

Echinoderes hakaiensis sp. nov.: a new mud dragon (Kinorhyncha, Echinoderidae) from the northeastern Pacific Ocean with the redescription of *Echinoderes pennaki* Higgins, 1960

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Received: 8 February 2017 / Revised: 24 April 2017 / Accepted: 2 May 2017
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Abstract Surveys of kinorhynch diversity in the northeastern Pacific Ocean are rare, and only eight species have been described from the region so far. We explored the diversity of kinorhynchs from a pristine coastal habitat of British Columbia (Calvert Island) and discovered a new subtidal species, *Echinoderes hakaiensis* sp. nov. We also redescribed one of the oldest described echinoderid species, *E. pennaki*. Both species were characterized with high-resolution light microscopy (LM), scanning electron microscopy (SEM), and DNA sequences from the mitochondrial cytochrome c oxidase subunit 1 (COI) gene. *Echinoderes hakaiensis* sp. nov. can be differentiated from other species by a unique pattern of spines and tubes (i.e., four pairs of tubes on segment 2 combined with three middorsal spines). *Echinoderes pennaki* shows a configuration of traits on segment 2 that questions the reliability of conventional morphological characters used to distinguish different genera within the Echinoderidae. Reevaluation of these characters will require a resolved molecular phylogenetic context including a more comprehensive sampling of species within the group.

Keywords British Columbia · Meiofauna · Cyclorhagida · COI · Systematics

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Communicated by S. Gollner

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Introduction

The coastal margins of British Columbia are considered one of the most productive and biologically diverse areas, not only within Canada but also of the world. The presence of an array of ecosystems, including island archipelagos, forests, glaciers, deep fiords, shallow mudflats, and estuaries, together with strong tidal currents and massive upwellings, contribute to the production of organic and inorganic nutrients enriching and stimulating marine food webs. Studies focused on the diversity of the Kinorhyncha have not been accomplished in British Columbia until very recently, when Herranz and Leander (2016) redescribed *Echinoderes kozloffii* Higgins, 1977 and *Echinoderes ohtsukai* Yamasaki and Kajihara, 2012. *Echinoderes kozloffii*, described from the San Juan Islands (Washington, USA), has become one of the most common kinorhynch species found along the coasts of British Columbia (Herranz pers. obs.), and *E. ohtsukai*, originally described from Japan, is now considered a potential introduced species in British Columbia associated with the commercial exportation of the Pacific oyster (Herranz and Leander 2016).

Knowledge of kinorhynchs in the northeastern Pacific Ocean is scarce, limited to only samples collected from the San Juan Archipelago (Washington, USA), Southwest Vancouver Island, and California by Robert P. Higgins and collaborators several decades ago (Brinkhurst 1987; Higgins 1960, 1961, 1967, 1977, 1986). The kinorhynch species list from Canada is quite short, limited by the scattered samplings performed over time by Higgins (1960, 1961), Jørgensen and Kristensen (1991), and more recently by Herranz and Leander (2016), all of them restricted to a few localities. The list includes eleven species, nested in five different genera: *Cristaphyes cryopygus* (Higgins and Kristensen, 1988); *Pycnophyes ilyocryptus* (Higgins, 1961); *Pycnophyes sanjuanensis* Higgins, 1961; *Pycnophyes frequens* Blake,

1930; *Higginsium cataphractus* (Higgins, 1961); *Krakenella canadensis* (Higgins & Korczynski, 1989); *Krakenella borealis* (Higgins & Korczynski, 1989); *Echinoderes aquilonius* Higgins & Kristensen, 1988; *Echinoderes tubilak* Higgins & Kristensen, 1988; *Echinoderes kozloffii* and *E. ohtsukai*.

Echinoderes is currently the largest genus of kinorhynchs, including 98 described species distributed worldwide and representing more than one-third of the total diversity in the group. However, there are just three *Echinoderes* species reported from the entire northeast Pacific coast: *Echinoderes pennaki* Higgins, 1960, *E. kozloffii*, and *E. ohtsukai*. Here, we describe a new echinoderid species, *Echinoderes hakaiensis* sp. nov. and redescribe *E. pennaki* (originally described from the San Juan Archipelago) providing, for the first time, high-resolution light and scanning electron micrographs, as well as molecular data. Both species were collected in subtidal muddy sediments off the coasts of Calvert Island, a pristine area in the central coast of British Columbia. *Echinoderes hakaiensis* sp. nov. increase the number of described kinorhynchs species in Canada to a total of 12.

Materials and methods

Sampling

Specimens of *Echinoderes* were collected in June 2015 and April 2016 on the coast of Calvert Island, located east of Queen Charlotte Sound in the Central Coast Region District of British Columbia, about 100 km north of Vancouver Island (Fig. 1a). Subtidal samples were taken from a boat using a grab sampler along Kwakshua Channel at depths ranging from 80 to 350 m (Fig. 1b). Details of the sampling stations are provided in Table 1.

Microscopy

Kinorhynchs were extracted from the sediment using the Higgins bubbling technique (Higgins 1964a; Sørensen and Pardos 2008) and fixed in 4% paraformaldehyde. Specimens prepared for light microscopy (LM) were dehydrated through a graded series of ethanol and transferred to glycerin prior to mounting in Fluoromount-G®. The specimens were examined and photographed using a Zeiss Axioplan 2 microscope with differential interference contrast (DIC) optics equipped with a Zeiss Axiocam 503 color camera. Measurements were made using ZEN 2 software (Zeiss, Germany). Specimens for scanning electron microscopy (SEM) were ultrasonically cleaned by exposing them to ultrasound intervals of 5–10 s and posteriorly dehydrated

through a graded series of ethanol and critical point dried. The dried specimens were mounted on aluminum stubs, sputter coated with platinum-palladium, and imaged with a Hitachi S4700 field emission scanning electron microscope. Coating and SEM imaging were performed at the Bioimaging Facility at the University of British Columbia (UBC).

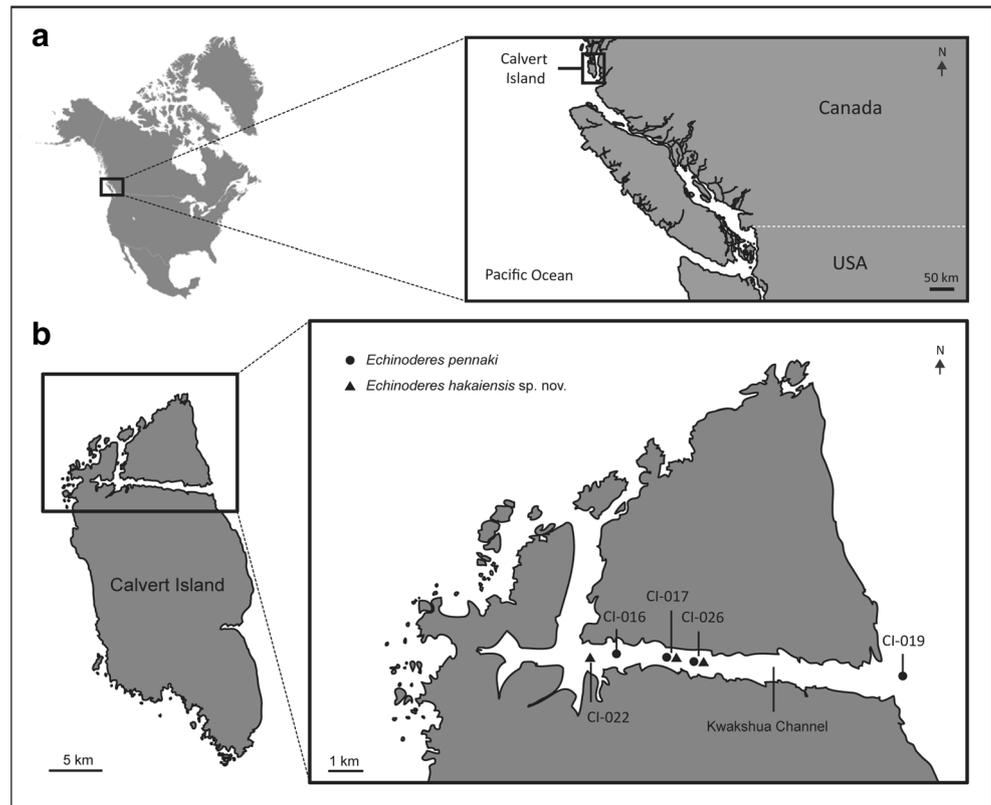
DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from single specimens of *E. hakaiensis* sp. nov. and *E. pennaki*, from Calvert Island, all preserved in 99% ethanol, using a DNeasy Blood and Tissue Kit (Qiagen, Tokyo), following the protocol described by Yamasaki et al. (2013). One cuticular voucher of *E. pennaki* was recovered from the lysis buffer and mounted in Fluoromount-G® using a regular glass slide and deposited at the Natural History Museum of Denmark, under catalog number NHMD-115708. DNA was extracted from isolated individuals to amplify and sequence the mitochondrial cytochrome c oxidase subunit 1 (COI) gene. Polymerase chain reaction (PCR) assays were performed using the PuReTaQ Ready-To-Go PCR Beads kit (GE Healthcare, Buckinghamshire, UK), using the following primers: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAA AAATCA-3') (Folmer et al. 1994). PCR cycling conditions were: 95 °C for 1 min; 35 cycles of (95 °C for 30 s, 49 °C for 1 min 30 s, and 72 °C for 3 min); and 72 °C for 7 min. The amplified fragments were gel purified using the UltraClean DNA Purification Kit (MO BIO, Carlsbad, CA) and sequenced. Nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit (Applied Biosystems). Sequence fragments were edited and assembled using Geneious version (9.1.6) (<http://www.geneious.com>; Kearse et al. 2012). After assembly, the sequences were identified using BLAST and deposited in GenBank (NCBI) under accession numbers KY550655 for *E. pennaki* and KY550656 for *E. hakaiensis* sp. nov.

Comparative material examined

For comparison with *Echinoderes pennaki*, type material from *E. angustus* (including holotype USNM-233200 and seven paratypes USNM-233202 to 233205) was loaned from the Smithsonian Institution, United States National Museum. Light micrographs used for the comparison of segment 2 in *E. aquilonius*, *E. eximus*, *E. aureus*, and *E. obtuspinosus* were kindly provided by M.V. Sørensen from paratypes from the collection of the National History Museum of Denmark (NHMD).

Fig. 1 Maps showing the sampling locations along the northeastern Pacific coast. **a** Close-up of the British Columbia area, showing the position of Calvert Island (black square). **b** Calvert Island area, showing a close-up of Kwakshua Channel with the sampling stations of *Echinoderes pennaki* and *E. hakaensis* sp. nov.



Results

Taxonomy

Class Cyclorhagida (Zelinka, 1896)
 Order Echinorhagata Sørensen et al., 2015
 Family Echinoderidae Zelinka, 1894
Genus *Echinoderes* Claparède, 1863

Emended diagnosis

Echinoderidae with segment 2 composed of a single tergal plate which may or may not show partial midventral divisions or folds. These divisions can be superficial and/or involving a partial pachycyclus split in the anterior part of the segment. The midventral divisions might show intraspecific variation.

Type species: *Echinoderes dujardini* Claparède, 1863 (see Higgins 1964b; Neuhaus 2013)

***Echinoderes pennaki* Higgins, 1960** (Figs. 2, 3, 4, 5, 6, and 7, Tables 2 and 3).

Emended diagnosis

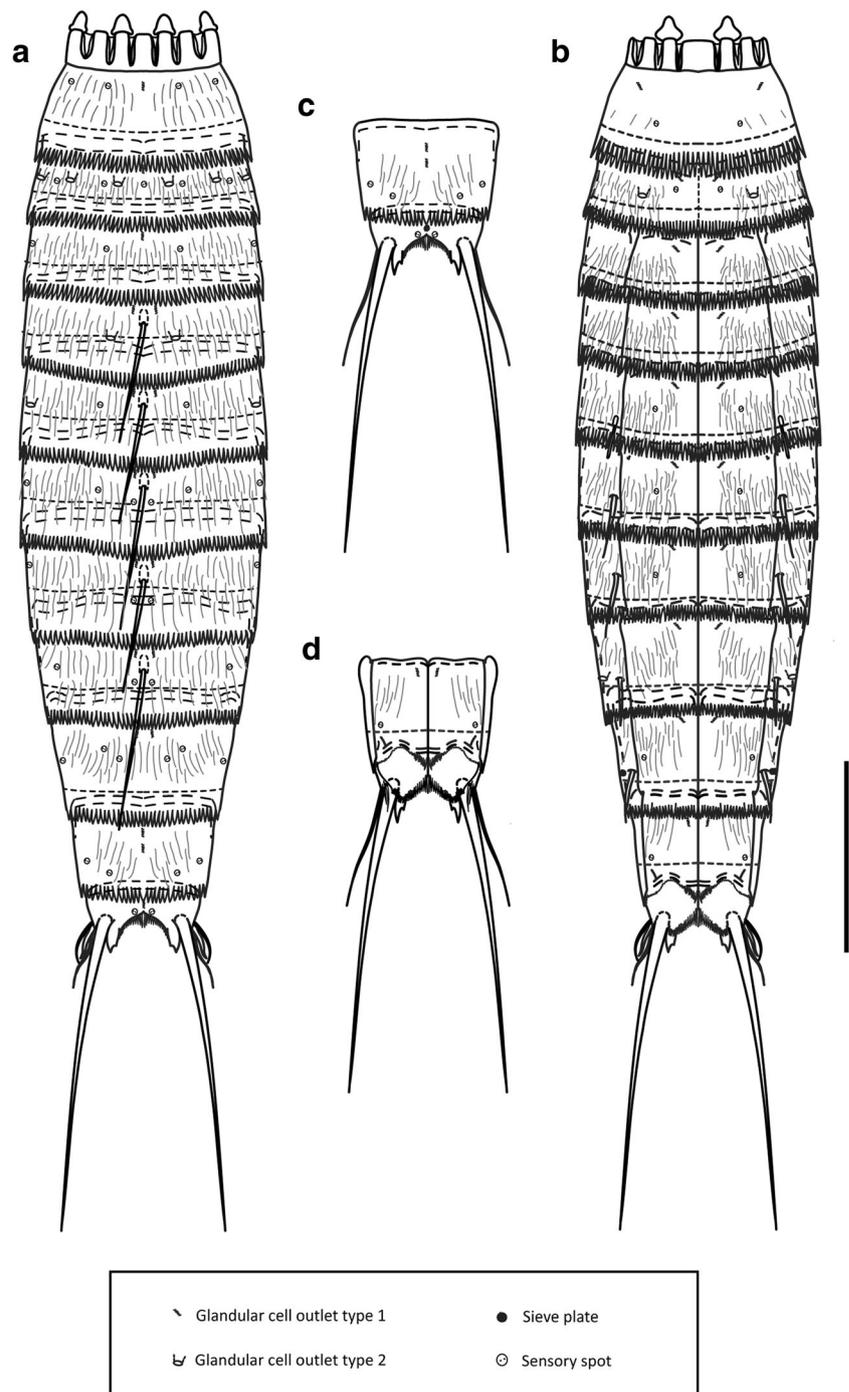
Echinoderes with five middorsal spines on segments 4–8 slightly increasing in length posteriorly showing protuberant bases. Lateroventral spines present on segments 6–9. Tubes present in lateroventral position on segment 5, males and females lacking tubes on segment 10. Glandular cell outlets type 2 in subdorsal, laterodorsal, midlateral, and ventrolateral positions on segment 2; subdorsal position on segment 4; midlateral position on segment 5; and sublateral on segment

Table 1 Summary of samples collected along Kwakshua Channel (Calvert Island)

Station	Date	Position	Depth (m)	Sediment	Species	Mounting
CI-016	Jun. 6, 2015	51° 39.248' N 128° 04.305' W	100	Mud	<i>E. pennaki</i>	LM (NHMD-115706), SEM
CI-017	Jun. 6, 2015	51° 39.178' N 128° 02.673' W	140	Muddy sand	<i>E. pennaki</i> <i>E. hakaensis</i> sp. nov.	LM (NHMD-115707) , SEM
CI-019	Jun. 7, 2015	51° 39.024' N 127° 57.147' W	350	Green mud mixed with gray silt	<i>E. pennaki</i>	LM (DNA voucher, NHMD-115708), SEM
CI-022	Jun. 8, 2015	51° 39.245' N 128° 04.827' W	88	Sandy mud	<i>E. hakaensis</i> sp. nov.	LM (holotype NHMD-115709), SEM
CI-026	Apr. 7, 2016	51° 39.187' N 128° 02.716' W	92	Sandy mud	<i>E. pennaki</i> <i>E. hakaensis</i> sp. nov.	LM (paratype NHMD-115710) , SEM

In **bold** is the material deposited at the National History Museum of Denmark when two species are present in the same sample

Fig. 2 Illustrations of *Echinoderes pennaki* from Calvert Island. **a** Male, dorsal view. **b** Male, ventral view. **c** Female, detail of segments 10–11, dorsal view. **d** Female, detail of segments 10–11, ventral view. The legend shows the cuticular characters represented in the line art excluding spines, tubes, and cuticular hairs. Scale bar = 100 μ m



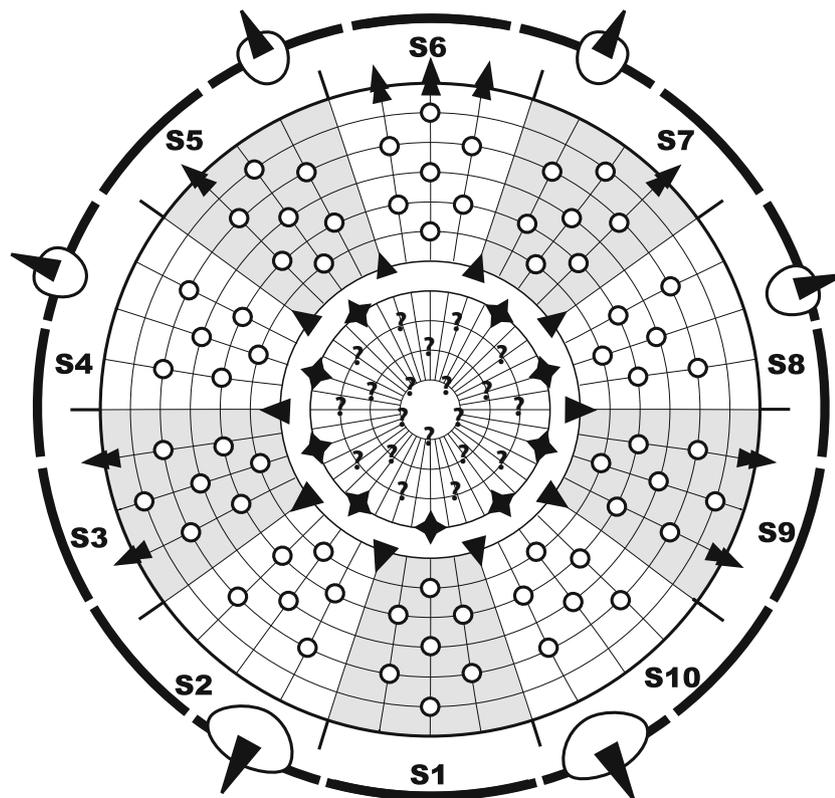
8. Segment 1 showing strongly developed pectinate fringe on the ventral side, while less developed in the dorsal side.

Material examined

Female holotype USNM-29746 was loaned from the Smithsonian Institution, United States National Museum, and examined with light microscopy equipped with DIC optics. No allotype is available. The type specimens originate from Orcas Island, San Juan Archipelago, Washington, USA. Additional material includes nine specimens collected in Calvert Island

(BC), Canada. One adult male extracted from subtidal mud on June 2015 at station CI-016 in Kwakshua Channel (Fig. 1, Table 1) and one adult female collected at station CI-017 from subtidal muddy sand. Both specimens were mounted in Fluoromount-G® and deposited at the Natural History Museum of Denmark, under catalog numbers NHMD-115706 and NHMD-115707, respectively. One adult female collected at station CI-019 was preserved in 99% ethanol and used for DNA extraction; the cuticle was kept as a DNA voucher and deposited

Fig. 3 Diagram of the mouth cone, introvert, and placids showing the distribution of oral styles, scalids, and trichoscalid plates in *Echinoderes pennaki*. The table below shows the scalid arrangement by sector and summarizes scalid numbers by rings and sectors. “Double diamonds” are marked in the table with double lines and quincunxes are marked with dotted lines. *ls* leaf-like scalid; *oos* outer oral style; *psp* primary spinoscalid; *sp* spinoscalid; *tr* trichoscalid. The *question marks* indicate uncertain positions



Scalid and style arrangement

Ring/Sector	1	2	3	4	5	6	7	8	9	10	Total
00 oos ◆	1	1	1	1	1	0	1	1	1	1	9
01 psp ▼	1	1	1	1	1	1	1	1	1	1	10
02 sp ○	1	1	1	1	1	1	1	1	1	1	10
03 sp ○	2	2	2	2	2	2	2	2	2	2	20
04 sp ○	1	1	1	1	1	1	1	1	1	1	10
05 sp ○	2	2	2	2	2	2	2	2	2	2	20
06 sp ○	1	0	1	0	1	1	1	0	1	0	6
07 ls ▼	0	0	2	0	1	3	1	0	2	0	9
08 tr ○	0	1	0	1	1	0	1	1	0	1	6
Total scalids	7	7	9	7	9	10	9	7	9	7	91

at the Natural History Museum of Denmark, under catalog number NHMD-115708. The mitochondrial cytochrome c oxidase subunit 1 (COI) gene was sequenced (GenBank accession number KY550655). Additionally, six females from different localities (Table 1) were mounted on SEM stubs and stored in the authors’ personal reference collection at the University of British Columbia.

Description

Adults with head, neck, and 11 trunk segments (Figs. 2, 4a, 5a, b, and 7a). Measurements and dimensions are given in Table 2. A summary of sensory spots, spines, sieve plates, tubes, and glandular cell outlet positions is provided in Table 3. The head consists of a retractable mouth cone and an introvert (Figs. 3 and 6a, b). Outer armature of the mouth cone formed by nine outer oral styles divided into two

subunits, alternating in size between five longer ones situated according to uneven sectors of the introvert and four shorter ones situated according to even sectors (Figs. 3 and 6c), mid-dorsal outer oral style is missing. Outer oral styles with a long fringe at their bases composed of 6–7 rigid and pointy tips (Fig. 6c). Inner armature of the mouth cone was not observed.

The introvert has seven rings of cuticular spinoscalids and one additional ring of trichoscalids that are associated with the placids (Figs. 3 and 6a). Ring 01 with ten primary spinoscalids consisting of a basal sheath and a distal end piece. The basal sheath has a very long and conspicuous proximal fringe, situated next to the insertion point, showing seven long and pointy tips, followed by a second fringe with four flexible long tips (Fig. 6b, d). The distal pieces of the primary spinoscalids have a flexible long fringe with 10–12 tips and are long and round in cross-section, ending

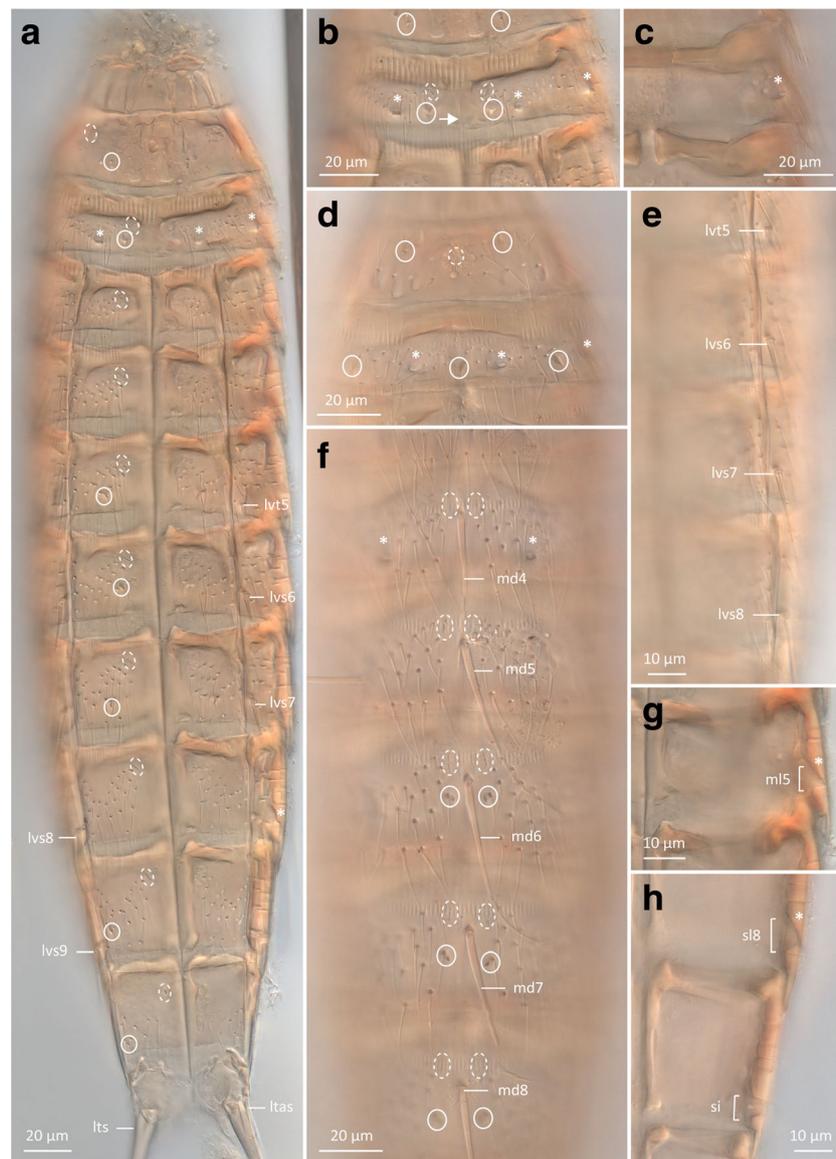


Fig. 4 Light micrographs (differential interference contrast, DIC) showing traits in *Echinoderes pennaki* holotype USNM-29746. **a** Ventral overview. **b** Detail of segment 2, ventral view. **c** Detail of segment 2, focus is on the laterodorsal area showing the glandular cell outlet type 2 opening. **d** Detail of segments 1 and 2, dorsal view. **e** Detail of segments 5–8, left side, focus is on the lateroventral area, where there is the insertion of tubes and spines. **f** Detail of segments 4–8, dorsal view. **g** Detail of segment 5, focus is on midlateral position to show the glandular outlet type 2 opening. **h** Detail of segments 8 and 9, focus is on sublateral

position to show the glandular type 2 opening on segment 8 and the sieve plate on segment 9. *lts* lateroterminal spine; *ltas* lateral terminal accessory spine; *lvt* lateroventral tube; *lvs* lateroventral spine; *md* middorsal spine; *ml* midlateral; *si* sieve plate; *sl* sublateral. The *dashed circles* indicate the position of glandular cell outlets type 1. The *solid circles* indicate the position of sensory spots. The *asterisks* mark the position of glandular cell outlets type 2. The *arrow* indicates the position of the midventral articulation on segment 2. Digits after abbreviations refer to segment numbers

in a blunt tip. Ring 02 is composed of ten laterally compressed spinoscalids, all formed by a long smooth basal part which ends in a short and thin fringe (Fig. 6b, d). Ring 03 consists of 20 spinoscalids with the same appearance of those from ring 02 but showing a basal part with a conspicuous spiny projection situated next to the articulation point (Figs. 3 and 6d). Rings 04 and 05 consist of 10 and 20 spinoscalids, respectively; all resemble those of ring 02 (Figs. 3 and, 6d). Ring 06 has six spinoscalids with the

same appearance but shorter than those on previous rings. Ring 07 has nine short leaf-like scalids showing a wide and hairy base from where several flexible elongations arise (Fig. 6d). Six long and hairy trichoscalids attached to trichoscalid plates are situated ventrally in sectors 2 and 10 and dorsally in sectors 4, 5, 7, and 8 (Figs. 3 and 6a, d). See Fig. 3 for a polar diagram that summarizes the location and arrangement of oral styles, scalids, and placids.

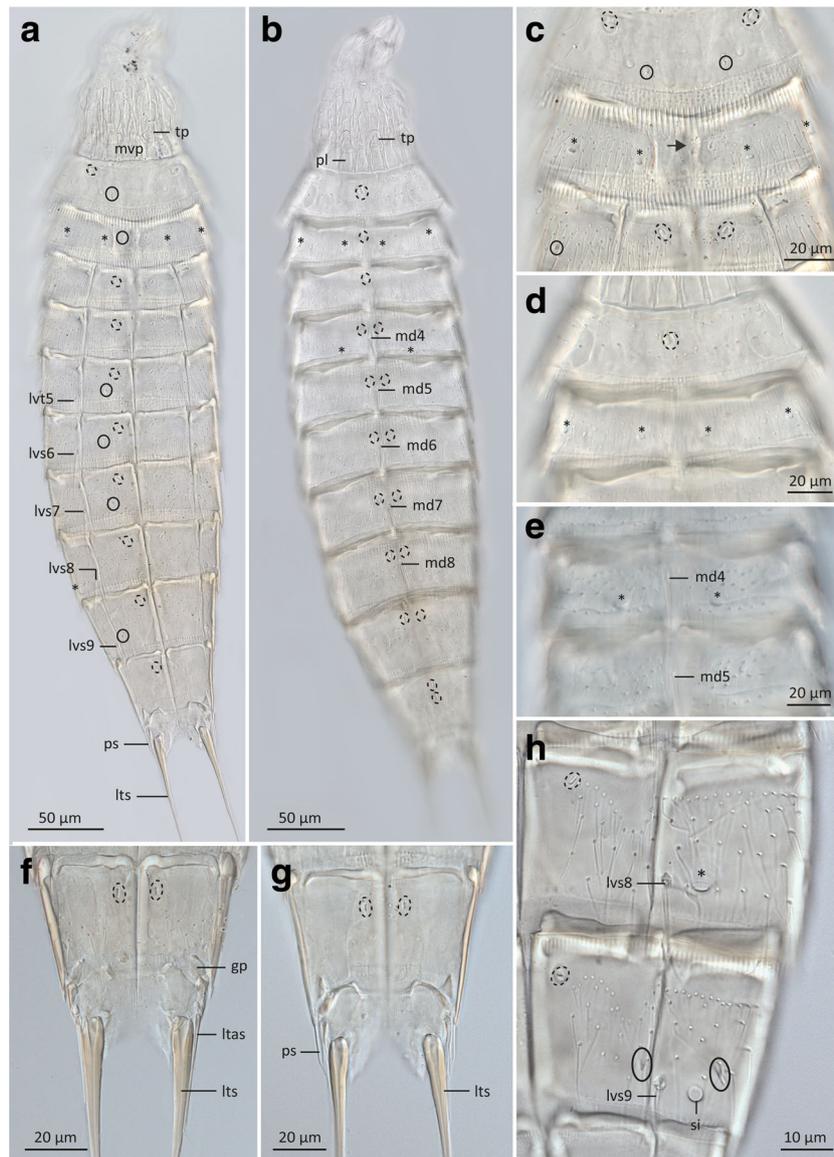


Fig. 5 Light micrographs (DIC) showing traits in *Echinoderes pennaki* collected from Calvert Island, male NHMD-115706 (**a–d, g**), female NHMD-115707 (**e, f**), and female NHMD-115708 (**h**). **a** Male overview, ventral view. **b** Male overview, dorsal view. **c** Detail of segments 1–3, ventral view. **d** Detail of segments 1 and 2, dorsal view. **e** Detail of segments 4 and 5, dorsal view. **f** Female, detail of segments 10 and 11, ventral view. **g** Male, detail of segments 10 and 11, ventral view. **h** Female, detail of segments 8 and 9 lateroventral view. *gp* gonopore; *lts*

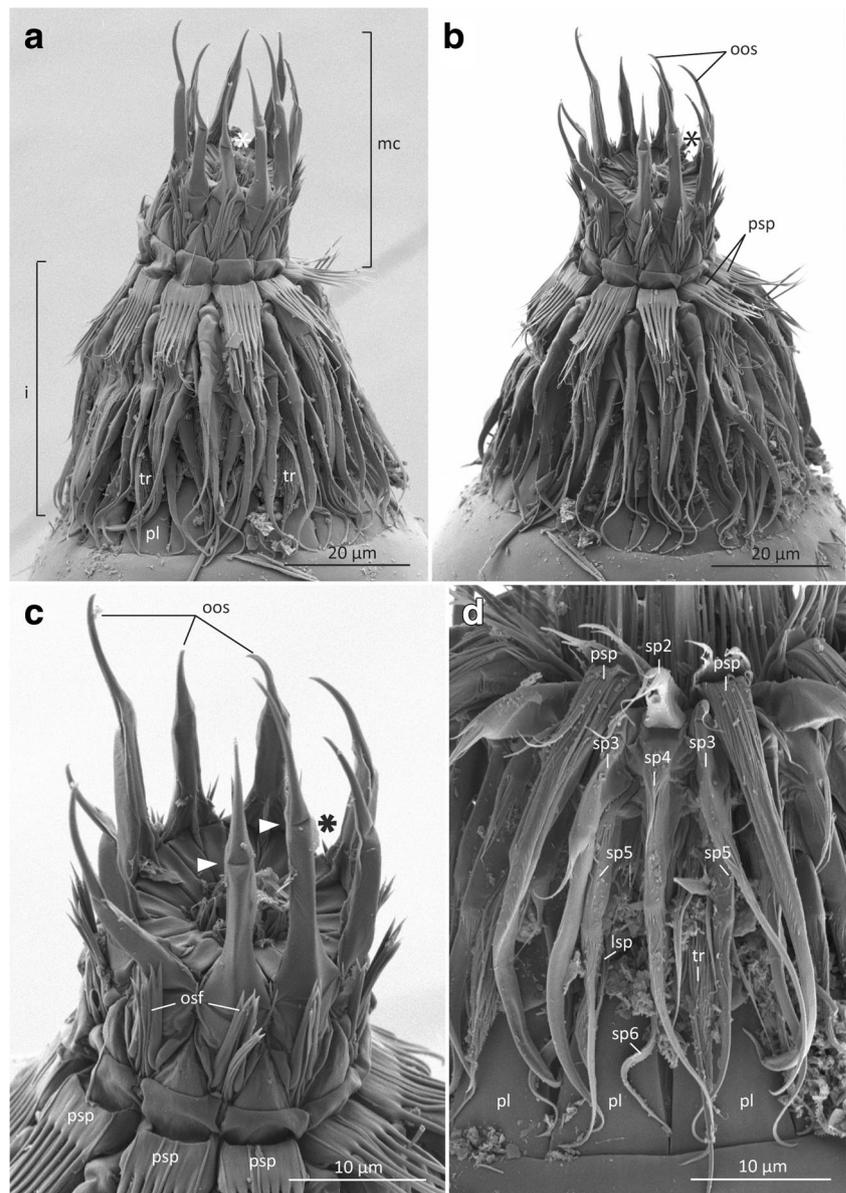
lateroterminal spine; *ltas* lateral terminal accessory spine; *lvt* lateroventral tube; *lvs* lateroventral spine; *md* middorsal spine; *mvp* midventral plate; *pl* placid; *ps* penile spines; *si* sieve plate; *tp* trichoscalid plate. The *dashed circles* indicate the position of glandular cell outlets type 1. The *solid circles* indicate the position of sensory spots. The *asterisks* mark the position of glandular cell outlets type 2. The *arrow* indicates the position of the midventral articulation on segment 2. Digits after abbreviations refer to segment numbers

The neck is composed of 16 elongated placids, with the midventral placid being rectangular and wider, measuring ca. 17 μm at the base (from LM), while the remaining ones are narrower and trapezoid-shaped, measuring ca. 9 μm at the base (from LM) (Figs. 4a, 5a, b, and 6a, b, d). All placids articulate with the first trunk segment. Trichoscalid plates bearing trichoscalids appear dorsally on placids 6, 8, 10, and 12 and ventrally on placids 2 and 16 (Figs. 3 and 5a, b). Ventral trichoscalid plates are wide and triangular-shaped with

rounded edges, while dorsal trichoscalid plates are more oval and smaller (Figs. 2a, b, 3, and 5a, b).

The trunk is divided into 11 segments (Figs. 2a, b, 4a, 5a, b, and 7a). Segment 1 is composed of a closed ring (Figs. 2a, b, 4a, d, 5a, b, and 7a, b), segment 2 is composed of a ring with a partial midventral division (Figs. 2b, 4b, 5a, c, and 7b), while segments 3–11 are composed of one tergal and two sternal plates (Figs. 2a, b, 4a, 5a, b, and 7a). Glandular cell outlets type 1 situated in the anterior part of the segments usually

Fig. 6 Scanning electron microscopy (SEM) images showing the introvert and mouth cone morphology of *Echinoderes pennaki* collected from Calvert Island. **a** Extended head, lateroventral view. **b** Extended head, lateral view. **c** Detail of the mouth cone, lateroventral view. **d** Introvert showing sector 7, laterodorsal view. *i* introvert; *lsp* leaf-like spinoscalid; *mc* mouth cone; *oos* outer oral styles; *osf* outer oral style fringe; *pl* placid; *psp* primary spinoscalids; *sp1–sp6* spinoscalids, number refers to the rows; *tr* trichoscalid. The asterisks mark the middorsal position. The arrowheads mark the position of the outer oral style articulation, which differs between contiguous styles



hidden under the posterior part of the previous segment. Dorsal outlets are elongated, single, and middorsal on segments 1–3 and 11, appearing as two middorsal outlets on segment 10, paired and paradorsal on segments 4–9 (Figs. 2a, 4f, and 5b). Ventral outlets are also elongated, lateroventral on segment 1 and ventromedial on segments 2–10 (Figs. 2b, 4a, and 5a). Primary pectinate fringe well developed on all segments, especially on segment 1, where the fringe tips become remarkably larger and pointy in the ventral side (Figs. 2a, b and 7a, b). Transition from long to short pectinate fringes in segment 1 occurs at midlateral position (Fig. 7b). Remaining segments with uniform short pectinate fringes with smaller fringe tips (Figs. 2a, b and 7a). Secondary pectinate fringe composed of minute short and homogeneous fringe tips (Fig. 7d–f). Cuticular hairs emerging from round

perforation sites on segment 1 (Fig. 7b) and slightly oval on segments 2–10 (Fig. 7d, f–g).

Segment 1 consists of a closed cuticular ring (Figs. 2a, b, 4a, d, 5a–d, and 7a, b). Three pairs of sensory spots are located in subdorsal, laterodorsal, and ventrolateral positions. Dorsal sensory spots located very close to the anterior segment margin, whereas the ventrolateral ones located in the middle to posterior most part of the segment close to the *ij*-line (Figs. 2a, b, 4a, b, 5c, and 7b). All of the sensory spots in this and the following segments are round and extremely small (ca. 1 µm from SEM), composed of six short papillae surrounding a central pore with a cilium (e.g., Fig. 7d, f). Cuticular hairs long and scarce, distributed forming a couple of lines on the dorsal side while reduced to a single line close to the pectinate fringe on the ventral side (Figs. 2a, b and 7b).

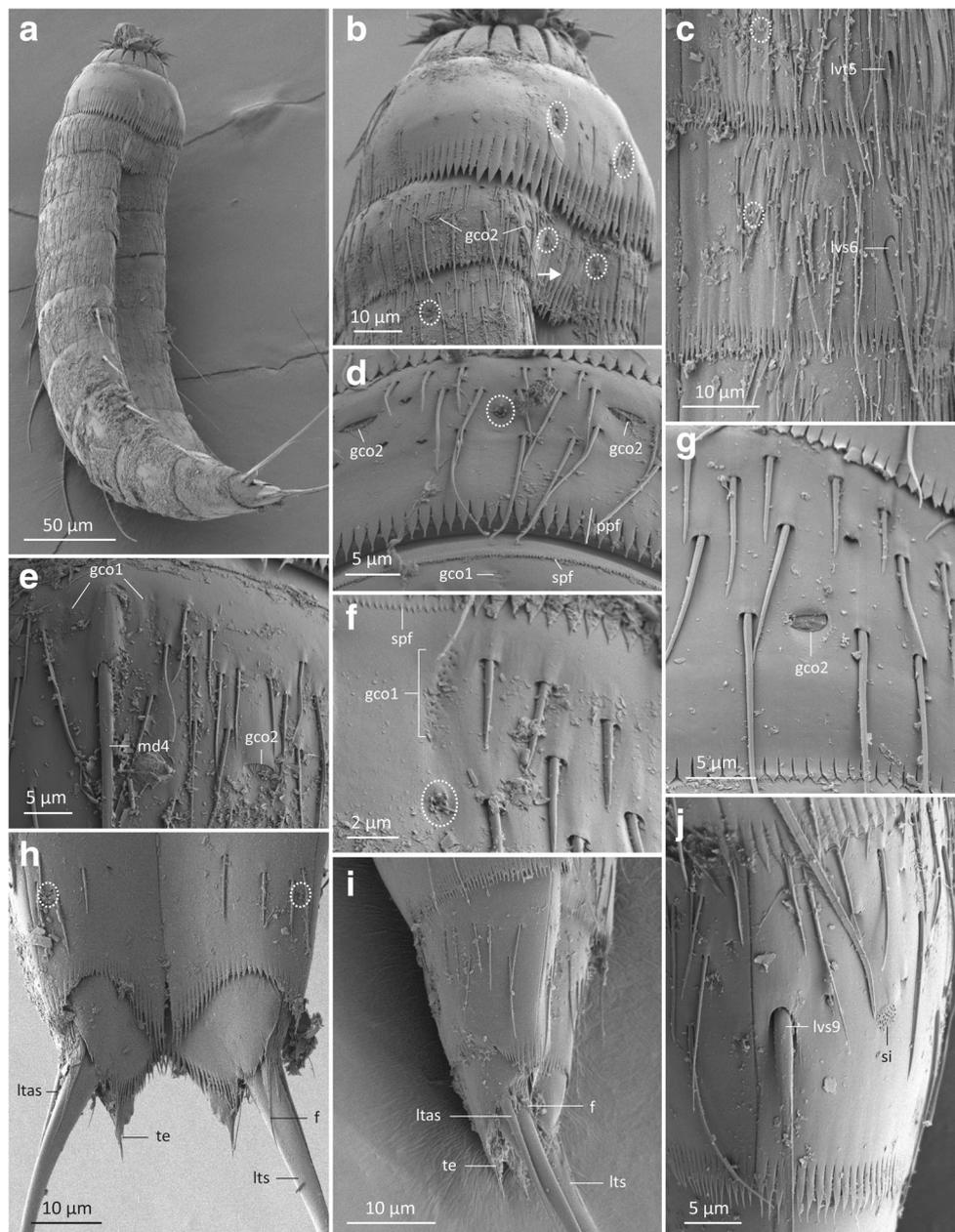


Fig. 7 SEM images showing overviews and details of *Echinoderes pennaki* collected from Calvert Island. **a** Male, lateroventral overview. **b** Detail of segments 1 and 2, lateroventral view. **c** Detail of segments 5 and 6, ventral view. **d** Detail of segment 2, showing two glandular cell outlets type 2 and a sensory spot, middorsal view. **e** Detail of anterior part of segment 4, middorsal view. **f** Detail of anterior part of segment 7, ventral view. **g** Detail of segment 5, midlateral view. **h** Female, detail of segments 10 and 11, ventral view. **i** Female, detail of segments 10 and 11, right side,

lateral view; **j** detail of segment 9, lateroventral view. *f* fringe-like extension; *gco1*, *gco2* glandular cell outlets types 1 and 2; *lts* lateroterminal spine; *ltsa* lateral terminal accessory spine; *lvs* lateroventral spine; *lvt* lateroventral tube; *md* middorsal spine; *si* sieve plate; *spf* secondary pectinate fringe; *te* tergal extension. *dashed circles* indicate the position of sensory spots. The *arrow* marks the midventral division of segment 2. Digits after abbreviations refer to segment numbers

Segment 2 composed of a single cuticular plate with a partial midventral articulation visible in both LM and SEM (Figs. 4a, b, 5a, c, and 7b). Four pairs of glandular openings type 2 are situated in subdorsal, laterodorsal, midlateral, and ventrolateral positions (Figs. 2a, b, 4a–d, 5a–d, and 7b, d). Three pairs of round sensory spots with the same appearance as those on segment 1 present in subdorsal, laterodorsal, and

ventromedial positions, plus an additional middorsal unpaired one (Figs. 2a, b, 4a, b, d, 5a, and 7b, d). Cuticular hairs covering the surface, forming a continuous belt interrupted in the ventromedial position (Figs. 2b and 7b).

Segment 3 and remaining trunk segments consist of a tergal and two sternal plates (Figs. 2a, b, 4a, 5a, b, and 7a). Three pairs of sensory spots with the same appearance as on

Table 2 Measurements (in μm) of adult *Echinoderes pennaki*

Character	<i>n</i>	Range	Mean	SD
TL	4	398–519	460	50
MSW (8)	3	67–71	69	2.3
MSW/TL (%)	3	15–17	16	1
SW	3	53–60	57	3.75
SW/TL (%)	3	13	13	0
S1	4	44–51	47	3.26
S2	4	35–38	37	1.57
S3	4	34–37	35	2.09
S4	4	38–41	39	1.47
S5	4	42–46	43	1.72
S6	4	47–59	51	5.87
S7	4	47–61	52	6.57
S8	4	50–63	55	6.30
S9	4	51–61	55	4.39
S10	4	40–54	48	6.03
S11	4	30–35	33	2.14
MD4	3	38–52	47	0.05
MD5	4	45–65	54	6.47
MD6	4	51–66	59	4.35
MD7	4	51–77	64	13.92
MD8	4	60–84	70	9.83
LVT5	3	10–21	16	5.77
LVS6	4	24–37	33	7.31
LVS7	4	28–40	33	6.33
LVS8	4	32–42	37	5.49
LVS9	4	29–38	34	3.88
LTS11	4	152–196	178	18.22
LTAS11	2	67–68	67	0.42

LTAS lateral terminal accessory spine; *LTS* lateral terminal spine; *LVS* lateroventral spine; *LVT* lateroventral tube; *MD* middorsal spine; *MSW* maximum sternal width; *n* number of specimens; *SD* standard deviation; *SW* standard width; *S1–S11* segment lengths of trunk segments 1–11; *TL* trunk length. Numbers, where inserted, indicate segment number

previous segments present in the subdorsal and midlateral positions. Cuticular hairs abundant and distributed in several lines, forming a belt interrupted in the laterodorsal and ventromedial areas.

Segment 4 with a middorsal acicular spine (Figs. 2a, 4f, 5b, e, and 7e). The insertion of the spine underneath the cuticle forms a very conspicuous protuberance also present in the middorsal spines of segments 5–8 (Fig. 7e). A pair of large glandular cell outlets of type 2 is present in subdorsal position (Figs. 2a, 4f, 5b, and 7e). No sensory spots present. Cuticular hairs as described on previous segments.

Segment 5 with a middorsal acicular spine slightly longer (4–6 μm) and with the same appearance as the one on the previous segment (Figs. 2a, 4a, e, and 5b, e). A pair of very

thin and inconspicuous tubes with the standard composition (basal part and tubular wing-like distal part) is present in lateroventral position (Figs. 2b and 7c). One pair of type 2 glandular cell outlets present in midlateral position (Figs. 2b, 4g, and 7g). Two pairs of sensory spots present on the subdorsal and ventromedial positions (Figs. 2a, b, 4a, 5a, and 7c). Cuticular hairs as described on previous segments.

Segment 6 showing a middorsal acicular spine slightly longer than the one on the previous segment (Figs. 2a, 4f and 5b), and a pair of rigid lateroventral acicular spines (Figs. 2b, 5a, and 7c). Pairs of sensory spots in paradorsal, subdorsal, midlateral, and ventromedial positions (Figs. 2a, b, 4a, f, 5a, and 7c). Cuticular hairs as described on previous segments.

Segment 7 with the same configuration as segment 6 but lacking the subdorsal pair of sensory spots (Figs. 2a, b, 4a, f, and 5a).

Segment 8 with a middorsal acicular spine slightly longer than the one on the previous segment (Figs. 2a and 5b) and a pair of acicular spines in lateroventral position (Figs. 2b, 4e, and 5a, h). Sensory spots present in paradorsal and laterodorsal positions, a pair of wide sublateral glandular openings type 2 is situated at the same level as the ventrolateral spine (Figs. 2b, 4h, and 5a, h). Cuticular hairs as described in previous segments.

Segment 9 with a pair of rigid lateroventral acicular spines (Figs. 2b, 4a, 5a, h, and 7j). Two pairs of sensory spots present in subdorsal position, one pair in midlateral and one pair in ventrolateral positions (Figs. 2a, b and 5a, h). A pair of round sieve plates (ca. 2 μm from SEM) with 20–30 holes present in the sublateral position (Figs. 2b, 4h, 5h, and 7j). Cuticular hairs as described on previous segments.

Segment 10 with three pairs of sensory spots present in subdorsal, laterodorsal, and ventrolateral positions (Figs. 2a, b, 4a, and 7h). Posterior edge of the segment straight along the tergal plate, whereas extending posteriorly in a V-shape towards the paraventral/ventromedial area in the sternal plates (Figs. 2a, b and 7h). Males and females lacking laterodorsal tubes (Figs. 2a–d and 7i). Cuticular hairs as described on previous segments.

Segment 11 with lateral terminal spines (Figs. 2a, b, 4a, 5a, b, and 7a, h, i). Females with a pair of lateral terminal accessory spines (Figs. 2c, d, 4a, 5f, and 7h) and males with three pairs of long penile spines (Figs. 2a, b and 5a, g). Two of the penile spines (p1 and p3) are flexible and elongated, while p2 is shorter and wider (Fig. 5g). One pair of sensory spots is present on the tergal plate, in paradorsal position. Tergal extensions pointy and longer than the sternal plates (Fig. 2a–d and 7h, i). Sternal plates triangular shaped with rounded edges, showing long cuticular hair-like fringes in the lateroventral/ventrolateral position (Fig. 7h, i). Segment covered with small cuticular hairs without perforation sites just in the posterior edge showing a smooth appearance (Fig. 2a–d and 7h, i).

Table 3 Summary of the nature and location of sensory spots, glandular cell outlets, tubes, and spines arranged by series in *Echinoderes pennaki*

Position segment	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM
1	gco1		ss	ss				gco1	ss	
2	gco1, ss		ss, gco2	ss, gco2	gco2				gco2	ss, gco1
3	gco1		ss		ss					gco1
4	ac	gco1	gco2							gco1
5	ac	gco1	ss		gco2			tu		ss, gco1
6	ac	ss, gco1	ss		ss			ac		ss, gco1
7	ac	ss, gco1			ss			ac		ss, gco1
8	ac	ss, gco1		ss		gco2		ac		gco1
9		gco1	2ss		ss	si		ac	ss	gco1
10	2gco1		ss	ss					ss	gco1
11	gco1	ss			pe (♂)		ltas (♀)	lts		

LA lateral accessory; LD laterodorsal; LV lateroventral; MD middorsal; ML midlateral; PD paradorsal; SD subdorsal; SL sublateral; VL ventrolateral; VM ventromedial; ac acicular spine; gco1/gco2 glandular cell outlet types 1/2; ltas lateral terminal accessory spine; lts lateral terminal spine; pe penile spines; si sieve plate; ss sensory spot; tu tube; (♀) female and (♂) male conditions of sexually dimorphic characters

Echinoderes hakaiensis sp. nov. (Figs. 8, 9, 10, 11, 12, and 13, Tables 4 and 5).

Diagnosis

Echinoderes with three middorsal spines on segments 4, 6, and 8 increasing in length posteriorly, lateroventral spines on segments 6–9; tubes present on the subdorsal, laterodorsal, sublateral, and ventrolateral positions on segment 2, lateroventral position on segment 5, and sublateral position on segment 8, males furthermore with well-developed tubes in the laterodorsal position on the posterior margin of segment 10. Tergal extensions short and pointy.

Etymology

The species is dedicated to the Hakai Institute, which supports scientific research in the coastal margin of British Columbia, including meiofaunal research. The name *hakai* also makes reference to the largest marine protected area on the BC coast, situated 400 km north of Vancouver, where the species was collected.

Type material

Holotype, adult male collected from subtidal sandy mud on June 2015 at station CI-022 in Kwakshua Channel (Calvert Island, BC) (Fig. 1, Table 1). Paratype, adult male collected at station CI-026 from subtidal sandy mud on April 2016. Both specimens were mounted in Fluoromount-G® and deposited at the Natural History Museum of Denmark under catalog numbers NHMD-115709 and NHMD-115710, respectively.

Additional non-type material collected at the same localities as the previous specimens includes one specimen from CI-017, preserved in 99% ethanol and used for DNA extraction; the cuticle could not be recovered from the lysis buffer. The mitochondrial cytochrome c oxidase subunit 1 (COI) gene was sequenced (GenBank accession number KY550656).

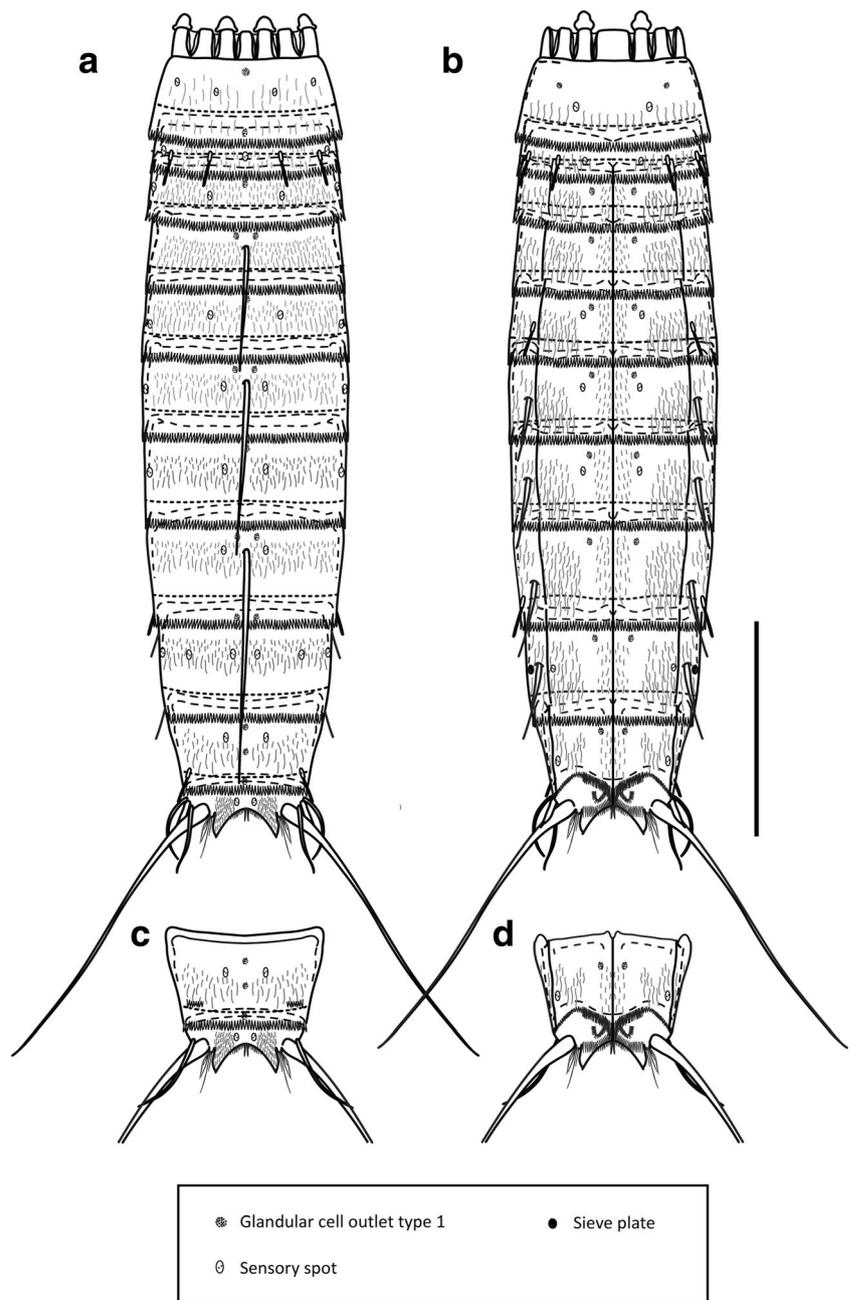
One female and five males from different localities (Table 1) were mounted on SEM stubs and stored in the authors' personal reference collection.

Description

Adults with head, neck, and 11 trunk segments (Figs. 8a, b, 10a, 11a, and 12a). Measurements and dimensions are given in Table 4. A summary of cuticular structures positions (sensory spots, spines, sieve plates, tubes, and glandular cell outlets) is provided in Table 5. The head consists of a retractable mouth cone and an introvert (Fig. 11a, b). Outer armature of the mouth cone formed by nine outer oral styles divided into two subunits slightly alternating in size between five longer ones situated according to uneven sectors of the introvert and four shorter ones situated according to even sectors (Fig. 11b, d); middorsal outer oral style is missing. Each outer oral style has a long fringe at its base, showing 10–12 flexible fringe tips (Fig. 11d). The number and arrangement of the inner armature of the mouth cone could not be studied.

The introvert has seven rings of cuticular spinoscalids and one additional ring of trichoscalids that are associated with the placids (Figs. 9 and 11b, c). Ring 01 with ten primary spinoscalids consisting of a short basal sheath and a distal end piece. The basal sheath has a proximal long fringe situated very close to the insertion point, with ten flexible and elongated fringe tips, followed by a smooth part that bears another fringe composed of eight to ten long and flexible tips (Fig. 11b, c). The distal piece of the primary spinoscalids is round in cross-section and bears a fringe composed of at least six very thin flexible fringe tips. Ring 02 is composed of ten laterally compressed spinoscalids, all formed by a long smooth basal part, ending in a delicate distal fringe (Figs. 9 and 11c). Ring 03 has 20 spinoscalids which show a well-developed sheath with a proximal flexible spine and a distal short fringe. Rings 04 and 05 consist of 10 and 20

Fig. 8 Illustrations of *Echinoderes hakaensis* sp. nov. **a** Male, dorsal view. **b** Male, ventral view. **c** Female, detail of segments 10 and 11, dorsal view. **d** Female, detail of segments 10 and 11, ventral view. The legend shows all the cuticular characters represented in the line art excluding spines, tubes, and cuticular hairs. Scale bar = 100 μm

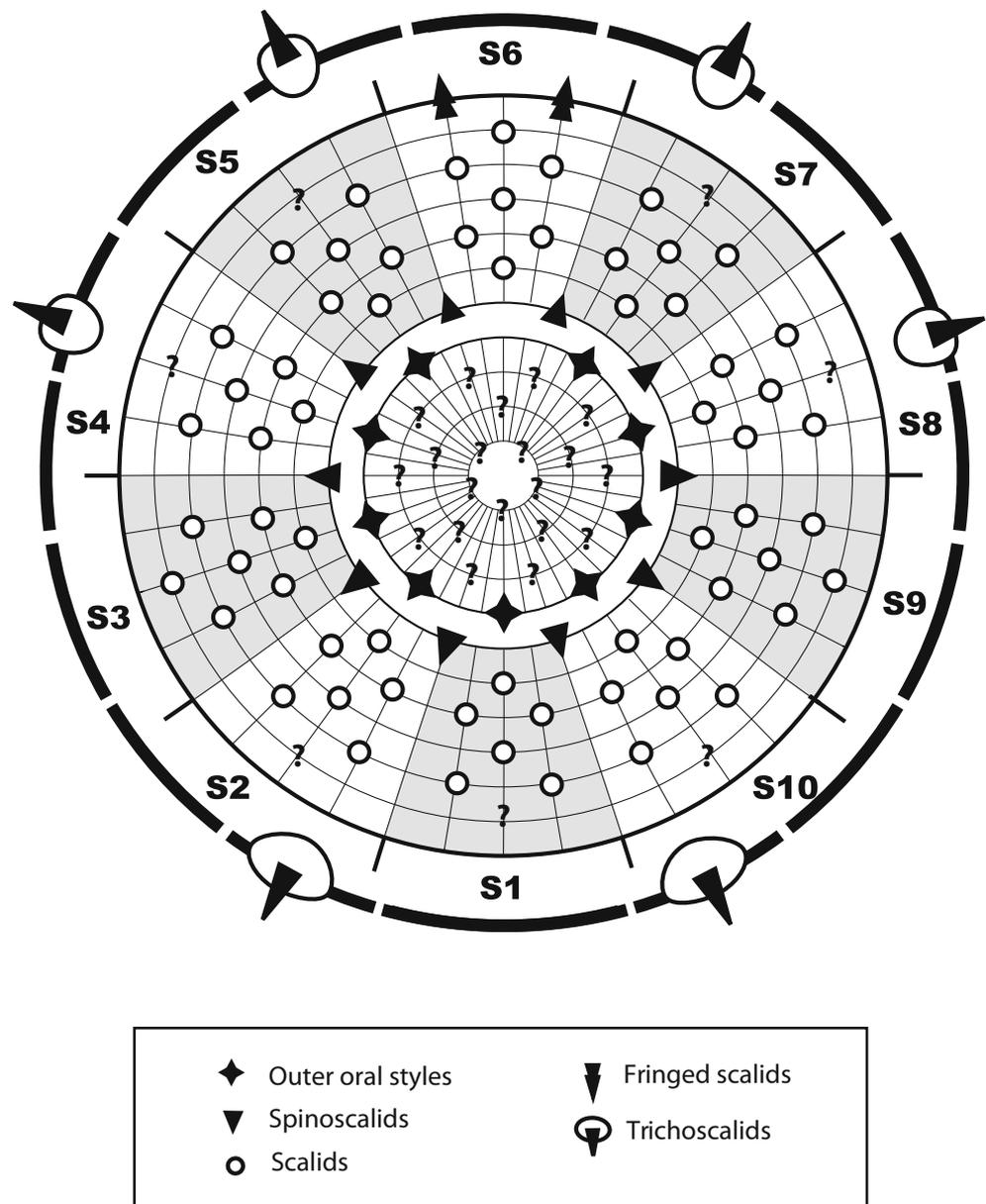


spinoscalids, respectively; all resemble those of ring 03 but instead of a spine, they have a fringed area (Figs. 9 and 11c). Ring 06 has at least three spinoscalids with the same appearance as those on previous rings but shorter; the arrangement of the scalids of the remaining sectors could not be confirmed (represented as question marks in Fig. 9). Ring 07 with a couple of fringed scalids on sector 6 showing a wide and hairy base from where several flexible elongations arise; the remaining sectors could not be checked. Six long and hairy trichoscalids attached to small trichoscalid plates are situated in sectors 2, 4, 5, 7, 8, and 10 (Fig. 9).

The neck consists of 16 placids numbered clockwise from the midventral placid 1 (Figs. 8a, b and 10a, b). Placids 2–16 are trapezoidal, measuring ca. 14 μm (from LM) at the base, while the midventral placid is more rectangular and wider, measuring ca. 7 μm (from LM) (Fig. 10a). All placids articulate with the first trunk segment. Ventral trichoscalid plates are triangular with rounded edges, while dorsal trichoscalid plates are rounded and smaller (Figs. 8a, b, 9, and 11c).

The trunk is divided into 11 segments (Figs. 8a, b, 10a, 11a, and 12a). Segments 1 and 2 consist of a closed cuticular ring, segments 3–11 are composed of one tergal and two sternal

Fig. 9 Diagram of mouth cone, introvert, and placids showing the distribution of oral styles, scalids, and trichoscalid plates in *Echinoderes hakaensis* sp. nov. A table summarizing the scalid arrangement by sector was omitted because of the lack of information on the number and arrangement of the last scalid rows. The question marks indicate uncertain positions



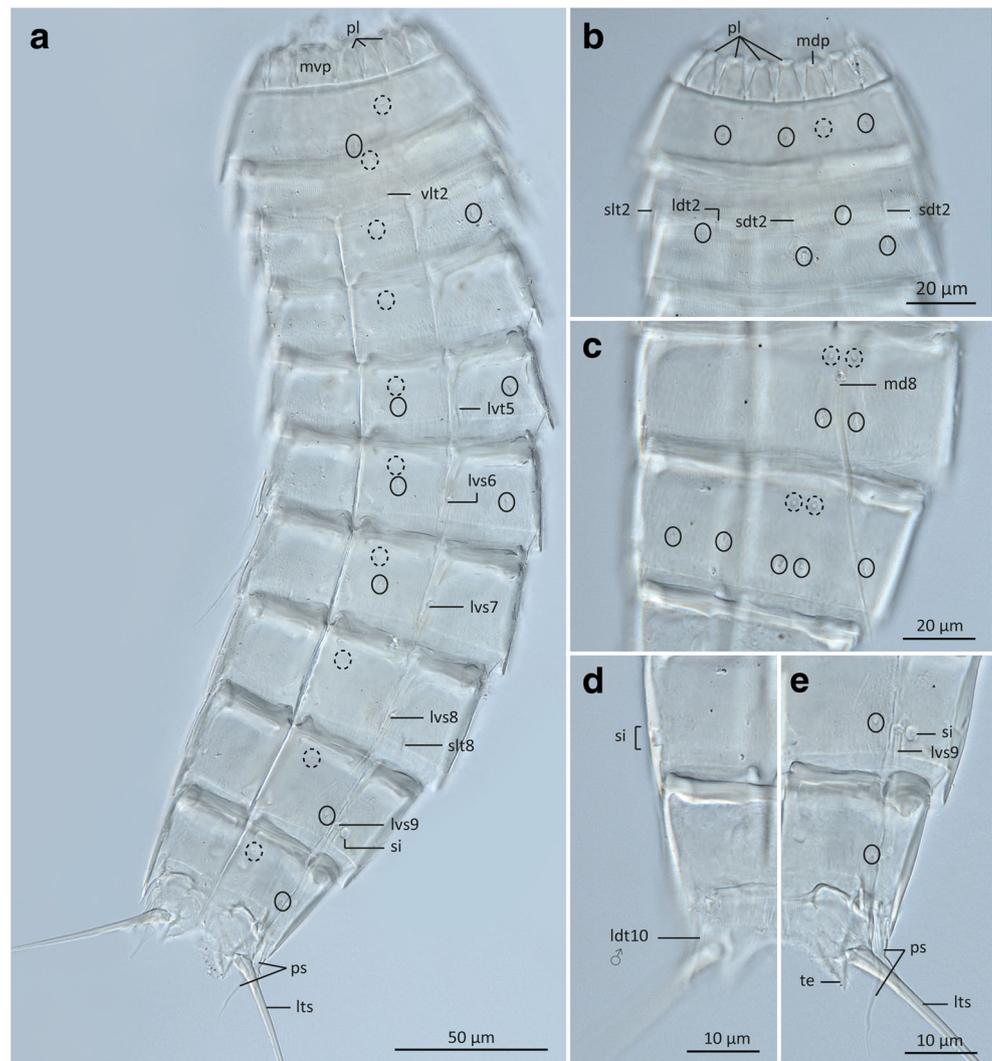
plates. Type 1 glandular cell outlets are round, consisting of numerous small pores located at the anterior part of every segment usually covered by the preceding segment. Dorsal outlets are unpaired and located middorsally on segments 1–3, 5, 7, and 11; dorsal outlets are paired and paradorsal on segments 4, 6, 8, and 9 (Figs. 8a and 10b, c). Segment 10 has two glandular cell outlets aligned in a middorsal position (Fig. 8a). Ventral outlets are paired and ventrolateral on segment 1, ventromedial on segments 2–10 (Figs. 8b and 10a). A primary pectinate fringe is well developed on all segments, showing a uniform length between segments 2–9, while having longer tips on segment 10 (Figs. 8a, b and 12a–i). Segment 10 has a regular pectinate fringe on the dorsal side, but the ventral one varies, showing ventromedial conspicuously

elongated fringe tips (Figs. 8b and 12g). Secondary pectinate fringes consist of a single belt of regular long and flexible teeth usually hidden under the primary pectinate fringe. A secondary pectinate fringe is absent on segment 1.

Segment 1 is composed of a closed cuticular ring. Sensory spots are small and oval-shaped with short papillae (Fig. 12c). There are three pairs of sensory spots located in subdorsal, laterodorsal, and ventromedial positions (Figs. 8a, b, 10a, b, and 12c). Cuticular hairs emerging from round perforation sites are arranged in a belt covering the dorsal side, narrowing towards the ventral side (Figs. 8a, b and 12a, c).

Segment 2 is conspicuously short and composed of a closed cuticular ring. Four pairs of long tubes are located in subdorsal, laterodorsal, sublateral, and ventrolateral positions (Figs. 8a, b,

Fig. 10 Light micrographs (DIC) showing details in male holotype, NHMD-115709 of *Echinoderes hakaiensis* sp. nov. **a** Ventral overview. **b** Detail of the neck and segments 1–3, dorsal view. **c** Detail of segments 8 and 9, dorsal view. **d** Detail of left half of segments 9–11, dorsal view. **e** Detail of left half of segments 9–11, ventral view. *ldt* laterodorsal tube; *lvt* lateroventral tube; *lvs* lateroventral spine; *lts* lateral terminal spine; *md* middorsal spine; *mdp* middorsal placid; *mvp* midventral placid; *pl* placid; *ps* penile spines; *sdt* subdorsal tube; *si* sieve plate; *slt* sublateral tube; *te* tergal extension; *vlt* ventrolateral tube. *dashed circles* indicate the position of glandular cell outlets of type 1. *solid circles* indicate the presence of sensory spots. Digits after abbreviations refer to segment numbers



10a, b, and 12b, c). One of the specimens studied with SEM showed an extra tube in laterodorsal position (Fig. 12b). The tubes in this and the following segments show the typical configuration composed of a short basal part and a long distal part with two small wing-like lateral projections. Paired sensory spots are in laterodorsal and ventromedial positions; a single sensory spot is present middorsally (Figs. 8a, b and 10b). Sensory spots on this and the following segments have longer papillae in their posteriormost area in comparison to more uniform papillae on those from segment 1 (Fig. 12b, c). Hairs with perforation sites are generally abundant and forming a continuous belt interrupted ventromedially; paraventral hairs are shorter than the rest, with no perforation sites forming two round paraventral patches (Fig. 12c). Perforation sites are bracteated in this and the following segments.

Segment 3 and the following segments are composed of one tergal and two sternal plates (Figs. 8a, b, 10a, and 11a). Two pairs of sensory spots are located in subdorsal and midlateral positions (Figs. 8a, 10a, b, and 12b). Hair distribution follows

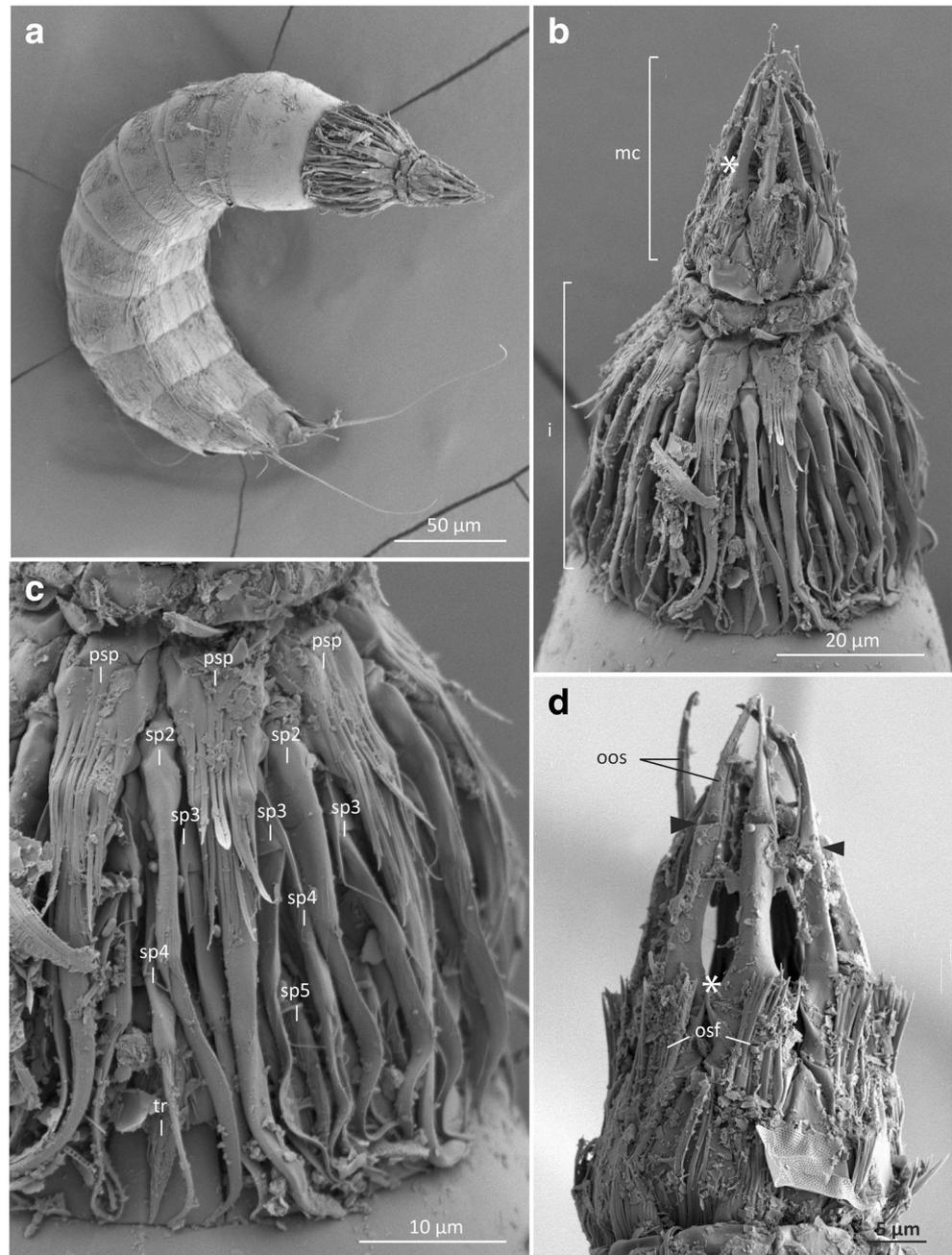
the same pattern of segment 2 having a more conspicuous hairless patch in ventromedial area. Paraventral areas showing a patch of cuticular hairs without perforation sites. This pattern is observed throughout the remaining segments, excluding segment 11 (Figs. 8b and 12c, d).

Segment 4 is characterized by a presence of a middorsal acicular spine (Figs. 8a and 12b). No sensory spots present. Other characters are similar to the previous segment.

Segment 5 has a pair of long tubes in lateroventral position (Figs. 8b, 10a, and 12d). Three pairs of sensory spots are present in subdorsal, midlateral, and ventromedial positions (Figs. 8a, b, 10a, and 12d). Other characters are similar to the previous segment.

Segment 6 has one middorsal acicular spine longer than the one of segment 4 (ca. 60 μm from LM) and a pair of acicular spines in lateroventral position (Figs. 8a, b, 10a, and 12d, e). Three pairs of sensory spots are present in subdorsal, midlateral, and ventromedial positions (Figs. 8a, b, 10a, and 12e, d). Other characters are similar to previous segments.

Fig. 11 SEM images showing overviews and traits of the introvert and mouth cone of *Echinoderes hakaiensis* sp. nov. **a** Male overview, lateroventral view, head extended. **b** Overview of the mouth cone and introvert, laterodorsal view. **c** Detail of introvert sector 8. **d** Detail of the mouth cone showing the outer oral styles, dorsal view. *i* introvert; *mc* mouth cone; *oos* outer oral styles; *osf* outer oral style fringe; *psp* primary spinoscalids; *sp1–sp5* spinoscalids rows 1–5; *tr* trichoscalid. *asterisks* indicate the middorsal position. The *arrowheads* mark the position of the outer oral style articulation, which differs among styles



Segment 7, except for the absence of middorsal acicular spine and the presence of a middorsal glandular cell outlet type 1 (Fig. 8a), the remaining characters are identical to segment 6.

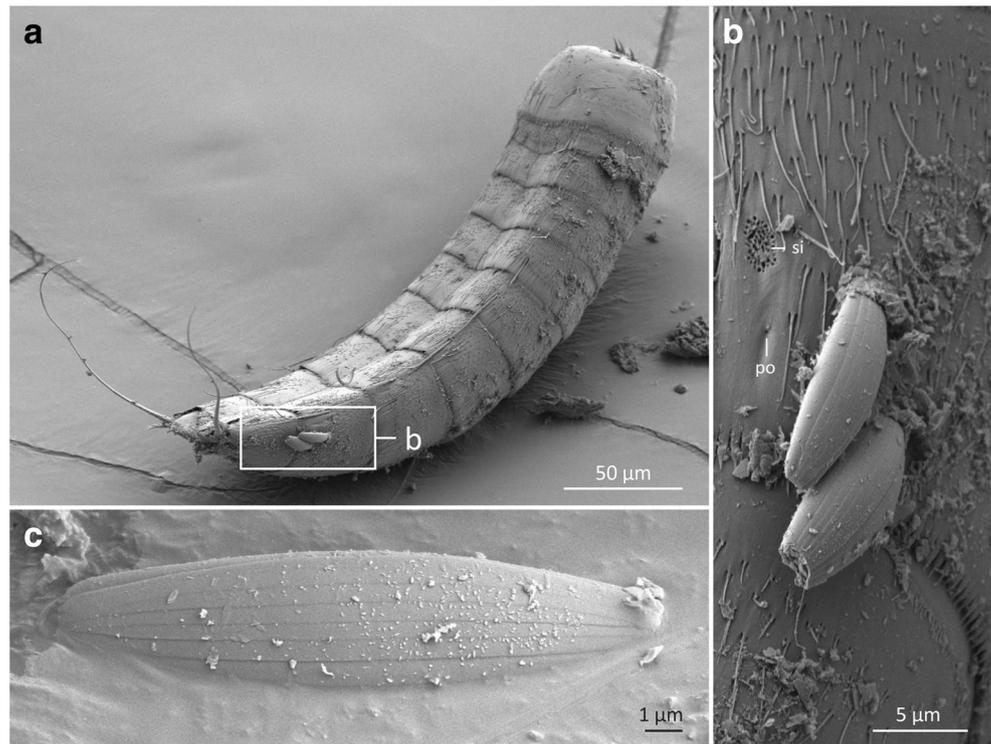
Segment 8 has one middorsal acicular spine longer than the one of segment 6 (ca. 80 μm from LM) and a pair of acicular spines in lateroventral position with long sublateral tubes (Figs. 8b, 10a, c, and 12i). One pair of sensory spots is present in subdorsal position (Fig. 10c). Other characters are similar to previous segments.

Segment 9 with a pair of lateroventral acicular spines (Figs. 8b, 10a, e, and 12f). Four pairs of sensory spots

present in subdorsal, laterodorsal, midlateral, and ventrolateral positions (Figs. 8a, b and 10a, c). A pair of round sieve plates (ca. 4 μm) is present in lateral accessory position (Figs. 8b, 10a, e, and 12f). The sieve plates are composed of an oval, perforated field with more than 30 holes plus an additional posterior pore (Fig. 12). Other characters are similar to previous segments.

Segment 10 with two pairs of sensory spots in subdorsal and ventrolateral positions (Figs. 8a, b, 10a, e, and 12h). The posterior edge of the sternal plates extends in the ventromedial and paraventral positions, forming a V-shape (Figs. 8b and 12g). The pectinate fringe is also conspicuously longer (three

Fig. 13 SEM images showing details of possible epibiontic growth on the surface of *Echinoderes hakaiensis* sp. nov. **a** Overview of a male carrying two putative epibionts on the lateral side of segment 9 next to the sieve plate (area within the white rectangle). **b** Detail of the marked area in **a**, showing the putative epibionts attached next to the sieve plate (excretory opening). **c** Detail of one of the putative epibionts that detached during the preparation of the specimen for SEM. *si* sieve plate; *po* sieve plate pore



one (*p*₂) is thicker and shorter (Fig. 12g). A pair of sensory spots is present in paradorsal position. Tergal extensions are short and pointy, extending further than the sternal plates (Fig. 12g, h). Sternal plates form a rounded triangular shape, showing more than five elongated and flexible cuticular projections exiting the segment borders in the ventromedial/ventrolateral position (Fig. 12g). The segment is completely devoid of cuticular hairs but has hair-like extensions and fringes covering the margins of the tergal and sternal plates (Figs. 8a–d and 12g–h).

One of the specimens of *E. hakaiensis* sp. nov. examined with SEM had a pair of epibionts attached to the lateral side of segment 9, close to the sieve plate that we interpret as protozoan epibionts (Fig. 13a, b). The epibiont bodies were approximately 10 μm long and adorned with an external structure consisting of 7–10 longitudinal furrows per side (Fig. 13b, c). They were attached from one of the ends to the cuticle of the kinorhynch (Fig. 13b).

Discussion

Composition of segment 2 in *Echinoderes*

Echinoderes is a specious genus accommodating more than one-third of the described species of kinorhynchs. Even though *Echinoderes* was originally differentiated from other genera by the presence of a ring-shaped segment 2, indications

of intracuticular midventral divisions or midventral folds have been described for some species, including: *Echinoderes angustus* Higgins & Kristensen, 1988; *E. aquilonius*; *Echinoderes aureus* Adrianov et al., 2002; *Echinoderes eximus* Higgins & Kristensen, 1988; *Echinoderes obtuspinosus* Sørensen et al., 2012; *Echinoderes peterseni* Higgins & Kristensen, 1988; *Echinoderes setiger* Greeff, 1869; *Echinoderes truncatus* Higgins, 1983; and *E. tubilak* (Herranz et al. 2012; Sørensen et al. 2015) (Fig. 14, Table 6). In some cases, the fissure was only visible in the anterior half of the midventral pachycycli (e.g., *E. tubilak* and *E. setiger*; see Neuhaus 2013) or weak and superficial as in *E. angustus*, *E. aquilonius*, and *E. eximus* (Herranz et al. 2012; Neuhaus 2013; Neuhaus and Blasche 2006; Sørensen et al. 2015). The reexamination and redescription of *E. pennaki* demonstrated a partial midventral articulation with a clear thickening or apodeme in the anteriormost part of segment 2 (Fig. 14a). Additionally, reexaminations of paratypes from other *Echinoderes* with traces of midventral divisions of segment 2 revealed a very weak or absent midventral split in *E. eximus* (Fig. 14c) and *E. peterseni*, meaning possible intra-specific variation when compared with the holotype LM pictures shown in Higgins and Kristensen (1988) and paratype KIN-27 in Neuhaus and Blasche (2006). However, the partial midventral division seems more consistent in *E. aquilonius*, *E. angustus*, and *E. tubilak* being weak and superficial in *E. obtuspinosus* and *E. aureus* (check Fig. 14 for comparisons). We interpret the variable arrangements of segment 2 in *Echinoderes* as intermediate stages between a single

Table 4 Measurements (in μm) of adult *Echinoderes hakaiensis* sp. nov.

Character	<i>n</i>	Range	Mean	SD
TL	2	266–382	324	81.76
MSW (8)	2	62	62	0.31
MSW/TL (%)	2	16–23	20	5
SW	2	46–47	46	0.76
SW/TL (%)	2	12–17	15	3.49
S1	2	26–37	32	7.54
S2	2	17–27	22	7.27
S3	2	26–28	27	1.24
S4	2	31–32	32	0.82
S5	2	32–33	33	0.37
S6	2	35	35	0.07
S7	2	35–38	37	1.07
S8	2	40–45	42	3.59
S9	2	40–43	42	1.88
S10	2	39–41	40	1.55
S11	2	24–27	26	2.05
MD4	1	40	–	–
MD6	1	60	–	–
MD8	2	75–80	77	3.17
LDT2	2	17	17	–
SDT2	2	16	16	–
SLT2	1	17	–	–
VLT2	2	17	17	–
LVT5	1	16	–	–
LVS6	2	30–31	31	1.57
LVS7	2	30–34	32	2.97
LVS8	2	25–34	30	5.82
LAT8	1	16	–	–
LVS9	2	27–33	30	4.07
LTS11	1	128	–	–
LTAS11	2	35–38	36	1.64

LAT lateral accessory tube; LDT laterodorsal tube; LTAS lateral terminal accessory spine; LTS lateral terminal spine; LVS lateroventral spine; LVT lateroventral tube; MD middorsal spine; MSW maximum sternal width; *n* number of specimens; SD standard deviation; SDT subdorsal tube; SLT sublateral tube; SW standard width; S1–S11 segment lengths of trunk segments 1–11; TL trunk length; VLT ventrolateral tube. Numbers, where inserted, indicate segment number

closed ring and a segment differentiated into one tergal and two external plates. Interestingly, all the species showing these variations have five middorsal spines. However, based on additional morphological characters, they do not seem to be each other's closest relatives, which might mean that the composition of segment 2 can be homoplastic. This weakens the main key character for the recognition of *Echinoderes* and supports our reasoning to emend the original genus diagnosis, now including the range of partial midventral divisions of segment 2. Outside *Echinoderes*, the variety in the configuration of

segment 2 presents similar challenges in the newest echinoderid genus *Meristoderes* (see discussion in Sørensen et al. 2015). Moreover, the most recent analyses of kinorhynch phylogeny based on a combination of morphological and molecular data could not confirm the monophyly of the echinoderid genera (Sørensen et al. 2015). This suggests that the diagnostic characters for each genus need to be reconsidered, especially those regarding the plate configuration of segment 2, which might end up being less phylogenetically informative than initially thought. In this context, a thorough revision of the Echinoderidae with a complete phylogenetic analysis is needed.

Diagnostic features of *E. pennaki*

Echinoderes pennaki was originally described by Higgins in 1960 from several locations in the San Juan Archipelago Washington (USA). This description was based on observations from traditional light microscopy and represented one of Higgins' first studies when the total number of described *Echinoderes* was less than 20 (Higgins 1960). Seventeen years later, in 1977, Higgins redescribed this species, providing more accurate drawings and measurements, but still lacking other important information, such as LM and SEM micrographs (see Higgins 1977). Based on the spine formula, *E. pennaki* belongs to the most common group of *Echinoderes* bearing five middorsal spines and showing lateroventral tubes/spines on segments 5–9. Currently, there are 47 described *Echinoderes* with five middorsal spines, 13 of them sharing identical spine/tube pattern as *E. pennaki*. In order to facilitate the identification of *E. pennaki* from its congeners, it was necessary to find additional diagnostic characters. In the last two years, the redescription of several *Echinoderes* species (Grzelak and Sørensen 2017; Herranz and Leander 2016; Landers and Sørensen 2016; Sørensen et al. 2016; Pardos et al. 2016a, b) has provided new valuable morphological details stressing the importance of the glandular cell outlets type 2 as a key taxonomic character within the genus. Over the years, these glands have been mostly overlooked or mistakenly interpreted as tubes, also named cuticular scars (e.g., Higgins and Kristensen 1988, see discussion in Sørensen et al. 2016). The identification and mapping of the glandular cell outlets type 2 is especially relevant for those species belonging to the “five middorsal *Echinoderes* group” that show a very stereotypical arrangement of spines and tubes (e.g., lateroventral spines/tubes segments 5–9 + ventrolateral tubes on segment 2 or lateroventral spines/tubes segments 5–9 + absence of tubes on segment 2). The revision of type material from “old *Echinoderes* descriptions” revealed in most cases unseen type 2 glandular outlets among other morphological details (see Sørensen et al. 2016 and Grzelak and Sørensen 2017 for a list of the species). This was also the case for *E. pennaki*; after examination of the

Table 5 Summary of the nature and location of sensory spots, glandular cell outlets, tubes, and spines arranged by series in *Echinoderes hakaiensis* sp. nov.

Position segment	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM
1	gcol		ss	ss					gcol	ss
2	ss, gcol		tu	ss, tu		tu			tu	ss, gcol
3	gcol		ss		ss					gcol
4	ac	gcol								gcol
5	gcol		ss		ss			tu		ss, gcol
6	ac	gcol	ss		ss			ac		ss, gcol
7	gcol		ss		ss			ac		ss, gcol
8	ac	gcol	ss			tu		ac		gcol
9		gcol	ss	ss	ss		si	ac	ss	gcol
10	2gcol		ss	tu (♂)					ss	gcol
11	gcol	ss			pe(♂)		ltas (♀)	lts		

LA lateral accessory; *LD* laterodorsal; *LV* lateroventral; *MD* middorsal; *ML* midlateral; *PD* paradorsal; *SD* subdorsal; *SL* sublateral; *VL* ventrolateral; *VM* ventromedial; *ac* acicular spine; *gcol* glandular cell outlet type 1; *ltas* lateral terminal accessory spine; *lts* lateral terminal spine; *pe* penile spines; *si* sieve plate; *ss* sensory spot; *tu* tube; (♀) female and (♂) male conditions of sexually dimorphic characters

Fig. 14 Light micrographs (DIC) comparing the configurations of segment 2 in reexamined *Echinoderes* species. All pictures show a ventral view of segments 1–3. **a** *E. pennaki*. **b** *E. angustus* paratype ZMUC KIN-24. **c** *E. eximus* paratype ZMUC KIN-28. **d** *E. aquilonius* non-type ZMUC KIN-39. **e** *E. obtuspinosus* holotype NIBR-INBRIV0000245084. **f** *E. aureus* non-type. The arrowheads mark the midventral fissure of segment 2. Note that *E. eximus* (**c**) does not show any trace of division, just a thickening of the pachycyclus. Scale bars = 20 μm

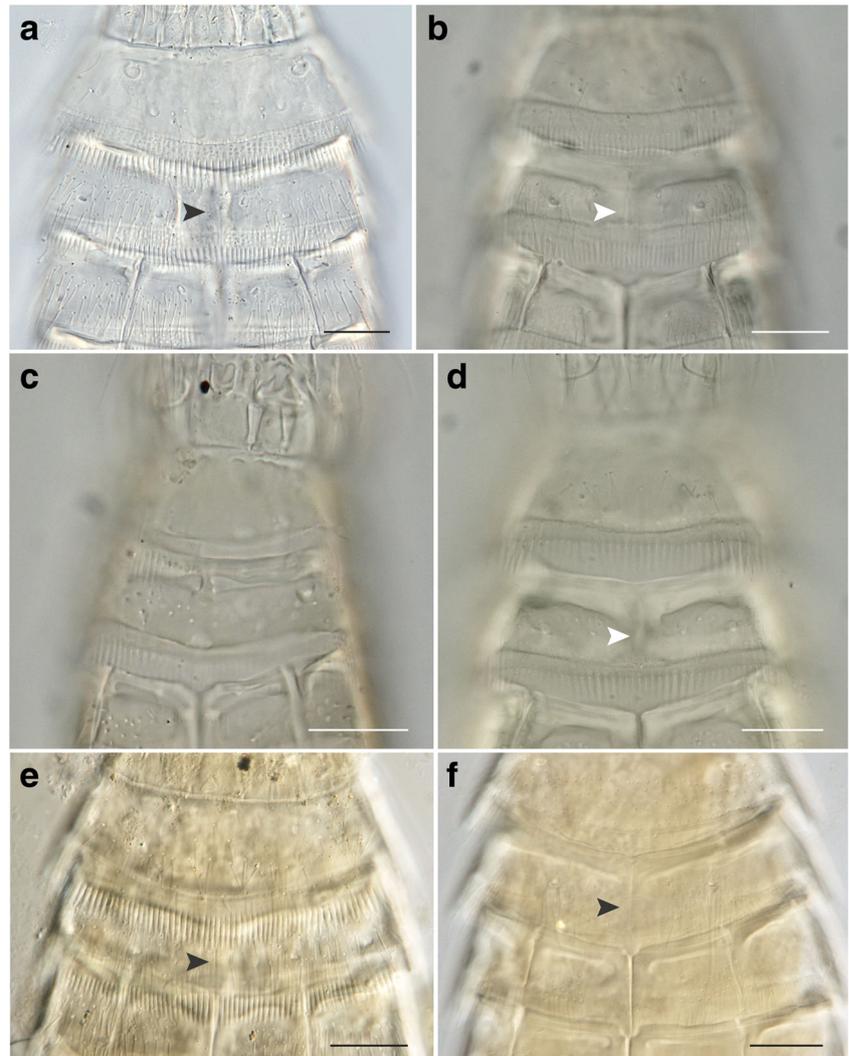


Table 6 Summary of all *Echinoderes* species described with traces of midventral articulations/fissures on segment 2

Species	Nature of midventral articulation	Graphic material available	References
<i>E. angustus</i>	Indications of intracuticular thickening with midventral partial fissure and superficial fold	LM, SEM	Higgins and Kristensen (1988) Neuhaus and Blasche (2006) Grzelak and Sørensen (2017) Present study
<i>E. aquilonius</i>	Indications of intracuticular thickening with midventral partial fissure and superficial fold	LM, SEM	Higgins and Kristensen (1988) Neuhaus and Blasche (2006) Grzelak and Sørensen (2017) Present study
<i>E. aureus</i>	Weakly developed	LM	Adrianov et al. (2002) Present study
<i>E. eximus</i>	Weak superficial fold/intracuticular thickening*	LM, SEM	Higgins and Kristensen (1988) Neuhaus and Blasche (2006) Grzelak and Sørensen (2017) Present study
<i>E. obtuspinosus</i>	Weak and superficial	LM, SEM	Sørensen et al. (2012) Present study
<i>E. pennaki</i>	Indications of intracuticular thickening with midventral partial fissure and superficial fold	LM, SEM	Present study
<i>E. peterseni</i>	Weak superficial fold/intracuticular thickening*	LM, SEM	Higgins and Kristensen (1988) Neuhaus and Blasche (2006) Grzelak and Sørensen (2017) Present study
<i>E. setiger</i>	Intracuticular thickening	LM	Neuhaus (2013)
<i>E. truncatus</i>	Weak and superficial	LM, SEM	Higgins (1983) Sørensen (2006)
<i>E. tubilak</i>	Indications of intracuticular thickening with midventral partial fissure and superficial fold	LM	Higgins and Kristensen (1988) Neuhaus and Blasche (2006) Neuhaus (2013)

SEM references are in **bold**

*Intraspecific variation of this character

type material, Sørensen et al. (2016) found three pairs of previously unnoticed *gco2* on segment 2, which became new diagnostic characters for the species. In the present study, we reexamined *E. pennaki* type material, finding extra glandular outlets type 2 in the laterodorsal and midlateral positions on segment 2, midlateral on segment 5, and sublateral on segment 8. In these particular positions, the glands are very difficult to detect if the specimen is mounted in a dorsoventral position, usually just identified as an area where the cuticle gets thinner (see Fig. 4g, h). The comparison of *E. pennaki* type material with the specimens collected from Calvert Island allowed us to identify the new material as *E. pennaki*. The specimens share identical cuticular characters, including the conspicuous pectinate fringe on the ventral side of segment 1, the midventral partial division on segment 2, and the spine, tube, gland, and sensory spot patterns. The only differences between the two populations relate to the length of the trunk and length of the terminal spines: on average, *E. pennaki* from Calvert Island is larger than *E. pennaki* from San Juan (trunk length 460 μm vs. 410 μm in *E. pennaki* from San Juan), with longer lateral terminal spines (178 μm vs. 156 μm in *E. pennaki* from San Juan) (Higgins 1960). We interpret these differences as intraspecific variations.

Echinoderes pennaki share identical spine/tube and number and distribution of glandular cell outlets type 2 (subdorsal, laterodorsal, midlateral, and ventrolateral positions on segment 2; subdorsal positions on segment 4; midlateral positions on segment 5; and sublateral positions on segment 8) with *E. tubilak*, a species described from the Arctic (see Grzelak and Sørensen 2017). Nonetheless, *E. tubilak* can be easily distinguished by the presence of a conspicuous and irregular midventral pectinate fringe on segment 2; it also has longer lateroventral spines and remarkably shorter lateroterminal spines when compared with *E. pennaki* (147 μm in *E. tubilak* vs. 172 μm in *E. pennaki* on average) (Higgins and Kristensen 1988). *Echinoderes angustus*, another Arctic species recently revised by Grzelak and Sørensen (2017), showed striking morphological similarities to *E. pennaki*, including spines, tubes, glandular outlets, sensory areas, pectinate fringes, and midventral partial division of segment 2. The only differences we could find relate to the presence of an extra pair of laterodorsal glandular cell outlets on segment 10 in *E. angustus* and the length of the lateroventral and mid-dorsal spines being around 20% longer in *E. angustus* (Higgins and Kristensen 1988). The identification of the *gco2* on segment 10 is extremely challenging and requires

clean SEM specimens (see Grzelak and Sørensen 2017). We could not confirm the presence of these glands in the studied SEM specimens of *E. pennaki*. This combined with the remarkable similarities between *E. pennaki* and *E. angustus* indicate that both species are very closely related and could potentially be synonymized in the future if molecular analyses support it.

Other *Echinoderes* species that resemble *E. pennaki* are *E. aquilonius*, *Echinoderes cernunnos* Sørensen et al., 2012, *Echinoderes romanoi* Landers & Sørensen, 2016, and *Echinoderes obtuspinosus*; all of these share the same spine/tube pattern and the presence of four pairs of glandular cell outlets type 2 on segment 2 (Sørensen et al. 2012; Landers and Sørensen 2016). However, none of these species share identical glandular patterns with *E. pennaki*. Of them, *E. romanoi* is the species that most resembles *E. pennaki* but it lacks glandular cell outlets type 2 on segment 4, the lateroventral and middorsal spines are notably shorter, the pectinate fringe of segment 1 is not different from the remaining segments, and the trunk size is half of the size of *E. pennaki* (Landers and Sørensen 2016). *Echinoderes obtuspinosus* differs from *E. pennaki* in the absence of midlateral glandular outlets type 2 on segment 5 and the presence of very short lateral terminal spines (Sørensen et al. 2012). *Echinoderes cernunnos* is easy to distinguish by the long and spiniform tergal extensions and the lack of glandular outlets type 2 in the subdorsal position on segment 4. Instead, it shows a pair in the midlateral position on segment 7. *Echinoderes aquilonius* lacks the laterodorsal and sublateral glandular outlets type 2 on segment 2 but instead it shows a midlateral one and an extra pair on the laterodorsal position on segment 10 (Grzelak and Sørensen 2017).

There are only two other *Echinoderes* species described from the northeastern Pacific coast: *E. ohtsukai* and *E. kozloffii*, both recently redescribed by Herranz and Leander (2016). *Echinoderes ohtsukai* belongs to the so-called “*Echinoderes coulli* group” and is easily distinguished showing a very different spine formula, with only a minute middorsal spine on segment 4, numerous fringed tubes distributed in the trunk, and a conspicuously large sieve plate (Yamasaki and Kajihara 2012; Herranz and Leander 2016). *Echinoderes kozloffii* belongs to the “five middorsal *Echinoderes* group” but shows a different spine/tube formula to *E. pennaki*. *Echinoderes kozloffii* has a pair of ventrolateral tubes on segment 2 and just presents glandular cell outlets type 2 on segment 8. Also, the length of the spines is significantly shorter (Herranz and Leander 2016).

Diagnostic features of *E. hakaiensis* sp. nov.

Echinoderes hakaiensis sp. nov. shares the presence of three middorsal spines on segments 4, 6, and 8 with 16 other species, but can be distinguished by a unique pattern of spines and

tubes. The lateroventral/lateral accessory arrangement of tubes and spines on segments 5–9, including additional tubes on segment 8, is fairly common, but the presence of four pairs of tubes (subdorsal, laterodorsal, sublateral, and ventrolateral) on segment 2 combined with three middorsal spines has never been described in any other *Echinoderes* before. Eight species of *Echinoderes* are similar to *E. hakaiensis* sp. nov.: *Echinoderes belenae* Pardos, Herranz & Sánchez, 2016; *Echinoderes rociae* Pardos, Sánchez & Herranz, 2016; *Echinoderes higginsii* Huys & Coomans, 1989; *Echinoderes hispanicus* Pardos et al., 1998; *Echinoderes intermedius* Sørensen, 2006; *Echinoderes newcaledoniensis* Higgins, 1967; *Echinoderes peterseni* Higgins & Kristensen, 1988; and *Echinoderes riedli* Higgins, 1978. Of these, *E. belenae*, *E. rociae*, *E. hispanicus*, *E. intermedius*, and *E. newcaledoniensis* show additional pairs of tubes on different segments (mainly situated on segments 2, 6–8, but also on segments 3, 5–9 in *E. belenae*), which makes them easy to discriminate from *E. hakaiensis* sp. nov. (Higgins 1967; Pardos et al. 1998, 2016a, b; Sørensen 2006). *Echinoderes belenae* and *E. rociae* also show very short and stout lateral terminal spines (Pardos et al. 2016a, b). The *Echinoderes* species that most resembles *E. hakaiensis* sp. nov. are: *E. peterseni*, *E. higginsii*, and *E. riedli*. *Echinoderes higginsii* and *E. riedli* are easily distinguished from *E. hakaiensis* sp. nov. by the presence of a single pair of tubes in a ventrolateral position on segment 2 (Higgins 1978; Higgins and Kristensen 1988; Huys and Coomans 1989). *Echinoderes higginsii* also has conspicuously long and pointy tergal extensions. *Echinoderes peterseni* shares a nearly identical tube/spine formula with *E. hakaiensis* sp. nov. but lacks the laterodorsal pair of tubes on segment 2 and the tubes on segment 8 are in the lateral accessory position; it also has significantly longer lateral terminal spines compared with *E. hakaiensis* sp. nov. (160 µm in *E. peterseni* vs. 104 µm in *E. hakaiensis* sp. nov.) (Higgins and Kristensen 1988).

Putative epibiontic growth on *E. hakaiensis* sp. nov.

The fusiform bodies attached to the lateral side of segment 9 on a specimen of *E. hakaiensis* sp. were reminiscent of diatoms (Fig. 14). The presence of epibionts on kinorhynchs has been reported previously, but rarely described with any detail (Adrianov and Higgins 1996; Herranz and Leander 2016; Ostmann et al. 2012). Kinorhynchs produce abundant mucus through several glandular openings distributed along the trunk. This mucus could provide lubrication to reduce the friction between segments, but also to protect the cuticle from the abrasion of the surrounding sediment (Neuhaus 2013). The presence of this mucus may favor the attachment of particles and bacterial films on the cuticle, so epibionts could take advantage of a habitat with a high microbial activity. It is also hypothesized that the mucus secreted may act first as a trap and afterwards as a

microbial garden, possibly used as a food source (Adrianov and Malakhov 1994). Some of the organisms commonly found as epibionts are diatoms, bacteria, cyanobacteria, fungi, and ciliates (Adrianov and Higgins 1996; Neuhaus 2013; Ostmann et al. 2012). These have been found in adults of different species and genera (e.g., *Condyloderes*, *Echinoderes*, and *Pycnophyes*); however, no epibionts have been found in juveniles and recently hatched adults (Neuhaus 2013). Most of the reported epibionts seem to concentrate in the posteriormost segments of the kinorhynchs and/or attaching to the terminal spines, where the amount of secretions/excretions is expected to be more abundant due to the proximity of the gonopores (between segments 10 and 11) and the anus (segment 11) (see Ostmann et al. 2012; Neuhaus 2013).

Conclusions

During our survey of kinorhynchs in a pristine region of British Columbia, in the northeastern Pacific Ocean, we discovered one new subtidal species and redescribed a previously named species, both belonging to the genus *Echinoderes*: *E. hakaiensis* sp. nov. and *E. pennaki*. The especial configuration of segment 2 shown in *E. pennaki* and shared by several *Echinoderes* species reopens the debate about the reliability of morphological characters used to discriminate echinoderid genera from one another. We emended the genus *Echinoderes* to alleviate this problem. However, a reevaluation of diagnostic characters within a resolved molecular phylogenetic context including a more comprehensive sampling of species within the Echinoderidae is needed.

Acknowledgments The authors are grateful to Niels Van Steenkiste for his help during the sampling. Martin V. Sørensen is acknowledged for kindly providing graphic material of different *Echinoderes* species. We wish to thank the staff at the Bioimaging Facility at the University of British Columbia (UBC) for their help and the staff at Hakai Institute, especially Wayne Jacobs and the Coastal Sand Ecosystems Project members. This work was supported by a grant to BSL from the Tula Foundation (Centre for Microbial Diversity and Evolution), the National Sciences and Engineering Research Council of Canada (NSERC 2014-05258), and the Canadian Institute for Advanced Research, Program in Integrated Microbial Biodiversity.

References

- Adrianov AV, Higgins RP (1996) *Pycnophyes parasanjuanensis*, a new kinorhynch (Kinorhyncha: Homalorhagida: Pycnophyidae) from San Juan Island, Washington, U.S.A. *Proc Biol Soc Wash* 109(2): 236–247
- Adrianov AV, Malakhov VV (1994) Kinorhyncha: structure, development, phylogeny and taxonomy. Nauka Publishing, Moscow
- Adrianov AV, Murakami C, Shirayama Y (2002) *Echinoderes aureus* n. sp. (Kinorhyncha: Cyclorhagida) from Tanabe Bay (Honshu Island), Japan, with a key to the genus *Echinoderes*. *Spec Div* 7(1):47–66
- Blake CH (1930) Three new species of worms belonging to the order Echinodera. *Biol Survey Mt Desert Region* 4:3–10
- Brinkhurst RO (1987) Distribution and abundance of macroscopic benthic infauna from the continental shelf off southwestern Vancouver Island, British Columbia. Canadian Technical Report of Hydrography and Ocean Sciences no. 85, 86 pp
- Claparède ARE (1963) Zur Kenntnis der Gattung *Echinoderes* Duj. Beobachtungen über Anatomie und Entwicklungsgeschichte wirbelloser Thiere: an der Küste von Normandie angestellt. Verlag von Wilhelm Engelmann, Leipzig, pp 90–92, 119, pls XVI–XVII
- Folmer O, Black M, Hoeh W et al (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3:294–299
- Greiff R (1869) Untersuchungen über einige merkwürdige Thiergruppen des Arthropoden- und Wurm-Typus. *Arch Naturgesch* 35:71–121
- Grzelak K, Sørensen MV (2017) New species of *Echinoderes* (Kinorhyncha: Cyclorhagida) from Spitsbergen, with additional information about known Arctic species. *Mar Biodiv* (in press)
- Herranz M, Leander BS (2016) Redescription of *Echinoderes ohtsukai* Yamasaki and Kajihara, 2012 and *E. kozloffii* Higgins, 1977 from the northeastern Pacific coast, including the first report of a potential invasive species of kinorhynch. *Zool Anz* 265:108–126
- Herranz M, Thormar J, Benito J et al (2012) *Meristoderes* gen. nov., a new kinorhynch genus, with the description of two new species and their implications for echinoderid phylogeny (Kinorhyncha: Cyclorhagida, Echinoderidae). *Zool Anz* 251:161–179
- Higgins RP (1960) A new species of *Echinoderes* (Kinorhyncha) from Puget Sound. *Trans Am Microsc Soc* 79:85–91
- Higgins RP (1961) Three new homalorhage kinorhynchs from the San Juan Archipelago, Washington. *J Elisha Mitchell Sci Soc* 77:81–88
- Higgins RP (1964a) A method for meiobenthic invertebrate collection. *Amer Zool* 4(3):291
- Higgins RP (1964b) Redescription of the kinorhynch *Echinoderes remanei* (Blake, 1930) Karling, 1954. *Trans Am Microsc Soc* 83(2):243–247
- Higgins RP (1967) The Kinorhyncha of New-Caledonia. In: Expédition Française sur les Recifs coralliens de la Nouvelle-Calédonie 2. Fondation Singer-Polignac, Paris, pp. 75–90
- Higgins RP (1977) Redescription of *Echinoderes dujardinii* (Kinorhyncha) with descriptions of closely related species. *Smithson Contrib Zool* 248:1–26
- Higgins RP (1978) *Echinoderes gerardi* n. sp. and *E. riedli* (Kinorhyncha) from the Gulf of Tunis. *Trans Am Microsc Soc* 97(2):171–180
- Higgins RP (1983) The Atlantic Barrier Reef ecosystem at Carrie Bow Cay, Belize, II: Kinorhyncha. *Smithson Contrib Mar Sci* 18:1–131
- Higgins RP (1986) A new species of *Echinoderes* (Kinorhyncha: Cyclorhagida) from a coarse-sand California beach. *Trans Am Microsc Soc* 105(3):266–273
- Higgins RP, Korczynski RE (1989) Two new species of *Pycnophyes* (Homalorhagida, Kinorhyncha) from the Canadian coast of the Beaufort Sea. *Can J Zool* 67:2056–2064
- Higgins RP, Kristensen RM (1988) Kinorhyncha from Disko Island, West Greenland. *Smithson Contrib Zool* 458:1–56
- Huys R, Coomans A (1989) *Echinoderes higginsi* sp. n. (Kinorhyncha, Cyclorhagida) from the southern North Sea with a key to the genus *Echinoderes* Claparède. *Zool Scr* 18(2):211–221
- Jørgensen M, Kristensen RM (1991) Meiofauna investigations from Igloodik, N.W.T. Arctic Canada. In: Jørgensen M (ed) Arctic biology course 1989, Igloodik, N.W.T., Canada. University of Copenhagen Zoological Museum, pp 61–80
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B,

- Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12):1647–1649
- Landers SC, Sørensen MV (2016) Two new species of *Echinoderes* (Kinorhyncha, Cyclorhagida), *E. romanoi* sp. n. and *E. joyceae* sp. n., from the Gulf of Mexico. *ZooKeys* 594:51–71
- Neuhaus B (2013) Kinorhyncha (=Echinodera). In: Schmidt-Rhaesa A (ed) *Handbook of zoology: Gastrotricha, Cycloneuralia and Gnathifera*. Volume 1: Nematomorpha, Priapulida, Kinorhyncha, Loricifera. Walter de Gruyter, Berlin, pp 1–168
- Neuhaus B, Blasche T (2006) *Fissuroderes*, a new genus of Kinorhyncha (Cyclorhagida) from the deep sea and continental shelf of New Zealand and from the continental shelf of Costa Rica. *Zool Anz* 245:19–52
- Ostmann A, Nordhaus I, Sørensen MV (2012) First recording of kinorhynchs from Java, with the description of a new brackish water species from a mangrove-fringed lagoon. *Mar Biodivers* 42:79–91
- Pardos F, Higgins RP, Benito J (1998) Two new *Echinoderes* (Kinorhyncha, Cyclorhagida) from Spain, including a reevaluation of Kinorhynch taxonomic characters. *Zool Anz* 237:195–208
- Pardos F, Sánchez N, Herranz M (2016a) Two sides of a coin: the phylum Kinorhyncha in Panama. I) Caribbean Panama. *Zool Anz* 265:3–25
- Pardos F, Herranz M, Sánchez N (2016b) Two sides of a coin: the phylum Kinorhyncha in Panama. II) Pacific Panama. *Zool Anz* 265:26–47
- Sørensen MV (2006) New Kinorhynchs from Panama, with a discussion of some phylogenetically significant cuticular structures. *Meiofauna Mar* 15:51–77
- Sørensen MV, Dal Zotto M, Rho HS et al (2015) Phylogeny of Kinorhyncha based on morphology and two molecular loci. *PLoS One* 10:e0133440
- Sørensen MV, Herranz M, Landers SC (2016) A new species of *Echinoderes* (Kinorhyncha: Cyclorhagida) from the Gulf of Mexico, with a redescription of *Echinoderes bookhouti* Higgins, 1964. *Zool Anz* 265:48–68
- Sørensen MV, Pardos F (2008) Kinorhynch systematics and biology—an introduction to the study of kinorhynchs, inclusive identification keys to the genera. *Meiofauna Mar* 16:21–73
- Sørensen MV, Rho HS, Min WG, Kim D, Chang CY (2012) An exploration of *Echinoderes* (Kinorhyncha: Cyclorhagida) in Korean and neighboring waters, with the description of four new species and a redescription of *E. tchefouensis* Lou, 1934. *Zootaxa* 3368:161–196
- Yamasaki H, Kajihara H (2012) A new brackish-water species of *Echinoderes* (Kinorhyncha: Cyclorhagida) from the Seto Inland Sea, Japan. *Spec Div* 17:109–118
- Yamasaki H, Hiruta SF, Kajihara H (2013) Molecular phylogeny of kinorhynchs. *Mol Phylogenet Evo* 67:303–310
- Zelinka C (1894) Über die Organisation von *Echinoderes*. *Ver Dtsch Zool Ges* 4:46–49
- Zelinka C (1896) Demonstration der Tafeln der *Echinoderes*—Monographie. *Ver Dtsch Zool Ges* 6:197–199