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1 **A new sexually dimorphic *Diploechiniscus* species (Tardigrada: Echiniscidae) from**
2 **Calvert Island (British Columbia, Canada)**

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22
23 **Abstract**

24 An important, but generally overlooked source in intraspecific variability of characters used in
25 tardigrades taxonomy is sexual dimorphism. Dioecious tardigrades species with unisexual or
26 bisexual populations are common, but external sexual dimorphism is rarely observed. The
27 discovery and integrative analysis of a *Diploechiniscus* species population with high sexual
28 dimorphism from Calvert Island (Canada) has allowed its description as a new species.
29 *Diploechiniscus dimorphus* n. sp. is characterized by a different chaetotaxy in males and
30 females, and by enlarged cephalic sensory appendages in the males. The discovery of this new
31 species shows that it is important to consider sexual dimorphism as a source of intraspecific
32 variability in tardigrade taxonomy due to its implications on the use of traits commonly used
33 to delineate species such as chaetotaxy. Lastly, the finding of individuals of *Diploechiniscus*
34 *horningi*, a species previously synonymized with *Diploechiniscus oihonnae*, allows us to prove
35 that it is genetically a separate species and to reinstate it.

36
37 **Keywords**

38 Echiniscidae, Tardigrada, sexual dimorphism, British Columbia, integrative taxonomy

39
40 **Introduction**

41
42 Tardigrades are a phylum of microinvertebrates known for their ability to resist desiccation.
43 However, our knowledge of tardigrade alpha diversity remains incomplete and new species are
44 discovered every year, in particular due to advances in our understanding of their intra- and
45 interspecific variability. Tardigrades are found all over the world in marine and limno-
46 terrestrial habitats (Schill 2018). Currently, there are around 1400 officially recognized

47 tardigrade species (Guidetti and Bertolani 2005; Degma and Guidetti 2007, 2023), and this
48 number increases annually. In particular, the number of tardigrade species reported using
49 integrative taxonomy has also increased in recent years. The collection of data from such
50 integrative investigations allowed for a more in-depth examination of phylogenetic
51 relationships and species delimitations, and its utility has been repeatedly demonstrated (e.g.,
52 Surmacz et al. 2019; Bochnak et al. 2020; Kayastha et al. 2020; Tumanov 2020; Guidetti et
53 al. 2021; Vecchi et al. 2023a).

54 The history of tardigrade taxonomy in Canada is summarized in Vecchi et al. (2022a,b) and
55 Massa et al. (2024). This first Canadian surveys were published in the early twentieth-century.
56 Richters (1908), identified three species collected from Vancouver, British Columbia during a
57 Pacific expedition from 1896 to 1897 and Murray (1910), collected 31 species in Canada,
58 although opportunities for collecting were limited due to a hurried journey across that part of
59 the North American continent. To date, 60 species are known from British Columbia
60 (Kaczmarek et al. 2016; Kayastha et al. 2021; Vecchi et al. 2022a,b), of which three
61 Echiniscidae (Heterotardigrada) have their type locality in the province: *Echiniscus canadensis*
62 Murray, 1910, *Echiniscus sylvanus* Murray, 1910, and *Echiniscus reymondi* Marcus, 1928
63 (originally identified by Murray 1910 as “*Echiniscus*, sp. ? (Plate XX. Fig. 45)” and described
64 by Marcus). Other echiniscids have been found: Murray (1910) identified *Hypechiniscus*
65 *gladiator* (Murra 1905), *Bryochoerus intermedius* (Murray 1910), *Diploechiniscus oihonnae*
66 (Richters 1903), *Echiniscus bisetosus* (now synonymized with *Echiniscus blumi* Richters
67 1903), and finally two undescribed *Echiniscus* species (“*Echiniscus*, sp. ? (Plate XX. Fig. 44)”
68 and (“*Echiniscus*, sp. ? (Plate XX. Fig. 46)”). Years later, Kathman and Dastych (1990) and
69 Kathman and Cross (1991) performed a faunistic survey of tardigrades in Vancouver Island
70 and recorded *Diploechiniscus horningi* (Schuster and Grigarick 1971), *Claxtonia mauccii*
71 (Ramazzotti 1956), *Claxtonia wendti* (Richters 1903), *Acanthechiniscus goedeni* (Grigarick,
72 Mihelčič and Schuster 1964), *Echiniscus quadrispinosus* Richters 1902, *Pseudechiniscus*
73 *suillus* (Ehrenberg 1853), and *Testechiniscus laterculus* (Schuster, Grigarick and Toftner
74 1980). Most recently, Vecchi et al. (2022b) and Vecchi et al. (2023b) reported
75 *Acanthechiniscus goedeni*, *Diploechiniscus* sp., and *Hypechiniscus gladiator*.

76 *Diploechiniscus* Vicente, Fontoura, Cesari, Rebecchi, Guidetti, Serrano and Bertolani 2013 is
77 a genus of armoured tardigrades (Echiniscidae) erected for the species *Echiniscus oihonnae*
78 Richters 1903 by Vicente et al. (2013) based on both morphological (dark eyes, double
79 sculptured cuticle, median plates m1 and m2 subdivided) and molecular characters. In the same
80 paper, *Echiniscus multispinosus* Da Cunha 1944 was designated as a junior synonym of *D.*
81 *oihonnae*. Up to date *Diploechiniscus* contains three species *D. oihonnae*, *D. polygonalis* (Ito
82 1993) and *D. laterosetosus* (Ito 1993), recently added to the genus (Gąsiorek 2023). The genus
83 was also briefly populated by another species, *D. horningi*, after Gąsiorek et al. (2019) moved
84 it from *Echiniscus* to *Diploechiniscus*. This situation did not last long, as Kaczmarek et al.
85 (2021) synonymized *D. horningi* with *D. oihonnae* and established a neotype for the latter
86 species. Both Vicente et al. (2013) and Kaczmarek et al. (2021) pointed out the variability of
87 chaetotaxy (i.e. the arrangement and shape of the body thin appendages, e.g. spines, filaments)
88 of *D. oihonnae*, both in the presence/absence of a spine in B^d (the character originally used to
89 differentiate *E. oihonnae* from *E. multispinosus*), not linked to any developmental stage or sex

90 (Kaczmarek et al. 2021), and in the presence of anormal individuals (e.g., without any
91 dorsolateral filaments; Kaczmarek et al. 2021).

92 During a tardigrade survey of Calvert Island (British Columbia, Canada), we found individuals
93 of two new species genetically clearly affiliated with *Diploechiniscus*, but morphologically
94 well differentiated from all other species in the genus. One of the new species is also
95 remarkable in showing sex-specific differences in chaetotaxy. We also found individuals from
96 Vancouver Island morphologically similar to *D. oihonnae*, but belonging to a different species
97 according to COI sequences.

98 In this paper, we present an integrative description of a new sexually dimorphic
99 *Diploechiniscus* species from Calvert Island (British Columbia) based on DNA sequencing and
100 morphological examination, and we reinstate *D. horningi* based on individuals from Vancouver
101 Island (British Columbia).

102

103 **Material and Methods**

104

105 *Samples and specimens*

106 Tardigrade specimens were recovered from moss samples growing on a vertical rock outcrop
107 near the Hakai Institute Calvert Island Field Station in 2021 and 2022 (Table 1). The Hakai
108 field Station is located within the Hakai Lúxvbális Conservancy of the Province of British
109 Columbia on the central coast of British Columbia. The central coast of British Columbia
110 extends from approximately the north end of Aristazabal Island at ~52°49' N to the entrance
111 to Queen Charlotte Strait at ~50°59' N, excluding the offshore Haida Gwaii archipelago
112 (Lindstrom et al. 2021). Additional moss samples were obtained during a survey of the Mount
113 Arrowsmith region, Vancouver Island by the Royal BC Museum Department of Botany.

114 Samples were examined for tardigrades using the protocol by Degma (2018) and the animals
115 were divided into three groups for specific analyses, i.e., morphological analysis in light
116 microscopy (LM) using phase contrast (PhC), SEM (Scanning Electron Microscopy) and DNA
117 sequencing (see results for details).

118

119 **Table 1.** Sampling data

Sample	Locality	Coordinates	Collection date	Collector	Sample type
S1911	Calvert Island, Lookout	51.651144 - 128.138404	29/06/2021	Gillian Sadlier- Brown	Moss on ground
S2075	Vancouver Island, Mount Arrowsmith	49.233972 - 124.608917	08/08/2022	Kendrick Marr	Moss on tree bark
S2085	Calvert Island, Lookout	51.651144 - 128.1384038	23/06/2022	Kelly Fretwell	Moss on stone

120

121 *Microscopy and Imaging*

122 The specimens for LM were mounted on microscope slides in a small drop of Hoyer's medium,
123 secured with a cover slip and dried at 60 °C for a week. The slides were examined with an
124 Olympus BX53 or a Leica DM RB microscope (both with PhC) associated with an AmScope
125 MU1803 digital camera, the figures were assembled in Adobe Photoshop CS4 Extended
126 (Adobe System Corporation).

127

128 *Morphometrics and morphological nomenclature*

129 Structures were measured according to Kaczmarek et al. (2021) and only if their orientation
130 was suitable. Body length was measured from the anterior extremity to the posterior end of the
131 body, excluding the hind legs. The morphometric data were handled using the Echiniscoidea
132 ver. 1.4 template available from the Tardigrada Register, <http://tardigrada.net/register> modified
133 to include the claw spur insertion height (DeMilio et al. 2022). Raw measurements are available
134 in cjz-2024-0040suppla and cjz-2024-0040supplb.

135

136 *Genotyping*

137 DNA was extracted from individual animals following the Chelex® 100 resin extraction
138 method (BioRad) by Casquet et al. (2012) with modifications described in detail in Stec et al.
139 (2020). Four DNA fragments, three nuclear (18S rRNA, 28S rRNA, ITS1) and one
140 mitochondrial (COI) were sequenced. Fragments were amplified and sequenced according to
141 the primers and protocols described in Stec et al. (2020) (18S rRNA, 28S rRNA, COI) and
142 Gąsiorek (2022) (ITS1). Sequencing products were read with an ABI 3130xl sequencer at the
143 Department of Biological and Environmental Sciences (University of Jyväskylä, Finland).

144

145 *Phylogenetic reconstruction*

146 The phylogenetic analyses were conducted using concatenated
147 18SrRNA+28SrRNA+COI+ITS1 sequences. The GenBank accession numbers of the newly
148 generated sequences and those used in the phylogenetic reconstruction are presented in Table
149 2. Few *Diploechiniscus oihonnae* 18S rRNA sequences (GenBank accession numbers
150 JX114910 and JX676181-6) and a 28S rRNA sequence (JX114869) were not considered as
151 they did not overlap with the other sequences in the alignment. The 18S rRNA, 28S rRNA,
152 and ITS1 sequences were aligned with MAFFT ver. 7 (Katoh 2002; Katoh and Toh 2008) with
153 the G-INS-i method (thread=4, threadb=5, threadit=0, reorder, adjustdirection, anysymbol,
154 maxiterate=1000, retree 1, globalpair input). The COI sequences were aligned according to
155 their amino acid sequences (translated using the invertebrate mitochondrial code) with the
156 MUSCLE algorithm (Edgar 2004) in MEGA11 (Tamura et al. 2021) with default settings (all
157 gap penalties=0, max iterations=8, clustering method=UPGMB, lambda=24). Alignments were
158 visually inspected and trimmed in MEGA7. Sequences were concatenated with the R package
159 ‘concatipede’ v1.0.0 (Vecchi and Bruneaux 2021). The Maximum Likelihood (ML)
160 phylogenetic reconstruction was performed on the partitioned alignment with IQ-tree
161 (Trifinopoulos et al. 2016) on the W-IQ-TREE online web server with default settings. For the
162 Bayesian Inference (BI) phylogenetic reconstruction, model selection was performed for each
163 alignment partition (six in total: 18S rRNA, 28S rRNA, ITS1 and three COI codons) with
164 PartitionFinder2 (Lanfear et al. 2016). BI phylogenetic reconstruction was done with MrBayes
165 v3.2.6 (Ronquist et al. 2012). Two runs with one cold chain and three heated chains were run
166 for 50 million generations with a burning of 5 million generations, sampling a tree every 50000
167 generations. Posterior distribution sanity was checked with the Tracer v1.7 (Rambaut et al.,
168 2018). MrBayes input file with the input alignment (and partitions models) is available as
169 Supplementary Materials (cjz-2024-0040supplc). The phylogenetic trees were visualized with
170 FigTree v1.4.4 (Rambaut 2007) and the image was edited with Inkscape 0.92.3 (Inkscape

171 Project 2020). The complete phylogenetic trees are available in cjz-2024-0040suppld and cjz-
 172 2024-0040supple.

173

174 **Table 2.** GenBank accession numbers of the sequences used in the phylogenetic reconstruction.

Tip name	SSU	LSU	COI	ITS1	References
<i>Acanthechiniscus goedeni</i> (Grigarick, Mihelčič & Schuster, 1964)	OP730 698	OP730 697	OP729 912		Vecchi et al. (2023b)
<i>Antechiniscus lateromamillatus</i> (Ramazzotti, 1964)	HM19 3370		HM19 3404		Jørgensen et al. (2011)
<i>Barbaria bigranulata</i> (Richters, 1907)	MZ820 792	MZ820 810	MZ820 846	MZ820 828	Gąsiorek et al. (2022)
<i>Barbaria danieli</i> (Meyer, Tsaliki & Sorgee, 2017)	MZ820 800	MZ820 818	MZ820 853	MZ820 836	Gąsiorek et al. (2022)
<i>Barbaria madonnae</i> (Michalczyk & Kaczmarek, 2006)	MZ820 803	MZ820 821	MZ820 854	MZ820 839	Gąsiorek et al. (2022)
<i>Barbaria ollantayamboensis</i> (Nickel, Miller & Marley, 2001)	MZ820 804	MZ820 822	MZ820 855	MZ820 840	Gąsiorek et al. (2022)
<i>Barbaria paucigranulata</i> Wilamowski, Vončina, Gąsiorek & Michalczyk, 2022	MZ820 808	MZ820 826	MZ820 859	MZ820 844	Gąsiorek et al. (2022)
<i>Barbaria weglarskae</i> Gąsiorek, Wilamowski, Vončina & Michalczyk, 2022	MZ820 809	MZ820 827	MZ820 860	MZ820 845	Gąsiorek et al. (2022)
<i>Bryodelphax australasiaticus</i> Gąsiorek, Vončina, Degma & Michalczyk, 2020a	MT333 468	MT333 460		MT333 477	Gąsiorek et al. (2020a)
<i>Bryodelphax decorates</i> Gąsiorek, Vončina, Degma & Michalczyk, 2020a	MT333 469	MT333 462		MT333 478	Gąsiorek et al. (2020a)
<i>Bryodelpha maculatus</i> Gąsiorek, Stec, Morek, Marnissi & Michalczyk, 2017	MT333 471	MT333 464		MT333 479	Gąsiorek et al. (2020a)
<i>Bryodelphax nigripunctatus</i> Degma, Gąsiorek, Vončina & Michalczyk, 2020	MT333 472	MT333 465			Gąsiorek et al. (2020a)
<i>Cornechiniscus cornutus</i> (Richters, 1907)	MT420 869	MT420 853	MT420 439	MT420 860	Gąsiorek and Michalczyk, (2020)
<i>Cornechiniscus imperfectus</i> Gąsiorek & Michalczyk, 2020	MT420 872	MT420 856	MT420 451	MT420 865	Gąsiorek and Michalczyk, (2020)
<i>Cornechiniscus madagascariensis</i> Maucci, 1993	MT420 871	MT420 855	MT420 450	MT420 863	Gąsiorek and Michalczyk, (2020)
<i>Cornechiniscus subcornutus</i> Maucci & Ramazzotti, 1981	MT420 873	MT420 857	MT420 457	MT420 867	Gąsiorek and Michalczyk, (2020)
<i>Diploechiniscus oihonnae</i> 1 Type loc.	MT416 481	MT416 552	MT418 513		Kaczmarek et al. (2021)
<i>Diploechiniscus oihonnae</i> 2			MW71 5563		Morek et al. (2021)
<i>Diploechiniscus oihonnae</i> 3	MW13 6887	MW13 6923		MT374 201	Bochnak et al. (2020); Morek et al. (2021)
<i>Diploechiniscus oihonnae</i> 4			MG06 3724		Gąsiorek et al. (2017)
<i>Diploechiniscus oihonnae</i> 5			JX676 198		Vicente et al. (2013)
<i>Diploechiniscus oihonnae</i> 6			JX676 197		Vicente et al. (2013)
<i>Diploechiniscus oihonnae</i> 7			JX676 196		Vicente et al. (2013)
<i>Diploechiniscus oihonnae</i> 8			JX676 195		Vicente et al. (2013)
<i>Diploechiniscus oihonnae</i> 9			JX676 194		Vicente et al. (2013)
<i>Diploechiniscus oihonnae</i> 10			JX676 193		Vicente et al. (2013)
<i>Diploechiniscus oihonnae</i> 11			JX676 192		Vicente et al. (2013)
<i>Diploechiniscus oihonnae</i> 12			JX676 191		Vicente et al. (2013)
<i>Echiniscus aonikenk</i> Gąsiorek, Bochnak, Vončina & Michalczyk, 2021	MZ467 756	MZ467 762	MZ444 670	MZ467 821	Gąsiorek et al. (2021a)
<i>Echiniscus azoricus</i> Fontoura, Pilato & Lisi, 2008	MK52 9673	MK52 9701			Gąsiorek et al. (2019)
<i>Echiniscus dariae</i> Kaczmarek & Michalczyk, 2010	MK52 9677	MK52 9705			Gąsiorek et al. (2019)
<i>Echiniscus insularis</i> Gąsiorek, Vončina & Kiosya, 2021	MW18 0887	MW18 0879	MW17 8242	MW18 0910	Kiosya et al. (2021)

<i>Echiniscus masculinus</i> Gąsiorek, Vončina & Michalczyk, 2020	MT106 621	MT106 620	MT106 223	MT106 623	Gąsiorek et al. (2020b)
<i>Echiniscus pellucidus</i> Gąsiorek, Bochnak, Vončina & Michalczyk, 2021	MZ467 759	MZ467 765	MZ444 675	MZ467 828	Gąsiorek et al. (2021a)
<i>Echiniscus succineus</i> Gąsiorek & Vončina, 2019	MK67 5903	MK67 5914	MK64 9675	MT374 198	Bochnak et al. (2020)
<i>Echiniscus tantulus</i> Gąsiorek, Bochnak, Vončina & Kristensen, 2020	MT126 785	MT126 765	MT107 427	MT108 138	Bochnak et al. (2020)
<i>Echiniscus testudo</i> (Doyère, 1840)	MK52 9686	MK52 9716		MT374 199	Gąsiorek et al. (2019); Bochnak et al. (2020)
<i>Hypechiniscus cataractus</i> Gąsiorek, Oczkowski, Kristensen & Michalczyk, 2021	MT809 235	MT809 198		MT809 182	Gąsiorek et al. (2021b)
<i>Hypechiniscus daedalus</i> Gąsiorek, Oczkowski, Bartels, Nelson, Kristensen & Michalczyk, 2021	MT809 237	MT809 200		MT809 187	Gąsiorek et al. (2021b)
<i>Hypechiniscus exarmatus</i> (Murray, 1907)	MT809 238	MT809 201		MT809 190	Gąsiorek et al. (2021b)
<i>Hypechiniscus flavus</i> Gąsiorek, Oczkowski, Suzuki, Kristensen & Michalczyk, 2021	HM19 3377		HM19 3410		Jørgensen et al. (2011)
<i>Hypechiniscus geminus</i> Gąsiorek, Oczkowski, Suzuki, Kristensen & Michalczyk, 2021	HM19 3378		HM19 3411		Jørgensen et al. (2011)
<i>Hypechiniscus gladiator</i> (Murray, 1905)	MT809 243	MT809 203			Gąsiorek et al. (2021b)
<i>Kristenseniscus tessellatus</i> (Murray, 1910)	MK52 9690	MK52 9720			Gąsiorek et al. (2019)
<i>Kristenseniscus walteri</i> (Pilato & Lisi, 2003)	MK52 9691	MK52 9721			Gąsiorek et al. (2019)
<i>Mopsechiniscus granulatus</i> Mihelčič, 1967	HM19 3379		HM19 3412		Jørgensen et al. (2011)
<i>Nebularmis auratus</i> Gąsiorek & Michalczyk, 2021	MW18 0881	MW18 0904	MW17 8237	MW18 0893	Gąsiorek et al. (2021c)
<i>Nebularmis burmensis</i> Gąsiorek & Vončina, 2021	MW18 0882	MW18 0905	MW17 8238	MW18 0894	Gąsiorek et al. (2021c)
<i>Nebularmis cirinoi</i> (Binda & Pilato, 1993)	MK52 9692	MK52 9722			Gąsiorek et al. (2019)
<i>Nebularmis indicus</i> Gąsiorek, Ciosek & Michalczyk, 2021	MW18 0886	MW18 0909	MW17 8240	MW18 0897	Gąsiorek et al. (2021c)
<i>Oreella chugachii</i> Calloway, Miller, Johansson & Whiting, 2011	GU552 458				Calloway et al. (2011)
<i>Oreella mollis</i> Murray, 1910	EU266 962				Sands et al. (2008)
<i>Parechiniscus chitonides</i> Cuénot, 1926	HM19 3380		HM19 3413		Jørgensen et al. (2011)
<i>Proechiniscus hannaee</i> (Petersen, 1951)	HM19 3381		HM19 3414		Jørgensen et al. (2011)
<i>Pseudechiniscus angelusalas</i> Roszkowska, Grobys, Bartylak & Kaczmarek, 2020	MW03 2006	MW03 2096	MW03 1217	MW03 2186	Gąsiorek et al. (2021f)
<i>Pseudechiniscus dreyeri</i> Gąsiorek, Vončina, Kristensen & Michalczyk, 2021	OK048 615	OK048 633	OK047 278	OK048 646	Gąsiorek et al. (2021d)
<i>Pseudechiniscus quadrilobatus</i> Iharos, 1969	MW03 1946	MW03 2035	MW03 1178	MW03 2125	Gąsiorek et al. (2021f)
<i>Pseudechiniscus shintai</i> Vončina, Kristensen & Gąsiorek, 2020	MT645 084	MT645 082	MT644 270	MT645 086	Vončina et al. (2020)
<i>Pseudechiniscus totoro</i> Gąsiorek, Vončina, Kristensen & Michalczyk, 2021	OK048 616	OK048 635		OK048 648	Gąsiorek et al. (2021d)
<i>Testechiniscus spitzbergensis spitzbergensis</i> (Scourfield, 1897)	MH27 9664	MH28 6188		MH28 6189	Gąsiorek et al. (2018a)
<i>Testechiniscus spitzbergensis tropicalis</i> Gąsiorek, Stec, Zawierucha, Kristensen & Michalczyk, 2018	MH27 9665	MH28 6187		MH28 6191	Gąsiorek et al. (2018a)
<i>Testechiniscus laterculus</i> (Schuster, Grigarick & Tofner, 1980)	OQ029 311		OQ029 483		Massa et al. (2024)
<i>Viridiscus</i> aff. <i>viridianus</i>	MZ868 197	OK094 230	MZ852 064	OK094 211	Gąsiorek et al. (2021e)
<i>Viridiscus perviridis</i> (Ramazzotti, 1959)	MK52 9696	MK52 9726			Gąsiorek et al. (2021e)
<i>Viridiscus viridissimus</i> (Péterfi, 1956)	MZ868 194	OK094 225	MZ852 051	OK094 195	Gąsiorek et al. (2021e)
<i>Diploechiniscus</i> aff. <i>dimorphus</i> [Ca. 1 OR859978] S2010 v06	OQ029 306		OQ029 482		Massa et al. (2024)
<i>Diploechiniscus</i> aff. <i>dimorphus</i> [Ca. 1 OR859978] S2010 v03	OQ029 307				Massa et al. (2024)
<i>Diploechiniscus</i> aff. <i>dimorphus</i> [Ca. 1 OR859978] S2085 T4	OR853 682		OR859 977		This study

<i>Diploechiniscus</i> aff. <i>dimorphus</i> [Ca. 1 OR859978] S1911 Dpl1	OR853 684	OR853 686	OR859 978		This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 Dpl2	OR853 683	OR853 687	OR859 979		This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 Dpl5			OR859 980		This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 v1m			OR859 981	OR853 688	This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 v3m			OR859 982	OR853 692	This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 v4f			OR859 983	OR853 693	This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 5m			OR859 984	OR853 690	This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 v6f			OR859 985	OR853 694	This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 v7f			OR859 986	OR853 689	This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 v8f			OR859 987	OR853 691	This study
<i>Diploechiniscus dimorphus</i> n. sp. S1911 v11m			OR859 988	OR853 695	This study
<i>Diploechiniscus horningi</i> S2075 Dpl1	OR853 685		OR859 976		This study

175

176

177 *Species delimitation analysis*

178 A subset of the COI alignment (only sequences from *Diploechiniscus*, plus *Testechiniscus*
179 Kristensen 1987 as outgroup) was used for species delimitation. Species delimitation was
180 performed with ASAP (Puillandre et al., 2021) and bPTP (Zhang et al. 2013). ASAP species
181 delimitation was performed on the p-distance matrix of the alignment on the ASAP online
182 server (Puillandre et al. 2021) with default parameters (complete results are available as cjz-
183 2024-0040supplaf). bPTP species delimitation was performed on the bPTP online server with
184 default parameters using as input a phylogenetic tree obtained from the COI reduced alignment
185 following the same pipeline as above (complete results are available as cjz-2024-0040supplg).
186 Additionally, p-distances between the sequences used in the species delimitation analyses were
187 calculated with MEGA11 (with pairwise deletion) (Tamura et al. 2021) (cjz-2024-0040supplh).
188

189 *Other comparative material*

190 Slides with *Echiniscus* cf. *robertsi* (Slides S2010_SL1 – C5084s1 and S2010.Ech.V03-6 –
191 C5084v1-2) and *Diploechiniscus* sp. (Slide S2013_SL1 – C5087s1) (Massa et al. 2024) from
192 Haida Gwaii Island (British Columbia) were examined.
193

194 **Results**

195

196 *Morphology*

197 From samples S1911 and S2085, Echiniscidae with constant dorsal plates ornamentation
198 (described in detail below in the taxonomic account section) but variable chaetotaxy were
199 identified. The individuals were divided in six morphotypes based on their chaetotaxy and claw
200 types: α) Individuals with only dorsolateral spicules D; β) Individuals with only lateral
201 filaments D; γ) Individuals with both dorsolateral spicules D and lateral filaments D; δ)
202 Individuals with lateral filaments in B (not always), C and D; ε) Individuals without
203 appendages (other than in A) and four claws per leg; ζ) Individuals without appendages (other

204 than in A) and two claws per leg. The most common morphotypes were α and β , whereas the
 205 others were less represented (Table 3). All the sequenced specimens with α morphotype
 206 resulted to be females, whereas those with the β morphotypes were males.

207

208 **Table 3.** Number of individuals found for each main morphotype in samples S1911 and
 209 S2085 (individuals mounted on stubs for SEM are not included in this count). The letters in
 210 parenthesis refer to the chaetotaxy of the specimens.

Morphotype (chaetotaxy)	Females	Males	Juveniles*	Undetermined
α (A-D ^d)	19		2	
β (A-D ^l)		27	1	
γ (A-D ^d -D ^l)		1	1	4
δ (A-(B ^l)-C ^l -D ^l)	2	2		
ϵ (A)				8
ζ (A) ^o			2	

211 * Individuals without gonopore; ^o this morphotype corresponds to 1st instar (or 2-clawed) larvae.

212

213

214 *DNA extraction, phylogenetic reconstruction and species delimitation*

215 The DNA extraction and amplification was successful for 13 individuals (11 from sample
 216 S1911, one from sample S2085, one from sample S2075). However, the sequencing, recovery
 217 and mounting of carcasses were only successful for eight individuals from the α and β
 218 morphotypes from sample S1911 and for the individual from S2075.

219 The phylogenetic reconstructions (Fig. 1) recovered the Echiniscidae from S1911 and S2085
 220 in the *Diploechiniscus* clade together *D. oihonnae* and *D. horningi* and with *Diploechiniscus*
 221 specimens previously sequenced by Massa et al. (2024) (*Echiniscus* cf. *robertsi*; sample
 222 S2010). Specimens identified as *D. horningi* (from sample S2075 – see *Diploechiniscus*
 223 *horningi* section below) were recovered as separate species by both ASAP and bPTP species
 224 delimitation analyses.

225 The newly analyzed specimens of *Diploechiniscus* belonged to three distinct clades, A, B, and
 226 D; Fig. 1): A and B clades were sister groups to each other, while D (*D. horningi* specimen)
 227 was sister group of clade C (*D. oihonnae* specimens). *Diploechiniscus* of clades A (highly
 228 supported) and B were recovered as separate species by bPTP and ASAP, however both
 229 methods recover one individual from clade A (S1911-5m) as a different species, however this
 230 was probably not reliable as this individual COI distance with the other members of clade A
 231 was on average ~2.42% (cjz-2024-0040supplh). Clade A includes two sequences from
 232 GenBank (S2010-v03 and S2010-v06) (from Massa et al. 2024) for which a voucher of the
 233 carcass is available, and originally classified as *Echiniscus* cf. *robertsi* (Massa et al. 2024).

234

235 *Diploechiniscus horningi*

236 Two individuals from sample S2075 were attributed to *Diploechiniscus horningi* based on their
 237 morphology and previous records of this species on Vancouver Island (Kathman and Dastych
 238 1990) (the same island from which sample S2075 came from). One individual was used for
 239 DNA extraction and sequencing and the carcass was recovered and mounted on slide, whereas
 240 the second animal was mounted on slide. Both the phylogenetic reconstruction (Fig. 1) and the

241 species delimitation analyses (Fig. 1) indicated that *D. horningi* is a separate species respect to
242 the other species of *Diploechiniscus* including *D. oihonnae*.

243

244 Discussion

245 We identified members of the genus *Diploechiniscus* which deviate from the typical
246 morphology of the genus by having fewer number of filaments and spines, a less evident
247 double sculpture of the dorsal plates and not having visible black eyes in mounted specimens
248 (black eyes are visible in mounted specimens in *D. oihonnae*) (Vicente et al. 2013; Kaczmarek
249 et al. 2021; Gąsiorek 2023). However, these individuals are confidently assigned to this genus
250 based on their phylogenetic position and other morphological characters (see taxonomic
251 account). We are temporarily refraining from erecting a new genus for these animals due to the
252 lack of evident morphological traits that would allow its clear definition. However, this remains
253 a possibility in the future should more populations be identified, allowing for a much more
254 detailed morphological analysis.

255 Two *Diploechiniscus* species corresponding to clade A and B (Fig. 1), which co-occur
256 in one particular sample (S1911), are present based on COI species delimitation; however, due
257 to the few issues explained below, we can confidently describe only one of them (clade A) as
258 new to science, naming it *Diploechiniscus dimorphus* n. sp. (see taxonomic account).

259 For *D. dimorphus* n. sp. (clade A), we managed to obtain a good number of voucher specimens
260 linked to COI sequences, of both males and females. These individuals show a striking sex
261 dimorphism related to chaetotaxy (females with α morphotype and males with β morphotype;
262 Tab. 3). For the clade B, we could not obtain new informative voucher specimens linked to
263 COI sequences. Given that these two *Diploechiniscus* species can be mixed in a sample (i.e.
264 S1911), it is challenging to morphologically differentiate the two species (clades A and B) with
265 present available information. However, Fig. 1 shows that specimens of S1911 and S2085
266 samples of clade B are conspecific with two individuals from Haida Gwaii (sample S2010;
267 Massa et al. 2024) for which vouchers are available (even if not in great condition). The Haida
268 Gwaii individuals was found together with four other individuals with identical morphology
269 (Massa et al. 2024), thus it is possible to use these individuals to obtain reliable information on
270 the *Diploechiniscus* of clade B. Reanalysis of the specimens from Haida Gwaii (sample S2010)
271 showed that both males and females have the β morphotype, thus allowing to discriminate
272 between the two species (clades A and B) based on the females morphology only. There are
273 insufficient specimens and data to formally describe a new species from specimens of clade B
274 (4 individuals and 2 carcasses from Haida Gwaii; Massa et al. 2024), although the molecular
275 data clearly identified it as separate clade; for these reasons we identify this clade as
276 Unconfirmed Candidate Species (UCS) naming it as *Diploechiniscus* aff. *dimorphus* [Ca1
277 OR859978] (also reported as *Echiniscus* cf. *robertsi* in Massa et al. 2024).

278 From individuals of all the other morphotypes (γ , δ , ϵ) we could not get DNA sequences, and
279 as they are present in low number, for the moment we do not consider them in the taxonomic
280 description. Those specimens probably represent examples of intraspecific variability in A
281 and/or B as variability in chaetotaxy in *Diploechiniscus* is known to occur (Kaczmarek et al.
282 2021; Gąsiorek 2023). In not considering them, we are aware that we are underestimating the
283 variability of the new species. However, we prefer to be cautious pending analysis of more
284 specimens (measurements of those individuals not assigned to the new species are available as

285 cjz-2024-0040supplb). As the males of clades A and B are indistinguishable and the proportion
286 of animals of clade B is low (1 over 11 individuals) in sample S1911 (where all animals of
287 clade A are present) and based on DNA data, the morphological description of the males (and
288 juveniles/second stage larvae) of *Diploechiniscus dimorphus* n. sp. was made including all the
289 males from sample S1911. The morphotype ζ , corresponding to first stage larvae, it tentatively
290 attributed to *D. dimorphus* n. sp. because the specimens of this species are more abundant in
291 the sample (as evidenced by the molecular data), therefore the two first stage larvae found very
292 probably correspond to this species.

293 We showed that within *Diploechiniscus* there is a species (that we identified with *D.*
294 *horningi*) morphologically indistinguishable from *D. oihonnae* but well distinct from it based
295 on molecular data (both the phylogenetic reconstruction and the species delimitation analyses
296 confirm these differences, and the COI p-distances of the *D. horningi* to *D. oihonnae*
297 individuals range from 13 to 15%). Kaczmarek et al. (2021) synonymized *Diploechiniscus*
298 *horningi* to *D. oihonnae* based on morphological characters. In light of our results, together
299 with the previous record of *D. horningi* in our sampling site (Mount Arrowsmith, Vancouver
300 Island; Kathman and Dastych 1990) and the relatively close geographical proximity of our
301 sampling site to the type locality of the species in the Pacific Northwest (i.e. Silver Falls State
302 Park, OR, USA; Schuster and Grigarick 1971), we propose the reinstatement of *D. horningi* as
303 a valid species.

304 The presence of bisexual species in Echiniscidae is relatively rare, and generally there
305 are no evident sexual dimorphic characters between males and females, other than the shape of
306 the gonopore (“six-petal rosette” around the pore in female, crescent-shaped pore at the
307 extremity of a short papilla-like structure in male). In the family, other common differences
308 among sexes are related to not so evident phenotypic characters as body size (larger in female,
309 in relation to the development of larger gonads; Guidetti et al. 2007) and/or slightly differences
310 in morphometry of some structures, as claws, cephalic and trunk appendages (e.g. Dastych
311 1987; Kristensen 1987; Claxton 1996, 2001; Guidetti et al. 2007; Mitchell and Romano 2007;
312 Rebecchi et al. 2008; Gąsiorek et al. 2018a,b; Cesari et al. 2020; for a more information on
313 sexual dimorphism in the Echiniscidae see also Gasiorek et al. 2020).

314 Other sexual dimorphic characters are extremely rare. Differences in chaetotaxy
315 between sexes, as we found in *D. dimorphus* n. sp., have been previously recorded in only three
316 species among the 14 genera of Echiniscoidea with dioecious species. In particular, *Echiniscus*
317 *masculus* Gąsiorek, Vončina and Michalczyk 2020 females have a D^d spine which is absent
318 in male, *Antechiniscus parvisetus* (Horning and Schuster 1983) females have a spine in D
319 replaced by a filament in males (Claxton 2001) and *Antechiniscus moscali* Claxton 2001
320 females have small triangular spines in B, C and D often missing in males.

321 Within *Diploechiniscus*, *D. oihonnae* shows very reduced and not easily detectable
322 morphological differences between males and females, i.e. males are slender than females and
323 have a larger granulation of the genital plates than in other parts of ventral side, and there are
324 morphometric differences in few cuticular structures between sexes (Kaczmarek et al. 2021).
325 The new *Diploechiniscus* species challenges the morphological homogeneity of
326 *Diploechiniscus* as currently defined, indicating that much work is still needed to have a clear
327 and complete comprehension of armored tardigrades evolution and variability. The differences
328 in chaetotaxy in males and females of *D. dimorphus* n. sp., show that caution should be applied

329 when using chaetotaxy as a diagnostic trait in species differentiation and classification due to
330 intraspecific variation.

331

332

333 **Taxonomic account**

334

335 ***Diploechiniscus dimorphus* n. sp.**

336

337 *Diploechiniscus* sp. in Vecchi et al. (2022b)

338 *Diploechiniscus* sp. in Massa et al. (2024)

339

340 urn:lsid:zoobank.org:act:12D4E509-1F06-44ED-B2EC-0BA9A7991ECA

341

342 Figures 2-5, Tables 4-6, cjz-2024-0040suppla

343 **Type locality:** Lookout, Calvert Island, British Columbia, Canada. 51.651144 -128.138404.

344 **Etymology:** The specific epithet comes from the pronounced sexual dimorphism of this
345 species.

346 **Type material:** Holotype and 50 paratypes (on slides): two specimens of the 1st instar larvae
347 (no anus, no gonopore, two claws per leg); three specimens of 2nd instar larvae (anus, no
348 gonopore, four claws per leg); 45 adults (gonopore present, four claws per leg), of which 27
349 identified as males and 18 identified as females. In addition, 16 paratypes (six females, seven
350 males, two 2nd stage larvae, one 1st stage larvae) on stub for SEM observation.

351 **Type depositories:** The holotype (S1911-C5010.s17) and four paratypes are deposited in the
352 Tardigrada collection of the Natural History Museum of Verona, Italy (Catalogue codes of
353 slides C5010-s22,-s24,-s25,-s53), nine paratypes in Bertolani collection, University of Modena
354 and Reggio Emilia, Modena, Italy (Catalogue codes of slides C5010s1,-s31,-s35,-s38,-s61,-
355 V4,-V6,-V7,-V8), four paratypes are deposited in the tardigrade collection of the Institute of
356 Systematics and Evolution of Animals (Polish Academy) of Sciences, Kraków, Poland
357 (Catalogue codes of slides CA.075.01 – 6), and six paratypes are deposited in the Invertebrate
358 Zoology Collections of the Royal BC Museum, Victoria, Canada (Catalogue codes of slides
359 024-00019-001 – 4).

360

361 **Description**

362 Animals (measurements and statistics in Tables 4-6)

363 *Females.* (measurements and statistics in Table 4). Body orange. Eyes not visible in animals
364 mounted in Hoyer's medium. Complete set of head appendages present: internal and external
365 cirri; large, drop-shaped cephalic papillae; cirrus A; large, finger-like clava near the base of
366 cirrus A (Figs. 2A-2B). Cuticle on dorsal plates appears constituted by polygonal granules (i.e.
367 head of epicuticular pillars; about 1-1.5 µm in diameter), larger in dorsal-central portion of the
368 body (about 1 µm) (Fig. 2A); each granule generally surrounded by five to seven other granules
369 (Fig. 3A); in the scapular and terminal plates of several animals these granules are connected
370 by thin line (striae). A line of pores (that appear in LM-PhC as white circle of about 0.8 µm
371 above the polygonal dark granules) is present along the posterior and anterior margins of the
372 scapular plate (aka dorsal plate I) and anterior margin of terminal plate, few other pores are

<i>Cirrus internus</i>	11	7.7	–	14.5	30.1	–	52.3	11.8	42.0	2.3	7.9	13.1	49.0
Cephalic papilla	11	3.6	–	6.0	15.5	–	20.1	5.0	17.7	0.7	1.5	5.3	20.1
<i>Cirrus externus</i>	10	8.4	–	15.8	36.2	–	57.7	13.2	47.0	2.5	7.6	15.4	57.7
Clava	8	4.1	–	5.1	13.1	–	18.9	4.7	16.4	0.4	2.0	5.0	18.9
<i>Cirrus A</i>	9	46.2	–	72.0	164.8	–	259.1	57.0	203.0	7.3	25.9	54.9	206.3
<i>Cirrus A</i> /Body length ratio	6	36%	–	45%		–		41%	–	4%	–	38%	–
Body appendages lengths													
<i>Cirrus D^d</i>	6	1.5	–	3.2	5.4	–	9.6	2.0	6.9	0.6	1.6	1.6	5.9
<i>Cirrus E</i>	1	1.7	–	1.7	6.3	–	6.3	1.7	6.3	?	?	?	?
Spine on leg I length	5	2.3	–	3.1	8.0	–	11.2	2.7	9.0	0.3	1.3	?	?
Papilla on leg IV length	7	2.9	–	3.9	8.7	–	14.0	3.2	11.3	0.4	1.6	3.1	11.6
Number of teeth on the collar	7	5	–	7		–		5.9	–	0.7	–	7	–
Claw I heights													
Branch	7	7.5	–	8.3	24.4	–	30.0	7.8	27.3	0.3	2.0	7.8	29.1
Spur insertion	7	2.1	–	2.4	6.6	–	8.2	2.2	7.6	0.1	0.6	2.1	8.0
Spur	7	1.5	–	2.1	5.5	–	7.6	1.8	6.4	0.2	0.8	1.5	5.7
Spur/branch height ratio	7	20%	–	25%		–		23%	–	2%	–	20%	–
Claw II heights													
Branch	7	6.5	–	7.8	23.5	–	28.1	7.4	25.8	0.4	1.9	7.4	27.9
Spur insertion	7	1.6	–	2.1	5.5	–	7.8	1.8	6.4	0.2	0.9	2.1	7.8
Spur	7	1.4	–	1.8	4.7	–	6.3	1.6	5.5	0.1	0.5	1.4	5.3
Spur/branch height ratio	7	19%	–	23%		–		21%	–	1%	–	19%	–
Claw III heights													
Branch	7	6.8	–	7.9	23.8	–	28.6	7.4	25.9	0.4	1.9	7.5	28.0
Spur insertion	6	1.5	–	2.0	5.4	–	7.3	1.8	6.2	0.2	0.7	?	?
Spur	5	1.5	–	1.8	4.4	–	6.3	1.6	5.5	0.1	0.7	?	?
Spur/branch height ratio	5	19%	–	23%		–		21%	–	2%	–	?	–
Claw IV heights													
Branch	7	7.7	–	9.1	25.8	–	33.2	8.5	29.6	0.5	2.7	8.9	33.2
Spur insertion	6	2.2	–	2.7	7.1	–	9.7	2.5	8.7	0.2	1.1	?	?
Spur	6	1.7	–	2.3	6.3	–	8.1	2.0	6.9	0.2	0.6	?	?
Spur/branch height ratio	6	22%	–	26%		–		24%	–	1%	–	?	–

411

412

413 *Males.* (measurements and statistics in Table 5). The males (Figs. 2C-D, 3B, 3D, 3F, 5) are
414 similar to females, there are differences in the shape of the gonopore (short papilla-like
415 structure; Figs 3D, 5E), in the presence of proportionally larger cephalic papillae (Figs 2C, 3F)
416 and larger rugose areas (i.e. genital plates; Figs 3D, 5E) beside the gonopore, and in chaetotaxy
417 (Figs 2C-D, 3B). Males have long lateral filament (or long spine) in D position (absent in
418 females) and generally (but not always) lack the small spines in the E' position of terminal
419 plate (Figs 2C, 2D).

420

421 **Table 5.** Measurements of selected morphological structures of adult males of *Diploechiniscus*
 422 *dimorphus* n. sp. mounted in Hoyer's medium. N – number of specimens/structures measured,
 423 RANGE refers to the smallest and the largest structure among all measured specimens; SD –
 424 standard deviation; sp – the proportion between the length of a given structure and the length
 425 of the scapular plate.

CHARACTER	N	RANGE		MEAN		SD	
		μm	sp	μm	sp	μm	sp
Body length	12	112 – 145	442 – 595	129	526	11	41
Scapular plate length	16	20.3 – 32.5	–	25.1	–	2.7	–
Head appendages lengths							
<i>Cirrus internus</i>	15	10.8 – 22.1	33.3 – 83.6	16.0	64.8	3.0	13.8
Cephalic papilla	13	5.8 – 9.7	18.0 – 40.2	7.7	31.3	1.1	6.3
<i>Cirrus externus</i>	15	12.2 – 21.6	44.7 – 87.9	17.0	68.5	2.6	11.2
Clava	15	4.8 – 8.8	14.8 – 34.5	6.8	27.4	1.1	5.4
<i>Cirrus A</i>	13	39.7 – 64.2	175.9 – 229.2	51.3	202.8	6.9	17.5
<i>Cirrus A</i> /Body length ratio	10	34% – 49%	–	39%	–	5%	–
Body appendages lengths	0						
<i>Cirrus D</i>	12	23.9 – 36.9	112.8 – 149.8	30.5	124.6	3.4	12.8
Spine on leg I length	5	1.5 – 2.9	5.7 – 12.5	2.2	9.2	0.5	2.6
Papilla on leg IV length	12	2.2 – 4.1	7.8 – 16.5	3.0	12.4	0.5	2.2
Number of teeth on the collar	12	4 – 6	–	5.1	–	0.7	–
Claw I heights							
Branch	12	6.9 – 8.0	24.7 – 33.8	7.4	30.4	0.3	2.5
Spur insertion	11	1.6 – 2.2	6.5 – 10.2	1.9	7.9	0.2	1.0
Spur	10	1.6 – 1.8	6.1 – 8.2	1.7	7.0	0.1	0.7
Spur/branch height ratio	10	21% – 25%	–	23%	–	1%	–
Claw II heights							
Branch	12	6.6 – 7.7	24.3 – 32.4	7.1	29.3	0.3	2.3
Spur insertion	11	1.6 – 2.1	6.4 – 8.9	1.7	7.1	0.2	0.7
Spur	10	1.3 – 1.7	5.4 – 6.6	1.5	6.1	0.1	0.4
Spur/branch height ratio	10	20% – 24%	–	21%	–	1%	–
Claw III heights							
Branch	12	6.3 – 7.7	24.9 – 32.0	7.1	29.0	0.4	2.0
Spur insertion	12	1.5 – 2.0	6.2 – 7.6	1.7	6.9	0.1	0.4
Spur	11	1.2 – 1.7	5.3 – 6.9	1.5	6.0	0.1	0.5
Spur/branch height ratio	11	17% – 23%	–	21%	–	2%	–
Claw IV heights							
Branch	9	7.6 – 9.5	27.5 – 38.3	8.5	34.9	0.7	3.2
Spur insertion	8	2.3 – 2.9	9.3 – 11.9	2.5	10.3	0.2	1.0
Spur	7	2.0 – 2.1	7.4 – 10.1	2.0	8.5	0.1	1.0
Spur/branch height ratio	7	21% – 27%	–	24%	–	2%	–

427

428 *Larvae.* (measurements and statistics in Table 6). Specimens with four claws per leg and
 429 without gonopore (2nd stage larva according to Bertolani et al., 1984) were found and attributed
 430 to this species due to the large number of specimens of this species respect to those of the clade
 431 A in the sample. The larvae present the same characters of the adults but with two types of
 432 chaetotaxy. One type with lateral filament in D position (ascribable to juvenile males), the other
 433 with small dorsal spine in D^d position and very small spine on the margins of the notches of
 434 terminal plate (ascribable to juvenile females). Two 2nd stage larvae were found with very small
 435 spines in D^d and filaments in D. Three 1st stage larvae (according to Bertolani et al., 1984) (72-
 436 82 µm long) with two claws per leg and without gonopore were found, showing no dorsal or
 437 lateral appendages.

438

439 *Eggs.* Not found.

440

441 **Distribution:** The species has been found only in Canada; other than the type locality (Calvert
 442 Island, Lookout, British Columbia), it was recorded also in Graham Island (Haida Gwaii,
 443 British Columbia) (Massa et al. 2024).

444

445

446 **Table 6.** Measurements of selected morphological structures of juveniles (2nd stage larvae) of
 447 *Diploechiniscus dimorphus* n. sp. mounted in Hoyer's medium. N – number of
 448 specimens/structures measured, RANGE refers to the smallest and the largest structure among
 449 all measured specimens; SD – standard deviation; sp – the proportion between the length of a
 450 given structure and the length of the scapular plate.

CHARACTER	N	RANGE		MEAN		SD	
		µm	sp	µm	sp	µm	sp
Body length	2	133 – 147	509 – 529	140	519	10	14
Scapular plate length	2	25.1 – 28.8	–	26.9	–	2.6	–
Head appendages lengths							
<i>Cirrus internus</i>	2	14.2 – 18.2	49.3 – 72.5	16.2	60.9	2.9	16.4
Cephalic papilla	2	5.6 – 8.7	19.4 – 34.6	7.1	27.0	2.2	10.7
<i>Cirrus externus</i>	2	13.0 – 18.2	45.3 – 72.6	15.6	58.9	3.7	19.3
Clava	2	4.5 – 7.6	15.7 – 30.1	6.0	22.9	2.1	10.1
<i>Cirrus A</i>	2	44.1 – 64.8	175.5 – 225.1	54.4	200.3	14.6	35.0
<i>Cirrus A</i> /Body length ratio	2	33% – 44%	–	39%	–	8%	–
Body appendages lengths							
<i>Cirrus D</i>	1	28.1 – 28.1	112.0 – 112.0	28.1	112.0		
<i>Cirrus D^d</i>	1	3.4 – 3.4	11.6 – 11.6	3.4	11.6		
Spine on leg I length	1	2.7 – 2.7	9.4 – 9.4	2.7	9.4		
Papilla on leg IV length	2	2.7 – 3.5	10.7 – 12.1	3.1	11.4	0.6	1.0
Number of teeth on the collar	2	5 – 5	–	5.0	–	0.0	–
Claw I heights							
Branch	2	7.3 – 7.5	26.1 – 29.1	7.4	27.6	0.1	2.1

Spur insertion	2	2.1 – 2.2	7.5 – 8.2	2.1	7.8	0.1	0.5
Spur	2	1.8 – 1.8	6.2 – 7.0	1.8	6.6	0.0	0.6
Spur/branch height ratio	2	24% – 24%	–	24%	–	0%	–
Claw II heights							
Branch	2	6.6 – 7.2	25.1 – 26.4	6.9	25.7	0.4	0.9
Spur insertion	2	1.6 – 1.8	6.2 – 6.3	1.7	6.2	0.1	0.0
Spur	2	1.3 – 1.5	5.1 – 5.1	1.4	5.1	0.1	0.0
Spur/branch height ratio	2	19% – 20%	–	20%	–	1%	–
Claw III heights							
Branch	2	6.5 – 7.1	24.6 – 26.0	6.8	25.3	0.4	1.0
Spur insertion	1	1.7 – 1.7	5.8 – 5.8	1.7	5.8		
Spur	1	1.5 – 1.5	5.1 – 5.1	1.5	5.1		
Spur/branch height ratio	1	21% – 21%	–	21%	–		–
Claw IV heights							
Branch	2	7.8 – 8.6	30.0 – 31.1	8.2	30.6	0.6	0.8
Spur insertion	1	1.9 – 1.9	7.4 – 7.4	1.9	7.4		
Spur	1	1.9 – 1.9	7.4 – 7.4	1.9	7.4		
Spur/branch height ratio	1	24% – 24%	–	24%	–		–

451

452 **Remarks**

453 A small number of individuals (10 in total) with plates ornamentation compatible with
454 *Diploechiniscus dimorphus* n. sp., but atypical chaetotaxy were found in the same samples (A-
455 D^d-D^l; A-(B^l)-C^l-D^l; A). Due to the absence of genetic data for these morphologies, they were
456 not included in the species description for precaution (see Discussion). Measurements of those
457 individuals are available in cjz-2024-0040supplb.

458

459 **Differential diagnosis.**

460 The new species differs from:

- 461 • *Diploechiniscus oihonnae* (Richters 1903), based on the redescription of Kaczmarek et
462 al. (2021), by several characters, in particular it differs for the very reduced number and
463 size of the body appendages (e.g. in female absence of lateral appendages in B, C, D,
464 and E, dorsal appendages in C, and dorsolateral spines in B, C and D), the lower number
465 and not uniformly distributed cuticular pores, the fewer and larger teeth in the dentate
466 collar, the absence of spurs in the external claws IV. *Diploechiniscus horningi* is
467 considered by Kaczmarek et al. (2021) as morphologically indistinguishable from
468 *Diploechiniscus oihonnae*.
- 469 • *Diploechiniscus laterosetosus* (Ito 1993) by several characters; in particular it differs
470 for the very reduced number and size of the body appendages (e.g. in female absence
471 of lateral appendages in B, C, D, and E, dorsal appendages in C, and dorsolateral spines
472 in B, C and D), the lower number and not uniformly distributed cuticular pores, the
473 absence of spurs in the external claws IV.
- 474 • *Diploechiniscus polygonalis* (Ito 1993) by several characters; in particular it differs for
475 the very reduced number and size of the body appendages (e.g. in female absence of

476 lateral appendages in B, C, D, and E, dorsal appendages in C, and dorsolateral spines
477 in B, C and D), the lower number and not uniformly distributed cuticular pores, the
478 absence of spurs in the external claws IV.

479
480

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482

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488 processing of field samples. Collection of specimens was conducted in the frameworks of the
489 activities of the Hakai Institute and of the Royal BC Museum in accordance with applicable
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491 (#314219 and #335759).

492

493 **Competing interests**

494 The authors declare there are no competing interests.

495

496 **Author contribution statement**

497 Conceptualization: MV, RG, HC

498 Data curation: MV, RG, JV

499 Formal analysis: MV, RG

500 Funding acquisition: SC

501 Investigation: MV, RG, JV

502 Methodology: MV, RG, JV

503 Project administration: RG, HC, SC

504 Resources: RG, HC, SC

505 Software: MV

506 Supervision: MV, RG

507 Validation: MV, RG, JV, HC, SC

508 Visualization: MV, RG

509 Writing – original draft: MV, RG

510 Writing – review and editing: MV, RG, JV, HC, SC

511

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513 This work was supported by the Academy of Finland Fellowship to SC (#314219 and
514 #335759).

515

516 **Data availability statement**

517 Genetic data generated and analyzed during this study are available in GenBank
518 (<https://www.ncbi.nlm.nih.gov/genbank/>) repository (see main text for GenBank accession

519 numbers). Morphometric data generated and analyzed during this study are available and are
 520 provided in full within the published article and its supplementary materials.

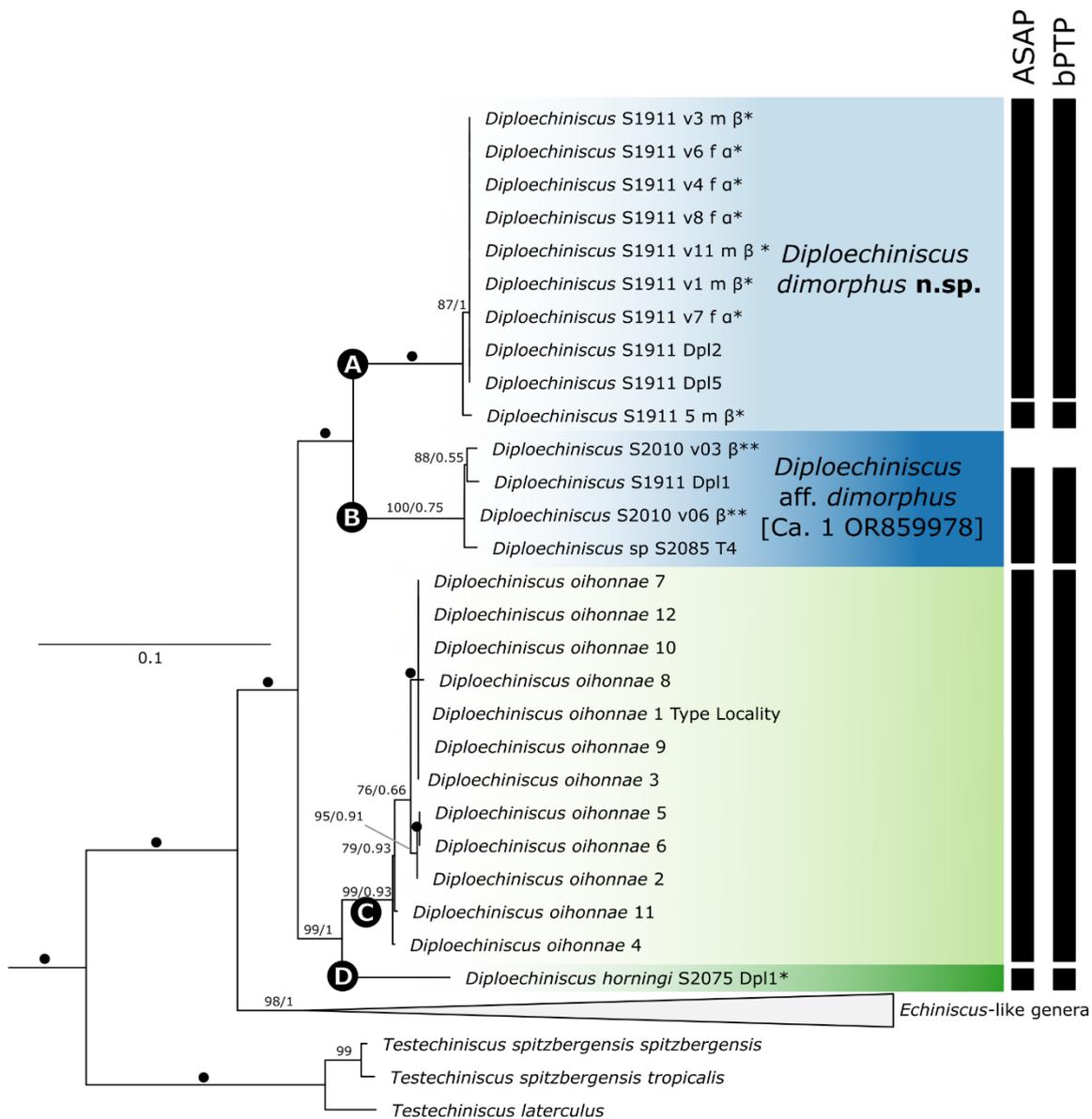
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523 **Figures captions**

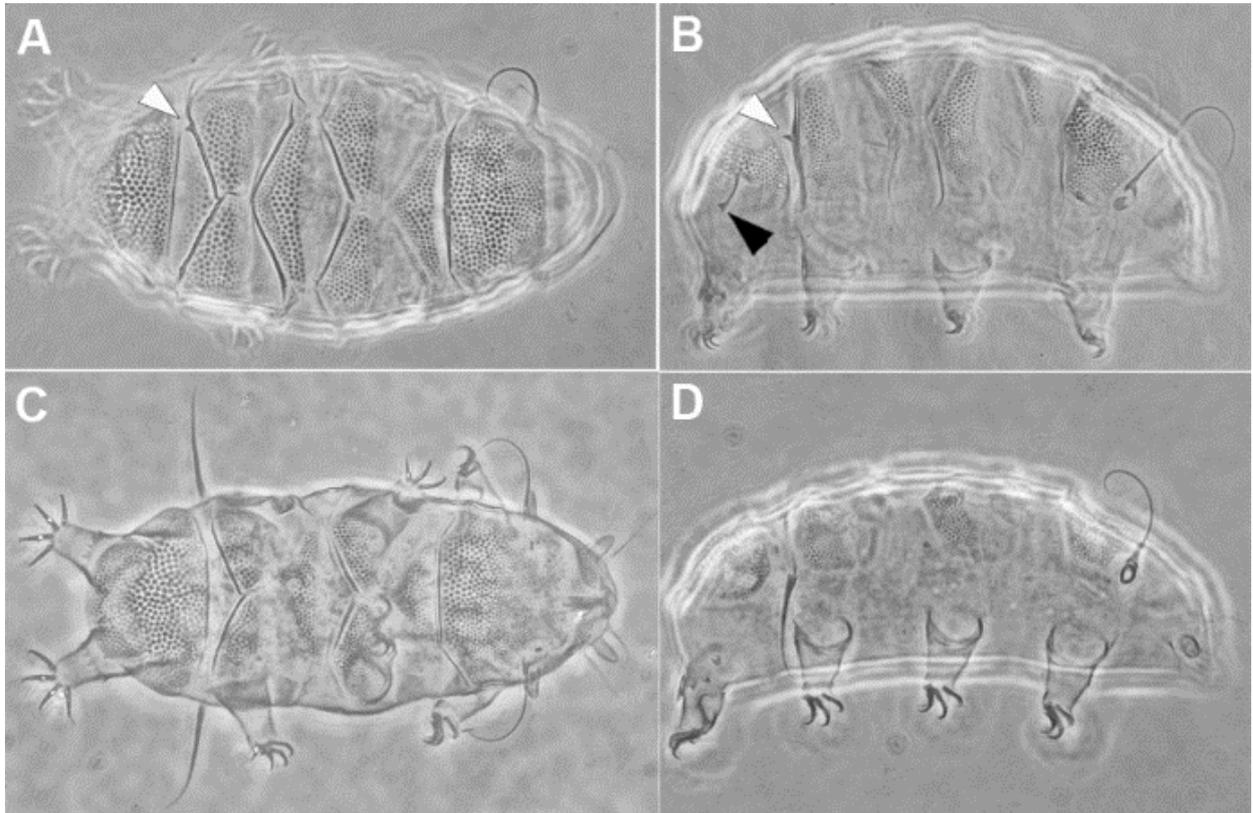
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525 **Figure 1:** Maximum likelihood phylogenetic reconstruction of *Diploechiniscus* and related
 526 genera, along with species delimitation analyses results. (*: hologenophore voucher from this
 527 study, **: hologenophore voucher from Massa et al. 2024). Individual S2010v03 was not
 528 included in species delimitation analyses as COI sequence is not available. Scale bar indicates
 529 substitutions/site. Number above nodes indicate ML/BI support values. ● indicates support
 530 values of 100/1. Shaded boxes indicate the identified *Diploechiniscus* species based on
 531 molecular and morphological data combined.



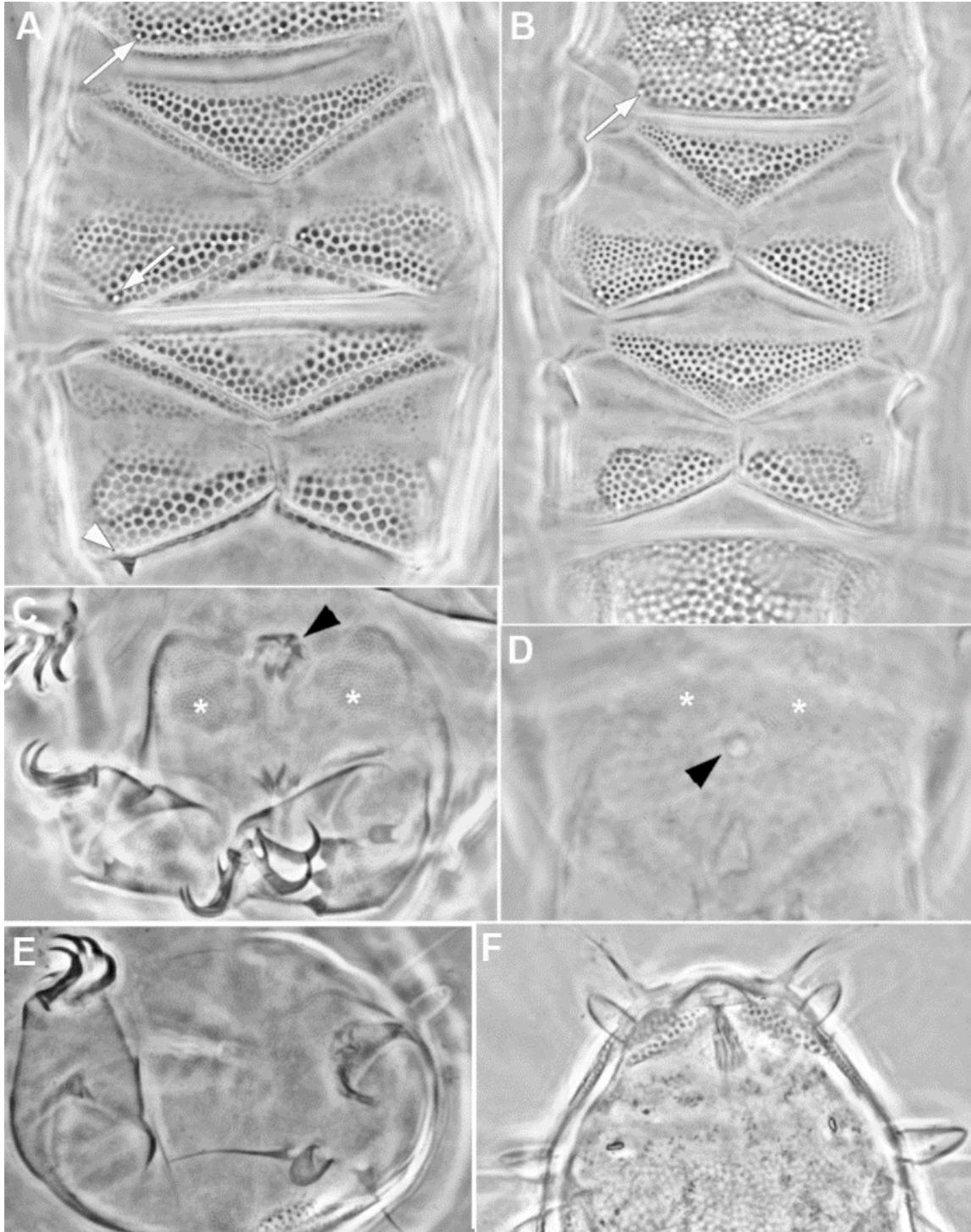
532

533 **Figure 2.** *Diploechiniscus dimorphus* n.sp. (LM). A. Female (holotype), dorsal view
534 (arrowhead: small D^d spine). B. Female, lateral view (white arrowhead: small D^d spine; black
535 arrowhead: small E' spine). C. Male, dorsal view. D. Male, lateral view. Scale bars: 20 μm.



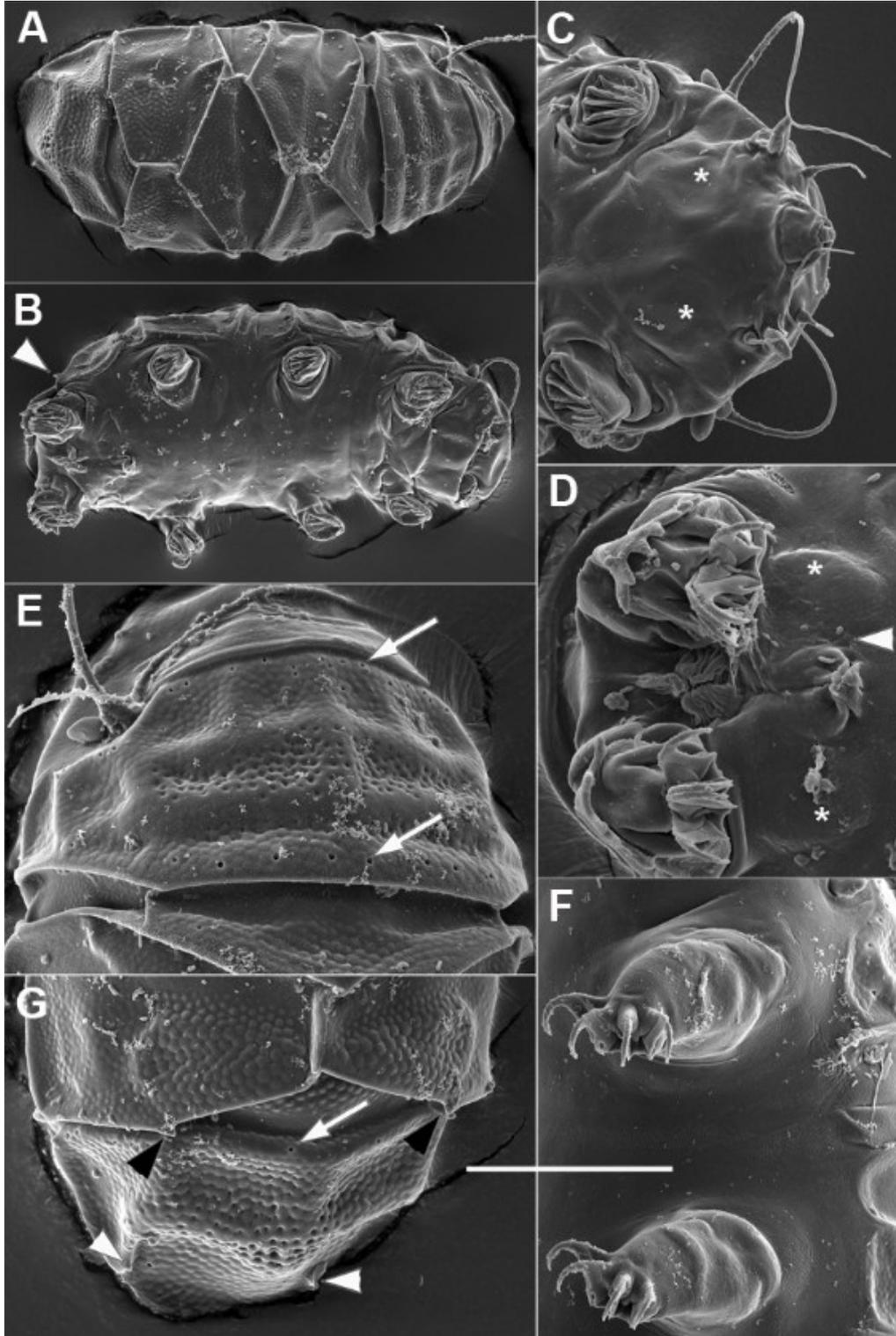
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558 **Figure 3.** *Diploechiniscus dimorphus* n.sp. (LM). A. Female, dorsal view (arrows: holes in
559 the cuticle; arrowhead: small D^d spine). B. Male, dorsal view (arrow: hole in the cuticle). C.
560 Female gonopore (arrowhead), genital plates (asterisks). D. Male gonopore (arrowhead),
561 genital plates (asterisks). E. Ventral surface of a female head cuticle with small dots.
562 of a male with eyes and cephalic sensory organs. Scale bars: A-B = 20 μm, C-F = 10 μm.

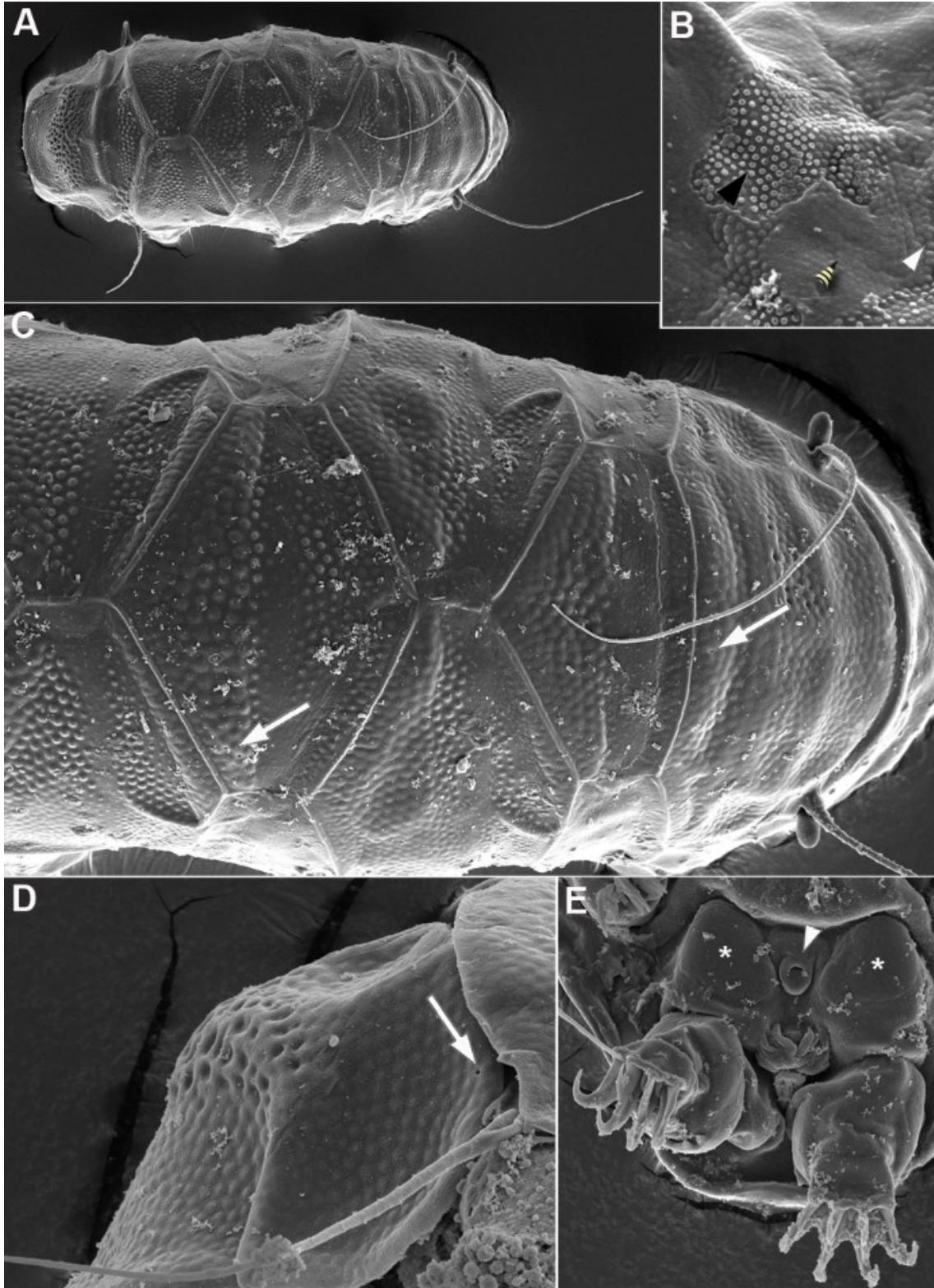


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567 **Figure 4.** *Diploechiniscus dimorphus* n.sp. female (SEM). A. Dorsal view. B. Ventral view
 568 (arrowhead: small spine in E'). C. Ventral surface of the head cuticle with small dots and
 569 cephalic plates (asterisks). C. Ventral surface of the head cuticle with small dots and cephalic
 570 plates (asterisks). D. Gonopore (arrowhead), genital plates (asterisks). E. Scapular plate with
 571 lines of small pores (arrows). F. Legs of the second and third pairs. G. Second paired plates
 572 and terminal plates (arrow: small hole; black arrowhead: small D^d spine; white arrowhead:
 573 small spine in E). Scale bars: A-B = 20 μm, C-F = 10 μm.



575 **Figure 5.** *Diploechiniscus dimorphus* n.sp. male (SEM). A. Dorsal view. B. Ventral cuticle
576 (black arrowhead: cuticular pillars; white arrowhead: granules; striped arrowhead: granules
577 with depressions). C. Dorsal cuticle (arrows: small holes). Terminal plate (arrow: small hole).
578 E. Gonopore (arrowhead), genital plates (asterisks). Scale bars: A = 50 μ m, B = 5 μ m, C-E =
579 10 μ m.



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856 **Supplementary material**

857 cjz-2024-0040suppla. Raw measurements of *Diploechiniscus dimorphus* n. sp. type series
858 from S1911
859 cjz-2024-0040supplb. Raw measurements of *Diploechiniscus* spp. from S1911
860 cjz-2024-0040supplc. MrBayes input file with alignment and partition models
861 cjz-2024-0040suppld. Complete ML phylogenetic tree
862 cjz-2024-0040supple. Complete BI phylogenetic tree
863 cjz-2024-0040supplf. ASAP results
864 cjz-2024-0040supplg. bPTP results
865 cjz-2024-0040supplh. COI p-distances table