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A new leaf litter dwelling *Adropion* species (Tardigrada; Eutardigrada; Itaquasconinae) from the Northern Apennines (Italy)

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Short communication

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Tardigrades are known to inhabit a variety of substrates, including leaf litter. In this article, I describe a new tardigrade species, *Adropion fagineum* n.sp. that inhabits beech leaf litter in the Italian Northern Apennines. Due to having long and thin macroplacoids, the new species belongs to the species of the *belgicae-scoticum* complex; however, it can be differentiated from other species by the number of macroplacoids (two in the new species), the presence of cuticular bars in the legs (present in the new species) and by the relative length of the flexible pharyngeal tube compared to the rigid buccal tube.

Key words: Taxonomy, new species, water bear, biodiversity, faunistic.

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Tardigrades are a group of microscopic (<1 mm) animals found all over the world in substrates that experience the presence of at least a film of liquid water (Nelson *et al.* 2018). Nearly 1500 tardigrade species have been described up until now (Degma & Guidetti 2007, 2024; Guidetti & Bertolani 2005), with new species being described each year (Degma & Guidetti 2024; Guil & Cabrero-Sañudo 2007). Tardigrades are typically found in mosses and lichens (Nelson *et al*. 2018), and most of the research on their biodiversity has focused on these habitats. However, another habitat colonised by tardigrades that has received less attention is leaf litter, which can host rich and diverse tardigrade communities (Czerneková *et al.* 2018; Guidetti *et al.* 1999; Guil & Sanchez-Moreno 2013; Hallas & Yeates 1972; Hinton *et al.* 2010; Nelson & Bartels 2013), and from which new species have been described (e.g., Bertolani *et al.* 1994; Vecchi & Stec 2021).

The genus *Adropion* Pilato, 1987 was established by Bertolani *et al.* (2014) to accommodate the species formerly included in the subgenus *Diphascon* (*Adropion*) (Pilato 1987). This genus is characterised by the presence of a long and thin annulated flexible pharyngeal tube following the buccal tube, without a drop-like thickening between them (Bertolani *et al.* 2014), as well as by claws of the *Hypsibius* type (Pilato & Binda 2010). Since its original description as a subgenus, the species composition of *Adropion*, as well as its definition, has changed over time (Bertolani *et al.* 2014; Gąsiorek *et al.* 2023; Gąsiorek & Michalczyk

2020). Today, the genus contains 11 species (Degma & Guidetti 2024), with its latest addition being *A. camtchaticum* Tumanov & Kalimullin, 2024. Two of the species included in the genus have problematic taxonomic statuses: *A. gani* (Sun, Li & Feng 2014) is considered *nomen inquirendum*; and *A. marcusi* (Rudescu 1964) is considered *species dubia* (Gąsiorek *et al.* 2023). In this study I describe a new species, *Adropion fagineum* n. sp. inhabiting beech leaf litter collected in the Italian Northern Apennines through morphology, morphometry and DNA sequencing.

Materials and Methods

Sampling and tardigrades extraction

A beech leaf litter sample (about 1 litre in volume, sample code IT.232) was collected on 11/04/2024 by the author in Monchio delle Corti, Parma, Italy (44°21′22.2″N 10°05′40.0″E; 1616 m a.s.l.). The leaf litter was collected from the side of a small temporary rivulet. The sample was processed within 6 hours of its collection, by washing the leaf litter with water and collecting the sediment flowing out of it. The sediment was then inspected under a stereomicroscope, and the tardigrades were individually removed with an Irwin loop. The sample was collected under Sampling Permit N.0001671/2020 from Parco Nazionale Appennino Tosco-Emiliano (Italy).

Microscopy and imaging

The specimens used for light microscopy were mounted on microscope slides in a small drop of Hoyer's medium, secured with a cover slip and dried at 50°C for a week. The slides were examined under a Leica DMLB light microscope with phase contrast (PCM), associated with a digital camera. For structures that could not be satisfactorily focused in a single light microscope photograph, a stack of 2-5 images was taken with an equidistance of ca. 0.2 µm and were assembled manually into a single deep-focus image in GIMP v.2-10 (GIMP Development Team 2019). The figures were assembled in Figure J (Mutterer & Zinck 2013).

Taxa identification

Taxa identification was done up to the species level by using the relevant literature; in particular, the identification relied mostly on Bingemer & Hohberg (2017), Gąsiorek *et al.* (2023), Guidetti *et al.* (2022), Hansen *et al.* (2017), Pilato & Binda (2010) and Ramazzotti & Maucci (1983).

Morphometrics and morphological nomenclature

All measurements are given in micrometres (µm). Structures were measured only if their orientation was suitable. Body length was measured from the anterior extremity to the posterior end of the body, excluding the hind legs. Buccal tube length and the level of the stylet support insertion point were measured according to Pilato 1981. The *pt* index is the ratio of the length of a given structure to the length of the buccal tube (Pilato 1981). Claws were measured according to Stec *et al.* (2018) and the ratios were calculated according to Vecchi *et al.* (2023). The nomenclature of the leg structures follows Gąsiorek *et al.* (2023). The morphometric data was handled using the 'Parachela' ver. 1.7 template available from the Tardigrada Register (Michalczyk & Kaczmarek 2013). The raw morphometric data is provided as Supplementary Material (SM.01).

Genotyping and molecular analyses

DNA was extracted from two individual animals following a Chelex® 100 resin (BioRad) extraction method, with modifications as described in detail in Stec *et al.* (2020). The animals were observed and photographed in water under a light microscope to ensure their identity. Three DNA fragments – two nuclear (18S rRNA, 28S rRNA) and one mitochondrial (COI) – were sequenced. All the fragments were amplified and sequenced according to the primers (18S: 18S_Tar_1Ff + 18S_Tar_1Rr; 28S: 28S_Eutar_F + 28SR0990; COI: LCO1490 + HCO2198) and protocols described in Stec *et al.* (2020). The sequencing products were read with the ABI 3130xl sequencer at the Genomed company (Warsaw, Poland).

The obtained DNA sequences were searched against the Tardigrada (taxid:42241) sequences in the NCBI core_nt database (Sayers *et al.* 2022) with the blastn algorithm (Zhang *et al.* 2000). The blastn results are available as SM.02.

Results and Discussion

In the examined sample, 63 tardigrades and 10 eggs were found. The identified taxa are presented in Table 1.

Based on morphological characteristics, 14 of the identified *Adropion* individuals were attributed to

Table 1

Tardigrade taxa found in the sample IT.232.

Taxa	Notes
Adropion fagineum n.sp.	14 animals. See the taxonomic account below.
Adropion sp.	2 animals. See the remarks section below.
Bertolanius weglarskae (Dastych 1972)	20 animals + 10 eggs. Already found in beech leaf litter by Guidetti *et al.* (1999).
Dianea sattleri (Richters 1902)	1 animal. Already found in beech leaf litter by Guidetti *et al.* (1999).
Diphascon gr. pingue	1 animal.
Hypsibius gr. dujardini	5 animals.
Mesobiotus sp.	19 animals. Due to the lack of eggs, it was not possible to provide a species level identification.
Paramurrayon meieri Guidetti, Giovannini, Del Papa, Ekrem, Nelson, Rebecchi & Cesari 2022	1 animal. Despite the lack of eggs, the animal matches the original species description. This is the first confirmed record from beech leaf litter. Guidetti *et al.* (1999) identified *Paramurrayon* cf. *dianae* in beech leaf litter, which could potentially represent a previous record of *P. meieri* in beech leaf litter.

a new species, which is here formally described (see the Taxonomic account below). Of the two *Adropion* individuals processed for DNA amplification and sequencing, only one of them (Adr.sp._IT.232.02) provided a successful amplification.

Taxonomic account

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Adropion fagineum n. sp. Vecchi, 2024

Type locality. Monchio delle Corti, Parma, Italy (44°21′22.2″N 10°05′40.0″E; 1616 m a.s.l.)

Material examined. Holotype (Slide IT.232.08) and 11 paratypes (Slides IT.232.03, IT.232.07, IT.232.08) mounted on slides in Hoyer's medium. Two individuals (Adr.sp._IT.232.01 and Adr.sp._IT.232.02) were processed for DNA extraction.

Material repository. The slides with the type series are deposited in the Tardigrada collection of the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016, Kraków, Poland.

Etimology. From *Fagus* (Beech), referring to the habitat where the new species has been found (beech leaf litter).

Morphological description

Body medium-sized to large (Measurements presented in Table 2), elongated, of a similar width over its entire length (Fig. 1A). Body colour whitish, but

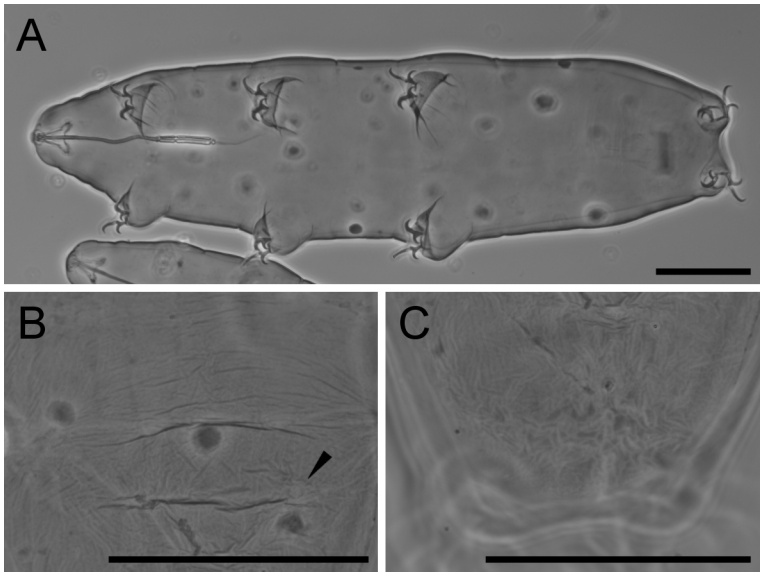

Fig. 1. A*dropion fagineum* n.sp. *in toto* and cuticle under PCM. A – Holotype. B-C – Dorso-caudal wrinkled cuticle. The arrowhead indicates a cribrous area. Scale bars 50 µm.

Table 2

Morphometric measurements of *Adropion fagineum* n. sp.

CHARACTER	N	RANGE						MEAN		SD		Holotype	
		µm			*pt*			µm	*pt*	µm	*pt*	µm	*pt*
Body length	12	252	–	631	*1421*	–	*2204*	438	*1841*	100	*230*	362	*1806*
Buccopharyngeal tube													
Buccal tube length	12	17.7	–	28.6		–		23.6	–	3.2	–	20.0	–
Pharyngeal tube length	12	37.0	–	67.2	*185.0*	–	*242.1*	49.7	*211.8*	7.4	*19.3*	43.9	*219.3*
Buccopharyngeal tube length	12	54.7	–	95.8	*285.1*	–	*342.1*	73.3	*311.8*	10.1	*19.3*	63.9	*319.0*
Buccal/pharyngeal tube length ratio	12	41%	–	54%		–		48%	–	4%	–	46%	–
Stylet support insertion point	12	13.9	–	20.4	*64.5*	–	*78.5*	17.4	*74.1*	1.9	*4.2*	15.1	*75.5*
Buccal tube external width	12	2.0	–	3.6	*9.1*	–	*13.2*	2.6	*10.8*	0.5	*1.2*	2.2	*10.8*
Buccal tube internal width	12	0.9	–	2.0	*4.6*	–	*7.4*	1.4	*6.0*	0.3	*0.9*	1.2	*6.0*
Placoid lengths													
Macroplacoid 1	12	5.5	–	14.5	*30.9*	–	*57.2*	10.2	*42.8*	2.5	*6.9*	8.3	*41.3*
Macroplacoid 2	12	13.4	–	32.0	*72.4*	–	*111.9*	21.7	*91.3*	4.7	*10.9*	18.0	*89.8*
Microplacoid	12	1.1	–	2.5	*4.5*	–	*10.2*	1.7	*7.3*	0.5	*1.8*	1.4	*7.1*
Macroplacoid row	12	19.1	–	47.5	*107.7*	–	*165.9*	32.6	*137.6*	7.3	*17.5*	26.8	*133.7*
Placoid row	12	21.0	–	52.2	*116.8*	–	*182.4*	35.3	*148.7*	8.0	*18.9*	29.1	*145.2*
Claw I heights													
External base	7	3.5	–	8.2	*19.9*	–	*28.8*	5.9	*24.7*	1.6	*3.4*	5.3	*26.2*
External primary branch	7	6.1	–	13.9	*34.2*	–	*52.4*	10.5	*44.4*	2.4	*7.1*	10.0	*49.7*
External secondary branch	7	3.5	–	9.5	*19.9*	–	*37.9*	7.1	*29.8*	1.9	*5.6*	7.6	*37.9*
External cbt ratio	7	46.4	–	70.0		–		56.2	–	7.4	–	52.7	–
External *br* ratio	7	58.2	–	82.6		–		67.3	–	9.1	–	–	–
External total	7	5.8	–	19.1		–		13.4	–	4.7	–	–	–
Internal base	8	3.3	–	7.3	*14.8*	–	*25.6*	5.2	*21.8*	1.3	*3.3*	4.2	*21.2*
Internal primary branch	8	4.9	–	8.8	*17.7*	–	*37.4*	6.8	*29.3*	1.5	*6.4*	6.3	*31.3*
Internal secondary branch	7	3.9	–	7.2	*19.6*	–	*29.4*	5.5	*24.1*	1.2	*3.4*	4.5	*22.5*
Internal cbt ratio	8	39.6	–	124.3		–		78.8	–	24.0	–	67.7	–
External *br* ratio	7	67.5	–	97.5		–		78.7	–	9.6	–	–	–
Internal total	8	7.1	–	13,3		–		10.3	–	1.9	–	–	–
Claw II heights													
External base	7	3.3	–	8.5	*18.6*	–	*30.1*	6.5	*27.3*	1.8	*4.1*	5.4	*27.0*
External primary branch	7	9.0	–	17.7	*49.5*	–	*61.7*	12.9	*54.9*	2.7	*4.7*	11.3	*56.3*
External secondary branch	7	5.2	–	10.3	*26.2*	–	*39.9*	8.0	*33.9*	1.7	*5.0*	8.0	*39.9*
External cbt ratio	7	36.5	–	60.8		–		49.8	–	7.4	–	48.0	–
External *br* ratio	7	48.3	–	70.8		–		61.9	–	7.9	–	–	–
External total	7	12.2	–	24.7	*68.8*	–	*86.2*	17.8	*75.5*	4.0	*6.7*	14.7	*73.6*
Internal base	4	5.3	–	8.6	*23.6*	–	*29.9*	6.7	*26.5*	1.4	*2.6*	5.3	*26.4*
Internal primary branch	4	6.5	–	11.3	*26.1*	–	*39.5*	8.6	*34.1*	2.1	*6.2*	7.7	*38.4*
Internal secondary branch	4	5.9	–	9.4	*25.0*	–	*32.8*	7.1	*28.1*	1.6	*3.7*	5.9	*29.5*
Internal cbt ratio	4	68.7	–	100.0		–		79.2	–	14.2	–	68.7	–
External *br* ratio	4	76.7	–	95.7		–		83.4	–	8.6	–	–	–
Interna total	4	10.2	–	14.9	*42.9*	–	*51.9*	12.3	*48.5*	1.9	*4.0*	10.2	*50.7*

Table 2 Cont.

Morphometric measurements of *Adropion fagineum* n. sp.

CHARACTER	N	RANGE						MEAN		SD		Holotype	
		µm			*pt*			µm	*pt*	µm	*pt*	µm	*pt*
Claw III heights													
External base	7	3.4	–	9.3	*19.0*	–	*32.5*	6.8	*28.3*	1.9	*4.6*	6.0	*29.7*
External primary branch	7	8.8	–	17.1	*49.1*	–	*61.4*	13.1	*55.7*	2.5	*5.7*	12.1	*60.3*
External secondary branch	7	5.2	–	10.2	*29.5*	–	*38.7*	8.2	*34.7*	1.6	*3.5*	7.7	*38.2*
External cbt ratio	7	38.4	–	63.2		–		50.9	–	7.9	–	49.3	–
External *br* ratio	7	59.4	–	69.4				62.5		3.4			
External total	7	12.0	–	24.3	*67.3*	–	*86.2*	18.2	*77.7*	3.7	*8.1*	16.8	*83.9*
Internal base	6	3.8	–	9.3	*20.4*	–	*32.5*	6.2	*25.9*	1.8	*4.6*	5.3	*26.5*
Internal primary branch	6	5.8	–	10.9	*31.8*	–	*42.5*	8.6	*36.6*	1.7	*5.0*	8.5	*42.5*
Internal secondary branch	6	4.3	–	9.8	*24.1*	–	*34.0*	6.7	*28.4*	1.8	*4.2*	6.1	*30.5*
Internal cbt ratio	6	62.3	–	85.3		–		71.0	–	9.5	–	62.3	–
External *br* ratio	6	71.7	–	89.4				77.9		6.3			
Interna total	6	7.6	–	15.8	*42.6*	–	*58.3*	12.1	*51.1*	2.7	*6.8*	11.1	*55.2*
Claw IV heights													
Anterior base	6	5.4	–	9.0	*25.8*	–	*33.2*	7.3	*29.8*	1.3	*3.1*	5.4	*27.1*
Anterior primary branch	6	8.1	–	14.5	*34.7*	–	*50.6*	10.3	*42.0*	2.3	*5.7*	8.1	*40.6*
Anterior secondary branch	6	6.6	–	9.1	*26.6*	–	*39.7*	8.0	*32.8*	1.2	*4.3*	6.7	*33.4*
Anterior cbt ratio	6	62.2	–	79.1		–		71.3	–	6.2	–	66.7	–
External *br* ratio	6	62.5	–	85.5				78.4		8.3			
Anterior total	6	9.6	–	16.9	*43.3*	–	*61.6*	13.4	*54.6*	2.7	*7.6*	9.6	*48.1*
Posterior base	6	6.4	–	8.3	*27.3*	–	*36.3*	7.5	*30.8*	0.7	*3.3*	6.4	*32.1*
Posterior primary branch	6	11.5	–	18.2	*51.1*	–	*70.4*	14.4	*59.1*	2.5	*7.1*	11.5	*57.2*
Posterior secondary branch	6	7.5	–	10.1	*30.2*	–	*40.4*	8.7	*35.7*	0.9	*4.3*	8.1	*40.4*
Posterior cbt ratio	6	43.4	–	59.6		–		52.4	–	5.4	–	56.1	–
External *br* ratio	6	55.4	–	70.7				60.6		5.4			
Posterior total	6	17.1	–	23.7	*72.1*	–	*97.0*	20.4	*84.0*	2.3	*8.2*	17.1	*85.5*

larger animals are brownish (at least in the caudal part and more evident after mounting). Cuticle without pores, wrinkled in the dorso-caudal part with cribrous areas visible (Fig. 1B-C). Eyes absent in live animals. Buccopharyngeal apparatus of the *Adropion s.l.* type (Fig. 2A, *sensu* Table 1 in Gąsiorek & Michalczyk 2020). Well-developed mouth cone present around the mouth opening (Fig. 2B).

The OCA is not visible under PCM (Fig. 2C). Dorsal and ventral apophyses for the insertion of stylet muscles (AISM) in the shape of 'semilunar hooks' (Fig. 2D, Pilato & Binda, 2010). Chitinous pharyngeal bars (definition according to Massa *et al.* 2024) present in the anterior part of the pharynx (Fig. 2E). Pharyngeal apophyses and drop-like dorsoposterior apodeme of the buccal tube (DABT; Gąsiorek *et al.* 2023) absent (Fig. 2F). Furcae of the *Hypsibius* type. Pharyngeal apophyses absent (Fig. 2G). Pharynx oval and broad. Macroplacoid length sequence 1 < 2; macroplacoids elongated and thin (Fig. 2G). The second macroplacoid twice as long as the first, with a weakly swollen terminal part and a weakly visible constriction at about one third of its length (Fig. 2G). Microplacoid present (Fig. 2G). Claws of the *Hypsibius* type, large and robust, with divergent accessory points. Claw septa sometimes visible, dividing the claw into three portions (basal tract, primary branch and secondary branch). Pseudolunulae weakly visible at the base of the claws (Fig. 3A). Median and internal cuticular bars on legs I-III present (Fig. 3A), and posterior cuticular bar on legs IV present (Fig. 3B). Eggs not found.

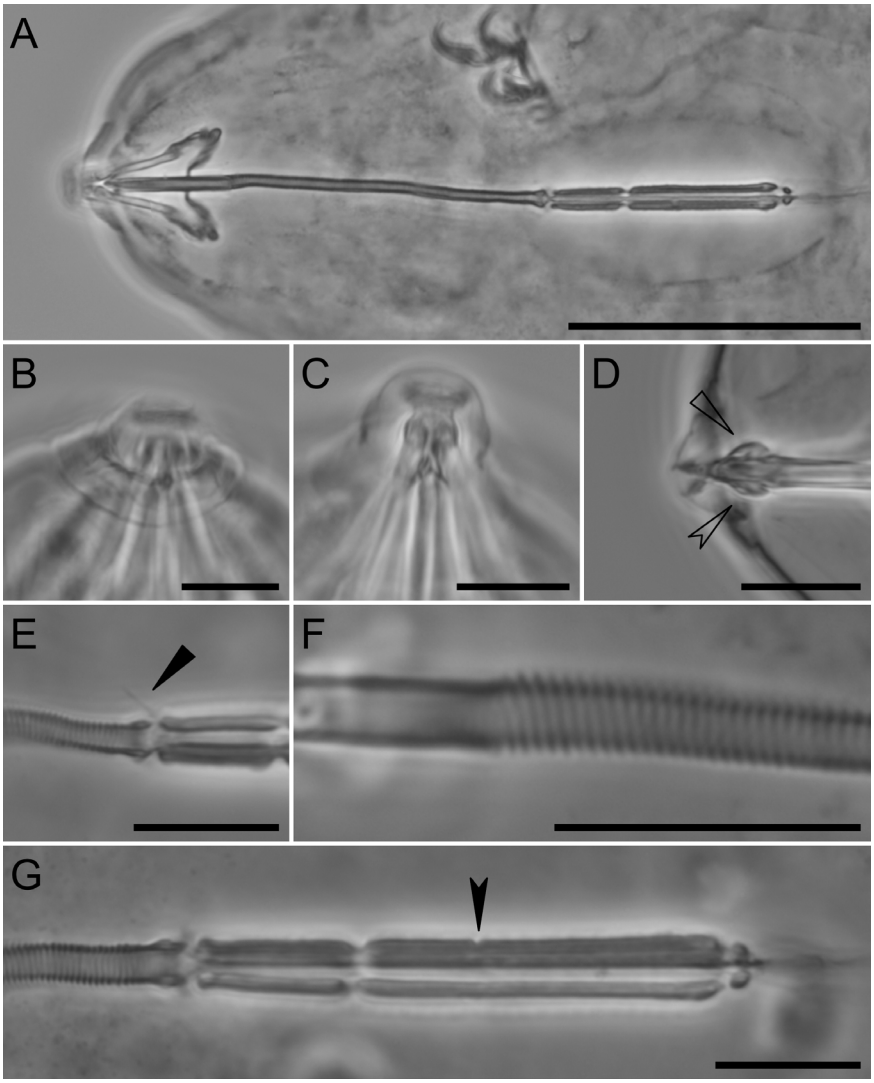

Fig. 2. *Adropion fagineum* n.sp. buccopharyngeal apparatus under PCM. A – Buccopharyngeal apparatus *in toto*. B – Buccal cone. C – Ventral view of the oral cavity. D – Anterior portion of the buccal apparatus in a lateral view showing the AISM. E – Chitinous pharyngeal bars in the anterior part of the pharynx. F – Connection between the buccal and pharyngeal tube showing the absence of DABT. G – Placoids. The empty arrowhead indicates the dorsal hook of the AISM. The empty indented arrowhead indicates the ventral hook of the AISM. The filled arrowhead indicates the cuticular rods in the anterior part of the pharynx. The filled indented arrowhead indicates the constriction of the second macroplacoid. Scale bars A: 50 µm, B-G: 10 µm.

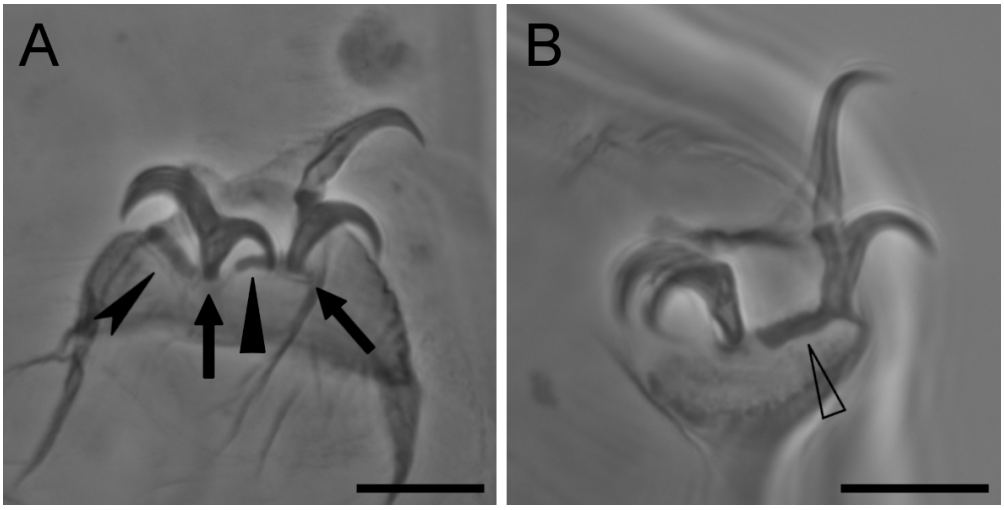

Fig. 3. *Adropion fagineum* n.sp. claws under PCM. A – Claws II. B – Claws IV. Arrows indicate weakly visible pseudolunules. The indented arrowhead indicates the internal cuticular bar. The arrowhead indicates the median cuticular bar. The empty arrowhead indicates the posterior cuticular bar. Scale bars 10 µm.

DNA sequences.

18S: PQ240639

28S: PQ240638

COI: PQ246915

The blastn search (SM.02) of the 18S and 28S markers against the Tardigrada sequences in the NCBI core_nr database confirmed the new species as belonging to the genus *Adropion* (with the 18S sequence having the highest identity (98.44%) with *Adropion* sp. TW.008 [OR693186]; and with the 28S having the highest identity (96.50%) with *Mesocrista spitzbergensis* [KX347533] and with *Adropion scoticum* (96.36%) [OP035749]).

The blastn search (SM.02) of the COI sequences did not find any close match, with the most similar sequence from *Adropion* being *A. scoticum* [MT107465] with a 76.25% identity.

Remarks

In addition to the individuals conforming to the new species description, two animals with three macroplacoids were found. These individuals matched *A. fagineum* n.sp. for all other traits. The presence of three macroplacoids in these individuals could be due to the splitting of the second macroplacoid at the point corresponding to its constriction (already observed by Guidetti *et al.* 1999). Those individuals were not used for the species description, nor were they included in the type series. The distinctiveness of the new species from *A. scoticum* is without doubt, as the COI sequences of *A. scoticum* from neotype locality [MT107465] and *A. fagineum* n. sp. have a 76.25% identity, which is clearly below any intraspecific threshold. However, the new species is morphologically very similar to *A. scoticum,* except for the difference in the placoids number, which can show some level of variability due to breakages. Thus, caution should be used when providing positive identifications of either *A. scoticum* and *A. fagineum* n. sp. when few individuals are available without any molecular data from the COI marker.

One individual (Slide IT.232.07-SM.03) was found to be heavily infected by a fungus, with its body cavity filled with zygospores, possibly of *Ballocephala* sp. (Vecchi *et al.* 2016).

Differential diagnosis

There are only two other *Adropion* species with two elongate macroplacoids and a microplacoid in the pharynx: *A. diphasconiellum* (Ito 1991) and *A. belgicae* (Richters 1911). However, *Adropion fagineum* n. sp. differs from:

– *A. belgicae* (Richters 1911) by the presence of internal and median cuticular bars in the legs (absent in *A. belgicae* vs. present in *A. fagineum* n. sp.).

– *A. diphasconiellum* (Ito 1991) by the relative length of the rigid buccal tube to the flexible pharyngeal tube (60-113% in *A. diphasconiellum* vs. 41-54% in *A. fagineum* n. sp.).

Due to the observed potential breakage of the second macroplacoid in some animals, which gives the impression of three macroplacoids, the new species should also be compared to *Adropion* species with three elongated macroplacoids and a microplacoid:

– *A. ommatophorum* (Thulin,1991) by the absence of eyes (present in *A. ommatophorum* vs. absent in *A. fagineum* n. sp.).

– *A. marcusi* (Rudescu, 1964) by the presence of internal and median cuticular bars in the legs (absent in *A. marcusi* vs. present in *A. fagineum* n. sp.).

– *A. scoticum* (Murray, 1905) by the bigger size (up to 404 µm in *A. scoticum* vs. up to 631 µm in *A. fagineum* n. sp.) and by the presence of cribriform areas visible in the LM (absent in *A. scoticum* vs. present in *A. fagineum* n. sp.).

Conclusions

In this study, I have provided an integrative description of *Adropion fagineum* n. sp. collected from beech leaf litter. The identification of this new species, along with the finding of six other taxa from all four Eutardigrada superfamilies in just one sample, highlights the biodiversity that is found in this particular substrate.

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Conflict of Interest

The author declares no conflict of interest.

Supplementary Materials

Supplementary Materials to this article can be found online at:

http://www.isez.pan.krakow.pl/en/folia-biologica.html

Supplementary files:

SM.01. Raw morphometric data for *Adropion fagineum* n.sp.

SM.02. Results of the blastn search of the newly produced sequences against the NCBI nore_nt Tardigrada database.

SM.03. Photographs *Adropion fagineum* n.sp. (Slide IT.232.07) infected with possibly *Ballocephala* sp.

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