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Bernardius lineatus gen. n., sp. n. (Enoplida: Enchelidiidae), a remarkable nematode with ornamented cuticle from a Brazilian tropical sandy beach

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Summary – *Bernardius lineatus* gen. n., sp. n. is described from a sandy beach at Tamandaré Bay, Brazil. It possesses the main characteristics of the family Enchelidiidae (Nematoda: Enoplida), but is unique by the presence of a body cuticle ornamented with longitudinal ridges formed by small rods, an entirely different character to all other members of the order Enoplida. The new genus is also characterised by a long, subdivided, buccal cavity with two subventral teeth and numerous denticles and the arrangement of the anterior sensilla in two crowns, the anterior crown being papilliform and the posterior crown consisting of six external labial and four cephalic sensilla, setiform. The relationships of the genera within the Enchelidiidae are discussed and an emended family diagnosis provided.

Keywords – description, morphology, morphometrics, new genus, new species, phylogeny, SEM, taxonomy.

According to Lorenzen (1981, 1994) the order Enoplida Filipjev, 1929 is characterised by two synapomorphies: the presence of metanemes and a smooth or only weakly striated cuticle. The families Enchelidiidae Filipjev, 1918 and Oncholaimidae Filipjev, 1916 together belong to the superfamily Oncholaimoidea (Oncholaimina). According to Smol and Coomans (2006), one of the main characters to distinguish these two families is the pharynx which gradually enlarges posteriorly (sometimes crenate or with many bulbs) in the former but is cylindrical and never crenate in the latter. The family Enchelidiidae comprises 17 valid genera classified into two subfamilies; the Enchelidiinae Filipjev, 1918 and the Aronematinae Fadeeva & Belogurov, 1988. The present work describes *Bernardius lineatus* gen. n., sp. n. within the Enchelidiidae.

Materials and methods

Nematodes were collected in Tamandaré Bay, 110 km south of Recife, Pernambuco, Brazil (08°45′58″N, 35°05′96″W). This sandy beach is characterised by quartz sediments composed of fine to medium grain size and the subtidal area presents coral reef formations running parallel to the coast (Maida & Ferreira, 1997). The tidal amplitude in the area is *ca* 2.5 m and the cycle is semidiurnal. The climate is hot and humid and corresponds to type Aws according to the Köppen system. The region has two seasons with a dry season extending from September to February and a rainy season from March to August, half of the annual rainfall occurring between May and July (SEMA – Science, Technology and Environment Department of Pernambuco State). The daylight period in the area is approximately 12-13 h/day.

Samples were collected in May, July, September and November 1991 over a period of 24 h at 1 h intervals. At each sampling, six meiofauna cores were collected using a PVC tube (3.7 cm inner diam. and 10 cm deep) and fixed immediately in 4% formaldehyde solution. In the laboratory the samples were extracted using routine methods for meiofauna (wet sieving and manual centrifu-

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gation) as suggested by Elmgren (1973) and the nematodes were counted and sorted using Dollfus plates under a stereoscopic microscope.

Individuals of *B. lineatus* gen. n., sp. n. were picked out, processed and mounted on permanent Cobb slides (Cobb, 1917) using the method of De Grisse (1969). Measurements were done with a *camera lucida* on an Olympus CX31. Drawings were done using a Reichert Polyvar light-microscope. Scanning electron-microscopic pictures (SEM) were taken with a Jeol JSM.

Family Enchelidiidae Filipjev, 1918

DIAGNOSIS (emended after Smol & Coomans, 2006)

Oncholaimoidea. Cuticle smooth or transversely striated and ornamented with longitudinal ridges (*Bernardius*). Buccal cavity with one, two or three unequal teeth, with or without rows of denticles. When two ventrosublateral teeth present, right tooth often larger, exceptionally either left or right ventrosublateral tooth can be larger (*e.g.*, in *Calyptronema*). Sexually dimorphism in buccal cavity and pharynx present or absent (reduced in males of *Calyptronema*, *Polygastrophora* and *Symplocostoma*). Pharynx crenate, gradually enlarging posteriorly or possessing several bulbs in females and in other genera. Amphidial fovea either non-spiral or dorsally spiral; possibly sexually dimorphic. Females didelphic-amphidelphic (monodelphic-opisthodelphic only in *Calyptronema sabulicola*). Demanian system always absent. Males with or without precloacal supplements, papilliform or winged.

TYPE GENUS

Enchelidium Ehrenberg, 1836 (for a discussion of the type genus see Lorenzen, 1994 and Smol and Coomans, 2006).

OTHER GENERA

Abelbolla Huang & Zhang, 2004
Aronema Fadeeva & Belogurov, 1988
Bathyeurystomina Lambshead & Platt, 1979
Belbolla Andrássy, 1973
Bernardius gen. n.
Calyptronema Marion, 1870
Ditlevsenella Filipjev, 1927
Eurystomina Filipjev, 1921
Ledovitia Filipjev, 1927
Lyranema Timm, 1961
Megeurystomina Luc & De Coninck, 1959
Pareurystomina Micoletzky, 1930
Polygastrophora de Man, 1922
Symplocostoma Bastian, 1865
Symplocostomella Micoletzky, 1930
Thoonchus Cobb, 1920

Bernardius[*] gen. n.

DIAGNOSIS

Body elongate, tapering anteriorly. Cuticle with fine transverse striae except at level of head and ornamented with longitudinal ridges starting just posterior to amphidial fovea and running onto tail. Anterior sensilla arranged according to pattern $6 + (6 + 4)$, with anteriormost circle papilliform, other sensilla setiform with four cephalic setae clearly shorter than external labial setae. Lips fused into a wide oral ring ornamented with bars (only visible in SEM). Buccal cavity with cheilostom and gymnostom broad and cylindrical, pharyngostom tubiform. Cheilostom thin walled in LM, gymnostom and pharyngostom with well sclerotised walls (rhabdia). Two hollow, bifid teeth present, anterior one larger; both teeth inserted close to mid-ventral line. In SEM numerous additional denticles visible at transition of cheilostom and gymnostom. Pharynx gradually enlarging posteriorly, surrounded by nerve ring at mid-region. Amphidial apertures located posterior to buccal cavity, sexually dimorphic in shape with fovea pocket-shaped in male and an incomplete, broad, dorsal spiral in female and juvenile. Male reproductive system with single anterior outstretched testis situated on right side of intestine. Spicules cephalated, gubernaculum with dorsal apophyses. Minute precloacal papillae present. Female reproductive system didelphic, ovaries antidromously reflexed, located on right side of intestine. Tail terminus with sclerotised spinneret.

RELATIONSHIPS

Bernardius gen. n. is easily differentiated from all other taxa of the Enchelidiidae by having the cuticle ornamented with longitudinal ridges (visible at low magnification) and not smooth (Table 1). *Bernardius* gen. n. is most similar to the genus *Aronema* Fadeeva & Belogurov, 1988. Both possess a comparable structure of buccal cavity and

[*] Genus named in honour of Dr Bernard Genevois, husband of Dr Verônica Fonsêca-Genevois.

Table 1. *Comparison of major morphological features of* Bernardius *gen. n. and other genera within the Enchelidiidae.*

Reference	Cuticle	Sexual dimorphism	Teeth	Pharynx	Amphids	Ocelli	Tail shape	Caudal glands	Gubernaculum	Supplements
Abellbola Huang & Zhang, 2004	smooth	–	3 with the right ventro-lateral largest	cylindrical, without bulbs	not known	absent	long conical cylindrical	absent	with apophysis	2 winged or absent
Aronema Fadeeva & Belogurov, 1988	smooth	amphid shape	2 subventral + denticles	cylindrical, without bulbs	M: loop-shaped F: pocket-shaped	absent	short conical	present	with apophysis	3-4 weak papillae
Bathyeurystomina Lambshead & Platt, 1979	smooth	–	1 right ventro-sublateral* + denticles	cylindrical, without bulbs	faint, crescentic	absent	conical-flagellate	absent	with well developed apophysis	2 sclerotised tubes with cap
Belbolla Andrássy, 1973	smooth	–	3 with the ventrosublateral largest	7-10 bulbs	dorsally spiral	absent	elongate conical	present	absent or with apophysis	winged (partly)
Calyptronema Marion, 1870	smooth	anterior end and buccal cavity	F: 3 with right or left ventrosublateral largest + denticles M: absent	cylindrical, without bulbs	non-spiral	present or absent	short or elongate conical	present	without apophysis	many, papilliform
Ditlevsenella Filipjev, 1927	smooth	–	3 with left or right ventrosublateral largest	cylindrical, without bulbs	dorsally spiral	absent	short conical or clavate	present	with apophysis	6-8 papillae
Eurystomina Filipjev, 1921	smooth	–	3 with right ventrosublateral largest + denticles	with bulbs	dorsally spiral	present or absent	short conical	present	with or without apophysis	2 winged
Ledovitia Filipjev, 1927	smooth	–	3 with right ventrosublateral largest + denticles	cylindrical, without bulbs	oval	absent	long conical or cylindrical	present	absent or without apophysis	2 winged (weakly developed)
Lyranema Timm, 1961	smooth	–	mandibles lyre-shaped	cylindrical, without bulbs	bowl-shaped	absent	conical cylindrical	not observed	?	?
Megeurystomina Luc & De Coninck, 1959	smooth	–	1 right ventrosublateral tooth+denticles	cylindrical, without bulbs	dorsally spiral	absent	conical digitate	present	?	?

Table 1. *(Continued).*

Reference	Cuticle	Sexual dimorphism	Teeth	Pharynx	Amphids	Ocelli	Tail shape	Caudal glands	Gubernaculum	Supplements
Pareurystomina Micoletzky, 1930	smooth	–	3 with the ventro-sublateral largest + denticles	cylindrical, without bulbs	dorsally spiral	absent	conical, sharp tip	absent	with or without apophysis	2 winged
Polygastrophora de Man, 1922	smooth	–	1 large ventro-sublateral and 2 smaller teeth	several bulbs	dorsally spiral	present	conical cylindrical	present	with or without apophysis	minute papillae or setae
Symplocostoma Bastian, 1865	smooth	anterior end and buccal cavity	F: 3 with the ventro-sublateral largest + denticles M: absent	cylindrical, without bulbs	M: pocket-shaped F: not observed	present	conical cylindrical	present	with or without apophysis	papilliform
Symplocostomella Micoletzky, 1930	smooth	anterior end and buccal cavity	F: 3 with the ventro-sublateral largest + denticles M: absent	cylindrical, without bulbs	M: pocket-shaped F: not observed	absent	conical cylindrical	present	with or without apophysis	papilliform
Thoonchus Cobb, 1920	smooth	–	3 with right ventro-sublateral largest + denticles	cylindrical, without bulbs	large	absent	short cylindrical	present	with expanded corpus and with or without apophysis	1-2 papilliform
Bernardius gen. n.	with longitudinal ridges	amphid shape	2 subventral + ring of denticles	cylindrical, without bulbs	M: pocket-shaped F: loop-shaped	absent	short conical	present	with apophysis	2 papilliform

? = Males unknown, * = two (?) small projections (teeth) are located opposite the large tooth (Lambshead & Platt, 1979). M = male, F = female.

armature, sharing the shape and posterior position of the amphidial fovea and have similar sexual dimorphism in shape. They also show similarities in the structure of the pharynx (cylindrical without bulbs), the long cervical setae, the shape of the tail (short conical) and presence of a gubernaculum with apophyses. *Bernardius* gen. n. differs in the structure of the teeth, *i.e.*, two bifid teeth (a large anterior tooth and a short posterior one) positioned close to the mid-ventral line whereas *Aronema crinita* Fadeeva & Belogurov, 1988 was described as having one long subventral tooth with an anterior guiding part. This armature is herein interpreted as referring to a long posterior and a strong anterior tooth, similar that for *A. setifera* (Gerlach, 1953) which has a long, slender, left subventral styletiform tooth inserted at the base of the buccal cavity and a strong, short, right subventral tooth. Both *Aronema* species also possess a small dorsal tooth at the level of the denticles but this is absent in the new genus.

TYPE AND ONLY SPECIES

Bernardius lineatus gen. n., sp. n.

Bernardius lineatus* **gen. n., sp. n.**
(Figs 1, 2)

MEASUREMENTS

See Table 2.

DESCRIPTION

Male

Body slender, distinctly tapering in pharyngeal region; head diam. 20-22% of body diam. at base of pharynx. Cuticle with fine transverse striations (except at level of head), 14-16 longitudinal ridges starting just posterior to amphidial fovea and running onto tail. Each longitudinal ridge composed of a row of rods. In SEM each bar extending over *ca* seven fine transverse striations. Anterior sensilla arranged according to pattern 6 + (6 + 4), anteriormost circle papilliform and postlabial, external labial and cephalic sensilla setiform; four cephalic setae clearly shorter than external labial sensilla. Set of two 11 μm long cervical setae present at level of amphidial fovea, two additional sets of postfoveal cervical setae present, anteriormost set composed of four 12 μm long setae, most posterior one of two 16 μm long setae. Other somatic setae also present along body but frequently broken or missing. Amphidial fovea pocket-shaped with posterior border of aperture thickened. Lips fused, anterior part forming ring around wide mouth opening. Buccal cavity elongate, subdivided into cheilostom, gymnostom and pharyngostom. Cheilostom and gymnostom broad, cylindrical, pharyngostom tubiform with well sclerotised walls (rhabdia). Two hollow, bifid, teeth present, a large anterior and a shorter posterior one, both teeth in same position close to mid-ventral line. In SEM, numerous additional denticles visible at transition of cheilostom-gymnostom. Pharynx gradually enlarging posteriorly. Nerve ring located at mid-pharynx level. Neither secretory-excretory gland nor outlet visible. Reproductive system with single outstretched anterior testis located to right of intestine. Spicules cephalated, equal in shape and size, *ca* one anal body diam. long. Gubernaculum short with dorsal apophyses, surrounding retracted spicule at about midpoint. Two minute precloacal papillae located 103 and 189 μm anterior to cloacal opening; quite difficult to observe in some males. Two adcloacal pairs of setae, one precloacal (6 μm long) and one postcloacal (11 μm long). Tail 2.0 (1.9-2.7) anal body diam. long, terminal sclerotised, spinneret present. Three caudal glands extending anteriorly beyond cloacal aperture. Tail tip with four subterminal setae of same length (4 μm).

Female

Similar to male in most morphological aspects, including cuticle. Striking sexual dimorphism in amphid structure with amphidial fovea a derived spiral visible as an incomplete, broad, dorsal spiral- or loop-shape. Reproductive system didelphic, ovaries antidromously reflexed, both branches located on right side of intestine. Vulva short, slightly posterior to mid-body, surrounded by weakly developed constrictor muscles; no common ovejector opposite vagina.

Intersex

One gravid (one uterus with an egg) female specimen with normally developed female reproductive system also showed male characteristics, *i.e.*, two spicules. However, spicules and gubernaculum much shorter than in normal males, respectively 20 *vs* 32-38 μm and 7 *vs* 17-27 μm. Neither testis nor precloacal papillae observed.

Juveniles

Similar to adults in most morphological aspects, including buccal armature. Amphidial fovea in form of incom-

* Specific epithet refers to the longitudinal ridges on the cuticle.

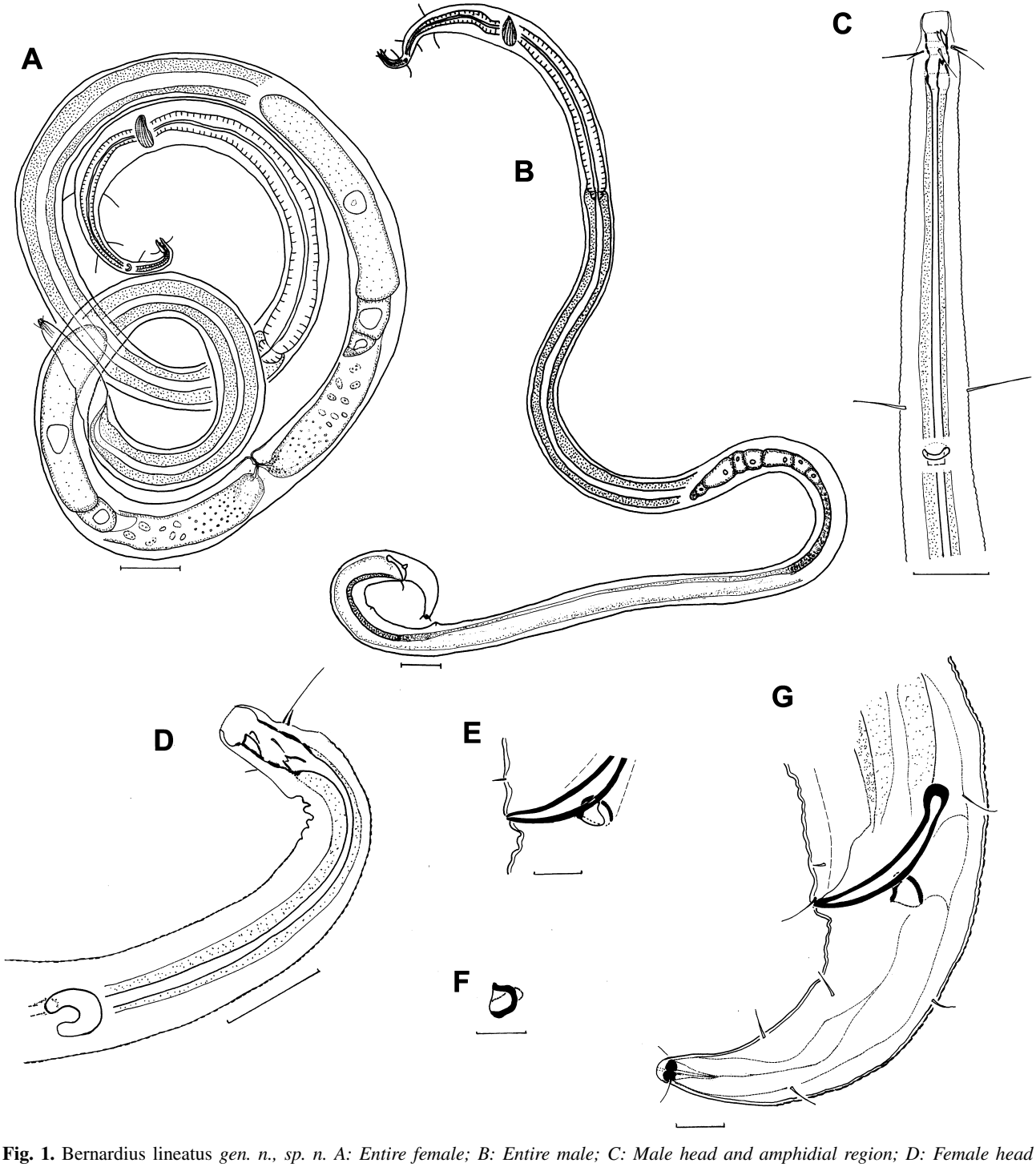

Fig. 1. Bernardius lineatus *gen. n., sp. n. A: Entire female; B: Entire male; C: Male head and amphidial region; D: Female head and amphidial region; E: Copulatory apparatus; F: Detail of gubernaculum; G: Male tail region. (Scale bars: A, B = 50 μm; C-G = 10 μm.)*

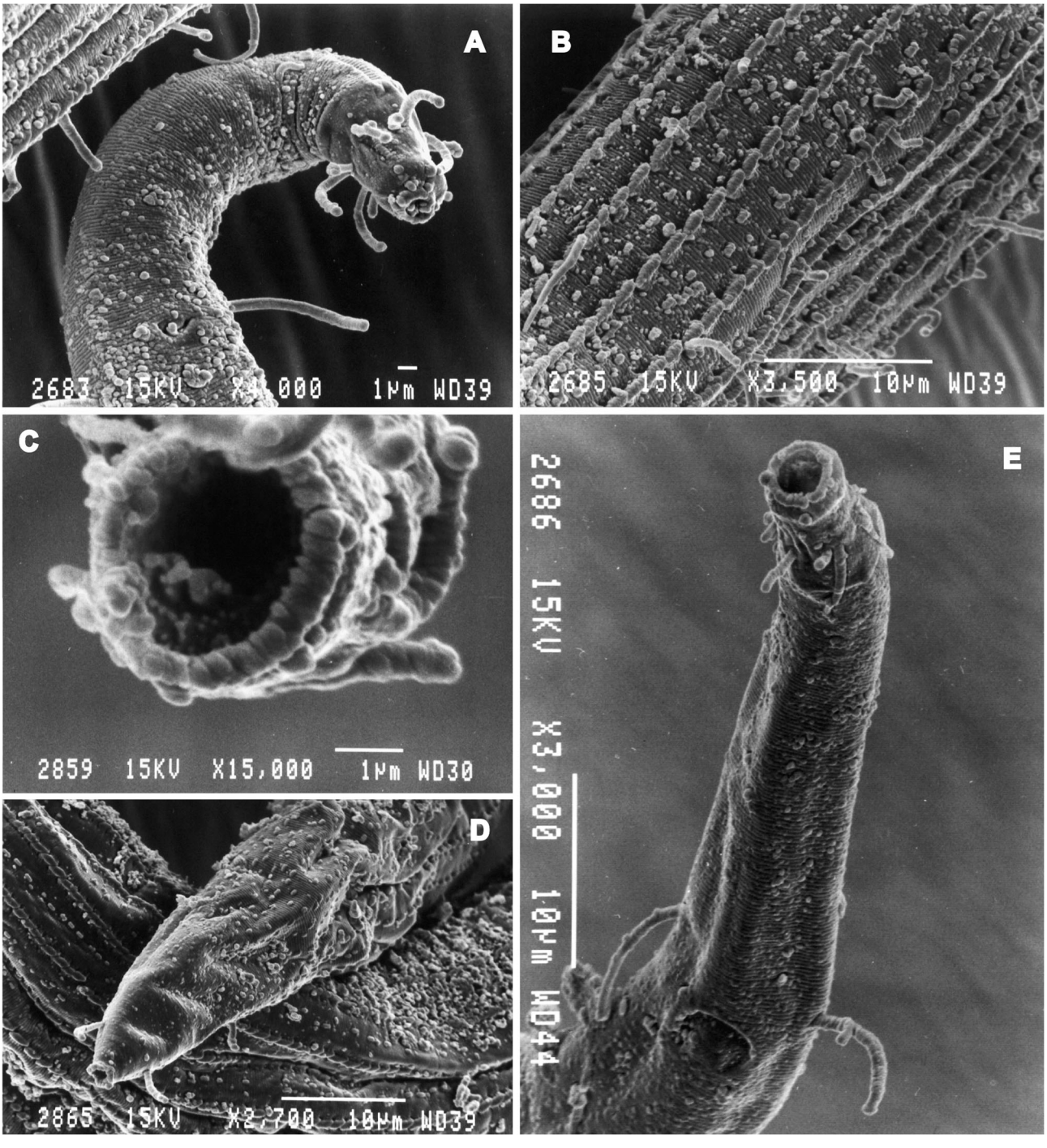

Fig. 2. Bernardius lineatus *gen. n., sp. n. SEM studies. Male. A-D. A: Head and amphidial region; B: Cuticle; C: Buccal cavity; D: Tail region. Female. E: Head and amphidial region.*

Table 2. *Morphometrics of* Bernardius lineatus *gen. n., sp. n. All measurements in μm and in the form: mean (range).*

Parameter	Male		Female	Intersex	Juveniles
	Holotype	Paratypes	Paratypes	Paratype	Paratypes
n	–	8	11	1	4
L	1770	1942 (1823-2150)	1950 (1770-2080)	2025	1679 (1348-1921)
a	45.5	48.1 (40.3-55.9)	42.8 (39.3-46.1)	45.0	54.1 (51.8-56.8)
b	4.5	4.5 (4.1-5.0)	4.3 (3.8-4.8)	4.8	4.5 (4.0-5.0)
c	25.6	26.4 (22.9-29.9)	27.5 (23.2-33.1)	28.5	25.1 (19.8-31.1)
V	–	–	63 (59-66)	–	–
Max. body diam.	39	41.2 (35-46)	46 (43-48)	45	32.5 (26-35)
Tail length	69	75 (63-90)	70 (61-83)	71	74 (68-78)
Anal body diam.	35	33 (29-38)	26 (22-30)	33	23 (19-28)
Buccal cavity diam.	4	3.9 (3-4)	3.8 (3-4)	4	3.2 (2-4)
Buccal cavity length	11	9.1 (8-11)	9.6 (9-11)	11	7 (6-8)
Head diam.	7	7.5 (6-10)	7.2 (6-11)	7	6 (5-7)
Pharynx length	390	433 (390-474)	457 (402-491)	423	369 (334-384)
Nerve ring from anterior end	179	198 (174-225)	195 (181-211)	171	213 (156-265)
Amphids from anterior end	40	48 (40-60)	47 (41-55)	45	42 (35-45)
Body diam. at amphidial aperture	8	11 (8-13)	11 (9-13)	10	9 (8-9)
Spicule length	38	32 (30-38)	–	20	–
Gubernaculum length	27	20 (17-27)	–	7	–
Longitudinal lines in cuticle	14	14-16	14-16	14	10-13
Length of cephalic setae	5	5.2 (5-6)	6.4 (5-8)	6	3 (2-4)
Number of supplements	2	2	–	–	–

plete dorsal spiral as in female. Apart from one exception, all juvenile specimens with well developed female reproductive system, apparently representing fourth stage juveniles. Body cuticle ornamented with longitudinal ridges as in adults but fewer in number and starting more posteriorly, *i.e*., at mid-pharynx. More anteriorly longitudinal ridges unclear, consisting of rows of rods covered by particulate detritus.

Type habitat and locality

Marine intertidal sandy sediment collected at Tamandaré Beach, Tamandaré-PE, Brazil.

Type material

Holotype male (slide UGMD104061) and paratype female (slide UGMD104067) deposited at the Museum voor Dierkunde, Ghent University, Ghent, Belgium. One intersex paratype (slide 71 NM), eight male paratypes (72-79 NM), ten female paratypes (slides 80-89 NM) and four juvenile paratypes (slides 98-101 NM) deposited at the Laboratory of Meiofauna, Department of Zoology, Federal University of Pernambuco, Recife, Brazil.

Diagnosis

Bernardius lineatus gen. n., sp. n. is characterised by its habitus, body strongly anteriorly tapered in neck region, body cuticle ornamented with 14-16 longitudinal rows of rods, sexual dimorphism of amphidial fovea, male with two papilliform precloacal supplements, short spicules with marked manubrium and short gubernaculum with apophyses and female with a short vagina located posterior to mid-body and absence of a common ovejector.

Enchelidiidae: analyses of relationships between genera

To analyse the phylogenetic relationships within the Enchelidiidae a data matrix (Table 3) was used consisting of 17 ingroup taxa and 13 characters. The polarity of the characters was determined on the basis of outgroup analyses, ontogenetic criteria, commonality principle and

Table 3. *Data matrix of taxa studied and polarity of morphological characters.*

Taxa	Feature												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	0
Abelbolla 1	0	0	0	0	0	0	0	0	0	0	1	1	0
Abelbolla 2	0	0	0	0	0	0	0	0	0	0	1	1	1
Aronema	0	0	1	0	1	0	1	0	1	0	0	0	0
Bathyeurystomina	0	0	0	0	1	0	2	0	0	0	1	1	1
Belbolla	0	1	0	0	0	0	0	0	0	1	1	0	1
Bernardius	1	0	1	0	1	0	1	0	1	1	0	0	0
Calyptronema	0	0	1	1	1	0	0	1	0	0	1	0	0
Ditlevsenella	0	0	0	0	0	0	0	0	0	1	1	0	0
Eurystomina	0	0	0	0	1	0	0	0	0	1	1	0	1
Ledovitia	0	0	0	0	1	0	0	0	0	0	1	0	1
Lyranema	0	0	1	0	0	1	3	0	0	0	1	?	?
Megeurystomina	0	0	0	0	1	0	2	0	0	1	1	0	1
Pareurystomina	0	0	0	0	1	0	0	0	0	1	1	1	1
Polygastrophora	0	1	0	0	0	0	0	1	0	1	1	0	0
Symplocostoma	0	0	0	1	1	0	0	1	0	0	1	0	0
Symplocostomella	0	0	0	1	1	0	0	0	0	0	1	0	0
Thoonchus	0	0	0	0	1	0	0	0	0	0	1	0	0

concept of complexity. A hypothetical ancestor with all the plesiomorphic characters was chosen.

The phylogenetic reconstruction was based on the Wagner parsimony program (Hennig86, version 1.5; Farris, 1989) with heuristic search (mh*, bb*), characters considered additive and non-additive and with the number of trees and consensus tree retained by the program.

Morphology and character evaluation – explanation of polarity

For the analysis of relationships between the taxa of the Enchelidiidae, 13 of the diagnostic characters used for the genera of this family (based on Smol and Coomans, 2006) are considered. Some of these characters were also used by Lorenzen (1981, 1994) and Fadeeva and Belogurov (2006) for understanding relationships at subfamily and genus level. Table 4 gives an overview of the morphological characters considered with indication of their polarity. The plesiomorph character states are coded 0 and the apomorph states scored 1 or more. When a character showed variability (not an exception) the taxon was included twice, *i.e*., with each character state.

Body cuticle (character 1)

A body cuticle ornamented with longitudinal ridges composed of rods is considered an apomorphy within the family based upon commonality principle and concept of complexity. It represents an autapomorphy for the new genus *Bernardius*.

Pharynx (character 2)

A non-differentiated pharynx is considered primitive compared with a more complex pharynx with multiple bulbs based upon outgroup comparison, concept of complexity and commonality principle within the family (Lorenzen, 1981, 1994).

Pharyngeal lumen (character 3)

A pharyngeal lumen in female appearing dilated in the relaxed condition is considered apomorphic based on outgroup comparison with the sister group Oncholaimidae within the Oncholaimoidea.

Sexual dimorphism in buccal cavity and amphidial fovea (characters 4 and 9)

The presence of sexual dimorphism in the structure of the buccal cavity and in the amphids is considered a derived character based on ontogenetic evidence (Lorenzen, 1981, 1994).

Denticles in buccal cavity (character 5)

The presence of denticles in the buccal cavity is considered apomorphic based upon ontogenetic evidence

Table 4. *Polarity of diagnostic morphological characters.*

1. Body cuticle
   - 0 without ornamentation
   - 1 with longitudinal ridges
2. Pharynx
   - 0 pharynx gradually enlarged posteriorly, crenate outline but lacking many bulbs
   - 1 pharynx with many bulbs
3. Pharyngeal lumen in female (relaxed condition)
   - 0 closed
   - 1 dilated
4. Sexual dimorphism in buccal cavity
   - 0 absent
   - 1 present
5. Denticles in buccal cavity
   - 0 absent
   - 1 present
6. Armature of buccal cavity
   - 0 tooth/teeth
   - 1 mandibles
7. Number of teeth in buccal cavity of female when present
   - 0 three teeth
   - 1 two teeth
   - 2 one tooth
   - 3 absent
8. Ventrosublateral tooth
   - 0 not movable
   - 1 movable
9. Sexual dimorphism in amphid
   - 0 absent
   - 1 present
10. Amphidial fovea in female
    - 0 non-spiral (pocket, oval, bowl, crescent)
    - 1 dorsally spiral
11. Position of amphidial fovea
    - 0 clearly posterior to stoma
    - 1 at level of stoma
12. Caudal glands
    - 0 present
    - 1 absent
13. Male precloacal supplements
    - 0 absent or papilliform
    - 1 more complex sclerotised or winged

(absent or reduced in number in earlier developmental stages) and concept of complexity.

Buccal armature (character 6)

The absence of teeth but presence of a buccal armature formed by mandibles is considered a derived character within the family based upon outgroup comparison and forms an autapomorphy for the genus *Lyranema*.

Number of teeth (character 7)

The number of teeth or absence of teeth was interpreted as a multistate character; the polarity of the character states was determined using commonality principle with the presence of three teeth as common within the family. The absence of teeth (= replacement by mandibles) was considered as the most advanced stage. It is not clear if we are dealing with a transformation series with gradual loss

of teeth or parallel development from the plesiomorphic stage to any of the more advanced stages.

Movable tooth (character 8)

The presence of a movable ventrosublateral tooth is considered as apomorphic based on the commonality principle and concept of complexity and outgroup comparison.

Amphidial fovea in female (character 10)

Concerning the structure of the amphidial fovea in the female, a non-spiral fovea is considered plesiomorphic based on outgroup comparison and commonality (Lorenzen, 1981, 1994).

Position amphidial fovea (character 11)

In general, the position of the amphidial fovea posterior to the lip region is considered to be plesiomorphic (Chitwood & Chitwood, 1950; Lorenzen, 1981, 1994) and according to postembryonic data amphids never move posteriorly. The more anterior position of the fovea within the family is considered more advanced.

Caudal glands (character 12)

The presence of three caudal glands is a holapomorphy of nematodes (Lorenzen, 1981, 1994). The loss of caudal glands within the Enchelidiidae is considered at a secondary level and interpreted as more derived.

Precloacal supplements in male (character 13)

The presence of complex (= winged) precloacal supplements in the male is considered apomorphic based upon commonality principle, concept of complexity and outgroup comparison.

Results and discussion

The phylogenetic reconstruction with characters considered additive resulted in seven equally parsimonious trees (length = 29). The consistency index of the strict consensus tree was 51 and the retention index was 63 (Fig. 3). The reconstruction with characters considered to be non-additive resulted in two equally parsimonious trees (length = 25) with a consistency index of 60 and a retention index of 69 (Fig. 4).

The phylogenetic tree with additive characters provides only restricted information on relationships within the family. *Aronema* and *Bernardius* gen. n. cluster together, apparently based on the possession of two teeth in the buccal cavity. Another possible clade is formed by *Calyptronema* clustering with *Symplocostoma* and both together with *Symplocostomella*, all three taxa being characterised by a pharynx with many bulbs. No resolution is given for

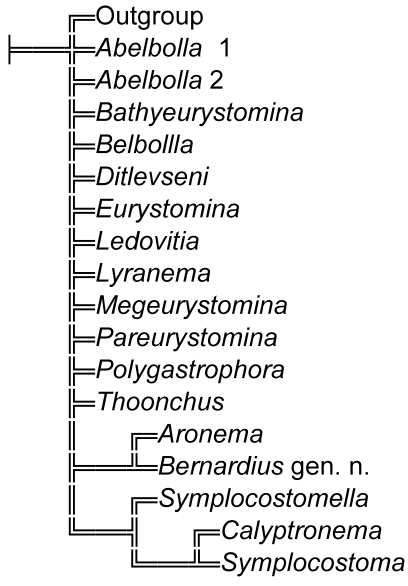

Fig. 3. *Phylogenetic consensus tree based on the Wagner parsimony program (Hennig86, version 1.5; Farris, 1989) with characters considered as additive.*

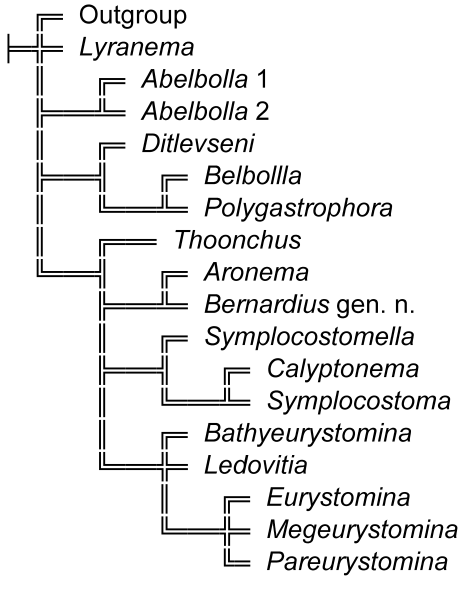

Fig. 4. *Phylogenetic consensus tree based on the Wagner parsimony program (Hennig86, version 1.5; Farris, 1989) with characters considered as non-additive.*

the relationship of the two clades and the other taxa considered.

When characters are considered non-additive, some further resolution is observed. *Belbolla* clustered with *Polygastrophora* and both genera also with *Ditlevsenella*, a grouping apparently based upon the presence of a pharynx with many bulbs. A second clade is formed by the other taxa of the family with the exception of *Lyranema*, *Abelbolla* and the outgroup. Within this second clade, we observe four groups: *i*) *Aronema* clustered with *Bernardius* gen. n.; *ii*) a cluster formed by the three taxa having a pharynx with many bulbs (*Calyptronema*, *Symplocostoma* and *Symplocostomella*); *iii*) *Eurystomina*, *Megeurystomina* and *Pareurystomina* together form a cluster with a second cluster formed by *Bathyeurystomina* and *Ledovita*; and *iv*) *Thoonchus* appears as a separate genus from the others. Both phylogenetic reconstructions have two clades in common: *Aronema* + *Bernardius* gen. n. and *Calyptronema* + *Symplocostoma* and *Symplocostomella*.

Following Smol and Coomans (2006), the family Enchelidiidae at present comprises 17 genera and, until now, no holapomorphy had been established for the family. Moreover, Lorenzen (1981, 1994) was unable to recognise the holophyly of any of the five introduced subfamilies Eurystominae Chitwood, 1935, Enchelidiinae Filipjev, 1918, Thoonelinae Gerlach & Riemann, 1974, Belbollinae Andrássy, 1976, Pareurystominae Andrássy, 1976 and synonymised them with the original subfamily Enchelidiinae (*Aronema* and Aronematinae were not treated in Lorenzen, 1994). Smol and Coomans (2006) omitted further discussion on the remaining two subfamilies. Fadeeva and Belogurov (1988) classified *Aronema* within their new subfamily Aronematinae, mainly because of the presence of a single, large, movable subventral tooth without additional teeth and the posterior position of the amphidial fovea, characters different from any other taxon within the family. Fadeeva and Belogurov (1988) included a discussion on phylogenetic relationships at subfamily level based mainly on the absence/presence and degree of development of a stoma and stomatal armature and position of the amphidial apertures. They did not include a profound discussion on the polarity of these characters nor a sister group analyses. In the current study, we considered the "thorn-like structure" located anteriorly in the buccal cavity in *A. crinita* as a second ventrosublateral tooth in agreement with the interpretation by Gerlach (1953) of *Calyptronema setifer* Gerlach, 1953, a species transferred to *Aronema* by Fadeeva and Belogurov (1988).

As the resolution of relationships between the taxa of the family Enchelidiidae is still largely unresolved, the introduction of subfamilies is best avoided. Therefore, we provisionally accept the subfamily Aronematinae and classify *Bernardius* gen. n. within it, although support for a separate subfamily within the family is very weak.

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