<u>PENSOFT</u>.



An overview of the sexual dimorphism in *Echiniscus* (Heterotardigrada, Echiniscoidea), with the description of *Echiniscus masculinus* sp. nov. (the *virginicus* complex) from Borneo

Piotr Gąsiorek¹, Katarzyna Vončina¹, Łukasz Michalczyk¹

1 Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland

http://zoobank.org/48BDE4B7-B052-4A00-AF36-BF2F5C7E7285

Corresponding author: Piotr Gąsiorek (piotr.lukas.gasiorek@gmail.com)

Academic editor: Martin Husemann • Received 8 January 2020 • Accepted 25 February 2020 • Published 20 March 2020

Abstract

Members of the genus *Echiniscus* C.A.S. Schultze, 1840 are mostly unisexual, with thelytokously reproducing females. Therefore, every newly described dioecious species in the genus is particularly interesting. Here, we describe *Echiniscus masculinus* **sp. nov.** from Gunung Kinabalu, the highest peak of Borneo and the entire Southeast Asia. The new species belongs in the predominantly parthenogenetic *E. virginicus* complex, and its females are confusingly similar to females of the pantropical *E. lineatus* Pilato et al., 2008, another member of this group. However, genetic evidence and noticeable sexual dimorphism clearly delineate the new species. Males of *E. masculinus* **sp. nov.** are unlike females in the body proportions, cuticular sculpturing, and appendage configuration. The new discoveries provide a justification to review the current knowledge about evolution and forms of sexual dimorphism within *Echiniscus*.

Key Words

bisexual, clavae, dioecious, Echiniscidae, endemic, Gunung Kinabalu, limno-terrestrial life cycle, tropics

Introduction

A swiftly increasing number of tardigrade species is currently at circa 1300 species (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Degma et al. 2009-2019), which have already approached the conservative estimate of Bartels et al. (2016). This number has also exceeded the mean estimate of circa 1150 (upper 95% CI >2100) limno-terrestrial tardigrade species based on a protocol by Mora et al. (2011). Recent works have included DNA barcoding in modern tardigrade taxonomy, disclosing numerous species complexes in various phylogenetic lineages of the phylum (e.g. Stec et al. 2018; Guidetti et al. 2019; Cesari et al. 2020). On the other hand, many tardigrade groups contain a significant number of dubious or synonymic taxa (e.g. Gasiorek et al. 2019b). Within the class Heterotardigrada, the greatest progress in solving taxonomic and phylogenetic problems has been made regarding a fascinating group of armoured limno-terrestrial tardigrades, the family Echiniscidae (Kristensen 1987; Jørgensen et al. 2011, 2018; Vicente et al. 2013; Vecchi et al. 2016; Gąsiorek et al. 2018a, 2018b, 2019b; Cesari et al. 2020). Recently, Gąsiorek et al. (2019a) demonstrated synonymy within the *Echiniscus virginicus* complex, reducing the number of valid species from five to four, and for the first time presenting an integrative evidence for a pantropical tardigrade species. Currently, only one member of this group, *E. clevelandi* (Beasley 1999), is dioecious.

Gunung Kinabalu, together with the Crocker Range located farther south, constitute the highest prominence in the northern part of Borneo. Due to the remarkable geological and climatic conditions, an altitudinal zonation of flora is present on Gunung Kinabalu (Kitayama 1992), which is a characteristic of many high mountain peaks in the Indomalayan region (van Steenis 1984; Ohsawa et al. 1985). Consequently, these mountains harbour unparalleled animal diversity associated with rich plant vegetation, even for the extraordinarily speciose faunae of the Malay Archipelago (Lohman et al. 2011; de Bruyn et al. 2014). On the other hand, as in many tropical areas, some animal groups remain barely known. This is the case with tardigrades, the subject of only two Bornean papers (Pilato et al. 2004; Gąsiorek 2018). Given the recent explosion of hidden species diversity in several tardigrade genera, it is more than likely that Bornean rainforests hold numerous undescribed tardigrade species.

In this contribution, by using morphological and phylogenetic methods, we describe *Echiniscus masculinus* sp. nov. from a high elevation in Gunung Kinabalu. The new species sheds light on the evolution of the *E. virginicus* complex and raises questions about the prevalent type of speciation (sympatric vs allopatric) in this group. Finally, the sexual dimorphism within *Echiniscus* is compared to that of other echiniscids, and the apparent morphological stasis in females of the *E. virginicus* complex is discussed.

Methods

Sample collection and specimen preparation

A total of 52 animals representing the new species was extracted from a moss sample collected in Northern Borneo by Maciej Barczyk on 29 June 2016 (sample code MY.026). The air-dried sample, stored in a paper envelope, was rehydrated in water for several hours, and the obtained sediment was poured into Petri dishes to search for microfauna under a stereomicroscope with dark field illumination. Individuals isolated from the sample were used for two types of analysis: imaging in light microscopy (morphology and morphometry; 44 specimens) and DNA sequencing + phylogenetics (eight specimens).

Imaging, morphometrics, and terminology

Individuals for light microscopy and morphometry were first air-dried on microscope slides, and then 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. All figures 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 2-12 images was taken every circa 0.1 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 not damaged and if their orientations were 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 (Dastych 1999). Morphometric data were handled using the Echiniscoidea ver. 1.3

template available from the Tardigrada Register, http:// www.tardigrada.net/register (Michalczyk and Kaczmarek 2013). The terminology follows Kristensen (1987) and subsequent changes proposed in Gąsiorek et al. (2019b). For qualitative differential diagnoses, species descriptions and amendments of the four taxa constituting the *Echiniscus virginicus* group were studied (Riggin 1962; Moon and Kim 1990; Beasley 1999; Abe et al. 2000; Pilato et al. 2008; Kaczmarek and Michalczyk 2010; Gąsiorek et al. 2019a).

Genotyping and phylogenetics

The DNA was extracted from eight individual animals following a Chelex 100 resin (Bio-Rad) extraction method by Casquet et al. (2012) with modifications described in detail in Stec et al. (2015). All specimens were mounted in water on temporary slides and examined under PCM before DNA extraction to ensure correct taxonomic identifications. One hologenophore cuticle (Pleijel et al. 2008) was retrieved from an Eppendorf tube, mounted on a permanent slide, and deposited in the Institute of Zoology and Biomedical Research in Kraków. We sequenced four nuclear and one mitochondrial DNA fragments: the small and the large ribosome subunit 18S rRNA and 28S rRNA (918 bp and 728 bp, respectively), the internal transcribed spacers ITS-1 and ITS-2 (642 and 484 bp, respectively), and the cytochrome oxidase subunit I COI (632 bp). 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 1. Sequences were aligned using default settings of MAFFT7 (Katoh et al. 2002; Katoh and Toh 2008) under G-INS-i strategy. Uncorrected pairwise distances were calculated using MEGA7 (Kumar et al. 2016) and are included as the Suppl. material 2.

To ensure that the topologies of the trees reconstructed on the basis of genetic markers were identical, we calculated Bayesian inference (BI) marginal posterior probabilities using MrBayes ver. 3.2 (Ronquist and Huelsenbeck 2003) for each of the three markers (COI, ITS-1, and ITS-2) separately. 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 that the two independent analyses had converged. The program Tracer ver. 1.3 (Rambaut et al. 2014) was then used to ensure that 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, and a consensus tree was obtained after summarizing the resulting topologies and discarding the 'burn-in'. Trees were rooted on Echiniscus succineus. Clades recovered with a posterior probability (PP) between 0.95 and 1.00 were considered well supported, those with a PP between 0.90 and 0.94 were considered moderately supported, and those with a low-

DNA fragment	Primer name	Primer direction	Primer sequence (5'–3')	Primer source	PCR programme*
18S rRNA	18S_Tar_Ff1	forward	AGGCGAAACCGCGAATGGCTC	Stec et al. (2018)	Zeller (2010)
	18S_Tar_Rr2	reverse	CTGATCGCCTTCGAACCTCTAACTTTCG	Gąsiorek et al. (2017)	
28S rRNA	28S_Eutar_F	forward	ACCCGCTGAACTTAAGCATAT	Gąsiorek et al. (2018a)	Mironov et al. (2012)
	28SR0990	reverse	CCTTGGTCCGTGTTTCAAGAC	Mironov et al. (2012)	
ITS-1	ITS1_Echi_F	forward	CCGTCGCTACTACCGATTGG	Gąsiorek et al. (2019a)	Wełnicz et al. (2011)
	ITS1_Echi_R	reverse	GTTCAGAAAACCCTGCAATTCACG		
ITS-2	ITS3	forward	GCATCGATGAAGAACGCAGC	White et al. (1990)	
	ITS4	reverse	TCCTCCGCTTATTGATATGC		
COI	bcdF01	forward	CATTTTCHACTAAYCATAARGATATTGG	Dabert et al. (2008)	
	bcdR04	reverse	TATAAACYTCDGGATGNCCAAAAAA		

Table 1. Primers and references for specific protocols for amplification of the five DNA fragments sequenced in the study.

* All PCR programmes are also provided in Stec et al. (2015).

er PP were considered unsupported. All final consensus trees were viewed and visualized using FigTree ver. 1.4.3 (available at: https://tree.bio.ed.ac.uk/software/figtree).

Data deposition

Raw morphometric data are placed as the Suppl. material 1 and in the Tardigrada Register under http://www. tardigrada.net/register/0062.htm. Type DNA sequences are deposited in GenBank.

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 masculinus sp. nov.

http://zoobank.org/99CA96E7-D111-4E07-A0A2-4133F54755C9 Figures 1–3, Tables 2–5

Description. Mature females (i.e. from the third instar onwards; measurements and statistics in Table 2). Body cylindrical, orange with minute red eyes present in live specimens; colours disappearing soon after mounting in Hoyer's medium. *Echiniscus*-type cephalic papillae (secondary clavae) and (primary) clavae; cirri growing out from bulbous cirrophores (Figure 1A). The body appendage configuration is *A*-*C*-*D*-*D*^{*d*}-*E*, with all trunk appendages always symmetrical and smooth. Spine *C*^{*d*} rudimentarily developed in two females (one with an asymmetrical spicule [2 µm], the other normally formed [8 µm]).

Dorsal plates with the mixed type of sculpturing, with an evident layer of endocuticular pillars visible as black dots under PCM, and an upper layer of greyish epicuticular matrix forming the ornamented pattern together with pseudopores, enhanced as dark belts on the anterior por-



Figure 1. Morphology of *Echiniscus masculinus* sp. nov. (PCM). **A.** Adult female (holotype, dorsolateral view); **B.** Juvenile (paratype, dorsolateral view); **C.** Subcephalic plates; **D.** Genital plates enclosing male gonopore; **E.** First leg pair with claws and spine I. All scale bars in μm.

tions of the paired segmental plates (Fig. 1A). Generally, the epicuticular sculpture is poorly developed and gives way to large pillars, especially on the cephalic and scapular plates, and also on the central portion of the median plate I and centroposterior portions of segmental plates. The cephalic plate is relatively large whereas the cervical (neck) plate is barely demarcated from the scapular plate,

Table 2. Measurements $[in \mu m]$ of selected morphological structures of the adult females of *Echiniscus masculinus* 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	sp	μm	sp	μm	sp	μm	sp
Body length	10	159–192	432-492	175	453	11	21	178	444
Scapular plate length	10	32.6-43.7	-	38.8	-	3.2	-	40.1	-
Head appendages lengths									
Cirrus internus	9	9.7-15.5	25.1–38.8	12.6	32.4	2.3	4.9	14.6	36.4
Cephalic papilla	10	5.9-7.8	15.1-19.2	6.7	17.3	0.5	1.3	6.4	16.0
Cirrus externus	8	12.3-18.8	37.7-47.0	16.6	42.7	1.9	3.7	18.8	46.9
Clava	10	4.7-6.4	11.4-17.1	5.5	14.2	0.6	1.6	5.6	14.0
Cirrus A	10	23.3-42.3	69.6-105.5	32.8	84.7	4.7	10.7	33.1	82.5
Cirrus A/Body length ratio	10	15%-24%	_	19%	-	3%	-	19%	-
Body appendages lengths									
Spine C	10	10.9-21.6	33.4-56.3	16.6	43.0	3.1	8.3	15.1	37.7
Spine D	10	11.2-21.6	29.5-57.9	16.0	41.3	3.1	8.3	13.8	34.4
Spine D ^d	10	2.9-16.8	8.9-45.0	11.7	30.1	4.1	10.9	9.7	24.2
Spine E	10	13.6-23.3	33.9-60.7	18.6	48.3	2.5	8.2	13.6	33.9
Spine on leg I length	10	3.0-3.9	8.0-11.0	3.4	8.8	0.3	0.9	3.3	8.2
Papilla on leg IV length	10	3.6-5.3	9.9-12.9	4.4	11.3	0.6	1.1	4.6	11.5
Number of teeth on the collar	9	8–12	-	10.1	_	1.3	_	9	-
Claw I heights									
Branch	8	8.8-10.7	23.5-27.6	9.7	25.3	0.6	1.7	9.7	24.2
Spur	8	2.2-3.2	6.7-8.5	2.8	7.3	0.3	0.6	2.7	6.7
Spur/branch height ratio	8	24%-33%	_	29%	-	2%	-	28%	-
Claw II heights									
Branch	9	8.4-10.4	21.5-25.9	9.4	24.4	0.6	1.4	10.0	24.9
Spur	9	2.1-3.1	6.4-8.2	2.8	7.1	0.3	0.5	2.6	6.5
Spur/branch height ratio	9	25%-33%	-	29%	_	3%	_	26%	_
Claw III heights									
Branch	10	8.4-10.2	22.7-26.2	9.5	24.5	0.6	1.2	9.9	24.7
Spur	10	2.0-3.1	6.1-7.2	2.6	6.6	0.3	0.4	2.9	7.2
Spur/branch height ratio	10	24%-31%	-	27%	_	2%	-	29%	-
Claw IV heights									
Branch	7	9.4-12.1	24.9-30.3	10.9	27.4	0.9	2.4	?	?
Spur	7	2.3-3.2	6.1-8.6	3.0	7.4	0.3	0.9	?	?
Spur/branch height ratio	7	24%–29%	-	27%	-	1%	-	?	-

formed only as thin grey belt without pillars. The scapular plate large, with additional lateral sutures separating narrow rectangular lateral portions with poorly developed pillars. Paired segmental plates divided into a smaller, much narrower anterior and a dominant posterior part by a smooth, wide transverse stripe (Fig. 1A). The caudal (terminal) plate with short incisions and fully developed epicuticular layer. Median plate I unipartite, whereas median plate II divided into weakly defined parts, with a wide rhomboidal smooth space between them (Fig. 1A). Median plate III small but with a well-developed epicuticular layer. Ventral cuticle with minute endocuticular pillars distributed throughout the whole venter, and a pair of oval subcephalic (Fig. 1C) and trapezoid genital plates. Sexpartite gonopore placed between genital plates, and a trilobed anus between legs IV.

Pedal plates I–III absent, pedal plate IV developed as a dark matrix without pillars, bearing a typical dentate collar (Figure 1A). Distinct pulvini on all legs (Fig. 1A). A small spine on leg I (Fig. 1E) and a papilla on leg IV present. Claws IV slightly higher than claws I–III (Table 2). External claws on all legs smooth (Figure 1E). Internal claws with large spurs positioned at circa 1/3 of the claw height and bent downwards. Buccal apparatus short, with a rigid, stout tube and a spherical pharynx. Stylet supports absent.

Mature males and sexually dimorphic traits (i.e. from the third instar onwards; measurements and statistics in Tables 3, 4). Generally resembling females, but a closer observation reveals two qualitative differences (body appendage configuration and dorsal plate sculpturing) and numerous morphometric dissimilarities between males and females (all summarised in Table 4). Densely punctuated areas in the central leg portions present (Fig. 2A). Male genital plates are always clearly visible (of identical shape as female plates), and dark densely arranged pillars are present in the entire genital zone, extending between the plates (Fig. 1D).

Juveniles (i.e. the second instar, measurements and statistics in Table 5). Clearly smaller than adult females and males, with the body appendage configuration A-C-D- D^d -E. Endocuticular pillars well developed in all plates, the largest pillars present in the posterior portion of the scapular plate and in the central part of the caudal (terminal) plate. Epicuticular ornamented pattern absent, although lighter and darker parts of the scapular plate can be distinguished under PCM (Fig. 1B), constituting presumably the developing epicuticular layer.

Larvae. Unknown.

Table 3. Measurements $[in \mu m]$ of selected morphological structures of the adult males of *Echiniscus masculinus* 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		Allotype	
		μm	sp	μm	sp	μm	sp	μm	sp
Body length	10	142–170	464-527	161	493	9	23	167	527
Scapular plate length	10	30.3-35.7	-	32.6	-	1.5	-	31.7	-
Head appendages lengths									
Cirrus internus	10	10.2-19.2	31.0-58.9	15.3	47.2	2.3	7.7	15.0	47.3
Cephalic papilla	10	7.7–9.3	23.4-30.0	8.6	26.6	0.6	2.2	8.6	27.1
Cirrus externus	10	16.0-21.0	47.3-67.3	18.8	57.8	1.6	6.0	17.5	55.2
Clava	10	6.1-7.5	19.2-22.8	6.8	20.8	0.4	1.1	6.1	19.2
Cirrus A	8	28.4-36.2	84.6-111.0	31.9	98.0	2.8	9.1	30.0	94.6
Cirrus A/Body length ratio	8	18%-24%	_	20%	_	2%	_	18%	_
Body appendages lengths									
Spine C	10	19.9-26.9	63.7-77.9	23.1	70.9	2.3	5.5	24.7	77.9
Spine D	10	17.6-29.7	54.0-83.2	23.0	70.4	3.4	8.5	25.0	78.9
Spine E	10	19.4-30.5	59.1-92.7	24.5	75.1	4.1	12.0	27.7	87.4
Spine on leg I length	10	2.0-3.7	6.5-11.3	3.1	9.6	0.5	1.4	2.8	8.8
Papilla on leg IV length	10	3.8-5.3	12.4-16.2	4.6	14.2	0.5	1.1	4.1	12.9
Number of teeth on the collar	9	7–12	_	9.4	_	1.7	-	12	_
Claw I heights									
Branch	10	8.4-10.7	26.5-33.0	9.4	28.9	0.7	2.1	8.4	26.5
Spur	10	2.2-3.1	6.9–9.9	2.7	8.3	0.3	0.8	2.2	6.9
Spur/branch height ratio	10	23%-32%	-	29%	-	3%	-	26%	-
Claw II heights									
Branch	9	8.4-10.4	24.9-32.1	9.2	28.4	0.6	2.5	8.6	27.1
Spur	9	1.9-2.7	5.8-8.9	2.4	7.5	0.3	1.0	2.6	8.2
Spur/branch height ratio	9	20%-31%	-	26%	-	3%	-	30%	-
Claw III heights									
Branch	8	8.5-10.1	25.8-31.4	9.2	28.3	0.6	2.0	8.7	27.4
Spur	8	2.3-2.8	7.0-8.5	2.5	7.7	0.2	0.5	2.3	7.3
Spur/branch height ratio	8	24%-30%	-	27%	-	2%	-	26%	-
Claw IV heights									
Branch	4	9.5-10.4	28.1-34.0	10.1	31.1	0.4	3.2	?	?
Spur	4	2.7-3.1	8.3–9.2	2.9	8.8	0.2	0.4	?	?
Spur/branch height ratio	4	26%-30%	-	28%	-	2%	-	?	-

Table 4. Sexual dimorphism in qualitative and quantitative traits in *Echiniscus masculinus* sp. nov., with results of one-tailed Student's *t*-tests in case of overlapping ranges in morphometric traits (all statistically significant at the α -level of $p_{BH} < 0.05$ adjusted with the Benjamini-Hochberg correction).

Qualitative traits	<u></u>	රීරී	Remarks
Body appendage configuration	A-C-D-D ^d -E	A-C-D-E	a single male had an asymmetrically developed spine D ^d [8 μm long]
Cuticular sculpturing	epicuticular ornamentation poor	epicuticular ornamentation pronounced	compare Figures 1, 2A, B and 3A, but see also Figures 2C and 3B for an atypically poor sculpturing in a male
Quantitative traits	우우: x ± SD, N = 10	ೆ ೆ: x̄ ± SD, N = 10	t, p
Body proportions: bs ratio	0.54–0.57 (= body larger and plump)	0.48–0.49 (= body smaller and slender)	non-overlapping ranges; see also Fig. 2
Body length	175 ± 11	161 ± 9	t ₁₈ = 3.27; p = 0.002
Scapular plate length	38.8 ± 3.2	32.6 ± 1.5	$t_{18} = 5.51; p < 0.001$
Head appendages lengths			
Cephalic papilla	17.3 ± 1.3	26.6 ± 2.2	$t_{18} = -11.47; \ \rho < 0.001$
Clava	14.2 ± 1.6	20.8 ± 1.1	$t_{18} = -10.44; \ \rho < 0.001$
Body appendage lengths			
Spine C	43.0 ± 8.3	70.9 ± 5.5	t ₁₈ = −8.90; <i>p</i> < 0.001
Spine D	41.3 ± 8.3	70.4 ± 8.5	t ₁₈ = -7.75; <i>p</i> < 0.001
Spine E	48.3 ± 8.2	75.1 ± 12.0	$t_{18}^{10} = -5.79; p < 0.001$
Claw branch heights			
Claw I	25.3 ± 1.7	28.9 ± 2.1	$t_{16} = -3.99; \ \rho < 0.001$
Claw II	24.4 ± 1.4	28.4 ± 2.5	t_{16}^{-1} = -4.36; $\rho < 0.001$
Claw III	24.5 ± 1.2	28.3 ± 2.0	$t_{16} = -5.01; p < 0.001$



Figure 2. Morphology of males of *E. masculinus* sp. nov. (PCM). **A.** allotype (dorsolateral view, arrowheads indicate areas with densely packed pillars in legs); **B.** paratype with fully developed sculpturing (dorsal view); **C.** paratype with poorly developed epicuticular layer of sculpturing (dorsal view). See Table 4 for the phenotypic comparison between females and males. All scale bars in μm.

Eggs. Up to two round, yellow eggs per exuvia were found.

Genetic markers and phylogenetic position. The 18S rRNA, 28S rRNA and ITS-2 were characterised by single haplotypes (GenBank accession numbers: MT106621, MT106620, MT106622, respectively), but three haplotypes were detected in the case of ITS-1 (MT106623–5), and five in COI (MT106223–7). All three DNA-based phylogenetic reconstructions revealed *E. masculinus* sp. nov. as the sister species to the clade *E. lineatus* + *E. vir*-



Figure 3. Close-up on the details of sculpturing of *E. masculinus* sp. nov. (PCM). **A.** evident epicuticular layer, endocuticular pillars of various sizes; **B.** remnants of epicuticular layer on the scapular and caudal (terminal) plates, endocuticular pillars densely packed and of equal, minute size. All scale bars in µm.

ginicus with a maximum support (Fig. 4). The divergence between the new species and the other two congeners was notably larger in COI compared to the ITS markers (compare Fig. 4A and 4B, C). The differences are congruent with the *p*-distances (see SM.2).

Type material. Holotype (mature female, slide MY.026.05), allotype (mature male, slide MY.026.07) and 42 paratypes on slides MY.026.01–09. Moreover, one voucher specimen (hologenophore) mounted on the slide MY.026.14. In total: 21 females, 14 males, and nine juveniles. Slides MY.026.01–07 are deposited in the Institute of Zoology and Biomedical Research, Jagiellonian University, Poland; slide MY.026.08 (4QQ, 3dd, one juvenile) is deposited in the Natural History Museum of Denmark, University of Copenhagen, Denmark; slide MY.026.09 (4QQ, 2dd, 2 juveniles) is deposited in the Catania University, Sicily, Italy. Found together with a new species of *Echiniscus* and a new species of *Pseudechiniscus* (descriptions in preparation).

Type locality. Ca 6°05'N, 116°32'E, ca 3500 m a.s.l.: Malaysia, Borneo, Sabah, Gunung Kinabalu; subalpine vegetation zone with single *Leptospermum* and *Rhododendron ericoides* bushes, moss on a stunted tree trunk.

Etymology. From Latin *masculinus* = male (an adjective in the nominative singular). The name underlines the presence of males in the new species, in contrast to closely related parthenogenetic *E. lineatus* and *E. virginicus*.

Table 5. Measurements [in μ m] of selected morphological structures of the juveniles of *Echiniscus masculinus* 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		Me	ean	SD		
		μm	sp	μm	sp	μm	sp	
Body length	5	115–148	431-477	129	454	13	18	
Scapular plate length	5	26.0-34.4	-	28.4	_	3.6	-	
Head appendages lengths								
Cirrus internus	5	7.4-12.3	27.1-35.8	8.8	30.7	2.0	3.5	
Cephalic papilla	5	3.8-6.4	13.1-19.6	4.9	17.3	1.0	2.7	
Cirrus externus	4	8.8-14.2	33.3-41.3	10.8	38.0	2.3	3.4	
Clava	5	3.7-5.4	13.7-16.7	4.3	15.2	0.7	1.2	
Cirrus A	5	19.5-28.6	74.1-83.5	22.8	80.1	3.5	3.7	
Cirrus A/Body length ratio	5	16%-19%	-	18%	-	1%	_	
Body appendages lengths								
Spine C	5	8.1-20.3	30.8-59.0	12.9	44.6	4.6	10.7	
Spine D	5	7.4-17.5	28.5-50.9	11.6	39.9	4.1	9.3	
Spine D ^d	5	7.1-16.1	27.0-46.8	10.4	35.9	3.4	7.3	
Spine E	5	10.8-18.0	40.2-52.3	12.7	44.3	3.0	4.9	
Spine on leg I length	4	1.9-2.7	7.2-9.1	2.2	7.9	0.4	0.9	
Papilla on leg IV length	5	3.2-3.8	10.8-14.4	3.4	12.2	0.3	1.5	
Number of teeth on the collar	5	7–8	-	7.6	-	0.5	-	
Claw I heights								
Branch	5	6.3–9.3	24.0-27.0	7.3	25.6	1.2	1.2	
Spur	5	1.5-2.7	5.2-8.1	1.9	6.8	0.5	1.2	
Spur/branch height ratio	5	21%-31%	-	27%	-	4%	-	
Claw II heights								
Branch	4	6.1-6.8	23.2-25.0	6.5	24.0	0.3	0.9	
Spur	4	1.4-1.9	5.4-7.2	1.7	6.2	0.2	0.8	
Spur/branch height ratio	4	22%-31%	-	26%	-	4%	-	
Claw III heights								
Branch	4	6.3-8.9	23.0-25.9	7.1	24.3	1.2	1.2	
Spur	4	1.7-2.5	5.8-7.3	2.0	6.7	0.4	0.6	
Spur/branch height ratio	4	25%-29%	-	28%	-	1%	-	
Claw IV heights								
Branch	4	6.7-9.1	25.4-27.7	7.6	26.3	1.0	1.0	
Spur	4	1.8-2.8	6.8-8.5	2.2	7.7	0.4	0.8	
Spur/branch height ratio	4	25%-33%	-	29%	_	4%	-	

Differential diagnosis. There are four known members of the *E. virginicus* complex: *E. clevelandi* Beasley, 1999, *E. hoonsooi* Moon & Kim, 1990, *E. lineatus* Pilato et al., 2008, and *E. virginicus* Riggin, 1962 (Gąsiorek et al. 2019a). *Echiniscus masculinus* sp. nov. can be differentiated from (body appendage configuration given collectively for both sexes):

- E. clevelandi, recorded from China, the only other dioecious representative of this group, by the body appendage configuration (A-C-D-(D^d)-E in E. masculinus sp. nov. vs A-B-C-C^d-D-D^d-E in E. clevelandi) and dorsal sculpturing (faint and poorly visible epicuticular layer with pseudopores in E. masculinus sp. nov. vs well-developed epicuticular layer with bright and large pores in E. clevelandi; see Pilato et al. 2008).
- E. hoonsooi, recorded from Korea, by the body appendage configuration (A-C-D-(D^d)-E in E. masculinus sp. nov. vs A-(C)-(D)-E in E. hoonsooi), homomorphic spurs on all legs (heteromorphic spurs I–III and IV in E. hoonsooi; see Abe et al. 2000), and by the presence of males.
- 3. E. lineatus, distributed widely in the tropical and subtropical zone, by the body appendage configu-

ration $(A-C-D-(D^d)-E \text{ in } E. masculinus \text{ sp. nov. vs} A-(B)-C-C^d-D-D^d-E \text{ in } E. lineatus)$, and by the presence of males.

4. E. virginicus, native to the eastern Nearctic realm, by the body appendage configuration (A-C-D-(D-^d)-E in E. masculinus sp. nov. vs A-(B)-C-C^d-D-D^d-E in E. virginicus), dorsal plate sculpturing (pseudopores in E. masculinus sp. nov. vs pores in E. virginicus), and by the presence of males.

Discussion

The *Echiniscus virginicus* complex contains species with well-defined geographical ranges: *E. lineatus* is pantropical, *E. clevelandi* and *E. hoonsooi* are known from Far East Asia, and *E. virginicus* has been recorded only from the Nearctic (Gąsiorek et al. 2019a). Phylogenetic analyses inferred the new species as sister to the clade *E. lineatus* + *E. virginicus*, with the latter two more closely related to each other than to *E. masculinus* sp. nov. (Fig. 4). This is surprising for two reasons: the same place of origin of *E. masculinus* sp. nov. and *E. lineatus*, the tropics, as both occur only there, and the morphological similarity of these two species, since they both have pseudopores.



Figure 4. Bayesian phylogenetic trees showing the relationships between members of the *E. virginicus* complex; *E. succineus* was used as an outgroup, and branches within species-specific clades were collapsed. Bayesian posterior probability values are given above tree branches. Phylogenetic analyses were run on the subsequent DNA markers to assure that the tree topology was congruent: COI, ITS-1, and ITS-2.

As it is generally assumed that dioecy is ancestral, and parthenogenetic thelytoky is an advanced character within Echiniscidae (e.g. Kristensen 1987), the presence of males within populations of *E. masculinus* sp. nov. is probably a retained plesiomorphy of the entire complex. Given that the new species is described from a very peculiar habitat, namely a prominent mountain peak with high levels of endemism characterising many groups of animals (Merckx et al. 2015), the isolated locality suggests a contracted, relictual geographic range of *E. masculinus* sp. nov. and its potentially restricted area of occurrence (only Gunung Kinabalu or maybe also other high mountains of Borneo).

In contrast to arthrotardigrades, usually ancestrally dioecious (Fontoura et al. 2017), echiniscoidean taxa are more diversified in terms of reproductive modes and many groups embrace both parthenogenetic and dioecious species. Echiniscoididae and Oreellidae are bisexual (Kristensen and Hallas 1980; Dastych et al. 1998; Møbjerg et al. 2016), but sexual dimorphism is not well-marked in either of the two. The first observations on sexual dimorphism within Echiniscidae were documented by Dastych (1987) and Kristensen (1987). At present, males have been reported for 14 echiniscid genera: Antechiniscus (Claxton 2001), Barbaria (Miller et al. 1999; Michalczyk and Kaczmarek 2007), Bryodelphax (Gasiorek and Degma 2018), Claxtonia (Kaczmarek and Michalczyk 2002; Mitchell and Romano 2007), Cornechiniscus (Dastych 1979), Diploechiniscus (Vicente et al. 2013), Hypechiniscus (Kristensen 1987), Mopsechiniscus (Dastych 2001), Novechiniscus (Rebecchi et al. 2008), Proechiniscus (Kristensen 1987), Pseudechiniscus (Cesari et al. 2020), Stellariscus (Gasiorek et al. 2018b), Testechiniscus (Gasiorek et al. 2018a), and Echiniscus. Sexual dimorphism can be obvious, as in Mopsechiniscus, or restricted to different gonopore shapes (e.g. in Cornechiniscus). Until now, males have been reliably discovered only in 11 Echiniscus spp. (Degma et al. 2009-2019): E. clevelandi (the virginicus complex), E. curiosus Claxton, 1996 and E. merokensis Richters, 1904 (the merokensis complex), E. duboisi Richters, 1902 and E. siticulosus Gasiorek & Michalczyk, 2020 (the spinulosus complex), E. ehrenbergi Dastych & Kristensen, 1995 and E. rodnae Claxton, 1996 (the testudo complex), E. jamesi Claxton, 1996 (the granulatus complex), E. lentiferus Claxton & Dastych, 2017 (the quadrispinosus complex), E. marleyi Li, 2007 (the blumi-canadensis complex), E. nepalensis Dastych, 1975 (the lapponicus complex). The differences between the sexes are often minor (Dastych 1975; Dastych and Kristensen 1995; Miller et al. 1999), but some authors emphasised notable disparities in morphometric traits (Beasley 1999; Claxton 1996; Claxton and Dastych 2017; Gasiorek and Michalczyk 2020). These encompass mainly differences in body proportions, and dimensions of claws, cephalic and trunk appendages (Claxton 1996; Gasiorek and Michalczyk 2020). The sex ratio varies greatly even between populations of a single species (Miller et al. 1999), indicating that there may be seasonal variations in the presence of males within Echiniscus populations, as was observed for other micrometazoans (Gilbert and Williamson 1983).

Originally, the "Gondwanan" hypothesis was postulated to explain the distribution of dioecious *Echiniscus* spp. (Miller et al. 1999). In fact, except for the cosmopolitan *E. merokensis* and East Palaearctic *E. marleyi*, other dioecious *Echiniscus* spp. inhabit exclusively post-Gondwanan lands. Additionally, males are generally absent or present in almost negligible proportions in European and Central Asian populations of *Echiniscus* (Jørgensen et al. 2007; Guil and Giribet 2009). The evolutionary causes of this phenomenon are, however, still unknown.

The sexual dimorphism of E. masculinus sp. nov., evidenced in both quantitative and qualitative traits (Table 4) is interesting in the context of usually poorly marked sexual differences in dioecious Echiniscus spp., and the fact that females of E. lineatus, E. virginicus, and E. masculinus sp. nov. are confusingly similar to each other. In fact, females are a good example of profound evolutionary stasis in morphology, which led, for example, to a description of a synonymous species in the complex (E. dariae synonymised with E. lineatus by Gąsiorek et al. 2019a). In contrast, males of E. masculinus sp. nov. and E. clevelandi can be easily distinguished based on the differences in dorsal sculpturing and appendage configuration (compare Beasley 1999 and the present study). Consequently, a question arises: why do females of the virginicus complex tend to diverge morphologically at a slower rate than males? The acquisition of genetic data for E. clevelandi and E. hoonsooi could help to resolve this conundrum, as the putative, basal, character of E. clevelandi and E. masculinus sp. nov. within the *virginicus* clade would support the hypothesis that asexually reproducing species are young and poorly phenotypically differentiated from each other and from the ancestral female phenotype. Finally, considering that the sexually reproducing E. masculinus sp. nov. is a sister taxon to the asexual E. lineatus + E. virginicus clade, we hypothesise that the males were originally present in the ancestor of the clade. Moreover, given the overall similarity of males of E. clevelandi and E. masculinus sp. nov., we also hypothesise that males in the ancestral lineage leading to E. lineatus and E. virginicus were phenotypically similar to males of E. masculinus sp. nov.

Conclusions

The description of sexually dimorphic *E. masculinus* sp. nov. elucidates the evolution of the *virginicus* complex and raises new questions about the phenotype evolution in tardigrades. Females of three species (*E. lineatus*, *E. virginicus* and *E. masculinus* sp. nov.) represent an exemplary case of delusively similar taxa (i.e. almost identical under PCM but easily identifiable with SEM analysis). The tardigrade fauna of the Indomalayan region requires more sampling effort to uncover its diversity and uniqueness.

Acknowledgements

We are most grateful to Maciej Barczyk (Senckenberg Biodiversity and Climate Research Centre, Goethe University Frankfurt, Germany) for the collection of the sample. We would also like to thank Diane Nelson, Reinhardt M. Kristensen, and an anonymous reviewer, who contributed to the improvement of this manuscript. The study was supported by the Polish Ministry of Science and Higher Education via the *Diamond Grant* (DI2015 014945 to PG, supervised by ŁM) and by the *Sonata Bis* programme of the Polish National Science Centre (grant no. 2016/22/E/NZ8/00417 to ŁM). We owe our sincere thanks to the Museum für Naturkunde, Berlin, for covering the publication charge.

References

- Abe W, Ito M, Takeda M (2000) First record of *Echiniscus hoonsooi* (Tardigrada: Echiniscidae) from Japan. Species Diversity 5: 103– 110. https://doi.org/10.12782/specdiv.5.103
- Bartels PJ, Apodaca JJ, Mora C, Nelson DR (2016) A global biodiversity estimate of a poorly known taxon: phylum Tardigrada. Zoological Journal of the Linnean Society 178: 730–736. https://doi. org/10.1111/zoj.12441
- Beasley CW (1999) A new species of *Echiniscus* (Tardigrada, Echiniscidae) from Northern Yunnan Province, China. Zoologischer Anzeiger 238: 135–138.
- Casquet J, Thebaud C, Gillespie RG (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: 136–141. https://doi.org/10.1111/j.1755-0998.2011.03073.x
- Cesari M, Montanari M, Kristensen RM, Bertolani R, Guidetti R, Rebecchi L (2020) An integrated study of the biodiversity within the *Pseudechiniscus suillus–facettalis* group (Heterotardigrada: Echiniscidae). Zoological Journal of the Linnean Society 188: 717– 732. https://doi.org/10.1093/zoolinnean/zlz045
- Claxton SK (1996) Sexual dimorphism in Australian *Echiniscus* (Tardigrada, Echiniscidae) with descriptions of three new species. Zoological Journal of the Linnean Society 116: 13–33. https://doi. org/10.1111/j.1096-3642.1996.tb02330.x
- Claxton SK (2001) Antechiniscus in Australia: Description of Antechiniscus moscali sp. n. and redescription of Antechiniscus parvisentus (Horning & Schuster, 1983) (Heterotardigrada: Echiniscidae). Zoologischer Anzeiger 240: 281–289. https://doi.org/10.1078/0044-5231-00035
- Claxton SK, Dastych H (2017) A new bisexual species of *Echiniscus* C.A.S. Schultze, 1840 (Heterotardigrada: Echiniscidae) from Tasmania, Australia. Entomologie Heute 29: 105–119.
- Dastych H (1975) Some Tardigrada from the Himalayas (Nepal) with a description of *Echiniscus (E.) nepalensis* n. sp. Memorie dell'Istituto Italiano di Idrobiologia 32 (Supplement): 61–68.
- Dastych H (1979) Tardigrada from Afghanistan with a description of *Pseudechiniscus schrammi* sp. nov. Bulletin de la Société des Amis des Sciences et des Lettres de Poznań, Série D, sciences biologiques 19: 99–108.
- Dastych H (1987) Two new species of Tardigrada from the Canadian Subarctic with some notes on sexual dimorphism in the family Echiniscidae. Entomologische Mitteilungen aus dem Zoologischen Museum Hamburg 8: 319–334.
- Dastych H (2001) Notes on the revision of the genus *Mopsechiniscus* (Tardigrada). Zoologischer Anzeiger 240: 299–308. https://doi.org/10.1078/0044-5231-00037
- Dastych H, Kristensen RM (1995) Echiniscus ehrenbergi sp. n., a new water bear from the Himalayas (Tardigrada). Entomologische Mitteilungen aus dem Zoologischen Museum Hamburg 11: 221–230.
- Dastych H, McInnes SJ, Claxton SK (1998) Oreella mollis Murray, 1910 (Tardigrada): a redescription and revision of Oreella. Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 95: 89–113.

- de Bruyn M, Stelbrink B, Morley RJ, Hall R, Carvalho GR, Cannon CH, van den Bergh G, Meijaard E, Metcalfe I, Boitani L, Maiorano L, Shoup R, von Rintelen T (2014) Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. Systematic Biology 63: 879–901. https://doi.org/10.1093/sysbio/syu047
- Degma P, Bertolani R, Guidetti R (2009–2019) Actual checklist of Tardigrada species. Ver. 36: 01-09-2019. https://doi.org/10.25431/11380 1178608
- Degma P, Guidetti R (2007) Notes to the current checklist of Tardigrada. Zootaxa 1579: 41–53. https://doi.org/10.11646/zootaxa.1579.1.2
- Fontoura P, Bartels PJ, Jøgensen A, Kristensen RM, Hansen JG (2017) A dichotomous key to the genera of the marine heterotardigrades (Tardigrada). Zootaxa 4294: 1–45. https://doi.org/10.11646/zootaxa.4294.1.1
- Gąsiorek P (2018) New *Bryodelphax* species (Heterotardigrada: Echiniscidae) from Western Borneo (Sarawak), with new molecular data for the genus. Raffles Bulletin of Zoology 66: 371–381.
- Gąsiorek P, Degma P (2018) Three Echiniscidae species (Tardigrada: Heterotardigrada) new to the Polish fauna, with the description of a new gonochoristic *Bryodelphax* Thulin, 1928. Zootaxa 4410: 77–96. https://doi.org/10.11646/zootaxa.4410.1.4
- Gąsiorek P, Jackson KJ, Meyer HA, Zając K, Nelson DR, Kristensen RM, Michalczyk Ł (2019a) *Echiniscus virginicus* complex: the first case of pseudocryptic allopatry and pantropical distribution in tardigrades. Biological Journal of the Linnean Society 128: 789–805. https://doi.org/10.1093/biolinnean/blz147
- Gąsiorek P, Michalczyk Ł (2020) *Echiniscus siticulosus* (Echiniscidae: *spinulosus* group), a new tardigrade from Western Australian scrub. New Zealand Journal of Zoology 47: 87–105. https://doi.org/10.108 0/03014223.2019.1603166
- Gąsiorek P, Morek W, Stec D, Michalczyk Ł (2019b) Untangling the *Echiniscus* Gordian knot: paraphyly of the "*arctomys* group" (Heterotardigrada: Echiniscidae). Cladistics 35: 633–653. https://doi. org/10.1111/cla.12377
- Gąsiorek P, Stec D, Zawierucha K, Kristensen RM, Michalczyk Ł (2018a) Revision of *Testechiniscus* Kristensen, 1987 (Heterotardigrada: Echiniscidae) refutes the polar-temperate distribution of the genus. Zootaxa 4472: 261–297. https://doi.org/10.11646/zootaxa.4472.2.3
- Gąsiorek P, Suzuki AC, Kristensen RM, Lachowska-Cierlik D, Michalczyk Ł (2018b) Untangling the *Echiniscus* Gordian knot: *Stellariscus* gen. nov. (Heterotardigrada: Echiniscidae) from Far East Asia. Invertebrate Systematics 32: 1234–1247. https://doi.org/10.1071/IS18023
- Gilbert JJ, Williamson CE (1983) Sexual dimorphism in zooplankton (Copepoda, Cladocera, and Rotifera). Annual Review of Ecology and Systematics 14: 1–33. https://doi.org/10.1146/annurev. es.14.110183.000245
- Guidetti R, Cesari M, Bertolani R, Altiero T, Rebecchi L (2019) High diversity in species, reproductive modes and distribution within the *Paramacrobiotus richtersi* complex (Eutardigrada, Macrobiotidae). Zoological Letters 5: 1. https://doi.org/10.1186/s40851-018-0113-z
- Guidetti R, Bertolani R (2005) Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification Zootaxa 845: 1–46. https://doi.org/10.11646/zootaxa.845.1.1
- Guil N, Giribet G (2009) Fine scale population structure in the *Echinis-cus blumi-canadensis* series (Heterotardigrada, Tardigrada) in an Iberian mountain range – When morphology fails to explain genetic structure. Molecular Phylogenetics and Evolution 51: 606–613. https://doi.org/10.1016/j.ympev.2009.02.019
- Jørgensen A, Kristensen RM, Møbjerg N (2018) Phylogeny and integrative taxonomy of Tardigrada. In: Schill O (Ed.) Water Bears: The

zse.pensoft.net

Biology of Tardigrades. Zoological Monographs, Springer, 95–114. https://doi.org/10.1007/978-3-319-95702-9_3

- Jørgensen A, Møbjerg N, Kristensen RM (2007) A molecular study of the tardigrade *Echiniscus testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. Journal of Limnology 66(S1): 77–83. https://doi.org/10.4081/jlimnol.2007.s1.77
- Jørgensen A, Møbjerg N, Kristensen RM (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(S1): 6–16. https://doi.org/10.1111/j.1439-0469.2010.00592.x
- Kaczmarek Ł, Michalczyk Ł (2002) Echiniscus barbarae, a new species of tardigrade from Cuba Island (Tardigrada: Heterotardigrada, Echiniscidae, 'arctomys group'). Zootaxa 53: 1–4. https://doi. org/10.11646/zootaxa.53.1.1
- 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: 91–106.
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066. https://doi. org/10.1093/nar/gkf436
- Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286– 298. https://doi.org/10.1093/bib/bbn013
- Kitayama K (1992) An altitudinal transect study of the vegetation on Mount Kinabalu, Borneo. Vegetatio 102: 149–171. https://doi. org/10.1007/BF00044731
- Kristensen RM (1987) Generic revision of the Echiniscidae (Heterotardigrada), with a discussion of the origin of the family. In: Bertolani R (Ed.) Biology of Tardigrades. Selected Symposia and Monographs U.Z.I., Modena, 261–335.
- Kristensen RM, Hallas TE (1980) The tidal genus *Echiniscoides* and its variability, with erection of Echiniscoididae fam. n. (Tardigrada). Zoologica Scripta 9: 113–127. https://doi. org/10.1111/j.1463-6409.1980.tb00657.x
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054
- Li X (2007) Tardigrades from the Tsinling Mountains, central China with descriptions of two new species of Echiniscidae (Tardigrada). Journal of Natural History 41: 2719–2739. https://doi. org/10.1080/00222930701711046
- Lohman DJ, de Bruyn M, Page T, von Rintelen K, Hall R, Ng PKL, Shih H-T, Carvalho GR, von Rintelen T (2011) Biogeography of the Indo-Australian Archipelago. Annual Review of Ecology, Evolution, and Systematics 42: 205–226. https://doi.org/10.1146/annurev-ecolsys-102710-145001
- Merckx VSFT, Hendriks KP, Beentjes KK, Mennes CB, Becking LE, Peijnenburg KTCA, Afendy A, de Boer ANH, Biun A, Buang MM, Chen P, Chung AYC, Dow R, Feijen FAA, Feijen H, Feijen-van Soest C, Geml J, Geurts R, Gravendeel B, Hovenkamp P, Imbun P, Ipor I, Janssens SB, Jocqué M, Kappes H, Khoo E, Koomen P, Lens F, Majapun RJ, Morgado LN, Neupane S, Nieser N, Pereira JT, Rahman H, Sabran S, Sawang A, Schwallier RM, Shim P, Smit H, Sol N, Spait M, Stech M, Stokvis F, Sugau JB, Suleiman M, Sumail S, Thomas DC, van Tol J, Tuh FYY, Yahya BE, Nais J, Repin R, Lakim M, Schilthhuizen M (2015) Evolution of endemism on a young tropical mountain. Nature 524: 347–350. https://doi.org/10.1038/nature14949

- Michalczyk Ł, Kaczmarek Ł (2007) Echiniscus ganczareki, a new species of Tardigrada (Heterotardigrada: Echiniscidae, bigranulatus group) from Costa Rica. Zootaxa 1471: 15–25. https://doi. org/10.11646/zootaxa.1471.1.2
- Miller WR, Claxton SK, Heatwole HF (1999) Tardigrades of the Australian Antarctic Territories: Males in the genus *Echiniscus* (Tardigrada: Heterotardigrada). Zoologischer Anzeiger 238: 303–309.
- Mitchell CR, Romano FA (2007) Sexual dimorphism, population dynamics and some aspects of life history of *Echiniscus mauccii* (Tardigrada; Heterotardigrada). Journal of Limnology 66 (Supplement 1): 126–131. https://doi.org/10.4081/jlimnol.2007.s1.126
- Moon SN, Kim HS (1990) A new species of *Echiniscus* (Tardigrada: Echiniscidae) from Korea. Korean Journal of Systematic Zoology 6: 231–234.
- Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B (2011) How many species are there on Earth and in the ocean? PLoS Biology 9: e1001127. https://doi.org/10.1371/journal.pbio.1001127
- Møbjerg N, Kristensen RM, Jørgensen A (2016) Data from new taxa infer *Isoechiniscoides* gen. nov. and increase the phylogenetic and evolutionary understanding of echiniscoidid tardigrades (Echiniscoidea: Tardigrada). Zoological Journal of the Linnean Society 178: 804–818. https://doi.org/10.1111/zoj.12500
- Ohsawa M, Nainggolan PHJ, Tanaka N, Anwar C (1985) Altitudinal zonation of forest vegetation on Mount Kerinci, Sumatra: with comparisons to zonation in the temperate region of east Asia. Journal of Tropical Ecology 1: 193–216. https://doi.org/10.1017/S0266467400000286
- Pilato G, Binda MG, Lisi O (2004) Famelobiotus scalicii, n. gen. n. sp., a new eutardigrade from Borneo. New Zealand Journal of Zoology 31: 57–60. https://doi.org/10.1080/03014223.2004.9518359
- Pilato G, Fontoura P, Lisi O, Beasley C (2008) New description of *Echiniscus scabrospinosus* Fontoura, 1982, and description of a new species of *Echiniscus* (Heterotardigrada) from China. Zootaxa 1856: 41–54. https://doi.org/10.11646/zootaxa.1856.1.4
- Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander C, Sundberg P, Thollesson M (2008) Phylogenies without roots? A plea for the use of vouchers in molecular studies. Molecular Phylogenetics and Evolution 48: 369–371. https://doi.org/10.1016/j.ympev.2008.03.024
- Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. https://beast.bio.ed.ac.uk/Tracer
- Rebecchi L, Altiero T, Eibye-Jacobsen J, Bertolani R, Kristensen RM (2008) A new discovery of *Novechiniscus armadilloides* (Schuster, 1975) (Tardigrada, Echiniscidae) from Utah, USA with considerations on non-marine Heterotardigrada phylogeny and biogeography. Organisms, Diversity & Evolution 8: 58–65. https://doi. org/10.1016/j.ode.2006.11.002
- Richters F (1902) Beiträge zur Kenntnis der Fauna der Umgebung von Frankfurt a. M. Bericht über die Senckenbergische Naturforschende Gesellschaft in Frankfurt am Main, 23–26.
- Richters F (1904) Arktische tardigraden. Fauna Arctica 3: 495–511.
- Riggin GT (1962) Tardigrada of the Southwest Virginia: with the addition of a description of a new marine species from Florida. Virginia Agricultural Experiment Station Technical Bulletin 152: 1–147.
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180
- Stec D, Morek W, Gąsiorek P, Michalczyk Ł (2018) Unmasking hidden species diversity within the *Ramazzottius oberhaeuseri* complex, with an integrative redescription of the nominal species for the family Ramazzottiidae (Tardigrada: Eutardigrada: Parachela).

Systematics and Biodiversity 16: 357–376. https://doi.org/10.1080/14772000.2018.1424267

- Stec D, Smolak R, Kaczmarek Ł, Michalczyk Ł (2015) An integrative description of *Macrobiotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya. Zootaxa 4052: 501–526. https://doi.org/10.11646/zootaxa.4052.5.1
- van Steenis CGGJ (1984) Floristic altitudinal zones in Malesia. Botanical Journal of the Linnean Society 89: 289–292. https://doi. org/10.1111/j.1095-8339.1984.tb02560.x
- Vecchi M, Cesari M, Bertolani R, Jönsson KI, Rebecchi L, Guidetti R (2016) Integrative systematic studies on tardigrades from Antarctica identify new genera and new species within Macrobiotoidea and Echiniscoidea. Invertebrate Systematics 30: 303–322. https://doi. org/10.1071/IS15033
- Vicente F, Fontoura P, Cesari M, Rebecchi L, Guidetti R, Serrano A, Bertolani R (2013) Integrative taxonomy allows the identification of synonymous species and the erection of a new genus of Echiniscidae (Tardigrada, Heterotardigrada). Zootaxa 3613: 557–572. https://doi. org/10.11646/zootaxa.3613.6.3

Supplementary material 1

Raw morphometric data for the type population

- Authors: Piotr Gąsiorek, Katarzyna Vončina, Łukasz Michalczyk
- Data type: morphometric data
- Explanation note: The dataset comprises individual measurements provided separately for all life stages.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons. org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/zse.96.49989.suppl1

Supplementary material 2

Uncorrected pairwise distances

- Authors: Piotr Gąsiorek, Katarzyna Vončina, Łukasz Michalczyk
- Data type: genetic data
- Explanation note: p-distances between haplotypes of fastly evolving DNA fragments (ITS-1, ITS-2, COI) provided for the members of the *virginicus* complex.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons. org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/zse.96.49989.suppl2

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: Zoosystematics and Evolution

Jahr/Year: 2020

Band/Volume: 96

Autor(en)/Author(s): Gasiorek Piotr, Voncina Katarzyna, Michalczyk Lukasz

Artikel/Article: An overview of the sexual dimorphism in Echiniscus (Heterotardigrada, Echiniscoidea), with the description of Echiniscus masculinus sp. nov. (the virginicus complex) from Borneo 103-113