

EUBOSTRICHUS TOPIARIUS SP. N., A NEW FREE-LIVING, MARINE SPECIES OF STILBONEMATINAE (NEMATODA: DESMODORIDAE) FROM A SHALLOW SUBTIDAL SAND BOTTOM

BY

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Eubostrichus topiarius sp. n. is a new species of the small subfamily Stilbonematinae, whose members are obligatorily associated with ectosymbiotic bacteria. It is recorded from the Bay of Vestar (northern Adriatic Sea) where it inhabits the interstitial space of a moderately sorted, coarse subtidal sand. The new species is much larger than, and of different proportions from the previously described species. The unispiral amphid is comparatively small and close to the anterior end; the cephalic region shows circles of 6 inner and 6 outer labial sensillae; the 4 cephalic sensillae are elongated; the anterior circle of 8 subcephalic setae inserts at the posterior margin of the amphid; the females are larger than the males showing up to 7 eggs in their uterus. This is a contribution to a revision of a very interesting genus to which a number of inadequately described specimens have been assigned.

Keywords: marine meiofauna, symbiosis, sulphur bacteria, taxonomy, Adriatic Sea.

The Stilbonematinae Chitwood, 1936, is a subfamily of the Desmodoridae Filipjev, 1922 (Nematoda; classification after Gerlach & Riemann, 1973). Members are known to be obligatorily associated with bacterial epigrowth (Ott & Novak, 1989; Ott *et al.*, 1991; Polz *et al.*, 1992). Due to the nature of their association and their sulphidic habitat, they are of special interest. During ecological and ecophysiological investigations (Ott & Novak, 1989; Schiemer *et al.*, 1990; Berger & Ott, in prep.) several species of Stilbonematinae were encountered.

The new species described in this paper and four further species belonging to three other genera (*Laxus cosmopolitus* Ott *et al.*, 1995, one undescribed species of *Catanema* Cobb, 1894, and 2 undescribed species of *Leptonemella* Cobb, 1920) occur regularly but not abundantly in the deeper layers of a shallow, subtidal, moderately well-sorted, coarse sand bottom in 3-4 m water depth in the Bay of Vestar, Croatia, northern Adriatic Sea.

MATERIALS AND METHODS

Several buckets of sand were collected and transported to the laboratory at the University of Vienna in spring and summer 1991. The animals were anaesthetized with $MgCl_2$ isotonic to seawater for 15 min and decanted through a 35 μm sieve. For light microscope observations, specimens were fixed with 4% formaldehyde in seawater for at least three days. Whole nematodes were transferred into glycerol. Bacteria were carefully removed by gently shaking in glycerol or brushing off with an eyelash.

For scanning electron microscopy (SEM), the bacteria were removed from their host with brief ultrasonic pulses. The aposymbiotic nematodes were then fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and postfixed with 2% OsO_4 . The specimens were dehydrated with ethanol, critical point dried with a POLARON E-3000, subsequently sputtered with a thin layer of gold with a TECHNICS HUMMER JR, and examined with a JEOL JSM-35CF.

Drawings from whole mounts in pure glycerol were made on a Reichert Diavar and a Reichert Polyvar, equipped with a camera lucida. Interference contrast photos were made on the Reichert Polyvar. All morphometric data of the nematodes were obtained by measurements of light microscope drawings; the bacteria were examined and measured on SEM-photographs.

TAXONOMY

Order Desmodorida sensu Gerlach & Riemann, 1973

Family Desmodoridae Filipjev, 1922

Subfamily Stilbonematinae Chitwood, 1936

Genus *Eubostrichus* Greeff, 1869

Eubostrichus topiarius, sp. n.

(Figs 1-5; Table I)

Synonymy. *Eubostrichus* cf. *parasitiferus* (Ott & Novak, 1989; Ott *et al.*, 1991; Polz *et al.*, 1992; Ott, 1993). *Eubostrichus* sp. (Urbancik *et al.*, 1996a, b).

Etymology. Species name from the Latin *topiarius* = horticulturist (especially of ornamental gardens).

Type material. Types are deposited in the Evertebrata Varia Collection of the Natural History Museum (EVC-NHM) at Vienna. Length is given in μm . Where applicable the mean \pm standard deviation is added.

TABLE I

Biometrical data of Eubostrichus topiarius sp. n. Measurements in μm ; mean \pm standard deviation

	Holotype	Allotype	Males <i>n</i> = 11	Females <i>n</i> = 10
body length (μm)	6300	6060	5780 \pm 546	6781.7 \pm 846
a	210.0	135	176.8 \pm 14	157.4 \pm 24.5
b	47.7	54	49.5 \pm 5	56.4 \pm 6
c	50.0	45	48.8 \pm 4	54.7 \pm 8
head diameter	20.0		20.2 \pm 1	22.5 \pm 2
maximum diameter	30.0		32.7 \pm 2	43.4 \pm 3
cephalic setae (<i>l</i>)	21.5		21.3 \pm 1	21.6 \pm 3
subcephalic setae 1(<i>l</i>)	16.0		17.2 \pm 1	16.6 \pm 1
subcephalic setae 2(<i>l</i>)	11.8		14.1 \pm 1.5	12.7 \pm 2.4
distance between subcephalic setae	10.0		9.2 \pm 1	9.5 \pm 1
subventr. cervical setae 1(<i>l</i>)	16.0		14.3 \pm 2	
subventr. cervical setae 2(<i>l</i>)	17.2		13.0 \pm 2.5	
distance of cv1 to anterior end	55.0		47.9 \pm 4	
distance of cv2 to anterior end	105.0		97.4 \pm 6	
buccal cavity (<i>l</i>)	5.0		7.7 \pm 2	9.0 \pm 1.3
buccal cavity (diameter)	3.0		3.0 \pm 1	4.3 \pm 1
amphid (<i>l</i>)	6.0		5.2 \pm 0.7	5.3 \pm 1.3
amphid (width)	9.0		8.1 \pm 1	8.6 \pm 1
amphid distance to anterior end	3.0		3.3 \pm 1	3.8 \pm 1
amphid c.b.d.	20.0		19.3 \pm 1	21.7 \pm 2
distance of nerve ring to anterior			73.7 \pm 5.5	72.2 \pm 7.5
nerve ring c.b.d.			34.0 \pm 1.5	37.4 \pm 4
pharynx (<i>l</i>)	132.0		116.6 \pm 7	120.2 \pm 9
pharynx (width)	7-8		7-10	8-10
posterior bulb (<i>l</i>)	22.0		20.0 \pm 1.5	21.7 \pm 3
posterior bulb (width)	22.0		22.2 \pm 3	23.1 \pm 3
posterior bulb c.b.d.	32.0		32.8 \pm 2.6	35.0 \pm 4
anal body diameter	40.0		37.8 \pm 6	28.2 \pm 2
tail (<i>l</i>)	126.0		120.0 \pm 4	124.6 \pm 10.4
tail length related to body length	3.2		3.3 \pm 0.5	4.4 \pm 0.4
cardia (<i>l</i>)	4.0		4.1 \pm 1.3	5.1 \pm 1.4
vas deferens %	63.0		61.3 \pm 2.5	
testis to anterior	1300.0		1730 \pm 303	
testis %	20.6		31.3 \pm 6	
spiculum (<i>l</i>)	58.0		55.4 \pm 3.5	
gubernaculum (<i>l</i>)	26.0		27.6 \pm 3	
terminal setae (<i>l</i>)	8.0		6.5 \pm 1	
anal subventral setae:				
distance to spic. (ps1)	9.6		8.6 \pm 2.5	
distance to spic. (ps2)	59.0		47.8 \pm 16	
distance to spic. (ps3)	75.0		70.6 \pm 9	
distance to spic. (ps4)	96.0		96.3 \pm 9	

TABLE I
(Continued)

	Holotype	Allotype	Males <i>n</i> = 11	Females <i>n</i> = 10
ps1 (<i>l</i>)	6.0		7.8 ± 1.5	
ps2 (<i>l</i>)	10.0		8.4 ± 2	
ps3 (<i>l</i>)	8.0		9.9 ± 1.2	
ps4 (<i>l</i>)	11.0		9.0 ± 2	
V %				49.7 ± 1
distance of vulva to anterior end				3306.4 ± 424
vulva c.b.d.				46.6 ± 4.4
female gonad (one part; <i>l</i>)				1298.2 ± 275
ovary (<i>l</i>)				307.6 ± 92
uterus (<i>l</i>)				404.8 ± 66.5
distance between somatic pores			12-15	10.0
distance between cervical pores			5-8	10.0
distance between tail pores			15-18	10-12
distance between vulva pores				4-6
egg (<i>l</i>)				130.0 ± 33
egg (width)				38.7 ± 8.3
number of eggs				1-7

Abbreviations used:

a, b, c	proportions of de Man,
c.b.d.	corresponding body diameter,
<i>l</i>	length,
testis %	positions of the anterior end of testis,
V %	positions of vulva as a percentage of total body length from anterior,
vas deferens %	positions of the anterior end of the vas deferens.

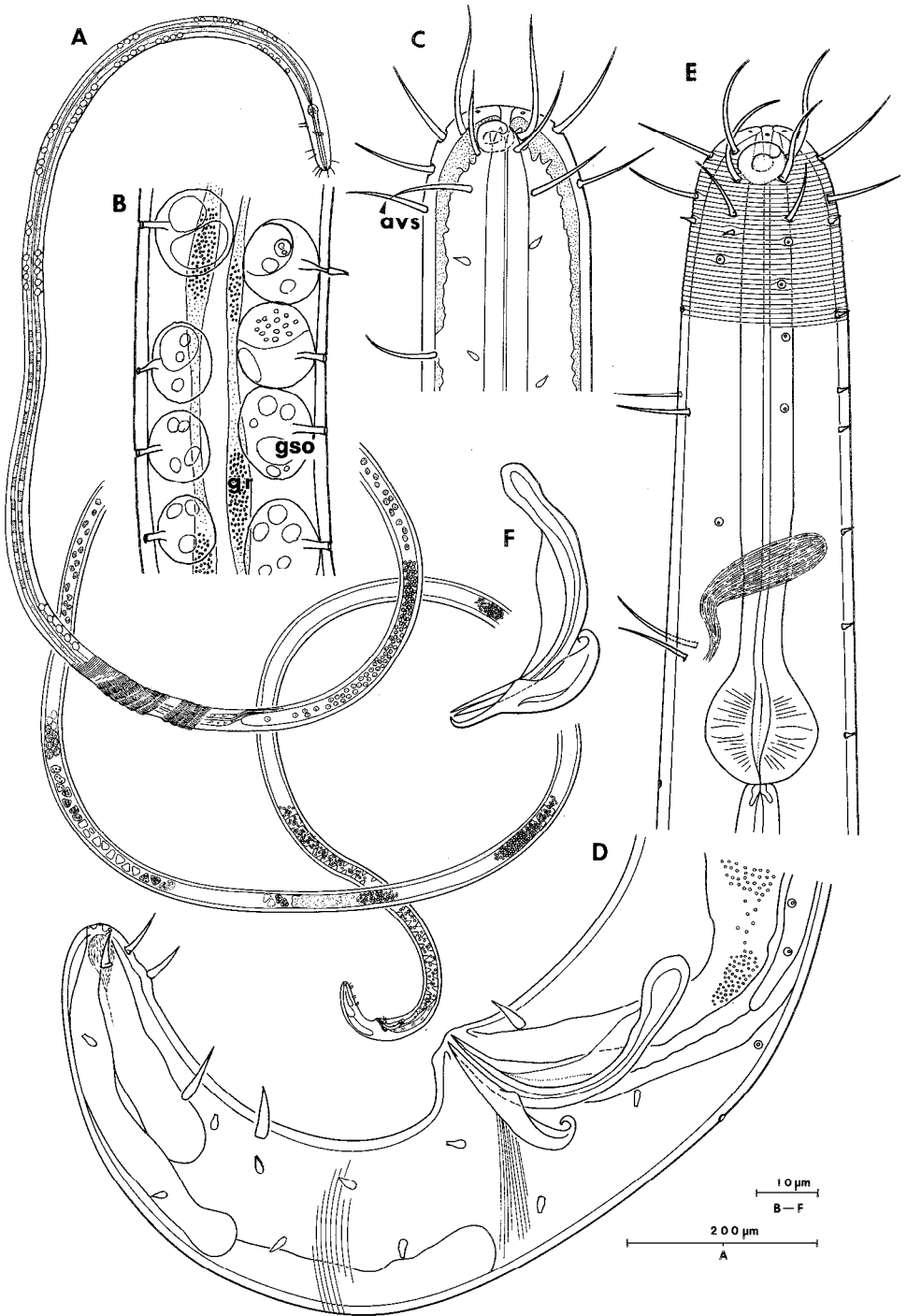
Holotype: male EVC-NHM Wien Number 3426 (measurements see Table I).

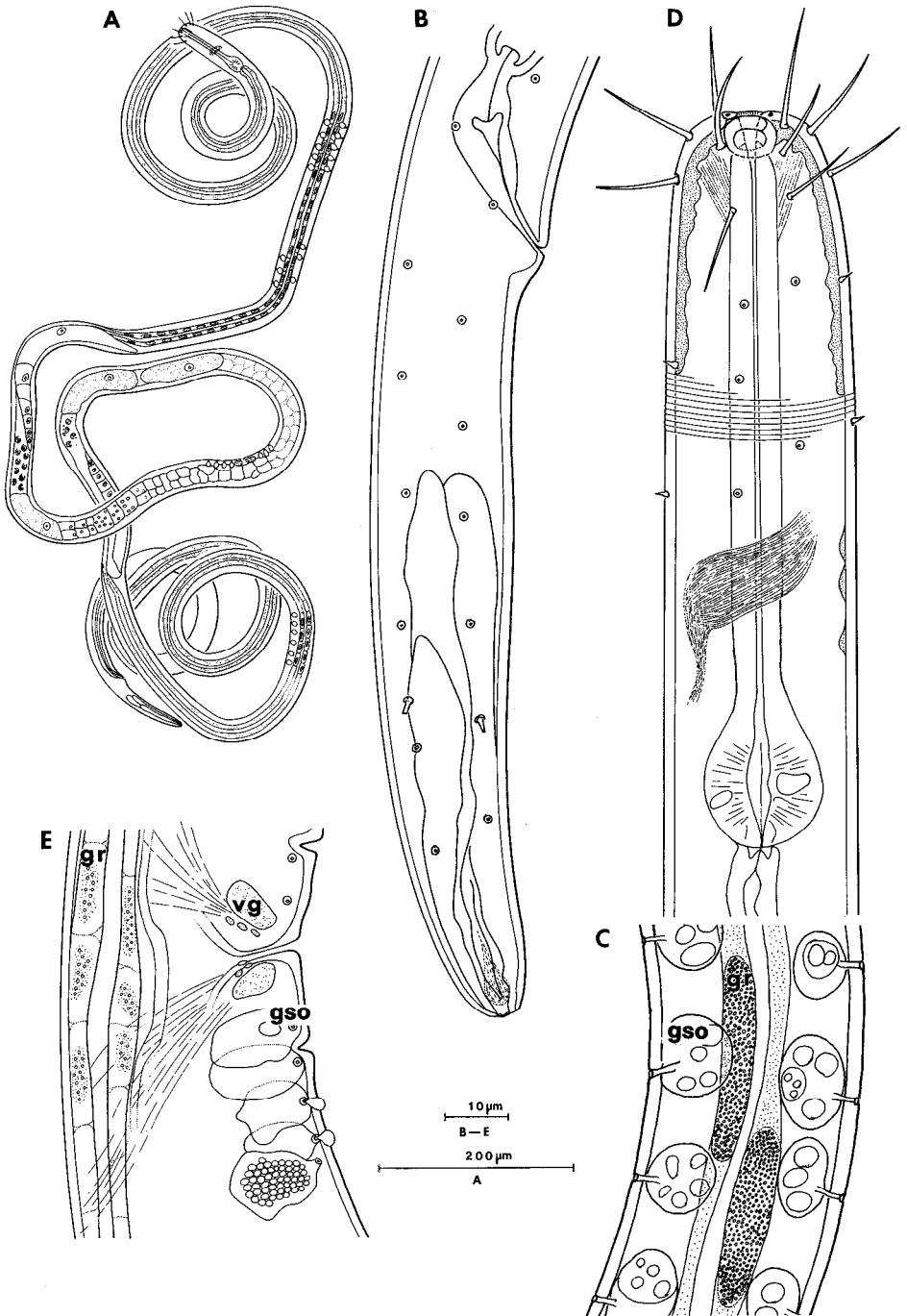
Allotype: female EVC-NHM Wien Number 3432 (measurements see Table I).

Paratypes: males EVC-NHM Wien Numbers 3427-3431: L = 5744 ± 473; a = 175 ± 7; b = 49 ± 4.2; c = 48 ± 4.2 (*n* = 5).

Paratypes: females EVC-NHM Wien Numbers 3432-3438: L = 7239 ± 533; a = 157 ± 27.3; b = 61 ± 2.6; c = 55.6 ± 1.3 (*n* = 7).

Fig. 1. *Eubostrichus topiarius* sp. n. (male). A) Lateral view of entire animal (paratype, #3427). B) Somatic region, showing glandular sensory organs (gso) and intestine with clusters of granular material (gr; paratype, #3427). C) Head with one additional ventral seta (avs; holotype). D) Tail (holotype). E) Head (paratype, #3428). F) Spicular apparatus (paratype, #3429). B-F) Left lateral view.





Type locality

Bay of Vestar (45°02'8"N, 13°41'1"E), Croatia (northern Adriatic Sea). Moderately well-sorted, coarse sand dominated by biogenic calcareous components low in organic matter; shallow subtidal at 3-4 m water depth; samples were collected in 1991.

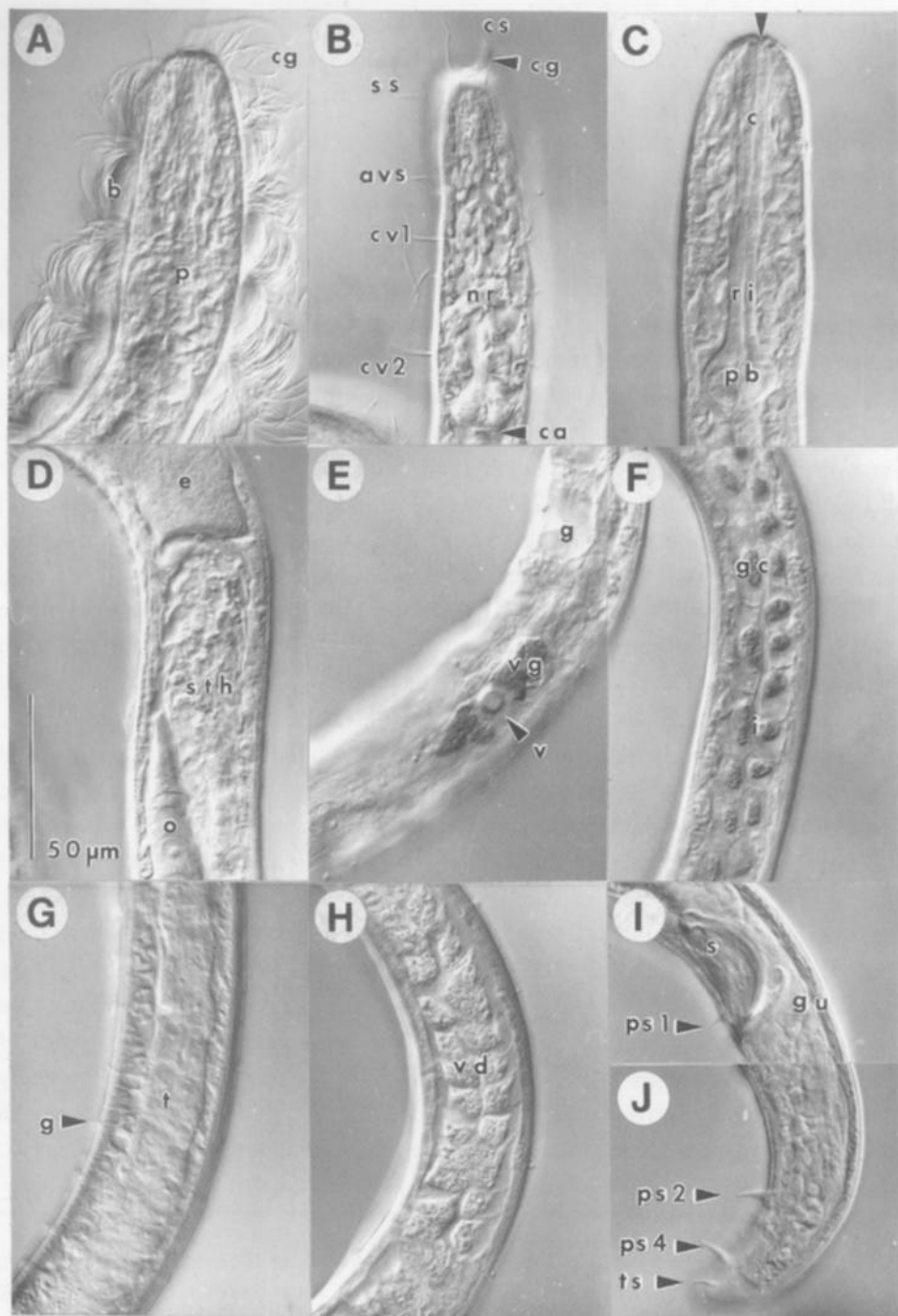
Species description

The filiform and transparent nematodes taper slightly at both ends (Figs 1A, 2A). The cuticle is usually covered with opaque, crescent-shaped bacteria, which are attached to their host with both ends forming a thick, highly ordered microbial coating (Figs 3, 4). Aposymbiotic specimens were occasionally found, but in general even the smallest first-stage juveniles are already associated with bacteria. The worms are usually tightly coiled, almost immobile, and highly thigmotactic. On average, adult females are larger than adult males. Worms have a slender and elongated habitus ($a = 160-210$ for males and $a = 106-192$ for females) and a relatively short pharynx and tail ($b = 49.5 \pm 4.6$ for males and $b = 56.4 \pm 6.1$ for females; $c = 48.8 \pm 4$ for males and $c = 54.7 \pm 7.8$ for females).

The cuticle is faintly annulated. The annuli (width approx. $1 \mu\text{m}$) begin at the level of the centre of the amphid (Urbancik *et al.*, 1996a; Fig. 4I) and extend to the tip of the tail. The regular pattern is interrupted where setae, branchings, and terminations of annuli and variations of their thickness are visible (Fig. 4C, G).

In general the body setae are short, hollow cylinders (approx. $1.8 \mu\text{m}$ long) which do not extend above the bacterial coat (Fig. 4A, B, E, F, G). In the cervical region they are arranged in 8 longitudinal rows (Figs 1E; 4A, E; two sublateral, one subdorsal and one subventral row on each side). Two circles of 8 long ($16 \mu\text{m}$) subcephalic setae each are present (Figs 1C, E; 2D; 4A, E), the anterior circle is situated at the level of the posterior margin of the amphid (Fig. 4I) and in females the posterior circle is approx. $10 \mu\text{m}$ further back (Figs 2D; 4E); in males the distance between the two circles of subcephalic setae can vary between 7 and $10 \mu\text{m}$ (Figs 1C, E; 4A). Males have two pairs of elongated subventral cervical setae (Figs 1E; 3B; 4A) situated in a diagonally opposed arrangement (Fig. 4C; $13-14 \mu\text{m}$ length). An additional elongated seta in ventral position is present in some of the males (including the holotype) (avs;

Fig. 2. *Eubostrichus topiarius* sp. n. (female). A) Lateral view of the entire animal (allotype). B) Tail (allotype). C) Somatic region, showing glandular sensory organs (gso) and intestine with clusters of granular material (gr; allotype). D) Head (paratype, #3434). E) Midbody region, showing vulva, glandular sensory organs (gso), and vaginal glands (vg; allotype). C, D) Left lateral view; B, E) Right lateral view.



Figs 1C; 3B) between the second circle of subcephalic setae and the anterior pair of elongated subventral cervical setae. Since the elongated setae easily break during cleaning of the worms, we cannot state with certainty whether they are not developed or just missing in the rest of the specimens. Posterior to the pharynx the submedian rows of somatic setae merge into one medioventral and one mediodorsal row (terminology after Coomans, 1979a). All somatic setae are the outlets of spherical glandular sensory organs (gso, Nebelsick *et al.*, 1992; Bauer-Nebelsick *et al.*, 1995), which likewise occur in eight (cervical) and six (somatic) rows (Figs 1B; 2C; 3E, G). In females the somatic setae and the gso are especially dense in the vulva region (Figs 2E; 4G).

The head is rounded, without cephalic capsule (Urbancik *et al.*, 1996b). On average the head diameter is 62% of the maximum body diameter in males and 52% in females. The cephalic sensillae consist of three separate circles (6 + 6 + 4) following a triradial symmetry (terminology after Coomans, 1979b). The anterior circle is represented by papilliform projections (Fig. 4H = ils): one mediolateral, and one subdorsal and subventral sensilla on each side. The second circle consists of short, nipple-like sensillae (Fig. 4H = ols): one lateral (shifted slightly towards dorsal), one laterodorsal, and one lateroventral sensilla on each side. The four cephalic sensillae are arranged in laterosubdorsal and laterosubventral position (Figs 1C, E; 2D; 3B; 4A, E) and insert at the anterior margin of the amphid (Fig. 4H, I). They are slightly longer than the head diameter in males and equal to the head diameter in females.

Fig. 3. Nomarski interference contrast micrographs of glycerine-mounted specimens of *Eubostrichus topiarius* sp. n. A) Anterior region of a female covered with bacteria (b), showing the pharynx (p) and the corpus gelatum (cg). B) Head of a male, showing cephalic sensillae (cs), corpus gelatum (cg), subcephalic seta (ss), nerve ring (nr), cardia (ca), one additional ventral seta (avs), one of the anterior subventral cervical setae (cv1), and one of the posterior subventral cervical setae (cv2). C) Anterior region of a female with the three-part pharynx consisting of corpus (c), region of isthmus (ri), and posterior bulb (pb); arrow indicates the buccal cavity. D) Gonad region of a female, showing part of an egg (e), spermatheca (sth), and reflexed part of germinal zone of the ovary (o). E) Vulva region of a female, with vulva (v), vaginal glands (vg), and a glandular sensory organ (g). F) Somatic region of a female, showing the intestinal cells (i) filled with clusters of granular material (gs). G) Somatic region of a male, showing the indistinct beginning of the testis (t) and a ventral row of glandular sensory organs (g). H) Posterior body region of the same individual with vas deferens (vd). I) Cloacal region of a male with the spicular apparatus consisting of spiculum (s) and gubernaculum (gu); arrow indicates one thorn-like, pre-anal, subventral seta (ps1). J) Caudal region of a male with thorn-like, postanal subventral setae (arrow indicates ps2 and ps4) and one terminal seta (ts). All photographs show a left lateral view; except (E) ventral view.

The circular amphid is unispiral and ventrally wound (Figs 1C, E; 2D; 4I). It's width amounts to 42% of the corresponding body diameter in males and 39% in females. In both sexes it lies close to the anterior end.

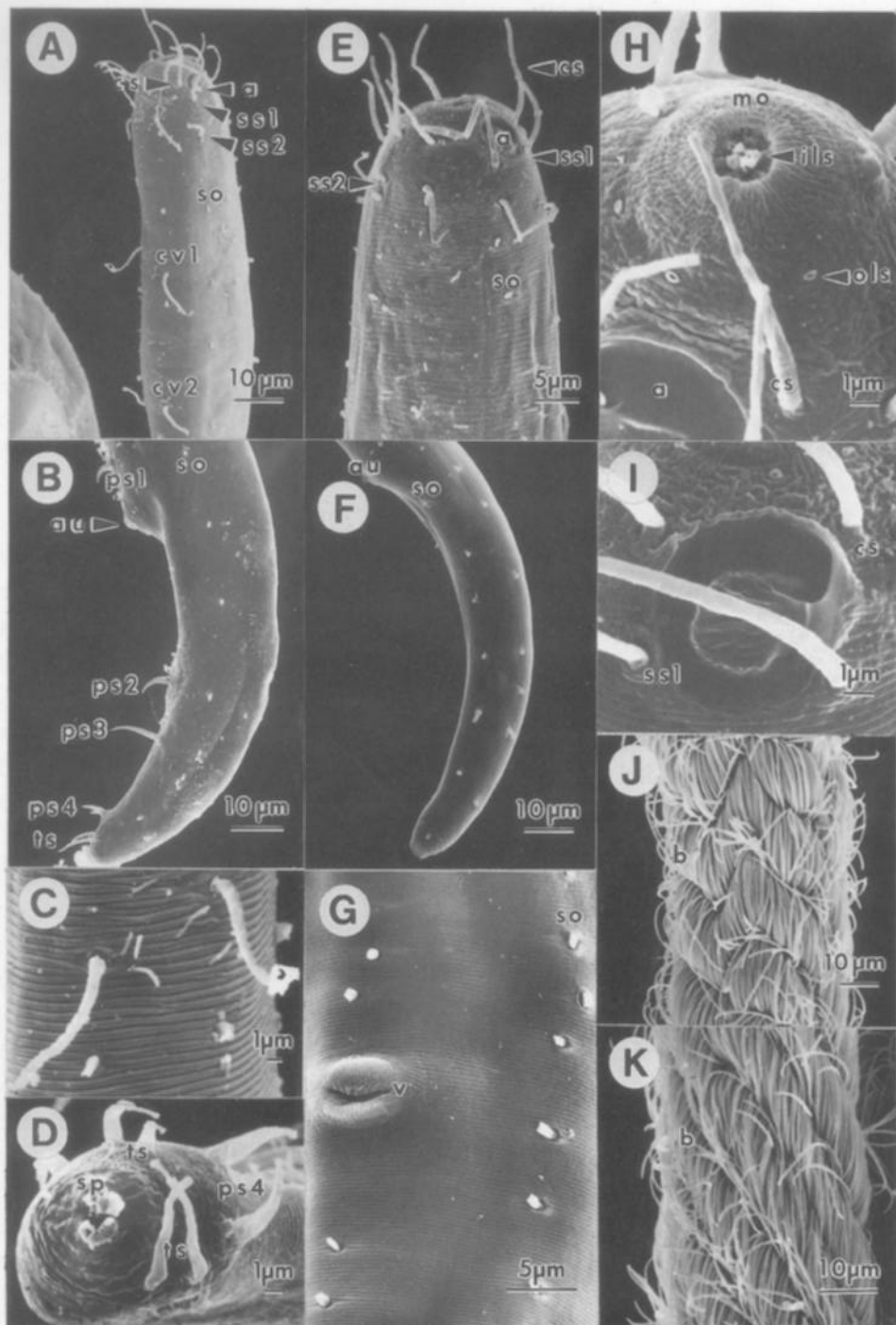
The buccal cavity is minute and unarmed (Figs 1C, E; 2D; 3C). The three-part pharynx (Maggenti, 1981) consists of a slightly swollen corpus, a thinner isthmus, and a spherical, muscular posterior bulb with two transverse breaks in the musculature (Figs 1E; 2D; 3C). A pharyngo-intestinal valve (cardia) is well developed (Figs 1E; 2D) and leads to a large-celled intestine with clusters of refractile granular material (Figs 1B; 2C, E; 3F). These structures are particularly large in females. In the gonad region the intestine is squeezed into a subdorsal position.

The nerve ring (Figs 1E; 2D; 3B) is located at 63% of pharynx length. No excretory pore or ventral gland could be detected.

The tail is conical and approx. 3.2 anal diameters long in males (Figs 1D; 3I, J; 4B) and about 4.5 anal diameters long in females (Figs 2B; 4F). It contains three caudal glands of different size (Figs 1D; 2B) which open separately at the terminal spinneret (Fig. 4D). Two pairs of terminal setae are located sublaterally (Figs 3J; 4D). Males possess one pair of preanal and three pairs of postanal, thorn-like setae (6 to 11 μm long) sublaterally, the second pair of the postanal setae being the longest (Figs 1D; 3J; 4B).

Males are monorchic, having an outstretched testis lying to the left of the intestine (Figs 1A; 3G, H). The indistinct testis starts at 30% of the body length

Fig. 4. SEM photographs of *Eubostrichus topiarius* sp. n. and symbiotic epibacteria. A-D) males. E-G) females. H-K) details. A) Head and cervical region of a male showing the amphid (a), cephalic sensillae (cs), the two circles of subcephalic setae (ss1 + ss2), the distribution of somatic setae (so) in longitudinal rows, and the two pairs of subventral cervical setae (cv1, cv2); left lateral view. B) Caudal region of a male showing the anus (au), one preanal subventral thorn-like seta (ps1), three postanal subventral thorn-like setae (ps2-ps4), one pair of terminal setae (ts), and longitudinal rows of somatic setae (so); left lateral view. C) One pair of subventral cervical setae showing the subopposite insertion; ventral view. D) Tip of the tail of a male showing spinneret (sp), two pairs of terminal setae (ts), and the last pair postanal subventral thorn-like setae (ps4); terminal view. E) Head region of a female showing the amphid (a), the four cephalic sensillae (cs1-cs4), the two circles of subcephalic setae (ss1 + ss2), and the distribution of somatic setae (so) in longitudinal rows; right subdorsal view. F) Caudal region of a female showing the anus (au) and the distribution of somatic setae (so) in longitudinal rows; left lateral view. G) Midbody region of a female showing vulva (v) and the close spacing of body setae; left subventral view. H) Frontal view of a female showing the mouth opening (mo) with inner labial sensillae (ils), the outer labial sensillae (ols), and the insertion of one cephalic sensilla (cs) at the anterior margin of the amphid (a). I) Amphid with insertions of cephalic sensillae (cs) and subcephalic setae (ss); left lateral view. J) Midbody region with crescent-shaped symbiotic bacteria (b) forming a spirally wound, zigzag pattern. K) Midbody region with crescent-shaped symbiotic bacteria (b) showing a regular, spirally wound mode of attachment.



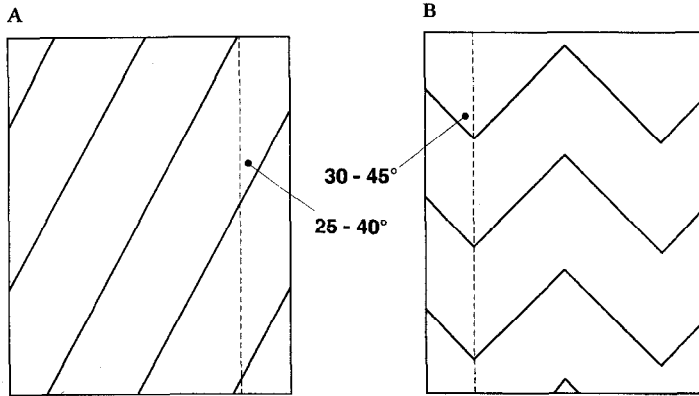


Fig. 5. Patterns of bacterial epigrowth in *Eubostrichus topiarius* sp. n. Lateral view, (A, straight-lined simple spiral; B, zigzag line) in relation to the longitudinal axis of the nematode (broken line).

and leads into a well-defined vas deferens (Fig. 3H) at about 60% of the body length. The spicula are arcuate with a knobbed to club-shaped capitulum, have an average length of 1.5 anal diameters, and bear a narrow velum that follows the curve of the lamina (Figs 1D, F; 3I). They are enveloped by a gubernaculum with a strong median piece which is twisted into a curl proximally (Fig. 3I).

Females have paired antidromously reflexed ovaries; on average the vulva is positioned at 50% of the body length (Figs 2A; 3D). The uterus is indistinct and may contain two to seven eggs per animal. The vulva is accompanied by two vaginal glands (Figs 2E; 3E).

The crescent-shaped symbiotic bacteria measured between 5 and approx. 30 μm in length, increasing in size from those closest to the worm's body to those situated in a more distal position (Fig. 3A; see details in Ott *et al.*, 1991; Polz *et al.*, 1992). The bacterial cells overgrow each other but are attached to the cuticle with both ends, constituting a monolayered microbial biofilm by definition. The large size and dense packing of the microbes, however, results in the worm's body effectively being surrounded by a bacterial coat several cell-layers thick (see cross sections in Ott *et al.*, 1991; Polz *et al.*, 1992). The bacterial coat appears in two main patterns, which occasionally show irregularities. The first pattern is that of a straight-lined, simple spiral at an angle between 25° and 40° relative to the longitudinal axis of the worm (Figs 4K; 5A). The second pattern is that of a spiralling zigzag line (Figs 4J; 5B). Both patterns can be present on the same individual and are independent of body width.

Diagnosis

E. topiarius sp. n. is filiform and elongated (males on average 5.5 mm, females on average longer than 6.0 mm); the amphid is small, unispiral, ventrally wound, close to the anterior end; the first circle of 8 elongated subcephalic setae insert at the level of the posterior margin of the amphid, the second circle of 8 setae posteriorly at a distance of 7-10 μm .

DISCUSSION

The genus *Eubostrichus* Greeff, 1869 was established with the type species *Eubostrichus filiformis* from the North Sea. His drawings and descriptions are rather inadequate (Baylis & Daubney, 1926; Chitwood, 1936; Hopper & Cefalu, 1973). Chitwood (1936) discovered a new species belonging to the genus and presented the first useful description of the genus for *E. parasitiferus* from Shackelford's Channel, Beaufort, North Carolina. Subsequently a number of specimens were assigned to this species (Gerlach, 1963, 1964; Hopper & Cefalu, 1973). Hopper & Cefalu (1973) presented a thorough revision and redefinition of the genus *Eubostrichus*, including a key to the known species, the description of the new species *E. dianeae* from Biscayne Bay, Florida and a precise redescription of *E. parasitiferus* from the same location. Recently Muthumbi *et al.* (1995) proposed to rename this as *E. hopperi*, since the specimens assigned to *E. parasitiferus* by Hopper & Cefalu (1973) differed from the preceding descriptions (Chitwood, 1936; Gerlach, 1963), and added new useful data to the genus with the description of two new species (*E. africanus* and *E. longosetosus*). We agree with this reassignment, which once more brings order into this genus. *Eubostrichus gerlachi* (Hopper & Cefalu, 1973) Platt & Zhang, 1982 (= *Catanema gerlachi* (Gerlach, 1963) Hopper & Cefalu, 1973 = *E. exilis* Gerlach, 1963) does not belong to this genus and is most probably a member of the genus *Laxus* (Ott *et al.*, 1995).

E. topiarius sp. n. differs from *E. parasitiferus* Chitwood, 1936 in length and size ratios. It is at least twice as long as the previously described species and has higher values of the de Man ratios, i.e. a more slender habitus and a relatively shorter pharynx. Structures similar to the inner and outer labial sensillae have not been described by the above authors, but might have been overlooked. The new species shows a definitely unispiral amphid, a character that is not uniformly described in the other publications: Chitwood (1936) observed an obscurely spiral amphid. Gerlach (1963) described sausage-shaped spirals with two turns, but then again a spiral amphid with one turn (Gerlach, 1964). Another difference is the deviating insertion of the two circles of subcephalic setae: Gerlach (1963)

described two circles, each with four subcephalic setae, but his drawings showed that this is most probably the posterior circle consisting of eight setae inserting at the posterior margin of the amphid; in his paper of 1964 the two circles showed the same position in *E. parasiticus* (= *E. parasitiferus*) as in *E. topiarius* sp. n.

E. hopperi (= *E. parasitiferus* in Hopper & Cefalu, 1973) is smaller and stouter than *E. topiarius* sp. n. Hopper & Cefalu (1973) were unable to ascertain the detailed structures of the amphid in male *E. hopperi*. According to their measurements, *E. hopperi* has a wider and longer amphid than the species described here. They also mentioned the orifice of the glandular sensory organs (as "hypodermal pore complexes"; Hopper & Cefalu, 1973) to be at the body surface, which is different from *E. topiarius* sp. n. This, however, could be due to broken-off setae, which is common during handling. Hopper & Cefalu (1973) described the first circle of the 8 subcephalic setae at the anterior margin and the second circle at a distance behind the posterior margin of the amphid. In *E. topiarius* sp. n. the first circle of subcephalic setae inserts at the posterior margin of the amphid.

E. topiarius sp. n. differs from *E. dianeae* in that it is much larger and less tapered towards the anterior end. Further *E. dianeae* differs from *E. topiarius* sp. n. by: the epigrowth with a fur-like appearance and only one end of the bacterial filaments is attached to the cuticle; the stouter body ($a = 50$); the shorter cephalic sensillae and subcephalic setae; the circles of the subcephalic setae are closer together. The same applies to the two pairs of elongated subventral cervical setae and the four to five pairs of subventral pre- and postanal setae in males. Finally, the spicules in *E. dianeae* are less curved with definitely thicker laminal structures and the gubernaculum is more simple and club-like.

The recently described species *E. africanus* and *E. longosetosus* (Muthumbi *et al.*, 1995) differ from *E. topiarius* sp. n. in that they are much smaller (3 to 4 mm long) with a less tapered head. The cephalic and cervical (= subcephalic setae in *E. topiarius* sp. n.) setae of both species are shorter and appear in a 4 + 4 + 4 constellation; in *E. topiarius* sp. n. they are arranged in a 4 + 8 + 8 pattern. The two distinct pairs of subventral cervical setae in males are missing. Both males have a testis that lies to the right of the intestine, where in *E. topiarius* sp. n. the testis lies on the left side. The arrangement of the thorn-like postanal subventral setae is similar in *E. topiarius* sp. n. and *E. longosetosus*; *E. africanus* has one more pair of postanal subventral setae.

The genus *Eubostrichus* was described on the basis of a single female individual of *E. filiformis* by Greeff (1869). The drawings are inaccurate and misleading. The author obviously confused anterior and posterior ends, as already pointed out by Chitwood (1936) and probably the caudal glands with what he considered

to be the pharynx. The structure he thought to be the intestine is most probably the reflexed part of the ovary. No positive identification with our species is possible. Most of the diagnostic characters (amphid, subcephalic setae, modified cervical and pre- and postanal setae) were certainly overlooked, as in the case of *E. phalacrus* (Greeff, 1869).

Supplementary to the genus diagnosis of Hopper & Cefalu (1973), the following morphological details can be added: mouth opening without lips, minute buccal cavity; cephalic sensillae consisting of three separate circles (6 + 6 + 4); short papilliform inner labial sensillae; nipple-like outer labial sensillae; elongated cephalic sensillae.

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ZUSAMMENFASSUNG

Eubostrichus topiarius sp. n., eine freilebende marine Art der Stilbonematinae (Nematoda: Desmodoridae) aus seichten, geschützten Sanden

Eubostrichus topiarius sp. n. (Nematoda: Desmodoridae) ist ein mariner, freilebender Nematode, der in seichten, geschützten Sanden der Nord-Adria zu finden ist. Es ist die fünfte Spezies dieser Gattung, die sich allgemein durch einen Bewuchs mit langen sichelförmigen oder fadenförmigen Bakterien auszeichnet. Die neue Art ist größer und anders proportionier als die nahverwandten bisher beschriebenen Arten. *Eubostrichus topiarius* sp. n. weist jeweils einen Kreis von 6 inneren und 6 äußeren labialen Sensillen auf; die 4 cephalen Sensillen sind verlängert; der erste Kreis der 8 subcephalen Setae inseriert am hinteren Rand des Amphids; die weiblichen Tiere sind größer als die Männchen und können bis zu 7 Eier im Uterus tragen.

REFERENCES

- BAUER-NEBELSICK, M., BLUMER, M., URBANCIK, W. & OTT, J. A. (1995). Glandular sensory organs of Desmodoridae (Nematoda)—an ultrastructural and phylogenetic analysis. *Invertebrate Biology* **114**, 211-219.
- BAYLIS, H. A. & DAUBNEY, R. (1926). *A synopsis of the families and genera of Nematoda*, 277 pp. London: British Museum (Natural History).
- CHITWOOD, B. G. (1936). Some marine nematodes from North Carolina. *Proceedings of the Helminthological Society of Washington* **3**, 1-16.
- COBB, N. A. (1894). *Tricoma* and other new nematode genera. *Proceedings of the Linnean Society of New South Wales* **8**, 389-421.
- COBB, N. A. (1920). One hundred new nemas (type species of 100 new genera). *Contributions to a Science of Nematology (Baltimore)* **9**, 217-343.

- COOMANS, A. (1979a). A proposal for a more precise terminology of the body regions of a nematode. *Annales de la Societe Royale Zoologique de Belgique* **108**, 115-117.
- COOMANS, A. (1979b). The anterior sensilla of nematodes. *Revue de Nématologie* **2**, 259-283.
- FILIPJEV, I. (1922) Enore sur les Nematodes libres de la Mer Noir. *Trudy Stavropol. Sel.-khoz. Institute* **1**, 88-184.
- GERLACH, S. A. (1963). Freilebende Meeresnematoden von den Malediven II. *Kieler Meeresforschungen* **19**, 67-103.
- GERLACH, S. A. (1964). Freilebende Nematoden aus dem Roten Meer. *Kieler Meeresforschungen* **20**, 18-34.
- GERLACH, S. A. & RIEMANN, F. (1973). The Bremerhaven checklist of aquatic nematodes. *Veröffentlichungen des Institutes für Meeresforschung in Bremerhaven*. Supplement **4**, 233.
- GREEFF, R. (1869). Untersuchungen über einige merkwürdige Formen des Arthropoden- und Wurmtypus. *Archiv für Naturgeschichte* **35**, 71-121.
- HOPPER, B. E. & CEFALU, R. C. (1973). Free-living marine nematodes from Biscayne Bay, Florida. V. Stilbonematinae: Contributions to the taxonomy and morphology of the genus *Eubostrichus* Greeff and related genera. *Transactions of the American Microscopical Society* **92**, 578-591.
- MAGGENTI, A. (1981). *General nematology*, pp. 106-113. Heidelberg: Springer Verlag.
- MUTHUMBI, A., VERSCHELDE, D. & VINCX, M. (1995). New Desmodoridae (Nematoda: Desmodoridae): three new species from *Cerriops* mangrove sediments (Kenya) and one related new species from the North Sea. *Cahiers de Biologie Marine* **36**, 181-195.
- NEBELSICK, M., BLUMER, M., NOVAK, R. & OTT, J. (1992). A new glandular sensory organ in *Catanema* sp. (Nematoda, Stilbonematinae). *Zoomorphology* **112**, 17-26.
- OTT, J. (1993). Lebensräume-Lebensformen: Nematoden und Bakterien. *Biologie in unserer Zeit* **2**, 127-132.
- OTT, J., BAUER-NEBELSICK, M. & NOVOTNY, V. (1995). The genus *Laxus* Cobb, 1894 (Stilbonematinae: Nematoda): Description of two new species with ectosymbiotic chemoautotrophic bacteria. *Proceedings of the Biological Society of Washington* **108**, 508-527.
- OTT, J. A. & NOVAK, R. (1989). Living at an interface: Meiofauna at the oxygen/sulfide boundary of marine sediments. In: *Reproduction, Genetics and Distribution of Marine Organisms*, pp. 415-422. Fredensborg, Denmark: Olsen & Olsen.
- OTT, J. A., NOVAK, R., SCHIEMER, F., HENTSCHEL, U., NEBELSICK, M. & POLZ, M. (1991). Tackling the sulfide gradient: A novel strategy involving marine nematodes and chemoautotrophic ectosymbionts. *P.S.Z.N.I: Marine Ecology* **12**, 261-279.
- POLZ, M. F., FELBECK, H., NOVAK, R., NEBELSICK, M. & OTT, J. A. (1992). Chemoautotrophic, sulfur-oxidizing symbiotic bacteria on marine nematodes: Morphological and biochemical characterization. *Microbial Ecology* **24**, 313-329.
- SCHIEMER, F., NOVAK, R. & OTT, J. (1990). Metabolic studies on thiotrophic free-living nematodes and their symbiotic microorganisms. *Marine Biology* **106**, 129-137.
- URBANCIK, W., BAUER-NEBELSICK, M. & OTT, J. A. (1996a). The ultrastructure of the cuticle of Nematoda. I. The body cuticle of Stilbonematinae (Adenophorea, Desmodoridae). *Zoomorphology* **116**, 51-64.
- URBANCIK, W., NOVOTNY, V. & OTT, J. A. (1996b). The ultrastructure of the cuticle of Nematoda. II. The cephalic cuticle of Stilbonematinae (Adenophorea, Desmodoridae). *Zoomorphology* **116**, 65-75.