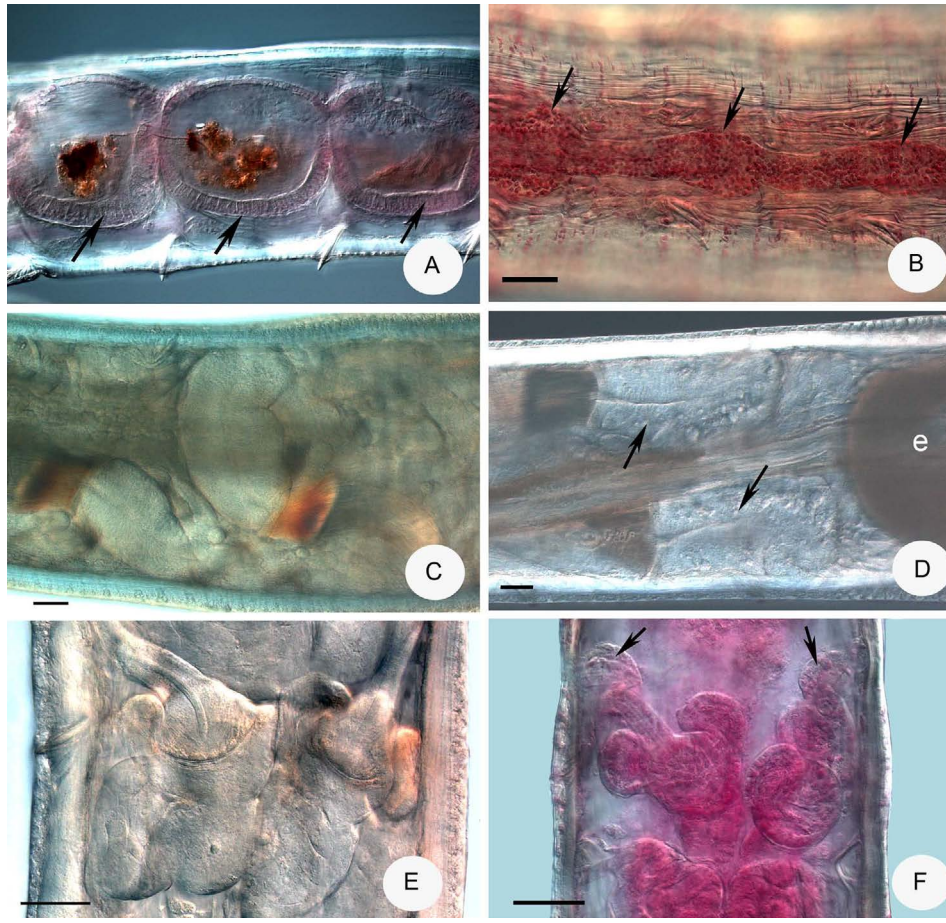

Fig. 2. Micrograph of *Fridericia alpica* sp. n. A = midgut pars tumida in XXX–XXXI, lateral view (marked with arrows), B = subneural glands in XIII–XV (marked with arrows), C–D sperm funnels (in D marked with arrows, e = egg), E–F spermathecae (in F the large ectal glands marked with arrows). (A–B, F fixed, stained, C–E *in vivo*, scale bars = 50 μm)

Table 2. Morphological comparison of *F. discifera* and *F. alpica* sp. n. Only similar traits are shown (*in vivo* values are bold, other values measured as fixed).

	F. discifera	*F. alpica sp. n.*
number of segments	38–49	38–52
length (mm)	**8.5–12** 7.5–10	**7–16** 4.7–11
diameter in VIII (μm)	**250–320** 270–380	**300–400** 270–380
diameter in XII (μm)	**280–370** 290–400	**340–400** 310–430
type of oesophagus appendages	a	a
dorsal vessel	XVI–XXII	XVI–XXI
chylus cells (occupying segments)	XIV–XVII (2)	XVI–XVII (2)
length of brain (mm) (length:diameter)	**140–160** (2:1)	**140–160** (2:1)
length of sperm funnel (mm) (length:diameter)	**150–250 (2-3**) 80–180 (1.5–2.2:1)	**150–280 (2–3)** 140–180 (1.5–2:1)
length of spermatozoa (μm)	**250–300** 110	**200–300** 150–220
length of sperm head (μm)	**100–120** 58–65	**70–150** 60–75
small male copulatory organ (μm)	**85–140** 70–90	**100–150** 80–20
bursal slits	T or Y-shaped	T or Y-shaped

ters are listed here. The maximum number of chaetae in a bundle is only 4 in *F. discifera*, but 5 or 6 in *F. alpica*. The body wall in both species has approximately the same width (in our *F. discifera* 30–40 μm, according to Schmelz (1999) only 20–25 μm, in *F. alpica* the same or slightly thicker: 30–60 μm), but the cuticle is in *F. discifera* much thicker, i.e. 5–9 μm (Fig. 3D) than in *F. alpica,* where it has only 1–1.5 μm (fixed). There are only 4 pairs of preclitellar nephridia in *F. discifera*, but 5 pairs in the new species. The mucocytes are very scarce and with hyaline matrix and small refractile vesicles at periphery in *F. discifera* (Fig. 3F) but numerous and of a-type (without refractile vesicle) in *F. alpica* (Fig. 1H). Lenticytes are of about the same length in both species. The pharyngeal glands are also slightly different: in *F. alpica* the first pair is connected dorsally and has small ventral lobes, the second pair is connected dorsally or free and has medium large ventral lobes, and the third pair is free dorsally, has large ventral lobes and projections backwards (Figs 2E–G). In *F. discifera*, the first pair of pharyngeal glands is of the same kind, the secondary

pair is also similar, but always connected dorsally, the third pair is also similar, but the projection absent (Fig. 3E). Both species have subneural glands, but in *F. alpica* in XIII–XV (in 3 segments) (Fig. 2B), whereas in *F. discifera* only in XIII–XIV (in 2 segments). The spermathecae are very similar (Figs 2E–F and 3I) either, except the ectal gland, which is larger (35–57 μm long, fixed) and stalked in *F. alpica* (Fig. 2F) but only 17–25 μm long and sessile in *F. discifera* (Fig. 3I).

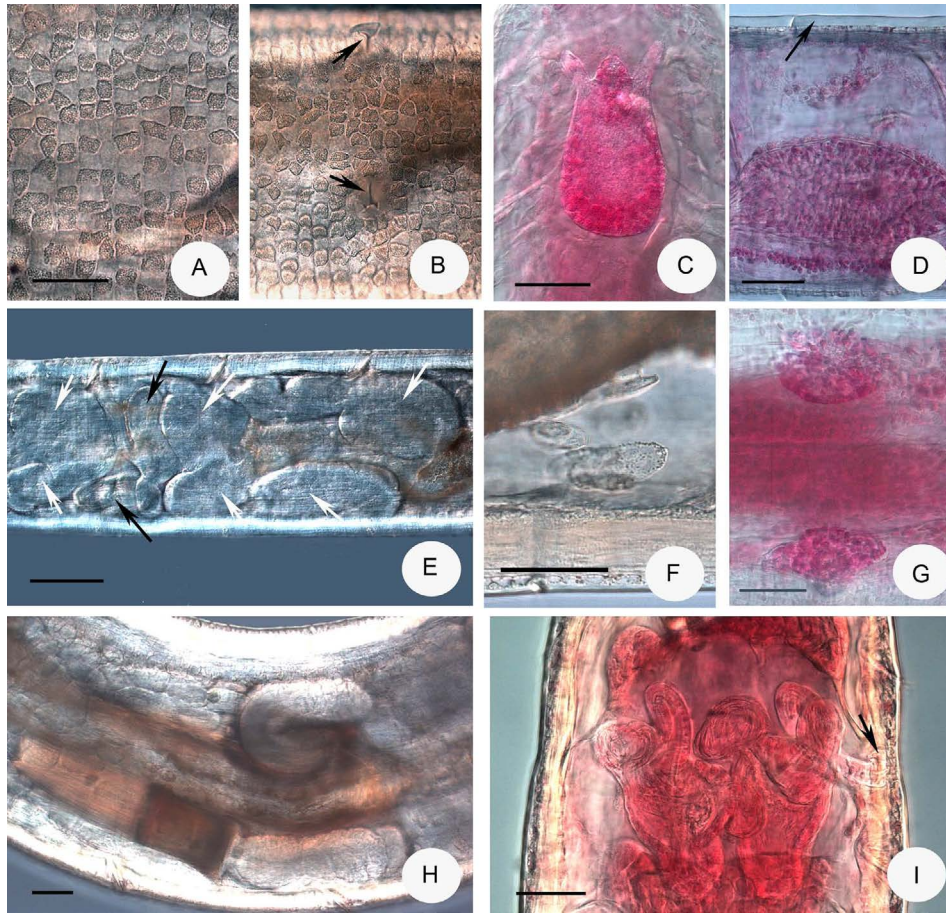

Fig. 3. Micrograph of *Fridericia discifera*. A = clitellar glands dorsally, B = clitellar glands ventrally (bursal slits marked with arrows), C = brain, D = body wall, thick cuticle (marked with arrow), E = pharyngeal glands dorsal view (marked with white arrows, no ventral projections, spermathaecae marked with black arrows), F = coelomocytes, scarce, a/b-type, G = male copulatory organs, H = sperm funnels, I = spermathaecae (small ectal gland marked with arrow). (A–B, E–F, H *in vivo*, C–D, G, I fixed, stained; scale bars = 50 μm)

Fridericia cf. *discifera* described by Rota (1995) from Tuscany and Umbria, Italy, differs considerably from *F. discifera* proper, therefore Schmelz (1999) had the opinion that this is another, yet undescribed species. However, it also differs from *F. alpica* sp. n. The main differences are as follows: the oesophageal appendages are branched whereas unbrached in *F. alpica*, the coelomocytes according to Figure 9C (Rota 1995) are c-type (according to Möller 1971) and larger (maximum of 55 μm, whereas a-type and only 29–42 μm long in the new species). The spermathecal ectal ducts are much longer 520–550 μm, whereas only 150–250 μm in *F. alpica*.

Fridericia raxiensis sp. n.
(Figs 4–5)

Type material – Holotype. F.27. slide No. 2125, Rax Mountain, close to the Rax cable car terminal, under *Pinus mu*go 47°43.036N, 15°46.024E, 1620 m a.s.l., leg. Farkas, J., 15.05.2012.

Paratypes. In total 12 specimens P.111.1.1.–111.1.4. slide No. 2229–2233 four specimens Rax Mountain, close to the Rax cable car terminal, under *Pinus mugo,* 47°43.23.3N, 15°45.164E 1612 m a.s.l., leg. Farkas, J., 15.05.2012; P.111.2.1–111.2.3 slide No. 2126–2128 three specimens Rax Mountain, close to the Rax cable car terminal, subalpine meadow, 47°43.172N, 15°45.218E, 1613 m a.s.l., leg. Farkas, J., 15.05.2012; P.111.3.1–111.3.2 slide No. 2227–2228 two specimens Rax Mountain, close to the Rax cable car terminal, under *Pinus mugo* 47°43.007N, 15°45.401E, 1613 m a.s.l., leg. Farkas, J., 15.05.2015; P.111.4.1–111.4.3 slide No. 2239–2241 three specimens Rax Mt. 47°71.666N, 15°77.305E, 1613 m a.s.l., under *Pinus mugo* leg. Bauer, R., 10.06.2008.

Etymology – Named after the Rax Mountain where this species was found.

Diagnosis – The new species can be recognized by the following combination of characters: (1) medium size (14–18 mm *in vivo*), segments 51–59; (2) maximum 5 chaetae per bundle; (3) clitellum girdle-shaped: hyalocytes and granulocytes arranged in transverse rows but weakly developed; (4) five preclitellar pairs of nephridia; (5) coelomo-mucocytes numerous, c/b-type (according to Möller 1971), scarce, 30–44 μm *in vivo*, lenticytes 8–10 μm long; (6) chylus cells in XIII–XVI (3–4 segments long); (7) bursal slit T-shaped, the transverse component is short; (8) seminal vesicle large; (9) a small subneural gland in XIII; (10) sperm funnel pear-shaped, approximately half as long as body diameter, collar narrower as funnel body, sperm 340–370 μm long, heads 75–85 μm *in vivo*; (11) spermatheca with long ectal duct, large ectal gland, ampulla entally separate, with about 8 sessile, sphaerical diverticula varying in size.

Description – Holotype 11.7 mm long, 380 μm wide at VIII and 380 μm at the clitellum (fixed), 51 segments. Body length of the paratypes 14–18 mm, width 350–410 μm at

VIII and 370–480 μm at the clitellum (*in vivo*). Length of fixed specimens 8–13 mm, width 350–440 μm at VIII and 380–470 μm at the clitellum. Segments 51–59. Chaetal formula: 2,3,4 – 4,3,2,(1) : 3,4,5 – 4,3,2. Chaetae in bundles arranged in pairs with the outer pair being longer and thicker than the inner pair: 45–60 by 5 μm against 35–40 by 2.5–3 μm. Chaetal lengths about the same also in postclitellar segments. From about XXX only two chaetae per bundle, but in one case (slide No. 2229) already from XXV, these about 60 μm long and 5 μm wide in terminal segments. Head pore at 0/I. Dorsal pores from VII. Epidermal gland cells in 5–9 transverse rows per segment (Fig. 4A). Body wall thick, about 50 μm, the cuticle 3–5 μm, so that internal organs are often difficult to investigate *in vivo*. Clitellum in XII–1/2XIII, girdle-shaped, glands arranged in transverse rows, weakly developed (Fig. 4B).

Brain egg-shaped, about 140–160 μm long, 2 times longer than wide *in vivo* and 120–160 μm and 1.5 times longer than wide in the fixed specimens (Fig.4C). Oesophageal appendages extending into V, without branches. Pharyngeal glands are very characteristic. All pairs connected dorsally (sometimes the third is free), ventral lobes absent in IV. Large additional ventral lobes in segment VII (Fig. 4G). Chloragocytes from V, brown *in vivo*. Dorsal vessel from XVII–XX, blood colourless. Midgut pars tumida not visible. Five pairs of preclitellar nephridia from 6/7 to 10/11 (Fig. 4D), large anteseptale, the length ratio anteseptale : postseptale 1:1.2–1.6, posteroventral origin of efferent duct. Coelomo-mucocytes oval, numerous, c/b-type, matrix fine granulous with some refractile grains, length 30–44 μm *in vivo* (Fig. 4E), but in the fixed specimens the matrix of the mucocytes (24–36 μm long) considerably granulous with well stained nucleus (Fig. 4F). Lenticytes scarce, 8–10 μm long. Chylus cells (Fig. 4H) between XIII–XVI, occupying 3–4 segments. Seminal vesicle large, in X–XI. Sperm funnels nearly pear-shaped, about 170–250 μm long and 1.5–2 times as long as wide (*in vivo*) (Fig. 5A). Funnel length in fixed specimens 120–190 μm and about 1.4 times longer than wide (Fig. 5B). Collar narrower than the funnel. Spermatozoa about 340–370 μm long, heads 75–85 μm *in vivo*, in fixed specimens 250–300 μm and 45–60 μm, respectively. Diameter of sperm ducts 6–8 μm (fixed). Male copulatory organs (Figs 4I, 5C) small, 140–160 μm long, 70–90 μm wide and 60 μm high (*in vivo*), (100–120, 60–90 and 40–60 μm in fixed specimens, respectively). Bursal slits T-shaped, but the transverse component is short and the longitudinal component at the two ends with two short transverse components too (Fig. 5C). One small subneural gland in XIII (Fig. 4I). Spermathecae (Figs 5D–F): one large, 40–60 μm long (*in vivo* and fixed equally) ectal gland at the orifice (Figs 5E–F). Ectal ducts about 320–390 μm long and 18–20 μm wide (250–330 μm long and 16–18 μm wide, fixed), projecting into ampullae, ental bulbs about 40 μm wide *in vivo*, canals not widened. About 6–9 sessile diverticula (mostly 8) of varying size: diameter (16)–30–50 μm (fixed). Sperm in a circle in lumen of ampullar distal part. Diameter of ampulla and diverticula together 90–120 μm. The epithelium of diverticula *in vivo* thick and warty (Fig. 5D), mostly no sperm in the diverticula. Separate openings into oesophagus dorso-laterally. One or two mature eggs at a time.

Distribution and habitat – Only known from the type locality.

Differential diagnosis – The number of valid *Fridericia* species with more than two diverticula per spermatheca is 18: *F. agilis* Smith, 1895; *F. agricola* Moore, 1895; *F. bernini* Dózsa-Farkas, 1988; F. *douglasensis* Welch, 1914; *F. dura* Eisen, 1879; *F. firma* Smith et Welch, 1913; *F. glandifera* Friend, 1911; *F. galba* (Hoffmeister, 1843); *F. gigantea* Dequal, 1912; *F. hegemon* (Vejdovksy, 1878); *F. minor* Friend, 1913; *F. oconeensis* Welch, 1914; *F. parаunisetosa* Xie, Liang et

Wang, 2000; *F. pyrenaica* Gianni, 1979; *F. regularis* Nielsen et Christensen, 1959; *F. terrarossae* Sesma et Dózsa-Farkas, 1993; *F. vixdiverticulata* Sesma et Dózsa-Farkas, 1993; *F. callosa* (Eisen, 1878) (type with diverticula).

The new species differs from all these species, leaving other characters out of consideration, by the presence of the additional large ventral lobes of pharyngeal glands in VII, which is a very rare character among the *Fridericia* species.

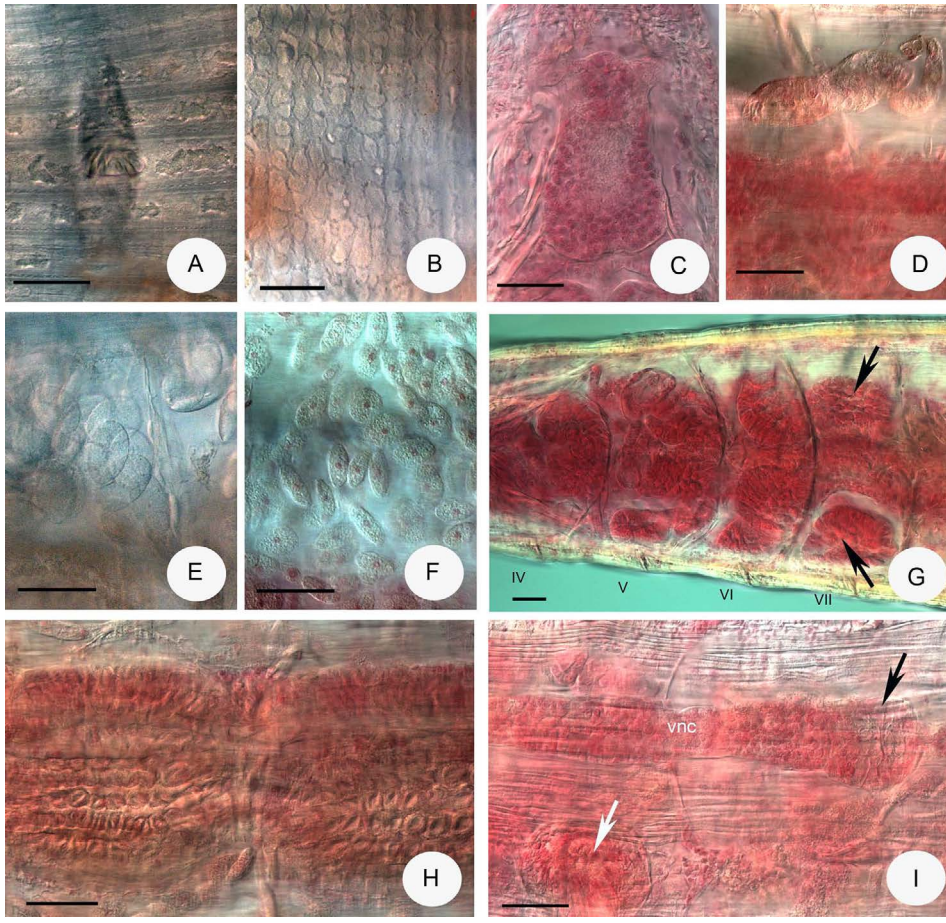

Fig. 4. Micrograph of *Fridericia raxiensis* sp. n. A = epidermal glands, B = clitellar glands dorsally (weakly developed), C = brain, D = second nephridium preclitellarly, E–F = coelomocytes, G = pharyngeal glands dorsal view (the additional ventral lobes in VII marked with arrows), H = chylus cells in XIV–XV of holotype, I = male copulatory organ (marked with white arrow) and the small subneural gland in XIII (marked with black arrow). (A–B, E *in vivo*, C–D, F–I fixed, stained; scale bars = 50 μm)

Molecular results

In total, 8, 10 and 12 sequences were obtained from various *Fridericia* specimens in the case of ITS, CO1 and H3, respectively, and some additional sequences obtained previously were also used for comparison (Table 3). Results of this molecular analysis confirmed that *F. discifera* and *F. alpica* sp. n. are distinct species, since they separated on the phylogenetic trees constructed based on the three studied regions (Fig. 6). Additionally, the other novel species, *Fridericia raxiensis* sp. n. also had a position on the trees distinct from any other similar species.

DISCUSSION

The enchytraeid fauna (41 species of 12 genera) of the Rax Mountain area is quite diverse, and consists mostly of species typical for the Northern and Central European fauna. An essential element of this fauna is *Euenchytraeus clarae* (Bauer, 1993), a typical species of the forest fauna of European mountains (Bretscher 1906, Bauer 1993, Schmelz & Collado 2010), so it is understandable, that this species was found only in the *Pinus mugo* and *Picea abies* zone.

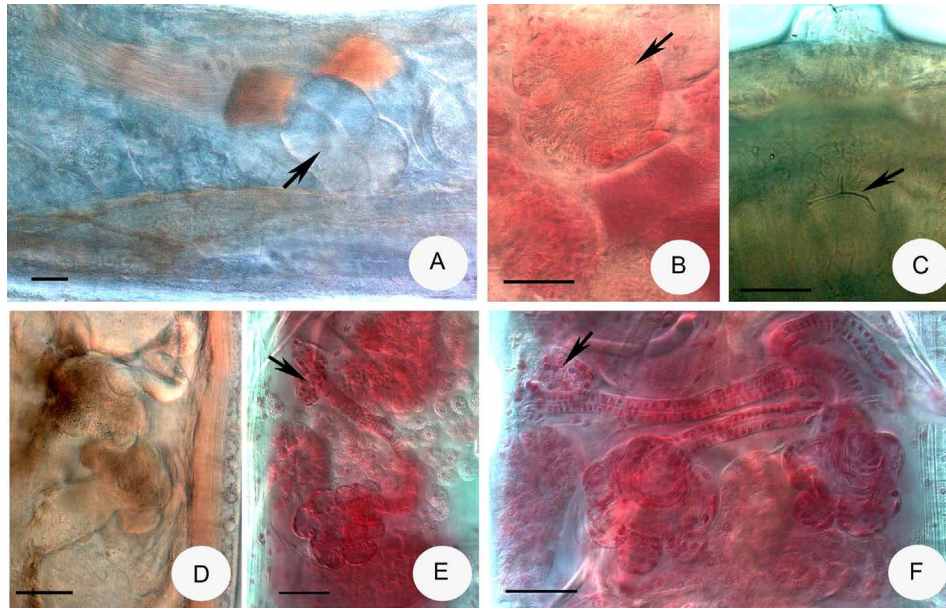

Fig. 5. Micrograph of *Fridericia raxiensis* sp. n. A–B = sperm funnels (marked with arrows), C = bursal slit (marked with arrow), D–F spermathecae (in E and F the large ectal glands marked with arrows). (A, C, D *in vivo*, B, E–F fixed, stained; scale bars = 50 μm)

Table 3. List of *Fridericia* specimens used for molecular taxonomic analyses with collection data and GenBank accession numbers (sequences determined in this study appear in bold).

Species	Collection source and collection date	Specimen ID	GenBank accession numbers		
			ITS	CO1	H3
F. alpica sp.n.	Austria, Rax Mt., 47°43.012N, 15°46.317E, 1560 m a.s.l., leg. J. Farkas & Z. Tóth, 16.06.2015.	834	**KX985873**	**KX985881**	**KX985887**
		835	**KX985874**	**KX985882**	–
F. benti	(see reference Dózsa-Farkas et al. 2015)	795	KR872365	–	KR872365
F. bisetosa	(see reference Dózsa-Farkas & Felföldi 2016)	522	KU586623	KU586588	KU58604
F. brunensis	Hungary, Zemplén Mts., hillside with beech trees, 48°46.222N, 21°38.833E, 341 m a.s.l., leg. K. Dózsa-Farkas, 21.04.2004.	24	–	**KX985879**	–
		25	**KX985870**	**KX985880**	–
F. connata	(see reference Dózsa-Farkas & Felföldi 2016)	519	KU586625	KU586594	KU586610
F. connatiformis	(see reference Dózsa-Farkas & Felföldi 2016)	516	KU586621	KU586591	–
	(see reference Dózsa-Farkas & Felföldi 2016)	517	–	–	KU586607
F. christeri	Hungary, Mezőföld, Felsőtengelic, meadow, 46°33.222N, 18°45.552E, 117 m a.s.l., leg. K. Dózsa-Farkas & J. Farkas, 13.11.2013.	768	–	–	**KX985888**
F. dura	Hungary, Danube-Dráva N.P., Páprád, Bükkhát Forest Reserve, riverine oak-elm-ash woodlands, 45°52.194N, 18°00.474E, 109 m a.s.l., leg. K. Dózsa-Farkas, A. Ortmann-Ajkai, J. Farkas, 28.03.2011.	716	–	–	**KX985896**
	Hungary, Őrség N.P., Gödörházi rétek, on the edge of alder swamp, woodlands with *Hemerocallis lilioasphodelus*, 46°44.782N, 16°21.227E, 229 m a.s.l., leg. Z. Tóth, 26.10.2015.	907	–	–	**KX985894**
F. glandifera	Hungary, Pilisszentlélek, beech woodland, 47°72.694N, 18°83.722E, 396 m a.s.l., leg. K. Dózsa-Farkas, G. Nagy & J. Novák, 11.07.2013.	883	–	–	**KX985889**
F. discifera	Austria, Rax Mt., under *Picea alba*, 47°43.012N, 15°46.317E, 1560 m a.s.l., leg. J. Farkas & Z. Tóth, 15.05.2015.	836	**KX985871**	**KX985878**	**KX985890**
		884	–	**KX985877**	**KX985891**
	Austria, Rax Mt., under *Pinus mugo*, 47°43.007N, 15°45.401E, 1613 m a.s.l., leg. J. Farkas & Z. Tóth, 15.05.2015.	840	**KX985872**	**KX985876**	**KX985892**

Table 3 (continued).

Species	Collection source and collection date	Specimen ID	GenBank accession numbers		
			ITS	CO1	H3
F. longiducta	(see reference Dózsa-Farkas & Felföldi 2016)	531	–	KU586585	KU586600
F. phaeostriata	(see reference Dózsa-Farkas & Felföldi 2016)	814	KU586614	KU586584	KU586601
F. ratzeli	Hungary, Őrség N.P., Gödörházi rétek, hay medow with *Salix rosmarinifolia,* 46°44.931N, 16°21.177E, 229 m a.s.l., leg. Z. Tóth, 26.10.2015.	844	**KX985875**	**KX985884**	**KX985895**
	Switzerland, Neuchûtel, lake shore, 47°00.722N, 6°99.444E, 445 m a.s.l., leg. G. Boros, 21.10.2015.	964	–	**KX985883**	**KX985893**
	(see reference Erseus et al. 2015)	CE782	–	GU902070	–
F. perrieri	Hungary, Bátorliget, Vámosatya, mesotrophic wet meadow 48°11.304N, 22°24.110E, 116 m a.s.l., leg. K. Dózsa-Farkas & J. Farkas, 13.05.2011.	867	**KX985869**	–	–
F. raxiensis	Austria, Rax Mt., alpine meadow, 47°43.172N, 15°45.218E, 1613 m a.s.l., leg. J. Farkas, 15.05.2012.	721	–	–	**KX985886**
	Austria, Rax Mt., under *Pinus mugo,* 47°43.007N, 15°45.401E, 1613 m a.s.l., leg. J. Farkas & Z. Tóth, 15.05.2015.	879	**KX985868**	**MG921590**	**KX985885**
F. zicsii	Hungary, Őrség N.P., Gödörházi rétek, on the edge of alder swamp woodlands with *Hemerocallis lilioasphodelus*, 46°44.782N, 16°21.227E, 229 m a.s.l., leg. K. Dózsa-Farkas, J. Farkas, Z. Tóth & F. Hoc, 31.03.2014.	870	KU586620	KU586587	KU586606
Hemifridericia parva (outgroup)	(see reference Dózsa-Farkas & Felföldi 2015)	511a	KM591939	KM591923	KM591931

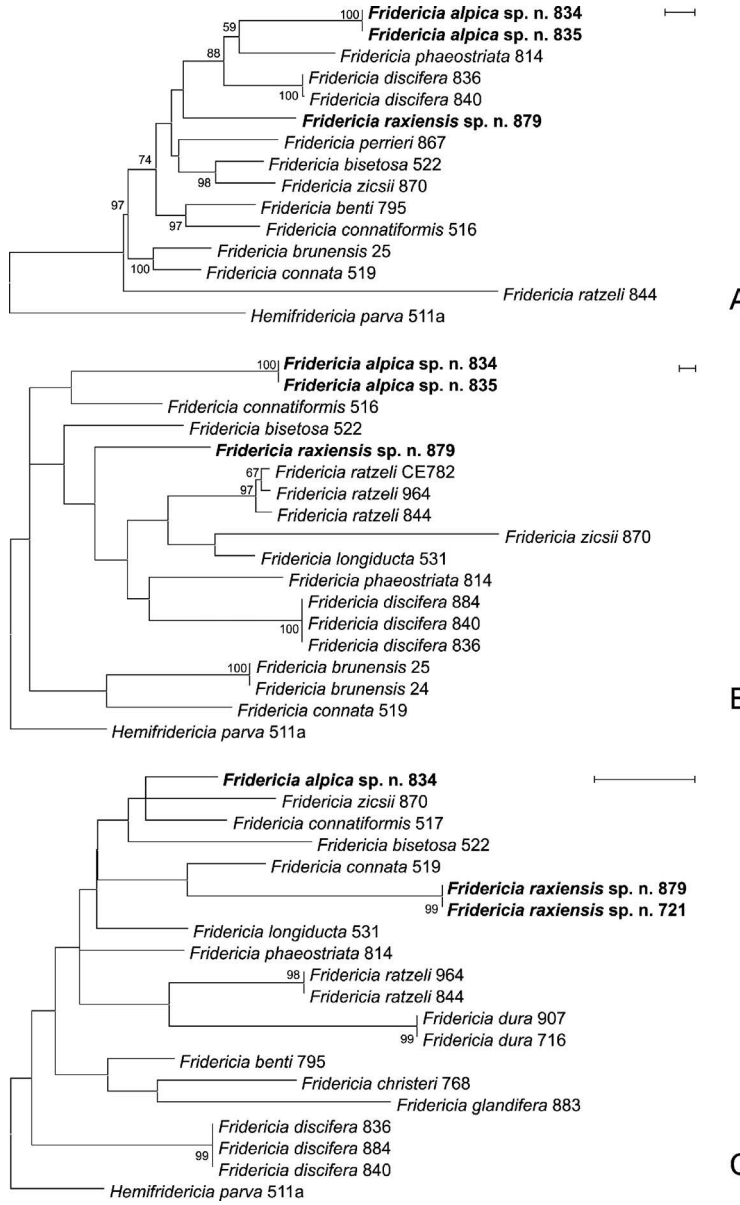

Fig. 6. Maximum likelihood (ML) trees of studied *Fridericia* species based on the ITS region (A), CO1 (B) and H3 genes (C). Bootstrap values greater than 50 are shown at the nodes. Sequences from new species described here appear in bold. A = ML tree of the ITS region based on 779 nucleotide positions using the GTR+G+I substitution model, B = ML tree of the CO1 gene based on 423 nucleotide positions using the GTR+I substitution model, C = ML tree of the H3 gene based on 176 nucleotide positions using the K2+G substitution model. Scale bars, 0.05 substitutions per nucleotide position

Regarding the species numbers of the investigated areas (Table 1), the fauna of *Pinus mugo* stands showed the highest value with 26 species (although it should be noted, that most samples were taken from this habitat). The habitat with *Larix decidua* showed low species richness with only 6 species present.

Using morphological characters, the two new species, *F. alpica* sp. n. and *F. raxiensis* sp. n. could easily be discriminated from other similar *Fridericia* species, and are well supported by the molecular results as well. Both new *Fridericia* species were found only in the highest region. *F. raxiensis* sp. n. seems to be a species with a narrower geographical distribution, but the other new species, *Fridericia alpica* sp. n., occupies a broader area, as it was found also in Rax Mt. and in Kőszeg Mountains (in Austria and Hungary), having an alpine or subalpine mesoclimate. Two presumably new species, *Fridericia* sp. and *Enchytraeus* sp., and the two species belonging to the *Fridericia aurita* species complex require further study before their descriptions can be published.

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