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Continued Exploration of Tanzanian Rainforests Reveals a New Echiniscid Species (Heterotardigrada)

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The Afrotropical tardigrade fauna is insufficiently studied, and consequently its diversity in this region is severely underestimated. Ongoing sampling in the Udzungwa Mountains, Morogoro Region of Tanzania has revealed a new representative of the genus *Echiniscus* C.A.S. Schultz, 1840 (Echiniscidae). *Echiniscus tantulus* sp. nov. belongs to the *spinulosus* group, but it stands out from other members of this speciose *Echiniscus* clade by having a heteromorphic sculpture of the dorsal plates and an uncommonly stable body appendage configuration A-C^d-D^d-E. The new species is characteristic by being equipped with long dorsal spines and very short lateral spicules, which so far have been found only in one other species of the group, *Echiniscus spinulosus* (Doyère, 1840). An updated checklist of Tanzanian Echiniscidae is provided, incorporating recent advances in their classification.

Key words: Biodiversity, Chaetotaxy, Cuticular sculpturing, The *spinulosus* group, Udzungwa Mountains.

BACKGROUND

The Eastern Arc Mountains of Kenya and Tanzania consist of numerous ranges covered with mostly isolated and fragmented Afromontane rainforest, which possesses a staggering species richness and remarkable endemism (Rodgers and Homewood 1982; Bjørndalen 1992; Newmark 2002; Burgess et al. 2007). Most of the system lies in Tanzania, and only Taita Hills are situated in Kenya. The boundaries of the Eastern Arc are delineated by volcanic mountains, e.g., Kilimanjaro and Meru, to the north, and the Southern Highlands at its southern extremity, close to the border of Zambia (Bjørndalen 1992). The Udzungwa Mountains, located to the south of the Tanzanian capital Dodoma, are among the largest in the Eastern Arc ranges, and constitute a primeval habitat for the numerous endemic

species (Scharff et al. 2015).

Tardigrades are generally poorly studied in Africa (McInnes et al. 2017), which, considering the diversity of specific habitats that they usually dominate, like cryofauna of glaciers (Zawierucha and Shain 2019), renders real tardigrade abundance obscure. The heterotardigrade family Echiniscidae has been the subject of several studies on animals inhabiting mosses and lichens from Tanzania (Van Rompu et al. 1991; Binda and Pilato 1993 1995; McInnes et al. 2017; Gąsiorek and Kristensen 2018). Jørgensen (2001) pointed out an overall under-sampling in Africa, and Gąsiorek and Kristensen (2018) conjectured that more undescribed species await discovery and formal description in Tanzania. Here, by applying classic light and scanning microscopy and DNA barcoding (e.g., Wang et al. 2018), we provide an integrative description

of *Echiniscus tantulus* sp. nov. from the Udzungwa Mountains (Fig. 1), and place it in the *Echiniscus* phylogeny. The composition of the Tanzanian echiniscid fauna is discussed in relation to the progress made in unravelling its taxonomy and biogeography.

MATERIALS AND METHODS

Sample collection, morphometry and terminology

Twenty-five animals representing the new species were extracted from fruticose lichen samples collected in the Udzungwa Mountains by Thomas Pape on 18th August 2018. The air-dried samples stored in unbleached

paper envelopes were rehydrated in water for several hours before being agitated and squeezed, and the obtained sediment was poured into Petri dishes to search for microfauna under a stereomicroscope with dark-field illumination. Specimens isolated from samples TZ.072 and TZ.073 were used for a number of analyses: (I) imaging with light microscopy (morphology and morphometry; nineteen specimens), (II) imaging with scanning electron microscopy (SEM – ultrastructure; three specimens), and (III) DNA sequencing + phylogenetics (three specimens). Specimens for light microscopy and morphometry were mounted in a small drop of Hoyer's medium and examined under a Nikon Eclipse 50i phase contrast microscope (PCM) associated with a Nikon Digital Sight DS-L2 digital camera.



Fig. 1. The type locality of the new species.

Specimens were prepared for SEM in accordance with the protocol provided by Stec et al. (2015). Images were assembled in Corel Photo-Paint X6, ver. 16.4.1.1281. For deep structures that could not be fully focused in a single light microscope photograph, a series of 2–4 images were taken every ca. 0.2 μm and then assembled into a single deep-focus image. All measurements are given in micrometres (μm) and were performed under PCM. Structures were measured only if they were undamaged and their orientations suitable. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. The *sp* ratio is the ratio of the length of a given structure to the length of the scapular plate (Dastyh 1999). Morphometric data were handled using the Echiniscoidea ver. 1.3 template, available from the Tardigrada Register, www.tardigrada.net/register (Michalczyk and Kaczmarek 2013). Raw morphometric data are included as supplementary material S1 and deposited in the Tardigrada Register under www.tardigrada.net/register/0067.htm. Morphological terminology follows Kristensen (1987) and subsequent changes proposed in Gąsiorek et al. (2019a).

Genotyping and phylogenetics

DNA was extracted following the Chelex[®] 100 resin (Bio-Rad) extraction method by Casquet et al. (2012) with modifications as detailed in Stec et al. (2015). All specimens were mounted on temporary water slides and examined under PCM before DNA extraction to ensure correct taxonomic identification. Two hologenophore cuticles (Pleijel et al. 2008) were

retrieved from Eppendorf tubes, mounted on permanent slides, and deposited in the Institute of Zoology and Biomedical Research in Kraków. Five markers of variable mutation rates were sequenced: 18S rRNA, 28S rRNA, ITS-1, ITS-2, and *COI*. All fragments were amplified and sequenced according to the protocols described in Stec et al. (2015). Primers and original references for specific PCR programmes are listed in table S1. Sequences were aligned with default settings of MAFFT ver. 7 (Kato et al. 2002; Kato and Toh 2008) under G-INS-i strategy. *COI* sequences were examined in MEGA7 (Kumar et al. 2016) to exclude possible pseudogenes. Uncorrected pairwise distances were calculated using MEGA7 and are enclosed in the table S2.

ITS-1 and ITS-2 sequences were used to reconstruct separate Maximum Likelihood (ML) phylogenies; GenBank accession numbers for the newly sequenced taxa and for sequences retrieved from GenBank are presented in table 1. Alignments were 724 bp (ITS-1) and 540 bp (ITS-2) long. ModelFinder (Kalyaanamoorthy et al. 2017) was used to choose the best-fit models—TIM2+F+G4 (ITS-1) and TPM2u+F+G4 (ITS-2)—according to the Bayesian information criterion. W-IQ-TREE was used for ML reconstruction (Nguyen et al. 2015; Trifinopoulos et al. 2016). One thousand ultrafast bootstrap (UFBoot) replicates were applied to provide support values for branches (Hoang et al. 2018). Trees were rooted on *Diploechiniscus oihonnae* (Richters, 1903). The final consensus trees were visualized using FigTree ver. 1.4.3 (available at: <http://tree.bio.ed.ac.uk/software/figtree/>).

Table 1. GenBank accession numbers for the sequences used in the present study. New sequences are in boldface

Species	<i>COI</i>	ITS-1	ITS-2	Origin of new sequences
<i>Echiniscus belloporus</i> Gąsiorek & Kristensen, 2018	MT374157	MT374193	MT374181	type population (TZ.030)
<i>Echiniscus blumi</i> Richters, 1903	EU046198	-	-	
<i>Echiniscus canadensis</i> Murray, 1910	MF459630	-	-	
<i>Echiniscus granulatus</i> (Doyère, 1840)	EU244600	-	-	
<i>Echiniscus lineatus</i> Pilato et al., 2008	MN548147	MN545741	MN545749	
<i>Echiniscus manuelae</i> da Cunha & do Nascimento Ribeiro, 1962	MT374158	MT374194	MT374182	population UG.004
<i>Echiniscus merokensis</i> Richters, 1904	FJ435813	-	-	
<i>Echiniscus ornamentatus</i> Gąsiorek & Kristensen, 2018	MT374159	MT374195	MT374183	type population (TZ.022)
<i>Echiniscus quadrispinosus</i> Richters, 1902	JX683821	-	-	
<i>Echiniscus siticulosus</i> Gąsiorek & Michalczyk, 2020	MT374160	MT374196–7	MK726308–9	type population (AU.080)
<i>Echiniscus succineus</i> Gąsiorek & Vončina, 2019	MK649675	MT374198	MK675925	type population (MG.005)
<i>Echiniscus tantulus</i> sp. nov.	MT107427	MT108138	MT108137	type population (TZ.072)
<i>Echiniscus testudo</i> (Doyère, 1840)	MG025605	MT374199	MG016456	neotype population (FR.057)
<i>Echiniscus trisetosus</i> Cuénot, 1932	MF459627	-	-	
<i>Echiniscus tristis</i> Gąsiorek & Kristensen, 2018	MT374161	MT374200	MT374184	type population (TZ.030)
<i>Echiniscus virginicus</i> Riggan, 1962	MN548176	MN545748	MN545756	
<i>Diploechiniscus oihonnae</i> (outgroup)	MG063724	MT374201	MT374185	population GB.098

RESULTS

TAXONOMY

Phylum Tardigrada Doyère, 1840
Class Heterotardigrada Marcus, 1927
Order Echiniscoidea Richters, 1926
Family Echiniscidae Thulin, 1928
Genus *Echiniscus* C.A.S. Schultze, 1840

***Echiniscus tantulus* sp. nov. Gąsiorek,
 Bochnak, Vončina & Kristensen**

(Figs. 2–5, Tables 2–3)

urn:lsid:zoobank.org:act:4CB6B6FC-A376-4464-8338-B434C82FD8D5

Description: Females (*i.e.*, from the third instar onwards; measurements and statistics in table 2): Body orange with minute red eyes present in live specimens; colours disappearing soon after mounting. Cylindrical, *Echiniscus*-type cephalic papillae (secondary clavae) and (primary) clavae; cirri growing out from bulbous cirrophores (Figs. 2A–B, 3B, 4A, 5A). The body appendage configuration is *A-C-C^d-D^d-E*, with trunk appendages formed as: spines (*C^d*, *D^d*) or spicules (*C*, *E*). Spicules formed precisely at the posterior edges of segmental plates, usually as a prolongation of the cuticular margin. All trunk appendages smooth (Figs. 2A–B, 3, 4A). Single cases of asymmetry in all positions (*e.g.*, Fig. 3A).

Dorsal plates with the *spinulosus* type of sculpturing (see Gąsiorek et al. 2019a), that can be easily subdivided into (I) typical pores present in the majority of plates, (II) more densely arranged pores at the anterior portions of the segmental plates and median plate 2, and the entirety of median plate 3 that are clearly seen under SEM and as darker areas under PCM (Figs. 2A–B, 3, 4), (III) very fine epicuticular wrinkling present on bands dividing the segmental plates and median plate 2, and the posteriormost part of median plate 3, which are visible only in SEM (Fig. 3B). Pores are smaller and more sparsely distributed in the lateral portions of all plates. Pores without endocuticular rings (Figs. 3, 4B–C). The cephalic plate with typical pores, divided into halves (Figs. 2A, 5A). The cervical (neck) plate poorly delineated from the scapular plate, formed as thin grey belt without pores (Figs. 2A, 3A, 4A). The scapular plate large, with poorly marked lateral sutures separating narrow rectangular lateral portions without pores (Figs. 2A–B, 3A, 4). Paired segmental plates divided into a smaller, much narrower anterior and a prominent posterior part by a light nonporous, transverse band (wrinkled in SEM, Figs. 2A–B, 3,

4A). Posterior parts heterogenic, with the anteriormost margins adjacent to the belts being identical to the anterior parts of the plate (Fig. 3). The caudal (terminal) plate with short incisions and horizontal and vertical epicuticular ridges, forming a cross, *i.e.*, dividing the plate into four facets (Figs. 2A–B, 3, 4C). Median plates 1 and 3 unipartite, whereas median plate II divided into a very narrow anterior and wide posterior portion (Fig. 3). Ventral cuticle with minute endocuticular pillars distributed evenly throughout the entire venter (Fig. 5B), with rudimentary subcephalic plates in the form of convex swellings (Figs. 4A, 5A). Sexpartite gonopore between genital plates, and a trilobed anus between legs IV.

Pedal plates seen as dark areas on the central leg portions under PCM, without pores; plate IV with a typical dentate collar composed of short teeth (Figs. 2A–B, 4A, 5D). Distinct pulvini on all legs (Figs. 2A, 4A). A small spine on leg I and a papilla on leg IV present (Figs. 2A–B, 4A). External claws on all legs smooth. Internal claws with large, acute spurs positioned at ca. 1/4–1/3 of the claw height and bent downwards (Fig. 5B–D).

Buccal apparatus short, with a rigid, stout tube and a roundish pharynx containing serrated, chitinous placoids. Stylet supports absent.

Males: Unknown (likely a parthenogenetic species).

Juveniles (*i.e.*, the second instar, measurements and statistics in Table 3): Clearly smaller than adult females, but with the trunk appendage configuration as in sexually mature individuals. In one individual, spines *Cd* displaced in the direction of the dorsolateral position (Fig. 2C). Pores fainter than in adult females; the differences in dorsal sculpturing between anterior and posterior portions of segmental plates not apparent. Lacking gonopore. No other significant disparities were found between the juvenile and mature life stages.

Larvae: Unknown.

Eggs: Up to five round, orange eggs per exuvia were found in two exuviae.

Molecular markers and phylogenetic position: All five genetic markers were represented by single haplotypes (GenBank accession numbers: 18S rRNA – MT126785, 28S rRNA – MT126765, *COI* – MT107427, ITS-1 – MT108138, and ITS-2 – MT108137). Both ITS-1 and ITS-2-based phylogenies reflected the topology of the *Echiniscus* clades from Gąsiorek et al. (2019a), with the *virginicus* complex as basal and *E. testudo* (Doyère, 1840) as sister to the *spinulosus* complex, which included *E. tantulus* sp. nov. According to the ITS-1 tree, *E. tantulus* sp. nov. is a sister species to the clade *E. succineus* Gąsiorek & Vončina, 2019 + *E. ornamentatus* Gąsiorek & Kristensen, 2018 (Fig. 6). In

the ITS-2 tree, *E. tantulus* sp. nov. is also most closely related to these two taxa, but their relationships are unclear as the tree is inconclusive. The *p*-distances were calculated for both ITS and *COI* markers on the dataset of selected sequences (see Table S2).

Type material: Holotype (slide TZ.072.01), and 16 paratypes on slides TZ.072.01–06. Three paratypes mounted on SEM stub no. 19.15. Moreover, two voucher specimens (hologenophores) mounted on the slides TZ.073.01–02. The slides TZ.072.02–03 (5 ♀ ♀) deposited in the Natural History Museum of Denmark, University of Copenhagen, Denmark; the slide TZ.072.04 (5 ♀ ♀) deposited in the Catania

University, Sicily, Italy. All remaining slides deposited in the Institute of Zoology and Biomedical Research, Jagiellonian University, Poland. The present species was found together with abundant populations of *E. tristis* Gąsiorek & Kristensen, 2018.

Additional material: Two females mixed with a population of *E. tristis* in an additional sample collected in the *locus typicus* (sample reference TZ.069).

Type locality: 7°49'04"S, 36°50'39"E, ca. 2100 m asl; Mwanihana Peak, Udzungwa Mountains, Tanzania; Afromontane rainforest, lichens growing on exposed bedrock.

Etymology: From Latin *tantulus* = “so small”. The

Table 2. Measurements [in μm] of selected morphological structures of the adult females of *E. tantulus* sp. nov. mounted in Hoyer’s medium. N, number of specimens/structures measured; Range, refers to the smallest and the largest structure among all measured specimens; SD, standard deviation; *sp*, the proportion between the length of a given structure and the length of the scapular plate

Character	N	Range		Mean		SD		Holotype	
		μm	<i>sp</i>	μm	<i>sp</i>	μm	<i>sp</i>	μm	<i>sp</i>
Body length	16	182–237	476–534	204	503	17	21	232	523
Scapular plate length	16	35.1–44.9	-	40.5	-	3.0	-	44.4	-
Head appendages lengths									
<i>Cirrus internus</i>	16	7.6–14.4	17.0–36.6	10.8	26.8	1.9	4.9	14.2	32.0
Cephalic papilla	15	4.6–8.0	11.0–20.1	6.6	16.2	0.8	2.0	7.4	16.7
<i>Cirrus externus</i>	16	11.4–19.0	29.7–47.0	15.5	38.3	2.1	4.5	19.0	42.8
(Primary) C lava	16	4.2–6.8	10.7–15.8	5.4	13.4	0.7	1.6	6.8	15.3
<i>Cirrus A</i>	16	27.2–40.3	68.7–99.0	34.1	84.2	3.8	7.4	39.9	89.9
<i>Cirrus A</i> /Body length ratio	16	14%–20%	-	17%	-	1%	-	17%	-
Body appendages lengths									
Spine C	15	2.5–6.1	6.4–14.3	4.3	10.6	1.0	2.2	6.1	13.7
Spine C ^d	16	12.4–18.9	29.1–46.7	15.9	39.3	2.0	5.1	16.6	37.4
Spine D ^d	16	15.2–29.9	38.7–67.3	21.8	54.0	3.7	8.7	29.9	67.3
Spine E	15	2.5–4.1	6.1–10.3	3.3	8.1	0.5	1.3	2.9	6.5
Spine on leg I length	16	2.3–3.4	5.8–9.4	2.9	7.0	0.3	0.8	3.4	7.7
Papilla on leg IV length	16	2.5–3.9	5.8–9.9	3.1	7.6	0.4	1.0	3.2	7.2
Number of teeth on the collar	16	9–16	-	11.3	-	1.9	-	13	-
Claw I heights									
Branch	16	10.3–13.3	26.5–31.4	11.6	28.6	1.0	1.5	13.3	30.0
Spur	14	1.8–3.2	4.3–7.8	2.7	6.6	0.4	0.9	3.2	7.2
Spur/branch length ratio	14	16%–26%	-	23%	-	3%	-	24%	-
Claw II heights									
Branch	16	9.9–12.9	26.0–29.9	11.2	27.7	0.8	1.2	12.9	29.1
Spur	15	2.1–3.2	4.7–7.6	2.6	6.5	0.4	0.8	2.8	6.3
Spur/branch length ratio	15	17%–28%	-	23%	-	3%	-	22%	-
Claw III heights									
Branch	16	9.8–13.7	25.4–30.9	11.4	28.0	1.1	1.7	13.7	30.9
Spur	14	2.1–3.5	5.6–7.9	2.6	6.4	0.4	0.7	2.5	5.6
Spur/branch length ratio	14	18%–28%	-	23%	-	3%	-	18%	-
Claw IV heights									
Branch	16	10.5–15.0	27.3–35.9	12.8	31.6	1.4	2.4	15.0	33.8
Spur	13	2.5–3.9	5.6–8.8	3.1	7.5	0.4	0.9	3.6	8.1
Spur/branch length ratio	13	19%–31%	-	24%	-	3%	-	24%	-

name refers to the microscopic size of the animal. An adjective in the nominative singular.

Differential diagnosis: The stable body appendage configuration *A-C-C^d-D^d-E*, with the dorsal spines several times longer than short lateral spicules, makes *E. tantulus* sp. nov. unique among the members of the *spinulosus* group (Gąsiorek et al. 2019a), which are typically highly variable in terms of the development of trunk spines and frequent asymmetries (e.g., see Pilato et al. 2008; Meyer 2016). There is only one other species showing a combination of long dorsal spines and short lateral spicules: *E. spinulosus* (Doyère, 1840). *Echiniscus canedoi* da Cunha & do Nascimento Ribeiro,

1962 also bears some resemblance to *E. tantulus* sp. nov. due to the dorsal spines *D^d* two times longer than the longest lateral spines *C*, yet the new species can be differentiated from:

E. canedoi, so far found only in Madeira, based on the trunk appendage configuration (*C-C^d-D^d-E* in *E. tantulus* sp. nov. vs *C-D-D^d* in *E. canedoi*), and dissimilarities in the dorsal sculpturing (markedly darker anterior portions of segmental plates, with densely arranged pores in *E. tantulus* sp. nov. vs anterior portions with sparsely arranged pores in *E. canedoi*); *E. spinulosus*, reliably reported only from numerous locales in Western Palaearctic (McInnes 1994), by

Table 3. Measurements [in μm] of selected morphological structures of the juveniles of *E. tantulus* sp. nov. mounted in Hoyer’s medium. N, number of specimens/structures measured; Range, refers to the smallest and the largest structure among all measured specimens; SD, standard deviation; *sp*, the proportion between the length of a given structure and the length of the scapular plate

Character	N	Range		Mean		SD	
		μm	<i>sp</i>	μm	<i>sp</i>	μm	<i>sp</i>
Body length	3	127–157	465–503	142	488	15	20
Scapular plate length	3	27.2–31.2	-	29.1	-	2.0	-
Head appendages lengths							
<i>Cirrus internus</i>	3	5.9–10.5	20.4–33.7	8.4	28.8	2.3	7.3
Cephalic papilla	3	3.7–5.5	12.8–17.6	4.4	14.9	1.0	2.5
<i>Cirrus externus</i>	3	8.9–12.3	32.7–40.8	11.0	37.7	1.8	4.3
(Primary) clava	3	3.7–4.0	12.8–14.0	3.8	13.2	0.2	0.7
<i>Cirrus A</i>	3	23.7–25.8	82.7–87.9	25.0	85.9	1.1	2.8
<i>Cirrus A</i> /Body length ratio	3	16%–19%	-	18%	-	1%	-
Body appendages lengths							
Spine <i>C</i>	3	2.6–3.2	8.3–11.8	2.8	9.7	0.3	1.8
Spine <i>C^d</i>	3	6.1–10.2	21.1–37.5	8.8	30.3	2.3	8.4
Spine <i>D^d</i>	3	13.2–16.6	46.4–53.2	14.4	49.4	1.9	3.5
Spine <i>E</i>	3	2.1–2.7	7.7–8.7	2.4	8.1	0.3	0.5
Spine on leg I length	3	1.7–2.3	5.9–7.4	1.9	6.5	0.3	0.8
Papilla on leg IV length	3	1.9–2.6	7.0–8.3	2.3	7.8	0.4	0.7
Number of teeth on the collar	3	9–11	-	9.7	-	1.2	-
Claw I heights							
Branch	3	7.9–9.0	28.8–29.8	8.5	29.2	0.6	0.5
Spur	3	1.9–2.1	6.7–7.3	2.0	7.0	0.1	0.3
Spur/branch length ratio	3	23%–24%	-	24%	-	1%	-
Claw II heights							
Branch	3	7.9–9.0	27.7–29.0	8.3	28.5	0.6	0.7
Spur	3	1.3–2.3	4.5–7.4	1.8	6.0	0.5	1.4
Spur/branch length ratio	3	16%–26%	-	21%	-	5%	-
Claw III heights							
Branch	2	7.4–7.9	27.2–27.3	7.7	27.3	0.4	0.1
Spur	2	1.6–1.6	5.5–5.9	1.6	5.7	0.0	0.2
Spur/branch length ratio	2	20%–22%	-	21%	-	1%	-
Claw IV heights							
Branch	3	8.6–10.3	29.8–33.0	9.2	31.7	0.9	1.7
Spur	2	1.9–2.0	6.9–7.0	2.0	7.0	0.1	0.0
Spur/branch length ratio	2	22%–23%	-	22%	-	1%	-

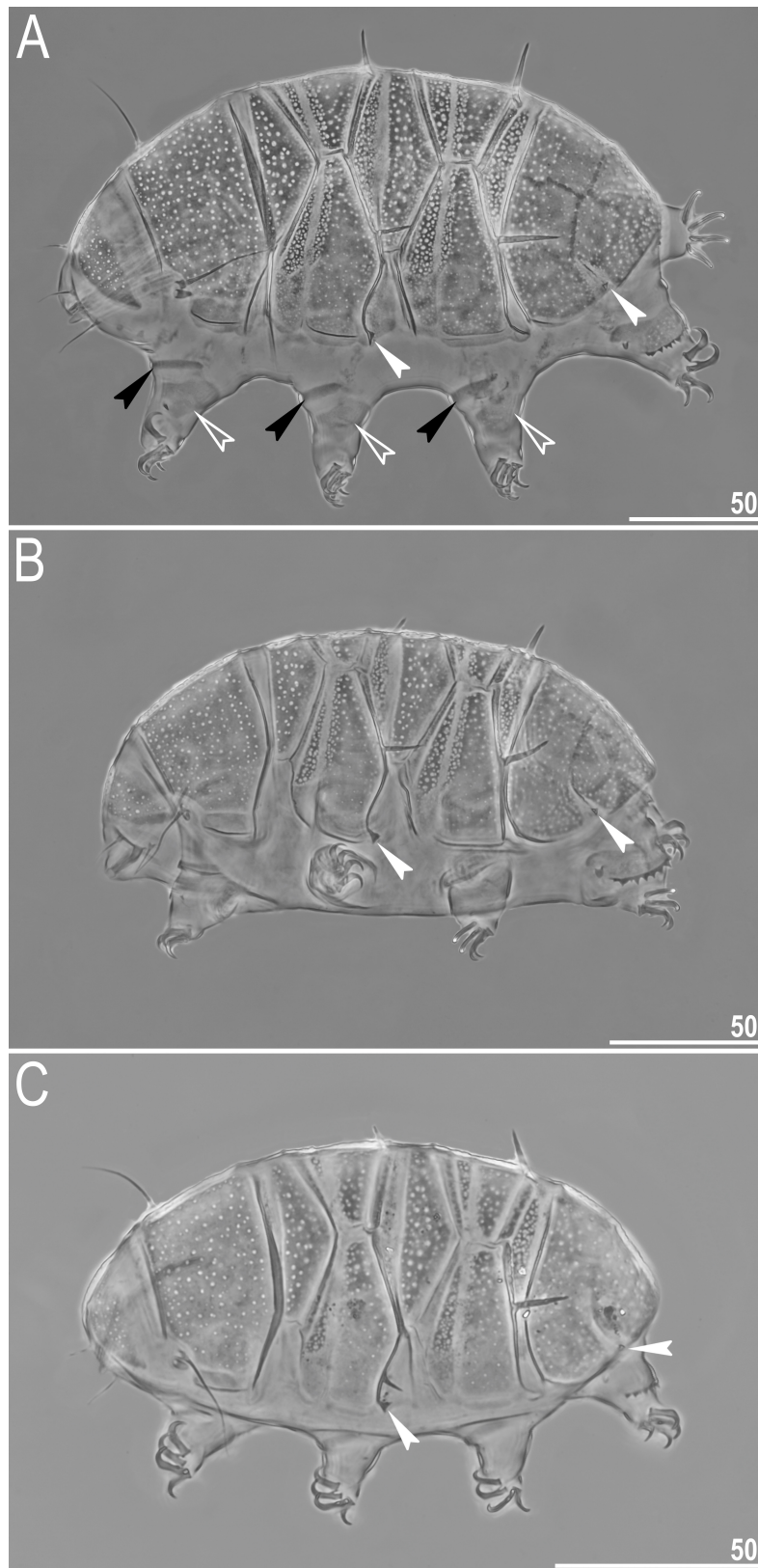


Fig. 2. Habitus of *Echiniscus tantulus* sp. nov. (PCM): A, adult female (holotype, dorsolateral view); B, adult female (paratype, lateral view); C, juvenile (paratype, dorsolateral view). White arrowheads indicate spicules in lateral positions C and E, black arrowheads indicate pulvini, and empty arrowheads point out pedal plates. All scale bars in μm .

the distinct trunk appendage configuration ($C-C^d-D^d-E$ in *E. tantulus* sp. nov. vs $(B)-C-C^d-D-D^d-E$ in *E. spinulosus*), dissimilarities in the dorsal sculpturing (markedly darker anterior portions of segmental plates, with densely arranged pores in *E. tantulus* sp. nov. vs uniform sculpturing in *E. spinulosus*, see Pilato et al. 2008), the presence of epicuticular ridges on the caudal

plate (forming a cross in *E. tantulus* sp. nov. vs absent in *E. spinulosus*, see Pilato et al. 2008), and the level of development of pedal plates (poorly developed and lacking pores in *E. tantulus* sp. nov. vs well-developed, with large pores identical to the pores present on the dorsal plates in *E. spinulosus*, see Gąsiorek and Degma 2018).

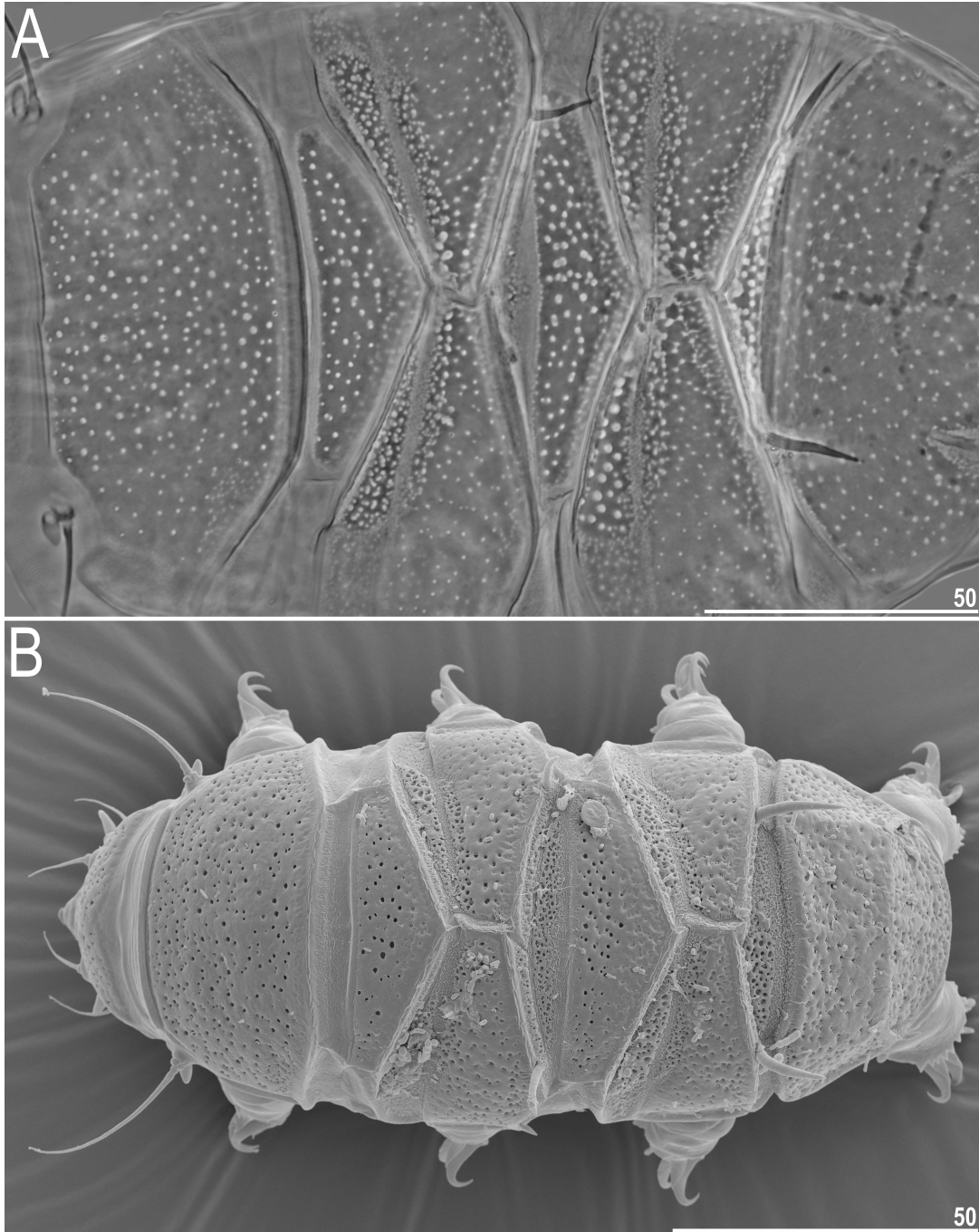


Fig. 3. Dorsal sculpturing of *E. tantulus* sp. nov.: A, in PCM (note one spine C^d asymmetrically lacking); B, in SEM (note wrinkled belts of cuticle between anterior and posterior portions of segmental plates and median plate 2, and in the posterior portion of median plate 3. All scale bars in μm).

DISCUSSION

To date, twelve echiniscid species have been reported from Mount Kilimanjaro and the Udzungwa Mountains (Binda and Pilato 1995; Gąsiorek and Kristensen 2018). Ten are representatives of the genus *Echiniscus*, and can be divided into three phyletic lineages: the *spinulosus* group (7 spp.: *E. angolensis* da Cunha & do Nascimento Ribeiro, 1964, *E. baius* Marcus, 1928, *E. belloporus* Gąsiorek & Kristensen, 2018, *E. ornamentatus*, *E. scabrospinus* Fontoura, 1982, *E. tantulus* sp. nov., *E. tristis*), the *africanus* group (2 spp.: *E. africanus* Murray, 1907 and *E. pusae* Marcus, 1928), and the *virginicus* group (*E. lineatus*

Pilato et al., 2008). In Gąsiorek and Kristensen (2018), *E. pusae* was misidentified and listed as *E. africanus* (first reported from Tanzania by Binda and Pilato 1995); however, a comparison of specimens from Tanzania, the Malay Archipelago and Australia (data in preparation) under PCM revealed a consistent low morphological variation within this species. Consequently, *E. pusae* is likely to be another pantropical echiniscid species (or at least one with a very wide range that extends from Africa to Australasia), along with *E. baius* (McInnes 1994) and *E. lineatus* (*E. lineatus* was listed as *E. dariae* Kaczmarek & Michalczyk, 2010 in Gąsiorek and Kristensen 2018, and later synonymised in Gąsiorek et al. 2019b).

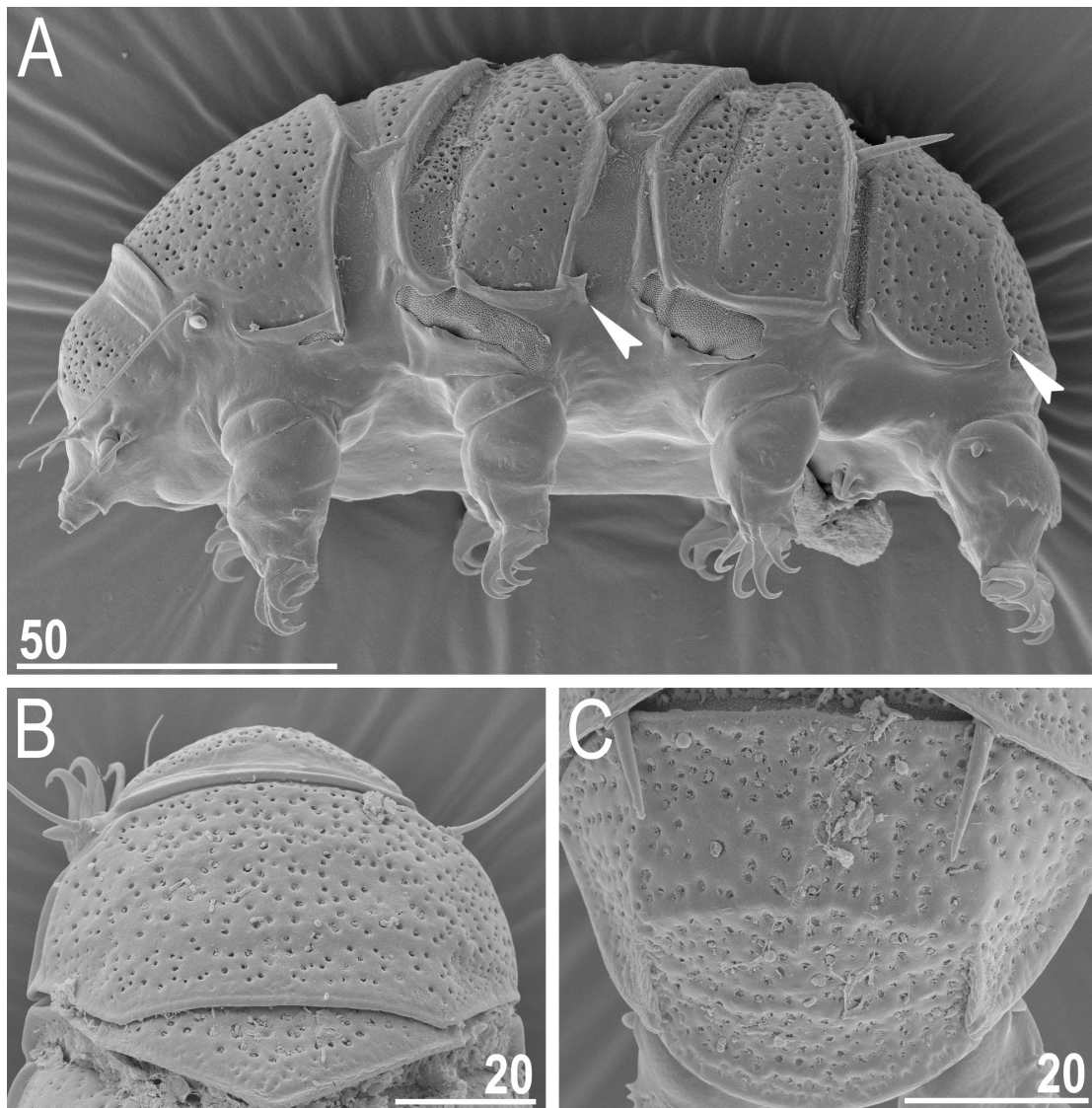


Fig. 4. Close-up on the details of sculpturing of *E. tantulus* sp. nov. (SEM): A, female in lateral view (note sparsely distributed pores on the lateral portions of all dorsal plates); B, scapular plate; C, caudal (terminal) plate. Arrowheads indicate spicules in lateral positions C and E. All scale bars in μm .

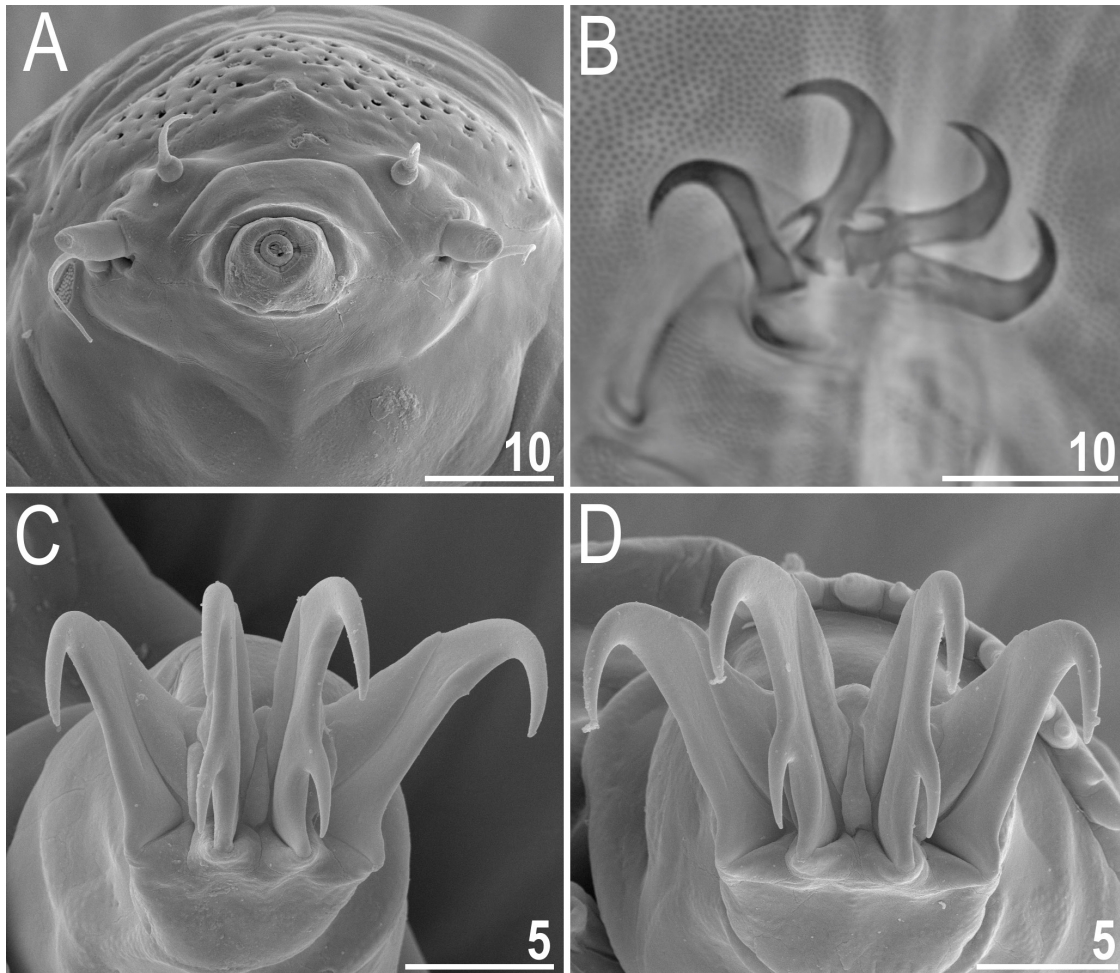


Fig. 5. Close-up on the details of head and claws of *E. tantulus* sp. nov.: A, anteriormost cephalic region with peribuccal cirri and cephalic papillae (SEM); B, claws III (PCM); C, claws I (SEM); D, claws IV with dentate collar (SEM). All scale bars in μm .

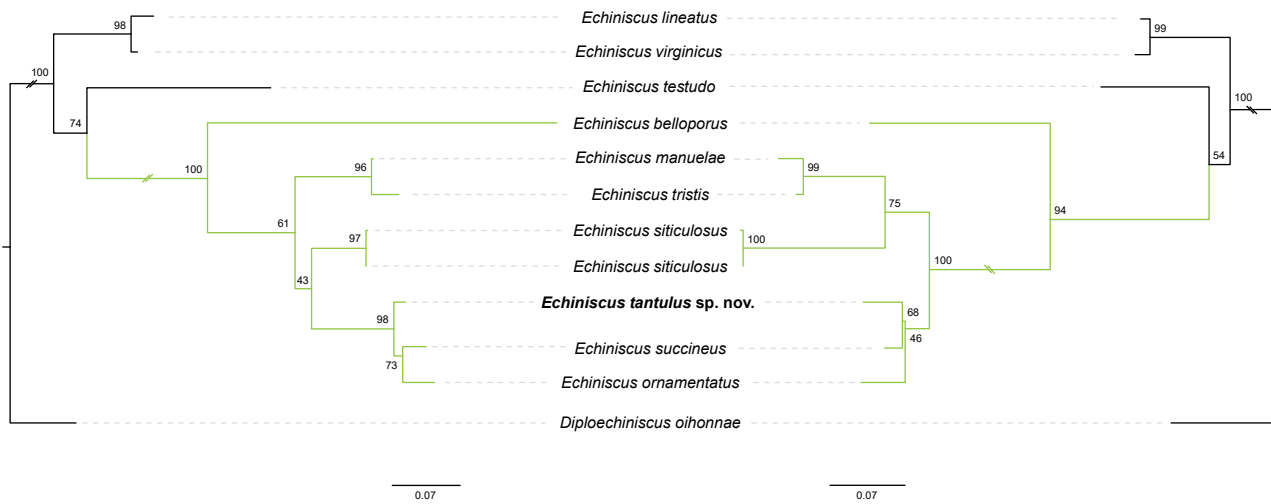


Fig. 6. Maximum Likelihood consensus phylogenetic trees (ITS-1-based on the left, ITS-2-based on the right) showing the position of *E. tantulus* sp. nov. between members of the *E. spinulosus* complex (green clade); *D. oihonnae* was used as an outgroup. ML bootstrap values are presented at the nodes.

The prevalence of the *spinulosus* group in Tanzania is indicative of a thermophilic preference among the members of this species complex. In the Western Palaearctic, the biogeographic region nearest to the Afrotropics, the *spinulosus* group is moderately species-rich, e.g., six species occur in the Mediterranean (*E. canedoi*, *E. carusoi* Pilato, 1972, *E. manuelae* da Cunha & do Nascimento Ribeiro, 1962, *E. scabrospinus*, *E. spiniger* Richters, 1904, *E. spinulosus*), but further north, in the lowlands of Central and Eastern Europe, only the last two species can be found, both of which are rare (McInnes 1994). *E. scabrospinus* probably has a wide geographic range: from the Iberian Peninsula to Tanzania (Binda and Pilato 1995; Pilato et al. 2008); *E. tristis* has recently been reported from Madagascar (Bartylak et al. 2019). Therefore, some echiniscid species inhabiting the Udzungwa Mountains have broad geographic distributions. The recent faunistic data for macrobiotids concur with this statement, as a *Minibiotus* species was reported both from Tanzania and the Neotropics (Stec et al. 2020).

Moreover, one species of the genus *Nebularmis* Gąsiorek & Michalczyk, 2019 (in Gąsiorek et al. 2019a), *N. cirinoi* (Binda & Pilato, 1993), occurs in Tanzania (Gąsiorek and Kristensen 2018). Finally, the Tanzanian records of *Pseudechiniscus suillus* (Ehrenberg, 1853) should be treated as unreliable and actually representing a new *Pseudechiniscus* species since the genus potentially incorporates an enormous number of species (Cesari et al. 2020). *Pseudechiniscus jiroveci* Bartoš, 1963, also reported from Tanzania, is now established as *nomen dubium* (Tumanov 2020), thus its records are invalidated. Consequently, the checklist is as follows:

I. Genus: *Echiniscus*

1. *E. africanus*
2. *E. angolensis*
3. *E. belloporus*
4. *E. baius*
5. *E. lineatus*
6. *E. ornamentatus*
7. *E. pusae*
8. *E. scabrospinus*
9. *E. tantulus*
10. *E. tristis*

II. Genus: *Nebularmis*

1. *N. cirinoi*

III. Genus: *Pseudechiniscus*

1. *P. suillus* (dubious record signifying other species).

CONCLUSIONS

Exploration of Tanzanian rainforests continues to reveal the presence of new species (see also Stec et al. 2018). The echiniscid fauna of this region is dominated by the *spinulosus* group, the most speciose lineage of *Echiniscus* (Gąsiorek et al. 2019a). The addition of molecular data for *E. tantulus* sp. nov. to an increasing dataset for all Echiniscidae is important given its unique morphological characters.

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Authors' contributions: RMK and PG conceived the study; MB isolated tardigrades from the samples, extracted DNA, performed qualitative and quantitative morphological analyses, and assembled the figures; KV isolated tardigrades from the samples and prepared permanent slides; PG sequenced specimens, performed phylogeny, delineated the new species and wrote the manuscript; all authors read, corrected and approved the final version of the manuscript.

Competing interests: The authors declare no conflict of interests.

Availability of data and materials: The key datasets of the manuscript are deposited as additional supplementary files. Morphometric data are deposited in the Tardigrada Register. Sequences are available from GenBank (MT107427, MT108137–8, MT126765, MT126785, MT374157–61, MT374181–5, MT374193–201).

Consent for publication: The authors give their consent to publish.

Ethics approval consent to participate: Ethics approval is not necessary.

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Supplementary materials

Supplementary Material S1. Raw morphometric data for the type population of *E. tantulus* sp. nov. (download)

Table S1. Primers and references for specific protocols for amplification of the five DNA fragments sequenced in the study. (download)

Table S2. Uncorrected pairwise distances. (download)