# Three new species of free-living marine nematodes of *Microlaimus* (Nematoda: Microlaimidae) from the continental shelf off northeastern Brazil (Atlantic Ocean) (#92535)



First submission

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# Three new species of free-living marine nematodes of *Microlaimus* (Nematoda: Microlaimidae) from the continental shelf off northeastern Brazil (Atlantic Ocean)

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Three new species of the *Microlaimus* genus (Nematoda: Microlaimidae) are described from sample sediments collected in the South Atlantic, along the Continental Shelf break of Northeastern Brazil. *Microlaimus paraundulatus* **sp. n.** presents four cephalic sensillae in the shape of thin setae, buccal cavity with three small teeth, arched and slender spicules and gubernaculum wave-shaped. *Microlaimus modestus* **sp. n.** is characterized by four small cephalic sensillae, buccal cavity with three teeth (one large dorsal tooth), cephalized spicules and a strongly arched gubernaculum in the distal region. *Microlaimus nordestinus* **sp. n.** is characterized by bring together of the following features: relatively long body, eight rows of hypodermal glands that extend longitudinally along of the body and gubernaculum funnel-shaped surrounding the spicules at the distal end. The amendment of the diagnosis is proposed for the genus.

# Three new species of free-living marine nematodes *Microlaimus* (Nematoda: Microlaimidae) from the continental shelf off northeastern Brazil (Atlantic Ocean)

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## Abstract

Three new species of the *Microlaimus* genus (Nematoda: Microlaimidae) are described from sample sediments collected in the South Atlantic, along the Continental Shelf break off Northeastern Brazil. ~~*Microlaimus paraundulatus*~~ **sp. n.** ~~presents four cephalic sensillae in the shape of thin setae,~~ a buccal cavity with three small teeth, arched and slender spicules and a wave-shaped gubernaculum. *Microlaimus modestus* **sp. n.** is characterized by four small cephalic sensillae, a buccal cavity with three teeth (one large dorsal tooth), ~~cephalized~~ spicules and a strongly arched gubernaculum in the distal region. *Microlaimus nordestinus* **sp. n.** is characterized by following set of features: relatively long body, eight rows of hypodermal glands that extend longitudinally along the body and a funnel-shaped gubernaculum surrounding the spicules at the distal end. The amendment of the diagnosis is proposed for the genus.

**Key words:** Marine nematodes, taxonomy, species description, South Atlantic, Nematode diversity.

## Introduction

The superfamily Microlaimoidea Micoletzky, 1922 currently comprises four families: Aponchiidae Gerlach, 1963; Molgolaimidae Jensen, 1978; Monoposthiidae Filipjev, 1934 and Microlaimidae Micoletzky, 1922 (Leduc *et al*. 2017), the latter of which comprises the largest

number of genera and species (Tchesunov *et al*. 2021). However, records of Microlaimidae species originally described from sample sediments from the South Atlantic are still scarce. In research carried out in Brazil in the 1950s, Gerlach described several new species of Nematoda for the hitherto "unexplored Brazilian coast". As part of their results, four species of the genus *Microlaimus* de Man, 1880 were described (*M*. *papillatus* Gerlach, 1956; *M*. *capillaris* Gerlach, 1957a; *M*. *spinosus* Gerlach, 1957b and *M*. *formosus* Gerlach, 1957b) from samples collected in mangroves and sandy beaches along the southeastern coast of Brazil. Later, *Aponema papillatum* Pastor de Ward, 1980 and *M*. *decoratus* Pastor de Ward, 1989 were described from the Ria Deseado (Santa Cruz, Argentina). Recently, Lima *et al*. (2022) described three species of *Microlaimus* (*M*. *campiensis*, *M*. *alexandri* and *M. vitorius*) from the continental shelf of the Campos Basin, southeastern Brazil.

*Microlaimus* is by far the most diverse genus of the family Microlaimidae (Leduc 2016). Nowadays, the genus includes 86 valid species (Nemys 2023). Marine representatives of this taxon are widely distributed, ranging from the interdial zone (Leduc & Wharton 2008) to the deepest areas of the ocean (Miljutin & Miljutina 2009). Due to their morphological similarity, species transfers between *Microlaimus* and other close genera belonging to the same family have been recorded several times in the literature (Techunov 2014; Leduc 2016; Lima *et al*. 2022). This is due to disagreements about which morphological ~~characteristics~~ should be used to establish differences between these genera (Leduc 2016). The main features used together to differentiate representatives of the genus *Microlaimus* from other genera of the same family are: annulated cuticle, some species also show punctations or longitudinal bars; head often slightly set off; fovea ~~amphidial~~ cryptocircular or unispiral; small to medium-sized buccal cavity, armed with three teeth with an often well-developed dorsal tooth; females are didelphic-amphidelphic with outstretched ovaries and males present a gubernaculum without dorso-caudal apophysis (Decraemer & Smol 2006; Techunov 2014; Leduc 2016; Lima *et al*. 2022).

In the present study, representatives of the genus *Microlaimus* were found from samples collected in the South Atlantic, along the break of the Continental Shelf in Northeast Brazil. Here we describe the first three new species of *Microlaimus* for this locality. We also propose to amend the diagnosis of the genus.

## Material and methods

**Study area (Table 1).** Sampling was carried out during an oceanographic campaign associated with the UFPE S.O.S. SEA project, in November and December 2019, on board the ship Vital de Oliveira. The sampling grid consisted of 23 collection stations arranged along the break of the Continental Shelf in Northeast Brazil, off the coast of the states of Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia. In Table 1, information of the collection stations related to the present study are indicated. A box-corer was used to collect sediments, and the meiofauna samples were obtained with a 10 $cm^2$ corer.

**Sampling and laboratory processing.** In the laboratory, sediment samples were sieved using a 500 μm mesh followed by a 45 μm mesh sieve which was used to retain the meiobenthic organisms. The samples remaining in the 45 μm mesh were extracted with colloidal silica (Somerfield *et al*. 2005).

Nematoda were counted (and removed) under a stereomicroscope using a Dolffus plate. All individuals were transferred to a small glass container containing a solution with 99% formaldehyde (4%) + 1% glycerin (Solution 1 – De Grisse 1969). Each animal's body was then impregnated with glycerin, followed by diaphanization, according to the method described by De Grisse (1969). Individuals were permanently mounted on glass slides, as an adaptation of the method described by Cobb (1920). The genus was identified using keys provided by Warwick *et al*. (1998) and Decraemer & Smol (2006). Species were identified through the comparison of their characteristics with those provided in the original descriptions. Drawings were made with the aid of an Olympus CX 31 optical microscope fitted with a drawing tube. Body measurements were taken using a mechanical mapmeter.

The holotype and one paratype (female) of each species are deposited in the Nematoda Collection of the Museum of Oceanography Prof. Petronio Alves Coelho (MOUFPE), Brazil. Other paratypes are deposited in the Meiofauna Laboratory, Zoology Department, Federal University of Pernambuco (NM LMZOO-UFPE).

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## Results

### SYSTEMATICS

### Class CHROMADOREA Inglis, 1983

### Subclass CHROMADORIA Pearse, 1942

### Order Microlaimida Leduc, Verdon & Zhao, 2017

### Superfamily Microlaimoidea Micoletzky, 1922

### Family Microlaimidae Micoletzky, 1922

### Genus *Microlaimus* de Man, 1880

Syn *Microlaimoides* Hoeppli, 1926; *Paracothonolaimus* Schulz, 1932

**Diagnosis.** (Emended from Lima *et al*. 2022): Cuticle transversely striated, punctuations or longitudinal bars may be present. Lateral differentiation in the form of lateral alae occurs in *M. falciferus* Leduc & Wharton, 2008. Cephalic region often set off. Presence or absence of association between hypodermal glands with pores or setae, small somatic setae occur in some species. Anterior sensilla arranged according to pattern 6 + 6 + 4: six inner labial setae, usually papilliform; six ~~external~~ labial setae, papilliform or setiform; and four cephalic setae. Amphidial fovea cryptocircular or unispiral (= cryptospiral). Presence or absence of sexual dimorphism in amphidial fovea size. Buccal cavity small to medium-sized, **armed (except in *M. nympha*) with two to five (three teeth in most species) small or well-developed teeth**, especially the dorsal

tooth. Transverse cuticularized band or ring may be present in buccal cavity. Most species have two testes extending in opposite directions; some with two anterior testes, others with only one testis, positioned anteriorly or posteriorly. Pre-cloacal supplements absent or present (papilliform, tubular, or small pores). Spicules usually short and arcuate, seldom long and slender. Gubernaculum usually present and without dorso-caudal apophysis. Female didelphic-amphidelphic, with outstretched ovaries. Tail predominantly conical.

**Type species:** *Microlaimus globiceps* de Man, 1880.

*Microlaimus paraundulatus* **sp. n.**

(Table 2; Fig 1–3)

**Material studied**. Holotype male (MOUFPE 0017), paratype female (MOUFPE 0017) and 1 male paratype (473 NM LMZOO-UFPE).

**Type locality**. Continental shelf off the State of Rio Grande do Norte, Brazil, station 2 (S 05°42'54.42" W 34°59'31.92"), November 28, 2019, 60 m. Paratypes found in the same locality.

**Etymology**. The shape of the gubernaculum (wave-shape) is similar to that of *Microlaimus undulatus* Gerlach, 1953. Greek para = similar; *undulatus* = *M. undulatus*.

**Holotype male**. Body cylindrical 457.5 µm long. Maximum body diameter corresponding to 2.1 times the head diameter. Cuticle striated posteriorly to cephalic setae insertion. Cuticular pores and somatic setae not observed. Six inner and six outer papilliform labial sensillae. Four cephalic sensillae in the shape of thin setae 3 µm long, corresponding to 43% of head diameter. Head slightly set off. Amphidial fovea cryptocircular, located 11 µm from anterior end (1.6 times the head diameter) and occupying 44% of corresponding body diameter. Buccal cavity weakly cuticularized. Cheilostoma rugae indiscernible under a light microscope. Three small teeth, difficult to see (a slightly larger dorsal tooth and two smaller ventrosublateral). Pharynx (82 µm long) with terminal oval bulb. Bulb occupying 79% of corresponding body diameter. Cardia embedded in intestine. Nerve ring situated at 57% of the pharynx length, from anterior end. Ventral gland and secretory-excretory pore not observed. Reproductive system with two testes extending in opposite directions. Spicules slender and arched. Gubernaculum curved and wave-

147 shaped. Precloacal supplements absent. Three caudal glands. Tail conical, about 4.9 times the
148 cloacal body diameter.
149 **Paratype female**. Similar to male. Body measuring 465 µm in length, with a maximum diameter
150 of 18 µm. Cephalic sensilla equivalent to 43% of head diameter. Amphidial fovea, occupying
151 56% of corresponding body width and located 13 µm from anterior end. Buccal cavity, teeth and
152 pharynx similar to that of the male. Basal bulb occupying 80% of the corresponding body
153 diameter. Nerve ring situated at 59% of the pharynx length, from anterior end. Vulva located
154 244.5 µm from anterior end, at 53% of body length. Reproductive system didelphic-
155 amphidelphic, with outstretched ovaries. Anterior ovary situated to the right side of intestine,
156 posterior ovary to the left side of intestine. Tail conical, about 5.5 times the anal body diameter.
157 **Diagnosis**. *Microlaimus paraundulatus* **sp. n.** characterized by its body length (439–465 µm).
158 Cuticle finely annulated. Head slightly set off. Four cephalic sensillae in the shape of thin setae
159 (3 µm long), corresponding to 43% of head diameter. Amphidial fovea occupying 44% of the
160 corresponding body diameter in the males and 56% in the female, located at about 1.6–1.9 times
161 the head diameter. Buccal cavity with three small teeth, one dorsal and two ventrosublateral, the
162 dorsal is slightly larger. Spicule arched and slender (1.8–2.3 times the cloacal body diameter)
163 with a wave-shaped gubernaculum. Tail conical which corresponds to 4–6 cloacal or anal body
164 diameter.
165
166 **Differential diagnosis** (**Table 3**). Firstly, it is important to establish that only males of each
167 species mentioned in this section were used in the comparisons with males of the new species.
168 More detailed information, such as some measurements and proportions, about females is absent
169 in the original descriptions of some of the species in question. The measurements and
170 proportions missing from the descriptions were obtained from available images.
171
172 *Microlaimus paraundulatus* **sp. n.** resembles *M. undulatus* Gerlach, 1953 mainly due to
173 the peculiar shape of the gubernaculum (wave-shaped). Additionally, in both species the
174 amphidial fovea occupies a similar proportion of the corresponding body diameter (44% in *M.*
175 *paraundulatus* **sp. n.** and 42% in *M. undulatus*) and the spicules are very similar in length (23–
176 27 µm in *M. paraundulatus* **sp. n.** and 27 µm in *M. undulatus*). Although, the species differ in
177 terms of cephalic setae length (3 µm in the new species vs 7 µm in *M. undulatus*), the position of

the amphidial fovea from the anterior end (1.6 times the diameter of the head in *M. paraundulatus* **sp. n.** vs 0.7 times the diameter head diameter in *M. undulatus*); spicule shape (thin in the new species vs robust in *M. undulatus*) and the ratio between spicule length and cloaca diameter (1.8–2.3 in *M. paraundulatus* **sp. n.** vs 1.1 in *M. undulatus*).

The other four species of the genus (*M. copulatus* Jensen, 1988; *M. limnophilus* Turpeenniemi, 1997; *M. porus* Bussau, 1993 and *M. zosterae* Allgén, 1930) that morphologically resemble *M. paraundulatus* **sp. n.** are included in Table 3**.** Males of these species share the combination of three features with the new species: cephalic setae with a length of less than 50% of the head diameter; amphidial fovea with anterior edge positioned between 1.6 and 1.9 times the head diameter in relation to the anterior end; amphidial fovea occupying less than 50% of the corresponding body diameter. Exceptionally, the percentage of the corresponding body diameter occupied by the amphidial fovea for *M. porus* was obtained from the drawing of the female paratype (proportion not reported and anterior end not drawn in the original description of the male holotype; sexual dimorphism absent). *Microlaimus undulatus*, like the other species of the genus, does not present these characteristics simultaneously and therefore the species was not included in Table 3.

The new species differs from *M. copulatus* with regard to index "a" (27–30.5 in *M. paraundulatus* **sp. n.** vs 18–20 in *M. copulatus*), the proportion (%) between gubernaculum length in relation to length of spicule along arc (48–56% in the new species vs 30% in *M. copulatus*) and the precloacal supplement (absent in *M. paraundulatus* vs a precloacal papilla in *M. copulatus*). Additionally, the gubernaculum has dorsal apophyses and an irregular shape in *M. copulatus* and is wave-shaped without apophyses in *M. paraundulatus* **sp. n.**

*Microlaimus paraundulatus* **sp. n.** differs from *M. limnophilus* in the proportion (%) between the cephalic setae and the head diameter (43% in *M. paraundulatus* **sp. n.** vs 23–25% in *M. limnophilus*), the shape of the gubernaculum (wave-shaped in *M. paraundulatus* **sp. n.** vs rod-like in *M. limnophilus*) and the proportion between the spicules in relation to the cloacal body diameter (2.3 in *M. paraundulatus* **sp. n.** vs 1.3 in *M. limnophilus*). Furthermore, *M. limnophilus*

presents two pore-like precloacal supplements, while in the new species the precloacal supplements are absent.

The new species differs from *M. porus* Bussau, 1993 with regard to the number of teeth (3 in *M. paraundulatus* vs 2 in *M. porus*), the shape of the gubernaculum (wave-shaped in the new species vs simple in *M. porus*) and the values of the indices "a" (30.5 in the new species vs 21.1 in *M. porus*) and "c" (8 in the new species vs 5.4 in *M. porus*). Additionally, *M. porus* has rows of pores distributed throughout the body. This feature is absent in the new species.

Based on the redescription of *M. zosterae* provided by Kovalyev & Tchesunov (2005), whose original description was based on females alone, *M. paraundulatus* **sp. n.** differs from the males of *M. zosterae* in terms of the shape of the gubernaculum (wave-shaped in the new species vs curved, tapering to the ends, wider in the middle part in *M. zosterae*) and the precloacal supplements that are absent in *M. paraundulatus* **sp. n.** vs present in *M. zosterae*.

*Microlaimus modestus* **sp. n.**

(Table 4; Figs 4–7)

**Material studied. Material studied**. Holotype male (MOUFPE 0018, paratype female (MOUFPE 0019), 1 male paratype (474 NM LMZOO-UFPE) and 4 female paratypes (475-478 NM LMZOO-UFPE).

**Type locality.** South Atlantic Ocean, Continental shelf off the State of Sergipe, Brazil, station 16 (S 10°44'59.28" W 36°25'32.88"), December 09, 2019, 58 m. Paratypes found in the same locality.

**Etymology.** Due to its relatively small body length. Latin modestus: short in length.

**Holotype male.** Body cylindrical 342 µm long. Maximum body diameter corresponding to 2.2 times the head diameter. Cuticle striated posteriorly to cephalic setae insertion. Cuticular pores and somatic setae not observed. Anterior sensilla arranged in the 6+6+4 pattern: six inner labial

papilliform sensilla, six outer labial papilliform sensilla and four short cephalic setae (2 μm long), corresponding to 22% of head diameter. Head slightly set off. Amphidial fovea cryptocircular, located 10 μm from anterior end and occupying 57% of corresponding body diameter. Buccal cavity cuticularized. Cheilostoma rugae indiscernible under a light microscope. Three cuticularized teeth, one large dorsal tooth and two smaller ventrosublateral teeth. Pharynx (82 μm long) with terminal oval bulb. Bulb occupying 88% of corresponding body diameter. Cardia embedded in intestine. Nerve ring situated at 62% of the pharynx length, from anterior end. Secretory-excretory pore located 56 μm from anterior end (about 68% of the pharynx length). Ventral gland not observed. Reproductive system with single anterior outstretched testis on the right side of intestine. Spicules arched, with ~~proximal portion cephalized~~. Gubernaculum simple with strongly arched distal region. Precloacal supplements absent. Three caudal glands. Tail conical, about 3.2 times the cloacal body diameter.

**Paratype female**. Similar to male. Body measuring 359 μm in length, and maximum diameter 23 μm. Cephalic sensilla equivalent to 24% of head diameter. Amphidial fovea, occupying 56% of corresponding body width and located 11 μm from anterior end. Buccal cavity, teeth and pharynx similar to that of males. Basal bulb occupying 84% of the corresponding body diameter. Nerve ring situated at 59% of the pharynx length, from anterior end. Secretory-excretory pore located 53 μm from anterior end (about 65% of the pharynx length). Vulva located 206 μm from anterior end, at 57% of body length. Reproductive system didelphic-amphidelphic, with outstretched ovaries. Anterior ovary situated to right side of intestine, posterior ovary to left side of intestine. Anterior and posterior ovary measuring respectively 49 and 64 μm. Tail conical, about 3.6 times the anal body diameter.

**Diagnosis**. *Microlaimus modestus* **sp. n.** is characterized by small body length (331.5–359 μm). Cuticle finely annulated. Head slightly set off. Inner and outer labial setae in the shape of papillae. Four short cephalic setae (2 μm long), corresponding to 22–25% of head diameter. Amphidial fovea accounting for 50–60% of the corresponding body diameter, located at about 1.1–1.2 times the head diameter in males and 1.1–1.5 in females. Buccal cavity with three teeth, one large dorsal and two smaller ventrosublateral. Paired spicules arched, with proximal portion cephalized. Gubernaculum strongly arched in distal region.

**Differential diagnosis** (**Table 5**). Firstly, it is important to establish that only the males of each species mentioned in this section were used in the comparisons with males of the new species. This is because only males of *M. acanthus* (Jayasree & Warwick, 1977) Kovalyev & Tchesunov, 2005 have been described. Measurements and proportions missing from the descriptions were obtained from the available images.

Two species of the genus (*M. acanthus* and *M. microseta* Gerlach, 1953) that morphologically resemble *M. modestus* **sp. n.** are included in Table 5**.** Males of these species share this combination of three features with the new species: cephalic setae with a length less than or equal to 50% of the head diameter; amphidial fovea occupying more than 50% of the corresponding body diameter; amphidial fovea with anterior edge positioned between 1.1 and 1.5 times the head diameter in relation to the anterior end. *M. acanthus* showed a greater variation in the relative position of the amphidial fovea (1.1–1.8 times the head diameter). Nevertheless, due to other similarities with the new species, it was included in the table for comparison purposes.

*M. modestus* **sp. n.** shares the ratio between gubernaculum length and spicule length (between 50–60% in the three species), as well as the de Man's ratio c' (between 3–4.25) with *M. acanthus* and *M. microseta*. However, the values of the other de Man's ratio (a, b and c) for *M. modestus* **sp. n.** are relatively low compared to *M. acanthus* and *M. microseta* (see Table 4). Furthermore, the ratio between the length of the spicule along the arc and cloacal body diameter is higher in *M. modestus* **sp. n.** (2–2.3) when compared to the ratios observed in *M. acanthus* and *M. microseta* (between 1.1–1.4). Additionally, the new species differs from *M. acanthus* and *M. microseta* with regard to the shape of the gubernaculum (simple with strongly arched distal region in *M. modestus* **sp n.** vs pointed and narrow proximally and expanded distally in *M. acanthus* vs narrow and simple in *M. microseta*). *M. acanthus* presents four to six prominent precloacal supplements in the form of robust setae, while in the new species precloacal supplements are absent.

*Microlaimus nordestinus* **sp. n.**

(Table 6; Figs 8–11)

**Material studied**. Holotype male (MOUFPE 0020), paratype female (MOUFPE 0021), 5 male paratypes (479–483 NM LMZOO-UFPE) and 1 female paratype (484 NM LMZOO-UFPE).

**Type locality**. South Atlantic Ocean, Continental shelf off the State of Alagoas, Brazil, station 11 (S 09°15'30.54" W 34°57'13.14"), November 26, 2019, 87 m.

**Locality of paratypes**. Paratype female 1: South Atlantic Ocean, Continental shelf off the State of Alagoas, Brazil, station 11 (S 09°15'30.54" W 34°57'13.14"), November 26, 2019, 87 m. Paratype males (1–3): South Atlantic Ocean, Continental shelf off the State of Alagoas, Brazil, station 12 (S 09°39'14.52" W 35°15'21.66"), November 25, 2019, 50 m. Paratype males (4 and 5): South Atlantic Ocean, Continental shelf off the State of Alagoas, Brazil, station 13 (S 09°56'55.68" W 35°39'51.78"), November 25, 2019, 44 m. Paratype female 2: South Atlantic Ocean, Continental shelf off the State of Rio Grande do Norte, Brazil, station 04 (S 06°27'06.06" W 34°45'53.64"), November 27, 2019, 56 m.

**Etymology**. Nordestinus is the latinized form of the term "nordestino". In Brazil, "nordestino" refers to something or someone originating from the northeastern region of the country.

**Holotype male**. Body cylindrical, 1450 µm long. Maximum body diameter corresponding to 2.8 times head diameter. Cuticle striated posteriorly to cephalic setae insertion. Four sublateral, two subventral and two subdorsal rows of hypodermal glands that begin after the amphidial fovea and extend longitudinally along the body. Hypodermal glands visible up to about 57% of the total length of the tail from the cloaca. Cuticular pores and somatic setae not observed. Anterior sensilla arranged in the 6+6+4 pattern: six inner labial papilliform sensilla, six outer labial papilliform sensilla and four cephalic sensilla (6 µm long), corresponding to 67% of head diameter. Head slightly set off. Amphidial fovea unispiral, located 17 µm from anterior end (1.9 times head diameter) and occupying 46% of corresponding body diameter. Buccal cavity weakly cuticularized. Cheilostoma rugae indiscernible under a light microscope. Three small teeth, difficult to see (a slightly larger dorsal tooth and two smaller ventrosublateral). Pharynx (108 µm long) with terminal oval bulb. Bulb occupying 67% of corresponding body diameter. Cardia embedded in intestine. Nerve ring situated at 66% of the pharynx length, from anterior end. Ventral gland and secretory-excretory pore not observed. Reproductive system with single anterior outstretched testis on the right side of intestine. ~~Sperm fusiform.~~ Spicules arched, with

proximal portion cephalized. Gubernaculum funnel-shaped surrounding the spicules at the distal end. Two precloacal papilla present. The closest being about 16 µm from the cloaca and the second at 24 µm. Three caudal glands. Tail conical with cylindrical terminal portion, 4.8 times the cloacal body diameter.

**Paratype female**. Similar to male. Body measuring 1404 µm in length, with a maximum diameter of 31 µm. Rows of hypodermal glands similar to the male. Hypodermal glands visible up to about 60% of the total length of the tail from the anus. Cephalic sensilla equivalent to 75% of head diameter. Amphidial fovea, occupying 36% of corresponding body width and located 17 µm from anterior end. Buccal cavity, teeth and pharynx similar to that of males. Basal bulb occupying 64% of the corresponding body diameter. Nerve ring situated at 58% of the pharynx length, from anterior end. Secretory-excretory located after the nerve ring and 74 µm from the anterior end. Ventral gland located immediately posterior to pharynx. Vulva located 756 µm from anterior end, at 54% of body length. Reproductive system didelphic-amphidelphic, with outstretched ovaries. In this paratype, the posterior ovary is apparently damaged. However, in Female paratype 1 it was possible to visualize the described pattern. Anterior ovary situated to the right side of intestine, posterior ovary to the left side of intestine. Three caudal glands. Tail conical, about 6.5 times the anal body diameter.

**Diagnosis**. *Microlaimus nordestinus* **sp. n.** characterized by its long body length (1080–1450.5 µm). Cuticle finely annulated. Head slightly set off. Cephalic setae 5–6 µm long and corresponding to 56–75% of head diameter. Amphidial fovea occupying 36–50% of the corresponding body diameter, located at about 1.6–2.1 times the head diameter. Buccal cavity with three small teeth, one of which is dorsal and two are ventrosublateral. Four sublateral, two subventral and two subdorsal rows of hypodermal glands that begin after the amphidial fovea and extend longitudinally along the body. Hypodermal glands visible up to about a half of the total length of the tail from the cloaca. Two precloacal papilla. Gubernaculum funnel-shaped surrounding the spicules at the distal end. Tail conical with cylindrical terminal portion (4.2–6.5 times the cloacal body diameter).

**Differential diagnosis.** The new species shares the following features with *Microlaimus cyatholaimoides* de Man, 1922: anterior sensilla arrangement, where the first two are circles of papilliform setae and the third is setiform; de Man's ratio c (9–12 in *M. cyatholaimoides* and 10–15 in M. *nordestinus* **sp. n.**); the presence of precloacal supplements and spicule length (33–34 µm in *M. cyatholaimoides* and 28–31 µm in *M. nordestinus* **sp. n.**). However, *M. cyatholaimoides* has a shorter total body length compared to the species described here (684–960 µm vs 1080–1450.5 µm in *M. nordestinus* **sp. n.**). Furthermore, the new specie differs from *M. cyatholaimoides* in terms of the shape of the gubernaculum (funnel-shaped surrounding the spicules at the distal end in the new specie vs lamellar in *M. cyatholaimoides*) and the presence of a conical tail with a cylindrical terminal portion vs conical tail in *M. cyatholaimoides*. Based on the illustrations provided by Man, 1922 the amphidial fovea of female *M. cyatholaimoides* is located 1 times the head diameter in relation to the anterior end, while in *M. nordestinus* **sp. n.** females this structure is 2.1 times the head diameter from the anterior end. Although both species have rows of hypodermic glands along the body, in *M. cyatholaimoides* these glands are longitudinally predominantly distributed along four sublateral rows (according to Hopper and Meyers, 1967). In *M. nordestinus* **sp. n.**, the glands are distributed longitudinally along eight rows: four sublateral, two subventral and two subdorsal.

The occurrence of rows of hypodermic glands has also been reported for the species *M. discolensis* Bussau, 1993, *M. porus* Bussau, 1993, *M. parviporosus* Miljutin & Miljutina, 2009 and *M. vitorius* Lima *et al*., 2022. For all the previously mentioned species, the occurrence of cuticular pores was also recorded. *M. sergeevae* Revkova, 2020 has rows of pores along the body, however the presence of rows of hypodermic glands was not mentioned. The occurrence of cuticular pores cannot be observed in any of the specimens of the new species.

*Microlaimus nordestinus* **sp. n.** differs from *M. discolensis* in terms of total body length (1080–1450.5 µm vs 425–565 µm in the latter species); external labial setae papilliform and cephalic setae setiform, while these structures are setiform and are about the same length in *M. discolensis* and with regard to de Man's ratio c' (4.2–6.5 in the new specie vs 2.3–3.3 in *M. discolensis*). Moreover, the tail is conical-shaped with a cylindrical terminal portion in the new species vs conical in *M. discolensis*.

The new species differs from *M. porus* in terms of total body length (2.4–3.8 times longer), the presence of two precloacal supplements vs absent in *M. porus* and the shape of the gubernaculum (funnel-shaped surrounding the spicules at the distal end in the new specie vs lamellar in *M. porus*). Moreover, *M. nordestinus* **sp. n.** has three small teeth vs two visible teeth in *M. porus*.

*Microlaimus nordestinus* **sp. n.** differs from *M. parviporosus* with regard to its cephalic setae which are much longer than the outer labial ones, whereas the outer labial setae and the cephalic setae are about the same length in *M. parviporosus*. Additionally, it differs from *M. parviporosus* in terms of the longer spicule (28–31 μm vs 16–18 μm), the shape of the gubernaculum (funnel-shaped surrounding the spicules at the distal end in the new specie vs rod-like, slightly bent anteriorly in *M. parviporosus*), the presence of precloacal supplements (vs absent in *M. parviporosus*) and body length, which is 2.6–4 times greater compared to that of *M. parviporosus*.

*Microlaimus nordestinus* **sp. n.** resembles *M. sergeevae* and *M. vitorius* in terms of the shape of the gubernaculum. In these species, this structure surrounds the spicule in its distal portion. Nevertheless, *M. nordestinus* **sp. n.** differs from *M. sergeevae* in terms of the absence of cervical setae (vs present in *M. sergeevae*), the tail (conical with cylindrical terminal portion without rows of setae in the new species vs conical with a slightly swollen final portion and a row of subventral setae in *M. sergeevae*) and the precloacal supplement (two papilla in *M. nordestinus* **sp. n.** vs eight thin channels in *M. sergeevae*). The new species differs from *M. vitorius* with regard to tail shape (conical with a cylindrical terminal portion in the new species vs conical in *M. vitorius*), shorter spicules and gubernaculum (spicules: 28–31 μm vs 45–55 μm; gubernaculum: 13–17 μm vs 20–27 μm), the presence of three small teeth in *M. nordestinus* **sp. n.** vs. three large teeth in *M. vitorius*, the position of the amphidial fovea (relatively further from the anterior end in the new species compared to *M. vitorius*: ratio between the distance from the amphidial fovea to the anterior end and the head diameter= 1.6–2.1 in *M. nordestinus* **sp. n.** vs 0.5–0.9 in *M. vitorius*) and the precloacal supplement (two papilla in *M. nordestinus* **sp. n.** vs three small pores in *M. vitorius*).

## Discussion

Although most species of the genus *Microlaimus* have three teeth in the buccal cavity, descriptive information on the species belonging to this genus varies with regard to this characteristic. Some species have an unarmed buccal cavity, as described for *M. nympha* Bussau, 1993; armed with two teeth, as described by Bussau 1993 and redescribed by Miljutin & Miljutina 2009 for *M. porus*; or with five teeth, as described for *M. alexandri* Lima *et al*, 2022. We added variability in the number of teeth present in the buccal cavity to the diagnosis of the genus.

Specific characteristics, such as the relationship between the length of the cephalic setae and head diameter (%), the diameter of the amphidial fovea in the corresponding region of the body (%) and its position in relation to the anterior end of the body, helped to approximate *M. paraundulatus* **sp. n.** and *M. modestus* **sp. n.** to the most morphologically similar known species. The use of this combination of characters is frequently used in descriptions of *Microlaimus* species to express similarity or to highlight differences between species (Kovalyev & Tchesunov 2005, Gagarin & Tu 2014, Revkova 2020, Lima *et al*. 2022). Taxonomic tools, such as de Man's ratios (a, b, c and c') and proportions between spicule length/cloacal body diameter, gubernaculum length/spicule length (%), as well as the presence and absence of cuticular pores and precloacal supplements, helped to highlight the differences between the new species and the known species that are most morphologically similar to them. Additionally, the presence of rows of hypodermic glands, such as those visualized in *M. nordestinus* **sp. n.**, which may or may not be associated with pores or/and setae, can also be used as a diagnostic feature to differentiate between *Microlaimus* species (Jensen 1978; Hopper & Meyers 1967; Muthumbi & Vincx 1999).

Our results recorded the first three species of the *Microlaimus* genus described from samples collected on the Continental Shelf off Northeast Brazil. The present study increases our knowledge on the species of this taxon present in the South Atlantic and significantly expands the available knowledge on the species richness of the genus, increasing the number of

*Microlaimus* species originally described from sediment samples collected on the coast of Brazil from seven to ten.

## References

Allgén, C.A. (1930) Über einige neue oder wenig bekannte Brachwasser-Nematoden von der Litoralzone des Öresunds. *Zoologischer Anzeiger*, 85: 58–72.

Bussau, C. (1993) *Taxonomische und ökologische Untersuchungen an Nematoden des Peru-Beckens*. Doctoral thesis, Kiel, 621 pp.

Cobb, N.A. (1920) One hundred new nemas (type species of 100 new genera). *Contributions to a science of nematology*, 9, 217–343.

De Grisse, A.T. (1969) Redescription ou modification de quelques techniques utilisées dans l'étude dês nématodes phytoparasitaires. *Mededelingen van de Rijksfakulteit Landbouwwetenschappen te Gent*, 34, 351–369.

de Man, J.G. (1880) Die einheimischen, frei in der reinen Erde und im süβen Wasser lebenden Nematoden monographisch bear-beitet. Vorläufiger Bericht und descriptiv-systematischer Theil. *Tijdschrift der Nederlandsche Dierkundige Vereenigung*, 5, 1–104.

de Man, J.G. (1922) Über einige marine Nematoden von der Küste von Walcheren, neu für die Wissenschaft und für unsere Fauna, unter welchen der sehr merkwürdige *Catalaimus Max Weberi* n. sp. *Bijdragen tot de Dierkunde (Feest-Nummer Max Weber)*, 22(1):117–124. https://doi.org/10.1163/26660644-02201017

Decraemer, W. & Smol, N. (2006) Orders Chromadorida, Desmodorida and Desmoscolecida. In: Eyualem, A., Traunspurger, W. & Andrassy, I. (Eds.), *Freshwater Nematodes: Ecology and Taxonomy*. CABI Publishing, Wallingford, pp. 497–573. https://doi.org/10.1079/9780851990095.0497

Filipjev, I.N. (1934) The classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithsonian Miscellaneous Collections*, 89 (6), 1–63, pl. 1–8.

Gagarin, V.G. & Tu, N.D. (2014) *Paracomesoma minor* sp. n. and *Microlaimus validus* sp. n. (Nematoda) from the coast of Vietnam. *Zootaxa*, 3856 (3): 366–374.
http://dx.doi.org/10.11646/zootaxa.3856.3.4

Gerlach, S.A. (1953) Die Nematodenbesiedlung des Sandstrandes und des Küstengrundwassers an der italienischen Küste I. Systematischer Teil. *Archivio Zoologico Italiano,* 37:517–640.

Gerlach, S.A. (1956) Brasilianische Meeres-Nematoden I. *Boletim do Instituto Oceanográfico, São Paulo Tomo*, 5 (1-2): 3–69.

Gerlach, S.A. (1957a) Marine Nematoden aus dem Mangrove-Gebiet von Cananéia (Brasilianische Meeres-Nematoden III). *Abhandlungen der mathematisch-naturwissenschaftlichen Klasse. Akademie der Wissenschaften und der Literatur in Mainz*, 5:129–176.

Gerlach, S.A. (1957b) Die Nematodenfauna des Sandstrandes an der Küste von Mittelbrasilien (Brasilianische Meerse-Nematoden IV). *Mitteilungen aus dem Zoologischen Museum in Berlin*, 33 (2): 411–459.

Gerlach, S.A. (1963) *Aponchium* Cobb, 1920, Typus einer neuen Familie freilebender Meeres-Nematoden. *Abhandlungen und Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg*, Neue Folge, 7, 157–166.

Hoeppli, R.J.C. (1926) Studies of free-living nematodes from the thermal waters of yellowstone park. *Transactions of the American Microscopical Society*, 45(3):234–255.

Hopper, B.E. & Meyers, S.P. (1967) Foliicolous marine nematodes on turtle grass, Thalassia testudinum König, in Biscayne Bay, Florida. *Bulletin of Marine Science*, 17(2):471–517.

Inglis, W.G. (1983) An outline classification of the Phylum Nematoda. *Australian Journal of Zoology*, 31(2):243–255.
https://doi.org/10.1071/ZO9830243

Jayasree, K. & Warwick, R.M. (1977) Free-living marine nematodes of a polluted sandy beach in the Firth of Clyde, Scotland. Description of seven new species. *Journal of Natural History*, 11, 289–302.

Jensen, P. (1978) Revision of Microlaimidae, erection of Molgolaimidae fam.n., and remarks on the systematic position of *Paramicrolaimus* (Nematoda, Desmodorida). *Zoologica Scripta*, 7 (3), 159–173.
https://doi.org/10.1111/j.1463-6409.1978.tb00599.x

Jensen, P. (1988) Four new nematode species, abundant in the deep-sea benthos of the Norwegian Sea. *Sarsia*, 73, 149–155.
https://doi.org/10.1080/00364827.1988.10420682

Kovalyev, S.V. & Tchesunov, A.V. (2005) Taxonomic review of microlaimids with description of five species from the White Sea (Nematoda: Chromadoria). *Zoosystematica Rossica*, 14, 1–16.
https://doi.org/10.31610/zsr/2005.14.1.1

Leduc, D. (2016) One new genus and three new species of deep-sea nematodes (Nematoda: Microlaimidae) from the Southwest Pacific Ocean and Ross Sea. *Zootaxa*, 4079 (2), 255–271.
https://doi.org/10.11646/zootaxa.4079.2.7

Leduc, D. & Wharton, D.A. (2008) Three new species of free-living nematodes from inter-tidal sediments in southern New Zealand. *Nematology*, 10(5):743–755.
https://doi.org/10.1163/156854108785787163

Leduc, D., Verdon, V. & Zhao, Z.Q. (2017) Phylogenetic position of the Paramicrolaimidae, description of a new *Paramicrolaimus* species and erection of a new order to accommodate the Microlaimoidea (Nematoda: Chromadorea). *Zoological Journal of the Linnean Society*, 183 (1), 1–18.
https://doi.org/10.1093/zoolinnean/zlx072

Lima, R.C., Neres, P.F., & Esteves, A.M. (2022) Three new species of *Microlaimus* (Nematoda: Microlaimidae) from the South Atlantic. *PeerJ*, (10): e12734.
https://doi.org/ 10.7717/peerj.12734

Micoletzky, H. (1922) Zur Nematodenfauna des Bodensees. *Internationale Revue der Gesamten Hydrobiologie und Hydrographie*, 10, 491–512.
https://doi.org/10.1002/iroh.19220100406

Miljutin, D.M. & Miljutina, M.A. (2009) Deep-sea nematodes of the family Microlaimidae from the Clarion-Clipperton Fractre Zone (North-Eastern Tropic Pacific), with the description of three new species. *Zootaxa*, 2096, 137–172.
https://doi.org/10.11646/zootaxa.2096.1.11

Muthumbi, A.W. & Vincx, M. (1999) Microlaimidae (Microlaimoidea: Nematoda) from the Indian Ocean: description of nine new and known species. *Hydrobiologia*, 397:39–58.
https://doi.org/10.1023/A:1003686212934

Nemys eds. (2023) Nemys: World Database of Nematodes. Accessed at https://nemys.ugent.be on 2023-11-23. doi:10.14284/366

Pastor de Ward, C.T. (1980) *Aponema papillatum* sp.nov., a new species of free-living marine nematodes from the Ria Deseado (Santa Cruz, Argentina). [*Aponema papillatum* sp. nov., nueva especie de nematode marino de puerto deseado, (Santa Cruz, Argentina)]. *Centro de Investigacion de Biologia Marina Contribucion Cientifica*, 160: 1–11.

Pastor de Ward, C. T. (1989) Nematodes marinos de la Ria Deseado (Microlaimoidea: Microlaimidae, Monoposthidae), Santa Cruz, Argentina. VIII. *Physis*, 47 (112): 1–12.

Pearse A.S. (1942) *An introduction to parasitology*. Springfield: Charles C. Thomas.

Revkova, T.N. (2020) A new species of *Microlaimus* de Man 1880 (Nematoda: Microlaimidae) from the Black Sea. *Zootaxa*, 4772 (1): 183–188.
https://doi.org/10.11646/zootaxa.4772.1.6

Schulz, E. (1932) Beiträge zur Kenntnis mariner Nematoden aus der Kieler Bucht. *Zoologische Jahrbücher (Systematik)*, 62:331–430.

Somerfield, P.J., Warwick, R.M. & Moens, T. (2005) Meiofauna techniques. In: Eleftheriou, A. & McIntyre, A. (Eds.), *Methods for the Study of Marine Benthos*. *3rd Edition*. Blackwell, Oxford, pp. 229–272.
https://doi.org/10.1002/9780470995129.ch6

Tchesunov, A.V. (2014) Order Desmodorida De Coninck, 1965. In: Shmidt-Rhaesa, A. (Ed.), *Handbook of Zoology Gastrotricha, Cyclioneura and Gnathifera. Vol. 2. Nematoda*. De Gruyter, Hamburg, pp. 399−434.

Tchesunov, A.V., Jeong, R. & Lee, W. (2021) A new genus and species of the family Microlaimidae (Nematoda: Chromadorea) from intertidal sand of the Jeju Island, South Korea. *Zootaxa*, 5020 (1): 130–140.
https://doi.org/10.11646/zootaxa.5020.1.6

Turpeenniemi, T. A. (1997) Four new nematode species from the Bothnian Bay, Northern Baltic Sea, with a redescription of *Microlaimus globiceps* de Man, 1880 (Nematoda). *Nematologica*, 43(1): 31–58.

Warwick, R.M., Platt, H.M. & Somerfield, P.J. (1998) *Free-living Marine Nematodes Part III. Monhysterids. Synopses of the British fauna (New Series)*. 53. Field Studies Council, Shrewsbury, VII + 296 pp. [ISBN 1-85153-260-9]

## Table 1(on next page)

Collection stations, their respective coordinates and depth. The samples were collected at the break of the continental shelf in Northeast Brazil, South Atlantic.

1

2

| Station | Latitude | Longitude | Depth |
|---|---|---|---|
| 2 | S 05°42'54.42" | W 34°59'31.92" | 60 m |
| 4 | S 06°27'06.06" | W 34°45'53.64" | 56 m |
| 11 | S 09°15'30.54" | W 34°57'13.14" | 87 m |
| 12 | S 09°39'14.52" | W 35°15'21.66" | 50 m |
| 13 | S 09°56'55.68" | W 35°39'51.78" | 44 m |
| 16 | S 10°44'59.28" | W 36°25'32.88" | 58 m |

3

## Table 2(on next page)

Morphometric data of *Microlaimus paraundulatus* sp. n. The measurements are expressed in micrometers, or if noted, as a percentage or ratio. Not applicable (*); not available for measurement (-); a, b, c, c’ = de Man’s ratios (1880).

1

2

| *Microlaimus paraundulatus* **sp. n.** | Holotype male | Paratype male | Paratype female |
|---|---|---|---|
| Body length | 457.5 | 439 | 465 |
| Cephalic setae length | 3 | 3 | 3 |
| Head diameter at level of the cephalic setae | 7 | 7 | 7 |
| Cephalic setae in relation to head diameter (%) | 43% | 43% | 43% |
| Distance from anterior end to amphidial fovea | 11 | 11 | 13 |
| Distance from anterior end to amphidial fovea in relation to head diameter | 1.6 | 1.6 | 1.9 |
| Amphidial fovea diameter (maximum width) | 4 | 4 | 5 |
| Body diameter at level of the amphidial fovea | 9 | 9 | 9 |
| % of the amphidial fovea diameter in relation to corresponding body diameter | 44% | 44% | 56% |
| Pharynx length | 82 | 83 | 84 |
| Position of nerve ring from anterior end | 47 | - | 49.5 |
| Nerve ring position in relation to pharynx length (%) | 57% | - | 59% |
| Pharyngeal bulb diameter | 11 | 11 | 12 |
| Body diameter at level of the pharyngeal bulb | 14 | 15 | 15 |
| % of basal bulb diameter in relation to corresponding body diameter | 79% | 73% | 80% |
| Maximum body diameter | 15 | 16 | 18 |
| Anal or cloacal body diameter | 12 | 13 | 10 |
| Tail lenth | 59 | 55.5 | 55 |
| Length of spicule along arc | 27 | 23 | * |
| Length of spicule along cord | 18 | 16 | * |
| Length of gubernaculum | 13 | 13 | * |
| Length of gubernaculum in relation to length of spicule along arc (%) | 48% | 57% | * |
| Length of spicule along arc in relation to cloacal body diameter | 2.3 | 1.8 | * |
| Distance from anterior end to vulva | * | * | 244.5 |
| Position of vulva from anterior end (%) | * | * | 53% |
| Body diameter in vulva region | * | * | 18 |
| Anterior ovary length | * | * | 110 |
| Posterior ovary length | * | * | 95 |
| Reproductive system | 303 | 224 | 205 |
| % of reproductive system in relation to body length | 66% | 51% | 44% |
| a | 30.5 | 27 | 26 |
| b | 6 | 5 | 5.5 |
| c | 8 | 8 | 8 |
| c' | 5 | 4 | 6 |

3

## Table 3(on next page)

Comparison of species *Microlaimus paraundulatus* sp. n. with morphologically similar species (only males).

Information (measurements/proportions) from males of the species *Microlaimus* that concomitantly have cephalic setae <50% of head diameter; amphidial fovea between 1.6 and 1.9 times the head diameter; amphidial fovea <50% of the head body diameter.

The measurements are expressed in micrometers, or if noted, as a percentage or ratio. Present (+) or absent (-); a, b, c, c’ = de Man’s ratios (1880); distance of amphidial fovea from anterior end in relation to head diameter (Amph/hd); percentage of the amphidial fovea diameter in relation to corresponding body diameter (amph%).

| | *M. copulatus* | *M. limnophilus* | *M. porus* | *M. zosterae** | *M. paraundulatus* |
|---|---|---|---|---|---|
| Body length | 320–330 | 401–470 | 380 | 618–621 | 439–457.5 |
| a | 18–20 | 22.8–29.3 | 21.1 | 27–29.4 | 27–30.5 |
| b | 4.1–4.6 | 5.6–6.5 | 4.9 | 6.7–7.2 | 5–6 |
| c | 6–7.2 | 7–8.8 | 5.4 | 9.4–9.9 | 8 |
| c' | 3.2 | 3.2–4.1 | 5.1 | 3.6–3.9 | 4–5 |
| Cephalic setae in relation to head diameter (%) | 17% | 23–25% | 38% | 20% | 43% |
| amph% | 40% | 33–35% | < 50%** | 43–45% | 44% |
| Amph/hd | 1.8 | 1.9 | 1.6 | 1.9 | 1.6 |
| Length of spicule along arc in relation to cloacal body diameter | 2.7–2.8 | 1.3 | 1.7 | 1.6 | 1.8–2.3 |
| Length of gubernaculum in relation to length of spicule along arc (%) | 30% | 47% | 42% | 60% | 48–56% |
| Precloacal supplements | + | + | - | + | - |
| Cuticular pores | - | - | + | - | - |

*Based on the redescription of the species *Microlaimus zosterae* Allgén, 1930 provided by Kovalyev & Tchesunov 2005.
**Proportion measured from the drawing of the female paratype. Missing information for the holotype male.

## Table 4(on next page)

Morphometric data of *Microlaimus modestus* sp. n. The measurements are expressed in micrometers, or if noted, as a percentage or ratio. Not applicable (*); not available for measurement (-); a, b, c, c’ = de Man’s ratios (1880).

1

2

| *Microlaimus modestus* **sp. n.** | Holotype male | Paratype male 1 | Females paratypes |
|---|---|---|---|
| Body length | 342 | 344 | 331.5–359 |
| Cephalic setae length | 2 | 2 | 2 |
| Head diameter at level of the cephalic setae | 9 | 9 | 8–9 |
| Cephalic setae in relation to head diameter (%) | 22% | 22% | 22–25% |
| Distance from anterior end to amphidial fovea | 10 | 11 | 10–12 |
| Distance from anterior end to amphidial fovea in relation to head diameter | 1.1 | 1.2 | 1.1–1.5 |
| Amphidial fovea diameter (maximum width) | 6 | 6 | 5–6 |
| Body diameter at level of the amphidial fovea | 10.5 | 11 | 10 |
| % of the amphidial fovea diameter in relation to corresponding body diameter | 57% | 55% | 50–60% |
| Pharynx length | 82 | 82 | 78–85.5 |
| Position of nerve ring from anterior end | 51 | 48 | 47–49 |
| Nerve ring position in relation to pharynx length (%) | 62% | 59% | 57%–63% |
| Pharyngeal bulb diameter | 15 | 17 | 13–17 |
| Body diameter at level of the pharyngeal bulb | 17 | 19 | 18–21 |
| % of basal bulb diameter in relation to corresponding body diameter | 88% | 89% | 65–84% |
| Position of secretory-excretory pore from anterior end | 56 | 56 | 53 |
| Maximum body diameter | 20 | 20 | 22–29.5 |
| Anal or cloacal body diameter | 15 | 16 | 13–16 |
| Tail lenth | 48 | 54 | 35–57 |
| Length of spicule along arc | 34 | 32 | * |
| Length of spicule along cord | 20 | 22 | * |
| Length of gubernaculum | 19 | 16 | * |
| Length of gubernaculum in relation to length of spicule along arc (%) | 56% | 50% | * |
| Length of spicule along arc in relation to cloacal body diameter | 2.3 | 2.0 | * |
| Distance from anterior end to vulva | * | * | 195–207 |
| Position of vulva from anterior end (%) | * | * | 57–59% |
| Body diameter in vulva region | * | * | 22–29 |
| Anterior ovary length | * | * | 49–65 |
| Posterior ovary length | * | * | 52.5–65 |
| Reproductive system length | 163 | 158 | 107.5–130 |
| % of reproductive system in relation to body length | 48% | 46% | 31–37% |
| a | 17 | 17 | 12–16 |
| b | 4 | 4 | 4.1–4.4 |
| c | 7 | 6 | 6–9 |
| c' | 3 | 3 | 3–4 |

3

4

## Table 5(on next page)

Comparison of the species Microlaimus modestus sp. n. with morphologically similar species (only males).

Information (measurements/proportions) from males of the species Microlaimus that concomitantly have cephalic setae ≤50% of the head diameter; amphidial fovea between 1.1 and 1.5 times the head diameter; amphidial fovea >50% of head diameter.

The measurements are expressed in micrometers, or if noted, as a percentage or ratio. Present (+) or absent (-); a, b, c, c’ = de Man’s ratios (1880); distance of amphidial fovea from anterior end in relation to head diameter (Amph/hd); percentage of the amphidial fovea diameter in relation to corresponding body diameter (amph%).

1

2

3

| | *M. acanthus* | *M. microseta* | *M. modestus* |
|---|---|---|---|
| L | 845–1175 | 1205 | 342–344 |
| a | 31.3–36.7 | 55 | 17 |
| b | 7.4–8 | 8.6 | 4 |
| c | 9.9–10.7 | 14.2 | 6–7 |
| c' | 3.5 | 4.25 | 3 |
| Cephalic setae in relation to head diameter (%) | 40–50% | 25% | 20% |
| amph% | 67–75% | 60% | 57–55% |
| Amph/hd | 1.1–1.8* | 1.1 | 1.1–1.2 |
| Length of spicule along arc in relation to cloacal body diameter | 1.1–1.4 | 1.3 | 2–2.3 |
| Length of gubernaculum in relation to length of spicule along arc (%) | 53–60% | 54% | 50–56% |
| Precloacal supplements | + | - | - |
| Cuticular pores | - | - | - |

4 **M. acanthus* showed a greater variation in the relative position of the amphidial fovea (1.1–1.8 times the head diameter). Despite
5 this, due to other similarities with the new species, we will include it in the table for comparison purposes.

## Table 6(on next page)

Morphometric data of Microlaimus nordestinus sp. n. The measurements are expressed in micrometers, or if noted, as a percentage or ratio. Not applicable (*); not available for measurement (-); a, b, c, c’ = de Man’s ratios (1880).

1

2

3

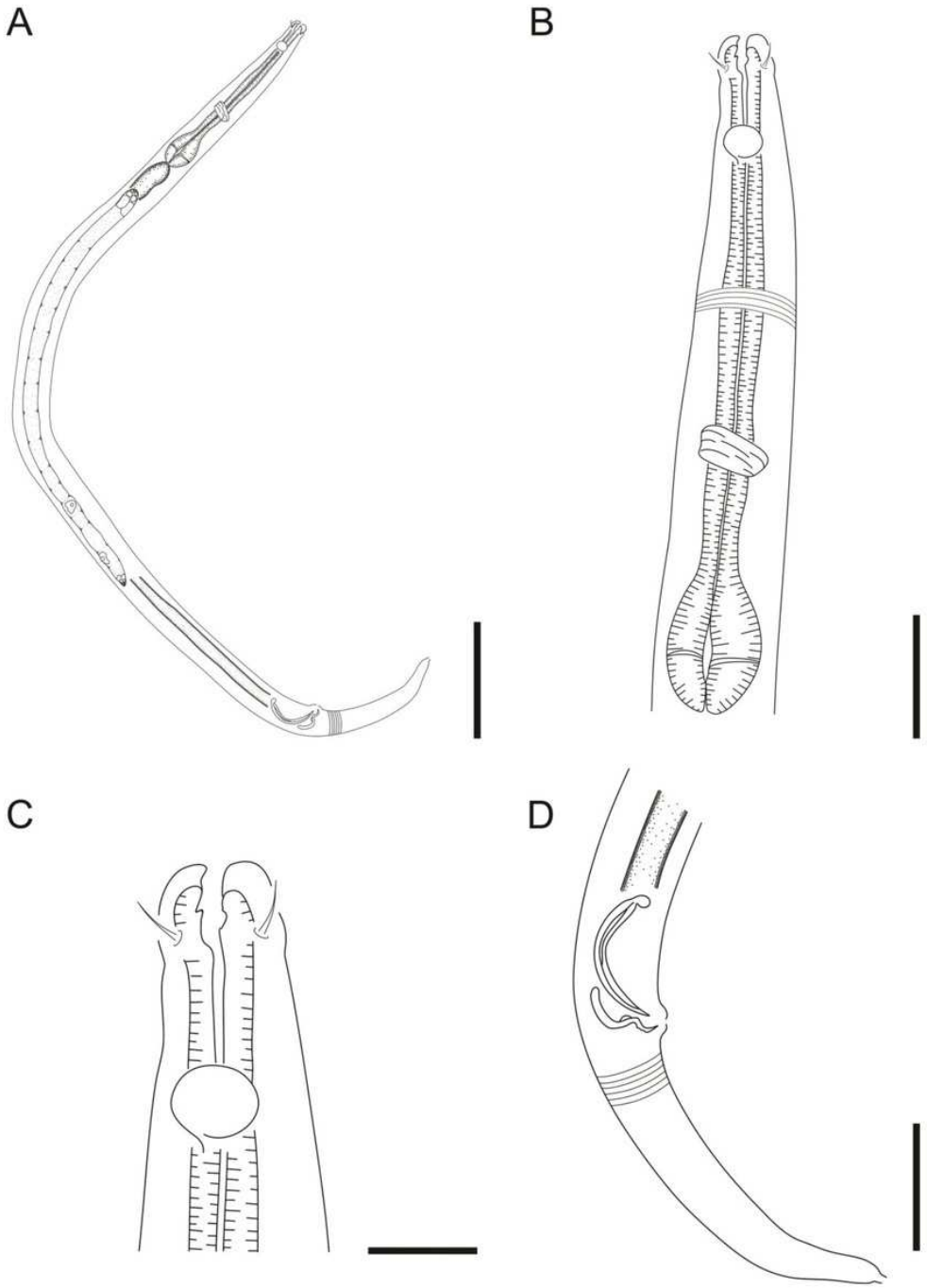
| *Microlaimus nordestinus* **sp. n.** | Holotype male | Males paratypes | Females paratypes |
|---|---|---|---|
| Body length | 1450 | 1273.5–1405.5 | 1080–1404 |
| Cephalic setae length | 6 | 5–6 | 6 |
| Head diameter at level of the cephalic setae | 9 | 8–10 | 8 |
| Cephalic setae in relation to head diameter (%) | 67% | 56–67% | 75% |
| Distance from anterior end to amphidial fovea | 17 | 14–19 | 17 |
| Distance from anterior end to amphidial fovea in relation to head diameter | 1.9 | 1.6–2.1 | 2.1 |
| Amphidial fovea diameter (maximum width) | 6 | 5–6 | 5 |
| Body diameter at level of the amphidial fovea | 13 | 12–15 | 12–14 |
| % of the amphidial fovea diameter in relation to corresponding body diameter | 46% | 36%–43% | 36–42% |
| Pharynx length | 108 | 98–107 | 97–103 |
| Position of nerve ring from anterior end | 66 | 58–68 | 60 |
| Nerve ring position in relation to pharynx length (%) | 61% | 54%–65% | 58% |
| Pharyngeal bulb diameter | 16 | 16% | 16 |
| Body diameter at level of the pharyngeal bulb | 24 | 23–24 | 25 |
| % of basal bulb diameter in relation to corresponding body diameter | 67% | 67%–70% | 64% |
| Maximum body diameter | 25 | 25–31 | 26–31 |
| Anal or cloacal body diameter | 21 | 20–25 | 16–17 |
| Tail lenth | 101 | 94–105 | 103.5–111 |
| Length of spicule along arc | 31 | 28–31 | * |
| Length of spicule along cord | 27 | 25.5–28 | * |
| Length of gubernaculum | 14 | 13–17 | * |
| Length of gubernaculum in relation to length of spicule along arc (%) | 45% | 42–55% | * |
| Length of spicule along arc in relation to cloacal body diameter | 1.5 | 1.2–1.5 | * |
| Precloacal supplement closest to cloaca | 16 | 13–17 | * |
| Precloacal supplement farthest from the cloaca | 24 | 21–28 | * |
| Distance from anterior end to vulva | * | * | 540–756 |
| Position of vulva from anterior end (%) | * | * | 50–54% |
| Body diameter in vulva region | * | * | 26 |
| Anterior ovary length | * | * | 130.5–246 |
| Posterior ovary length | * | * | 132 |
| Reproductive system length | 834 | 784 | 262.5 |
| % of reproductive system in relation to body length | 58% | 57% | 24% |
| a | 58 | 42–55 | 41.5–45 |
| b | 13 | 12.5–14 | 11–14 |
| c | 14 | 13–15 | 10–13 |
| c' | 5 | 4–5 | 6.5 |

4

# Figure 1

Microlaimus paraundulatus sp. n. Holotype male.

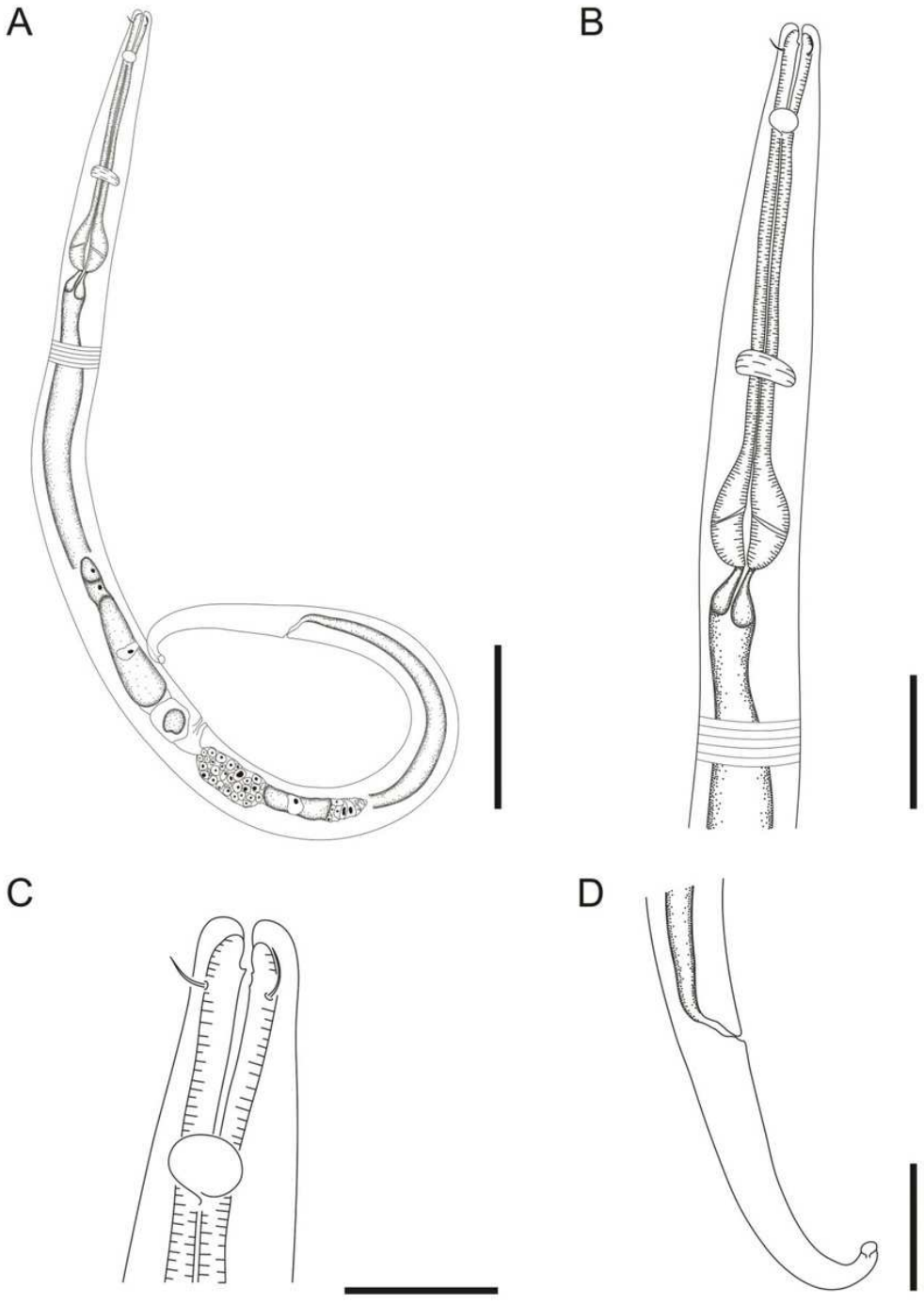
(A) overview, (B) anterior region, (C) anterior end, (D) posterior region. Scale bars: A= 50 µm, B= 15 µm, C= 5 µm and D = 20 µm.


A
B
C
D

# Figure 2

Microlaimus paraundulatus sp. n. Paratype female.

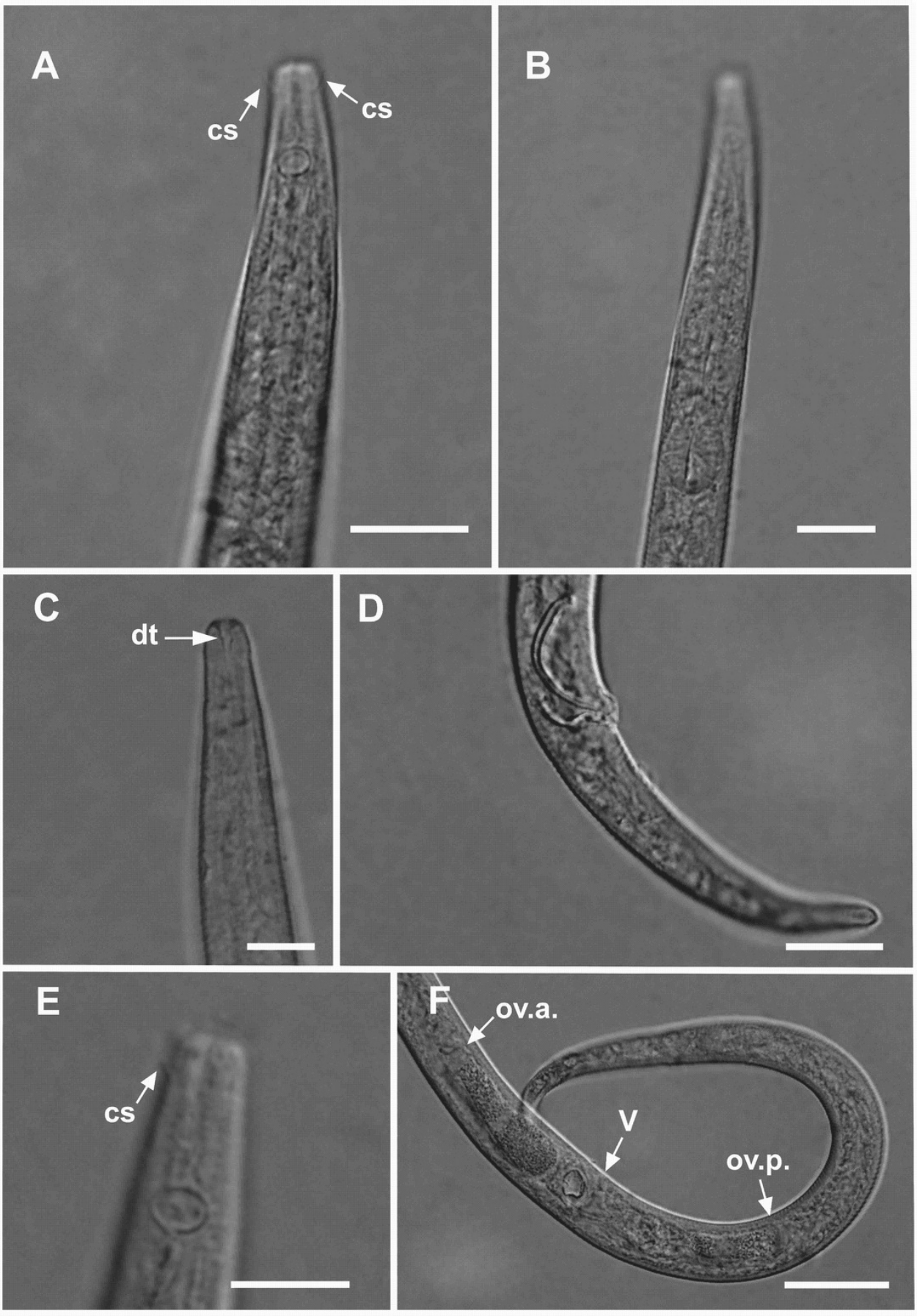
(A) overview, (B) anterior region, (C) anterior end, (D) tail. Scale bars: A= 50 µm, B and D= 20 µm C= 10 µm.


A
B
C
D

# Figure 3

Microlaimus paraundulatus sp. n. Holotype male, Paratype male and paratype female.

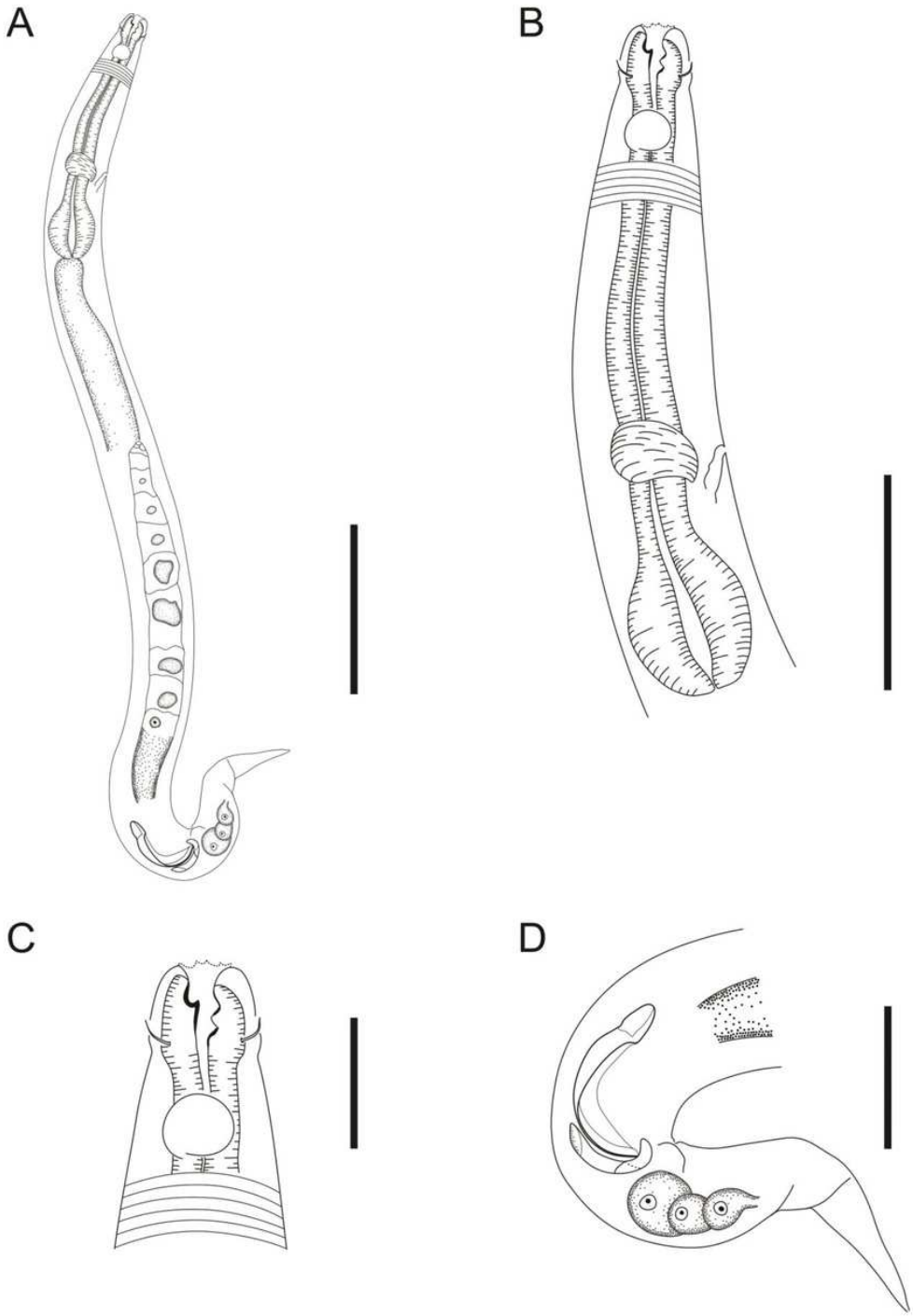
Holotype male: (A) anterior end (arrows indicating cephalic setae), (B) anterior region (pharynx and bulb). Paratype male: (C) anterior end (arrow indicating dorsal tooth). Holotype male: (D) spicule and gubernaculum. Paratype female: (E) anterior end (arrow indicating cephalic setae), (F) reproductive system (arrows indicating: V= vulva; ov.a.= anterior ovary; ov.p.= posterior ovary). Scale bars: A and B= 15 µm, C and E= 10 µm, D= 20 µm and F= 30 µm.


A
cs
cs
B
C
dt
D
E
cs
F
ov.a.
V
ov.p.

# Figure 4

Microlaimus modestus sp. n. Holotype male.

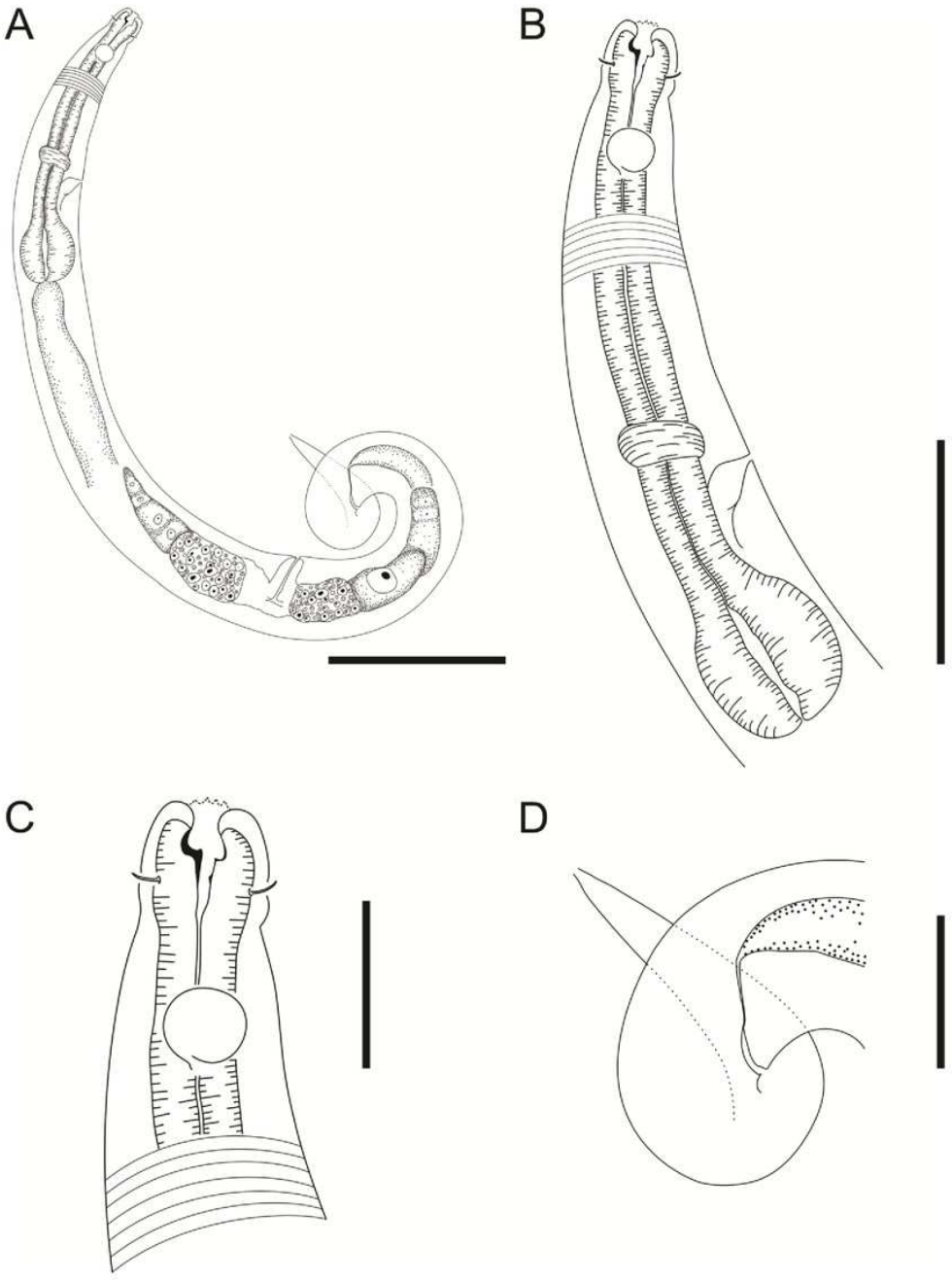
(A) overview, (B) anterior region, (C) anterior end, (D) posterior region. Scale bars: A= 50 µm, B= 25 µm C= 10 µm and D = 20 µm.


A
B
C
D

# Figure 5

Microlaimus modestus sp. n. Paratype female.

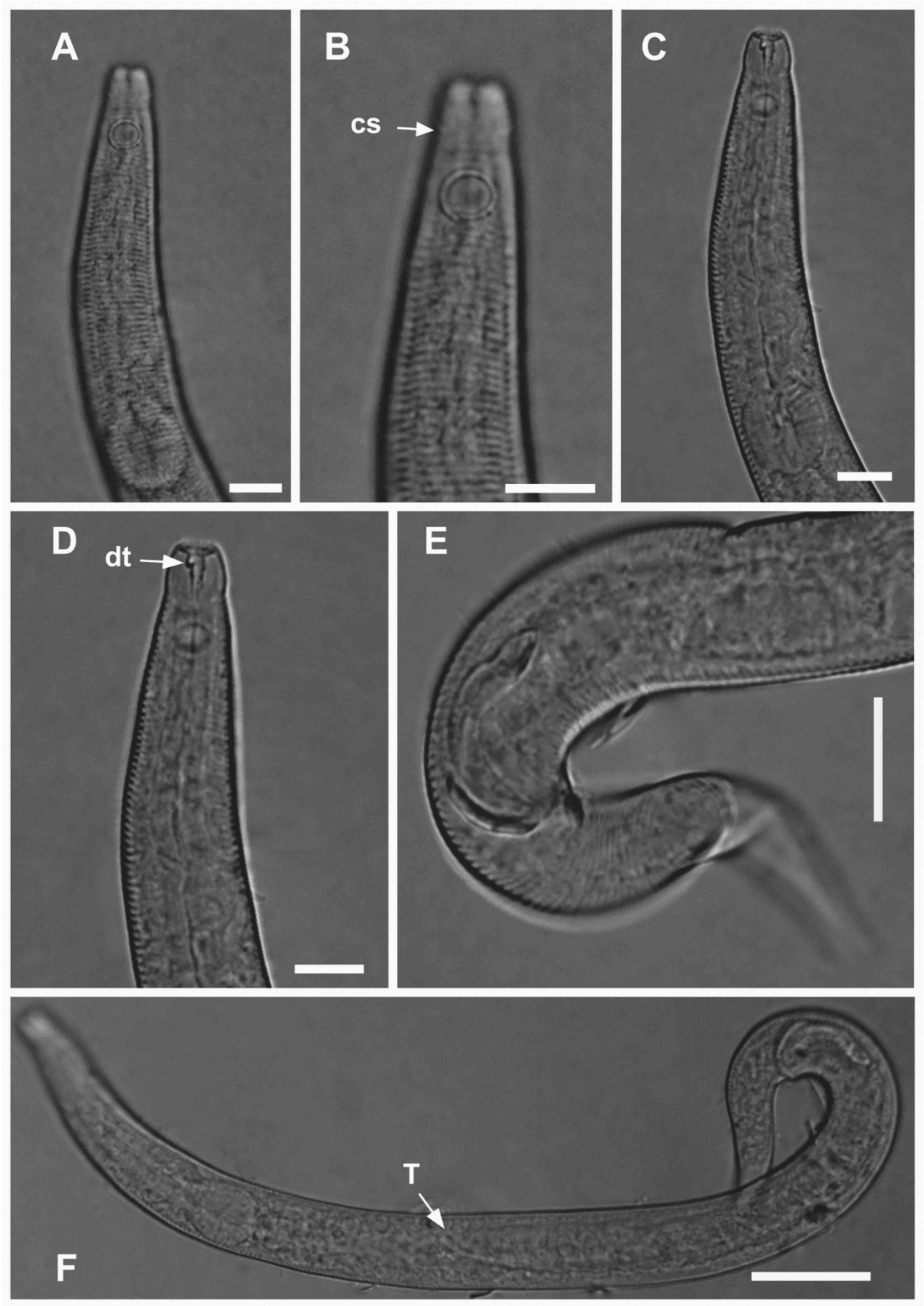
(A) overview, (B) anterior region, (C) anterior end, (D) posterior region. Scale bars: A= 50 µm, B= 25 µm C= 10 µm and D = 15 µm.


A
B
C
D

# Figure 6

Microlaimus modestus sp. n. Holotype male and paratype male

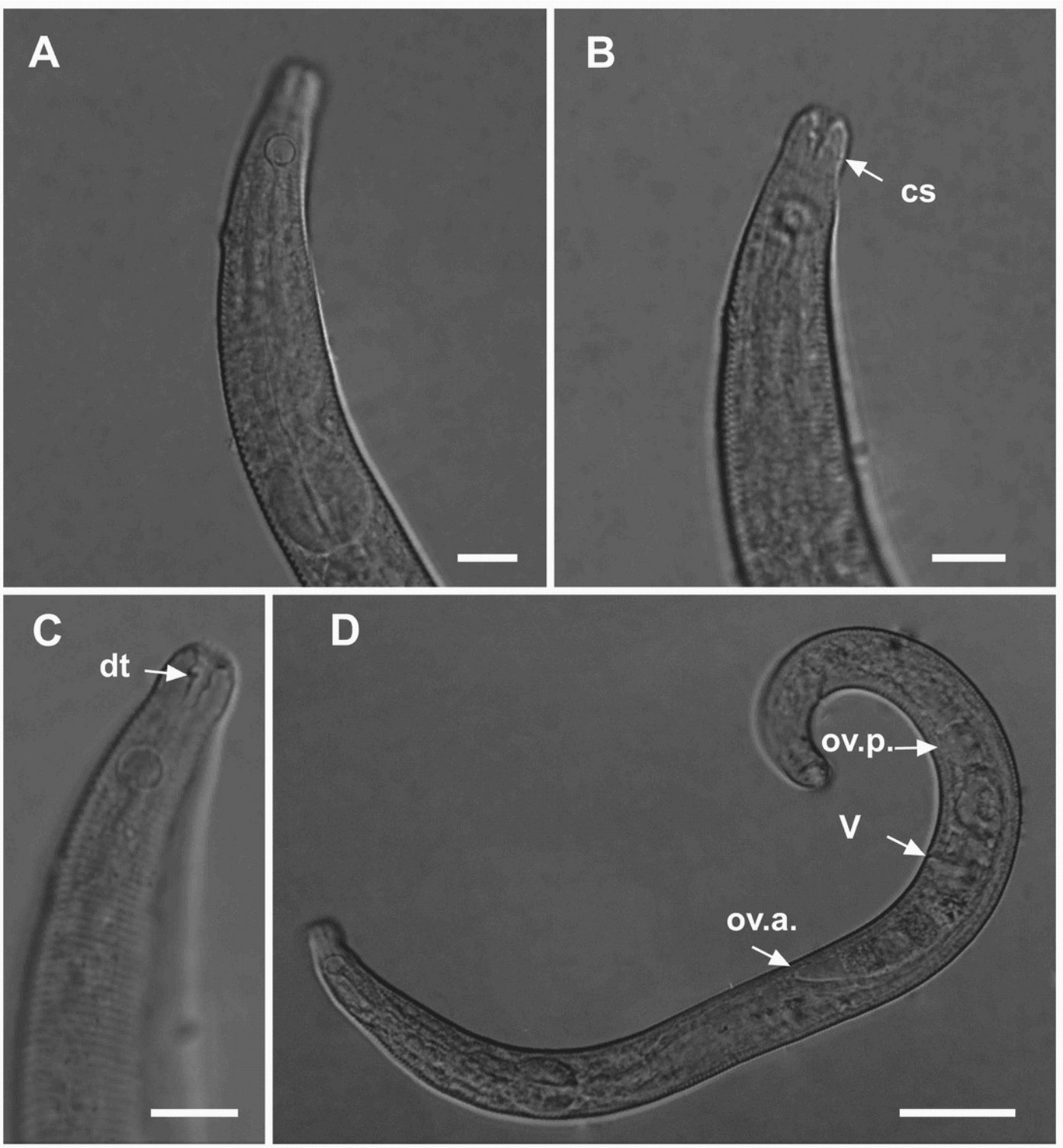
Holotype male (A) anterior region, (B) anterior region (arrow indicating cephalic setae), (C) anterior region (pharynx and bulb), (D) anterior end (arrow indicating dorsal tooth), (E) posterior end (spicule and gubernaculum). Paratype male: (F) habitus (arrow indicating testicle). Scale bars: A, B, C and D= 10 µm, E= 15 µm F= 30 µm.


A
B
cs
C
D
dt
E
T
F

# Figure 7

Microlaimus modestus sp. n. Paratype female and paratype female 2

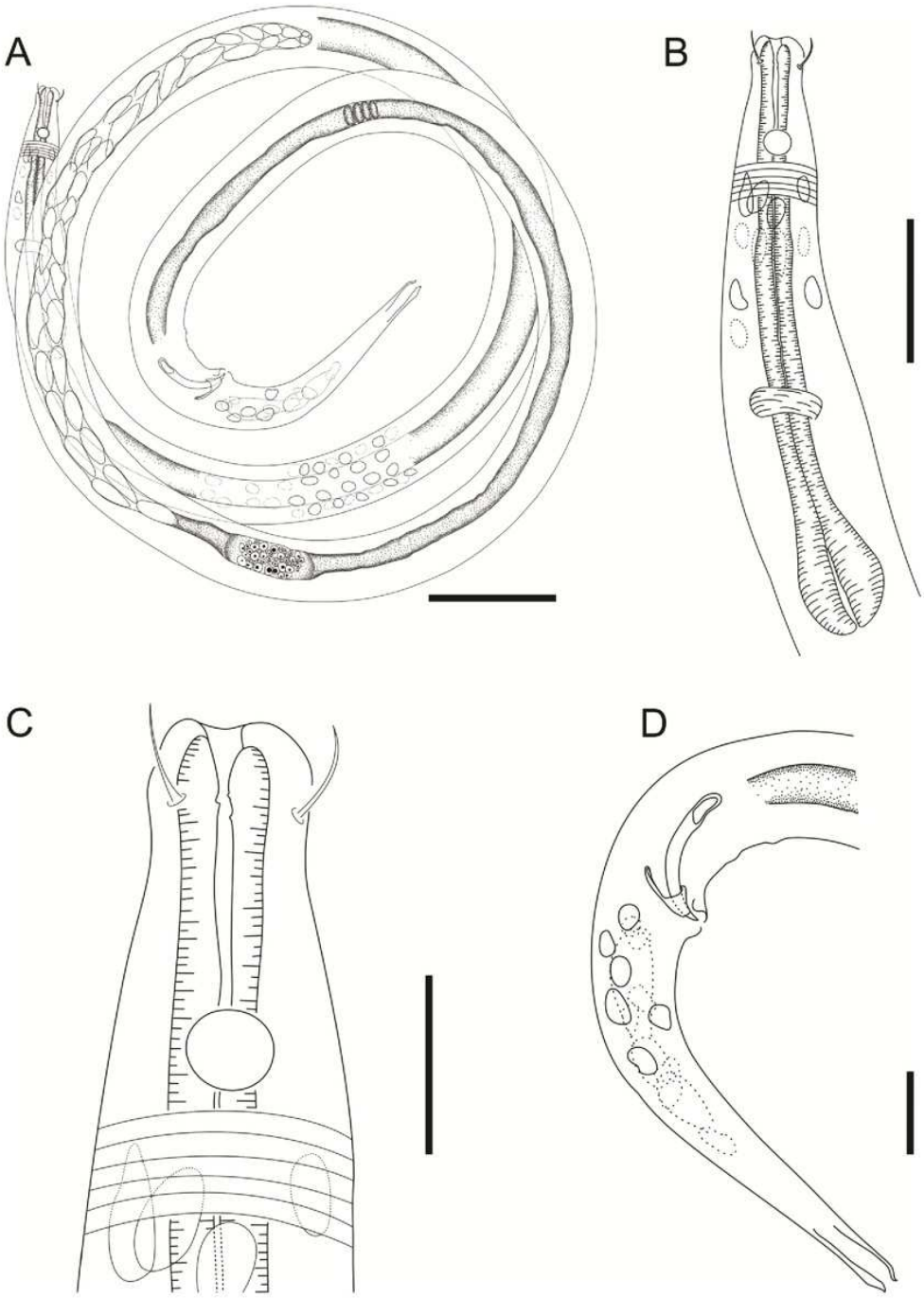
(A) anterior region, (B) anterior region (arrow indicating cephalic setae), (C) anterior end (arrow indicating dorsal tooth). Paratype female 2: (D) habitus (ov.a.= anterior ovary; ov.p.= posterior ovary; V= vulva). Scale bars: A, B and C= 10 µm, D= 30 µm.


A
B
cs
C
dt
D
ov.p.
V
ov.a.

# Figure 8

Microlaimus nordestinus sp. n. Holotype male.

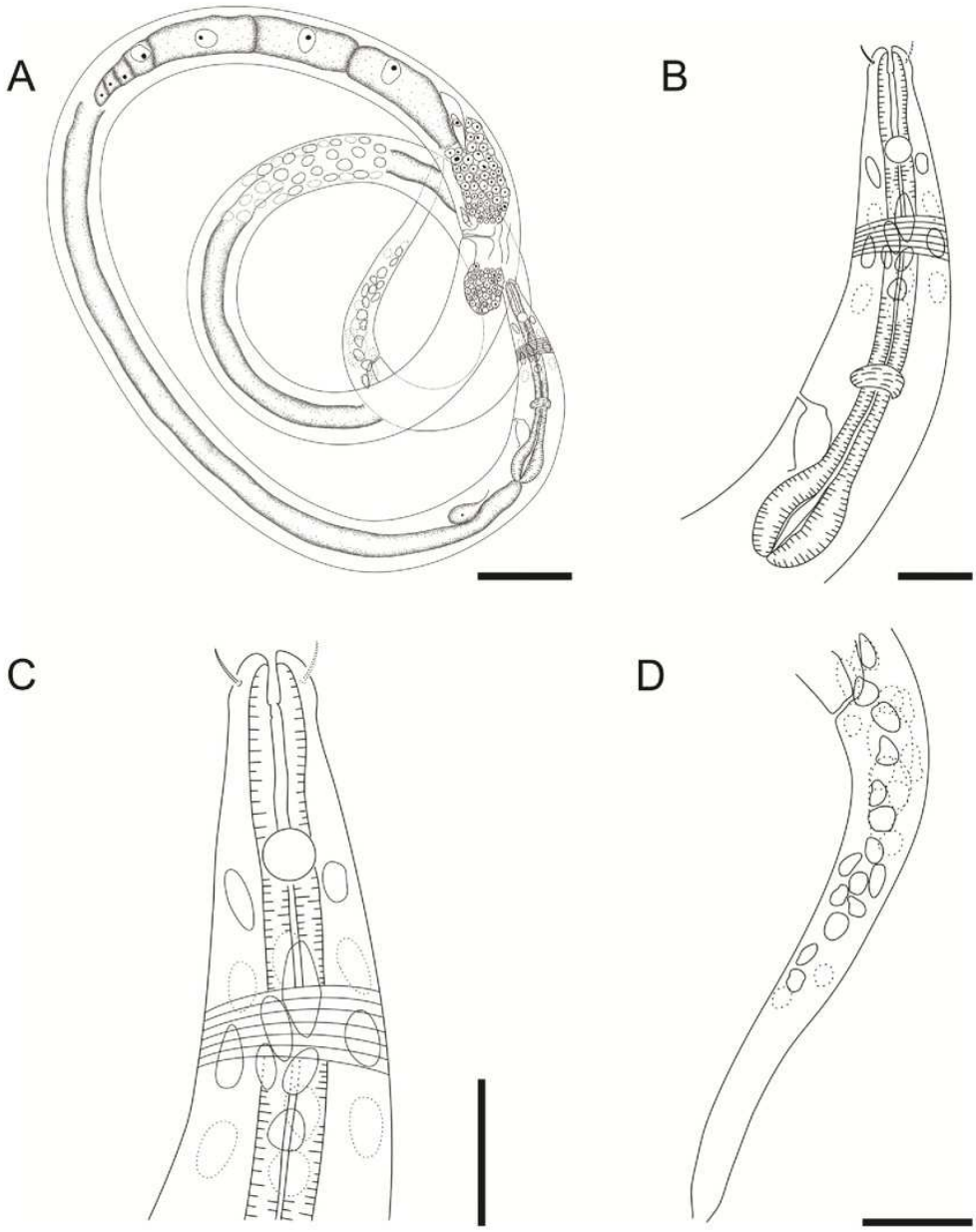
(A) overview, (B) anterior region, (C) anterior end, (D) posterior region. Scale bars: A= 50 µm, B= 25 µm, C= 10 µm and D = 20 µm.


A
B
C
D

# Figure 9

Microlaimus nordestinus sp. n. Paratype female.

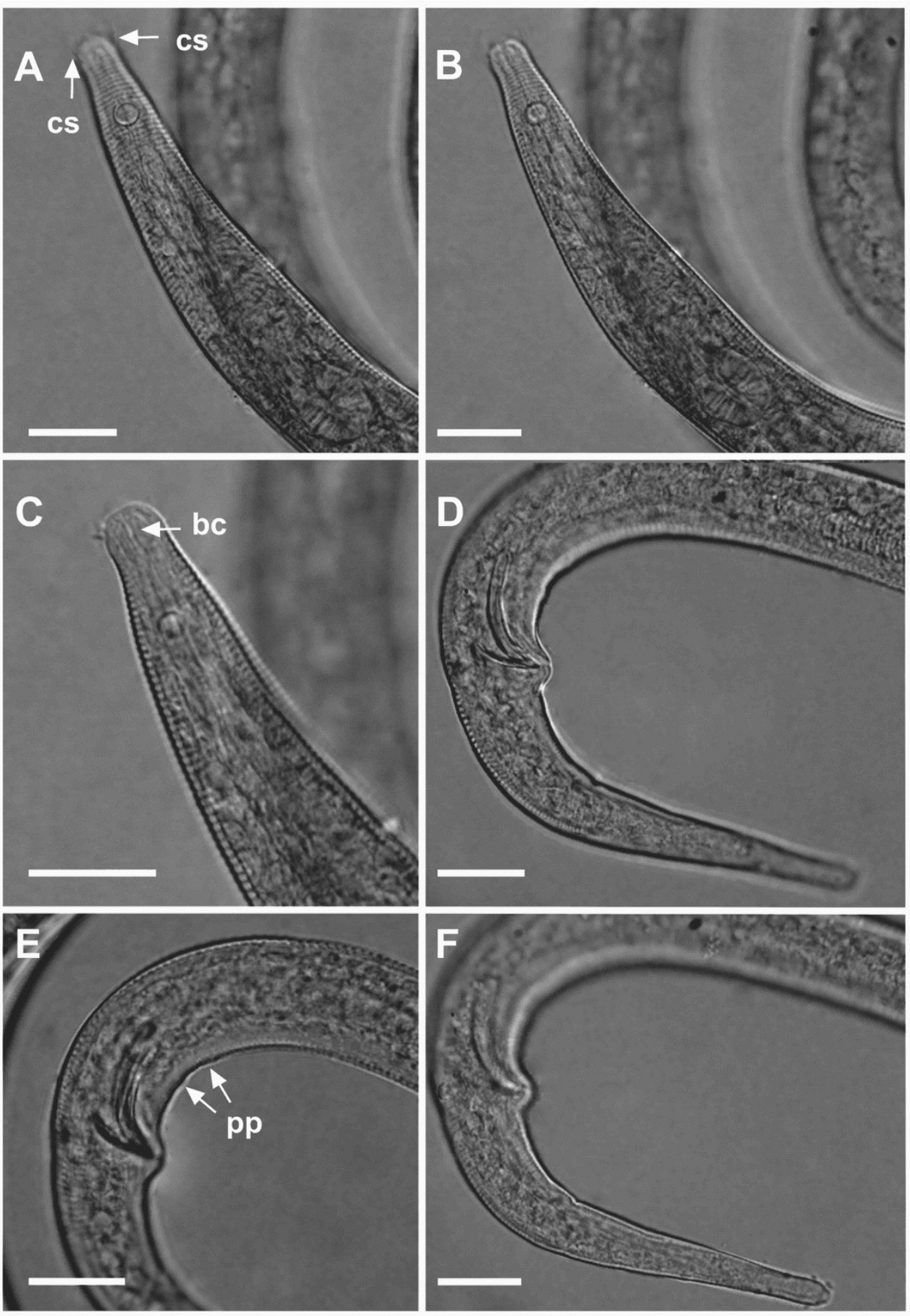
(A) overview, (B) anterior region, (C) anterior end, (D) posterior region. Scale bars: A= 50 µm, B and C= 15 µm and D = 20 µm.


A
B
C
D

# Figure 10

Microlaimus nordestinus sp. n. Holotype male.

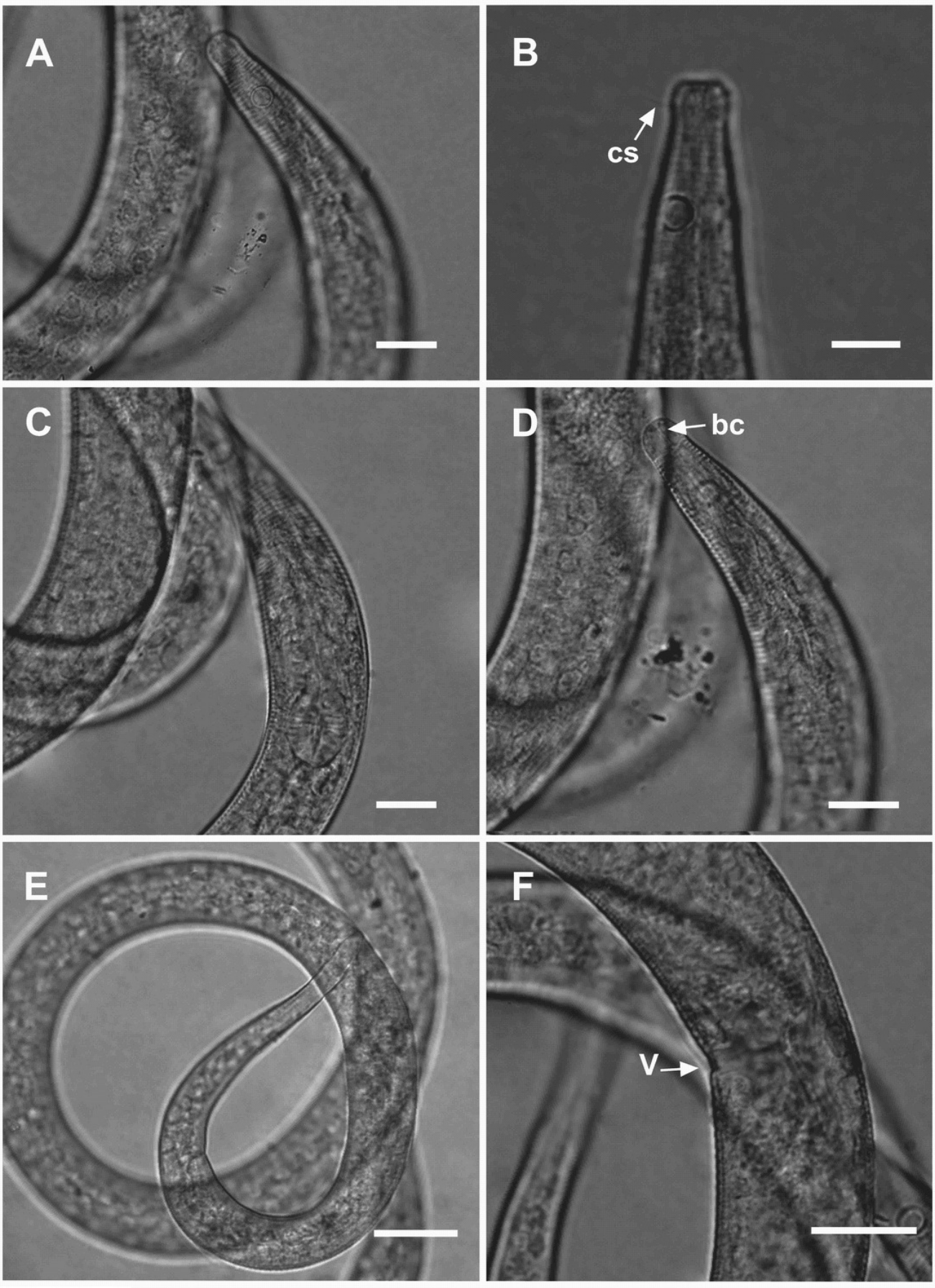
(A) anterior region (arrows indicating cephalic setae), (B) anterior region, (C) anterior end (arrow indicating buccal cavity), (D) posterior end (spicule and gubernaculum), (E) posterior end (arrow indicating precloacal papilla), (F) tail. Scale bars: A, B, C, D, E and F = 20 µm.


A
cs
cs
B
C
bc
D
E
pp
F

# Figure 11

Microlaimus nordestinus sp. n. Paratype female 1 and paratype female 2.

Paratype female 1: (A) anterior region. Paratype female 2: (B) anterior region (arrow indicating cephalic setae). Paratype female 1: (C) anterior region (pharynx and bulb), (D) anterior region (arrow indicating buccal cavity), (E) tail, (F) vulva. Scale bars: A, C and D= 15 µm, B= 10 µm, E= 30 µm and F= 20 µm.


A
B
cs
C
D
bc
E
F
V