



Title	Diexanthema hakuohmaruae sp. nov. (Copepoda: Siphonostomatoida: Nicothoidae) from the Hadal Zone in the Northwestern Pacific, with an 18S Molecular Phylogeny
Author(s)	Kakui, Keiichi; Fukuchi, Jun; Ohta, Mizuki
Citation	Acta Parasitologica, 68, 413-419 <a href="https://doi.org/10.1007/s11686-023-00676-z">https://doi.org/10.1007/s11686-023-00676-z</a>
Issue Date	2023-04-04
Doc URL	<a href="https://hdl.handle.net/2115/91482">https://hdl.handle.net/2115/91482</a>
Rights	This version of the article has been accepted for publication, after peer review (when applicable) and is subject to Springer Nature's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The Version of Record is available online at: <a href="http://dx.doi.org/10.1007/s11686-023-00676-z">http://dx.doi.org/10.1007/s11686-023-00676-z</a> .
Type	journal article
File Information	Acta Parasitologica.pdf



This version of the article has been accepted for publication, after peer review and is subject to Springer Nature's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The Version of Record is available online at: <https://doi.org/10.1007/s11686-023-00676-z>

1 ***Diexanthema hakuhomaruae* sp. nov. (Copepoda: Siphonostomatoida: Nicothoidae) from the hadal**  
2 **zone in the northwestern Pacific, with an 18S molecular phylogeny**

3

4 Keiichi Kakui<sup>1</sup> · Jun Fukuchi<sup>2</sup> · Mizuki Ohta<sup>3</sup>

5 <sup>1</sup>Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo, Japan

6 <sup>2</sup>Seto Marine Biological Laboratory, Kyoto University, Nishimuro, Japan

7 <sup>3</sup>Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwa, Japan

8

9 Corresponding author: Keiichi Kakui. Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan.

10 kakui@eis.hokudai.ac.jp

11

12 ORCID ids:

13 Keiichi Kakui: 0000-0003-4630-9065

14 Jun Fukuchi: 0000-0001-6857-9723

15 Mizuki Ohta: 0000-0003-1375-8876

16

17 Footnotes:

18 This article is registered in ZooBank under [https://zoobank.org/4A75D879-2540-46A8-AF47-](https://zoobank.org/4A75D879-2540-46A8-AF47-97C424A48B63)

19 97C424A48B63

20

21 **Acknowledgments**

22 We thank Shigeaki Kojima and Yasunori Kano for the opportunity to join R/V *Hakuho-maru* cruise KH-  
23 22-8, conducted as a part of the project “Comprehensive study of diversity and evolutionary mechanisms  
24 of deep-sea animals in trenches in the northwestern Pacific” supported by KAKENHI grant (JP19H00999)  
25 from the Japan Society for the Promotion of Science (JSPS); Captain Kazuhiko Kasuga and the crew of  
26 R/V *Hakuho-maru*, technicians from Marine Work Japan and MOL Marine & Engineering, and researchers  
27 aboard for support in collecting; Hiroshi Kajihara, Jamael Abato, and Tsuyoshi Takano for help in sorting  
28 samples; and Matthew H. Dick for reviewing the manuscript and editing the English. This study was  
29 supported in part by Atmosphere and Ocean Research Institute, The University of Tokyo, and by  
30 KAKENHI grants (JP19K06800 and JP22H02681) from JSPS.

31 **Abstract**

32 **Purpose** *Diexanthema* copepods are ectoparasites on deep-sea isopods. This genus currently contains six  
33 species, all reported from the North Atlantic. Our study describes a new species of *Diexanthema* found on  
34 isopods from 7184–7186 m depth in the Kuril-Kamchatka Trench, northwestern Pacific.

35 **Methods** We observed the copepod's morphology, made camera-lucida drawings, and compared our  
36 species with congeners. We determined partial sequences for its 16S rRNA and 18S rRNA genes and  
37 constructed an 18S-based maximum-likelihood copepod tree to place it phylogenetically. We identified the  
38 host isopod species through morphology and cytochrome *c* oxidase subunit I (COI, *cox1*) and 18S  
39 sequences.

40 **Results/conclusion** We described the copepod as *Diexanthema hakuohmaruae* **sp. nov.** and identified its  
41 host as *Eugerdella* cf. *kurabyssalis* Golovan, 2015 (Desmosomatidae). This is the first *Diexanthema*  
42 copepod from the Pacific and also from hadal depths. *Diexanthema hakuohmaruae* most closely resembles  
43 *D. bathydiaita* Richie, 1975, parasitic on *Nannoniscus* sp. (Nannoniscidae) in the Atlantic, but differs from  
44 the latter in having a smooth body surface and leg 5 in the ventrolateral region of the urosome. In the 18S  
45 tree, *D. hakuohmaruae* was the sister group to the *Rhizorhina* clade, which is consistent with the  
46 morphology-based hypothesis that they are closely related.

47

48 **Keywords** Crustacea · ectoparasite · integrative taxonomy · Japan · parasites

## 49 **Introduction**

50 Nicothoidae is a family of parasitic copepods, with 22 genera and about 140 species [1]. Nicothoids utilize  
51 various crustaceans as hosts, including Ostracoda, Leptostraca, Decapoda, Amphipoda, Cumacea, Isopoda,  
52 Mysida, and Tanaidacea [2]. Nicothoid morphology is highly diverse. *Rhizorhina* copepods, for example,  
53 have an unsegmented spherical body, whereas *Paranicothoe* copepods have a multi-segmented body  
54 consisting of a prosome and urosome. Recent molecular phylogenetic analyses have called into question  
55 the monophyly of this family [3, 4].

56         The nicothoid genus *Diexanthema* is characterized by the following female features: a spherical  
57 prosome lacking a head process; an unsegmented urosome; antennules with two or fewer articles;  
58 maxillipeds absent; and caudal rami shorter than the urosome, or absent [1, 5–7]. It currently contains six  
59 named species, *Diexanthema apoda* Boxshall and Harrison, 1988, *Diexanthema bathydiaita* Richie, 1975,  
60 *Diexanthema corrugatum* Boxshall and Harrison, 1988, *Diexanthema desistoma* Richie, 1975,  
61 *Diexanthema nudum* Boxshall and Harrison, 1988, and *Diexanthema ritchiei* Boxshall and Harrison, 1988,  
62 all of which are parasitic on deep-sea isopods in the North Atlantic Ocean [8, 9] (Table 1). Boxshall and  
63 Lincoln [10] placed this genus in the “*Rhizorhina* group,” along with the genera *Rhizorhina* and  
64 *Choniorhiza*. The same authors proposed two other groups in Nicothoidae, the “*Nicothoe* group” and the  
65 “*Sphaeronella* group,” containing five and eight genera, respectively. The validity of this grouping has not  
66 yet been tested with molecular data.

67         In 2022, we collected an undescribed *Diexanthema* species parasitic on a desmosomatid isopod

68 from the hadal zone in the Kuril-Kamchatka Trench, northwestern Pacific Ocean. This is the first  
69 *Diexanthema* copepod from outside the North Atlantic, and the first from hadal depths. Here we describe  
70 this species, provide partial sequences for multiple genes to aid future DNA barcoding, and infer its  
71 phylogenetic position in Siphonostomatoida based on an 18S-rRNA (18S) tree to test the validity of the  
72 “*Rhizorhina* group.”

73

## 74 **Materials and methods**

75 Five copepods, each from a different host individual, were collected on 6 October 2022 during cruise KH-  
76 22-8 of R/V *Hakuho-maru* (Japan Agency for Marine-Earth Science and Technology; JAMSTEC), with  
77 small plankton nets attached inside an Agassiz trawl (cf. fig. 2B in Akiyama *et al.* [11]), at depths of 7184–  
78 7186 m. Copepods were attached to the pereonite-3 sternite or the basis of pereopods 3, 4, or 6 of the host  
79 isopods. They were photographed and then fixed and preserved in 80% ethanol.

80 Three copepods were detached from their host with chemically sharpened needles, and two were  
81 retained intact on the host for future non-destructive observation or molecular analysis. Two of the three  
82 detached copepods were used for morphological observations and one for DNA extraction (see below).  
83 The former were transferred through an ethanol series (70, 60, 50, 40, and 30% ethanol, each step for ca. 5  
84 min) at room temperature, mounted on cavity slides (T8-R004; Toshin Riko, Japan) in 30% ethanol, and  
85 observed with an Olympus BX53 microscope. Illustrations were prepared with Adobe Illustrator CS6 from  
86 draft line drawings made with a camera lucida. In copepods, body length (BL) was measured from the

87 anterior to posterior ends of the body (prosome + urosome), and prosome width (PW) and urosome width  
88 (UW) at the widest portion of the prosome and urosome, respectively. In host isopods, BL (from the  
89 anterior edge of the cephalothorax to the tip of the pleotelson) and the pereonite-2 width (P2W) were  
90 measured. All measurements are presented in the text in micrometers unless noted otherwise. The  
91 specimens studied were deposited in the Invertebrate Collection of the Hokkaido University Museum  
92 (ICHUM), Sapporo, Japan, under catalog numbers ICHUM8451–ICHUM8455.

93 DNA was extracted from the whole body of one copepod and pereopod 1 of one host by using the  
94 NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). For the cytochrome *c* oxidase subunit I (COI)  
95 gene, PCR primers used for the amplification and cycle sequencing were LCO1490 and HCO2198 [12].  
96 For the 18S rRNA gene, amplification primers were SR1 and SR12 [13], and six primers (18S-b3F, 18S-  
97 b4R, 18S-b5F, 18S-b6F, 18S-a6R, and 18S-b8F [14, 15]) were used in cycle sequencing. For the copepod  
98 16S rRNA gene, the newly designed primers Copepod16S\_F (CGCCTGTTTATCAAARACWY) and  
99 Copepod16S\_R (TCGATTTGAACTCAAATCAWG) were used for amplification and cycle sequencing.  
100 PCR amplification conditions for COI with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) were  
101 as described by Munakata *et al.* [16]; those for 18S with KOD FX Neo (Toyobo, Japan) were as described  
102 by Okamoto and Kakui [17]; and those for 16S with KOD ONE PCR Master Mix (Toyobo) were 45 cycles  
103 of 98°C for 10 s, 50°C for 5 s, and 68°C for 1 s. PCR products for 16S were separated on a 2% agarose gel,  
104 excised with a micro spatula, and purified with the MagExtractor PCR & Gel Clean Up Kit (Toyobo). All  
105 nucleotide sequences were determined with a BigDye Terminator Kit ver. 3.1 and a 3730 DNA Analyzer

106 (Life Technologies, USA). Fragments were concatenated by using MEGA7 [18]. The sequences we  
107 determined were deposited in the International Nucleotide Sequence Database (INSD) through the DNA  
108 Data Bank of Japan.

109         The copepod 18S dataset for a phylogenetic analysis comprised the copepod dataset from Kakui  
110 and Munakata [4] and the one *Diexanthema* sequence we determined, representing 50 siphonostomatoid  
111 species and one outgroup taxon (*Misophthriopsis okinawensis*, a misophrioid). The sequences were aligned  
112 (1528 positions in the aligned dataset; see Online Resources 1 and 2) as described by Munakata *et al.* [19];  
113 methods for selecting the optimal substitution model (GTR+F+R3), the maximum likelihood (ML)  
114 analysis, and drawing the tree were as described by Kakui and Shimada [20].

115

## 116 **Results and Discussion**

### 117 **Host identification**

118 All host individuals were females with developing oostegites. An abbreviated description of their  
119 morphology is as follows. Pereonite 1 slightly longer than pereonite 2. Pleotelson with small posterolateral  
120 spines. Coxae I–II each with acute anterior projection (longer on coxa I longer than on coxa II). Pereopod  
121 1 stout: ischium with five robust distodorsal, unequally bifid setae; carpus enlarged, ventral margin convex  
122 in proximal two thirds but straight in distal third, with minute distal, unequally bifid, distally setulate seta  
123 (UBDS) at base of penultimate seta, and row of four robust UBDS of irregular size. Operculum truncate,  
124 slightly concave distally.

125           According to these character states, our individuals were *Eugerdella kurabyssalis* Golovan, 2015,  
126 described from the Northwest Pacific Basin east of the Kuril-Kamchatka Trench [21] and later reported  
127 also from the Kuril-Kamchatka Trench [22]. Jennings *et al.* [22] molecularly detected *Eugerdella cf.*  
128 *kurabyssalis* Golovan, 2015 among *Eugerdella* specimens collected from the Kuril-Kamchatka Trench. It  
129 closely resembles *E. kurabyssalis* but shows minor differences from the latter, such as a smaller, less  
130 pronounced coxa [22], and has been collected from deeper depths than *E. kurabyssalis* (*E. cf. kurabyssalis*  
131 from 7081–7123 m; *E. kurabyssalis* from 4830–6051 m [21, 22]). In a BLAST search [23] of the public  
132 database, the partial COI sequence (LC741552; 655 bp long) we determined for one host individual  
133 (ICHUM8452) was most similar to the COI sequence from *E. cf. kurabyssalis* (MN179516; query cover  
134 91%, identity score 99.83% [22]). Our isopods were collected from 7184–7186 m depth, similar to the  
135 depth range reported for *E. cf. kurabyssalis*. Although we could not judge whether the coxa in our  
136 specimens is smaller and less pronounced than in *E. kurabyssalis*, given the similarity in COI sequence and  
137 sampling depth, we concluded that the host isopods were *E. cf. kurabyssalis*.

138

## 139 **Taxonomy**

140 ***Diexanthema hakuhomaruae* sp. nov.**

141 (Figs. 1, 2)

142

143 *Diagnosis (females)*. Body smooth, lacking furrows or minute hairs; anterior hood absent; caudal ramus

144 present, with two spiniform setae (outer seta longer than inner); rod/horn-like antennule and antenna  
145 absent; semicircular irregular branching structure present; rootlet absent; mandible present; legs 1–4  
146 absent; leg 5 present, small lobe shape, with three spiniform setae in ventrolateral region of urosome.

147 *Type host.* *Eugerdella* cf. *kurabyssalis* Golovan, 2015 (Isopoda: Asellota: Desmosomatidae).

148 *Type locality.* Station A5, Kuril-Kamchatka Trench axis, northwestern Pacific (41°14.024' N  
149 145°01.931' E to 41°14.126' N 145°01.108' E), 7184–7186 m depth.

150 *Attachment sites.* Pereopod basis and pereon sternite.

151 *Material examined.* Holotype: female (ICHUM8451), BL 356, PW 346, UW 86, one vial  
152 containing extracted copepod and host (BL 1797, P2W 483). Paratypes: four females (ICHUM8452, BL  
153 397, PW 359, UW 96, used in DNA extraction, one slide and one vial containing host [BL 1925, P2W  
154 489]; ICHUM8453, BL 342, PW 326, UW 87, one vial containing extracted copepod and host [BL 1801,  
155 P2W 475]; ICHUM8454, BL 365, PW 385, UW 89, one vial containing copepod attached to host [BL  
156 1984, P2W 499]; ICHUM8455, BL 400, PW 366, UW 95, one vial containing copepod attached to host  
157 [BL 1820, P2W 490]). All specimens were collected at the type locality on 6 October 2022 by R/V

158 *Hakuho-maru.*

159 *Representative DNA sequences.* One 16S (INSD accession number LC741550; 424 bp long) and  
160 one 18S (LC741551; 1762 bp long) sequences were determined from paratype female ICHUM8452. One  
161 COI (LC741552; 655 bp long, encoding 218 amino acids) and one 18S (LC741553; 2174 bp long)  
162 sequences were determined from host female ICHUM8452.

163           *Etymology.* The specific name (a noun in the genitive case) is from R/V *Hakuho-maru*, the vessel  
164 from which the type specimens were collected.

165           *Description (female, based on the holotype).* Body (Figs. 1, 2) transparent (white in ethanol),  
166 smooth, lacking furrows or minute hairs; prosome globular but slightly flattened dorsoventrally, containing  
167 bulging ovaries (Fig. 1b); anterior hood absent; urosome wide, oblong. BL/PW 1.03, BL/UW 4.14,  
168 PW/UW 4.02. Caudal ramus present, small lobe shaped, with two spiniform setae (outer seta longer than  
169 inner). Rod/horn-like antennule and antenna absent; semicircular irregular branching structure present  
170 posterior to oral sucker. Rootlet absent. Mandible present, styliiform. Pore (maxillary gland pore?; arrow in  
171 Fig. 2e) present on ventrolateral swelling. Legs 1–4 absent. Leg 5 present, small lobe shaped, located in  
172 ventrolateral region of urosome, with three spiniform setae. Genital area without ornamentation; openings  
173 of seminal receptacles and genital apertures indistinct.

174           *Male and copepodid.* Unknown.

175           *Variation.* One female paratype (ICHUM8453) observed in addition to the holotype showed all  
176 diagnostic characters seen in the holotype. Ranges (mean with standard deviation in parentheses) of BL,  
177 PW, UW, BL/PW, BL/UW, PW/UW for five individuals in the type series were 342–400 (372±23), 326–  
178 385 (356±20), 86–96 (91±4), 0.95–1.11 (1.05±0.05), 3.93–4.20 (4.10±0.09), and 3.74–4.32 (3.94±0.22),  
179 respectively.

180

181   **Genetic divergence and phylogenetic analysis**

182 We attempted to determine COI, 16S, and 18S sequences for *D. hakuohomaru* **sp. nov.** but were unable to  
183 PCR-amplify the COI region. Among copepod 16S sequences in the INSD database, one from *Tripaphylus*  
184 *elongatus* (C. B. Wilson, 1932) (as *Paeon elongatus*, FJ447423 [24]) was most similar to our 16S sequence  
185 in a BLAST search, but the query cover and identity score were low (46% and 76.73%, respectively). To  
186 date, no other nicothoid 16S sequences have been deposited in public databases [25].

187 In the ML tree (Fig. 3), *D. hakuohomaru* formed a fully supported clade with the *Rhizorhina*  
188 clade. The other relationships were identical to those provided in Kakui and Munakata [4], except for  
189 minor differences in ultrafast bootstrap values.

190

#### 191 **Remarks**

192 *Diexanthema hakuohomaru* **sp. nov.** is the seventh species described in *Diexanthema*. Females lack  
193 segmented antennules, have mandibles, and lack legs 1–4, features shared with females of *D. bathydiaita*.  
194 The former differs from the latter in having a smooth body surface (body covered with minute hairs in *D.*  
195 *bathydiaita*) and leg 5 located ventrolaterally on the urosome (laterally in *D. bathydiaita*). Their host  
196 groups are different at the family level: Desmosomatidae for *D. hakuohomaru*, Nannoniscidae for *D.*  
197 *bathydiaita*.

198 *Diexanthema hakuohomaru* **sp. nov.** lacks rootlets observed in *D. apoda*. This species appears to  
199 use its oral sucker to attach to its host isopod.

200 Our 18S tree showed a close relationship between *Diexanthema* and *Rhizorhina*. This suggests

201 that, although we lacked *Choniorhiza* sequences, the “*Rhizorhina* group” proposed by Boxshall and  
202 Lincoln [10] may reflect close phylogenetic relationships among its members.

203       Individuals of *Diexanthema hakuohomaru* **sp. nov.** were, as with *Rhizorhina* individuals (KK  
204 unpublished data), easily deformed by changes in solution; for example, the body of one individual shrank  
205 when transferred into a 1:3:6 mixture of glycerin, absolute ethanol, and deionized water, making  
206 morphological observation difficult (transferred into 30% ethanol, it recovered its spherical shape). This  
207 suggests that differences in body form should be treated with caution in *Diexanthema* (and *Rhizorhina*)  
208 taxonomy. In addition, most appendages are strongly reduced or completely lacking in these nicothoid  
209 genera, and often few morphological differences are observable among congeners. As adopted in other  
210 parasitic groups [26], a “turbo taxonomy” [27] approach, i.e., providing concise morphological  
211 descriptions along with DNA-sequence and host data in establishing new species, may be advisable in the  
212 taxonomy of several character-poor nicothoid genera.

213

## 214 **Conclusions**

215 We described *Diexanthema hakuohomaru* **sp. nov.** parasitic on the desmosomatid *Eugerdella* cf.  
216 *kurabyssalis* Golovan, 2015 collected from a hadal depth in the Kuril-Kamchatka Trench, northwestern  
217 Pacific. This species represents the first *Diexanthema* species from the Pacific, and the first from hadal  
218 depths. Our 18S-based tree confirmed a close relationship between *Diexanthema* and *Rhizorhina*,  
219 previously suggested by morphology. Continued molecular studies, with wider taxon sampling and

220 additional molecular markers, should further elucidate the phylogenetic relationships among nicothoid  
221 copepods, with consequent advances in taxonomy.

222

## 223 **Supplementary Information**

224 **Online Resource 1** Aligned 18S sequences used in the maximum-likelihood analysis, with alignment-  
225 ambiguous sites retained.

226 **Online Resource 2** Aligned 18S sequences used in the maximum-likelihood analysis, reduced to 1528  
227 positions by removing alignment-ambiguous sites.

228

## 229 **Declarations**

230 **Author contributions:** KK conceived and designed the study, made morphological observations on the  
231 copepods, and conducted the molecular analysis; MO made morphological observations on the isopods;  
232 KK, JF, and MO collected samples, wrote the manuscript, and read and approved the final draft.

233 **Funding:** This study was supported in part by the Atmosphere and Ocean Research Institute, The  
234 University of Tokyo, and by KAKENHI grants (JP19K06800 and JP22H02681) from the Japan Society for  
235 Promotion of Science (JSPS). The funding agencies had no role in the study design, data collection,  
236 analysis, decision to publish, or preparation of the manuscript.

237 **Competing interests:** The authors have no competing interests to declare that are relevant to the content of  
238 this article.

239 **Ethics approval:** Not applicable.

240 **Consent:** Not applicable.

241 **Data availability:** The raw data (sampling locality; sampling date; museum deposition numbers, INSD  
242 accession numbers, and the depository for specimens) are included in the manuscript.

243

## 244 **References**

- 245 1. Walter TC, Boxshall G (2023) Nicothoidae Dana, 1852-1853.  
246 <https://www.marinespecies.org/aphia.php?p=taxdetails&id=135530>. Accessed 13 March 2023
- 247 2. Boxshall GA, Halsey SH (2004) An introduction to copepod diversity. The Ray Society, London
- 248 3. Kakui K (2016) Descriptions of two new species of *Rhizorhina* Hansen, 1892 (Copepoda:  
249 Siphonostomatoida: Nicothoidae) parasitic on tanaidacean crustaceans, with a note on their  
250 phylogenetic position. *Syst Parasitol* 93:57–68. <https://doi.org/10.1007/s11230-015-9604-x>
- 251 4. Kakui K, Munakata M (2022) A new *Sphaeronella* species (Copepoda: Siphonostomatoida:  
252 Nicothoidae) parasitic on *Euphilomedes* sp. (Ostracoda: Myodocopa: Philomedidae) from Hokkaido,  
253 Japan, with an 18S molecular phylogeny. *Syst Parasitol*. <https://doi.org/10.1007/s11230-022-10075-z>
- 254 5. Ohtsuka S, Boxshall GA, Harada S (2005) A new genus and species of nicothoid copepod (Crustacea:  
255 Copepoda: Siphonostomatoida) parasitic on the mysid *Siriella okadai* Ii from off Japan. *Syst Parasitol*  
256 62:65–81. <https://doi.org/10.1007/s11230-005-5483-x>
- 257 6. Bamber RN, Boxshall GA (2006) A new genus and species of the Langitanainae (Crustacea:

- 258 Peracarida: Tanaidacea: Tanaidae) bearing a new genus and species of nicothoid parasite (Crustacea:  
259 Copepoda: Siphonostomatoida: Nicothoidae) from the New Caledonia slope. Spec Divers 11:137–  
260 148. <https://doi.org/10.12782/specdiv.11.137>
- 261 7. Boyko CB (2009) Nomenclatural issues with *Paranicothoe* Carton, 1970 and *Pseudonicothoe* Avdeev  
262 & Avdeev, 1978 (Crustacea: Copepoda: Nicothoidae), with comments on the female isopod type  
263 specimen of *Paranicothoe cladocera* Carton, 1970. Proc Biol Soc Wash 122:206–211.  
264 <https://doi.org/10.2988/08-49.1>
- 265 8. Ritchie L (1975) A new genus and two new species of Choniostomatidae (Copepoda) parasitic on two  
266 deep sea isopods. Zool J Linn Soc 57:155–178. <https://doi.org/10.1111/j.1096-3642.1975.tb01415.x>
- 267 9. Boxshall GA, Harrison K (1988) New nicothoid copepods (Copepoda: Siphonostomatoida) from an  
268 amphipod and from deep-sea isopods. Bull Br Mus Nat Hist (Zool) 54:285–299
- 269 10. Boxshall GA, Lincoln RJ (1983) Some new parasitic copepods (Siphonostomatoida: Nicothoidae)  
270 from deep-sea asellote isopods. J Nat Hist 17:891–900. <https://doi.org/10.1080/00222938300770701>
- 271 11. Akiyama T, Shimomura M, Nakamura K (2008) Collection of deep-sea small arthropods: gears for  
272 collection and processing of samples on deck. TAXA 24:27–32. [https://doi.org/10.19004/taxa.24.0\\_27](https://doi.org/10.19004/taxa.24.0_27)  
273 [in Japanese with English abstract]
- 274 12. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of  
275 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol  
276 Biotechnol 3:294–299

- 277 13. Nakayama T, Watanabe S, Mitsui K, Uchida H, Inouye I (1996) The phylogenetic relationship  
278 between the Chlamydomonadales and Chlorococcales inferred from 18SrDNA sequence data. *Phycol*  
279 *Res* 44:47–55. <https://doi.org/10.1111/j.1440-1835.1996.tb00037.x>
- 280 14. Kakui K, Katoh T, Hiruta SF, Kobayashi N, Kajihara H (2011) Molecular systematics of Tanaidacea  
281 (Crustacea: Peracarida) based on 18S sequence data, with an amendment of suborder/superfamily-  
282 level classification. *Zool Sci* 28:749–757. <https://doi.org/10.2108/zsj.28.749>
- 283 15. Kakui K, Fukuchi J, Shimada D (2021) First report of marine horsehair worms (Nematomorpha:  
284 *Nectonema*) parasitic in isopod crustaceans. *Parasitol Res* 120:2357–2362.  
285 <https://doi.org/10.1007/s00436-021-07213-9>
- 286 16. Munakata M, Tanaka H, Kakui K (2021) *Heterocypris spadix* sp. nov. (Crustacea: Ostracoda:  
287 Cypridoidea) from Japan, with information on its reproductive mode. *Zool Sci* 38:287–296.  
288 <https://doi.org/10.2108/zs200127>
- 289 17. Okamoto N, Kakui K (2022) Integrative taxonomy of *Zeuxo* (Crustacea: Peracarida: Tanaidacea) from  
290 Japan, with the description of a new species. *Biologia* 77:2497–2506. [https://doi.org/10.1007/s11756-](https://doi.org/10.1007/s11756-022-01121-8)  
291 [022-01121-8](https://doi.org/10.1007/s11756-022-01121-8)
- 292 18. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0  
293 for bigger datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>
- 294 19. Munakata M, Tanaka H, Kakui K (2022) Taxonomy and natural history of *Cavernocypris hokkaiensis*  
295 sp. nov., the first ostracod reported from alpine streams in Japan. *Zoosyst Evol* 98:117–127.

- 296 <https://doi.org/10.3897/zse.98.80442>
- 297 20. Kakui K, Shimada D (2022) Dive into the sea: first molecular phylogenetic evidence of host  
298 expansion from terrestrial/freshwater to marine organisms in Mermithidae (Nematoda: Mermithida). *J*  
299 *Helminthol* 96:e33. <https://doi.org/10.1017/S0022149X22000256>
- 300 21. Golovan OA (2015) Description of two ubiquitous species of Desmosomatidae (Isopoda: Asellota)  
301 from the Northwest Pacific Basin east of the Kuril-Kamchatka Trench. *Zootaxa* 4039:201–224.  
302 <https://doi.org/10.11646/zootaxa.4039.2.1>
- 303 22. Jennings RM, Golovan O, Brix S (2020) Integrative species delimitation of desmosomatid and  
304 nannoniscid isopods from the Kuril-Kamchatka trench, with description of a hadal species. *Prog*  
305 *Oceanogr* 182:102236. <https://doi.org/10.1016/j.pocean.2019.102236>
- 306 23. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J*  
307 *Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- 308 24. Dippenaar SM (2009) Estimated molecular phylogenetic relationships of six siphonostomatoid  
309 families (Copepoda) symbiotic on elasmobranchs. *Crustaceana* 82:1547–1567.  
310 <https://doi.org/10.1163/001121609X12511103974538>
- 311 25. DDBJ (2022) DNA Data Bank Japan. <https://www.ddbj.nig.ac.jp/> Accessed 29 November 2022
- 312 26. Summers MM, Al-Hakim II, Rouse GW (2014) Turbo-taxonomy: 21 new species of Myzostomida  
313 (Annelida). *Zootaxa* 3873:301–344. <https://doi.org/10.11646/zootaxa.3873.4.1>
- 314 27. Butcher BA, Smith MA, Sharkey MJ, Quicke DLJ (2012) A turbo-taxonomic study of Thai *Aleiodes*

- 315        (*Aleiodes*) and *Aleiodes* (*Arcaleiodes*) (Hymenoptera: Braconidae: Rogadinae) based largely on COI
- 316        barcoded specimens, with rapid descriptions of 179 new species. *Zootaxa* 3457:1–232.
- 317        <https://doi.org/10.11646/zootaxa.3457.1.1>

318 **Figure and table legends**

319 **Fig. 1** *Diexanthema hakuhomaru* **sp. nov.** (females) attached to the host, *Eugerdella cf. kurabyssalis*

320 Golovan, 2015 (females with developing oostegites), fresh specimens. a, holotype on host (ICHUM8451);

321 b, same, enlarged view; c–f, paratypes on hosts (ICHUM8452–ICHUM8455). Abbreviation: *ov*, ovary

322

323 **Fig. 2** *Diexanthema hakuhomaru* **sp. nov.**, holotype, female (ICHUM8451). a, b, body, dorsal and

324 ventral views, respectively; c, d, urosome, dorsal and ventral views, respectively; e, head region, ventral

325 view (arrow, right maxillary gland pore?)

326

327 **Fig. 3** Maximum-likelihood (ML) tree based on 18S sequences (1528 positions). Numbers near nodes are

328 ultrafast bootstrap values. Black circles indicate 100% ultrafast bootstrap support. Clades containing more

329 than two confamilial terminal taxa were collapsed (terminal triangles), except for those in Nicotroidae

330 (shaded) and Asterocheridae. Group names follow Boxshall and Lincoln [10]. The scale bar indicates

331 branch length in substitutions per site

332

333 **Table 1** Information on six *Diexanthema* species from North Atlantic Ocean

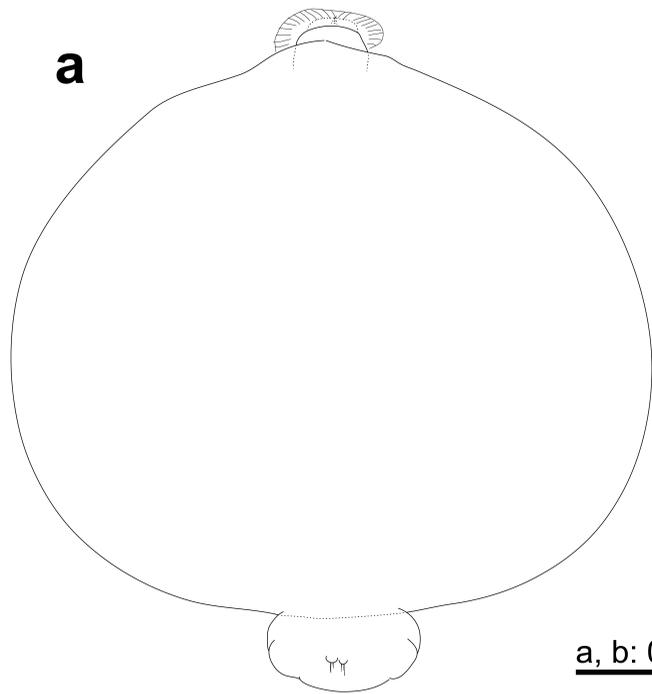
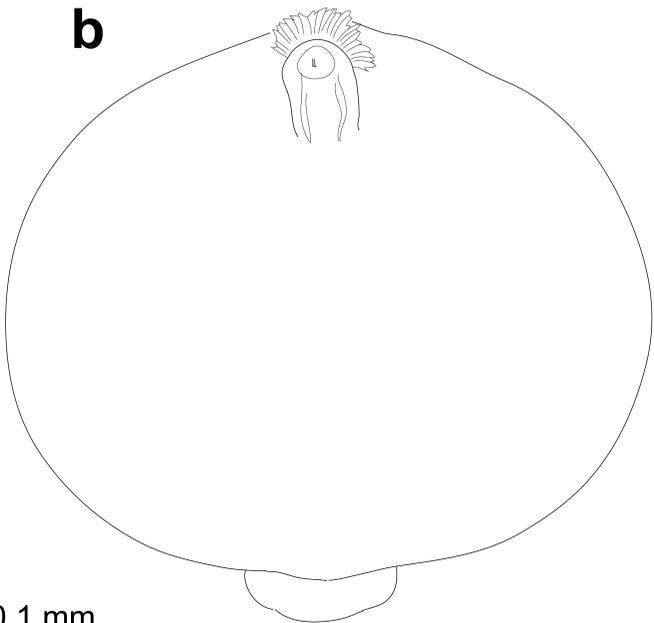
**a**

a, c-f: 0.5 mm

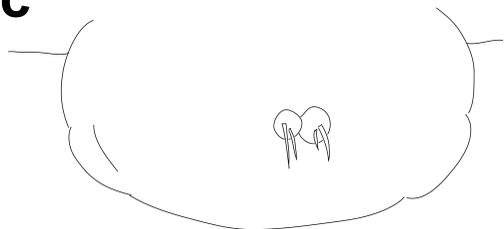
**b**

b: 0.1 mm

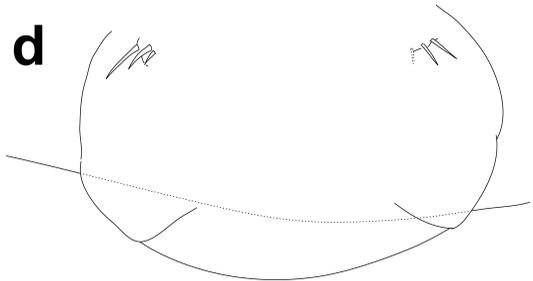
**c****d****e****f**

**a****b**

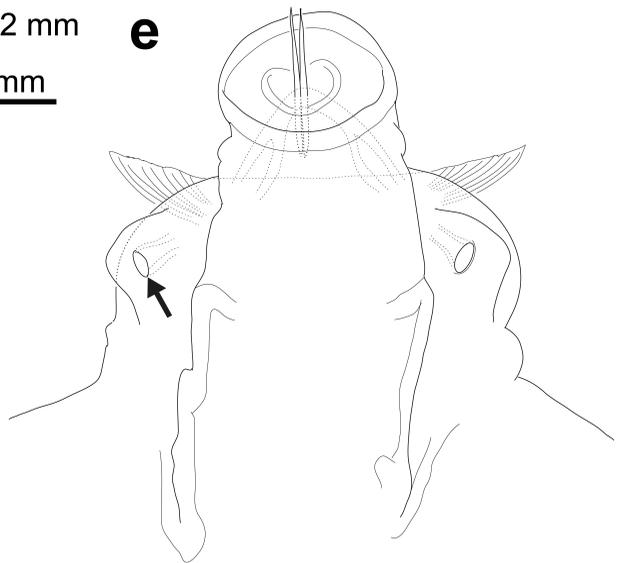
a, b: 0.1 mm

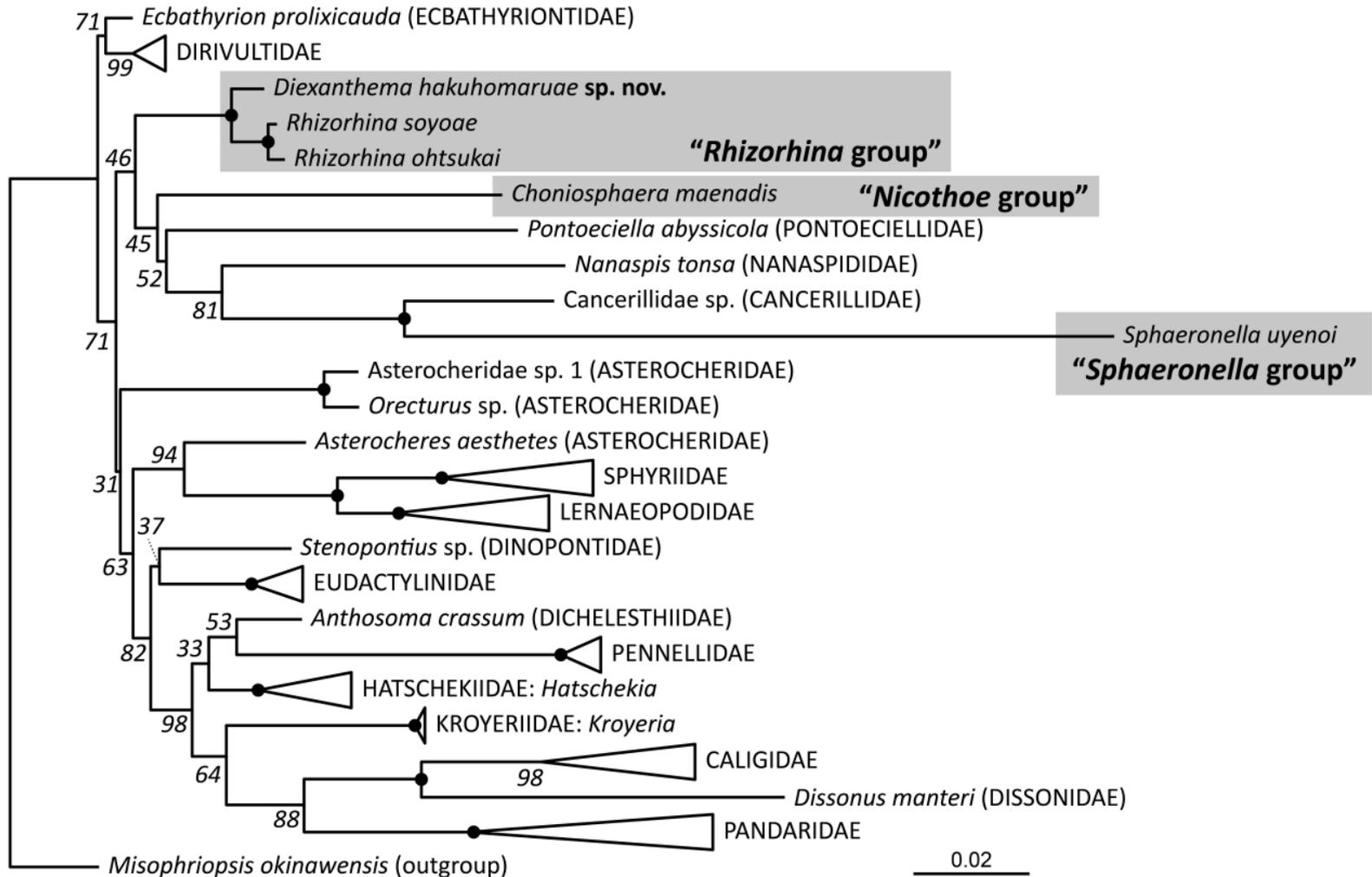
**c**

c, d: 0.02 mm

**d**

e: 0.02 mm

**e**



**Table 1** Information on six *Diexanthema* species from North Atlantic Ocean

Species	Isopod host	Locality	Depth (m)
<i>D. apoda</i>	Munnopsidae: <i>Bathybadistes spinosissima</i> (Hansen, 1916)	Rockall Trough, off Scotland	c. 2910
<i>D. bathydiaita</i>	Nannoniscidae: <i>Nannoniscus</i> sp.	Off North America	1624–1796
<i>D. corrugatum</i>	Munnopsidae: <i>Acanthocope</i> sp.	Rockall Trough	c. 2900
<i>D. desistoma</i>	Desmosomatidae: <i>Chelator insignis</i> (Hansen, 1916)	Off Africa	1330–1470
<i>D. nudum</i>	Desmosomatidae: <i>Mirabilicoxa</i> sp. (probably <i>M. acuminata</i> Hessler, 1970)	Rockall Trough	c. 2540
<i>D. ritchiei</i>	Ischnomesidae: <i>Gracilimesus tenuispinis</i> (Hansen, 1916)	Porcupine Seabight, off Ireland	3490–3550