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Pseudechiniscus in Japan: re-description of *Pseudechiniscus asper* Abe et al., 1998 and description of *Pseudechiniscus shintai* sp. nov.

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Abstract

The classification and identification of species within the genus *Pseudechiniscus* Thulin, 1911 has been considered almost a Sisyphean work due to an extremely high homogeneity of its members. Only recently have several contributions made progress in the taxonomy feasible through their detailed analyses of morphology and, crucially, by the re-description of the ancient, nominal species *P. suillus* (Ehrenberg, 1853). Herein, we focus on the Japanese representatives of this genus: *P. asper*, a rare species originally described from Hokkaido, and a new species *P. shintai*. Both taxa belong to the widespread *suillus-facettalis* complex. Detailed descriptions entailing DNA barcoding of four markers and illustrations of the ventral pillar patterns are indispensable for an accurate delineation of species within this genus.

Key Words

biodiversity, Echiniscidae, Heterotardigrada, morphology, sculpturing

Introduction

Tardigrades are poorly known micrometazoans famous for their ability to enter cryptobiosis (Møbjerg et al. 2011). This phylum is now widely accepted as a lineage within the superclade Ecdysozoa (Campbell et al. 2011) and related to the Onychophora and Arthropoda within the Panarthropoda (Giribet and Edgecombe 2017). In the last decade, tens of new species have been described, which reflects limited understanding of tardigrade diversity (Bartels et al. 2016). Studies on the Japanese tardigrades have a long history, resulting in over 150 species reported from this archipelago (Suzuki 2017). Amongst them, ca. 40 spp. (> 20%) belong to the limno-terrestrial heterotardigrade family Echiniscidae (Gąsiorek et al. 2018a, Suzuki et al. 2018), a distinct group characterised by the development of cuticular plates on the dorsal surface of the body (Kristensen 1987).

Recent advances in the taxonomy of one of the echiniscid genera, *Pseudechiniscus* Thulin, 1911, are a good illustration of the progress currently being made in the classification of tardigrades. Firstly, Tumanov (2020) discussed and re-organised the morphological nomenclature after a meticulous analysis of various members of *Pseudechiniscus* and he concluded that several species are unidentifiable, according to current taxonomic standards. Cesari et al. (2020) demonstrated high genetic variability amongst members of the speciose *suillus-facettalis* complex, implying that the species richness in the genus may be underestimated. Finally, the ability to confidently describe new *Pseudechiniscus* species was enabled by the modern diagnosis of *P. suillus* (Ehrenberg, 1853), one of enigmatic tardigrade taxa described in the 19th century (Grobys et al. 2020). In summary, better understanding of morphology, genetic disparities and ontogenetic shifts (Gąsiorek et al. 2019, Morek et al. 2019) has facilitated intensification in tardigrade research.

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

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

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

In this contribution, we concentrate on the Japanese *Pseudechiniscus* species. *Pseudechiniscus asper* Abe et al., 1998 is re-described and *P. shintai* sp. nov. is described, based on specimens from Aomori Prefecture (Northern Honshu). A brief review of Japanese *Pseudechiniscus* records is provided, concluding that they should be treated as unreliable and require formal confirmation through a new, large-scale sampling effort undertaken throughout Japan. Such a conclusion is in line with new discoveries of species complexes in numerous tardigrade genera (e.g. Guidetti et al. 2019, Stec et al. 2020, Roszkowska et al. 2020).

Materials and methods

Sample collection and processing

Specimens belonging to two species of the genus *Pseudechiniscus* were extracted from four moss samples (JP.012–5) collected from trees in Asamushi, Northern Honshu, Japan (ca. 40°54'03.6"N, 140°51'58"E, 30 m a.s.l.; R.M. Kristensen leg. on 24 July 2019). Samples were processed according to the protocol developed by Dastych (1980) with further amendments by Stec et al. (2015). The animals were used in two analyses: (I) qualitative and quantitative morphology, investigated under phase contrast microscopy (PCM) and (II) DNA sequencing (see descriptions for details). Each specimen was observed in a drop of distilled water on a temporary slide under a 400× magnification to confirm its identification prior to analysis.

Microscopy, imaging and morphometrics

Permanent microscope slides were made using Hoyer's medium and examined using an Olympus BX53 PCM associated with an Olympus DP74 digital camera. All figures were assembled in Corel Photo-Paint X7. All measurements are given in micrometres (µm) and were performed under PCM. Structures were measured only when not broken, deformed or twisted and 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 expressed as a percentage (Dastych 1999). Morphometric data were handled using the Echiniscoidea ver. 1.3 template, available from the Tardigrada Register, www.tardigrada.net (Michalczyk and Kaczmarek 2013). Importantly, all species designated as dubious or with insufficient descriptions (Grobys et al. 2020, Tumanov 2020), were discarded from the differential diagnoses.

Genotyping and genetic comparisons

DNA was extracted from individual animals following a Chelex 100 resin (Bio-Rad) extraction method (Casquet et al. 2012, Stec et al. 2015). Hologenophores were obtained for both species (Pleijel et al. 2008). Four DNA fragments were sequenced: three nuclear and one mitochondrial (Table 1) in the case of *P. shintai* sp. nov.; and three for *P. asper*. The COI fragment was amplifiable for *P. asper* but, due to a high number of double peaks, effective sequence cleaning was not possible. All fragments were amplified and sequenced, according to the protocols described in Stec et al. (2015). The obtained alignments were edited and checked manually in BioEdit v7.2.6.1 (Hall 1999) and ClustalW Multiple Alignment tool (Thompson et al. 1994) was used in the alignment of COI for *P. shintai* sp. nov. and other confidently identified species (Grobys et al. 2020, Roszkowska et al. 2020). MEGA7.0.26 (Kumar et al. 2016) was used for calculation of uncorrected pairwise distances (Srivathsan and Meier 2012).

Results

Systematic account

Phylum: Tardigrada Doyère, 1840
Class: Heterotardigrada Marcus, 1927
Order: Echiniscoidea Richters, 1926
Family: Echiniscidae Thulin, 1928
Genus: *Pseudechiniscus* Thulin, 1911

Pseudechiniscus asper **Abe, Utsugi & Takeda, 1998**

Figures 1, 2, 5A, Tables 2, 3

Locus typicus **and type material.** ca. 42°46'N, 141°24'E, 250 m a.s.l.; vicinity of the Lake Shikotsu (Chitose,

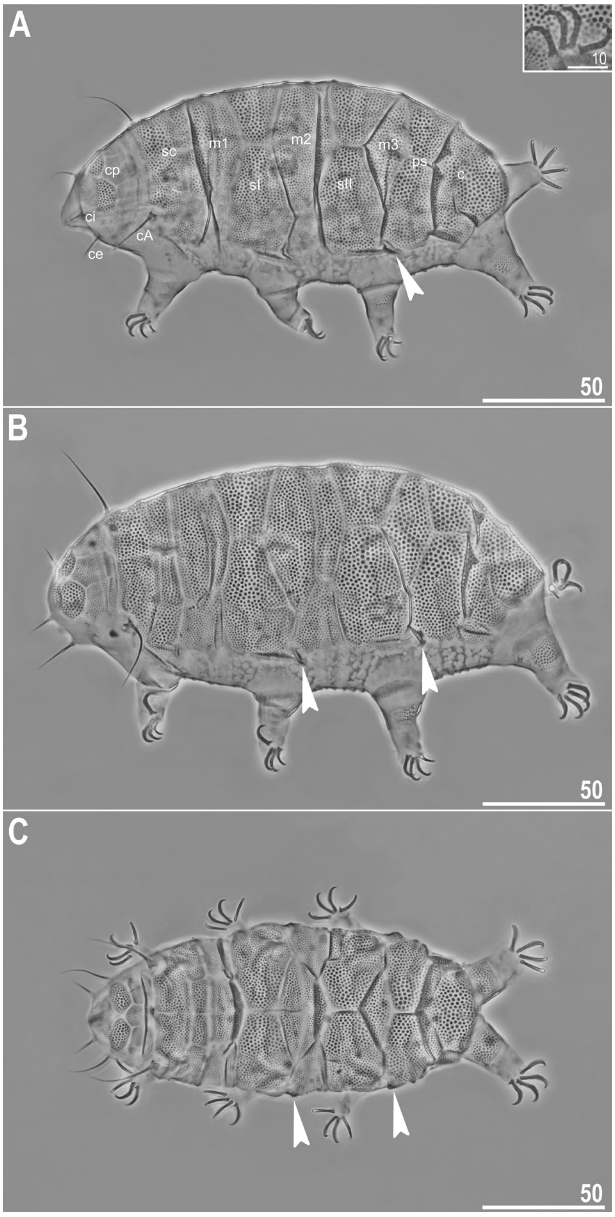

Figure 1. Habitus of *Pseudechiniscus asper* (PCM): **A, B** – females; **C** – male hologenophore. Insert shows claws III. Arrowheads indicate thickenings at the lateral positions *C* and *D*. List of abbreviations: c – caudal plate, cA – cirrus *A*, ce – *cirrus externus*, ci – *cirrus internus*, cp – cephalic plate, m1–3 – median plates, ps – pseudosegmental plate IV', sI–II – paired segmental plates, sc – scapular plate. Scale bars: in µm.

South-western Hokkaido, Japan); foliose lichen *Phaeophyscia imbricata* (Physciaceae) on the trunk of a maple (*Acer japonicum*). Collector: Kazuo Utsugi. Holotype: adult male on the slide NSMT-Tg 44 deposited in the National Museum of Nature and Science in Tokyo.

Additional material. Four females on the slides JP.012.01, JP.013.01–2, JP.014.01 and a male on the slide JP.012.04. Hologenophores: JP.012.01, 4, JP.013.02.

Etymology. From Latin *asper* = rough, referring to the irregular surface of dorsal plates. Adjective in the nominative singular.

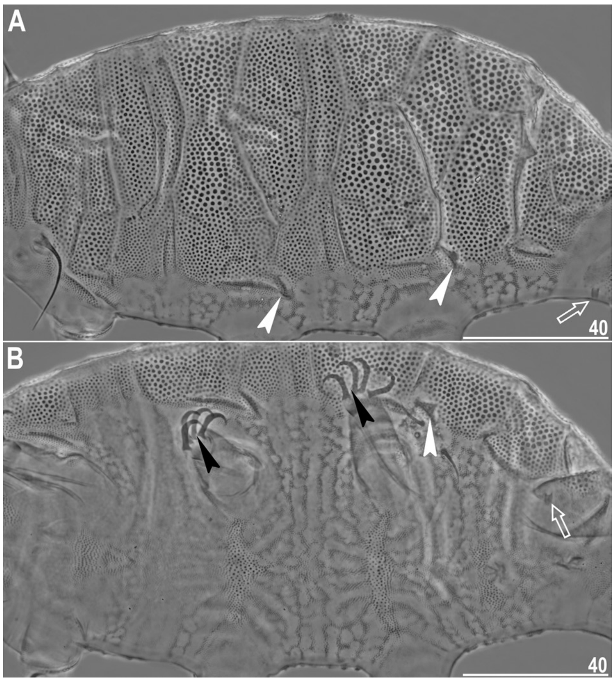

Figure 2. Sculpturing of *Pseudechiniscus asper* (PCM): **A** – dorsal; **B** – ventral. White arrowheads indicate thickenings at the lateral positions *C* and *D*, black arrowheads indicate claw spurs and empty arrows indicate papillae IV. Scale bars: in µm.

Description. Mature females (i.e. from the third instar onwards; measurements in Table 2). Body dark orange, with round black eyes present or dissolving soon after mounting (Fig. 1A, B). Member of the *suillus-facettalis* complex: dome-shaped (hemispherical) cephalic papillae (secondary clavae) and minute (primary) clavae; peribuccal cirri with poorly developed cirrophores. Cirrus *A* short, with cirrophore.

Dorsal plates well-sclerotised as for a *Pseudechiniscus* species, clearly demarcated from each other, with *Pseudechiniscus*-type sculpturing, i.e. large endocuticular pillars protruding through the epicuticle and visible as dark dots in PCM (Fig. 2A). *Striae* absent. The cephalic plate pentapartite, with the anterior bi-halved portion and three posterior portions, roughly equal in size (Fig. 1A, B). The cervical (neck) plate absent. The scapular plate with a transverse suture, separating a broader anterior portion and narrower posterior portion (Figs 1B, 2A). Three median plates: m1–2 bipartite, with much reduced, narrow posterior portions, m3 unipartite and large (Fig. 2A) with two pairs of lateral intersegmental platelets flanking the borders of m1–2. Two pairs of large segmental plates, their posterior portions exhibiting thickenings at positions *C* and *D* – the latter usually more pronounced (Figs 1A, B, 2). The pseudosegmental plate IV' divided by a median longitudinal suture; the posterior margin of the plate with a pair of short triangular projections (Figs 1A, B, 2A). The caudal (terminal) plate with short incisions that may be sclerotised (compare Fig. 1A with Fig. 1B).

Ventral cuticle with a faint species-specific pattern reaching the lateroventral sides of the body (Figs 2B, 5A),

Table 2. Measurements [in µm] of selected morphological structures of mature females of *Pseudechiniscus asper* mounted in Hoyer's medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure amongst all measured specimens; SD – standard deviation).

CHARACTER	N	RANGE		MEAN		SD	
		µm	*sp*	µm	*sp*	µm	*sp*
Body length	2	178 – 203	*712* – *810*	191	*761*	18	*70*
Scapular plate length	2	25.0 – 25.1	–	25.1	–	0.1	–
Head appendages lengths							
Cirrus *internus*	3	9.2 – 11.4	*42.0* – *45.4*	10.4	*43.7*	1.1	*2.4*
Cephalic papilla	3	3.5 – 4.7	*13.9* – *18.8*	4.1	*16.4*	0.6	*3.4*
Cirrus *externus*	3	14.8 – 19.4	*59.2* – *77.3*	16.6	*68.2*	2.5	*12.8*
Clava	3	4.3 – 5.9	*17.2* – *20.3*	5.1	*18.8*	0.8	*2.2*
Cirrus *A*	3	24.1 – 30.8	*96.4* – *122.7*	27.3	*109.6*	3.4	*18.6*
Cirrus *A*/Body length ratio	2	14% – 15%	–	14%	–	1%	–
Papilla on leg IV length	4	3.0 – 3.6	*12.0* – *13.9*	3.4	*13.0*	0.3	*1.4*
Claw 1 heights							
Branch	4	8.8 – 10.7	*40.2* – *40.4*	9.9	*40.3*	0.8	*0.1*
Spur	4	1.2 – 1.8	*4.8* – *6.4*	1.6	*5.6*	0.3	*1.1*
Spur/branch length ratio	3	12% – 20%	–	16%	–	4%	–
Claw 2 heights							
Branch	3	8.5 – 9.8	*38.0* – *39.0*	9.3	*38.5*	0.7	*0.7*
Spur	3	1.2 – 1.4	*4.8* – *5.2*	1.3	*5.0*	0.1	*0.3*
Spur/branch length ratio	3	13% – 16%	–	14%	–	2%	–
Claw 3 heights							
Branch	2	9.2 – 10.1	*36.8* – *40.2*	9.7	*38.5*	0.6	*2.4*
Spur	2	1.0 – 1.8	*4.0* – *7.2*	1.4	*5.6*	0.6	*2.2*
Spur/branch length ratio	2	11% – 18%	–	14%	–	5%	–
Claw 4 heights							
Branch	2	11.6 – 11.6	*46.2* – *46.2*	11.6	*46.2*	0.0	*?*
Spur	2	2.0 – 2.0	*8.0* – *8.0*	2.0	*8.0*	0.0	*?*
Spur/branch length ratio	2	17% – 17%	–	17%	–	0%	–

Table 3. Measurements [in µm] of selected morphological structures of mature males of *Pseudechiniscus asper* mounted in Hoyer's medium. Measurements of the holotype taken from Abe et al. (1998).

CHARACTER	♂		Holotype
	µm	*sp*	µm
Body length	159	*675*	166
Scapular plate length	23.5	–	?
Head appendages lengths			
Cirrus *internus*	11.4	*48.5*	8.0
Cephalic papilla	4.7	*20.0*	?
Cirrus *externus*	14.8	*63.0*	12.0
Clava	3.5	*14.9*	1.5
Cirrus *A*	19.4	*82.6*	20.0
Cirrus *A*/Body length ratio	12%	–	12%
Papilla on leg IV length	3.7	*15.7*	?
Claw 1 heights			
Branch	?	*?*	ca. 9.0
Spur	?	*?*	?
Spur/branch length ratio	?	–	?
Claw 2 heights			
Branch	11.1	*47.2*	ca. 9.0
Spur	0.9	*3.8*	?
Spur/branch length ratio	8%	–	?
Claw 3 heights			
Branch	10.9	*46.4*	ca. 9.0
Spur	?	*?*	?
Spur/branch length ratio	?	–	?
Claw 4 heights			
Branch	13.1	*55.7*	ca. 11.0
Spur	1.0	*4.3*	?
Spur/branch length ratio	8%	–	?

being a typical reticulum composed of large multiangular, longitudinal shapes connected by belts of pillars. Pillars are particularly poorly visible between legs I and II (Fig. 2B). The subcephalic zone with a wide patch of pillars (Fig. 5A). Sexpartite gonopore located anteriorly of legs IV and a trilobed anus between legs IV.

Pedal plates and dentate collar IV absent; instead, large patches of pillars are present centrally on each leg (Fig. 1A, B). Pulvini indistinct. No papilla or spine on leg I visible in PCM, a papilla on leg IV present (Figs 1B, 2). Claws IV higher than claws I–III; internal claws with needle-like spurs positioned at ca. 1/4–1/5 of the claw height (Fig. 1A, insert).

Mature males (i.e. from the second instar onwards; measurements in Table 3). Smaller than females, with slender body (Fig. 1C). *Cirri externi* approaching the length of cirri *A*. Pseudosegmental projections in the form of teeth or wide lobes. Gonopore circular.

Juveniles. Unknown.

Larvae. Unknown.

Eggs. Unknown.

DNA sequences. Single haplotypes in 18S rRNA (MT645083, 843 bp), 28S rRNA (MT645081, 716 bp) and ITS-1 (MT645085, 631 bp) were obtained.

Remarks. This is the third record of this very rare species, which, in addition to the type locality, has also been found on Mount Taibai, Shaanxi, China (Li et al. 2005). In the original description, only one male was found to possess triangular projections, ending with papillate tips, on the pseudosegmental plate (Abe et al. 1998). However, the variability in the shape of the pseudosegmental projections has previously been noted (Fontoura et al. 2010), thus the lobate form of these structures in the Chinese and Japanese (Honshu) specimens is not surprising. Moreover, Abe et al. (1998) did not illustrate the complete ventral pattern of this species (most likely because of the quality of the microscope used) and omitted the swelling or thickening of the armour at position *C*, which is weakly developed in this species. As Asamushi lies only ca. 200 km southwards from the shores of Lake Shikotsu (however, the Blakiston's Line was designated to separate faunae of large vertebrates of Honshu and Hokkaido; see Kawamura 2007), the formal amendments to the original description presented here are justified given that DNA barcodes compensate the scarcity of specimens used in morphometrics.

Phenotypic differential diagnosis. Taxa most similar to *P. asper*, i.e. those possessing pseudosegmental projections, can be easily distinguished from this species, based on the presence of *striae* (even rudimentary *striae* are absent in *P. asper*; see Fig. 2 in Fontoura et al. 2010 for microphotographs of other species), and/or by the lack of thickenings at the lateral positions (Abe et al. 1998).

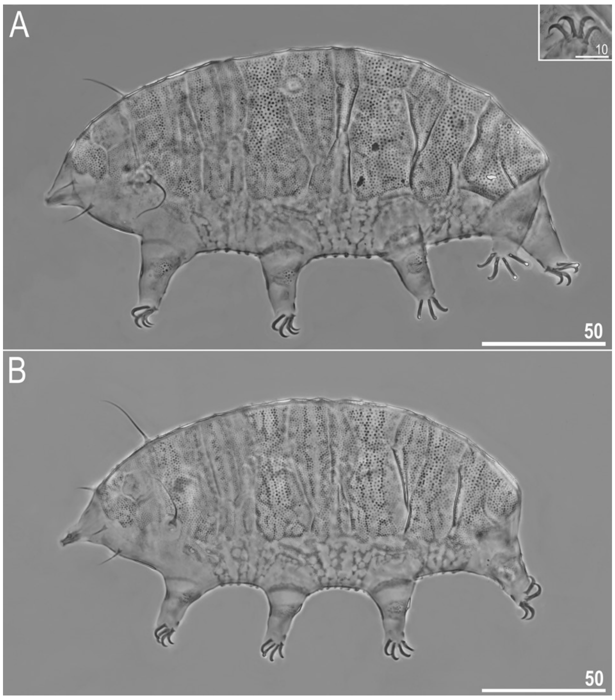

Figure 3. Habitus of *Pseudechiniscus shintai* sp. nov. (PCM): **A** – female (holotype); **B** – male (allotype). Insert shows claws I. Scale bars: in µm.

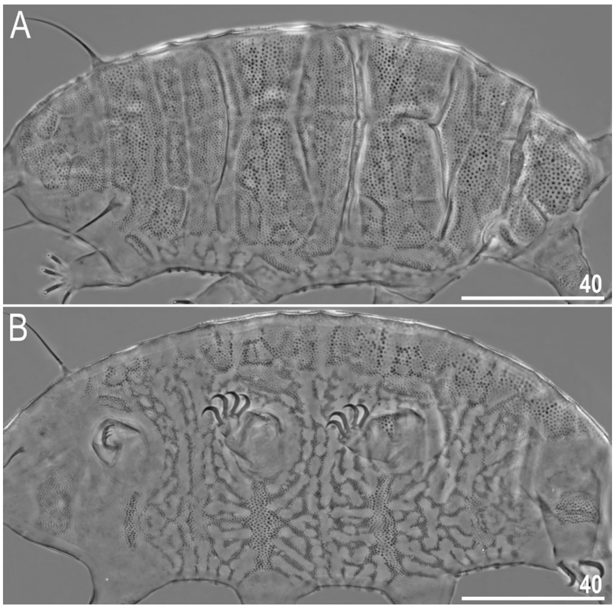

Figure 4. Sculpturing of *Pseudechiniscus shintai* sp. nov. (PCM): **A** – dorsal; **B** – ventral. Scale bars: in µm.

Pseudechiniscus shintai **sp. nov.**

http://zoobank.org/1BC6B3B3-16EF-4442-AD6D-CCD357C47C31

Figures 3, 4, 5B, Tables 4, 5

Locus typicus **and type material.** ca. 40°54'03.6"N, 140°51'58"E, 30 m a.s.l.; Asamushi-Onsen Forest Park (Aomori, Northern Honshu, Japan); mosses from tree trunks. Collector: R.M. Kristensen. Holotype and allotype: mature female and male on slide JP.013.01. Eight juveniles on the slides JP.012.02–3, JP.013.03–4, JP.014.01–3, JP.015.01. Hologenophores: JP.012.02–3, JP.013.03–4. Holotype, allotype and the majority of paratypes (slides JP.012.02–3, JP.013.01, JP.013.03–4 and JP.015.01) are deposited in the Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland; other paratypes (slides JP.014.01–3; NHMD–669705–7) are deposited in the Natural History Museum of Denmark, University of Copenhagen, Denmark.

Etymology. The name is a patronym honouring Shinta Fujimoto, an excellent Japanese tardigradologist specialising in marine Heterotardigrada. Noun in the genitive singular.

Description. Mature female (i.e. the third or latter instar; measurements in Table 4). Body orange, with minute, round black eyes that are absent after mounting (Figs 3A, 4A). Member of the *suillus-facettalis* complex: dome-shaped (hemispherical) cephalic papillae (secondary clavae) and minute (primary) clavae; peribuccal cirri with poorly developed cirrophores. Cirrus *A* short, with cirrophore.

Dorsal plates poorly sclerotised, but clearly demarcated from each other, with the *Pseudechiniscus*-type sculpturing, i.e. endocuticular pillars protruding through the epicuticle and visible as dark dots in PCM (Fig. 4A). *Striae* absent; epicuticular ornamentation visible as darker belts on all dorsal plates. The cephalic plate pentapartite, with the two anterior portions and three posterior portions approximately equal in size (Fig. 4A). The cervical (neck) plate absent. The scapular plate with sutures, separating a wide anterior portion from the four posterior portions (Fig. 4A). Three median plates: m1–2 bipartite;

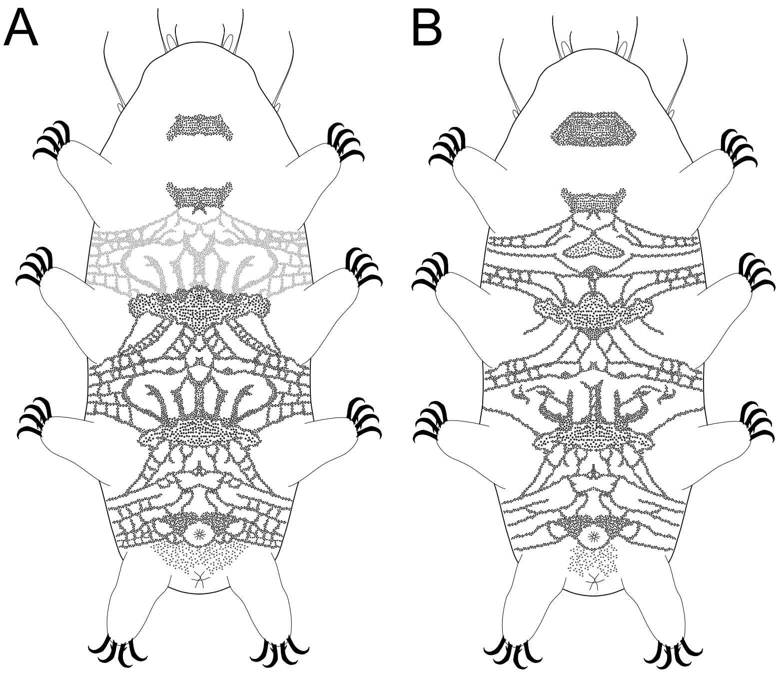

Figure 5. Schematic drawings of ventral sculpturing patterns: **A** – *Pseudechiniscus asper*; **B** – *Pseudechiniscus shintai* sp. nov.

Table 4. Measurements [in µm] of selected morphological structures of mature female (holotype) and male (allotype) of *Pseudechiniscus shintai* sp. nov. mounted in Hoyer's medium.

CHARACTER	Holotype ♀		Allotype ♂	
	µm	sp	µm	sp
Body length	196	*735*	178	*754*
Scapular plate length	26.7	–	23.6	–
Head appendages lengths				
Cirrus *internus*	6.8	*25.5*	8.2	*34.7*
Cephalic papilla	4.0	*15.0*	3.5	*14.8*
Cirrus *externus*	12.8	*47.9*	12.5	*53.0*
Clava	5.0	*18.7*	4.0	*16.9*
Cirrus *A*	23.1	*86.5*	25.3	*107.2*
Cirrus *A*/Body length ratio	12%	–	14%	–
Papilla on leg IV length	3.1	*11.6*	2.3	*9.7*
Claw 1 heights				
Branch	8.7	*32.6*	7.3	*30.9*
Spur	2.2	*8.2*	1.4	*5.9*
Spur/branch length ratio	25%	–	19%	–
Claw 2 heights				
Branch	8.7	*32.6*	7.0	*29.7*
Spur	2.0	*7.5*	1.6	*6.8*
Spur/branch length ratio	23%	–	23%	–
Claw 3 heights				
Branch	8.8	*33.0*	7.2	*30.5*
Spur	1.9	*7.1*	1.1	*4.7*
Spur/branch length ratio	22%	–	15%	–
Claw 4 heights				
Branch	9.2	*34.5*	8.8	*37.3*
Spur	1.7	*6.4*	1.8	*7.6*
Spur/branch length ratio	18%	–	20%	–

m3 unipartite (Figs 3A, 4A); four pairs of lateral intersegmental platelets flanking the borders of m1–2. Two pairs of large segmental plates. The pseudosegmental plate IV' divided by a median longitudinal suture; the posterior margin of the plate can be wide (Fig. 4A), but without lobes or teeth (Fig. 3A). The caudal (terminal) plate with short sclerotised incisions (Figs 3A, 4A).

Ventral cuticle with a pronounced species-specific pattern reaching the lateroventral sides of the body (Figs 4B and 5B), being a typical reticulum composed of large multiangular, longitudinal shapes joined by belts of pillars. The subcephalic zone with a wide belt of pillars. Sexpartite gonopore located anteriorly of legs IV and a trilobed anus between legs IV.

Pedal plates and dentate collar IV absent, instead large patches of pillars are present centrally on each leg (Fig. 3A). Pulvini indistinct. A papilla on leg I undetectable in PCM and a papilla on leg IV present, but scarcely visible. Claws I–IV of similar heights. External claws on all legs smooth. Internal claws with minuscule, thin spurs positioned at ca. 1/5 of the claw height. (Fig. 3A, insert).

Mature male (i.e. the second or latter instar; measurements in Table 4). No significant differences from females (Fig. 3B). Gonopore circular.

Juveniles (i.e. the second instar; measurements in Table 5). A morphometric gap exists between adult females and juveniles. Phenotypically similar to adults. Gonopore absent.

Larvae. Unknown.

Eggs. Unknown.

DNA sequences. Single haplotypes in 18S rRNA (MT645084, 900 bp), 28S rRNA (MT645082, 754 bp) and ITS-1 (MT645086, 622 bp), but two in COI (MT644270-1, 510 bp) were found.

Phenotypic differential diagnosis. The species was compared with the members of the *suillus-facettalis* complex (with hemispherical cephalic papillae) and other *Pseude-*

Table 5. Measurements [in µm] of selected morphological structures of juveniles of *Pseudechiniscus shintai* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure amongst all measured specimens; SD – standard deviation).

CHARACTER	N	RANGE						MEAN		SD	
		µm			*sp*			µm	*sp*	µm	*sp*
Body length	6	93	–	172	*597*	–	*730*	144	*682*	27	*49*
Scapular plate length	7	13.2	–	24.1		–		21.7	–	3.6	–
Head appendages lengths											
Cirrus *internus*	7	5.2	–	8.9	*22.7*	–	*37.4*	7.1	*31.2*	1.2	*5.2*
Cephalic papilla	6	2.0	–	4.3	*11.9*	–	*18.4*	3.0	*14.5*	0.8	*2.2*
Cirrus *externus*	7	6.1	–	12.1	*41.9*	–	*51.7*	10.0	*46.8*	1.9	*3.6*
Clava	2	3.9	–	4.4	*17.6*	–	*18.8*	4.2	*18.2*	0.4	*0.8*
Cirrus *A*	4	17.1	–	26.0	*84.2*	–	*111.1*	22.2	*96.8*	3.7	*11.0*
Cirrus *A*/Body length ratio	3	12%	–	16%		–		14%	–	2%	–
Papilla on leg IV length	4	2.9	–	3.9	*12.6*	–	*16.5*	3.3	*14.3*	0.5	*1.6*
Claw 1 heights											
Branch	5	5.9	–	7.8	*30.5*	–	*44.7*	7.1	*34.9*	0.7	*5.6*
Spur	5	0.9	–	1.7	*5.9*	–	*7.4*	1.4	*6.7*	0.3	*0.5*
Spur/branch length ratio	5	15%	–	23%		–		20%	–	3%	–
Claw 2 heights											
Branch	6	6.7	–	8.9	*30.1*	–	*37.2*	7.7	*33.2*	0.8	*2.3*
Spur	6	1.1	–	1.7	*5.1*	–	*7.1*	1.4	*5.9*	0.2	*0.8*
Spur/branch length ratio	6	16%	–	20%		–		18%	–	2%	–
Claw 3 heights											
Branch	6	5.3	–	9.0	*29.2*	–	*40.2*	7.2	*34.3*	1.2	*4.0*
Spur	6	1.0	–	1.8	*5.5*	–	*7.6*	1.4	*6.7*	0.3	*0.9*
Spur/branch length ratio	6	18%	–	21%		–		19%	–	1%	–
Claw 4 heights											
Branch	5	7.2	–	9.0	*35.5*	–	*39.3*	8.4	*37.2*	0.7	*1.4*
Spur	5	1.4	–	2.1	*6.0*	–	*9.2*	1.7	*7.5*	0.3	*1.2*
Spur/branch length ratio	5	16%	–	23%		–		20%	–	3%	–

chiniscus species lacking pseudosegmental projections. *P. shintai* sp. nov. is differentiated from:

1. *P. angelusalas* Roszkowska et al., 2020, described from Madagascar, by the shape of its cephalic papillae (hemispherical in *P. shintai* sp. nov. vs. dactyloid, elongated in *P. angelusalas*) and by the presence of *striae* (*striae* absent in *P. shintai* sp. nov. vs. present, but poorly developed in *P. angelusalas*);
2. *P. beasleyi* Li et al., 2007, described from Qinling Mountains (China), by much shorter claws (5.3–9.2 µm in *P. shintai* sp. nov. vs. 9.1–13.1 µm in *P. beasleyi*);
3. *P. chengi* Xue et al., 2017, described from Ningxia (China), by body colour (orange in *P. shintai* sp. nov. vs. brown in *P. chengi*) and by the distribution of pillars in the dorsal plates (sparsely distributed in *P. shintai* sp. nov. vs. densely arranged in *P. chengi*);
4. *P. dastychi* Roszkowska et al., 2020, described from the Argentine Islands (maritime Antarctic), by the shape of the cephalic papillae (hemispherical in *P. shintai* sp. nov. vs. dactyloid, elongated in *P. dastychi*) and by the presence of *striae* (*striae* absent in *P. shintai* sp. nov. vs. present in *P. dastychi*);
5. *P. ehrenbergi* Roszkowska et al., 2020, described from Northern Italy and reported from Mongolia (Cesari et al. 2020), by the subdivision of the scapular plate (without the median longitudinal suture in *P. shintai* sp. nov. vs. with the median longitudinal suture in *P. ehrenbergi*) and by the presence of a rudimentary papilla I (absent in *P. shintai* sp. nov. vs. present in *P. ehrenbergi*);
6. *P. indistinctus* Roszkowska et al., 2020, described from Norway, by the shape of its cephalic papillae (hemispherical in *P. shintai* sp. nov. vs. dactyloid, elongated in *P. indistinctus*) and by the presence of *striae* (*striae* absent in *P. shintai* sp. nov. vs. present in *P. indistinctus*);
7. *P. lacyformis* Roszkowska et al., 2020, described from Norway, by the length of its cephalic appendages: *cirrus internus* (5.2–8.9 µm in *P. shintai* sp. nov. vs. 10.6–14.0 µm in *P. lacyformis*), *cirrus externus* (6.1–12.8 µm in *P. shintai* sp. nov. vs. 14.1–19.4 µm in *P. lacyformis*) and cirrus *A* (17.1–26.0 µm in *P. shintai* sp. nov. vs. 26.5–35.0 µm in *P. lacyformis*);
8. *P. suillus* (Ehrenberg, 1853), reliably recorded only from Italy (Grobys et al. 2020), by the length of cirrus *A* (17.1–26.0 µm in *P. shintai* sp. nov. vs. 28.4–34.4 µm in *P. suillus*) and by the presence of males (present in *P. shintai* sp. nov. vs. absent in *P. suillus*);
9. *P. xiai* Wang et al., 2018, described from Ningxia (China), by the contrasting dorsal sculpturing (epicuticular ornamentation darker and more pronounced in *P. xiai*) and by the morphology of the pseudosegmental plate IV' (paired in *P. shintai* sp. nov. vs. unpaired in *P. xiai*).

Moreover, the ventral pattern distinguishes *P. shintai* sp. nov. from all other species for which this character has been described. We used morphometric differences for comparisons only as a last resort as sample sizes for the majority of the specimens in the type series were small. Importantly, although Roszkowska et al. (2020) included

P. angelusalas, *P. dastychi* and *P. indistinctus* in the *suillus-facettalis* complex, such combination is phylogenetically unjustified, as they all exhibit elongated (dactyloid) cephalic papillae, which is a distinguishing trait of *P. novaezeelandiae* (Richters, 1908) (see Pilato et al. 2005) and of the entire *novaezeelandiae* lineage (Cesari et al. 2020).

Genotypic differential diagnosis. *p*-distances between the new species and the remaining *Pseudechiniscus* spp., for which COI sequences are available, ranged between 18.6% (*P. suillus*) and 29.3% (*P. lacyformis*). Intraspecific distance was equal to 0.2%.

Discussion

The dorsal sculpturing of *P. asper* is particularly interesting morphologically, as large, roughly circular endocuticular pillars protrude through the epicuticle as isolated, solid bumps, unconnected by thin ridges – *striae*. In many other *Pseudechiniscus* species, *striae* are typical elements of the armour (e.g. Pilato et al. 2004, Pilato and Lisi 2006). Tumanov (2020) suggested that their presence may represent a phylogenetic signal and, as *striae* are absent in *P. suillus* (Grobys et al. 2020), this could mean that the absence of *striae* is a trait specific to the *suillus-facettalis* lineage (Cesari et al. 2020). The hypothesis would necessitate a comprehensive analysis of the sculpturing amongst the entire suite of species.

The recent studies on *Pseudechiniscus* imply that all previous records of putatively cosmopolitan species should be questioned and verified to ensure against misidentification (Grobys et al. 2020, Tumanov 2020). This is the case for almost all *Pseudechiniscus* spp. reported from Japan: *P. suillus*, *P. bartkei* Węglarska, 1962, *P. facettalis* Petersen, 1951, *P. pseudoconifer* Ramazzotti, 1943 and *P. ramazzottii* Maucci, 1952 (see Suzuki 2017). As the Japanese fauna of the four main islands is considered to be a part of the Palaearctic with high levels of endemism in many animal groups due to the isolation during glaciation periods (Motokawa 2017), it cannot be excluded that some of the above-mentioned species inhabit the Japanese archipelago (all but *P. bartkei* were described from the Western Palaearctic and Greenland). To confirm their status as native to Japan, re-descriptions must be prepared and an enhanced sampling effort is required in Japan.

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