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Leptonemella juliae sp.n. and *Leptonemella vestari* sp.n. (Stilbonematinae), two new free-living marine nematodes from a subtidal sand bottom

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Abstract

Two new species of Stilbonematinae (Desmodoridae), *Leptonemella juliae* sp.n., and *Leptonemella vestari* sp.n., are described from subtidal sand in the Bay of Veštar, Croatia, northern Adriatic Sea. *Leptonemella juliae* n.sp. is characterised by a finer striation of the cuticle and much longer cephalic setae than in previously described species of this genus. *Leptonemella vestari* n.sp. can be identified by a strongly annulated cuticle, a comparatively long pharynx, the number of cloacal setae, and the shape of the gubernaculum. Both species are covered by ectosymbiontic chemoautotrophic sulfur-oxidizing bacteria.

Key words: marine nematodes, Stilbonematinae (Desmodoridae), *Leptonemella*, Adriatic Sea.

Zusammenfassung

Leptonemella juliae sp.n. und *Leptonemella vestari* sp.n., zwei neue Arten der Stilbonematinae (Desmodoridae) aus sublitoralen Sanden der Bucht von Veštar (Kroatien, Nordadria), werden beschrieben. *Leptonemella juliae* unterscheidet sich durch eine feinere Ringelung der Kutikula und viel längere Kopfborsten von den bisher beschriebenen Arten dieser Gattung. *Leptonemella vestari* ist durch eine stark geringelte Kutikula, einen vergleichsweise langen Pharynx, die Zahl der Kloakalborsten und die Form des Gubernaculums gekennzeichnet. Beide Arten sind mit chemoautotrophen sulfid-oxidierend ektosymbiontischen Bakterien bedeckt.

Introduction

The Stilbonematinae are a subfamily within the family Desmodoridae, remarkable for the ectosymbiontic microorganisms that populate their entire body surface. This obligatory ectosymbiosis with sulfur-oxidizing bacteria, covering the host's cuticle with a species-specific coat (OTT 1995), is common for all members of the monophyletic (KAMPFER & al. 1998) taxon Stilbonematinae CHITWOOD, 1936. The nematodes feed on the microorganisms, which they supply with reduced sulfur compounds and oxygen as an electron acceptor by migrating through the chemocline (OTT & al. 1991).

Besides being characterised by the presence of the symbionts and a small buccal cavity without armature, the Stilbonematinae show considerable morphological variability in characters such as the cuticle (URBANCIK & al. 1996a,b), the amphid, or the structure of the pharynx. A conspicuous feature are numerous large epidermal glands (glandular sensory organ), which have a characteristic structure in the Stilbonematinae described by BAUER-NEBELSICK & al. (1995).

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The high variability regarding a number of taxonomically important characters complicates assessment of the relationship of the Stilbonematinae with other subfamilies of the desmodorids and between the various known genera. It is difficult to decide whether some characters have developed or have been modified/reduced independently more than once. There is, for example, a pronounced, highly developed corpus at the anterior end of the pharynx in the genera *Robbea* GERLACH, 1956 and *Catanema* COBB, 1920, whereas in other genera (*Leptonemella* COBB, 1920 and *Eubostrichus* GREEFF, 1869) there is only a slight or hardly any swelling. The reduction of the fovea of the amphid from the usual spiral shape to just a porus from which the corpus gelatum protrudes seems to have occurred independently at least three times (in the genera *Leptonemella, Stilbonema* COBB, 1920 and *Catanema*).

The two new *Leptonemella* species described in this paper occur regularly but not abundantly in the deeper layers of shallow sands in the northern Adriatic Sea.

Material & Methods

Sediment was collected in buckets from subtidal sand patches in 3-4 m depth. The animals were extracted from the sand by stirring and decanting through a 35 µm sieve following prior anaesthetisation with $MgCl_2$ isotonic to sea water for 15 min. For light microscope observations, specimens were sorted live under a stereomicroscope, fixed in 4% formaldehyde in sea water, transferred into glycerol:water 1 : 9, slowly evaporated and mounted in pure glycerol.

For scanning electron microscopy (SEM), the bacteria were removed from their host with brief ultrasonic pulses. The aposymbiontic nematodes were then fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) isotonic to sea water and postfixed in 2% OsO_4 over night. After dehydration in a graded ethanol series they were critical point dried with a POLARON E-3000, subsequently sputtered with a thin layer of gold and examined with a JEOL JSM-35CF.

Measurements and drawings were done on a Reichert Diavar, equipped with a camera lucida. A BX-50 Olympus microscope was used for the interference contrast micrographs.

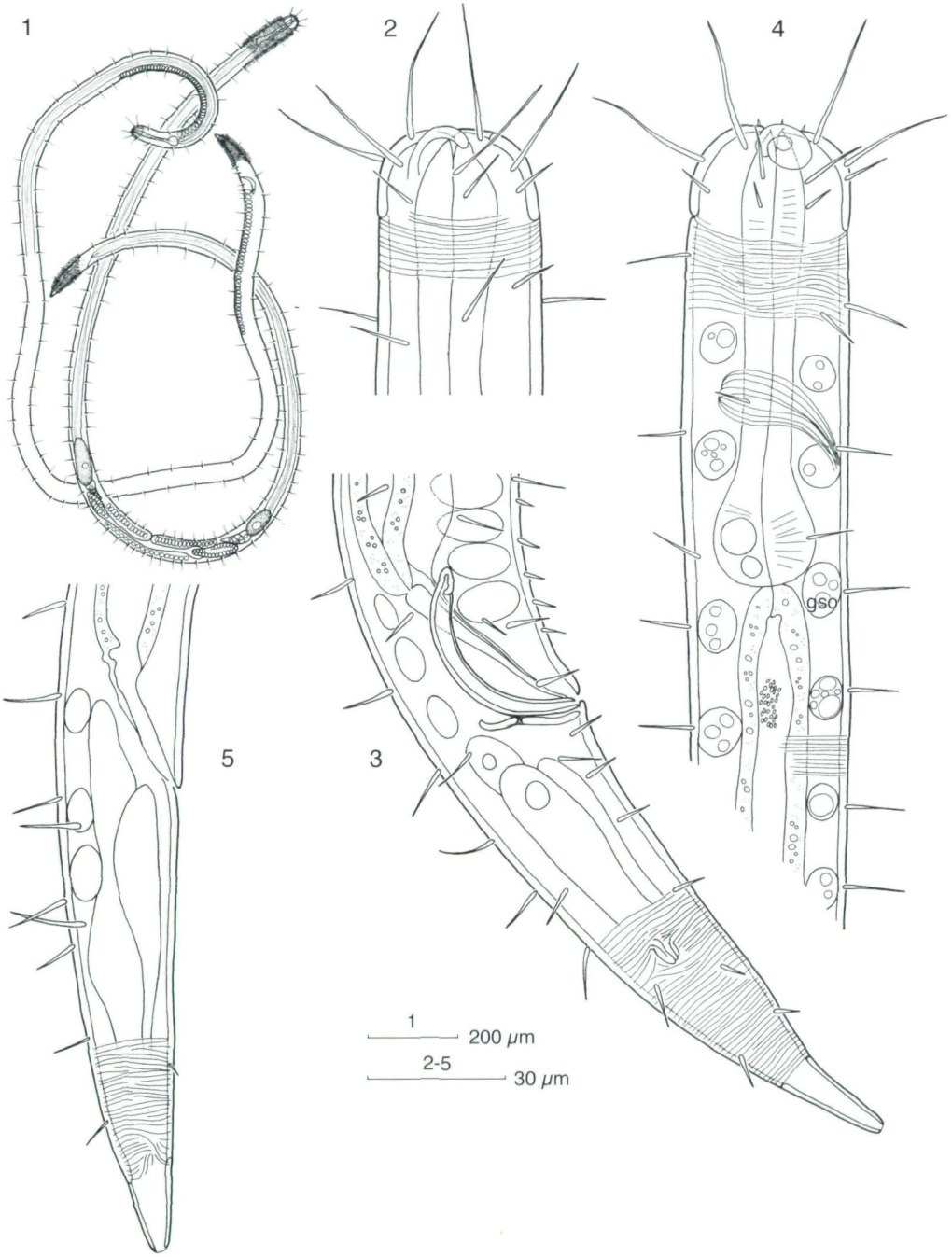
Types are deposited in the Evertebrata Varia collection of the Natural History Museum of Vienna, Austria (NHMW-EV).

Leptonemella juliae sp.n.

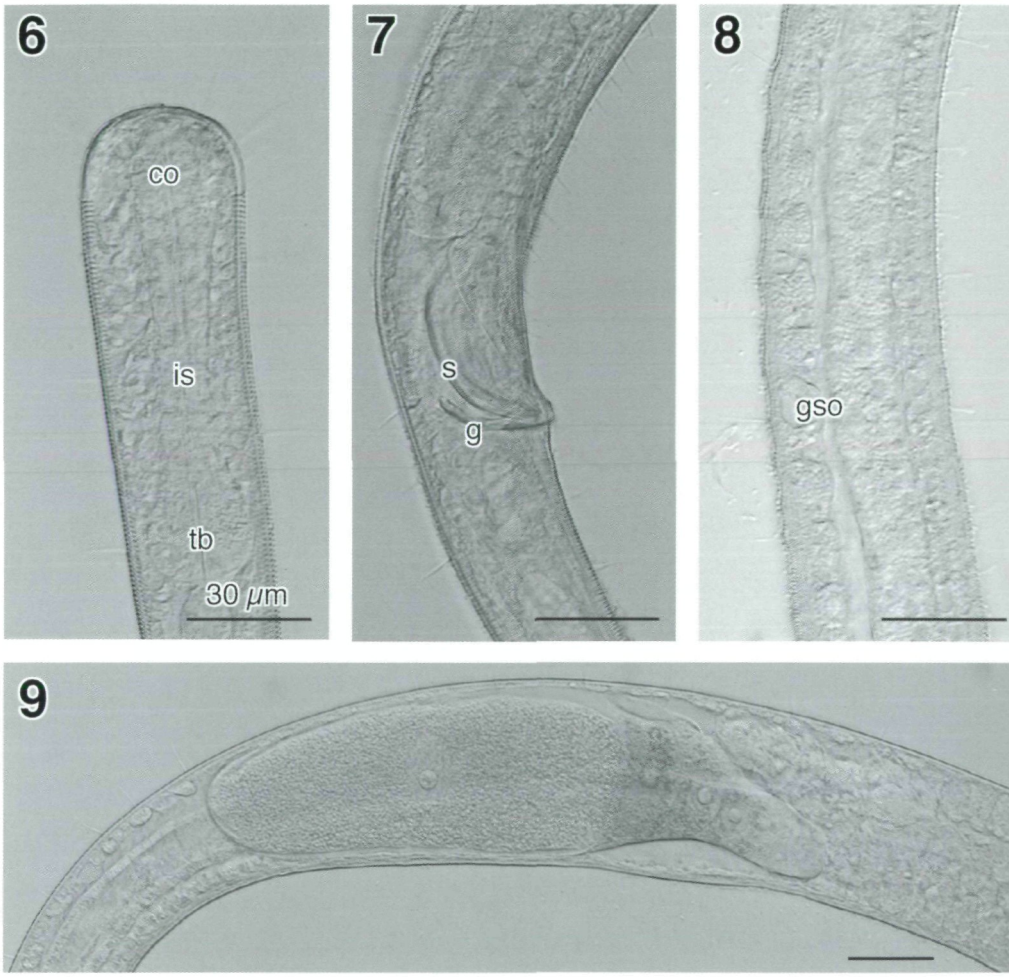
Type material: Holotype: male, L = 2784, a = 70, b = 26, c = 23, NHMW-EV 3825. **Allotype:** female, L = 2890, a = 88, b = 28, c = 26, NHMW-EV 3826. **Paratypes**: male, L = 2818, a = 70, b = 28, c = 25, NHMW-EV 3827; male (Rt Križ), L = 3350, a = 84, b = 29, c = 22, NHMW-EV 3831; female, L = 3153, a = 79, b = 29, c = 28, NHMW-EV 3828; female, L = 2952, a = 80, b = 30, c = 24, NHMW-EV 3829; female (Rt Križ), L = 3288, a = 82, b = 29, c = 22, NHMW-EV 3830; length (L) in µm.

Additional material: several specimens in the authors' collection and those used for SEM.

Type locality: Bay of Veštar, south of the town of Rovinj, Croatia (45°02'8"N, 13°41'1"E), 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.



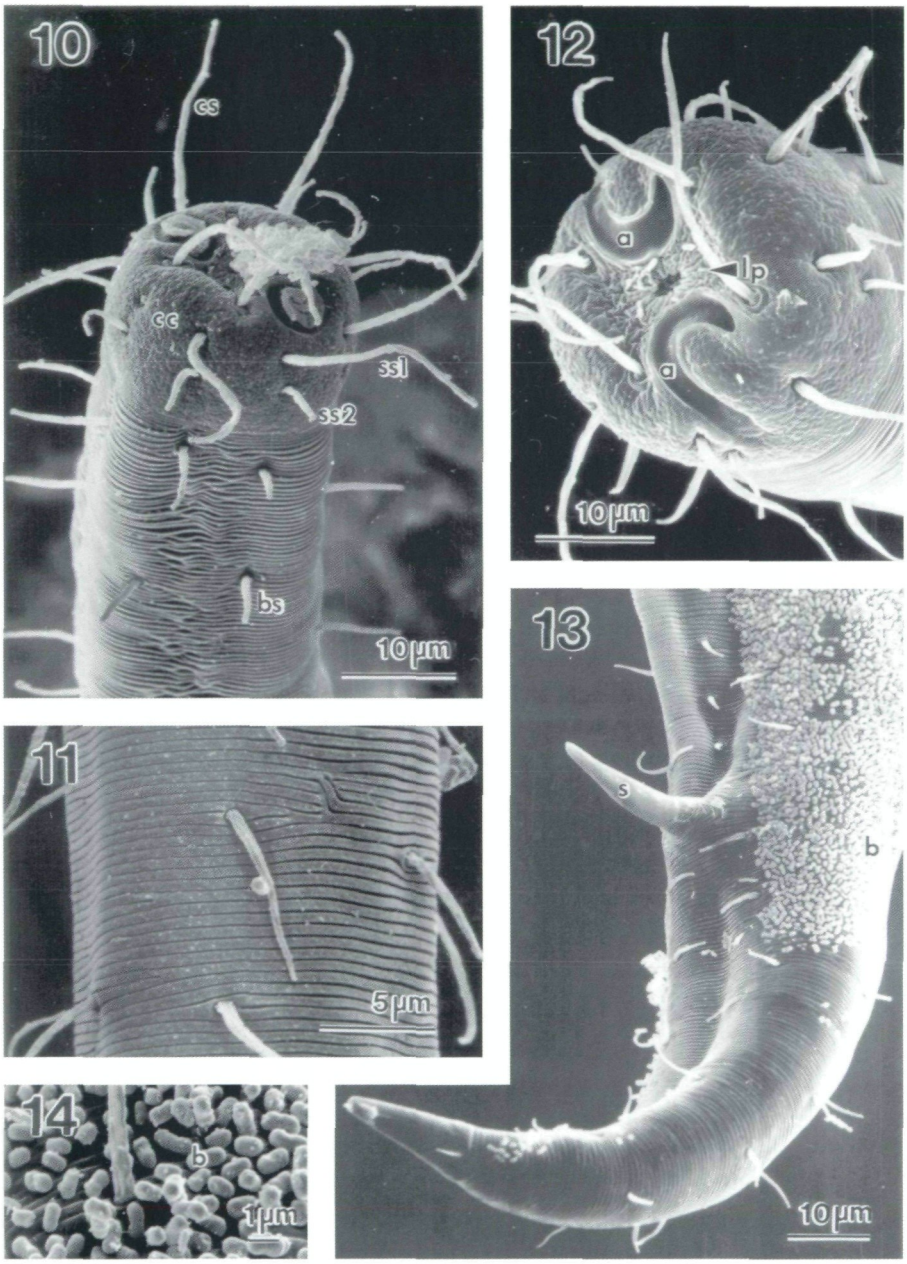
Figs 1-5: *Leptonemella juliae* sp.n. (1) total view, of holotype (NHMW-EV 3825) and allotype (NHMW-EV 3826); (2) head and (3) caudal region of holotype; (4) anterior end of allotype, showing the pharynx, glandular sense organs (gso) and cervical and postcervical setation; (5) caudal region of allotype.



Figs 6-9: *Leptonemella juliae* sp.n., interference contrast micrographs of (6) anterior region with the tri-partite pharynx consisting of corpus (co), isthmus (is), and terminal bulb (tb) and (7) cloacal region of male paratype (NHMW-EV 3831) with the spicular apparatus consisting of spiculum (s) and gubernaculum (g); (8) mid body region of holotype, showing the somatic setae which are the outlets of the spherical glandular sense organs (gso) (BAUER-NEBELSICK & al. 1995); (9) posterior gonad of a female paratype (NHMW-EV 3828), showing an egg.

In addition to the type locality, *L. juliae* was also found in coarse sand mixed with shell gravel in 8-10 m depth near Rt Križ, north of Rovinj, Croatia (45°06'8"N, 13°36'6"E), northern Adriatic Sea. The animals of this location agree in most characters with the specimens found in the Bay of Veštar. There is only one significant difference concerning the tail; with $\bar{x}$ = 149 ± 3 µm (n = 10) the specimens from Križ have a longer tail then those from Veštar ($\bar{x}$ = 120 ± 7 µm, n = 12).

Etymology: The species is dedicated to Julia Neider.



Figs 10-14: *Leptonemella juliae* sp.n., SEM photographs of (10) anterior body region of female, showing cephalic capsule (cc), circle of cephalic setae (cs), two circles of subcephalic setae (ss1+2), and somatic setae (bs); (11) midbody region, showing the annulated cuticle, note the interruption of the regular annulation; (12) frontal view of a male, showing amphids (a), mouth opening surrounded by six labial papillae (lp); (13) caudal region of a male, showing one spiculum (s) protruding from the cloaca, and a part of the bacterial coat; (14) symbiotic bacteria (b), covering the cuticle of the worm.

Description: Filiform nematodes with symbiotic bacteria, arranged as a multilayered coat of cocci (1-1.5 µm long) (Figs 13, 14). Bacteria-covered worm white in incident and dark in transmitted light. Body slender, cylindrical, tapering only slightly towards anterior end, tail conical. Solid non annulated cephalic capsule 18-20 µm long and 2 µm thick, slightly rounded and heavily cuticularised (Figs 2, 6, 10). Body cuticle finely annulated (Fig. 11), annuli 0.4-0.5 µm wide (21-24 annuli/10 µm) and curved with an overlapping portion. Annulation beginning approximately 23-26 µm from the anterior end and extending along the whole body, only the tip of the tail (18-23 µm) without annuli (Figs 3, 5, 13). The regular pattern interrupted where setae, branchings, and terminations of annuli are visible. The transition from the body cuticle to the head cuticle characterised by fusion of the anterior annuli with the posterior region of the cephalic capsule.

Large amphids (18-20 µm long) with deeply incised fovea at the anterior end of the cephalic capsule (Figs 2, 4, 12). Amphids with sexual dimorphism: spiral in females with 1.5 turns, shepherd's crook-shaped in males. Head with a circle of six very small labial papillae (1.4 µm long), surrounding the membranous buccal field (Fig. 12). A circle of four long cephalic setae (30-32 µm), followed by two circles of eight subcephalic setae, each. The first subcephalic circle situated at the level of the posterior margin of the amphid, setae 27-29 µm long, the second circle situated a short distance posterior with 9-13 µm long setae (Figs 2, 10). In both circles, the sublateral setae more posterior than the submedian setae. Somatic setae 10-13 µm long, arranged in six rows (except in the cervical region, where eight rows can be seen) over most of the body. Males with a single row of 5-6 small setae (approx. 5 µm) anterior to the cloaca (Fig. 3). Three caudal glands present (Figs 3, 5).

Buccal cavity small and tubular, tri-partite pharynx consisting of a corpus (30-35 µm long, 17µm wide), an narrow isthmus (40-45 µm long, 13 µm wide) and a terminal bulb (21-26 µm long, 24 µm wide) (Figs 4, 6). The nerve ring surrounds the isthmus 31-35 µm from the anterior end of the pharynx. Glandular sense organs present, forming rows (Figs 4, 8).

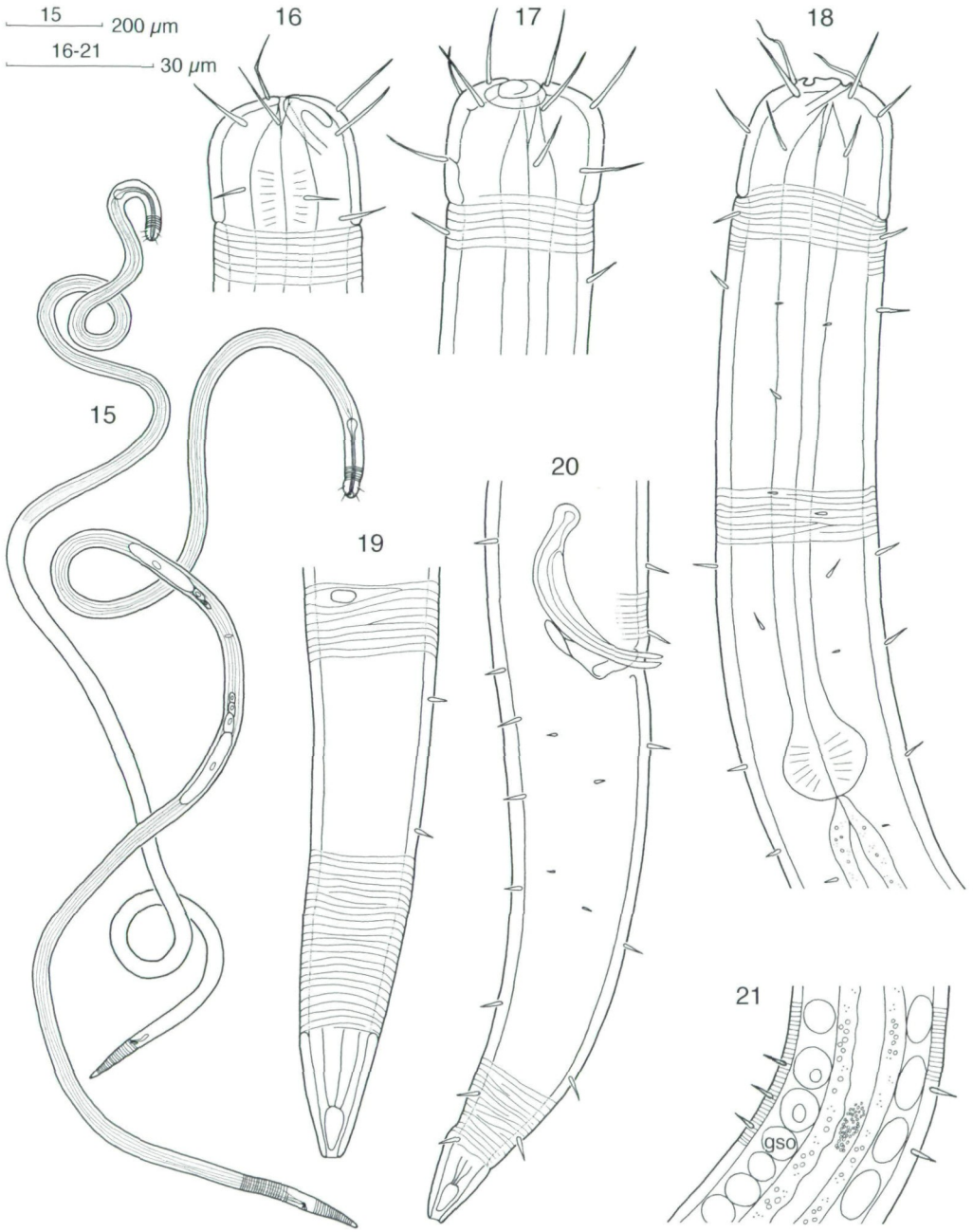
Male reproductive system monorchic, with outstreched testis. Testis starts at 30-35% of the body length, length 500-600 µm. Vas deferens filled with small granules. Spicula weakly cephalate proximally, arcuate, length 44-48 µm (chord) or 53-56 µm (arch), no velum visible. Gubernaculum consisting of two strongly cuticularised pieces joined by a membranous part, parallel to spicules, length 26-28 µm (Figs 3, 7). Supplements absent.

Female with two opposed antidromous ovaries of equal length, uteri containing large eggs (up to 143 µm long) (Fig. 9). Vulva a small transverse slit at 50% of body length.

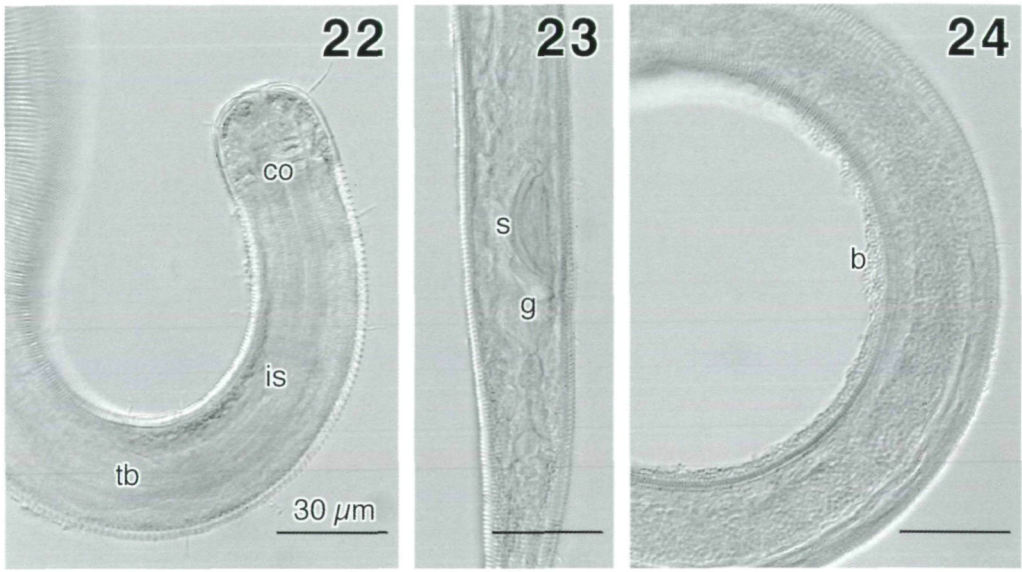
Leptonemella vestari sp.n.

Type material: Holotype: male, L = 3220, a = 80.5, b = 26, c = 27, NHMW-EV 3832; **Allotype:** female, L = 3556, a = 102, b = 30, c = 25, NHMW-EV 3833. **Paratypes**: male, L = 2952, a = 84, b = 20, c = 24, NHMW-EV 3834; male, L = 2751, a = 91, b = 20, c = 21, NHMW-EV 3835; length (L) in µm.

Additional material: several specimens in the authors' collection and those used for SEM.



Figs 15-21: *Leptonemella vestari* sp.n. (15) total view of holotype (NHMW-EV 3832) and allotype (NHMW-EV 3833); (16) head of male paratype (NHMW-EV 3835) with additional seta (as); (17) head of allotype; (18) anterior end and pharyngeal region of paratype (NHMW-EV 3834), showing cephalic capsule and pharynx; (19) caudal region of allotype; (20) caudal region of holotype with spicular apparatus and postcloacal setae; (21) midbody region, showing glandular sensory organs (gso) and intestine with clusters of granular material.



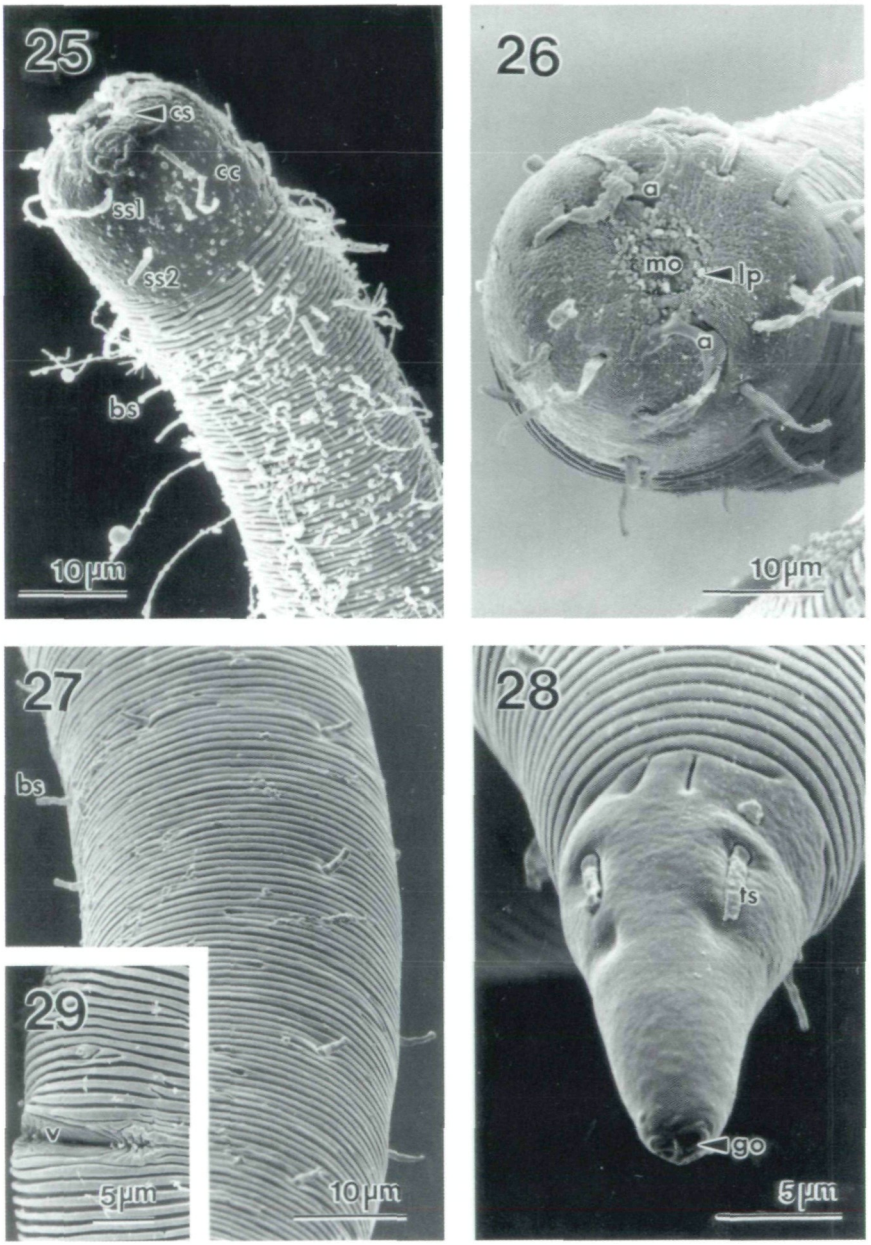
Figs 22-24: *Leptonemella vestari* sp.n., interference contrast micrographs of holotype (NHMW-EV 3832); (22) anterior region, with the tri-partite pharynx consisting of corpus (co), isthmus (is), and terminal bulb (tb); (23) cloacal region with the spicular apparatus consisting of spiculum (s) and gubernaculum (g), (24) midbody region, showing symbiotic bacteria (b).

Type locality: Bay of Veštar, south of the town of Rovinj, Croatia (45°02'8"N, 13°41'1"E), 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, rare compared to *L. juliae* sp.n..

Etymology: The species is named after the Bay of Veštar, the type locality.

Description: Body long, cylindrical, completely covered by a multilayered coat of cocci (1.5-1.8 µm long) (Fig. 24) except for head and tip of the conical tail (Figs 15, 19). Bacteria white in incident and dark in transmitted light. Cuticle strongly annulated, annuli 0.7-0.8 µm wide (13-15 annuli/10µm). Solid non annulated cephalic capsule 26-30 µm long and 2 µm thick, slightly rounded and heavily cuticularised.

Large amphids (24-26 µm long) with deeply incised fovea close to the anterior end of the cephalic capsule. Amphids with sexual dimorphism: spiral in females with 1.5 turns, loop-shaped in males (Figs 16, 17). Mouth opening surrounded by circle of six minute labial papillae (1 µm long), only seen in *en-face* view (Fig. 26). Arrangement of cephalic setae similar to that in *L. juliae* sp.n. but setae shorter and sometimes additional single setae posterior to the second circle of subcephalic setae (at the termination cephalic capsule and annulated body cuticle) are seen (Figs 16, 25). Four cephalic setae (15-17 µm), a circle of eight subcephalic setae (10-13 µm) at the level of the amphid. The second circle of eight subcephalic setae more posterior with 14-16 µm long setae. Short somatic setae, 7 µm long, in six rows (except in the cervical region, where eight rows can be seen) over most of the body (Fig. 27). Females without anal setae. Males with 3



Figs 25-29: *Leptonemella vestari* sp.n., SEM photographs of (25) anterior body region of a female, showing cephalic capsule with a circle of cephalic setae (cs), two circles of subcephalic setae (ss1+2), and somatic setae (bs); (26) *en-face* view of anterior end of male, showing amphids (a), mouth opening (mo) surrounded by six labial papillae (lp) and circles of cephalic and subcephalic setae: (27) midbody region, showing the annulation and rows of somatic setae (bs); (28) caudal region of female, showing the tip of the tail with terminal setae (ts) and caudal gland openings (go); (29) midbody region, showing the vulva (v).

short (5.5 µm long) precloacal setae and 4 postcloacal setae of the same size (Fig. 20). A pair of small, stout terminal setae (5 µm) on the tail tip (Fig. 28). The tail contains three caudal glands, which open separately at the terminal spinneret (Fig. 28).

Buccal cavity small and tubular, tri-partite pharynx consisting of a slightly swollen corpus (27-30 µm long, 13 µm wide), a very long, narrow isthmus (92-99 µm long, 9 µm wide) and a spherical terminal bulb (19-24 µm long, 20 µm wide) (Figs 18, 22). Nerve ring at approximately two-thirds of the length of the pharynx.

Single outstretched testis in males, starting at 25-30% of the body length, length 350-370 µm. Vas deferens filled with small granules. Spicula slightly cephalate proximally, arcuate, length 33-37 µm (chord) or 43-52 µm (arch), with a velum. Gubernaculum well developed, consisting of a proximal and a distal piece joined by a plate and forming an angel, length 16-19 µm (Figs 20, 23). Supplements absent.

Female reproductive system didelphic with two opposed antidromous ovaries (Fig. 15). Vulva a small transverse slit at 51% of body length (Fig. 29).

Discussion

The genus *Leptonemella* COBB, 1920, revised by GERLACH (1950) and by BOUCHER (1975), was established with the type species *Leptonemella cincta* COBB, 1920 collected from a sandy beach in Miami, Florida. GERLACH & RIEMANN (1973) recognised six species in the genus, but BOUCHER (1975) denoted *L. froeyensis* (ALLGEN, 1946) and *L. parabullata* (ALLGEN, 1929) as *species inquirendae* because of the incomplete descriptions. BOUCHER (1975) compared five species in his key, including *Leptonemella granulosa* described in that paper. PLATT & WARWICK (1988), not following the opinion of BOUCHER (1975), recognised seven species. We consider the following species as belonging to *Leptonemella*: *L. aphanothecae* GERLACH, 1950; *L. cincta* COBB, 1920; *L. gorgo* GERLACH, 1950 and *L. granulosa* BOUCHER, 1975. On the basis of material collected close to the type locality in the Maldive Islands we place *Leptonemella sigma* GERLACH, 1963 in the genus *Laxus* using the characters of the cephalic capsule as criteria, as defined in OTT & al. (1995) and URBANCIK & al. (1996 b).

Leptonemella cincta is distinguished from all other *Leptonemella* species by its slit-like amphid. *Leptonemella juliae* differs from *L. aphanothecae*, *L. gorgo* and *L. granulosa* in body size and proportions, especially the short pharynx, and the long somatic setae. The four cephalic setae of *L. juliae* as well as the eight subcephalic setae from the first circle are the longest measured setae from all known *Leptonemella* species. *L. juliae* is much thicker than *L. granulosa*. Moreover the somatic setae of *L. juliae* are twice as long as these setae from *L. granulosa*.

Leptonemella vestari differs from *L. aphanothecae* and *L. granulosa* in body size and proportions, and in the length of the setae in the second subcephalic circle, which are equal to that of the first. In this respect *L. vestari* is similar to *L. gorgo*. It differs, however, from the latter species in the shape of the gubernaculum, which is much more complex in *L. vestari*, and in the number of pre- and postcloacal setae in the midventral line of males, with *L. gorgo* having 5 precloacal and 8 postcloacal setae, *L. vestari* 3 and 4 respectively (Table 1).

Tab. 1. Differental diagnosis using selected features. * Cobb (1920); + Gerlach (1964); # personal observation on paratype R3774 AB (Muséum National d´Histoire Naturelle, Boucher 1975)

species	*L. aphanothecae*	*L. cincta*	*L. gorgo*	*L. granulosa*	*L. juliae*	*L. vestari*
amphid (female)	spiral	slit*,+	spiral	spiral	spiral	spiral
cephalic circle	short (15-20 µm)	medium (27 µm)+	medium (20-27 µm)	short (13-17 µm)	long (30-31 µm)	short (15-17 µm)
subcephalic circles	first circle longer (13-17 µm) than second (8-12 µm)	first circle longer (25 µm) than second (10 µm)+	nearly equal (first: 16-19 µm, second: 16-19 µm at least)	first circle longer (11-15 µm) than second (5-6 µm)	first circle longer (27-29 µm) than second (9-13 µm)	nearly equal (first: 10-13 µm, second: 14-16 µm)
somatic setae	short to medium (7-9 µm)	without*	short (7 µm)	short (3-4 µm)#	long (10-13 µm)	short (7 µm)
no. of pre- / postcloacal setae	0 / 0	0 / 0	5 / 8	3# / 6	5-6 / 0	3 / 4
gubernaculum	medium (15-20 µm); paired, median joined, with curved slender apophysis	medium (24 µm)+; single, one piece, slender, rod-shaped, parallel to spicules	short (17 µm); one rod-shaped piece without apophysis, parallel to spicules	short (15-16 µm); as open groove with two parallel apophyses parallel to spicules	long (26-28 µm); two pieces joined by a membranous part, parallel to spicules, without apophysis	short to medium (16-19 µm); two pieces joined by a plate, forming an angel, without apophysis

At present it is premature to give a revised definition of the genus *Leptonemella* and to formulate a new key. Several species await their description (F. Riemann, pers. comm., J.A. Ott, pers. obs.). The multilayered bacterial coat, the structure of body cuticle and cephalic cuticle - the latter with a dominant median zone (URBANCIK & al. 1996b) - the simple construction of the tri-partite pharynx, and - in those species where the amphid has not been reduced to a pore or slit - the sexual dimorphism of this organ can be used as distinctive characters for *Leptonemella*.

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References

BAUER-NEBELSICK, M., BLUMER, M., URBANCIK, W. & OTT, J.A. 1995: The glandular sensory organ of Desmodoridae (Nematoda) - an ultrastructural and phylogenetic analysis. – Invertebrate Biology 114(3): 211-219.

BOUCHER, G. 1975: Nématodes des sables fines infralittoraux de la Pierre Noire (Manche occidentale). I. Desmodorida. – Bulletin du Muséum national d'Histoire naturelle, Paris 3e série, no 285, Zoologie 195: 101-128.

COBB, N.A. 1920: One hundred new nemas (type species of new genera). – Contribution to a science of nematology, Baltimore 9: 217-343.

GERLACH, S.A. 1950: Über einige Nematoden aus der Familie der Desmodoriden. – Zoologischer Anzeiger, Neue Ergebnisse und Probleme der Zoologie (Klatt-Festschrift), Leipzig 1950 (Ergänzungsband zu Bd. 45): 178-198.

GERLACH, S.A. 1964: Freilebende Nematoden aus dem Roten Meer. – Kieler Meeresforschungen 20 (Sonderheft): 18-34

GERLACH, S.A. & RIEMANN, F. 1973: The Bremerhaven checklist of aquatic nematodes. A catalogue of Nematoda Adenophorea excluding the Dorylaimida. – Veröffentlichungen des Institutes für Meeresforschung in Bremerhaven, Suppl.4, Part 1 (1973) and Part 2 (1974): 736 pp.

KAMPFER, S., STURMBAUER, C. & OTT, J.A. 1998: Phylogenetic analysis of rDNA sequences from adenophorean nematodes and implications for the Adenophorea-Secernentea controversy. – Invertebrate Biolology 117(1): 29-36.

OTT, J.A. 1995: Sulfide symbioses in shallow sands. – In: ELEFTHERIOU, A., ANSELL, A.D. & SMITH, C.H.J. (Eds.): Biology and ecology of shallow coastal waters. Fredensborg, Olsen & Olsen: 143-147.

OTT, J.A., BAUER-NEBELSICK, M., NOVOTNY, V. 1995: The genus *Laxus* COBB, 1894: Description of two new species with ectosymbiontic chemoautotrophic bacteria. – Proceedings of the Biological Society of Washington 108(3): 508-527

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. – Pubblicazioni della Stazione Zoologica di Napoli I: Marine Ecology 12(3): 261-279.

PLATT, H.M., WARWICK, R.M. 1988: Free living marine nematodes. Part II: British chromadorids. Pictorial key to the world genera and notes for the identification of British species. – In: KERMACK, D.M. & BARNES, R.S.K. (Eds.): Synopsis of the British Fauna (New Series) No.38: 502 pp.

URBANCIK, W., BAUER-NEBELSICK, M. & OTT, J.A. 1996a: The ultrastructure of the cuticle of nematoda. I. The body cuticle within the 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.