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

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20

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Abstract

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

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

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

Keywords Crustacea · ectoparasite · integrative taxonomy · Japan · parasites

Introduction

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

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

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

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

Materials and methods

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

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

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

DNA was extracted from the whole body of one copepod and pereopod 1 of one host by using the NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). For the cytochrome *c* oxidase subunit I (COI) gene, PCR primers used for the amplification and cycle sequencing were LCO1490 and HCO2198 [12]. For the 18S rRNA gene, amplification primers were SR1 and SR12 [13], and six primers (18S-b3F, 18S-b4R, 18S-b5F, 18S-b6F, 18S-a6R, and 18S-b8F [14, 15]) were used in cycle sequencing. For the copepod 16S rRNA gene, the newly designed primers Copepod16S_F (CGCCTGTTTATCAAARACWY) and Copepod16S_R (TCGATTTGAACTCAAATCAWG) were used for amplification and cycle sequencing. PCR amplification conditions for COI with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) were as described by Munakata *et al*. [16]; those for 18S with KOD FX Neo (Toyobo, Japan) were as described by Okamoto and Kakui [17]; and those for 16S with KOD ONE PCR Master Mix (Toyobo) were 45 cycles 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, excised with a micro spatula, and purified with the MagExtractor PCR & Gel Clean Up Kit (Toyobo). All nucleotide sequences were determined with a BigDye Terminator Kit ver. 3.1 and a 3730 DNA Analyzer

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

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

Results and Discussion

Host identification

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

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

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

Male and copepodid. Unknown.

Variation. One female paratype (ICHUM8453) observed in addition to the holotype showed all diagnostic characters seen in the holotype. Ranges (mean with standard deviation in parentheses) of BL, PW, UW, BL/PW, BL/UW, PW/UW for five individuals in the type series were 342–400 (372±23), 326–385 (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), respectively.

Genetic divergence and phylogenetic analysis

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

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

Remarks

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

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

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

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

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

Conclusions

We described *Diexanthema hakuhomaruae* **sp. nov.** parasitic on the desmosomatid *Eugerdella* cf. *kurabyssalis* Golovan, 2015 collected from a hadal depth in the Kuril-Kamchatka Trench, northwestern Pacific. This species represents the first *Diexanthema* species from the Pacific, and the first from hadal depths. Our 18S-based tree confirmed a close relationship between *Diexanthema* and *Rhizorhina*, 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;
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Ethics approval: Not applicable.

Consent: Not applicable.

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

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317 https://doi.org/10.11646/zootaxa.3457.1.1

Figure and table legends

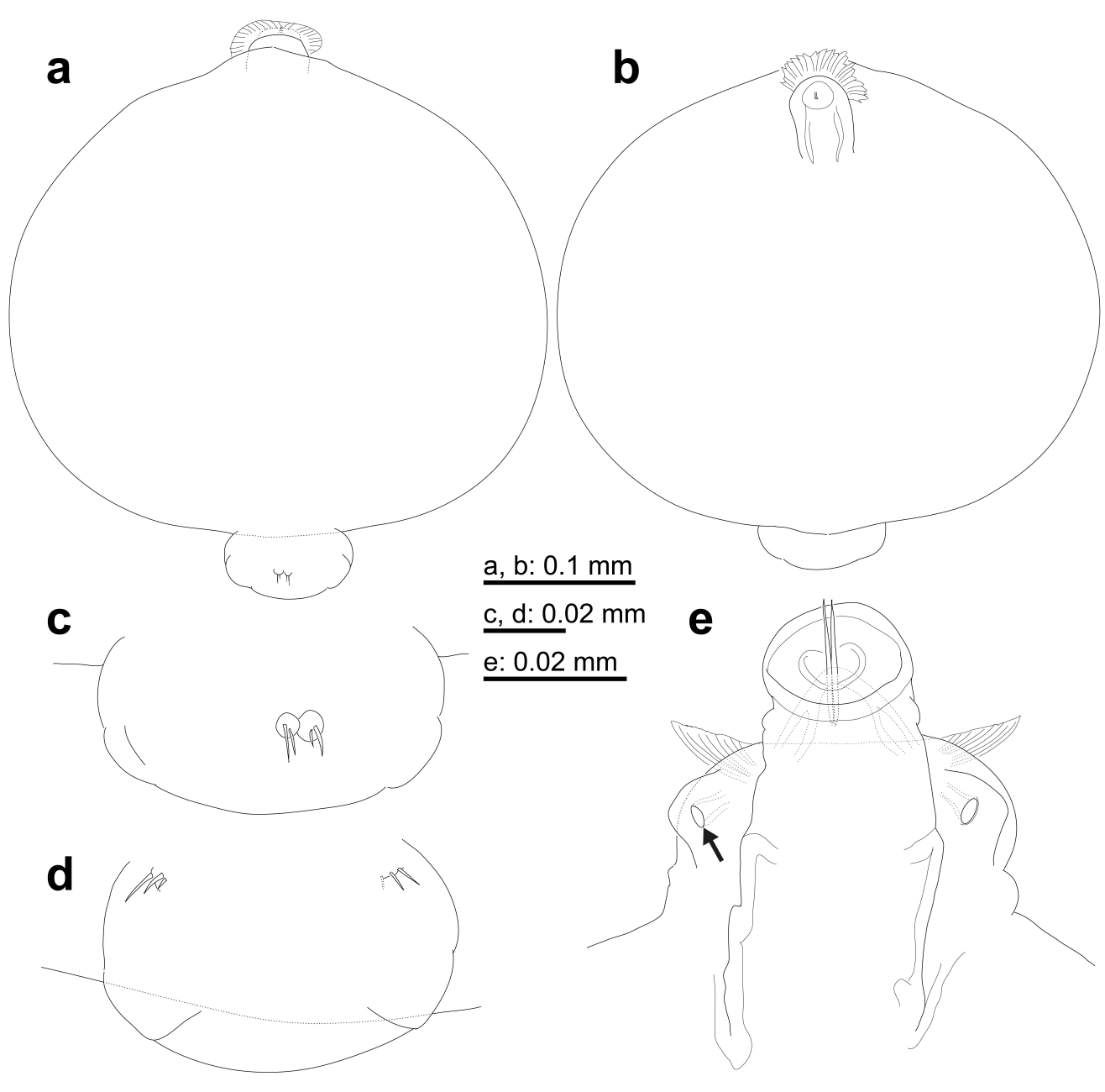
Fig. 1 *Diexanthema hakuhomaruae* **sp. nov.** (females) attached to the host, *Eugerdella* cf. *kurabyssalis* Golovan, 2015 (females with developing oostegites), fresh specimens. a, holotype on host (ICHUM8451); b, same, enlarged view; c–f, paratypes on hosts (ICHUM8452–ICHUM8455). Abbreviation: *ov*, ovary

Fig. 2 *Diexanthema hakuhomaruae* **sp. nov.**, holotype, female (ICHUM8451). a, b, body, dorsal and ventral views, respectively; c, d, urosome, dorsal and ventral views, respectively; e, head region, ventral view (arrow, right maxillary gland pore?)

Fig. 3 Maximum-likelihood (ML) tree based on 18S sequences (1528 positions). Numbers near nodes are ultrafast bootstrap values. Black circles indicate 100% ultrafast bootstrap support. Clades containing more than two confamilial terminal taxa were collapsed (terminal triangles), except for those in Nicothoidae (shaded) and Asterocheridae. Group names follow Boxshall and Lincoln [10]. The scale bar indicates branch length in substitutions per site

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

a
b
ov
a, c–f: 0.5 mm
b: 0.1 mm
c
d
e
f


a
b
a, b: 0.1 mm
c, d: 0.02 mm
e: 0.02 mm
c
e
d

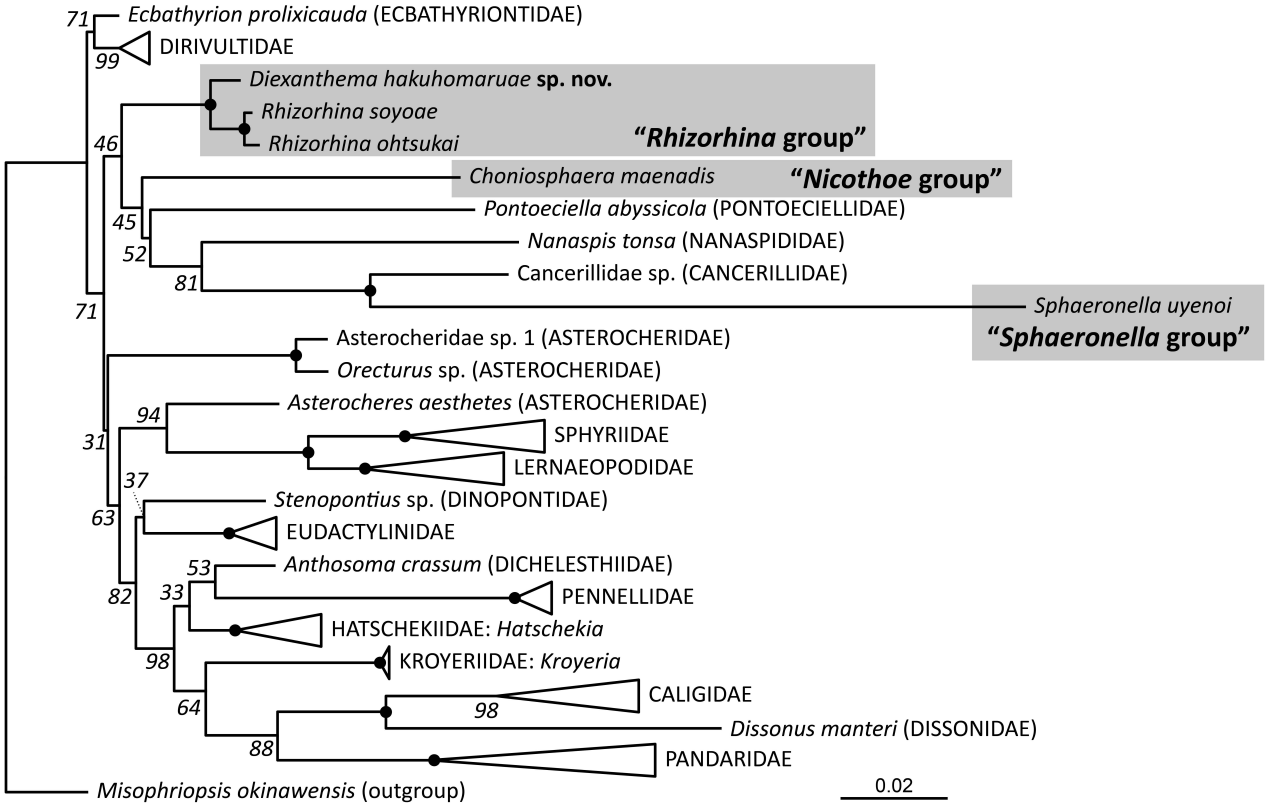

71
Ecbathyrion prolixicauda (ECBATHYRIONTIDAE)
DIRIVULTIDAE
99
Diexanthema hakuhomaruae sp. nov.
Rhizorhina soyoae
Rhizorhina ohtsukai
"Rhizorhina group"
46
Choniosphaera maenadis
"Nicothoe group"
Pontoeciella abyssicola (PONTOECIELLIDAE)
45
Nanaspis tonsa (NANASPIDIDAE)
52
Cancerillidae sp. (CANCERILLIDAE)
81
Sphaeronella uyenoi
"Sphaeronella group"
71
Asterocheridae sp. 1 (ASTEROCHERIDAE)
Orecturus sp. (ASTEROCHERIDAE)
Asterocheres aesthetes (ASTEROCHERIDAE)
94
SPHYRIIDAE
31
LERNAEOPODIDAE
37
Stenopontius sp. (DINOPONTIDAE)
63
EUDACTYLINIDAE
53
Anthosoma crassum (DICHELESTHIIDAE)
82
33
PENNELLIDAE
HATSCHEKIIDAE: Hatschekia
98
KROYERIIDAE: Kroyeria
64
CALIGIDAE
98
Dissonus manteri (DISSONIDAE)
88
PANDARIDAE
Misophriopsis okinawensis (outgroup)
0.02

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

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