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

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Received: 17 June 2007; revised: 27 June 2008
Accepted for publication: 27 June 2008

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 centrifugation).
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 *Symprocostoma*). 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
*Bathyurystomina* Lambshead & Platt, 1979
*Bellbolla* Andrássy, 1973
*Bernardius* gen. n.
*Calyptronema* Marion, 1870
*Ditlevsenella* Filipjev, 1927
*Eurystomina* Filipjev, 1921
*Eurystoma* Filipjev, 1927
*Ledovita* Filipjev, 1927

*Megeurystomina* Luc & De Coninck, 1959
*Pareurystomina* Micoletzky, 1930
*Polygastrophora* de Man, 1922
*Symprocostoma* Bastian, 1865
*Symprocostomella* 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.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cuticle</th>
<th>Sexual dimorphism</th>
<th>Teeth</th>
<th>Pharynx</th>
<th>Amphids</th>
<th>Ocelli</th>
<th>Tail shape</th>
<th>Caudal glands</th>
<th>Gubernaculum</th>
<th>Supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang &amp; Zhang, 2004</td>
<td>smooth</td>
<td>–</td>
<td>3 with the right ventro-lateral largest + denticles</td>
<td>cylindrical, without bulbs</td>
<td>not known</td>
<td>absent</td>
<td>long conical cylindrical</td>
<td>absent</td>
<td>with apophysis</td>
<td>2 winged or absent</td>
</tr>
<tr>
<td>Fadeeva &amp; Belogurov, 1988</td>
<td>smooth</td>
<td>amphid shape</td>
<td>2 subventral + denticles</td>
<td>cylindrical, M: loop-shaped</td>
<td>without bulbs</td>
<td>F: pocket-shaped</td>
<td>absent</td>
<td>short conical</td>
<td>present</td>
<td>with apophysis</td>
</tr>
<tr>
<td>Lambshead &amp; Platt, 1979</td>
<td>smooth</td>
<td>–</td>
<td>1 right ventrosublateral* + denticles</td>
<td>cylindrical, faint, crescentic</td>
<td>absent</td>
<td>conical-flagellate</td>
<td>absent</td>
<td>with well developed apophysis</td>
<td>absent or with apophysis</td>
<td>2 sclerotised tubes with cap winged (partly)</td>
</tr>
<tr>
<td>Andrássy, 1973</td>
<td>smooth</td>
<td>–</td>
<td>3 with the ventrosublateral largest + denticles</td>
<td>7-10 bulbs</td>
<td>dorsally spiral</td>
<td>absent</td>
<td>elongate conical</td>
<td>present</td>
<td>absent or with apophysis</td>
<td></td>
</tr>
<tr>
<td>Marion, 1870</td>
<td>smooth</td>
<td>anterior end and buccal cavity</td>
<td>F: 3 with right or left ventrosublateral largest + denticles</td>
<td>cylindrical, non-spiral</td>
<td>without bulbs</td>
<td>present or absent</td>
<td>short or elongate conical</td>
<td>present</td>
<td>without apophysis</td>
<td>many, papilliform</td>
</tr>
<tr>
<td>Filipjev, 1927</td>
<td>smooth</td>
<td>–</td>
<td>3 with left or right ventrosublateral largest + denticles</td>
<td>cylindrical, dorsally spiral</td>
<td>without bulbs</td>
<td>absent</td>
<td>short conical or clavate</td>
<td>present</td>
<td>with apophysis</td>
<td>6-8 papillae</td>
</tr>
<tr>
<td>Filipjev, 1921</td>
<td>smooth</td>
<td>–</td>
<td>3 with right ventrosublateral largest + denticles</td>
<td>with bulbs</td>
<td>dorsally spiral</td>
<td>present or absent</td>
<td>short conical</td>
<td>present</td>
<td>with or without apophysis</td>
<td>2 winged</td>
</tr>
<tr>
<td>Filipjev, 1927</td>
<td>smooth</td>
<td>–</td>
<td>3 with right ventrosublateral largest + denticles</td>
<td>cylindrical, oval</td>
<td>without bulbs</td>
<td>absent</td>
<td>long conical or cylindrical</td>
<td>present</td>
<td>absent or without apophysis</td>
<td>2 winged (weakly developed)</td>
</tr>
<tr>
<td>Timm, 1961</td>
<td>smooth</td>
<td>–</td>
<td>mandibles lyre-shaped</td>
<td>cylindrical, bowl-shaped</td>
<td>without bulbs</td>
<td>absent</td>
<td>conical cylindrical</td>
<td>not observed</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Luc &amp; De Coninck, 1959</td>
<td>smooth</td>
<td>–</td>
<td>1 right ventrosublateral tooth + denticles</td>
<td>cylindrical, dorsally spiral</td>
<td>without bulbs</td>
<td>absent</td>
<td>conical digitate</td>
<td>present</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Reference</td>
<td>Cuticle</td>
<td>Sexual dimorphism</td>
<td>Teeth</td>
<td>Pharynx</td>
<td>Amphids</td>
<td>Ocelli</td>
<td>Tail shape</td>
<td>Caudal glands</td>
<td>Gubernaculum</td>
<td>Supplements</td>
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<tr>
<td>Pareurystomina</td>
<td>smooth</td>
<td>–</td>
<td>3 with the ventro-sublateral largest + denticles</td>
<td>cylindrical, without bulbs</td>
<td>dorsally</td>
<td>absent</td>
<td>conical, sharp tip</td>
<td>absent</td>
<td>with or without apophysis</td>
<td>2 winged</td>
</tr>
<tr>
<td>Micoletzky,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>spiral</td>
<td></td>
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<tr>
<td>1930</td>
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<tr>
<td>Polygastrophora</td>
<td>smooth</td>
<td>–</td>
<td>1 large ventro-sublateral and 2 smaller teeth</td>
<td>several bulbs</td>
<td>dorsally</td>
<td>present</td>
<td>conical cylindrical</td>
<td>present</td>
<td>with or without apophysis</td>
<td>minute papillae or setae</td>
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<tr>
<td>de Man, 1922</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>spiral</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Symplocostoma</td>
<td>smooth</td>
<td>anterior end and buccal cavity</td>
<td>F: 3 with the ventro-sublateral largest + denticles</td>
<td>cylindrical, without bulbs</td>
<td>M: pocket-shaped</td>
<td>present</td>
<td>conical cylindrical</td>
<td>present</td>
<td>with or without apophysis</td>
<td>papilliform</td>
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<tr>
<td>Bastian, 1865</td>
<td></td>
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<tr>
<td>Symplocostomella</td>
<td>smooth</td>
<td>anterior end and buccal cavity</td>
<td>F: 3 with the ventro-sublateral largest + denticles</td>
<td>cylindrical, without bulbs</td>
<td>M: pocket-shaped</td>
<td>present</td>
<td>conical cylindrical</td>
<td>present</td>
<td>with or without apophysis</td>
<td>papilliform</td>
</tr>
<tr>
<td>Micoletzky,</td>
<td></td>
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<td></td>
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<td>1930</td>
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<tr>
<td>Thoonchus</td>
<td>smooth</td>
<td>–</td>
<td>3 with right ventro-sublateral largest + denticles</td>
<td>cylindrical, large without bulbs</td>
<td>absent</td>
<td>short cylindrical</td>
<td>present</td>
<td>with expanded corpus and with or without apophysis</td>
<td>1-2 papilliform</td>
<td></td>
</tr>
<tr>
<td>Cobb, 1920</td>
<td></td>
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<tr>
<td>Bernardius gen. n.</td>
<td>with longitudinal ridges</td>
<td>2 subventral ring of denticles</td>
<td>cylindrical, M: pocket-shaped bulbs</td>
<td>absent</td>
<td>short conical</td>
<td>present</td>
<td>with apophysis</td>
<td>2 papilliform</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

? = 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 sub-ventral 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.

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

**Female**

Similar to male in most morphological aspects, including cuticle. Striking sexual dimorphism in amphidial 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 ooejector 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-
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.)
Bernardius lineatus gen. n., sp. n. from Brazil

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).

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

Complete 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 particular detritus.

**Type habitat and locality**

Marine intertidal sandy sediment collected at Taman-daré 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
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
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 Polygastraphora and both genera also with Ditelvenella, 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, Megaeurystomina 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 Encheliidiidae 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, Encheliidiinae Filipjev, 1918, Thoenelinae Gerlach & Riemann, 1974, Belbolliinae Andrássy, 1976, Pareurystominae Andrásy, 1976 and synonymised them with the original subfamily Encheliidiinae (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 Encheliidiidae 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.

Acknowledgements

The authors thank Rita Van Driessche for her help with the SEM pictures and Luciana D. T. Sobral for the help with the line drawing figures. Virág Venekey also thanks CAPES for a postgraduate studentship.

References

FILIPJEV, I. (1918). [Free-living marine nematodes of the
Sevastopol area.] Trudy Osoboy zoologicheskoy laboratorii i
Sevastopol’skoy Biologicheskou stantsii Rossiykoy Akademii
Nauk (2) 4, 1-350.
FILIPJEV, I. (1921). [Free-living marine nematodes of the
Sevastopol area.] Trudy Osoboy zoologicheskoy laboratorii i
Sevastopol’skoy Biologicheskou stantsii Rossiykoy Akademii
Nauk (2) 4, 351-614.
FILIPJEV, I. (1927). Les nématodes libres des mers septentri-
onales appartenant à la famille des Enoplidae. Archiv für
Naturgeschichte 91A (6), 1-216.
FILIPJEV, I. (1929). Classification of freeliving Nematoda and
relations to parasitic forms. Journal of Parasitology 15, 281-
282.
GERLACH, S.A. (1953). Die Nematodenbesiedlung des Sand-
strandes und des Kustengrundwassers an der italienischen
Kuste. I. Systematischer Teil. Archivio Zoologico Italiano 37,
517-640.
species of free-living marine nematodes (Nematoda: Enop-
lida: Encheliidiidae) from the Yellow Sea, China. Cahiers de
Biologie Marine 45, 343-354.
LAMBSHEAD, P.J.D. & PLATT, H.M. (1979). Bathyeurysto-
mina, a new genus of free-living marine nematodes (Enche-
liidiidae) from the Rockall Trough. Cahiers de Biologie Ma-
rine 20, 371-380.
LORENZEN, S. (1981). Entwurf eines phylogenetischen Sys-
tems der freilebenden Nematoden. Veröffentlichungen des In-
stituts für Meeresforschung in Bremerhaven, Supplement 7,
1-472.
LORENZEN, S. (1994). The phylogenetic systematics of freeliv-
marins de la région de Roscoff. Archives de Zoologie Expéri-
mentale et Générale 98, 103-165.
MAIDA, M. & FERREIRA, B.P. (1997). Coral reefs of Brazil:
an overview. Proceedings of the 8th International Coral Reef
Symposium, 24-29 June 1996, Panama City, Panama, pp.
263-274.
DE MAN, J.G. (1922). Neue freilebende Nematoden aus
der Zündersee. Tijdschrift der Nederlandsche Dierkundige
Vereeniging (2) 18, 124-134.
MARION, A.F. (1870). Recherches zoologiques et anatomicques
sur des Nématoides non parasites, marins. Annales des
Sciences Naturelles 13 (14), 1-100.
den Sunda-Inseln. I. Enoplidae. (Papers from Dr. Th. 
Mortensen’s Pacific Expedition 1914-16.) Videnskabelige
Meddelelser fra Dansk Naturhistorisk Forening i København
87, 243-339.
Freshwater nematodes: ecology and taxonomy. Wallingford,
TIMM, R.W. (1961). The marine nematodes of the Bay of
Bengal. Proceedings of the Pakistan Academy of Sciences 1,
1-88.