

## New *Bryodelphax* species (Heterotardigrada: Echiniscidae) from Western Borneo (Sarawak), with new molecular data for the genus

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**Abstract.** Bornean fauna belongs to the most speciose on the world, however many animal groups remain largely or completely unstudied in this region. Microscopic water bears (Tardigrada) are a good example of such group. Members of the genus *Bryodelphax* Thulin, 1928 are the smallest land heterotardigrades. The new species found in a moss sample, *Bryodelphax arenosus* sp. nov., is among the tiniest representatives of this genus, with mature females not exceeding 100 µm in body length. It differs from other congeners by having contrasting ornamentation of some elements of the dorsal armature formed by densely arranged intra-cuticular pillars, deep faceting of the scapular plate, and minute claws. Additional 18S and 28S rRNA sequences for the new taxon and recently described *B. instabilis* Gąsiorek & Degma, 2018 unequivocally support earlier heterotardigrade phylogeny reconstructions, with *Bryodelphax* being not included in the *Echiniscus*-line [(*Echiniscus* + *Diploechiniscus*), *Testechiniscus*], *Hypochiniscus*]. The phyletic position of *Bryodelphax* is discussed in the light of present data.

**Key words.** biodiversity, *Bryodelphax arenosus* sp. nov., morphology, phylogeny, Sunda Archipelago, tropics

### INTRODUCTION

The Bornean fauna is among the richest animal communities on Earth (Myers et al., 2000). Borneo is a part of Sundaland region with strong zoogeographic affinities to continental South-East Asia (Mayr, 1944; MacKinnon et al., 1997), in contrast to Wallacean Sulawesi and Lesser Sunda Islands, which extend towards the east of Wallace's line (Stelbrink et al., 2012; Fig. 1) and are characterised by more pronounced levels of endemism in many groups of organisms (Glaubrecht & von Rintelen, 2003; Musser et al., 2010; von Rintelen et al., 2014). Nevertheless, the actual state of knowledge about such basic data as  $\alpha$ -diversity of a plethora of invertebrate groups inhabiting Borneo is far from being adequate, thus there is a certain risk of underestimation overall species diversity found in natural ecosystems (Robinson & Tuck, 1996). One of such groups are the tardigrades, microscopic ecdysozoans living literally everywhere (Nielsen, 2001), but reported from this gargantuan island only once (Pilato et al., 2004). Marine heterotardigrades exhibit a wide range of morphotypes (Fontoura et al., 2017) in comparison to less variable and mostly limno-terrestrial eutardigrades (Nelson et al., 2015). However, one heterotardigrade lineage, namely the bryo- and lichenophilous family Echiniscidae, is similarly morphologically homogenous since the dorsum is always covered by sclerotized plates in this group (Kristensen, 1987). Echiniscid genera *Bryodelphax* Thulin, 1928 and

*Bryochoerus* Marcus, 1936 are characterised by entangled history of systematic confusion due to the doubtful distinction between these taxa (Kristensen, 1987; Lisi et al., 2017; Xue et al., 2017). Two morphological groups are distinguished within the genus *Bryodelphax*: the *parvulus* group, whose members do not possess ventral armature (nine species), and the *weglarskae* group, with ventral plates (twelve species) (Degma et al., 2009–2017; Kristensen et al., 2010; Kaczmarek et al., 2012). The aim of this paper was to describe a new member of the echiniscid genus *Bryodelphax* found in Malaysian Western Borneo (Sarawak), provide novel molecular sequences for this genus, and briefly discuss its phylogenetic position in relation to the recent hypotheses (Jørgensen et al., 2011).

### MATERIAL & METHODS

**Sample processing and microscopy.** About 40 specimens of a new species were extracted from one moss sample, collected from tree in lowland rainforest in Bako Peninsula (see Fig. 1 for precise location) in August 2017. Dry material was placed and maintained in distilled water for 10 hours. After soaking and agitating, when all particles had settled onto the cylinder bottom, the top layer of water was discarded and the remaining ca. 20 ml was stirred and poured onto Petri dishes and scanned under a stereomicroscope in search of tardigrades. In order to obtain the highest possible number of specimens, the sample was soaked till even a single individual was extracted from remaining material. Specimens for light microscopy and morphometry were mounted on microscope slides in a small drop of Hoyer's medium following the protocol in Morek et al. (2016), and examined under a Nikon Eclipse 50i phase contrast microscope (PCM) associated with a Nikon Digital Sight DS-L2 digital camera or under

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Fig. 1. Map of the Malay Archipelago with the Wallace's line marked with red colour (signifies the western border of Wallacea). Asterisk = sampling locality (Bako Peninsula).

an Olympus BX51 PCM joined with a digital camera CCD ColorView III FW (operated with AnalySIS Five software). All figures were assembled in Corel Photo-Paint X6, ver. 16.4.1.1281 or in Adobe Photoshop CS3 Extended V.10.0 software. For deep structures that could not be fully focused in a single photograph, a series of 1–3 images were taken every ca. 0.5  $\mu\text{m}$  and then assembled into a single deep-focus image. Approximately 20 individuals were lost during the preparation for scanning electron microscopy.

Additionally, paratypes of *Bryodelphax asiaticus* Kaczmarek & Michalczyk, 2004 and representatives of *Bryodelphax parvulus* Thulin, 1928 from Öland, Sweden, were analysed under PCM. Unpublished photographs of *Bryodelphax aaseae* Kristensen et al., 2010 were also studied.

**Morphometrics and terminology.** All measurements are given in micrometres ( $\mu\text{m}$ ) and were performed under PCM with the Nikon Digital Sight DS-L2 software. Structures were measured only if their orientations were suitable. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. The density of pseudopores/pores was based on the measurements of the anterocentral portion of the scapular plate and the central portion of the caudal plate, using  $8.4 \times 8.4 \mu\text{m}$  grid, and subsequently calculated for  $100 \mu\text{m}^2$ . The *sc* ratio is the ratio of the length of a given structure to the length of the scapular plate (Fontoura & Morais, 2011; values italicised in the tables). Morphometric data were handled using the Echiniscoidea ver. 1.2 template available from the Tardigrada Register, [www.tardigrada.net/register](http://www.tardigrada.net/register) (Michalczyk & Kaczmarek, 2013). General taxonomy and morphological terminology follow Kristensen (1987) and Jørgensen et al. (2011).

**Molecular data.** DNA was extracted from six individuals of the new species and four individuals of *B. instabilis* Gašiorek & Degma, 2018 collected together with type

specimens of this species (all individuals were examined under  $40\times$  magnification, PCM prior to DNA extraction) following a Chelex® 100 resin (Bio-Rad) extraction method by Casquet et al. (2012) with modifications described in detail in Stec et al. (2015). Two conservative DNA fragments were sequenced: the small ribosome subunit 18S rRNA (primers 18S\_Tar\_Ff1 and 18S\_Tar\_Rf1 from Gašiorek et al., 2017b, PCR programme from Zeller, 2010) and the large ribosome subunit 28S rRNA (primers 28SF0001 and 28SR0990, and the PCR programme from Mironov et al., 2012). All fragments were amplified and sequenced according to the protocols described in Stec et al. (2015). Available 18S rRNA and 28S rRNA sequences were uploaded from GenBank to proceed in phylogenetic analyses (details in Table 1). Sequences were aligned using default settings of MAFFT version 7 (Katoh et al., 2002; Katoh & Toh, 2008). Alignments were processed by Gblocks 0.91b to eliminate uninformative gene fragments (Castresana, 2000; Dereeper et al., 2008). The obtained alignments were edited and checked manually in BioEdit v7.2.6.1 (Hall, 1999), and later concatenated using SequenceMatrix (Vaidya et al., 2010). Based on PartitionFinder version 2.1.1 (Lanfear et al., 2016) under the Bayesian Information Criterion (BIC) and applied greedy algorithm (Zhang et al., 2000), the best scheme of partitioning and substitution models were chosen for posterior phylogenetic analysis. The analysis was run to test all possible models implemented in the program. As the best-fit partitioning scheme, PartitionFinder suggested retaining the two predefined partitions separately. The best-fit model for both partitions was GTR+I+G. Bayesian inference (BI) marginal posterior probabilities were calculated using MrBayes v3.2 (Ronquist & Huelsenbeck, 2003). Random starting trees were used and the analysis was run for ten million generations, sampling the Markov chain every 1000 generations. An average standard deviation of split frequencies of 0.01 was used as a guide to ensure the two independent analyses had converged. The program Tracer

Table 1. List of the taxa and sequences used in the present study. New sequences are marked in bold.

Species	18S rRNA	28S rRNA	Reference
<i>Acanthechiniscus islandicus</i> (Richters, 1904)	AY582119	HM193400	Jørgensen & Kristensen (2004), Jørgensen et al. (2011)
<i>Antechiniscus lateromamillatus</i> (Ramazzotti, 1964)	HM193370	HM193386	Jørgensen et al. (2011)
<i>Bryodelphax arenosus</i> sp. nov.	MH414953	MH414964	<b>present study</b>
<i>B. instabilis</i> Gąsiorek & Degma, 2018	MH414963	MH414965	<b>present study</b>
<i>B. maculatus</i> Gąsiorek et al., 2017	KY609137	KY609138	Gąsiorek et al. (2017a)
<i>B. parvulus</i> Thulin, 1928	HM193371	HM193387	Jørgensen et al. (2011)
<i>Cornechiniscus lobatus</i> (Ramazzotti, 1943)	EU038079	HM193388	Guidetti et al. (2009), Jørgensen et al. (2011)
<i>Diploechiniscus oihonnae</i> (Richters, 1903)	JX114910, JX676186	JX114869	Guil et al. (2013), Vicente et al. (2013)
<i>Echiniscus bigranulatus</i> Richters, 1907	HM193373	JX114855	Jørgensen et al. (2011), Guil et al. (2013)
<i>E. blumi</i> Richters, 1903	HM193374	JX114851	Jørgensen et al. (2011), Guil et al. (2013)
<i>E. canadensis</i> Murray, 1910	FJ435714	FJ435786	Guil & Giribet (2012)
<i>E. granulatus</i> (Doyère, 1840)	DQ839606	–	Guidetti et al. (2009)
<i>E. merokensis</i> Richters, 1904	–	JX114864, JX114866	Guil et al. (2013)
<i>E. spiniger</i> Richters, 1904	HM193376	JX114859	Jørgensen et al. (2011), Guil & Giribet (2012)
<i>E. testudo</i> (Doyère, 1840)	MG016454	MG016455	Gąsiorek et al. (2017b)
<i>E. trisetosus</i> Cuénot, 1932	FJ435716	JX114852	Guil & Giribet (2012), Guil et al. (2013)
<i>E. viridissimus</i> Péterfi, 1956	AF056024	–	Jørgensen et al. (2011)
<i>E. wendti</i> Richters, 1903	–	JX114867	Guil et al. (2013)
<i>Hypechiniscus exarmatus</i> (Murray, 1907)	HM193377	HM193394	Jørgensen et al. (2011)
<i>H. gladiator</i> (Murray, 1905)	HM193378	HM193395	Jørgensen et al. (2011)
<i>Mopsechiniscus granulatus</i> Mihelčič, 1967	HM193379	HM193396	Jørgensen et al. (2011)
<i>Parechiniscus chitonides</i> Cuénot, 1926	HM193380	HM193397	Jørgensen et al. (2011)
<i>Proechiniscus hannaee</i> (Petersen, 1951)	HM193381	HM193398	Jørgensen et al. (2011)
<i>Pseudechiniscus facettalis</i> (Petersen, 1951)	–	FJ435788	Guil & Giribet (2012)
<i>P. novaezeelandiae</i> (Richters, 1908)	HM193380	HM193401	Jørgensen et al. (2011)
<i>P. suillus</i> (Ehrenberg, 1853)	–	JX114875	Guil et al. (2013)
<i>Testechiniscus spitsbergensis</i> (Scourfield, 1897)	EU266967	JX114870	Sands et al. (2008), Guil et al. (2013)
<i>Echiniscoides sigismundi</i> (M. Schultze, 1865)	GQ849021	JX114887	Jørgensen et al. (2011), Guil et al. (2013)

v1.3 (Rambaut et al., 2014) was then used to ensure Markov chains had reached stationarity and to determine the correct ‘burn-in’ for the analysis, which was the first 10% of generations. The ESS values were 200. A consensus tree was obtained after summarising the resulting topologies and discarding the ‘burn-in’. For the BI consensus tree, clades recovered with posterior probability (PP) between 0.95 and 1 were considered well supported, those with PP between 0.90 and 0.94 were considered moderately supported, and those with lower PP were considered unsupported. The final consensus tree was viewed and visualised by FigTree v.1.4.3 available from <http://tree.bio.ed.ac.uk/software/figtree>. Uncorrected pairwise distances for trimmed *Bryodelphax* spp. alignments (1028 bp – 18S rRNA, 570 bp – 28S rRNA) were calculated using MEGA7 (Kumar et al., 2016) and are presented in Table 3.

### TAXONOMY

**Phylum Tardigrada Doyère, 1840**  
**Class Heterotardigrada Marcus, 1927**  
**Order Echiniscoidea Richters, 1926**  
**Family Echiniscidae Thulin, 1928**  
**Genus *Bryodelphax* Thulin, 1928**

***Bryodelphax arenosus* new species**  
 (Figs. 2–4, Tables 2, 3)

**Material examined.** All individuals were mature females. Holotype (slide no. MY.813.01) and 14 paratypes (slides MY.813.01–11), six additional processed for DNA extraction and sequencing. Type locality: Bako Peninsula, lowland coastal rainforest close to the Tajor Waterfall (1°43'24"N, 110°27'50"E; 106 m asl), moss from a tree, coll. Piotr Gašiorek & Artur Oczkowski, 5<sup>th</sup> August 2017. Holotype and nine paratypes deposited in the Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland (slides MY.813.01–06), three paratypes deposited in the Natural History Museum of Denmark, Copenhagen (slides MY.813.07–09), and last two paratypes deposited in the Raffles Museum of Biodiversity Research, National University of Singapore (slides MY.813.10–11).

**Description of the new species.** Adults (i.e., from the third instar onwards, with developed gonopore; measurements in Table 2).

Body yellowish, pearly opalescent; eyes absent or not visible after preparation. Both primary and secondary clavae oval and small (Figs. 2, 3C, 4A). *Cirri interni* and *externi* with poorly developed cirrophores. Cirri *A* long (on average reaching  $\frac{1}{3}$  of the body length, whereas *Bryodelphax* typically has cirrus *A*/body length ratio around  $\frac{1}{4}$ ; Figs. 2, 3A). Dorsal plates with faint intra-cuticular pillars visible only under 100× magnification with immersion oil (Figs. 3C, 4), better visible on the scapular and caudal plates (Figs. 3C, 4A, C), pillars on the posterior portion of the caudal plate are also the largest (Figs. 3C, 4C). Distinct dark ridges are present on lateral portions of the scapular, paired, and caudal plates, forming characteristic and stable ornamented pattern (Figs. 3B, C,

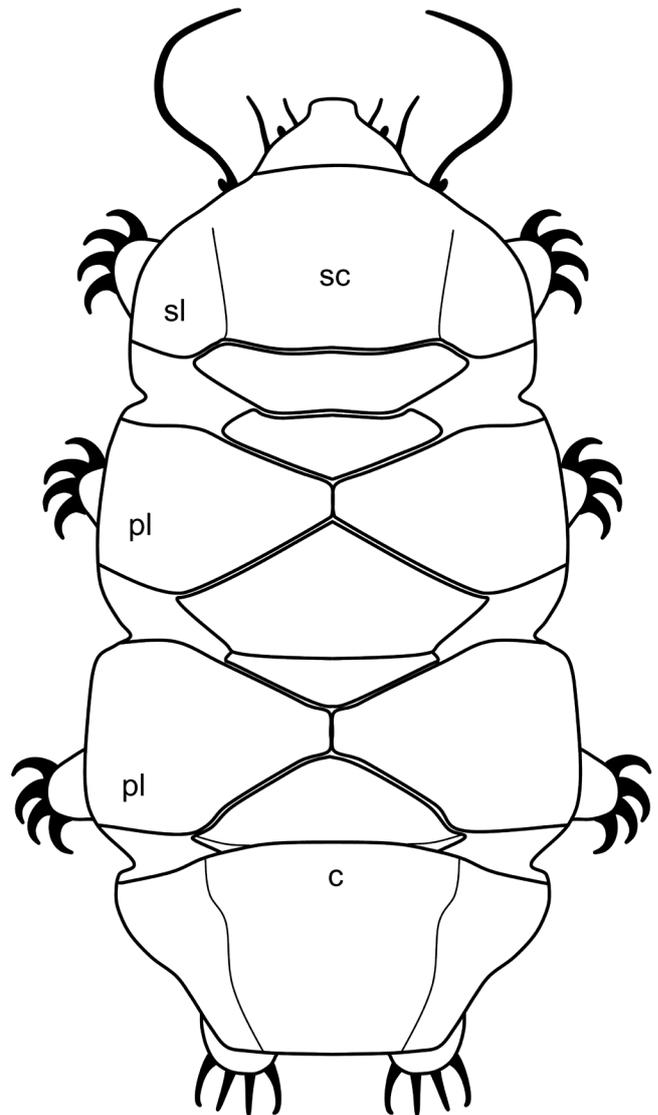


Fig. 2. Schematic arrangement of plates on dorsum of *Bryodelphax arenosus* sp. nov. Abbreviations: c = the caudal plate; pl = lateral portion of the paired plate; sc = central portion of the scapular plate; sl = lateral portion of the scapular plate.

4). Pseudopores or pores (SEM observations are needed to verify the morphology of the cuticular depressions) minute and barely visible (visible in PCM as bright dots with blurred margins; Figs. 3C, 4), sometimes absent. Pseudopores/pores distributed unevenly, i.e., on the anterocentral portion of the scapular plate 8–21 pseudopores/pores per 100  $\mu\text{m}^2$  were observed ( $\bar{x}$ =17, N=10; Fig. 4A), on the central portion of the caudal plate: 11–18 pseudopores/pores per 100  $\mu\text{m}^2$  ( $\bar{x}$ =15, N=10; Fig. 4C), whereas sparse and bleak pseudopores/pores occur rarely on other, especially paired, plates. Scapular plate deeply faceted by dark ridges, with six or eight lighter rectangular areas in the central portion (Figs. 3A, C, 4A). Scapular sutures clearly separate lateral portions from the central part, extending from the base of cirrophore *A* to the posterior margin of the plate (Figs. 3B, C, 4A). Paired plates divided into two roughly equal anterior and posterior parts by a transverse stripe (Figs. 3, 4B). Caudal plate mildly faceted in the central part by the same dark ridges as the scapular plate (Fig. 3A), but the faceting is fainter and often poorly visible (Figs. 3C, 4C). Median plates 1

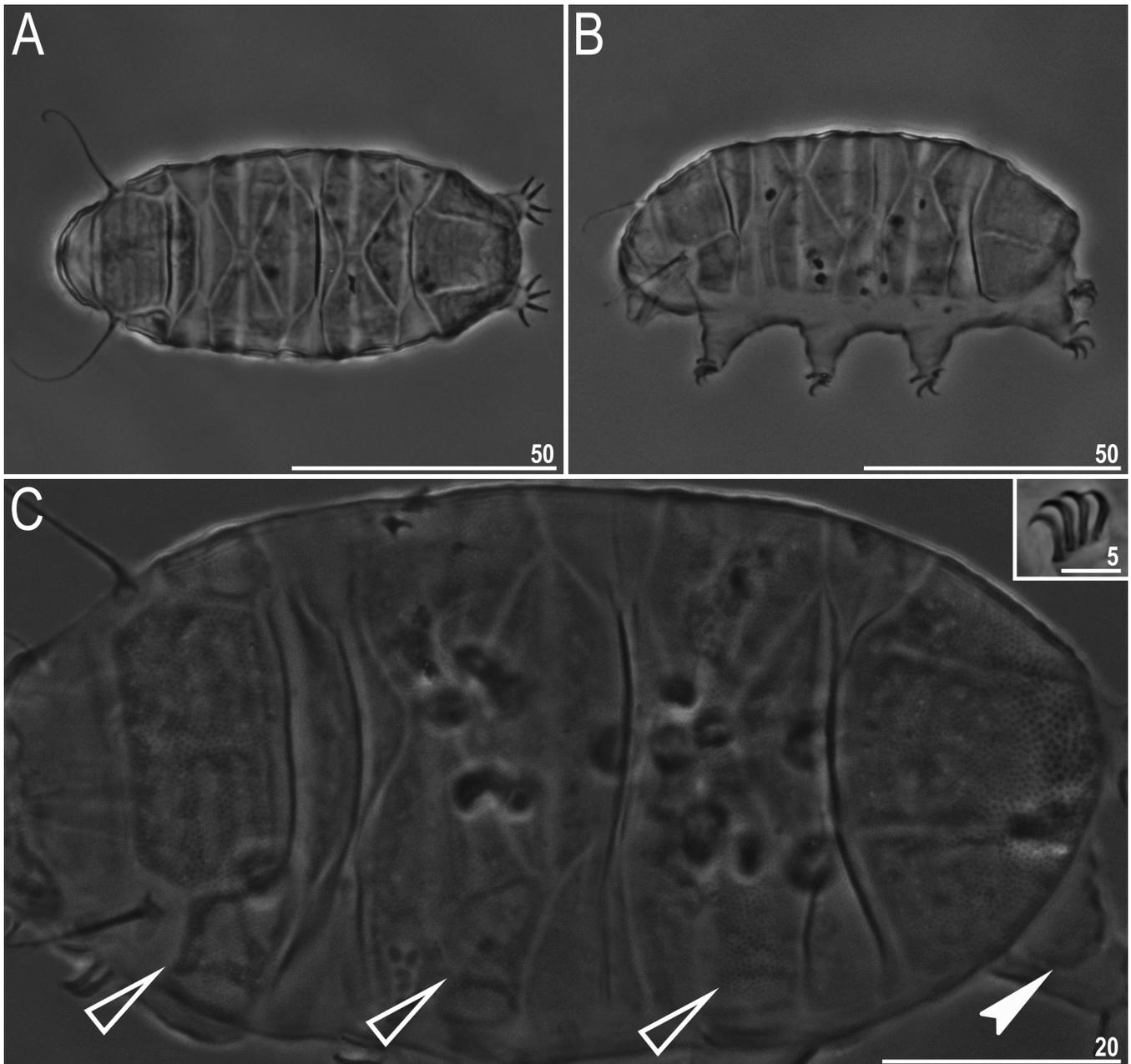


Fig. 3. *Bryodelphax arenosus* sp. nov., habitus (PCM). A, dorsal view (paratype); B, dorsolateral view (paratype); C, holotype dorsal armature in close-up (claws III inserted in the upper right corner). Empty arrowheads indicate ornamented portions of dorsal plates, whereas white arrowhead points pedal plate IV devoid of dentate collar. Scale bars in  $\mu\text{m}$ .

and 2 divided transversally into two unequal parts (Figs. 2, 3), and additionally the anterior portion of median 2 has a transverse suture (Fig. 3). Median plate 3 large, triangular in shape, and with a roundish posterior edge (Figs. 2, 3). Thin, non-sculptured supplementary lateral platelets present at the levels of median plates (three pairs of platelets on each body side: a pair between scapular plate and first pair of the segmental plates, a pair between paired plates, and a pair between second pair of segmental plates and caudal plate; Fig. 3B, C). Ventral cuticle smooth, without plates or intra-cuticular pillars. Papillae on legs I and IV absent or not detectable in PCM. Large, sclerotised pedal plates IV present, with irregular margin, but never forming a dentate collar (Fig. 3C, white arrowhead). External claws of all legs smooth, internal claws with minuscule bumps near their

bases, which can be spurs (Fig. 3C, insert). However, they are not measurable in PCM since barely divergent from the claw branch.

No juveniles, larvae (i.e., the second and first instars, respectively) or eggs were found.

**Remarks.** All found specimens were mature females, thus it is likely that the new species is parthenogenetic.

**DNA sequences.** New 18S and 28S rRNA sequences were obtained for two specimens; each fragment was represented by a single haplotype and is deposited in GenBank: MH414953 (18S rRNA, 1112 bp long), XX000000 (28S rRNA, 688 bp long). p-distances between sequenced species

Table 2. Measurements [in  $\mu\text{m}$ ] and *sc* indices [in % of the scapular plate length] of selected morphological structures of type mature females of *Bryodelphax arenosus* 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).

Character	N	Range				Mean		SD		Holotype			
		$\mu\text{m}$		$\mu\text{m}$	sc	$\mu\text{m}$	sc	$\mu\text{m}$	sc	$\mu\text{m}$	sc		
Body length	15	76	–	95	543	–	648	84	596	7	39	94	648
Scapular plate length	14	12.5	–	15.5		–		14.2	–	0.8	–	14.5	–
Head appendages lengths													
Cirrus internus	13	3.2	–	4.7	21.3	–	33.1	3.9	27.6	0.5	4.0	3.4	23.4
Cephalic papilla	10	2.0	–	3.1	14.8	–	24.8	2.6	18.3	0.3	2.8	2.7	18.6
Cirrus externus	13	5.4	–	8.1	34.8	–	60.4	6.5	46.0	0.9	7.3	7.5	51.7
Clava	13	1.6	–	2.6	11.9	–	18.4	2.2	15.8	0.3	2.1	2.6	17.9
Cirrus A	12	20.9	–	30.8	154.8	–	212.4	26.8	189.1	2.8	17.4	30.8	212.4
Cirrus A/body length ratio	12	27%	–	36%		–		32%	–	2%	–	33%	–
Claw branch lengths													
Claw 1	15	3.7	–	5.5	27.4	–	36.6	4.4	31.3	0.5	3.2	4.4	30.3
Claw 2	15	3.3	–	4.8	25.7	–	31.5	4.1	28.4	0.4	2.0	4.5	31.0
Claw 3	15	3.4	–	5.0	25.0	–	35.2	4.2	29.2	0.4	2.7	4.5	31.0
Claw 4	15	3.7	–	5.3	26.7	–	37.1	4.4	31.4	0.5	2.9	4.9	33.8

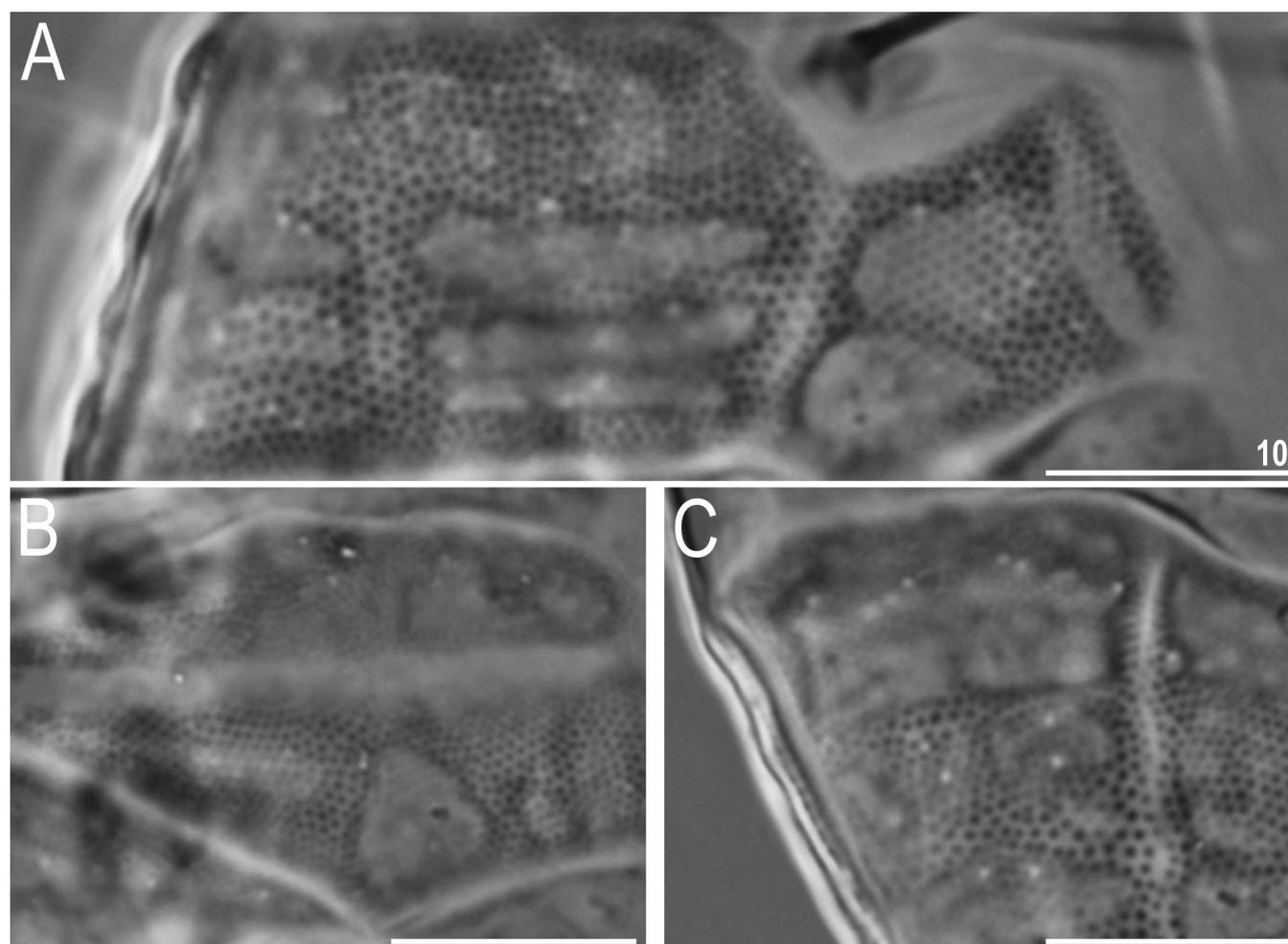


Fig. 4. Close-up on various parts of dorsal armature of *Bryodelphax arenosus* sp. nov. A, faceting of *sc* and ornamented pattern of *sl*; B, ornamented pattern of *pl*; C, faceting of *c*. Abbreviations are the same as for Figure 1. Scale bars in  $\mu\text{m}$ .

Table 3. Uncorrected pairwise distance (in %) for available *Bryodelphax* species.

Species	18S rRNA			28S rRNA		
	(1)	(2)	(3)	(2)	(3)	
(1) <i>Bryodelphax parvulus</i>						
(2) <i>Bryodelphax maculatus</i>	0.008					
(3) <i>Bryodelphax arenosus</i>	0.028	0.021		0.193		
(4) <i>Bryodelphax instabilis</i>	0.011	0.005	0.025	0.075	0.155	

of *Bryodelphax* ranged from 0.8% to 2.8% (18S rRNA), and from 7.5% to 19.3% (28S rRNA) (Table 3).

**Etymology.** From Latin (*h*)*arenosus* = sandy, inhabiting sand. The new species was found in coastal lowland rainforest with peculiarly sandy soil (arenosols), atypical for this biome.

## RESULTS AND DISCUSSION

**Differential diagnosis.** *Bryodelphax arenosus* sp. nov., by having no ventral plates, belongs to the *parvulus* group (Kristensen et al., 2010). The faceting of the scapular and caudal plates, together with specific ornamented pattern on lateral portions of plates, distinguishes the new species well from its known congeners. *Bryodelphax crossotus* Grigarick, Schuster & Nelson, 1983, described from Venezuelan mosses, also has sclerotised pedal plate IV devoid of dentate collar, but it is separated from *Bryodelphax arenosus* sp. nov. by a different body colour (yellowish with pearly opalescence in the new species vs. colourless in *B. crossotus*), the arrangement of pseudopores/pores on dorsal plates (faint, minute and scarcely visible almost exclusively on the scapular and caudal plates in the new species vs. evident and distributed on all plates in *B. crossotus*), *cirrus externus* length (5.4–8.1  $\mu\text{m}$  in the new species vs.  $\sim 10$   $\mu\text{m}$  in *B. crossotus*), papilla on legs IV visible under PCM (not visible under PCM, possibly absent, in the new species vs. clearly visible under PCM and large [4.8  $\mu\text{m}$ ] in *B. crossotus*), and by shorter claws (3.3–5.5  $\mu\text{m}$  in the new species vs.  $\sim 8$   $\mu\text{m}$  in *B. crossotus*).

The second species, *Bryodelphax ortholineatus* Bartoš, 1963, is chosen for the comparison because of the proximity of the type locality (reported from Java and Andaman Islands) and an insufficient description. Although relatively old, the original description provides some morphological details needed for the delineation of the new species: a different body colour (yellowish with pearly opalescence in the new species vs. colourless, transparent body in *B. ortholineatus*), the shape and size of the anterior portion of median 1 with regard to its posterior part (trapezoidal and of similar length in the new species vs. triangular and much smaller than the posterior part in *B. ortholineatus*), and by the morphology of caudal sutures (straight and reaching the anterior margin of the plate in the new species vs. sinusoidal and reaching  $\frac{2}{3}$  of the plate length in *B. ortholineatus*).

The two similar species, *Bryodelphax asiaticus* Kaczmarek & Michalczyk, 2004 from Mongolia and *Bryodelphax parvulus*

Thulin, 1928, from Lapland, have no dentate collar IV. They possess supplementary lateral platelets (Fig. 5A, C). However, both taxa have comparatively larger and numerous pores (Fig. 5). Moreover, *B. asiaticus* has longer buccal cirri (*cirrus internus* length 3.2–4.7  $\mu\text{m}$  + *cirrus externus* length 5.4–8.1  $\mu\text{m}$  in the new species vs. *cirrus internus* length 4.8–6.7  $\mu\text{m}$  + *cirrus externus* length 8.6–13.3  $\mu\text{m}$  in *B. asiaticus*) and claws (claws IV 3.7–5.3  $\mu\text{m}$  long in the new species vs. claws IV 5.7–8.6  $\mu\text{m}$  long in *B. asiaticus*). More discriminative criteria will be available when the redescription of *Bryodelphax parvulus* from Scandinavia is presented.

The validity of *cirrus A* length in the taxonomy of Echiniscidae was questioned by Fontoura & Morais (2011). Similarly, minute claw spurs in *Bryodelphax* can be easily overlooked or too small to be detected in PCM. Therefore, these two traits were not taken into consideration in the differential diagnosis.

**Phylogenetic position of *Bryodelphax*.** In agreement with recent phylogenies (Jørgensen et al., 2011; Guil et al., 2013; Vicente et al., 2013; Vecchi et al., 2016), the genus *Bryodelphax* is placed in polytomy with the *Pseudechiniscus*-like genera (provided with at least one pseudosegmental plate IV') and *Echiniscus*-line evolutionary clade, and clearly does not belong to the latter [(*Echiniscus* Schultze, 1840 + *Diploechiniscus* Vicente et al., 2013), *Testechiniscus* Kristensen, 1987), *Hypechiniscus* Thulin, 1928], which can be now re-defined as follows: echiniscids with paired (segmental) and unpaired (cephalic, cervical, median, and caudal [terminal]) dorsal plates, without pseudosegmental plates and with deep incisions (notches) on the caudal plate. The absence of pseudosegmental plates was the main reason to hypothesise the presence of the *Echiniscus*-line within Echiniscidae (Ramazzotti, 1972; Kristensen, 1987), and further research corroborated validity of this clade (Jørgensen et al., 2011) with the exclusion of *Bryodelphax* (Fig. 6). Five morphological traits support the recognition of a separate *Bryodelphax* (+ *Bryochoerus* Marcus, 1936, if the validity of the genus is demonstrated, see Kristensen, 1987) evolutionary lineage, namely: body miniaturisation, the lack of caudal incisions, significantly narrower tips of cirri *A*, the ring of ten peribuccal papulae, and the stylet supports encrusted by  $\text{CaCO}_3$ . Sexually mature individuals of *Bryodelphax* are typically below 200  $\mu\text{m}$ , but, in some species, they may not exceed 100  $\mu\text{m}$  (e.g., *B. arenosus* sp. nov., *B. dominicanus* Schuster & Toftner, 1982, *B. parvuspolaris* Kaczmarek et al., 2012). In contrast, in other echiniscid

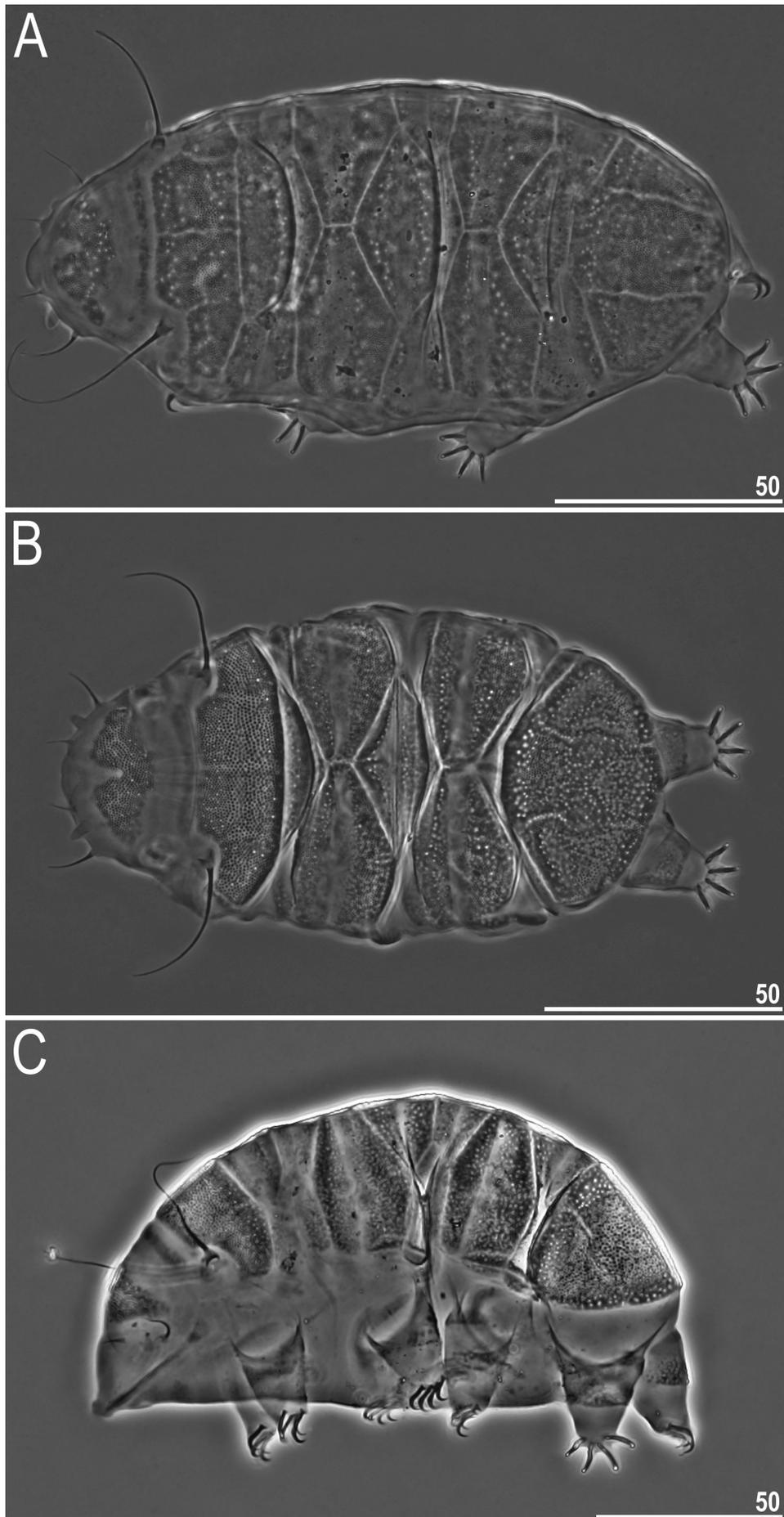


Fig. 5. Other *Bryodelphax* spp., habitus (PCM). A, *B. asiaticus* Kaczmarek & Michalczyk, 2004, dorsal view (paratype); B, *B. parvulus* Thulin, 1928, dorsal view (specimen from Öland, Sweden); C, *B. parvulus*, lateral view (specimen from Öland, Sweden). Scale bars in  $\mu\text{m}$ .

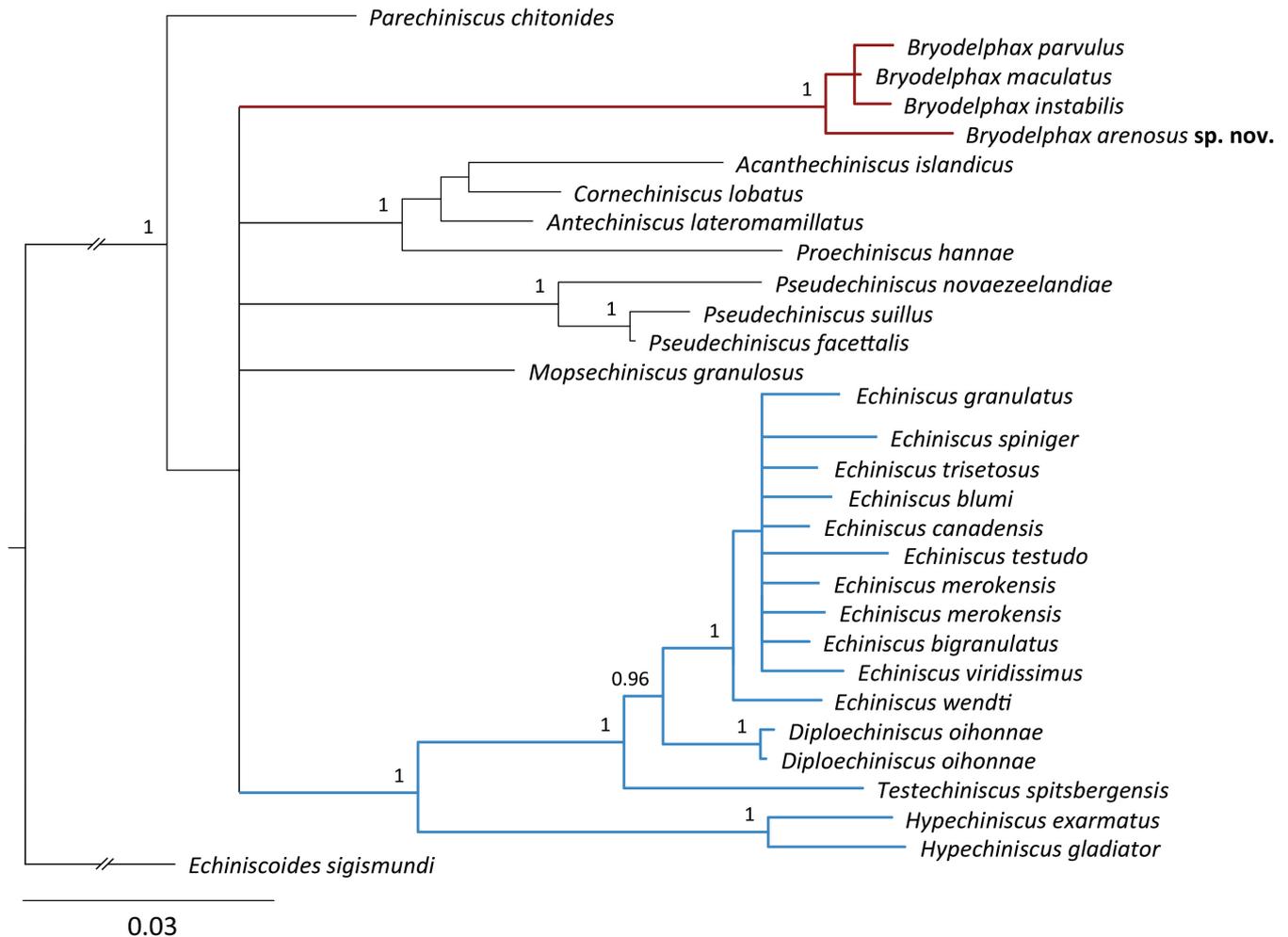


Fig. 6. The phylogenetic position of *Bryodelphax* Thulin, 1928 (red lineage) on the Bayesian Inference (BI) cladogram constructed from currently available 18S+28S rRNA sequences for Echiniscidae, with *Echiniscoides sigismundi* (M. Schultze, 1865) as the outgroup (see also Table 2). The *Echiniscus* evolutionary line is marked with blue colour. Values above branches indicate posterior probability values. Branches with support below 0.9 were collapsed.

genera, sexually mature individuals typically exceed 200  $\mu\text{m}$ . Moreover, instead of caudal incisions (notches), *Bryodelphax* species exhibit sutures (resembling in appearance sutures on the scapular plate), but it is currently impossible to assess if these structures constitute an intermediate stage between non-incised caudal plates and true incisions or should be considered autapomorphic (the earliest branches of the echiniscid tree, *Parechiniscus* Cuénot, 1926 and *Novechiniscus* Kristensen, 1987, both have caudal plates devoid of incisions and sutures, Kristensen, 1987). Thinner endings of cirri A, being absent in other genera, however, can be considered as an autapomorphy of *Bryodelphax*. This trait is visible only under SEM, and so far was shown for three species, including the type species *B. parvulus* Thulin, 1928 (Maucci, 1986; Gąsiorek et al., 2017a; Gąsiorek & Degma, 2018). However, it is also clearly present in *B. aaseae* Kristensen et al., 2010 (data not shown). Similarly, the ring of ten distinct peribuccal papulae is an autapomorphy of the genus. Finally, the calcium encrustation of the stylet supports is a retained plesiomorphy of *Bryodelphax*, being an exception within Echiniscidae, but frequent in marine arthrotardigrades (Kristensen, 1987). The final elucidation of the placement of *Bryodelphax* on the echiniscid tree will probably require additional sequences in poorly sampled

or not sampled genera to obtain more extensive genetic background (Hedtke et al., 2006).

#### ACKNOWLEDGEMENTS

Two Reviewers significantly improved this paper, and I feel indebted to them. Reinhardt M. Kristensen (University of Copenhagen, Denmark) kindly agreed to present unpublished photographs of *B. parvulus*. The study was supported by the Polish Ministry of Science and Higher Education via the Diamond Grant (DI2015 014945 to Piotr Gąsiorek, supervised by Łukasz Michalczyk, who also critically read the manuscript) and a grant from the European Commission's (FP6) Integrated Infrastructure Initiative programme SYNTHESYS (DK-TAF-6332). I owe sincere thanks to Daniel Stec for his mentorship in molecular laboratory, and to Artur Oczkowski for help in sorting out animals from the sample.

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