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An integrative redescription of *Echiniscus quadrispinosus quadrispinosus* Richters, 1902 (Heterotardigrada, Echiniscidae) from the terra typica in Taunus Mountain Range (Europe; Germany)

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Abstract. In the present study, we used an integrative taxonomy approach to redescribe a population of *Echiniscus quadrispinosus quadrispinosus* Richters, 1902 from the neotype locality in the Taunus Mountain Range (Germany). We found clear differences in the chaetotaxy formula between the life stages of *E. q. quadrispinosus*. The body appendages *B* are, in general, absent in juveniles. Moreover, in larvae all body lateral appendages, except for *E*, are absent. We also obtained DNA sequences of 18S rRNA, 28S rRNA, ITS-2, and COI of *E. q. quadrispinosus* from the neotype locality and three Norwegian populations. Comparison with the sequences available in GenBank showed low genetic differences between the neotypic population and specimens from other localities. Therefore, we decided to establish our specimens from Taunus Mountain Range as neotype and paraneotypes of *E. q. quadrispinosus*. We also discussed and amended the taxonomic status of three subspecies *E. q. brachyspinosus* Bartoš, 1934, *E. q. cribrosus* Murray, 1907 and *E. q. fissispinosus* Murray, 1907 and established them as junior

synonyms of *E. q. quadrispinosus*. Finally, we also confirmed *E. lichenorum* Maucci, 1983 as a valid species, clearly different from *E. q. quadrispinosus*.

Keywords. *E. lichenorum* Maucci, 1983, *E. q. brachyspinosus* Bartoš, 1934, *E. q. cribrosus* Murray, 1907, *E. q. fissispinosus* Murray, 1907, Tardigrada.

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Introduction

Tardigrades inhabit terrestrial and aquatic (freshwater and marine) environments from the highest mountains to the deepest oceans, from the polar regions to the tropics. They are found in mosses, lichens, soil, leaf litter, sediments and on aquatic plants (Nelson *et al.* 2020). Up to now, ca 1400 species of tardigrades have been described across the world (Guidetti & Bertolani 2005; Degma & Guidetti 2007; Degma *et al.* 2009–2021; Vicente & Bertolani 2013).

The genus *Echiniscus* C.A.S. Schultze, 1840 (amended by Gąsiorek *et al.* 2017) is characterized mainly by red eyes, a rigid buccal tube without stylet supports or with fine, fibrillar stylet supports, two pairs of paired plates, two or three median plates (sometimes transversally subdivided), notches on the terminal plate, pseudosegmental plates absent and ventral plates sometimes present. To date, 120 species and subspecies have been attributed to this genus, which makes it one of the most species-rich tardigrade genera (Degma *et al.* 2009–2021).

Echiniscus q. quadrispinosus Richters, 1902 was described from the Taunus Mountain Range (Germany) without either detailed diagnosis or specified type locality. Not long after, two varieties, i.e., *E. q. cribrosus* Murray, 1907 and *E. q. fissispinosus* Murray, 1907, were described from the Shetland Islands and Scotland, respectively (Murray 1907). Finally, Bartoš (1934) described a third variety *E. q. brachyspinosus* Bartoš, 1934 from the Carpathians. Ramazzotti & Maucci (1983) elevated *E. q. cribrosus* and *E. q. fissispinosus* to the subspecies level and suggested *E. q. brachyspinosus* to be a different form only. All these taxa differ from typical *E. q. quadrispinosus* by rather doubtful morphological characters. Gąsiorek *et al.* (2019a) included *E. q. quadrispinosus* into the *E. quadrispinosus* subgroup characterized by the presence of "plates with dominant circular pores, trunk appendages in the form of filiform cirri and additional spines". Two other species included in this group are *E. lentiferus* Claxton & Dastych, 2017 and *E. lichenorum* Maucci, 1983 nom. inq. (suggested as conspecific with *E. q. quadrispinosus*) (Gąsiorek *et al.* 2019a).

In this study, we applied an integrative taxonomy – a combination of classical morphology and molecular approach – to examine specimens of *E. q. quadrispinosus* collected in its terra typica, the Taunus Mountain Range (Germany). We also analysed morphological variability of different stages and sex of this species. Additionally, we enriched our study in DNA sequences (18S rRNA, 28S rRNA, ITS-2 and COI) of *E. q. quadrispinosus* from three Norwegian populations.

Material and methods

Sampling

Moss on stone was collected in a mixed forest in the Taunus Mountains Range (Germany) in September 2019 (sample code (SC): GR1, for more details see below). Additional samples were collected on 10 August 2017 by Torbjørn Ekrem *et al.* in Norway: 59°03′18″ N, 09°40′37″ E, 47 m a.s.l., Telemark, Porsgrunn, Blekebakken Nature Reserve, calcareous *Tilia cordata* Miller, 1768 forest, lichen from soil (SC: 169) and 59°05′38″ N, 09°38′58″ E, 34 m a.s.l., Telemark, Porsgrunn, Åsstranda Nature

Reserve, mixed deciduous with *Alnus* sp. forest, moss from soil (SC: 184 (moss species: *Frullania dilatata* Dumortier, 1835) and 187 (moss species: *Orthotrichum* sp. and *F. dilatata*)).

The samples were then packed in paper envelopes, dried at a temperature of ca 20ºC, and delivered to the Department of Animal Taxonomy and Ecology at the Faculty of Biology, Adam Mickiewicz University in Poznań, Poland. The tardigrade collection, extraction and mounting techniques followed the protocol of Dastych (1980).

Microscopy and imaging

A total of 112 specimens, including 36 females, 23 males, 35 juveniles, 3 larvae and 15 with undefined life-stage or sex (due to unfavourable orientation) were mounted on microscope slides in Hoyer's medium, and then examined under an Olympus BX41 Phase Contrast light Microscope (PCM) associated with an Olympus SC50 digital camera (Olympus Corporation, Shinjuku-ku, Japan).

Forty-five specimens were prepared for Scanning Electron Microscope (SEM) analysis according to the protocol in Roszkowska *et al.* (2018) and examined under high vacuum in Hitachi S3000N SEM.

All figures were assembled in Corel Photo-Paint 2017. For deep structures that could not be fully focused in a single photograph, a series of 2–50 images was taken every ca 0.5 µm depth and then manually assembled into a single deep-focus image in Corel Photo-Paint 2017.

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 end of the body, excluding the hind legs. Terminology used for the description of dorsal plates follows Kristensen (1987). Lengths of the claws were measured from the base of the claw (in its middle point) to its top. The *sp* index is the ratio of the length of a given structure to the length of the scapular plate expressed as a percentage (length of structure × 100 ⁄ length scapular plate) (Dastych 1999, and later independently proposed as the *psc* index by Fontoura & Morais 2011). Configuration and arrangement of body appendages (chaetotaxy) is given according to Gąsiorek *et al.* (2017).

Morphometric data were handled using the modified "Echiniscoidea" ver. 1.3 template available from the Tardigrada Register (Michalczyk & Kaczmarek 2013). Tardigrade taxonomy follows Gąsiorek & Michalczyk (2020a).

Species identification

Specimens were identified using the taxonomic key and morphological description in Ramazzotti & Maucci (1983) and compared with the original description (Richters 1902).

Genotyping

Three specimens of *E. q. quadrispinosus* from Germany (isolates numbers: GR5, GR8, GR10) and thirteen specimens of *E. q. quadrispinosus* from Norway (isolates numbers: 169/7, 169/8, 169/9, 184/2, 184/3, 184/4, 184/8, 187/1, 187/3, 187/4, 187/7, 187/8, 187/9) were preliminarily identified in vivo using light microscopy (LM) prior to DNA extraction for genotyping analysis. Specimens were placed separately in a 1.5 ml Eppendorf microcentrifuge tubes in 20 µl of sterile MQ H_2O and kept frozen at -80°C. Genomic DNA was extracted using a Chelex® 100 resin (Bio-Rad) method (Casquet *et al.* 2012) with modification in order to obtain tardigrade exoskeletons (Kaczmarek *et al.* 2019). To remove the exoskeleton, the obtained extract (40 µl of DNA) was examined under a stereo microscope. Then, the exoskeleton was mounted on a microscope slide in Hoyer's medium for further morphological analysis. All exoskeletons have been deposited in the collection of the Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań (Poland).

The polymerase chain reaction (PCR) amplification was carried out for four DNA fragments with different mutation rates. Fragments of cytoplasmic ribosome small and large subunit components (18S rRNA and 28S rRNA, respectively) were amplified using the following primers: SSU01_F (5'-AACCTGGTTGATCCTGCCAGT-3') and SSU82_R (5'-TGATCCTTCTGCAGGTTCACCTAC-3' (Sands *et al.* 2008)) for the 18S rRNA sequences and 28SF0001 (5'-ACCCvCynAATTTAAGCATAT-3') and 28SR0990 (5'-CCTTGGTCCGTGTTTCAAGAC-3' (Mironov *et al.* 2012)) for the 28S rRNA sequences. For amplification of the ITS-2 fragment we used primers: ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3' (White *et al.* 1990)). In turn, the COI sequences were amplified using universal primers: HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3'; Folmer *et al.* 1994). Amplification of mitochondrial and all nuclear sequences, agarose gel electrophoresis and sequencing were performed according to Kayastha *et al.* (2020).

Comparative molecular analysis

The obtained sequences were checked for quality and manually aligned in BioEdit ver. 7.2.5 (Hall 1999). The COI haplotypes were retrieved using the DnaSP ver. 5.10.01 software (Librado & Rozas 2009). All the COI haplotypes were translated into amino acid sequences using the EMBOSS-TRANSEQ application (Rice *et al.* 2000; Goujon *et al.* 2010) to check for internal stop codons and indels. The translation was successfully carried out with the invertebrate mitochondrial codon table and the -2th reading frame.

Basic Local Alignment Search Tool (BLAST, Altschul *et al.* 1990) searches with sequences deposited in the NCBI database were performed to verify the identity and homology of the amplified nuclear and mitochondrial barcode sequences. For molecular comparisons, single sequences of species belonging to the genus *Echiniscus* C.A.S. Schultze, 1840 were downloaded and aligned using the ClustalW Multiple Alignment tool (Thompson *et al.* 1994) implemented in BioEdit ver. 7.2.5. Only the available GenBank sequences that coincided with nrDNA and mtDNA fragments obtained in our study have been applied. Alignment sequences were trimmed to 685, 672, 276, and 510 bp for 18S rRNA (23 species), 28S rRNA (20 species), ITS2 (11 species) and COI (21 species) molecular markers, respectively. Calculation for the uncorrected p-distances was performed using the software MEGA X (Kumar *et al.* 2018). Uncorrected pairwise distances are provided as supplementary materials (Supp. file 1).

All obtained sequences have been deposited in GenBank under the following accession numbers: COI: MZ798397–MZ798404, 18S rRNA: MZ798389–MZ798396, 28S rRNA: MZ816972–MZ816979, ITS2: MZ816980–MZ816987 (see also Supp. file 1).

Results

Taxonomic account

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

Echiniscus quadrispinosus quadrispinosus Richters, 1902
Figs 1–7, Tables 1–4, Supp. file 2

Material examined

Neotype

GERMANY • Taunus Mountains Range, near Königstein im Taunus; 50°11′49″ N, 08°27′15″ E; 485 m a.s.l.; 2 Sep. 2019; moss from stone, mixed forest; Johenn Sholl leg.; slide GR1/5; Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań.

Paraneotypes

GERMANY • 33 ♀♀, 22 ♂♂, 35 juvs, 15 undefined, 3 larvae; same collection data as for neotype; slides GR 1/1, 1/2, 1/4, 1/5, 1/7; Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań • 9 ♀♀, 6 ♂♂, 4 juvs, 2 undefined, 1 larvae; same collection data as for neotype; slides GR 1/3, 1/6; Institute of Systematics and Evolution of Animals, Polish Academy of Sciences.

Remarks

Animals were mounted on microscope slides in Hoyer's medium, 45 animals prepared for SEM, and 15 prepared for molecular analyses. However, DNA sequences were obtained from two females and one male only (exoskeleton, No GR5/S, GR8/S and GR10/S) which was later mounted on microscope slide in Hoyer's medium.

Description of the neotypic population

Females (measurements and statistics in Table 1, Supp. file 2 and Figs 1–4)

Body orange in live specimens and transparent/light yellow after preparation. Eyes red. Apart from the head appendages which include internal and external cirri and cylindrical cephalic papillae (secondary clava), only appendage *A* with clava (primary clava) near its base is present (Fig. 1D). Dorsal and lateral appendages in the shape of short, and long filaments and/or spines are present at positions *A*-*B*-*C*-*C*^{d}-*D*-*D*^{d}-*E* (Fig. 1A–C). However, appendage *B* may be absent, at least on one side of the body, for more details see Morphological variability below.

Dorsal plates well developed. Head and scapular plates not faceted. Under PCM, lateral portions of the scapular plate appear to be detached from the dorsal plate, forming small additional plates (one on each side of the body) (Fig. 1E, arrowheads) divided from the lateral margin of the scapular plate by a thin bright stripe. This division is formed by the narrow stripe without pores, as it is clearly visible in SEM along with a small additional plate (Fig. 1C, arrowhead). Paired plates I and II are divided into two parts – narrow anterior part and a wider posterior part – by smooth stripe without sculpture (Fig. 2A, arrows). Anterior parts most often divided longitudinally into two parts (Fig. 2A–B, indented arrowheads). Median plate 1 and 2 divided into anterior part (Fig. 2A, asterisks) and posterior part (Fig. 2A, filled arrowhead; which is especially visible in lateral position), m3 undivided (Fig. 2A, empty arrow). The terminal plate with two notches (Fig. 2A, empty arrowhead).

All dorsal plates, covered with double sculpture under PCM (Fig. 2A–E), i.e., regular polygonal or roundish black 'granules', i.e., endocuticular pillars (0.6–1.0 μm in diameter on scapular plate and similar in size also on other plates) and white roundish or oval pores which especially in larger specimens may be merged (seen as white spots, 1.0–4.2 μm in diameter on scapular plate and similar in size also on other plates); but see below for more details. Pores absent on anterior parts of m1 and m2 (Fig. 2A, asterisks), with typical double sculpture present on the posterior parts (Fig. 2A, filled arrowheads). Central part of terminal and scapular plates with cross-like pattern, and on terminal plate a transverse line of the cross is an extension of notches (Fig. 2C–E). Sometimes, on the scapular plate, the transverse line is absent and then only the longitudinal line visible in the middle of this plate or an additional transverse line is present and the plate is poorly divided into six rectangles (Fig. 2C–D). Under SEM the plates with regularly distributed pores (Fig. 2F), which means that where the white pores visible under PCM are absent (i.e., neck plate, lateral portions of the scapular plate and anterior parts of paired plates) the plates appear to be smooth or with poorly visible granulation (Fig. 2F).

Two poorly marked ventral rectangular plates, arranged transversally, are present below the head (Fig. 3A, C, arrowheads). Another two rounded square plates are present on lateral sides of the gonopore

(Fig. 3B–C, arrows). Ventral cuticle possesses tiny and regular granulation (due to the presence of dense endocuticular pillars). Granulation is a little larger on the plates around the gonophore (0.3–0.4 μm diameter) than in other parts of the ventral cuticle, 0.1–0.2 μm diameter) (Fig. 3D).

Outer cuticle of legs I–III with clearly visible stripes of tiny and regular granulation (0.1–0.4 μm in diameter): a thin frontal stripe on the upper part of the leg (Fig. 4A, empty arrow), a wide stripe in the central part of the leg covering frontal and lateral side of the leg (Fig. 4A, empty arrowhead) and the most distal, thin stripe above claws on the ventral side of the leg (Fig. 4A, filled indented arrowhead); white pores absent. On legs IV only frontal stripe on the upper part of the leg (just above the plate with dentate collar) (Fig. 4C, E, empty arrows) and thin stripe above claws on the ventral side of the leg are present. Triangular spine on leg I (Fig. 4B–C, filled indented arrowhead) and finger-like papilla on leg IV, present (Fig. 4D–E, filled arrow). Legs IV with dentate collar with seven to ten sharp, triangular teeth and the plate with the same sculpture as dorsal plates (Fig. 4D–E). External claws of all legs I–IV smooth, internal with spurs directed downwards (Fig. 4C–F)). The gonopore with the typical six-petal rosette (Fig. 3C, asterisk).

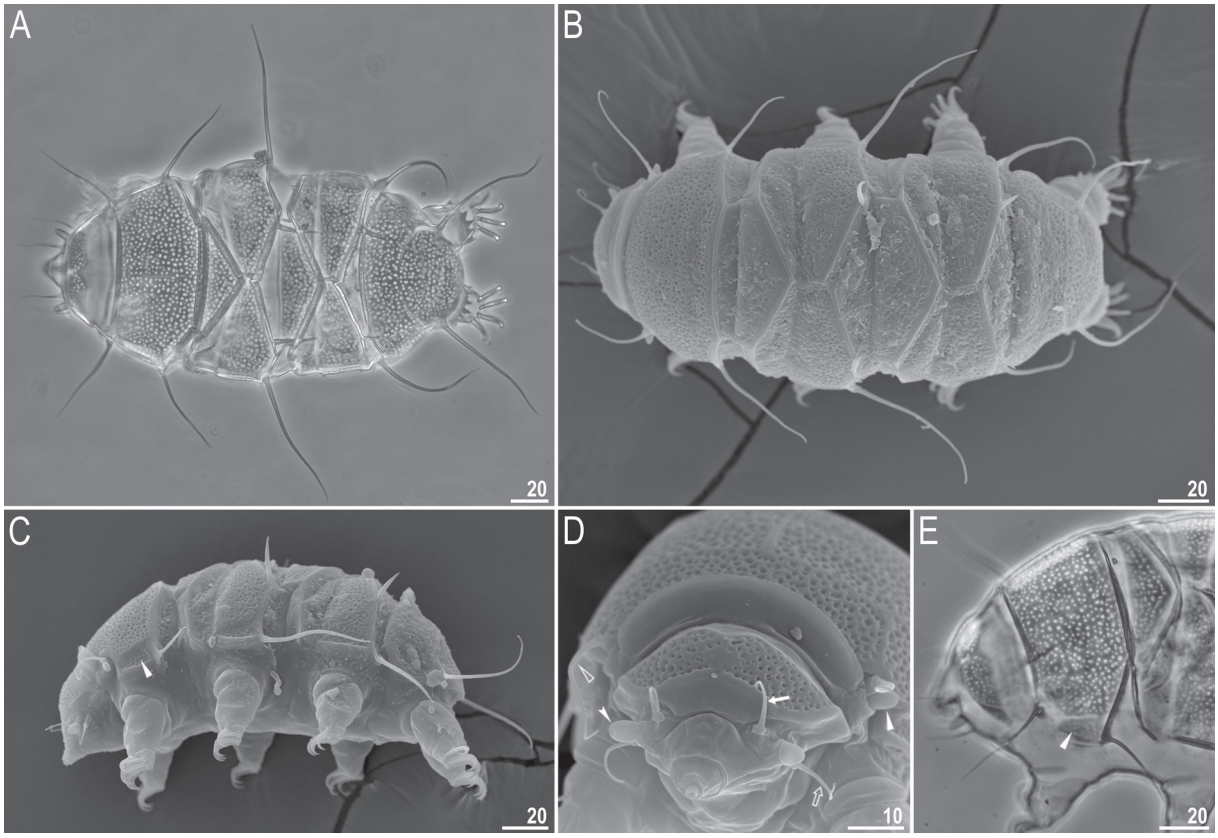

Fig. 1. *Echiniscus quadrispinosus* Richters, 1902, ♀, habitus. **A*–B**. Dorsal view of the entire animal with typical chaetotaxy *A-B-C-C^{d}-D-D^{d}-E* (PCM and SEM, respectively). **C**. Lateral view; arrowhead indicates additional plate divided from the lateral margin of the scapular plate (SEM). **D**. Head and scapular plates and head appendages visible in SEM; empty arrow indicates external cirri, filled arrow indicates internal cirri, indented arrowhead indicates cephalic papillae, empty arrowhead indicates appendage *A* and filled arrowhead indicates clava. **E**. Lateral view of head and scapular plates; arrowhead indicates additional plate divided from the lateral margin of scapular plate (PCM). * = manually assembled deep-focus image. Scale bars in micrometres (μm).

Males (measurements and statistics in Table 2, Supp. file 2 and Fig. 5)
Males are, in general, similar to females in the morphology of plates and chaetotaxy (Fig. 5A, C–D). However, there are differences in the lengths of some morphological structures (especially slightly shorter head appendages, i.e., cirri internal and external and body appendages) (compare values in Tables 1–2). On the dorsal side, regular polygonal or roundish black 'granules' 0.3–0.9 µm in diameter (on scapular plate and similar in size also on other plates) and white roundish or oval pores 1.0–3.1 µm

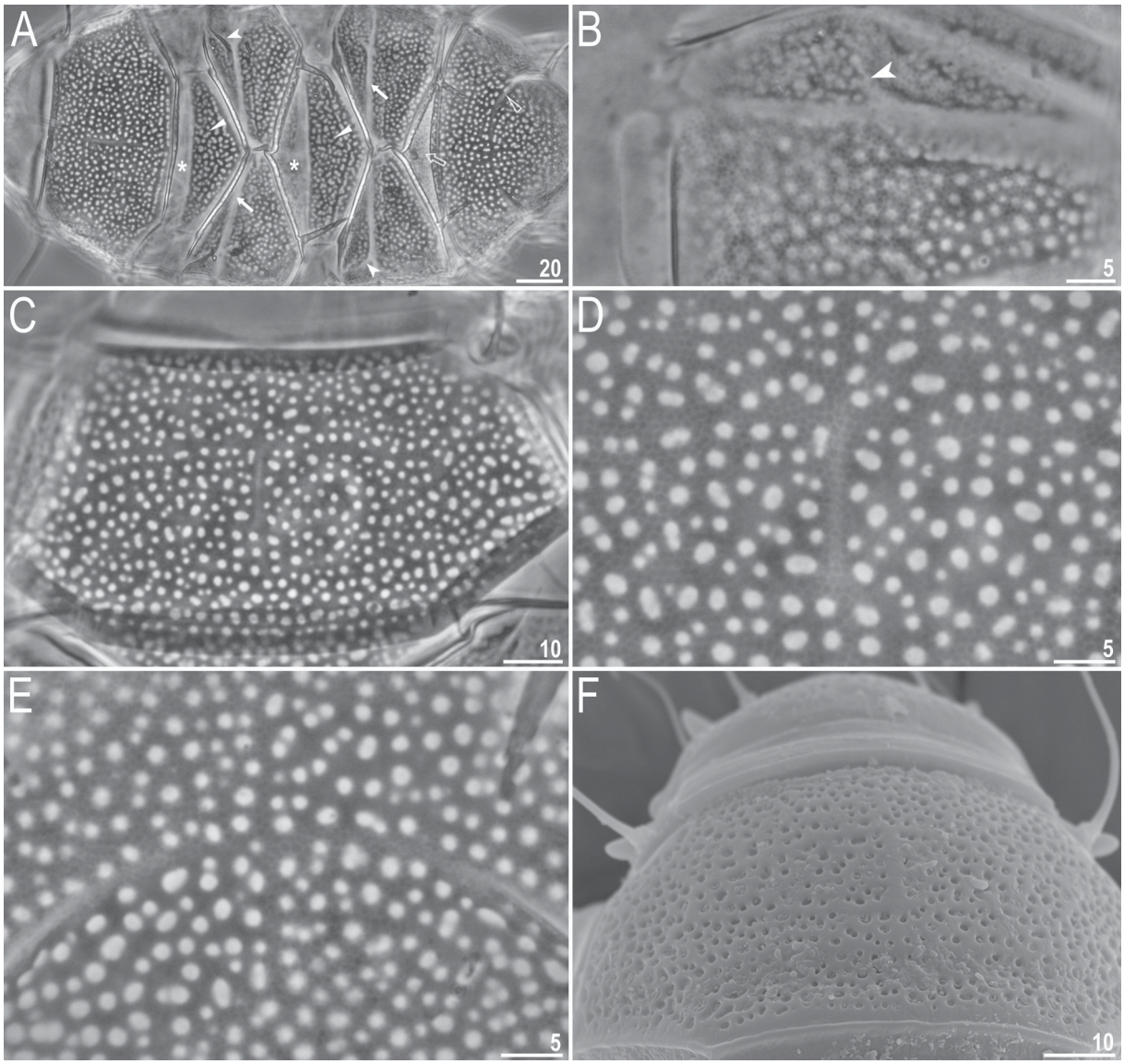

Fig. 2. *Echiniscus quadrispinosus* Richters, 1902, ♀. **A**. Close-up of the dorsal plates; arrows indicate smooth stripes dividing paired plates I and II into anterior and posterior part; indented arrowheads indicate longitudinal division of anterior part of paired plates; asterisks indicate anterior part of median plates I and II whereas filled arrowheads indicate posterior parts; empty arrow indicates median plate 3; empty arrowhead indicates notches on terminal plate (PCM). **B**. Close-up of the paired plate II; indented arrowhead indicates longitudinal division of anterior part of the plate (PCM). **C–D**. Close-up of the scapular plate sculpture (PCM). **E**. Close-up of the terminal plate sculpture (PCM). **F**. Close-up of the scapular plate (SEM). Scale bars in micrometres (µm).

Table 1. Measurements (in µm) and *sp* values of selected morphological structures of adult females from the neotype population of *Echiniscus q. quadrispinosus* Richters, 1902 mounted in Hoyer's medium. Abbreviations: N = number of specimens/structures measured; RANGE = refers to the smallest and the largest structure among all measured specimens; SD = standard deviation.

Character	N	Range		Mean		SD		Neotype	
		µm	*sp*	µm	*sp*	µm	*sp*	µm	*sp*
Body length	20	204–280	*450–562*	241	*502*	25	*24*	245	*507*
Scapular plate length	20	40.0–60.2	–	48.0	–	5.3	–	48.3	–
Head appendage lengths									
Cirrus internus	19	18.5–26.8	*42.2–55.2*	22.3	*46.8*	2.1	*4.5*	21.4	*44.4*
Cephalic papilla	20	6.4–10.3	*13.9–23.3*	8.7	*18.1*	1.2	*2.1*	9.6	*19.9*
Cirrus externus	20	23.2–32.0	*51.2–64.6*	26.9	*56.0*	3.1	*4.1*	29.6	*61.2*
Clava	20	5.6–7.4	*12.0–16.4*	6.6	*13.9*	0.5	*1.3*	7.0	*14.4*
Cirrus *A*	19	46.3–56.9	*87.6–121.4*	52.4	*109.6*	3.1	*9.8*	56.9	*117.8*
Cirrus *A*/Body length ratio	19	19%–25%	–	22%	–	2%	–	23%	–
Cirrus int/ext length ratio	19	71%–92%	–	84%	–	6%	–	72%	–
Body appendage lengths									
Cirrus *B*	18	35.8–77.9	*70.3–160.6*	57.1	*118.5*	10.7	*17.9*	57.3	*118.7*
Cirrus *C*	20	57.8–92.1	*125.5–181.9*	70.5	*147.1*	9.9	*15.5*	72.8	*150.6*
Cirrus C^d	20	17.1–30.6	*40.0–63.0*	24.2	*50.6*	2.6	*5.5*	22.4	*46.4*
Cirrus *D*	15	45.4–73.0	*93.9–137.5*	55.7	*118.5*	8.4	*12.2*	54.4	*112.7*
Cirrus D^d	19	10.8–22.8	*25.9–47.0*	17.0	*35.4*	2.7	*4.9*	17.3	*35.7*
Cirrus *E*	18	46.7–114.4	*109.4–190.0*	77.1	*159.0*	13.8	*16.0*	73.3	*151.8*
Spine on leg I length	19	3.4–5.4	*6.2–11.1*	4.3	*8.9*	0.6	*1.2*	4.3	*8.9*
Papilla on leg IV length	18	3.7–5.2	*7.7–10.7*	4.5	*9.4*	0.5	*1.0*	3.7	*7.7*
Number of teeth on the collar	19	7–10	–	8.1	–	1.0	–	8	–
Notch length	19	12.2–18.1	*28.1–34.6*	15.4	*32.4*	1.7	*1.7*	15.6	*32.3*
Claw 1 lengths									
Claw	19	12.0–18.4	*27.7–36.2*	14.9	*31.3*	1.7	*2.6*	16.0	*33.0*
Spur	15	1.9–4.4	*4.2–9.1*	2.9	*6.1*	0.7	*1.7*	4.4	*9.1*
Spur/claw length ratio	15	15%–28%	–	20%	–	5%	–	0	–
Claw 2 lengths									
Claw	17	10.2–16.9	*24.5–32.0*	13.8	*28.8*	1.9	*2.2*	14.2	*29.4*
Spur	11	2.1–3.0	*4.0–6.6*	2.5	*5.2*	0.2	*0.9*	?	*?*
Spur/claw length ratio	11	14%–25%	–	19%	–	4%	–	?	–
Claw 3 lengths									
Claw	20	11.1–17.5	*25.4–36.1*	14.1	*29.5*	2.0	*3.0*	14.2	*29.4*
Spur	15	1.8–3.1	*3.6–6.2*	2.4	*5.0*	0.3	*0.7*	?	*?*
Spur/claw length ratio	15	14%–21%	–	17%	–	3%	–	?	–
Claw 4 lengths									
Claw	20	13.3–19.3	*30.8–38.4*	16.4	*34.2*	1.6	*2.2*	16.4	*34.0*
Spur	13	2.1–4.2	*4.8–7.8*	2.8	*5.8*	0.6	*1.1*	?	*?*
Spur/claw length ratio	13	15%–24%	–	17%	–	4%	–	?	–

Table 2. Measurements (in µm) and *sp* values of selected morphological structures of adult males from the neotype population of *Echiniscus q. quadrispinosus* Richters, 1902 mounted in Hoyer's medium. Abbreviations: N = number of specimens/structures measured; RANGE = refers to the smallest and the largest structure among all measured specimens; SD = standard deviation.

Character	N	Range		Mean		SD	
		µm	*sp*	µm	*sp*	µm	*sp*
Body length	20	190–236	*469 –536*	219	*504*	12	*15*
Scapular plate length	20	39.3–46.2	–	43.4	–	1.9	–
Head appendage lengths	0						
Cirrus internus	20	15.8–20.4	*37.7 –45.0*	18.2	*41.9*	1.5	*2.4*
Cephalic papilla	20	6.7–8.8	*15.7 –20.6*	7.6	*17.4*	0.6	*1.5*
Cirrus externus	19	18.4–23.5	*43.9 –51.9*	20.8	*47.8*	1.5	*2.7*
Clava	20	4.4–6.8	*10.1 –16.2*	5.7	*13.3*	0.6	*1.5*
Cirrus *A*	20	40.2–52.7	*92.8 –120.6*	46.6	*107.3*	3.9	*9.0*
Cirrus *A*/Body length ratio	20	18%–24%	–	21%	–	2%	–
Cirrus int/ext length ratio	19	81%–95%	–	88%	–	4%	–
Body appendage lengths	0						
Cirrus *B*	19	19.3–43.7	*45.3 –97.9*	33.6	*77.5*	6.5	*15.5*
Cirrus *C*	20	42.7–67.4	*117.4–151.4*	56.6	*130.4*	4.5	*9.3*
Cirrus C^d	20	16.9–26.6	*39.5 –54.2*	20.8	*48.0*	2.0	*4.5*
Cirrus *D*	18	32.8–58.3	*83.7 –110.7*	42.9	*98.9*	5.0	*9.3*
Cirrus D^d	18	13.5–20.5	*30.3 –43.2*	15.9	*36.5*	1.8	*3.8*
Cirrus *E*	18	44.0–68.0	*118.9–158.8*	60.9	*139.7*	5.0	*12.6*
Spine on leg I length	16	3.1–4.6	*6.9 –10.5*	3.8	*8.6*	0.5	*1.1*
Papilla on leg IV length	18	3.3–4.9	*8.2 –10.7*	4.0	*9.2*	0.4	*0.8*
Number of teeth on the collar	18	7–10	–	8.4	–	1.0	–
Notch length	16	12.0–15.6	*27.0 –36.0*	13.5	*30.8*	1.1	*2.4*
Claw 1 lengths	0						
Claw	19	11.5–14.0	*26.2 –33.3*	12.6	*29.0*	0.7	*2.0*
Spur	12	1.3–2.2	*3.2 –4.8*	1.8	*4.2*	0.3	*0.5*
Spur/claw length ratio	12	11%–17%	–	15%	–	2%	–
Claw 2 lengths	0						
Claw	17	10.0–13.2	*23.7 –29.8*	12.0	*27.5*	0.6	*1.6*
Spur	9	1.5–2.3	*4.0 –5.4*	2.0	*4.5*	0.2	*0.4*
Spur/claw length ratio	9	14%–20%	–	17%	–	2%	–
Claw 3 lengths	0						
Claw	17	11.0–13.4	*25.1 –29.3*	12.1	*27.6*	0.4	*1.1*
Spur	9	1.7–2.1	*3.9 –4.6*	1.9	*4.4*	0.1	*0.3*
Spur/claw length ratio	9	13%–18%	–	16%	–	1%	–
Claw 4 lengths	0						
Claw	19	12.6–15.7	*30.2 –36.4*	14.3	*32.9*	0.8	*1.8*
Spur	5	1.6–2.6	*4.3 –5.8*	2.2	*5.0*	0.3	*0.6*
Spur/claw length ratio	5	13%–18%	–	16%	–	2%	–

in diameter (on scapular plate and similar in size also on other plates). Gonopore round and without the six-petal rosette (Fig. 5B).

Juveniles – four-clawed (measurements and statistics in Table 3, Supp. file 2 and Fig. 6)
In general, juveniles were similar to adults of both sexes in the morphology of plates (Fig. 6A). However, there are differences in chaetotaxy (Fig. 6A–D), in general, lack of filaments *B*. Moreover, appendages are shorter in juveniles than in adult females (compare values in Tables 1–3) (Fig. 6A–D). On the dorsal side, regular polygonal or roundish black 'granules' 0.4–0.9 µm in diameter (on scapular plate and similar in size also on other plates) and white roundish or oval pores 1.0–2.7 µm in diameter (on scapular plate and similar in size also on other plates). Gonopore absent.

Larvae – two-clawed (measurements and statistics in Table 4, Supp. file 2 and Fig. 7G–H)
All head and body appendages much shorter than in adults and juveniles (compare values in Tables 1–4) (Fig. 7G–H). Moreover, morphology and sculpture of plates are also different. Anterior parts of the paired plates not divided. Although, sculpture on dorsal plates composed of regular polygonal or roundish black 'granules' and white roundish or oval pores as in adults and juveniles. However, on head plate, anterior parts of paired plates and on entire median plate m3, pores completely absent and black 'granules' poorly marked. On scapular plate pores are distributed mainly on the margins of the plate and almost absent in the centre. On the dorsal side, regular polygonal or roundish black 'granules' 0.4–0.9 µm in diameter (on scapular plate) and white roundish or oval pores 0.7–1.4 µm in diameter (on

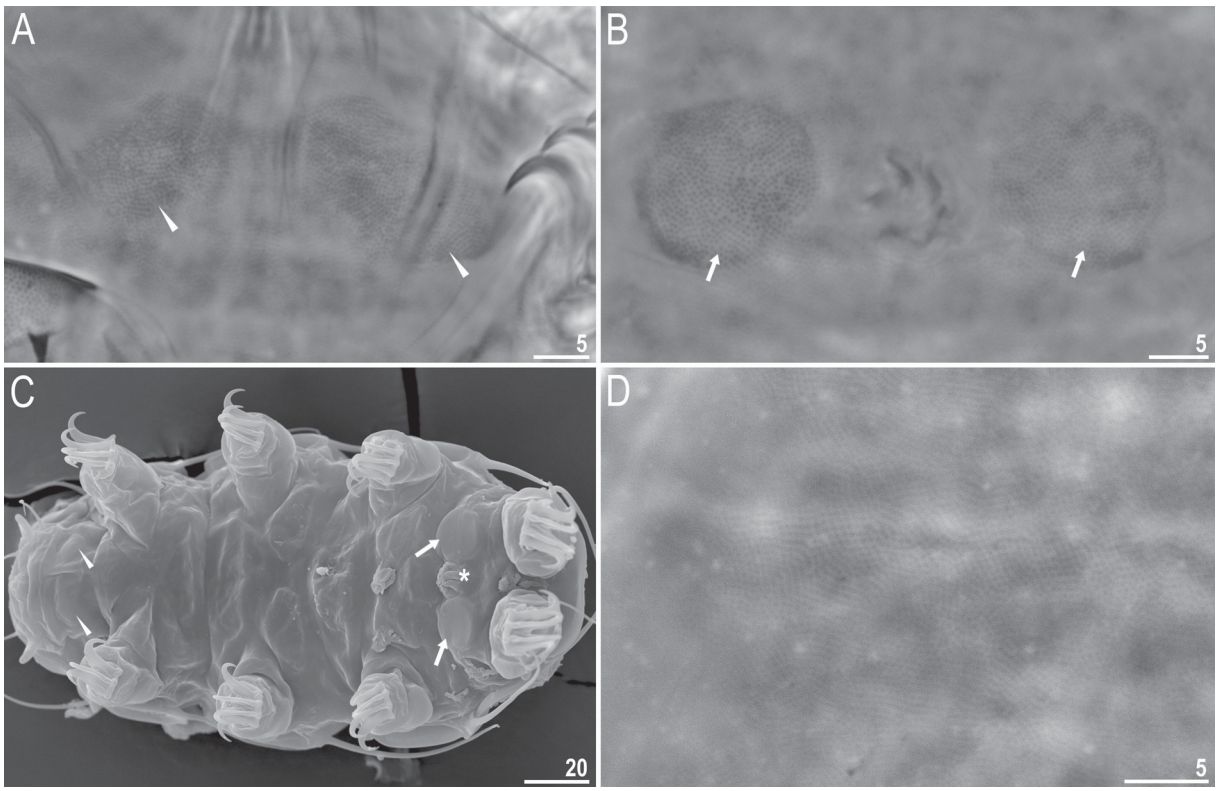

Fig. 3. *Echiniscus quadrispinosus* Richters, 1902, ♀. **A**. Two ventral plates below the head, arrowheads (PCM). **B**. Two ventral rounded plates on the lateral sides of the gonopore, arrows (PCM). **C**. Lateral view of the entire animal; arrowheads indicate plates below the head; arrows indicate plates on the lateral sided of the gonopore; asterisk indicates gonopore (SEM). **D**. Ventral sculpture visible in PCM. Scale bars in micrometres (µm).

Table 3. Measurements (in µm) and *sp* values of selected morphological structures of juveniles from the neotype population of *Echiniscus q. quadrispinosus* Richters, 1902 mounted in Hoyer's medium. Abbreviations: N = number of specimens/structures measured; RANGE = refers to the smallest and the largest structure among all measured specimens; SD = standard deviation.

Character	N	Range		Mean		SD	
		µm	*sp*	µm	*sp*	µm	*sp*
Body length	20	145–208	*457–546*	176	*492*	17	*28*
Scapular plate length	20	29.6–40.5	–	35.7	–	2.8	–
Head appendage lengths	0						
Cirrus internus	20	9.1–17.6	*30.8–44.7*	13.2	*36.8*	2.6	*5.0*
Cephalic papilla	19	4.7–8.0	*15.3–20.8*	6.3	*17.6*	1.0	*1.8*
Cirrus externus	20	10.4–19.8	*35.3–50.8*	15.4	*43.0*	2.9	*5.2*
Clava	19	3.4–6.5	*11.3–16.7*	5.0	*14.0*	0.8	*1.7*
Cirrus *A*	20	24.9–44.4	*83.9–117.6*	35.9	*100.4*	5.2	*10.1*
Cirrus *A*/Body length ratio	20	16%–25%	–	20%	–	3%	–
Cirrus int/ext length ratio	20	78%–94%	–	86%	–	6%	–
Body appendage lengths	0						
Cirrus *B*	6	9.9–24.4	*26.1–69.7*	20.6	*55.2*	5.8	*15.6*
Cirrus *C*	19	30.1–59.4	*91.0–152.8*	43.3	*119.2*	9.9	*22.9*
Cirrus C^d	20	11.2–20.0	*37.5–54.0*	16.1	*45.2*	2.2	*5.3*
Cirrus *D*	19	18.7–60.4	*53.7–155.6*	30.8	*84.5*	10.8	*25.8*
Cirrus D^d	19	9.1–18.1	*27.6–52.2*	13.2	*37.2*	2.3	*5.8*
Cirrus *E*	19	35.0–59.3	*106.2–162.5*	49.5	*136.7*	7.4	*16.2*
Spine on leg I length	15	2.0–3.9	*6.8–11.1*	3.3	*9.1*	0.5	*1.3*
Papilla on leg IV length	16	2.8–3.9	*8.0–10.9*	3.2	*9.1*	0.4	*0.9*
Number of teeth on the collar	16	5–8	–	6.0	–	1.1	–
Notch length	14	8.7–13.2	*24.8–34.7*	11.1	*30.4*	1.6	*3.2*
Claw 1 lengths	0						
Claw	20	8.4–12.4	*25.1–32.0*	10.2	*28.5*	1.2	*2.1*
Spur	8	1.2–2.7	*3.7–6.9*	1.9	*5.4*	0.5	*1.2*
Spur/claw length ratio	8	13%–23%	–	19%	–	4%	–
Claw 2 lengths	0						
Claw	20	7.7–12.8	*23.2–32.9*	9.6	*27.0*	1.3	*2.5*
Spur	8	1.3–2.6	*3.4–6.8*	1.9	*5.2*	0.4	*1.0*
Spur/claw length ratio	8	14%–23%	–	19%	–	3%	–
Claw 3 lengths	0						
Claw	18	7.5–12.5	*22.4–32.2*	9.7	*27.0*	1.5	*3.2*
Spur	9	1.3–2.4	*3.3–6.8*	1.8	*5.2*	0.4	*1.1*
Spur/claw length ratio	9	12%–22%	–	19%	–	4%	–
Claw 4 lengths	0						
Claw	19	8.9–15.2	*27.9–39.7*	11.3	*31.6*	1.8	*3.3*
Spur	11	1.5–2.2	*3.9–6.4*	1.9	*5.4*	0.2	*0.7*
Spur/claw length ratio	11	14%–21%	–	18%	–	3%	–

Table 4. Measurements (in µm) and *sp* values of selected morphological structures of larvae from the neotype population of *Echiniscus q. quadrispinosus* Richters, 1902 mounted in Hoyer's medium. Abbreviations: N = number of specimens/structures measured; RANGE = refers to the smallest and the largest structure among all measured specimens; SD = standard deviation.

Character	**N**	**Range**		**Mean**		**SD**	
		µm	***sp***	**µm**	***sp***	**µm**	***sp***
Body length	3	119–139	*503–551*	132	*523*	11	*25*
Scapular plate length	3	23.7–26.7	–	25.2	–	1.5	–
Head appendage lengths							
Cirrus internus	3	6.8–8.2	*25.6–34.4*	7.7	*30.8*	0.8	*4.6*
Cephalic papilla	3	3.9–4.6	*16.2–18.1*	4.3	*16.9*	0.4	*1.1*
Cirrus externus	3	8.5–9.8	*32.0–38.9*	9.2	*36.5*	0.6	*3.9*
Clava	3	3.0–3.4	*12.0–13.3*	3.2	*12.7*	0.2	*0.7*
Cirrus *A*	3	22.0–25.6	*92.9–98.7*	24.2	*95.9*	1.9	*2.9*
Cirrus *A*/Body length ratio	3	18%–19%	–	18%	–	0%	–
Cirrus int/ext length ratio	3	80%–88%	–	84%	–	4%	–
Body appendage lengths							
Cirrus C^d	3	9.6–11.0	*39.7–43.4*	10.4	*41.2*	0.7	*1.9*
Cirrus D^d	3	7.2–8.5	*30.2–33.3*	8.0	*31.7*	0.7	*1.6*
Cirrus *E*	3	20.5–27.3	*81.1–102.3*	23.6	*93.5*	3.4	*11.0*
Spine on leg I length	2	2.2–2.3	*8.2–9.8*	2.3	*9.0*	0.1	*1.2*
Papilla on leg IV length	3	2.2–2.8	*9.3–10.3*	2.5	*9.9*	0.3	*0.6*
Number of teeth on the collar	3	6–6	–	6.0	–	0.0	–
Notch length	3	8.0–8.6	*30.4–34.0*	8.2	*32.7*	0.3	*2.0*
Claw 1 lengths							
Claw	3	8.0–8.3	*31.1–33.8*	8.2	*32.4*	0.2	*1.3*
Spur	3	1.5–1.8	*5.6–7.1*	1.6	*6.4*	0.2	*0.7*
Spur/claw length ratio	3	18%–22%	–	20%	–	2%	–
Claw 2 lengths	0						
Claw	3	7.8–8.0	*30.0–33.4*	7.9	*31.4*	0.1	*1.8*
Spur	3	1.3–1.5	*4.9–6.3*	1.4	*5.7*	0.1	*0.8*
Spur/claw length ratio	3	16%–19%	–	18%	–	2%	–
Claw 3 lengths							
Claw	3	7.8–7.9	*29.6–33.4*	7.9	*31.3*	0.1	*1.9*
Spur	3	1.3–1.5	*4.9–6.3*	1.4	*5.7*	0.1	*0.8*
Spur/claw length ratio	3	16%–19%	–	18%	–	2%	–
Claw 4 lengths							
Claw	3	8.5–9.2	*32.2–36.3*	8.8	*34.8*	0.4	*2.3*
Spur	3	1.6–1.9	*6.0–8.0*	1.8	*7.0*	0.2	*1.0*
Spur/claw length ratio	3	19%–22%	–	20%	–	2%	–

scapular plate). Also, chaetotaxy different than adults and juveniles, i.e., *A*-*C*^{d}-*D*^{d}-*E* (Fig. 7G). Gonopore absent (Fig. 7H).

Eggs

Smooth, light orange and deposited in the exuviae up to 6 in one exuvium.

DNA sequences

We obtained good quality sequences for the applied molecular markers:

18S rRNA: GenBank: MZ798389-MZ798396, 771 bp long;
28S rRNA: GenBank: MZ816972-MZ816979, 715-747 bp long;
ITS-2: GenBank: MZ816980-MZ816987, 464 bp long;
COI: GenBank: MZ798397-MZ798404, 688 bp long.

Morphological variability

A strict chaetotaxy was analysed in 36 females, 23 males, 35 juveniles, 3 larvae. In all adult specimens, both females and males typical chaetotaxy, i.e., *A*-*B*-*C*-*C*^{d}-*D*-*D*^{d}-*E* was observed (Fig. 1A–B), whereas, in juveniles appendages *B* are most often absent (chaetotaxy: *A*-*C*-*C*^{d}-*D*-*D*^{d}-*E*). The other dorsal and lateral appendages were in general shorter in juveniles than in adults. In all studied larvae, chaetotaxy was always *A*-*C*^{d}-*D*^{d}-*E* and all appendages were much shorter than in juveniles and adults (compare values in Tables 1–4). Moreover, some modifications in chaetotaxy were observed in juveniles and adults.

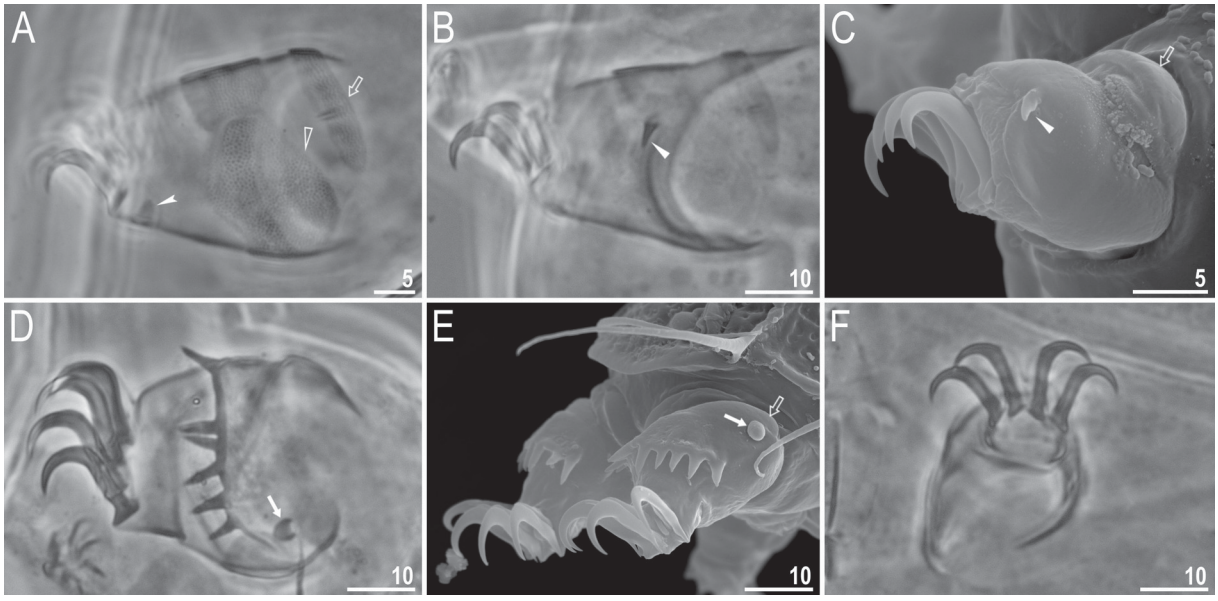

Fig. 4. *Echiniscus quadrispinosus* Richters, 1902, ♀. **A***. Leg I outer cuticle with clearly visible stripes of tiny and regular granulation: a thin frontal stripe on the upper part of the leg (empty arrow), a wide stripe in the central part of the leg covering frontal and lateral side of the leg (empty arrowhead) and the most distal, thin stripe above claws on the ventral side of the leg (filled indented arrowhead) (PCM). **B**. Spine on leg I (arrowhead) (PCM). **C**. Spine on leg I (arrowhead) and thin frontal stripe on the upper part of the leg (empty arrow) (SEM). **D***. Claws IV with dentate collar and finger-like papilla (filled arrow) (PCM). **E**. Vlaws IV with dentate collar and finger-like papilla (filled arrow); empty arrow indicates thin frontal stripe on the upper part of the leg (SEM). **F**. Claws of the II leg (PCM). * = manually assembled deep-focus image. Scale bars in micrometres (μm).

In two juveniles appendages *B* were present on both sides of the body and in five juveniles appendages *B* were present only on one side of the body (chaetotaxy: *A-B-C-C^d-D-D^d-E*) (Fig. 6A–B). Moreover, in one juvenile appendages *C^d* and *D^d* were present only on one side of the body, and in another one *D^d* was present on both sides and *C^d* only on one side (chaetotaxy: *A-C-C^d-D-D^d-E*) (Fig. 6C–D).

In two females appendages *B* were present only on one side of the body (chaetotaxy: *A-B-C-C^d-D-D^d-E*) (Fig. 7A). Two females had additional small spines near the base of normally developed appendages *B* (chaetotaxy: *A-B-C-C^d-D-D^d-E*) (Fig. 7B–D). In other two females only appendage *C^d* on one side of the body was present and appendages *D^d* were completely absent (chaetotaxy: *A-B-C-C^d-D-E*) (Fig. 7E–F). One female had very short appendage *B* on one side of the body (chaetotaxy: *A-B-C-C^d-D-D^d-E*). In another one a very short appendage *B* on one side of the body was present and appendages *C^d* and *D^d* were present only on one side of the body (chaetotaxy: *A-B-C-C^d-D-D^d-E*). Finally, in one female appendages *C^d* and *D^d* were present on only one side of the body (chaetotaxy: *A-B-C-C^d-D-D^d-E*).

In four males appendages *B* were present only on one side of the body (chaetotaxy: *A-B-C-C^d-D-D^d-E*) (Fig. 5C–D) and in two males appendages *B* were absent on both sides (chaetotaxy: *A-C-C^d-D-D^d-E*) (Fig. 5A).

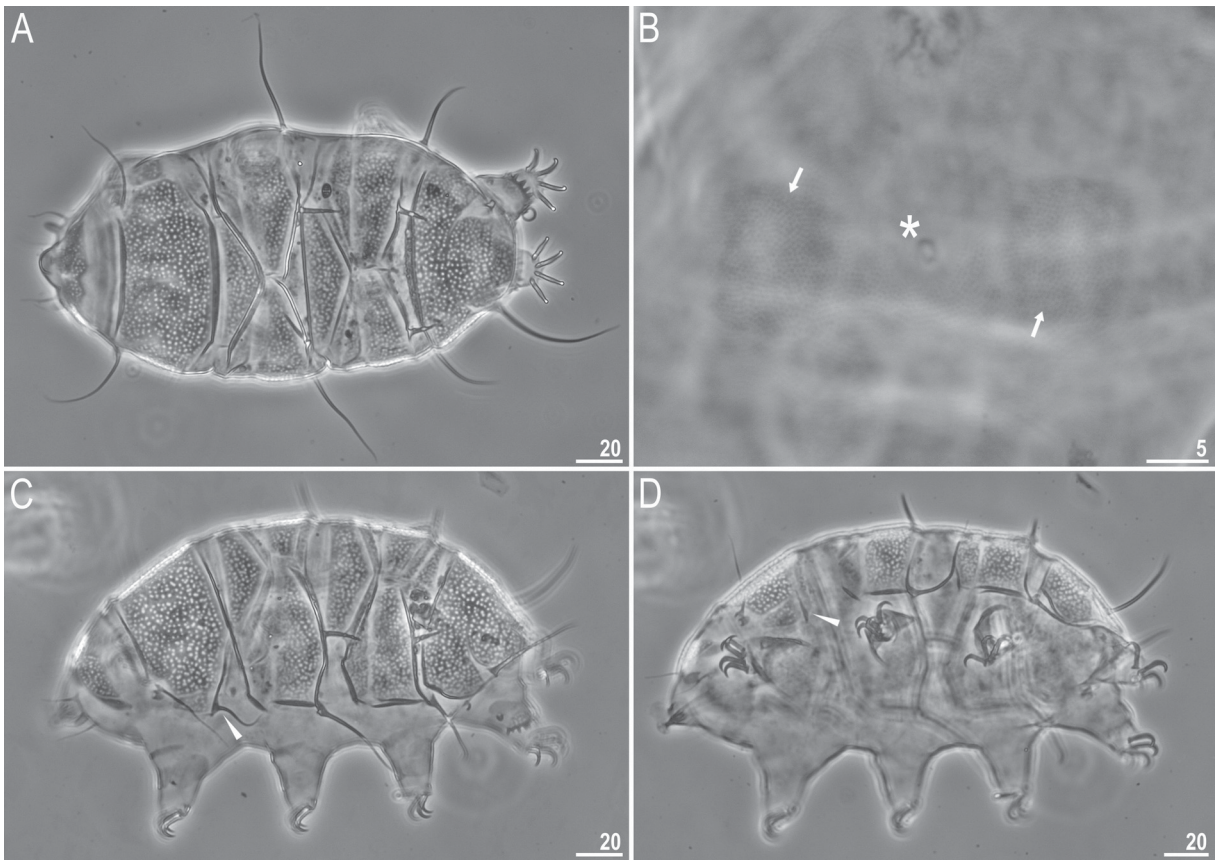

Fig. 5. *Echiniscus quadrispinosus* Richters, 1902, ♂. **A***. Dorsal view of the entire animal with appendages *B* absent on both sides (chaetotaxy: *A-C-C^d-D-D^d-E*). **B***. Two ventral rounded plates on the lateral sides of the gonopore (arrows); asterisk indicates gonopore. **C–D**. Lateral view of male with appendage *B* present only on one side of the body (chaetotaxy: *A-B-C-C^d-D-D^d-E*); arrowhead indicates presence and lack of appendage *B*. ***** = manually assembled deep-focus image. All PCM. Scale bars in micrometres (μm).

Based on these assumptions chaetotaxy formula for adults and juveniles of this species is in general *A-(B)-C-C^{d}-D-D^{d}-E* and for larvae *A-C^{d}-D^{d}-E*. The other observed aberrations in chaetotaxy are only accidental.

Genetic variability

The obtained eight COI sequences (GenBank accession numbers: MZ798397-MZ798404) of *E. q. quadrispinosus* consisted of four COI haplotypes. Haplotype 1 was found in the Norwegian (169/7 sequence, population code: 169) and German (GR8 and GR10 sequences, population code: GR) populations whereas haplotypes 2, 3 and 4 were identified in different Norwegian populations (haplotype 2 – 169/8 sequence, population code: 169; haplotype 3 – 184/3 and 184/8 sequences, population code: 184; haplotype 4 –187/4 and 187/7 sequences, population code: 187). The value of uncorrected genetic p-distances between obtained COI haplotypes ranged from 0.2% to 0.8%. In turn, the analysis of the p-distances between *E. q. quadrispinosus* and compared 20 taxa of the genus *Echiniscus* ranged

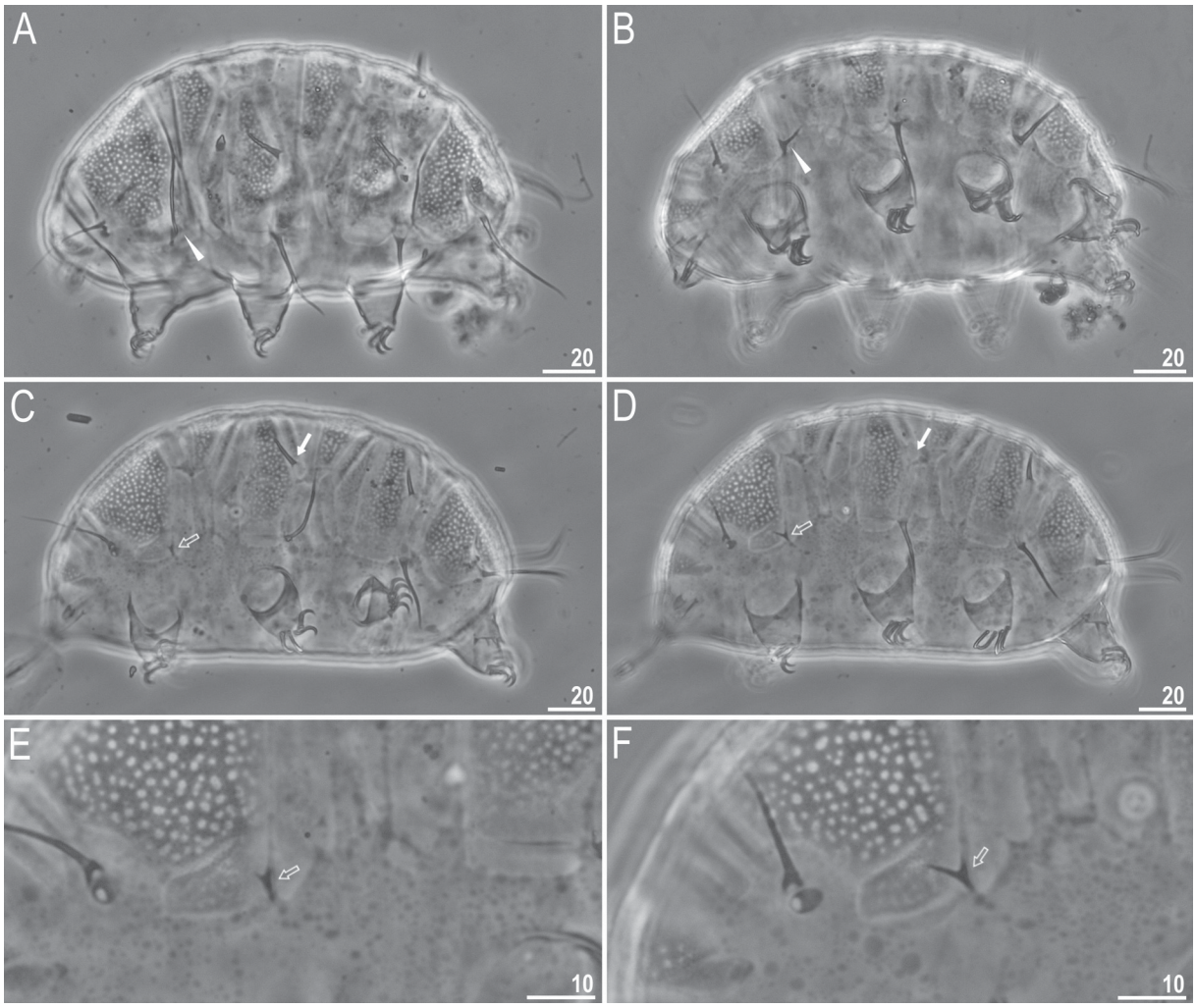

Fig. 6. *Echiniscus quadrispinosus* Richters, 1902, juvenile. **A–B**. Lateral view of the animal with appendage *B* present only on one side of the body (arrowhead) (chaetotaxy: *A-B-C-C^{d}-D-D^{d}-E*). **C–D**. Lateral view with appendage *C^{d}* present only on one side of the body (filled arrows) and two short spines present instead of appendage *B* (empty arrows). **E–F**. Close-up to the two short spines present instead of appendage *B* (empty arrows). All PCM. Scale bars in micrometres (μm).

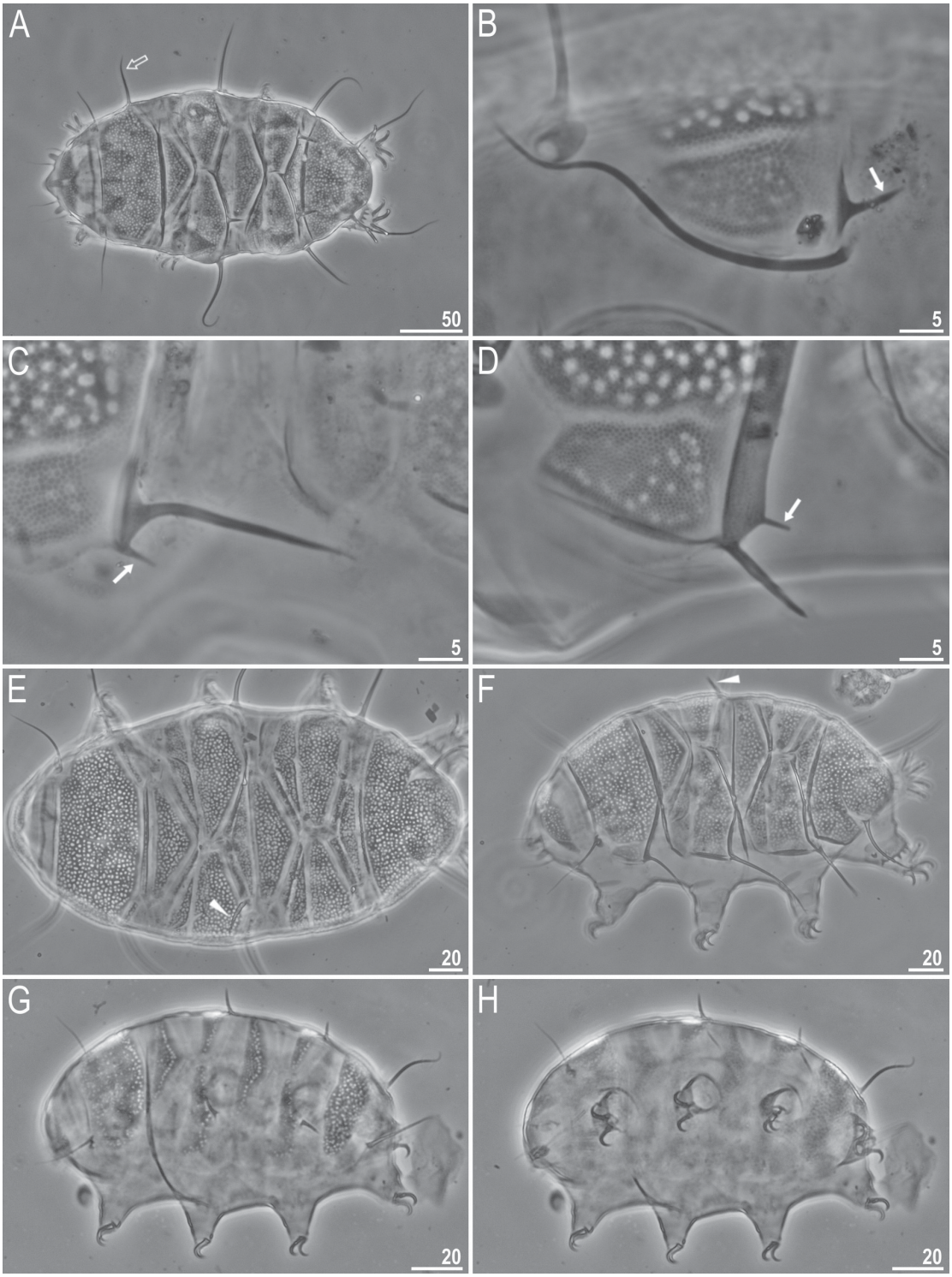

Fig. 7. *Echiniscus quadrispinosus* Richters, 1902. **A–F**. Examples of different chaetotaxy found in examined population. **A***. Female with appendage *B* present only on one side of the body (empty arrow) (chaetotaxy: *A-B-C-C*^{d}*-D-D*^{d}*-E*). **B–D**. Close-up to the spines near the base of normally developed appendages *B* (filled arrows). **E–F**. Presence of appendage *C*^{d} only on one side of the body (filled arrowheads) and lack of appendages *D*^{d} (chaetotaxy: *A-B-C-C*^{d}*-D-E*). **G**. Larvae, dorsal view (chaetotaxy: *A-C*^{d}*-D*^{d}*-E*). **H**. Larvae, ventral view. * = manually assembled deep-focus image. All PCM. Scale bars in micrometres (μm).

from the most similar 1.2% for *E. quadrispinosus* (GenBank accession number: JX683821, Vincente *et al.* 2013) to the least similar 21.8% for *E. tantulus* Gąsiorek, Bochnak, Vončina & Kristensen, 2020 (GenBank accession number: MT107427, Bochnak *et al.* 2020), with an average p-distance of 14.4%.

In the conservative 18S rRNA gene fragment we observed no differences between our eight sequences from the German and Norwegian populations (GenBank accession numbers: MZ798389-MZ798396) and sequences of *E. quadrispinosus* deposited in NCBI (GenBank accession number: MK529684). In turn, the uncorrected genetic p-distances between the other 21 taxa of the genus *Echiniscus* showed that the least similar was *E. belloporus* Gąsiorek & Kristensen, 2018 (GenBank accession number: MK529674, Gąsiorek *et al.* 2019a) with a genetic distance value of 3.1% and an average p-distance was 1.4%.

The analysis of the p-distances between our eight sequences of 28S rRNA from the German and Norwegian populations (GenBank accession numbers: MZ816972-MZ816979; two groups of sequences, i.e., the first consisted of GR8, GR10, 169/8, 169/9, 184/3, 184/8, 187/1 sequences and the second – one 187/8 sequence) indicated that the genetic distance was 1%. Comparison with other 19 taxa of the genus *Echiniscus*, for which GenBank sequences are available, are as follows: the most similar was *E. quadrispinosus* (GenBank accession number: MK529714, Gąsiorek *et al.* 2019a) with 1% value of the p-distance and the least similar was *E. belloporus* Gąsiorek & Kristensen, 2018 (GenBank accession numbers: MK529702, Gąsiorek *et al.* 2019a) – 5.4%, with an average p-distance of 2.5%.

No genetic differences were observed between our eight ITS2 sequences from the German and Norwegian populations (GenBank accession numbers: MZ816980-MZ816987). The ranges of uncorrected genetic p-distances between our sequences and the other 10 species of the genus *Echiniscus* indicated that the most similar was *E. virginicus* Riggin, 1962 (GenBank accession number: MN545756, Gąsiorek *et al.* 2019b) – 0.42% and the least similar was *E. blumi* Richters, 1903 (GenBank accession number: EF620383, Jørgensen *et al.* 2007) – 34.5%, with an average p-distance of 21.8%. There were no available ITS2 sequences of *E. quadrispinosus* in the GenBank database.

Establishing of the neotype and paraneotypes of E. q. quadrispinosus

The search for the type material of *E. q. quadrispinosus* in various collections did not bring positive results. We can probably assume that the type material of *E. q. quadrispinosus* no longer exists. Taking into consideration that accurate diagnoses of the species were poorly provided in the past, it is necessary to establish a neotype series of this species. For this reason, we designated the neotype and 108 paraneotypes of *E. q. quadrispinosus* which agree with the original description and were collected in the terra typica in the Taunus Mountain Range (Germany). The neotype series was deposited at the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University in Poznań and Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Poland. All the above-mentioned statements are in accordance with the International Commission on Zoological Nomenclature (ICZN) acts dedicated to the establishment of neotype series.

Discussion

In our study, we used integrative taxonomy to describe *E. q. quadrispinosus* specimens from its terra typica – Taunus Mountain Range (Germany). Our analysis has shown that our specimens are morphologically compatible with specimens described by Richters (1902). However, in the studied population we found morphological differences between adults and juveniles in chaetotaxy.

Intraspecific and interspecific variability of Echiniscidae has been studied for many years and is still problematic. However, despite the well-documented intraspecific variability in genera such as

Echiniscus, *Mopsechiniscus* du Bois-Reymond Marcus, 1944, or *Pseudechiniscus* Thulin, 1911, even in more recently published species descriptions concerning the variability, in relation to life stage or sex, was ignored (for a review see, e.g., Bartylak *et al.* 2019).

We analysed separately females, males, juveniles and larvae of *E. q. quadrispinosus* to check possible differences in chaetotaxy between different life stages and sex of the animals. We found a clear pattern related to the presence or absence of some filaments in different life stages, but not between sexes. This is similar to the *E. tristis* Gąsiorek & Kristensen, 2018, for which such differences in chaetotaxy were found to be connected to the life stages or sex of the animals (Bartylak *et al.* 2019). Such results underline how important it is to analyse a large number of specimens from any given species to avoid incorrect species identification or wrong/incomplete description of a new species, subspecies or forms as was done for *E. q. quadrispinosus*.

Apart from the nominal *E. q. quadrispinosus*, three other subspecies are known: *E. q. brachyspinosus*, *E. q. cribrosus* and *E. q. fissispinosus*. *Echiniscus q. brachyspinosus* was proposed as a variety only by Ramazzotti & Maucci (1983) who suggested that the described differences (body appendages in the form of short, wide spines in *E. q. brachyspinosus* instead of filaments in nominal species) should be rather considered as species variability and the difference between young and adult specimens. This is additionally confirmed by the fact that such spines were found only in small specimens which were part of the population of the typical *E. q. quadrispinosus*. Moreover, also Cuénot (1932) observed short spines *C* and *D* in small specimens of *E. q. quadrispinosus*. The same was observed by us in the present study and, what is more, such variability seems to be frequent in species of *Echiniscus* (e.g., Guil 2008; Bartylak *et al.* 2019). We think that in such a situation this form should be considered as young specimens of *E. q. quadrispinosus*.

Echiniscus q. cribrosus and *E. q. fissispinosus* differ from the nominal species by the absence of 'supplementary plates', which are in fact separated parts of paired plates I and II in *E. q. quadrispinosus*. Such pseudoplates are often present in other species of *Echiniscus* (see e.g., Claxton 1996; Kaczmarek & Michalczyk 2010; Claxton & Dastych 2017; Gąsiorek & Kristensen 2018; Bartylak *et al.* 2019; Gąsiorek & Vončina 2019; Gąsiorek & Michalczyk 2020b; Gąsiorek *et al.* 2020; Kiosya *et al.* 2021). Additionally, some differences were also reported in chaetotaxy (e.g., lacking of appendages *B*, reduced or absent lateral or dorsal appendages). Moreover, in *E. q. fissispinosus* lateral appendages *D* are doubled on one or both sides of the body. Since the visibility of 'supplementary plates' may depend on slide preparation or size of the specimens, differences in chaetotaxy (number of appendages, their shape and duplication) are typical in species of *Echiniscus* (for a review see, e.g., Guil 2008; Bartylak *et al.* 2019), we suggest that *E. q. cribrosus* should be considered a typical *E. q. quadrispinosus*.

Moreover, Gąsiorek *et al.* (2019a) suggested *E. lichenorum* as conspecific with *E. q. quadrispinosus* and proposed for this species a taxonomic status of nomen inquirendum. However, although we agree that dorsal plate sculpture in *E. lichenorum* is very similar or even identical as in *E. q. quadrispinosus*, both species are easy to differentiate because of the presence of appendages *E* in *E. q. quadrispinosus*. Based on this assumption, although we did not examine the type material of *E. lichenorum*, we think that this species should be considered as valid, even if some morphological details are not listed in the original description.

Echiniscus q. quadrispinosus is reported from many localities throughout the world, most of them in the Holarctic (McInnes 1994; Meyer 2013; Kaczmarek *et al.* 2016; McInnes *et al.* 2017). Due to its single reports from New Zealand, Central and South America, McInnes (1994) considered this species as "probably cosmopolitan". Although this is possible (see, e.g., the distribution of *E. testudo* by Gąsiorek *et al.* 2019c), in our opinion reports outside Holarctic need a confirmation.

Summarizing, we propose *E. lichenorum* as a valid species and the subspecies *E. q. brachyspinosus*, *E. q. cribrosus* and *E. q. fissispinosus* as part of the nominal *E. q. quadrispinosus*, meaning that the nomenclature of the nominal species needs to be changed to *E. quadrispinosus*.

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References

Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215 (3): 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Bartoš E. 1934. Prispevek k Poznani Tardigrad ČSR. *Časopis Československé Společnosti Entomologické* 31: 99–107.

Bartylak T., Kulpa A., Grobys D., Kepel M., Kepel A., Kmita H., Gawlak M., Grabiński W., Roszkowska M. & Kaczmarek Ł. 2019. Variability of *Echiniscus tristis* Gąsiorek & Kristensen, 2018—is morphology sufficient for taxonomic differentiation of Echiniscidae? *Zootaxa* 4701 (1): 1–24. https://doi.org/10.11646/zootaxa.4701.1.1

Bochnak M., Vončina K., Kristensen R.M. & Gasiorek P. 2020. Continued exploration of Tanzanian rainforests reveals a new echiniscid species (Heterotardigrada). *Zoological Studies* 59: 18. https://doi.org/10.6620/ZS.2020.59-18

Casquet J., Thebaud C. & Gillespie R.G. 2012. Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. *Molecular Ecology Resources* 12 (1): 136–141. https://doi.org/10.1111/j.1755-0998.2011.03073.x

Claxton S.K. 1996. Sexual dimorphism in Australian *Echiniscus* (Tardigrada, Echiniscidae) with description of three new species. *Zoological Journal of the Linnean Society* 116 (1–2): 13–33. https://doi.org/10.1111/j.1096-3642.1996.tb02330.x

Claxton S.K. & Dastych H. 2017. A new bisexual species of *Echiniscus* C.A.S. Schultze, 1840 (Heterotardigrada: Echiniscidae) from Tasmania, Australia. *Entomologie heute* 29: 105–119.

Cuénot L. 1932. Tardigrades. *In*: Lechevalier P. (ed.) *Faune de France* 24: 1–96.

Dastych H. 1980. Niesporczaki (Tardigrada) Tatrzańskiego Parku Narodowego. *Monografie Fauny Polski* 9: 1–232.

Dastych H. 1999. A new species of the genus *Mopsechiniscus* Du Bois-Reymond Marcus, 1944 (Tardigrada) from the Venezuelan Andes. *Acta Biologica Benrodis* 10: 91–101.

Degma P. & Guidetti R. 2007. Notes to the current checklist of Tardigrada. *Zootaxa* 1579 (1): 41–53. https://doi.org/10.11646/zootaxa.1579.1.2

Degma P., Bertolani R. & Guidetti R. 2009–2021. Actual checklist of Tardigrada species. (2009–2021, 40th Edition: 19–07–2021). Available from https://iris.unimore.it/retrieve/358743/Actual%20checklist%20of%20Tardigrada%2040th%20Edition%2019-07-21.pdf [accessed 4 May 2022].

Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3 (5): 294–299.

Fontoura P. & Morais P. 2011. Assessment of traditional and geometric morphometrics for discriminating cryptic species of the *Pseudechiniscus suillus* complex (Tardigrada, Echiniscidae). *Journal of Zoological Systematics and Evolutionary Research* 49 (s1): 26–33. https://doi.org/10.1111/j.1439-0469.2010.00594.x

Gąsiorek P. & Kristensen R.M. 2018. Echiniscidae (Heterotardigrada) of Tanzania and Uganda. *Tropical Zoology* 31 (3): 131–160. https://doi.org/10.1080/03946975.2018.1477350

Gąsiorek P. & Michalczyk Ł. 2020a. Revised *Cornechiniscus* (Heterotardigrada) and new phylogenetic analyses negate echiniscid subfamilies and tribes. *Royal Society Open Science* 7 (6): 200581. https://doi.org/10.1098/rsos.200581

Gąsiorek P. & Michalczyk Ł. 2020b. *Echiniscus siticulosus* (Echiniscidae: *spinulosus* group), a new tardigrade from Western Australian scrub. *New Zealand Journal of Zoology* 47 (2): 87–105. https://doi.org/10.1080/03014223.2019.1603166

Gąsiorek P. & Vončina K. 2019. New Echiniscidae (Heterotardigrada) from Amber Mountain (Northern Madagascar). *Evolutionary Systematics* 3 (1): 29–39. https://doi.org/10.3897/evolsyst.3.33580

Gąsiorek P., Stec D., Morek W. & Michalczyk Ł. 2017. An integrative redescription of *Echiniscus testudo* (Doyère, 1840), the nominal taxon for the class Heterotardigrada (Ecdysozoa: Panarthropoda: Tardigrada). *Zoologischer Anzeiger* 270: 107–122. https://doi.org/10.1016/j.jcz.2017.09.006

Gąsiorek P., Morek W., Stec D. & Michalczyk Ł. 2019a. Untangling the *Echiniscus* Gordian knot: paraphyly of the "*arctomys* group" (Heterotardigrada: Echiniscidae). *Cladistics* 35 (6): 633–653. https://doi.org/10.1111/cla.12377

Gąsiorek P., Jackson K.J., Meyer H.A., Zając K., Nelson D.R., Kristensen R.M. & Michalczyk Ł. 2019b. *Echiniscus virginicus* complex: the first case of pseudocryptic allopatry and pantropical distribution in tardigrades. *Biological Journal of the Linnean Society* 128 (4): 789–805. https://doi.org/10.1093/biolinnean/blz147

Gąsiorek P., Vončina K. & Michalczyk Ł. 2019c. *Echiniscus testudo* (Doyère, 1840) in New Zealand: anthropogenic dispersal or evidence for the 'Everything is Everywhere' hypothesis? *New Zealand Journal of Zoology* 46 (2): 174–181. https://doi.org/10.1080/03014223.2018.1503607

Gąsiorek P., Vončina K. & Michalczyk Ł. 2020. An overview of the sexual dimorphism in *Echiniscus* (Heterotardigrada, Echiniscoidea), with the description of *Echiniscus masculinus* sp. nov. (the *virginicus* complex) from Borneo. *Zoosystematics and Evolution* 96 (1): 103–113. https://doi.org/10.3897/zse.96.49989

Goujon M., Mcwilliam H., Li W., Valentin F., Squizzato S., Paern J. & Lopez R. 2010. A new bioinformatics analysis tools framework at EMBL–EBI. *Nucleic Acids Research* 38 (Suppl. 2): 695–699. https://doi.org/10.1093/nar/gkq313

Guidetti R. & Bertolani R. 2005. Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification. *Zootaxa* 845 (1): 1–46. https://doi.org/10.11646/zootaxa.845.1.1

Guidetti R., Schill R.O., Bertolani R., Dandekar T. & Wolf M. 2009. New molecular data for tardigrade phylogeny, with the erection of *Paramacrobiotus* gen. nov. *Journal of Zoological Systematics and Evolutionary Research* 47 (4): 315–321. https://doi.org/10.1111/j.1439-0469.2009.00526.x

Guil N. 2008. New records and within-species variability of Iberian tardigrades (Tardigrada), with comments on the species from the *Echiniscus blumi-canadensis* series. *Zootaxa* 1757 (1): 1–30. https://doi.org/10.11646/zootaxa.1757.1.1

Guil N. & Giribet G. 2012. A comprehensive molecular phylogeny of tardigrades — adding genes and taxa to a poorly resolved phylum-level phylogeny. *Cladistics* 28 (1): 21–49. https://doi.org/10.1111/j.1096-0031.2011.00364.x

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* 41: 95–98. Available from http://brownlab.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf [accessed 4 May 2022].

Jørgensen A., Møbjerg N. & Kristensen R.M. 2007. A molecular study of the tardigrade *Echiniscus testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. *Journal of Limnology* 66 (1s): 77–83. https://doi.org/10.4081/jlimnol.2007.s1.77

Jørgensen A., Faurby S., Hansen J.G., Møbjerg N., Mobjerg N. & Kristensen R.M. 2010. Molecular phylogeny of Arthrotardigrada (Tardigrada). *Molecular Phylogenetics and Evolution* 54 (3): 1006–1015. https://doi.org/10.1016/j.ympev.2009.10.006

Jørgensen A., Møbjerg N. & Kristensen R.M. 2011. Phylogeny and evolution of the Echiniscidae (Echiniscoidea, Tardigrada) – an investigation of the congruence between molecules and morphology. *Journal of Zoological Systematics and Evolutionary Research* 49 (1): 6–16. https://doi.org/10.1111/j.1439-0469.2010.00592.x

Kaczmarek Ł. & Michalczyk Ł. 2010. The genus *Echiniscus* Schultze 1840 (Tardigrada) in Costa Rican (Central America) rain forests with descriptions of two new species. *Tropical Zoology* 23 (1): 91–106.

Kaczmarek Ł., Michalczyk Ł. & McInnes S.J. 2016. Annotated zoogeography of non-marine Tardigrada. Part III: North America and Greenland. *Zootaxa* 4203 (1): 1–249. https://doi.org/10.11646/zootaxa.4203.1.1

Kaczmarek Ł., Grobys D., Kulpa A., Bartylak T., Kmita H., Kepel M., Kepel A. & Roszkowska M. 2019. Two new species of the genus *Milnesium* Doyère, 1840 (Tardigrada, Apochela, Milnesiidae) from Madagascar. *ZooKeys* 884: 1–22. https://doi.org/10.3897/zookeys.884.29469

Kayastha P., Berdi D., Mioduchowska M., Gawlak M., Łukasiewicz A., Gołdyn B., Jędrzejewski S. & Kaczmarek Ł. 2020. Description and molecular characterization of *Richtersius ziemowiti* sp. nov. (Richtersiidae) from Nepal (Asia) with evidence of heterozygous point mutation events in the 28S rRNA. *Annales Zoologici* 70 (3): 381–396. https://doi.org/10.3161/00034541ANZ2020.70.3.010

Kiosya Y., Vončina K. & Gąsiorek P. 2021. Echiniscidae in the Mascarenes: the wonders of Mauritius. *Evolutionary Systematics* 5 (1): 93–120. https://doi.org/10.3897/evolsyst.5.59997

Kristensen R.M. 1987. Generic revision of the Echiniscidae (Heterotardigrada), with a discussion of the origin of the family. *In:* Bertolani, R. (ed.) *Biology of Tardigrada. Selected Symposia and Monographs*: 261–335. U.Z.I. Mucci, Modena.

Kumar S., Stecher G., Li M., Knyaz C. & Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35 (6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Librado P. & Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25 (11): 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

McInnes S.J. 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. *Journal of Natural History* 28 (2): 257–352. https://doi.org/10.1080/00222939400770131

McInnes S.J., Michalczyk Ł. & Kaczmarek Ł. 2017. Annotated zoogeography of non-marine Tardigrada. Part IV: Africa. *Zootaxa* 4284 (1): 1–74. https://doi.org/10.11646/zootaxa.4284.1.1

Meyer H.A. 2013. Terrestrial and freshwater Tardigrada of the Americas. *Zootaxa* 3747 (1): 1–71. https://doi.org/10.11646/zootaxa.3747.1.1

Michalczyk Ł. & Kaczmarek Ł. 2013. The Tardigrada Register: a comprehensive online data repository for tardigrade taxonomy. *Journal of Limnology* 72 (S1): 175–181. https://doi.org/10.4081/jlimnol.2013.s1.e22

Mironov S.V., Dabert J. & Dabert M. 2012. A new feather mite species of the genus *Proctophyllodes* Robin, 1877 (Astigmata: Proctophyllodidae) from the Long-tailed Tit *Aegithalos caudatus* (Passeriformes: Aegithalidae) — morphological description with DNA barcode data. *Zootaxa* 3253 (1): 54–61. https://doi.org/10.11646/zootaxa.3253.1.2

Murray J. 1907. Scottish Tardigrada collected by the Lake Survey. *Transactions of the Royal Society of Edinburgh* 45 (3): 641–668. https://doi.org/10.1017/S0080456800011777

Nelson D.R., Guidetti R., Rebecchi L., Kaczmarek Ł. & McInnes S.J. 2020. Phylum Tardigrada. *In:* Damborenea C., Rogers D.C. & Thorp T. (eds) *Thorp and Covich's Freshwater Invertebrates 4th Ed., Vol. 5. Keys to Neotropical and Antarctic Fauna* 5: 505–522. https://doi.org/10.1016/B978-0-12-804225-0.00015-0

Ramazzotti G. & Maucci W. 1983. Il Phylum Tardigrada. III edizione riveduta e aggiornata. *Memorie dell'Istituto Italiano di Idrobiologia, Pallanza* 41: 1–1012.

Rice P., Longden I. & Bleasby A. 2000. EMBOSS: the European Molecular Biology Open Software Suite. *Trends in Genetics* 16: 276–277. https://doi.org/10.1016/S0168-9525(00)02024-2

Richters F. 1902. Neue Moosbewohner. *Bericht der Senckenbergischen Naturforschenden Gesellschaft in Frankfurt am Main* 1902: 23–26. Available from https://www.biodiversitylibrary.org/page/8478822 [accessed 4 May 2022].

Roszkowska M., Stec D., Gawlak M. & Kaczmarek Ł. 2018. An integrative description of a new tardigrade species *Mesobiotus romani* sp. nov. (Macrobiotidae: *harmsworthi* group) from the Ecuadorian Pacific coast. *Zootaxa* 4450 (5): 550–564. https://doi.org/10.11646/zootaxa.4450.5.2

Sands C.J., McInnes S.J., Marley N.J., Goodall-Copestake W.P., Convey P. & Linse K. 2008. Phylum Tardigrada: an "individual" approach. *Cladistics* 24 (6): 861–871. https://doi.org/10.1111/j.1096-0031.2008.00219.x

Schill R.O. & Steinbrück G. 2007. Identification and differentiation of Heterotardigrada and Eutardigrada species by riboprinting. *Journal of Zoological Systematics and Evolutionary Research* 45 (3): 184–190. https://doi.org/10.1111/j.1439-0469.2007.00409.x

Thompson J.D., Higgins D.G. & Gibson T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22 (22): 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Vecchi M., Newton I.L., Cesari M., Rebecchi L. & Guidetti R. 2018. The microbial community of tardigrades: environmental influence and species specificity of microbiome structure and composition. *Microbial Ecology* 76 (2): 467–481. https://doi.org/10.1007/s00248-017-1134-4

Vicente F. & Bertolani R. 2013. Considerations on the taxonomy of the Phylum Tardigrada. *Zootaxa* 3626 (2): 245–248. https://doi.org/10.11646/zootaxa.3626.2.2

Vicente F., Cesari M., Serrano A. & Bertolani R. 2013. The impact of fire on terrestrial tardigrade biodiversity: a first case-study from Portugal. *Journal of Limnology* 72 (S1): 152–159. https://doi.org/10.4081/jlimnol.2013.s1.e19

White T.J., Bruns T., Lee S. & Taylor J. 1990. 38 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis M.A., Gelfand D.H., Sninsky J.J. & White T.J. (eds) *PCR Protocols: A Guide to Methods and Applications*: 315–322. Academic Press, San Diego, CA. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

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Supplementary material

Supp. file 1. Estimates of evolutionary divergence between COI, 18S rRNA, 28S rRNA and ITS-2 sequences based on p-distances. https://doi.org/10.5852/ejt.2022.823.1819.7033

Supp. file 2. Raw measurements (in µm) and *sp* values of selected morphological structures of the specimens from neotype population of *Echiniscus q. quadrispinosus* Richters, 1902 mounted in Hoyer's medium. https://doi.org/10.5852/ejt.2022.823.1819.7035