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Author(s)	Kakui, Keiichi; Fukuchi, Jun; Ohta, Mizuki
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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 Kakui¹ · Jun Fukuchi² · Mizuki Ohta³

5 ¹Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo, Japan

6 ²Seto Marine Biological Laboratory, Kyoto University, Nishimuro, Japan

7 ³Atmosphere 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

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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 hakuohomaru* **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 hakuohomaru* 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. hakuohomaru* 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 hakuohmaruae* **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 hakuohmaruae* **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 nichthoid
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.

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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

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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 Nicotoidae

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

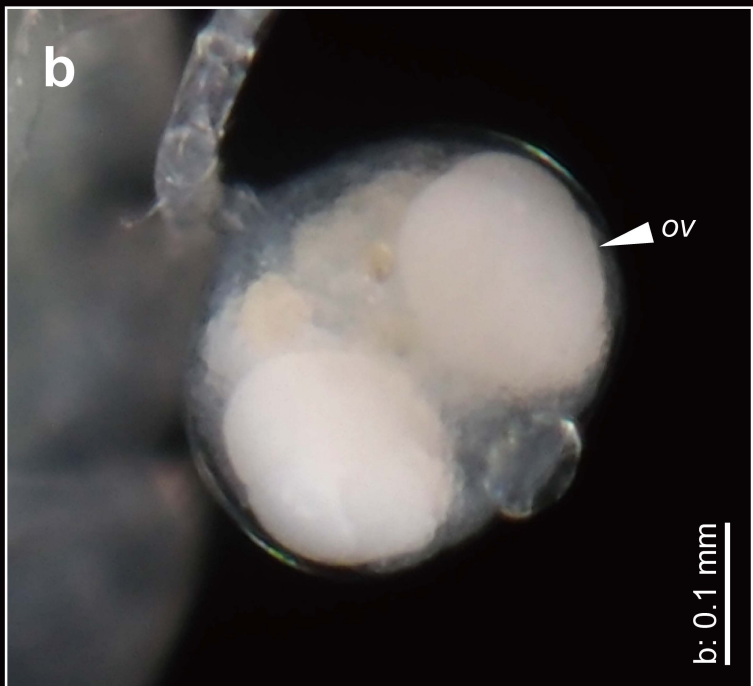
331 branch length in substitutions per site

332

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

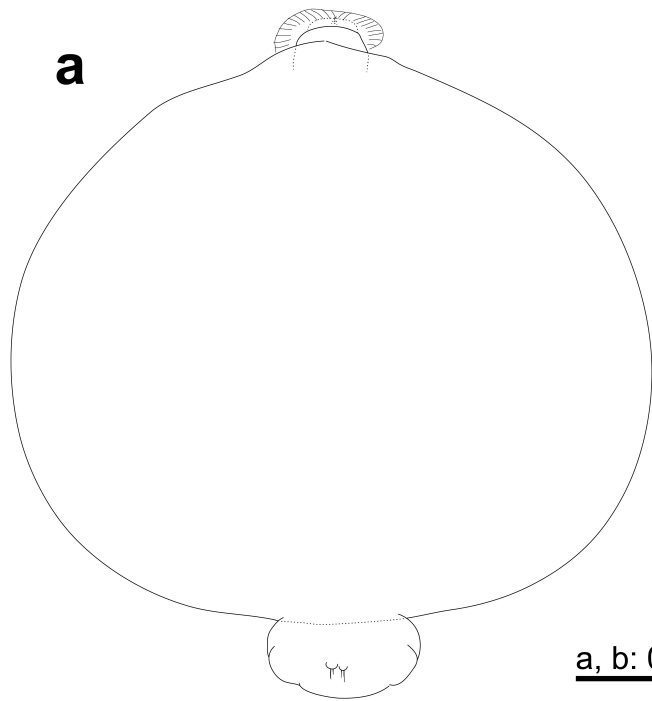
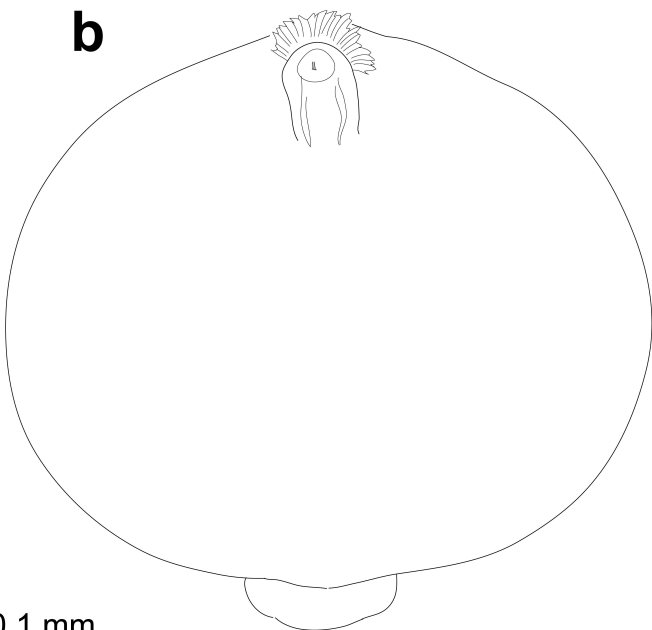
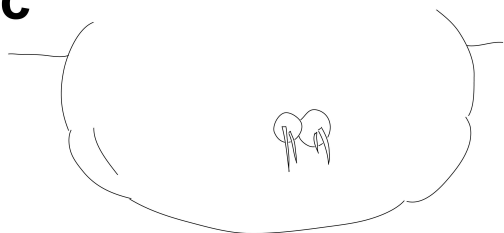
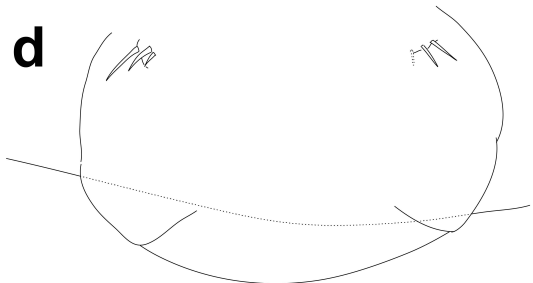
a

a, c-f: 0.5 mm

b

b: 0.1 mm

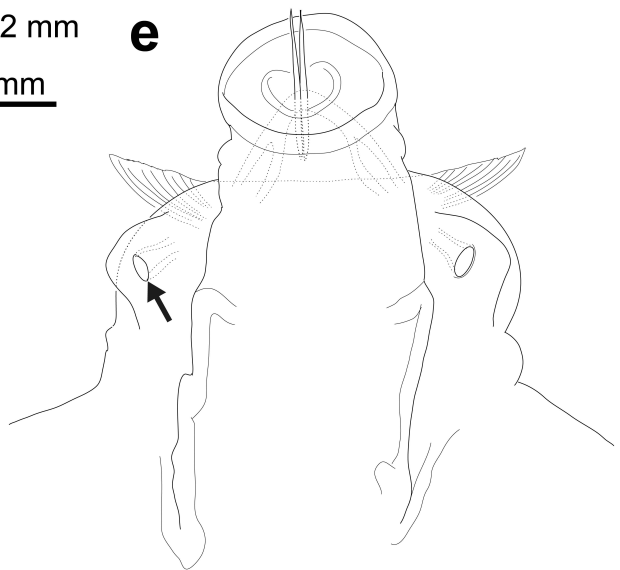
c**d****e****f**

a**b****c****d**

a, b: 0.1 mm

c, d: 0.02 mm

e: 0.02 mm

e

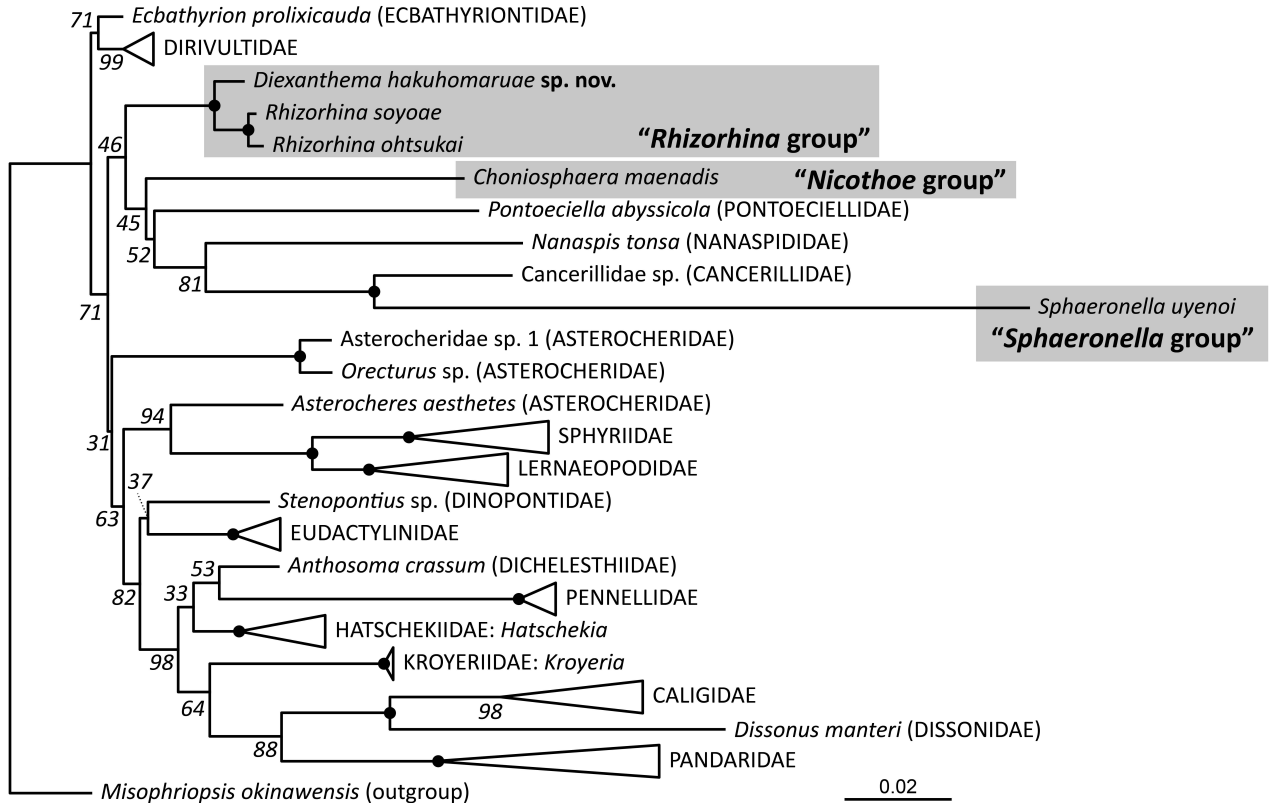


Table 1 Information on six *Diexanthema* species from North Atlantic Ocean

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