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Eubostrichopsis johnpearsei n. gen., n. sp., the first stilbonematid nematode (Nematoda, Desmodoridae) from the US West Coast

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Abstract

A new genus of the marine Stilbonematinae (Nematoda, Desmodoridae) is described from the Pacific coast of the United States of America. The worms inhabit the sulfidic sediment among the roots of the surfgrass *Phyllospadix* sp. in the rocky intertidal. The ectosymbiotic coat is of a new type for Stilbonematinae. It consists of rod-shaped bacteria pointed at both poles densely attached with one pole to the host cuticle. This is the first report of this symbiotic nematode subfamily from the US West Coast.

Key words: Stilbonematinae, marine free-living nematodes, thiobios, sulfide system, *Phyllospadix*, rocky intertidal, Oregon, California

Introduction

Thiobios (Boaden 1977) denotes organisms inhabiting anoxic and sulfidic aquatic sediments. It includes the microscopic interstitial fauna of the sulfide system (Fenchel & Riedl 1970), the ecosystem of reduced, microoxic to anoxic marine sediments. Taxa characteristic for this habitat are the Phylum Gnathostomulida, which exclusively occurs there and specialized representatives of the Ciliata, Platyhelminthes, Gastrotricha and Nematoda. Among the latter the Stilbonematinae Chitwood, 1936 (Desmodoridae), which live in symbiosis with sulfur-oxidizing chemoautotrophic bacteria are regularly found in the sulfide system.

The sulfide system is typically developed in sheltered intertidal and subtidal situations, such as mudflats, estuarine shores or backreef sediments. High-energy rocky shores are apparently not the place to search for thiobiotic organisms. Nonetheless, the sediment accumulated among the holdfasts of the rock-dwelling seagrass *Phyllospadix* spp. growing under wave-beaten conditions on the Pacific Coast of North America is reduced, sulfidic (Farris & Hooge 1998) and harbors typical elements of the thiobios, such as Gnathostomulida (Sterrer & Sorensen 2006) and at least one species of Stilbonematinae.

Here we describe a new species, *Eubostrichopsis johnpearsei* **n. gen, n. sp.**, from the coasts of Oregon and California, representing a new genus of Stilbonematinae that is remarkable for a novel type of ectosymbiotic bacterial coat.

Material and methods

Sediment among the holdfasts of *Phyllospadix* sp. was collected by hand at low tide in the intertidal near Yaquina Head (Oregon) and Santa Cruz (California) (Fig. 1). In the laboratory meiofauna was extracted from the sand by shaking and decanting through a 32 µm sieve and sorted under a stereo microscope. Worms were fixed in 4 % formaldehyde and subsequently transferred to a glycerol/water mixture 1:8 per volume, slowly evaporated and mounted in water-free glycerol.

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Drawings were made on a Diavar compound microscope (Reichert, Vienna, Austria) with the aid of a camera lucida. Light microscope photographs were made either on a Diavar microscope equipped with a Canon EOS 500D (Canon, Tokyo, Japan) or a Zeiss Imager A1 microscope (Carl Zeiss, Oberkochen, Germany) with a Zeiss AxioCam MRc 5 digital camera. All measurements were made from either drawings or photographs.

Finally, specimens mounted in glycerol on standard microscope slides were observed with a Leica SP5 II confocal scanning microscope (Leica Microsystems, Wetzlar, Germany). Confocal image stacks of autofluorescent signals were acquired with the LAS AF Software, 0.5 µm slice thickness, image size of 1024 x 568 pixels and using the argon laser utilizing the whole excitation bandwidth. The image software Amira 6.4 (Thermo Fisher Scientific, Waltham, MA, USA) was used for volume renderings utilizing the native Amira "volume rendering" and "volren" modules. Image processing was conducted in Adobe Photoshop CC 19.1.9 Release (Adobe Systems Incorporated, Mountain View, CA, United States) and the ImageJ package Fiji (Schindelin *et al*. 2012).

Enhanced contrast of images was obtained using the contrast limited adaptive histogram equalization (CLAHE) module in Fiji (Zuiderveld 1994). Dust particles that overlapped with the cuticle were removed in Photoshop with the "Spot Healing Brush Tool" and the "Healing Brush Tool".

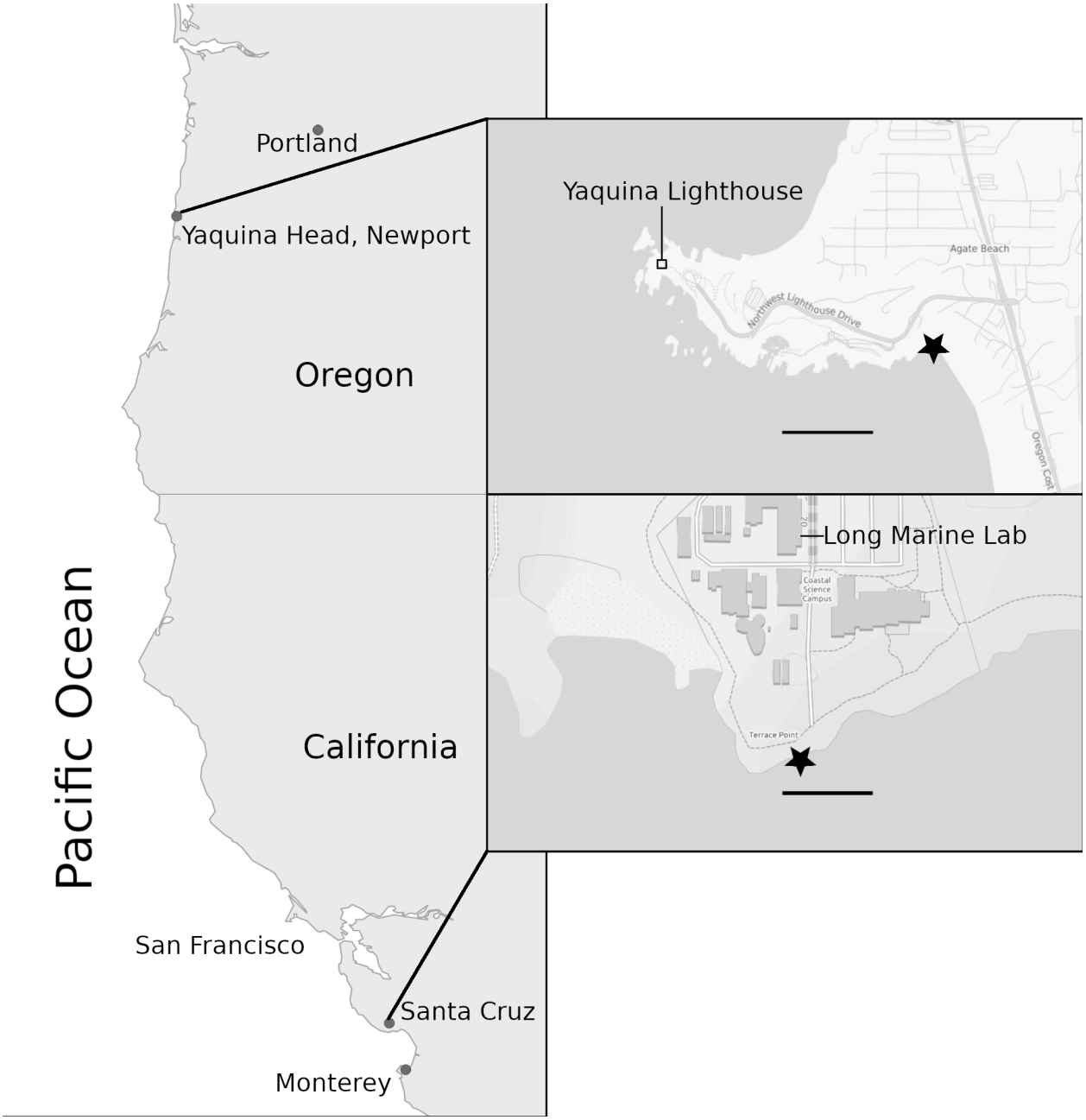

FIGURE 1. Sampling locations on the US West Coast in Oregon and California. Scalebars Yaquina Head: 400 m, Santa Cruz: 100 m.

Results

Class Chromadorea Inglis, 1983

Subclass Chromadoria Pearse, 1942

Order Desmodorida De Coninck, 1965

Suborder Desmodorina De Coninck, 1965

Superfamily Desmodoroidea Filipjev, 1922

Family Desmodoridae Filipjev, 1922

Subfamily Stilbonematinae Chitwood, 1936

Eubostrichopsis Ott & Pröts, gen. nov.

Type species. *Eubostrichopsis johnpearsei* Ott & Pröts, **sp. n.**

Genus definition. Stilbonematinae with a cylindical body. Anterior end with a distinct subglobular cephalic capsule. Tail conoid with spinneret. Six labial papillae, four cephalic setae directed forward, circles of 8 subcephalic setae each located on cephalic capsule. Cuticle transversely striated. Pharynx with round muscular bulbus, corpus not dilated. Male tail with pairs of thorn-like setae ventrally. Amphideal fovea in form of a ventrally wound spiral in extreme forward position. Fovea shape not sexually dimorphic. Spicula cephalate, curved, gubernaculum straight, directed dorsally, proximal end hook-shaped. Ectosymbiotic bacteria slender, slightly curved pointed rods, attached with one pole to the nematode cuticle. Symbiont coat dense, covering the whole body except the cephalic capsule and the tip of the tail.

Etymology: The genus name refers to its similarity to the genus *Eubostrichus* Greeff, 1869 with regard to the thorn-like like setae on the male tail and the shape of the symbiotic bacteria.

Species description

Eubostrichopsis johnpearsei sp. n.

(Figs. 2–5)

Type material: **Holotype** male, **paratype** female, **other paratypes:** male 2, females 2–3

Type specimens deposited in the collection of the Natural History Museum Vienna (Austria) Numbers Invertebrata varia Collection 5855 (holotype), 5856 (paratype male 2), 5857/1–3 (paratypes female 1–3). **Type locality:** Beach south of Yaquina Head, Newport (Oregon) (holotype male, paratype male 2, paratype female 1–2). Sampling date: April 25, 1986.

Other locality: beach in front of the Joseph M. Long Marine Laboratory, Santa Cruz (California) (paratype female 3). Sampling date: March 2, 1986.

Etymology: The species is named in honor of the late John Pearse, 1940–2020, eminent invertebrate biologist and the first author's friend and generous host during his sabbatical at the University of California Santa Cruz 1985–86, when the specimens were collected.

Measurements. Table 1.

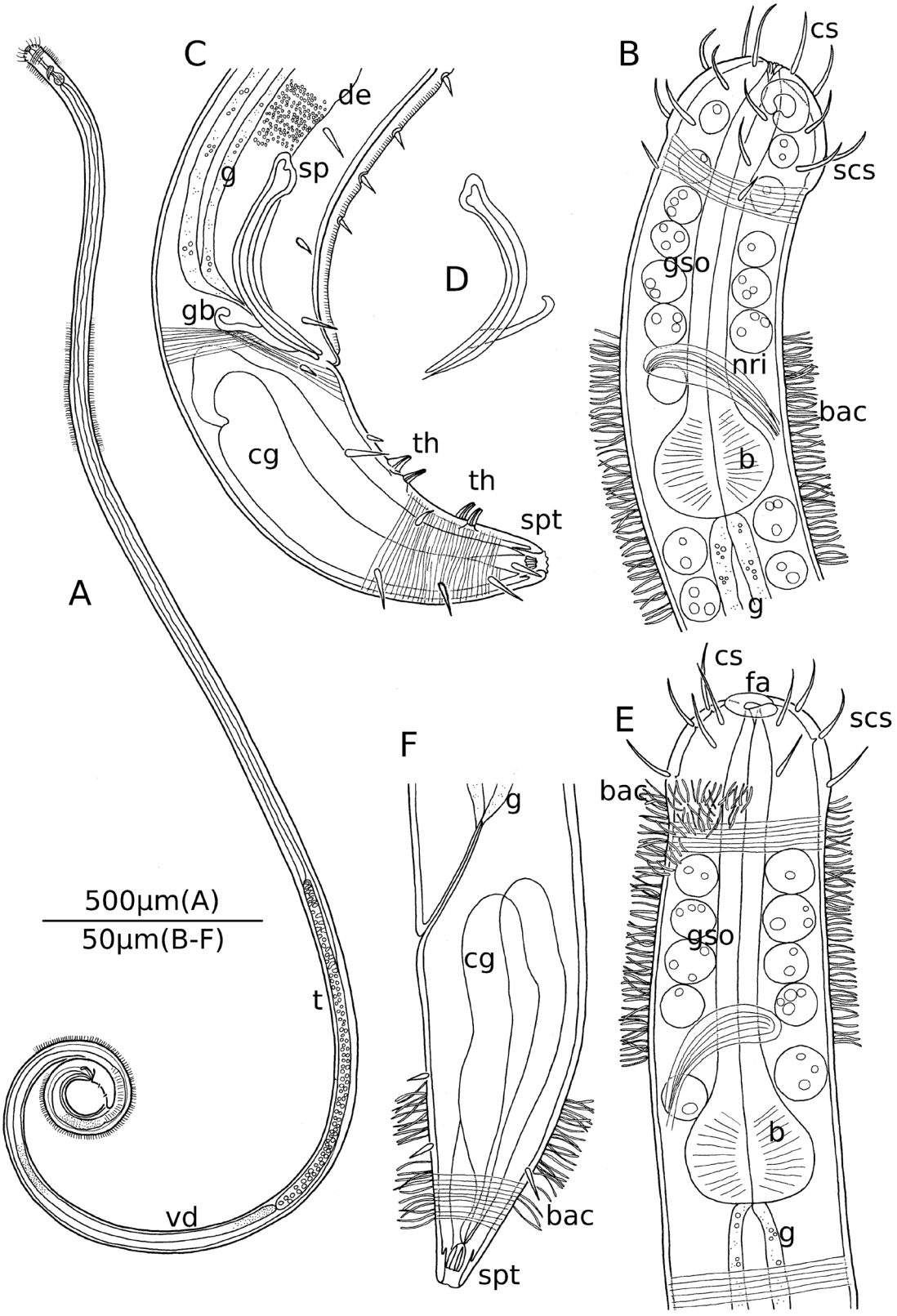

FIGURE 2. *Eubostrichopsis johnpearsei* **sp. n.** A. Holotype, total view. B. Anterior body region of male paratype. C. Cloacal and tail region of male paratype. D. Spiculum and gubernaculum of holotype. E. Anterior body region of female paratype 1. F. Tail of female paratype 1. b—bulbus, bac—bacteria, cs—cephalic setae, de—ductus ejaculatorius, fa—fovea amphidialis, g—gut, gb—gubernaculum, gso—glandular sensory organ, nri—nerve ring, scs—subcephalic setae, sp—spiculum, spt—spin neret, t—testis, tg—tail gland, th—thorn-like setae, vd—vas deferens.

TABLE 1. *Eubostrichopsis johnpearsei* **sp. n.** Morphometric data. All measurements in µm. Position of pairs of thorns on male tail given in % of tail length. (a, b, c—Demanian ratios, b'—pharynx length/diameter at end of pharynx, c'—tail length/anal diameter.)

	Holotype male	Paratype male	Paratype female	Paratype female	Paratype female
location	Oregon	Oregon	Oregon	Oregon	California
body length	4140	3675	5150	4540	3210
a	104	94.2	108	90.8	106
b	41.4	38.1	48.3	46.4	38.6
c	55.2	49	68.9	67.8	53.4
maximum diameter	40	39	48	50	30
pharynx length	100	95	107	98	83
tail length	75	75	75	67	60
b'	2.8	2.7	2.7	2.7	3.6
c'	2	2	2.1	2.1	3
cephalic setae length	15	10	13	12	10
subcephalic setae length	10	10	14	11	10
cephalic capsule length	20	22	27	25	16
fovea amphidialis	10	10	11	10	7.5
nerve ring % pharynx length	70	70	65	66	72
bulbus length/width	25/25	25/25	25/28	25/25	17/18
testis begin %	46.7	56	n.a.	n.a.	n.a.
vulva %	n.a.	n.a.	58	59.5	54
spicula arc/chord	55/45	50/45	n.a.	n.a.	n.a.
gubernaculum length	27	30	n.a.	n.a.	n.a.
1st thorns % tail length	40	47	n.a.	n.a.	n.a.
2nd thorns % tail length	75	80	n.a.	n.a.	n.a.

With the characters of the genus. Slender worms with a short pharynx and short tail. There are two circles of subcephalic setae on the cephalic capsule. Both the cephalic and subcephalic setae slightly curved inward. No somatic setae except for the precloacal region and tail of males. Cephalic capsule without block layer, surface largely unstriated, striation starting a few µm before level of posterior end of cephalic capsule. Striae 0.7–1 µm wide, striation extending over whole body, tip of tail with an unstriated terminal part, 15 µm long. Precloacal region of males with a medioventral membranous ridge, 340 µm (holotype) and 250 µm (paratype) long, 4 µm high, bearing a row of short (4 µm long) setae, 10–20 µm apart. Male tail with two pairs of thorn-like hollow setae (porids) at 40–47 and 75–80 % of tail length. Pharynx with cylindrical anterior part, spherical bulbus occupying 20 % of pharynx length, no cardia. Prominent nerve ring encircling pharynx directly anterior to bulbus. No excretory system present. Males monorchic, testis starting at midbody; spicular apparatus with prominent musculature. Females didelphic, amphidelphic, ovaries reflexed, anterior ovary left, posterior right of gut. Female 3 with two ripe eggs in each uterus 80 x 30 µm. Vulva not specially cuticularized, slightly posterior to midbody. Symbiont coat (Fig. 6) covers the whole striated part of the worms' body. Bacteria are 7–10 µm long, in center of cell 0.7 µm wide, tapering towards poles. Bacteria appear to divide longitudinally. Thicker (1.4–1.6 µm) cells show a distinct longitudinal groove. Approximately 170 cells per 100 μm^2 of host surface. A 4 mm long worm therefore carries about 850 000 bacterial cells. Total volume of symbiotic coat is more than 90 % of host volume or almost half of holobiont volume.

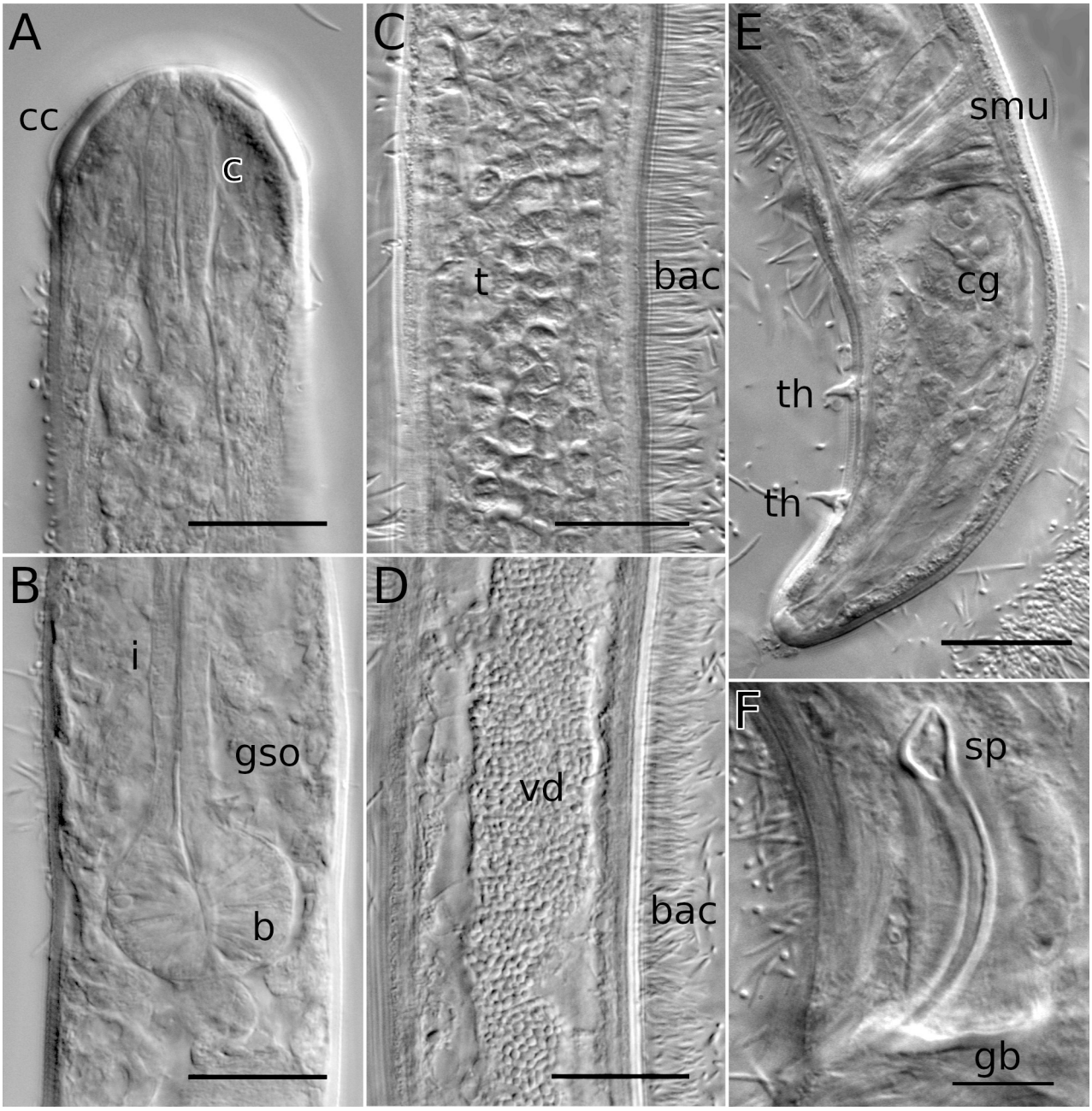

FIGURE 3. *Eubostrichopsis johnpearsei* **sp. n.** A. Head region of holotype. B. Pharynx bulbus of holotype. C. Testis region of paratype. D. Vas deferens of paratype. E Tail of holotype. F. Spiculum of holotype. DIC micrographs of preserved specimens. b—bulbus, bac—bacteria, cc—cephalic capsule, cg—caudal gland, gb—gubernaculum, gso—glandular sensory organ, i—isthmus, smu—muscles of the spicular apparatus, sp—spiculum, t—testis, th—thorn-like setae, vd—vas deferens. Scalebars A–E: 20 µm, F: 10 µm

Discussion

Taxonomy

The new genus is similar to *Eubostrichus* Greef, 1869 in several characters, such as the finely striated cuticle, the thorn-like setae (porids) on the male tail and the cylindrical anterior portion of the pharynx with undilated corpus. It differs from *Eubostrichus* in having a cylindrical body that does not taper towards the anterior end, a cephalic capsule and the spherical, muscular pharynx bulbus. In the absence of molecular data, the relationship to other genera of the Stilbonematinae remains unclear.

The shape and geometry of the arrangement of the symbiotic bacteria are genus-specific in Stilbonematinae and should be included into the definition of the respective genera (Scharhauser *et al*. 2020). The new genus is remarkable for a new type of symbiont coat consisting of up to 10 µm long slightly curved rods that are pointed at both poles. The rods stand upright, perpendicular to the host cuticle to which they are attached with one pole. Symbionts of similar shape are present in the genera *Adelphos* Ott, 1997 and *Eubostrichus* Greef, 1869 (except for *E. dianeae* Hopper & Cefalu, 1973). In the latter two genera, however, the symbionts are attached with both poles and arranged in a spiral manner. Pointed elongated rods are also present in the genus *Squanema* Gerlach, 1963, but they lie flat on the cuticle of the host, parallel to the worm's longitudinal axis.

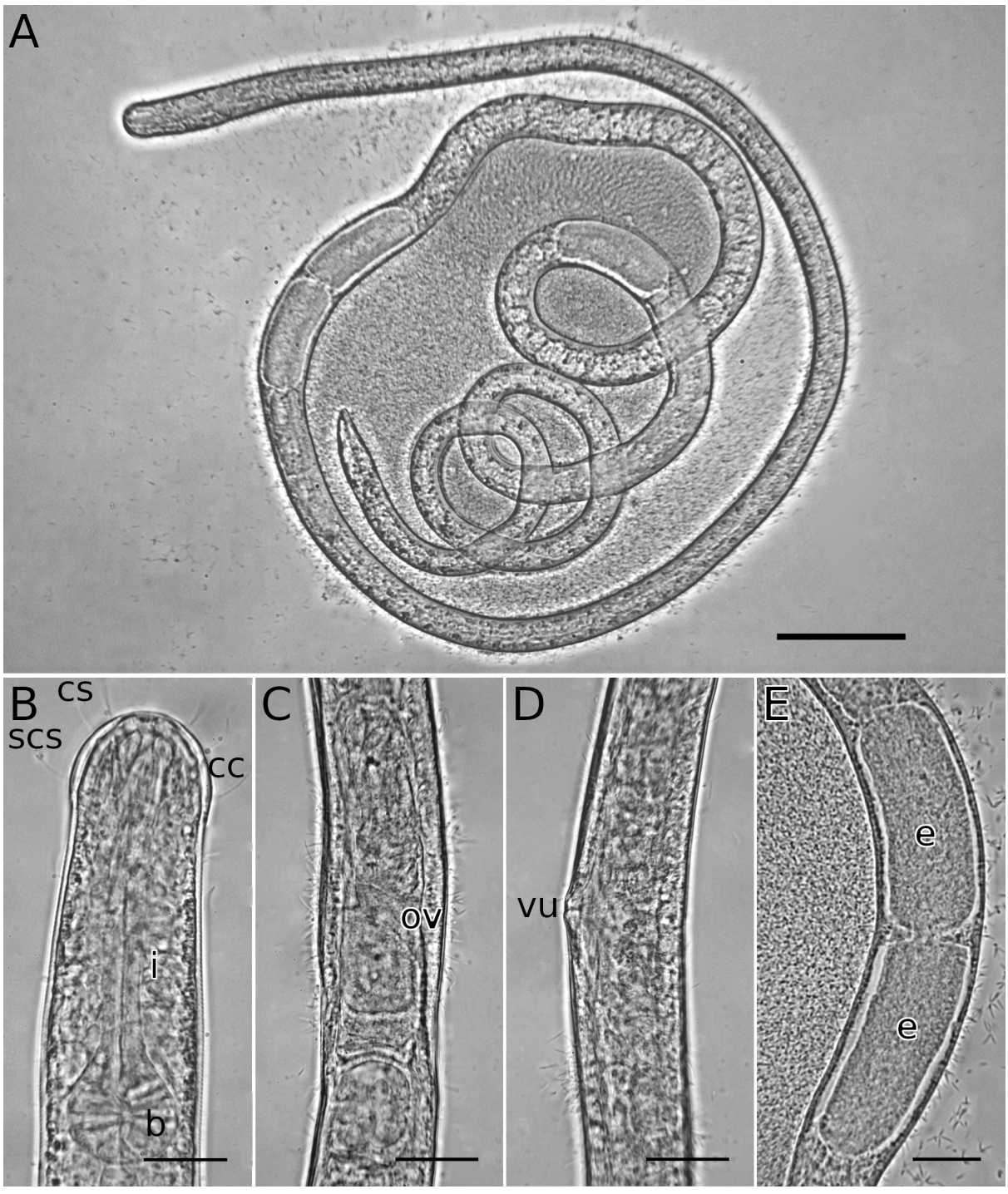

FIGURE 4. *Eubostrichopsis johnpearsei* **sp. n.** A. Female paratype 3, total view. B. Female paratype 1, anterior body region. C. Female paratype 1, anterior ovary. D. Female paratype 1, vulva region. F. Female paratype 3, eggs in uterus. LM micrographs of preserved specimens. b—bulbus, cc—cephalic capsule, cs—cephalic setae, e—egg, scs—subcephalic setae, ov—ovary, vu—vulva. Scalebars A: 100 µm, B–D: 20 µm, E: 10 µm.

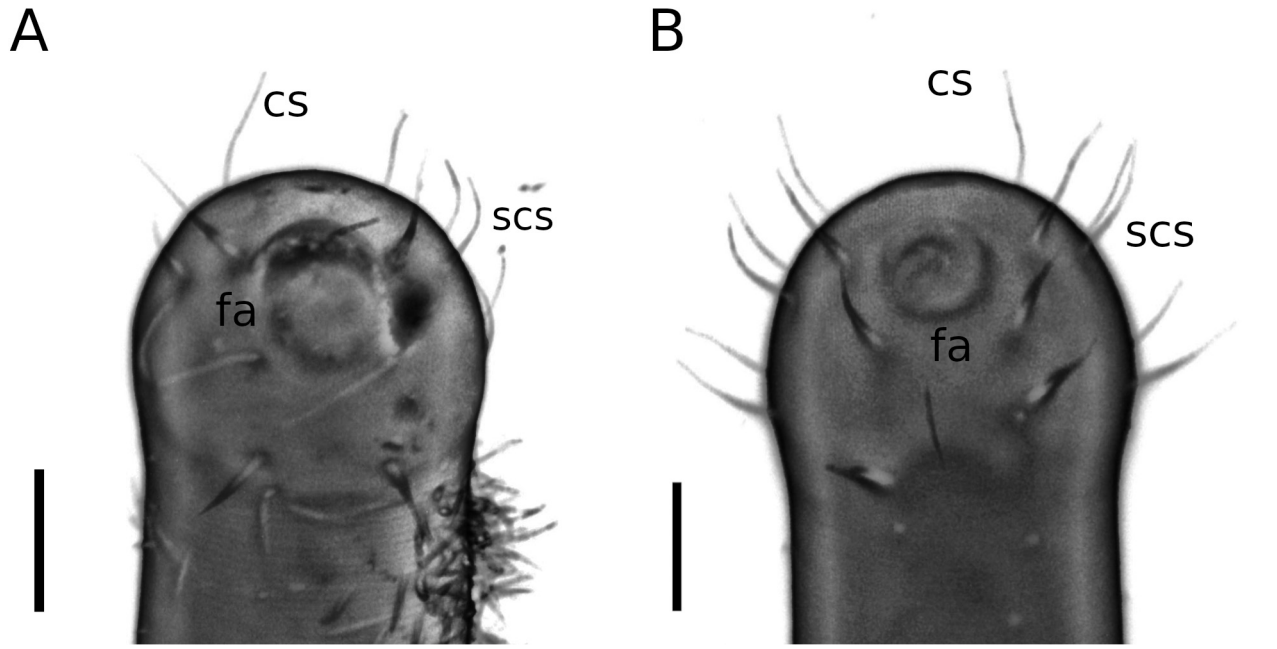

FIGURE 5. *Eubostrichopsis johnpearsei* **sp. n.** A. Head region of holotype. B. Head region of female paratype 1. Volume renderings based on image stacks of autofluorescence signal acquired with a confocal microscope. cs—cephalic setae, fa—fovea amphidialis, scs—subcephalic setae. Scalebars A–B: 15 µm.

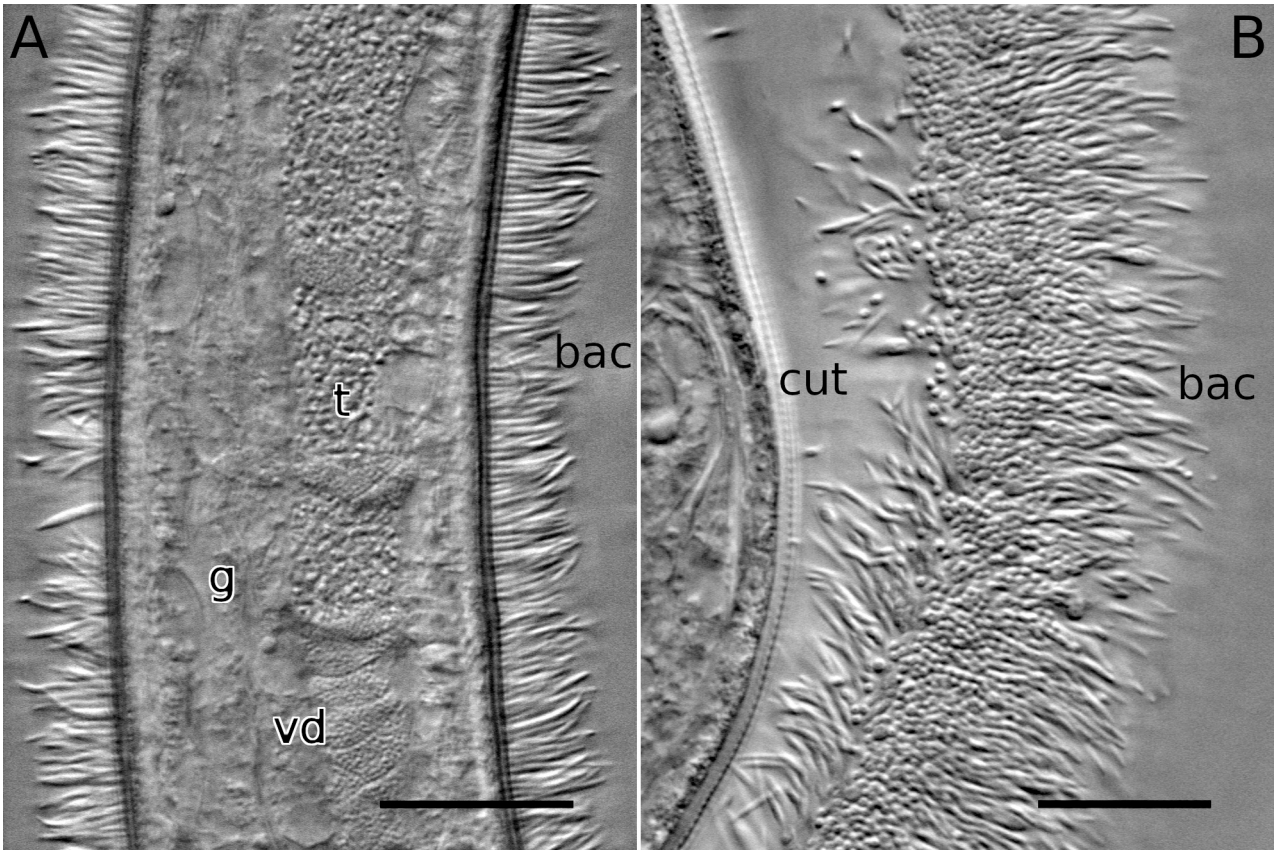

FIGURE 6. *Eubostrichopsis johnpearsei* **sp. n.** Symbiotic bacteria. A. Sagittal optical section through region of junction between testis and vas deferens in holotype. B. Detached symbionts showing an optical cross section through the dense bacterial coat. DIC micrographs of preserved specimens. bac—bacteria, cut—cuticle, g—gut, t—testis, vd—vas deferens. Scalebars A: 20 µm, B: 15 µm.

Ecology

Dense vegetation effectively reduces water movement inside the plant stands (Ott 1967, Fleeger *et al*. 1984). This can lead to oxygen depleted conditions in the basal sediment even under high ambient water movement. The sediment collected among the rhizomes and roots of surfgrass nevertheless contains a rich meiofauna, composed of nematodes as the dominant group, platyhelminths and gnathostomulids (Farris & Hooge, unpublished manuscript). Sterrer & Sorensen (2006) reported eight species of Gnathostomulida (including a new genus, *Chirognathia*), Gastrotricha and interstitial annelids from *Phyllospadix* sediment. Copepods are rare or lacking (Farris & Hooge 1998). Such a faunal composition is typical for the sulfide system (Fenchel & Riedl 1970).

Marine nematodes of the subfamily Stilbonematinae (Desmodoridae) are a characteristic element of sediments, where reduced, sulfidic conditions occur under an oxidized surface layer. The highest abundance and diversity of this taxon occurs in tropical to temperate shallow subtidal sediments where oxic and sulfidic sediments are separated by a layer containing neither oxygen nor sulfide (Ott *et al*. 2004). In sediments with sharp redox gradients, such as intertidal mudflats, stilbonematine worms are rare and less diverse. The sediments at the base of *Phyllospadix* stands are only a few mm thick and black under a thin oxidized surface. This may explain why we found only one species of Stilbonematinae and only in low numbers.

This is only the second report of this otherwise world-wide distributed symbiotic nematode subfamily for the East Pacific. The first findings of Stilbonematinae were reported by de Jesus-Navarrete (2007) from Socorro Island (Mexico) for the genus *Eubostrichus* Greef, 1869. Sulfidic systems have received little attention on the wave-beaten shores of the American West Coast. More studies, including such unexpected reduced habitats as the *Phyllospadix* holdfasts, will certainly bring forward more representatives of Stilbonematinae.

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