

Two new species of free-living marine nematodes of the Family Microlaimidae (Nematoda: Microlaimida) from the Continental Shelf off Northeastern Brazil (Atlantic Ocean) with emended diagnosis and dichotomous key (#99887)

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Two new species of free-living marine nematodes of the Family Microlaimidae (Nematoda: Microlaimida) from the Continental Shelf off Northeastern Brazil (Atlantic Ocean) with emended diagnosis and dichotomous key

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New species of the genera *Spirobolbolaimus* and *Ixonema* (family Microlaimidae) have been found in sediment samples collected in the South Atlantic, along the Continental Shelf break off Northeastern Brazil. *Spirobolbolaimus pernambucanus* sp. n. possesses six outer labial setae and four cephalic setae of approximately the same length. *Ixonema gracielea* sp. n. differs from other species of *Ixonema* in having somatic setae on peduncles. This is the first time that new species of these taxa have been described for the South Atlantic. An amendment of the diagnosis and a dichotomous key are proposed for both genera.

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Abstract

New species of the genera *Spirobolbolaimus* and *Ixonema* (Nematoda: Microlaimidae) have been found in sediment samples collected in the South Atlantic, along the Continental Shelf break off Northeastern Brazil. *Spirobolbolaimus pernambucanus* sp. n. possesses six outer labial setae and four cephalic setae of approximately the same length. *Ixonema gracielea* sp. n. differs from other species of *Ixonema* in having somatic setae on peduncles. This is the first time that new species of these taxa have been described for the South Atlantic. An amendment of the diagnosis and a dichotomous key are proposed for both genera.

Key words: Marine nematodes, taxonomy, species description, South Atlantic, *Spirobolbolaimus*, *Ixonema*.

Introduction

The family Microlaimidae Micoletzky, 1922 currently comprises 13 genera and about 150 valid species (Tchesunov, Jeong & Lee, 2021). From South Atlantic, the records of species belonging to this family are still scarce and are almost all related to the genus *Microlaimus* de Man, 1880 (Manoel, Neres & Esteves, 2024). The oldest records in which species related to any genus placed in Microlaimidae were originally described for this location date back to the 1950s, when Gerlach described four species of *Microlaimus* for southeastern coast of Brazil (Gerlach 1956, 1957a, 1957b). Later, Pastor de Ward (1980) described a species of *Aponema* and another, in 1989, for the genus *Microlaimus*, both found in the same region (Santa Cruz, Argentina). Lima, Neres & Esteves (2022), ~~after more than~~ three decades, described three species of *Microlaimus* from the continental shelf off the Campos Basin, Southeastern Brazil. Recently, Manoel, Neres & Esteves (2024) described three new species of *Microlaimus* for the Continental Shelf break off Northeastern Brazil.

For other genera of Microlaimidae, such as *Spirobolbolaimus* Soetaert & Vincx, 1988 and *Ixonema* Lorenzen, 1971, there are no records of species originally described for the South Atlantic. The genus *Spirobolbolaimus* was placed in Microlaimidae based on male (two opposite and outstretched testes) and female (two outstretched ovaries) gonads (Soetaert & Vincx, 1988). *Spirobolbolaimus* is mainly characterized by having outer labial setae longer than the cephalic

setae, multispiral amphidial fovea, postamphidial setae in six or eight longitudinal rows, buccal cavity armed with protruding teeth and pharynx with anterior and posterior bulbs (Soeretaet & Vincx, 1988; Gourbault & Vincx, 1990; Shi & Xu, 2016). The genus currently has three valid species: *S. bathyalis* Soeretaet & Vincx, 1988 (Mediterranean, Calvi Bay; 280–820 m depth); *S. boucherorum* Gourbault & Vincx, 1990 (Caribbean Sea, Guadeloupe; 2 m depth) and *S. undulatus* Shi & Xu, 2016 (China Sea, Nanji Islands; Dasha'ao sand beach).

Ixonema is the only representative of the family Microlaimidae that has three caudal glands with separate outlets, a feature considered rare in free-living marine nematodes (Lorenzen 1971, 1994). Often, representatives of this taxon are found with the cuticle covered by particles, such as algae, suggesting that these organisms are capable of carrying their own food (Steyaert *et al.* 1999). Additionally, this genus is characterized by a narrow and elongated anterior end, amphidial fovea far from the anterior end with a *corpus gelatum* projecting from the amphidial opening, a small mouth cavity and three minute teeth (Tchesunov, 2014). Only three species have been described for *Ixonema*: *I. sordidum* Lorenzen, 1971 (North Sea, German Bight; sublittoral region, depth unspecified); *I. powelli* Jensen, 1985 (Gulf of Mexico; 72 m depth) and *I. deleyi* Muthumbi & Vincx, 1999 (Indian Ocean, Kenyan coast; 21–2007 m depth).

Here we will describe two new species, one from the *Spirobolbolaimus* genus and another from the *Ixonema* genus, found along the break of the continental shelf off Northeastern Brazil. Amendments to the diagnosis and dichotomous keys are proposed for both genera.

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. The collection stations related to the present study are detailed in Table 1. A box-corer was used to collect sediments, and the meiofauna samples were obtained with a corer (dimensions 10 x 10 cm).

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, Warwick & Moens, 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). The methodology for impregnating each animal's body with glycerin was then applied, followed by diaphanization, according to the method described by De Grisse (1969). The individuals were mounted permanently on glass slides, as an adaptation of the method described by Cobb (1920). The genus was identified by using keys provided by Warwick, Platt & Somerfield (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 map meter. 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, 2018

Superfamily Microlaimoidea Micoletzky, 1922

Family Microlaimidae Micoletzky, 1922

Genus *Spirobolbolaimus* Soetaert & Vincx, 1988

Diagnosis. (Emended from Shi & Xu, 2016) Microlaimidae. Cuticle annulated. **Anterior sensilla in three circles: six inner labial setae papilliform; six stout outer labial setae (sometimes jointed); and four cephalic setae shorter or similar in length to the six outer labial setae. Buccal cavity large, armed with a well-developed dorsal tooth and a pair of ventrosublateral teeth. Additional lateral teeth may be present.** Amphidial fovea multispiral and ventrally wound, sclerotized. Postamphidial setae in six or eight longitudinal rows. Pharynx with anterior bulb. Oval posterior bulb present or absent. Females with two outstretched ovaries. Males with two opposed testes. Copulatory apparatus strongly sclerotized. Gubernaculum with or without apophysis. **Precloacal supplements (papilliform or small pores) present or absent. Oval, elongated or banana-shaped spermatozoa. Tail conical.**

Type species: *Spirobolbolaimus bathyalis* Soetaert & Vincx, 1988

List of valid species of *Spirobolbolaimus* Soetaert & Vincx, 1988

Spirobolbolaimus bathyalis Soetaert & Vincx, 1988
Spirobolbolaimus boucherorum Goubault & Vincx, 1990
Spirobolbolaimus pernambucanus sp. n.
Spirobolbolaimus undulatus Shi & Xu, 2016

Description of new species

Spirobolbolaimus pernambucanus sp. n.

(Table 2; Fig 1–4)

Material studied. Holotype male (MOUFPE 0022), paratype female (MOUFPE 0023), 1 male paratype (485 NM LMZOO-UFPE) and 2 female paratypes (486–487 NM LMZOO-UFPE).

Type locality. South Atlantic Ocean, Continental shelf off the State of Pernambuco, Brazil, station 8 (S 08°16'17.10" W 34°39'34.80"), November 26, 2019, 52 m.

Locality of paratypes. Female paratypes: South Atlantic Ocean, Continental shelf off the State of Paraíba, Brazil, station 6 (S 07°25'15.48" W 34°29'16.56"), November 27, 2019, 47 m. Paratype male 1: South Atlantic Ocean, Continental shelf of the State of Pernambuco, Brazil, station 8 (S 08°16'17.10" W 34°39'34.80"), November 26, 2019, 52 m.

Etymology. Due to the location where the holotype was collected. *Pernambucanus* is the Latinized form of the term "pernambucano". In Brazil, "pernambucano" refers to something or someone originating from the state of Pernambuco.

Holotype male. Body cylindrical (1716 µm long) and slightly narrow anteriorly. Maximum body diameter corresponding to 1.2 times the head diameter. Head blunt, slightly set-off. Cuticle striated from the posterior edge of the amphidial fovea. Cuticular pores not observed. Anterior sensilla arranged in the 6+6+4 pattern: six inner labial papillae (2 µm long), six outer labial setae with a broad base (7 µm long) and four slender cephalic setae about the same length as the outer labial setae. Cephalic setae corresponding to 21% of head diameter. Amphidial fovea distinctly sclerotized, multispiral, ventrally wound, about 3 turns, occupying 36% of corresponding body diameter and located 0.4 times the head diameter of the anterior end. Buccal cavity with a strong dorsal tooth and two ventrosublateral teeth located at the same level. Cheilostoma possesses 12 rugae. Eight rows of cervical setae starting 11 µm behind the amphid fovea. Pharynx (220 µm) with anterior bulb surrounding the buccal cavity. Basal bulb is oval and not very prominent, occupying 67% of corresponding body diameter. Cardia embedded in intestine. Nerve ring situated at 55% of the pharynx length, from anterior end. Ventral gland and secretory-excretory pore not observed. Reproductive system with two opposed and outstretched testes, both to the left of the intestine. Elongated sperm. Spicules sclerotized with a capitulum. Gubernaculum plate-like with dorsal apophysis. Twelve pore-like precloacal supplements present and arranged at irregular intervals. The closest supplement to the cloaca is located at 24.5 µm and the farthest at 258.5 µm. A pair of precloacal ventrosublateral setae located between the cloaca and the closest supplement to it. Three caudal glands. Tail conical (4 times the cloacal body diameter) with two rows of short ventrosublateral setae.

Paratype female. Similar to male. Body measuring 1752 μm in length and maximum diameter 46.5 μm (1.3 times the head diameter). Cuticle striated behind the posterior edge of the amphidial fovea. Anterior sensilla arranged in the 6+6+4 pattern: six inner labial papillae (2 μm long), six outer labial setae with a broad base (7 μm long) and four slender cephalic setae (6 μm long, corresponding to 17% of head diameter). Amphidial fovea distinctly sclerotized, multispiral, ventrally wound, about $2\frac{3}{4}$ turns, occupying 35% of corresponding body diameter and located 0.3 times the head diameter of the anterior end. Buccal cavity, teeth and pharynx similar to that of males. Basal bulb is oval and not very prominent, occupying 66% of corresponding body diameter. Nerve ring situated at 56% of the pharynx length, from anterior end. Vulva located 960 μm from anterior end, at 55% 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. Tail conical, about 4 times the anal body diameter.

Diagnosis. *Spirobolbolaimus pernambucanus* **sp. n.** it is characterized by its body length (1716–1848 μm). Cuticle striated. Head blunt, slightly set-off. Six outer labial setae with a broad base and four slender cephalic setae about the same length as the outer labial setae. Amphidial fovea sclerotized, multispiral, ventrally wound, about $2\frac{3}{4}$ –3 turns, occupying 31–36% of the corresponding body diameter, located at about 0.2–0.4 times the head diameter. Eight rows of cervical setae. Buccal cavity with a strong dorsal tooth and two ventrosublateral teeth. Spicules sclerotized with a capitulum (1.5–1.7 times the cloacal body diameter) and gubernaculum plate-like with dorsal apophysis. Twelve pore-like precloacal supplements. Tail conical which corresponds to 3–4 cloacal or anal body diameter.

Differential diagnosis. *Spirobolbolaimus pernambucanus* **sp. n.** shares the following features with *S. undulatus*: number of longitudinal rows of cervical setae (eight in both species), spicule length (53–55 μm in the new species and 51–55 μm in *S. undulatus*) and the ratio between spicule length and the cloacal body diameter (1.5–1.7 in *S. pernambucanus* **sp. n.** and 1.3–1.5 in *S. undulatus*). Nevertheless, *S. pernambucanus* **sp. n.** differs from *S. undulatus* in terms of body length (1686–1848 μm in the new species vs 2035–2558 μm in *S. undulatus*), the absence of jointed outer labial setae (vs outer labial setae jointed in *S. undulatus*), the presence of outer labial setae about the same length as the cephalic setae (vs outer labial setae longer than cephalic setae in *S. undulatus*) and the number of precloacal supplements (12 pore-like precloacal supplements in the new species vs 18–19 precloacal supplements in a series of mid-ventral elevations with pores on tops in *S. undulatus*). Furthermore, additional teeth are absent in *S. pernambucanus* **sp. n.** (vs present in *S. undulatus*).

Spirobolbolaimus pernambucanus **sp. n.** shares some features with adult specimens of *S. boucherorum*, such as: body length (1686–1848 μm in the new species vs 1460–1870 μm in *S. boucherorum*), amphidial fovea diameter (11–12 μm in *S. pernambucanus* **sp. n.** and 10–12 μm in *S. boucherorum*) and de Man's ratio c (13–16 in the new species and 12–17 in *S. boucherorum*). However, *S. pernambucanus* **sp. n.** differs from *S. boucherorum* in terms of the number of precloacal supplements (12 in the new species vs 7 in *S. boucherorum*), the length and shape of the gubernaculum (19–19.5 μm in *S. pernambucanus* vs 23–30 μm in *S. boucherorum*) and the presence of outer labial setae that are about the same length as the cephalic setae (vs outer labial setae longer than cephalic setae in *S. boucherorum*).

Dichotomous identification key for valid species of the genus *Spirobolbolaimus* Soetaert & Vincx, 1988

1. Six rows of cervical setae *S. bathyalis*
- Eight rows of cervical setae 2
2. Outer labial setae jointed *S. undulatus*
- Outer labial setae non-jointed 3
3. Seven pore-like precloacal supplements *S. boucherorum*
- Twelve pore-like precloacal supplements *S. pernambucanus* **sp. n.**

Genus *Ixonema* Lorenzen, 1971

Diagnosis. (Emended from Tchesunov, 2014) Microlaimidae. **Cuticle finely striated but can appear smooth** and may be covered with sediment particles. **Anterior sensilla arranged according to 6 + 6 + 4 pattern: six inner labial papillae; six outer labial papillae; and four cephalic setiform sensilla.** Anterior end narrowed and elongated. **Amphid fovea small circular or pocket-shaped, far posterior to the anterior end, with protruding rod-shaped *corpus gelatum* (not seen in some species, lost/broken or absent?).** Buccal cavity small, armed with a dorsal tooth and a pair of ventrosublateral teeth (additional small teeth posterior to ventrosublateral may be present). Somatic setae stout or on peduncles, sometimes jointed, arranged in rows along the body. Males with two opposite and outstretched testicles. **Precloacal supplements in the form of jointed or non-jointed setae may be present. Ventral supplements in the form of jointed setae located just behind the pharynx may be present. Gubernaculum present or absent, when present without apophyses. Female didelphic-amphidelphic, with outstretched ovaries. Tail conical. Each caudal gland with its own outlet on the tail tip.**

Type species: *Ixonema sordidum* Lorenzen, 1971

List of valid species of *Ixonema* Lorenzen, 1971

Ixonema deleyi Muthumbi & Vincx, 1999

Ixonema ***gracielea* sp. n.**

Ixonema powelli Jensen, 1985

Ixonema sordidum Lorenzen, 1971

Description of new species

Ixonema gracielea **sp. n.**

(Table 3; Fig 5–9)

Material studied. Holotype male (MOUFPE 0024), paratype female (MOUFPE 0025), 3 male paratypes (488–490 NM LMZOO-UFPE) and 2 female paratypes (491–492 NM LMZOO-UFPE).

Type locality. South Atlantic Ocean, Continental shelf off the State of Alagoas, station 12 (S 09°39'14.52" W 35°15'21.66"), November 25, 2019, 50m.

Etymology. The species name is a tribute to Graciele Mariza dos Santos Alves, wife of the first author.

Holotype male. Body cylindrical, short and plump, attenuating on both ends and thick in the middle (586.5 μm long). Maximum body diameter corresponding to 4.1 times the head diameter. Cuticle finely striated, the striations are so delicate that they are difficult to visualize (striations are most visible on the tail). Anterior sensilla arranged in the 6+6+4 pattern: six inner labial papillae, six outer labial papillae and four cephalic setae (6 μm long). Cephalic setae corresponding to 74% of head diameter. Amphidial fovea small and circular, occupying 25% of corresponding body diameter and located far posterior to the anterior end (4 times the head diameter of the anterior end). Protruding *Corpus gelatum* not observed. Buccal cavity with a dorsal tooth and two small ventrosublateral teeth. Cheilostoma rugae indiscernible under a light microscope. Four sublateral rows of stout cervical setae located between the posterior region of the cephalic setae and the base of the amphidial fovea. The first row is 24 μm from the anterior end and the second is at the same height of the amphidial fovea base, 33 μm from the anterior end. Six rows of cervical setae on peduncles located between the posterior region of the amphidial fovea and close to the bulb base: 4 sublateral rows where relatively smaller setae (4–7 μm) and long setae (10–16 μm) alternate; 2 lateral rows (about 4 μm). The first row is about 11 μm from the base of the amphidial fovea. After the cervical region, the somatic setae are distributed in two rows where the alternation between two smaller setae (about 5–9 μm) and a larger one (12–19 μm) usually occurs. Pharynx (104 μm) with prominent terminal bulb, occupying 73% of corresponding body diameter. Cardia embedded in intestine. Nerve ring situated at 60% of the pharynx length, from anterior end. Ventral gland and secretory-excretory pore not observed. Reproductive system with two opposed and outstretched testes on right side of intestine. Spicules curved (39 μm), about 2 times the cloacal body diameter. Gubernaculum slender and without apophysis. Four setiform ventral supplements: three precloacal supplements plus one located just behind the pharynx. The two setae closest to the cloaca (10.5 μm and 40 μm anterior to the cloaca, respectively) are jointed, measuring 7 μm and 6 μm respectively; the third precloacal seta is smaller (about 2 μm length), apparently non-jointed in light microscopy and far from the cloaca (62 μm). The setae located just behind the pharynx (about 7 μm length) are jointed (morphologically similar to the two setae closest to the cloaca) and are located 84 μm from the anterior end. Tail conical (73 μm long), with a blunt tip where the three caudal glands open through separate outlets into papilla-like extensions.

Paratype female. Similar to male. Body measuring 667.5 μm in length, and maximum diameter 36 μm (4.3 times the head diameter). Cuticle finely striated, the striations are so delicate that they are difficult to visualize. Anterior sensilla arranged in the 6+6+4 pattern: six inner labial papillae, six outer labial papillae and four cephalic setae (7 μm long, corresponding to 88% of head diameter). Amphidial fovea small and circular, occupying 21% of corresponding body diameter and located far posterior to the anterior end (3.9 times the head diameter of the anterior end). Protruding *corpus gelatum* not observed. Morphologies and distribution patterns of cervical and somatic setae are similar to the male. Jointed ventral seta located just behind the pharynx absent. Buccal cavity, teeth and pharynx similar to that of male. Bulb occupying 74% of corresponding body diameter. Nerve ring situated at 67% of the pharynx length, from anterior end. Vulva located 411 μm from anterior end, at 62% of body length. A pair of ventral papillae surround the vulva.

Reproductive system didelphic-amphidelphic, with outstretched ovaries. Anterior and posterior ovary to right side of intestine. Tail conical (67 μ m long), similar to the male.

Diagnosis. *Ixonema gracielea* **sp. n.** it is characterized by its finely striated cuticle. Amphidial fovea small and circular located far posterior to the anterior end. Protruding *corpus gelatum* not observed. Rows of cervical and somatic setae on peduncles along the body. Reproductive system with two opposed and outstretched testes in the males and didelphic-amphidelphic, with outstretched ovaries in the females. Spicules curved and gubernaculum without apophysis. Four setiform ventral supplements: three precloacal setae (the two closest to the cloaca are jointed and the furthest apparently non-jointed) and a jointed ventral seta located just behind the pharynx. Tail conical with a blunt tip where the three caudal glands open through separate outlets into papilla-like extensions.

Differential diagnosis. *Ixonema gracielea* **sp. n.** resembles the monospecific genus *Bathynox* (Bussau, 1993) Bussau & Vopel, 1999 due to the presence of the setae on peduncles distributed along the whole body. However, *I. gracielea* **sp. n.** differs from *Bathynox* in having three caudal glands opening through separate outlets in papilla-like extensions (vs three glands opening in a single outlet in *Bathynox*). Furthermore, the male reproductive system is different between the genera: male diorchic in *I. gracielea* **sp. n.** vs male monorchic in *Bathynox* and the gubernaculum lacks apophysis in *I. gracielea* **sp. n.** vs the presence of dorsal apophysis in *Bathynox*.

The presence of setae on peduncles in *I. gracielea* **sp. n.** differentiates it from other species of the genus. The new species shares the presence of a single jointed ventral seta located just behind the pharynx with *I. powelli*, as well as jointed precloacal setae in the males. Nonetheless, *I. gracielea* **sp. n.** differs from *I. powelli* in terms of the shape of the amphidial fovea (circular in *I. gracielea* **sp. n.** vs pocket-like in *I. powelli*), the distribution pattern and number of rows of cervical setae (6 rows of cervical setae in *I. gracielea* **sp. n.** vs 4 rows of cervical setae *I. powelli*) and gubernaculum length (11.5–14.5 μ m in *I. gracielea* **sp. n.** vs about 6 μ m in *I. powelli*).

Ixonema gracielea **sp. n.** differs from *I. sordidum* in having long somatic setae alternating with short somatic setae along the body and due to the presence of precloacal supplements, these characteristics are absent in the mentioned species. *I. gracielea* **sp. n.** differs from *I. deleyi* in terms of the presence of the gubernaculum (vs absent in *I. deleyi*) and the number and morphology of supplements (jointed ventral seta located just behind the pharynx + two jointed setae closer to the cloaca and a smaller non-jointed seta further away in *I. gracielea* **sp. n.** vs a single non-jointed ventral precloacal seta in *I. deleyi*).

Dichotomous identification key for valid species of the genus *Ixonema* Lorenzen, 1971

1. Precloacal supplements absent *I. sordidum*
– Precloacal supplements present 2
2. Gubernaculum absent *I. deleyi*
– Gubernaculum present 3
3. Amphidial fovea circular and somatic seta on peduncles *I. gracielea* **sp. n.**
– Amphidial fovea pocket-shaped and somatic seta jointed *I. powelli*

Discussion

Although there are no records of species originally described for the South Atlantic, the occurrence of *Spirobolbolaimus* and *Ixonema* was previously reported for this region. *Spirobolbolaimus* was found in the sublittoral of Pedra do Xaréu Beach, Pernambuco, Northeastern Brazil (Rocha *et al.*, 2006). This taxon was also found in sediment samples from the Grussai canyon and a point adjacent to it in the Campos Basin, Southeastern Brazil (Silva, 2012). *Ixonema* was identified in samples from the Campos Basin Slope, Southeastern Brazil (Moura, 2013).

Spirobolbolaimus pernambucanus **sp. n.** is the first species of the genus described for the South Atlantic. A comparison of the main characters of all valid species of *Spirobolbolaimus* is presented in Table 4. In all previously described species, the outer labial setae are longer than the cephalic setae. However, the outer labial setae of *S. pernambucanus* **sp. n.** are similar in length to the cephalic setae. We include this feature in the diagnosis of the genus. Based on the described species, we added the following characteristics to the diagnosis of the genus: the morphology of the buccal cavity and teeth that usually occur in species; the types of preloacal supplements; sperm and tail shape; occurrence of jointed outer labial setae. Jointed labial setae, as seen in *S. undulatus*, may be present in more genera or species than mentioned in the descriptions, since in some cases this characteristic may have been overlooked (Lorenzen, 1994).

Ixonema gracielea **sp. n.** can be easily confused and classified as belonging to the genus *Bathynox*, mainly due to the presence of rows setae on peduncles distributed along the body. However, *I. gracielea* **sp. n.** presents a combination of differential characteristics that typically only occur in representatives of the genus *Ixonema*: three caudal glands open through separate outlets into papilla-like extensions, two opposite and outstretched testes and gubernaculum without apophysis (Lorenzen, 1971; Jensen, 1985; Muthumbi & Vincx, 1999). Therefore, the presence of such characteristics invalidates the hypothesis of including this species in another genus and reinforces its taxonomic position. In the Microlaimidae family, the presence of caudal glands with independent outlets is unique for the genus *Ixonema*, and this feature is considered phylogenetically primitive (Lorenzen, 1971). *Ixonema gracielea* **sp. n.** is the first species of the genus described from the South Atlantic. The description of this new species strongly contributes to the knowledge and the variability of this genus.

Among the three valid species of *Ixonema*, it was possible to observe variations in some important characteristics for the identification of the genus that were absent in the last diagnosis of the genus provided by Tchesunov (2014). A comparison of the main characters of all valid species of *Ixonema* is presented in Table 5. The cuticle of the species *I. sordidum* and *I. powelli* was described as smooth (Lorenzen, 1971; Jensen, 1985). In both species, the cuticle was covered by a thin layer of particles, except in a *I. powelli* male, where it was possible to visualize subcuticular striae in the most anterior region of the pharynx. Electron microscopy analysis allowed the description of a very finely striated cuticle for *I. deleyi* (Muthumbi & Vincx, 1999). Tchesunov, Jeong & Lee (2021) included this variation in a comparative table between the genera of Microlaimidae but did not provide a complete diagnosis of *Ixonema*. In the new species, the cuticle is similar to that described for *I. deleyi*, and it is possible to visualize very fine striations on the tail of the analyzed specimens, even using optical microscopy. Therefore, the cuticle of

Ixonema is very finely striated, but can also appear smooth, especially when using light microscopy.

The amphidial fovea can vary from circular (*I. sordidum*, *I. deleyi* and *I. gracielea* **sp. n.**) to pocket-like (*I. powelli*). A protruding rod-shaped *corpus gelatum* is present in *I. sordidum* and *I. deleyi*. This characteristic was not observed in *I. powelli* and *I. gracielea* **sp. n.** When establishing the genus, Lorenzen (1971) argued that the walls of the gelatinous rods do not appear to be delicate structures. However, although *I. deleyi* presents a protruding *corpus gelatum*, in the electron microscopy analyzes provided in the original description of this species, it is possible to observe that this structure seems to have been lost during specimen preparation (see in Muthumbi & Vincx, 1999 - figure 7). This observation is not in line with Lorenzen's assumption (non-delicate structures). Therefore, we do not know for sure if this structure is absent in *I. powelli* and *I. gracielea* **sp. n.** or if it was lost/broken during sample processing and organism preparation, which commonly occurs with other structures such as the tail and setae.

The gubernaculum is present and lacks apophyses in the species *I. gracielea* **sp. n.**, *I. sordidum* and *I. powelli*. However, this structure is absent in *I. deleyi*. Supplements in the form of jointed setae in a ventral position are present just behind the pharynx and in front of the cloaca in *I. powelli* and *I. gracielea* **sp. n.** A non-jointed ventral seta located anterior to the cloacal opening is present in *I. deleyi*. Due to the similarity with *I. powelli* and *I. gracielea* **sp. n.** in relation to the position at which the seta is located, we will consider it as a precloacal supplement. Jointed somatic setae may be present (*I. powelli*). The occurrence of somatic setae on peduncles described for the new species is unprecedented for the genus. The variation of the characters discussed above, in addition to the new features found in the new species, were included in the diagnosis of the genus.

The present study increases the number of Microlaimidae species originally described from sediment samples collected in the South Atlantic. These results demonstrate that a great effort is still required in order to fully understand the real richness of the Microlaimidae assemblage present in the South Atlantic.

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Figure 1

Spirobolbolaimus pernambucanus sp. n. holotype male.

Holotype male: (A) overview; (B) anterior region; (C) anterior end (sensilla arranged, amphidial fovea and cervical setae); (D) anterior end (buccal cavity); (E) posterior region.

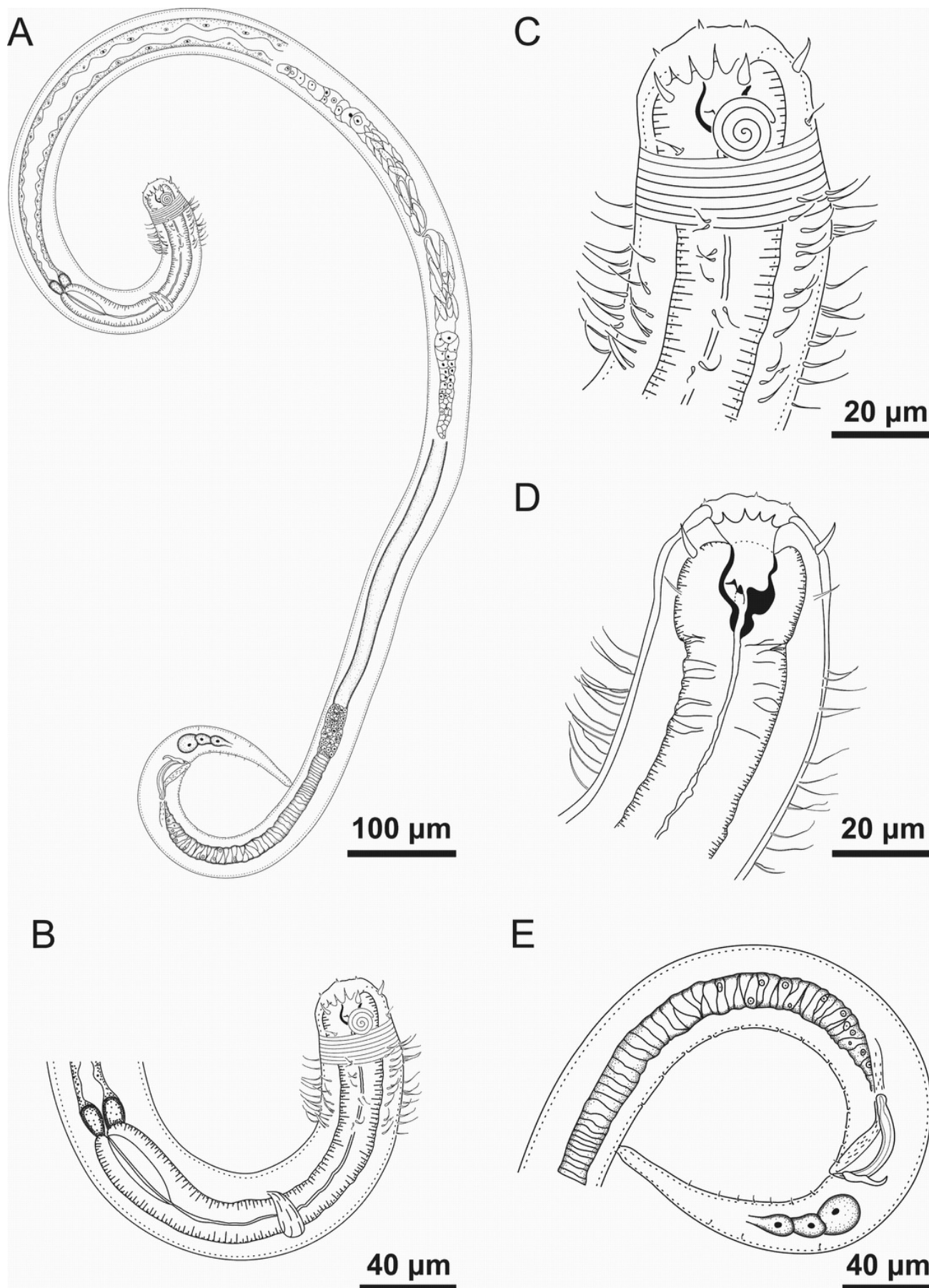


Figure 2

Spirobolbolaimus pernambucanus sp. n. paratype female.

Paratype female: (A) overview; (B) anterior region; (C) anterior end (sensilla arranged, amphidial fovea and cervical setae); (D) tail.

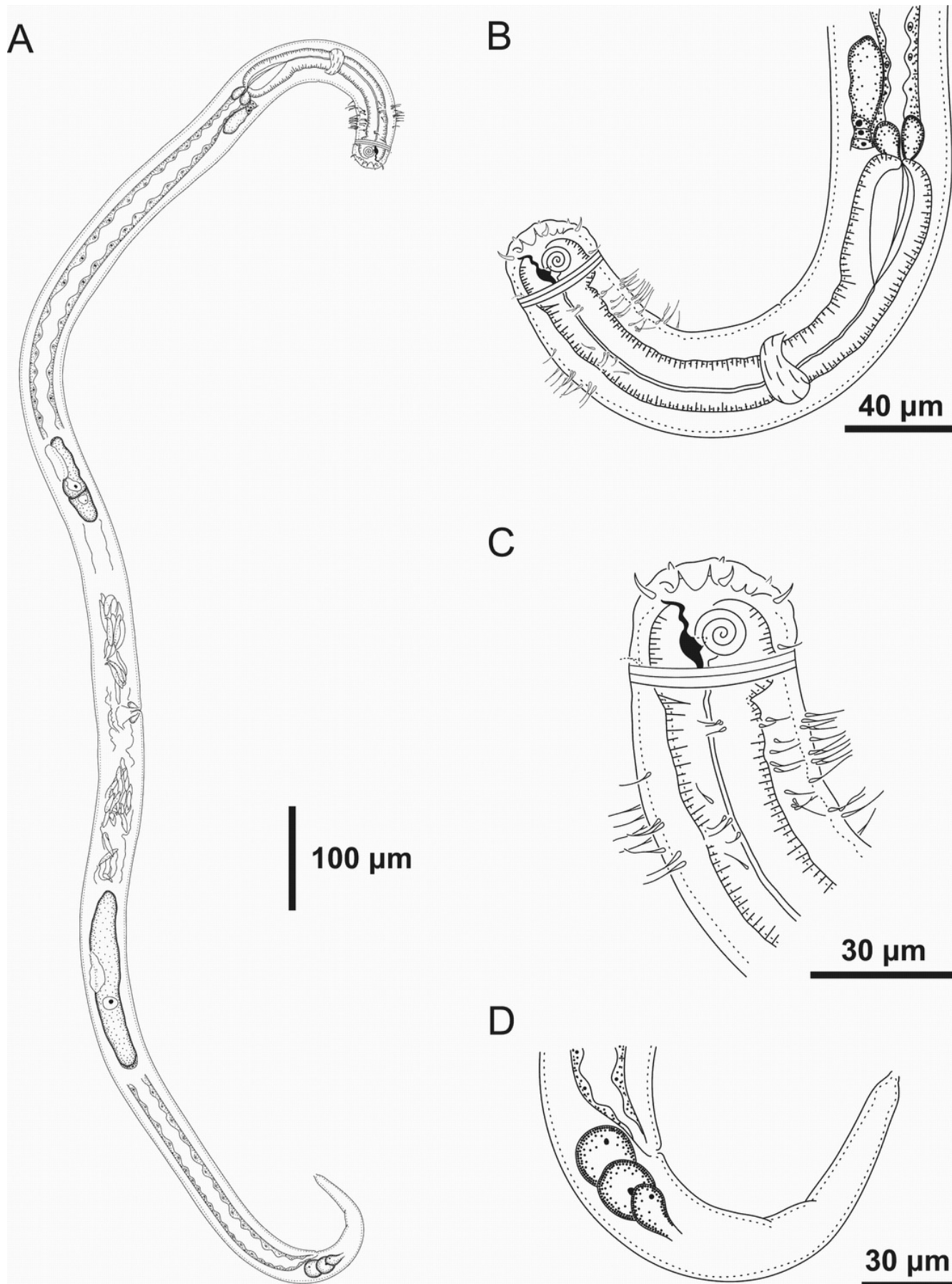


Figure 3

Spirobolbolaimus pernambucanus sp. n.: holotype male.

Holotype male: (A) anterior region: arrows indicating outer labial setae (l. ext.), cephalic setae (cs) and cervical setae (cer. s.); (B) anterior region (amphidial fovea); (C) anterior end: arrows indicating the dorsal tooth (dt) and pharynx (ph); (D) posterior end: arrows indicating pore-like precloacal supplements farthest from the cloaca; (E) posterior end: arrow indicating arrows indicating pore-like precloacal supplements closer to the cloaca; (F) posterior end: arrow indicating the spicule (spic), gubernaculum (gub) and rows of setae on the tail (r.t.).

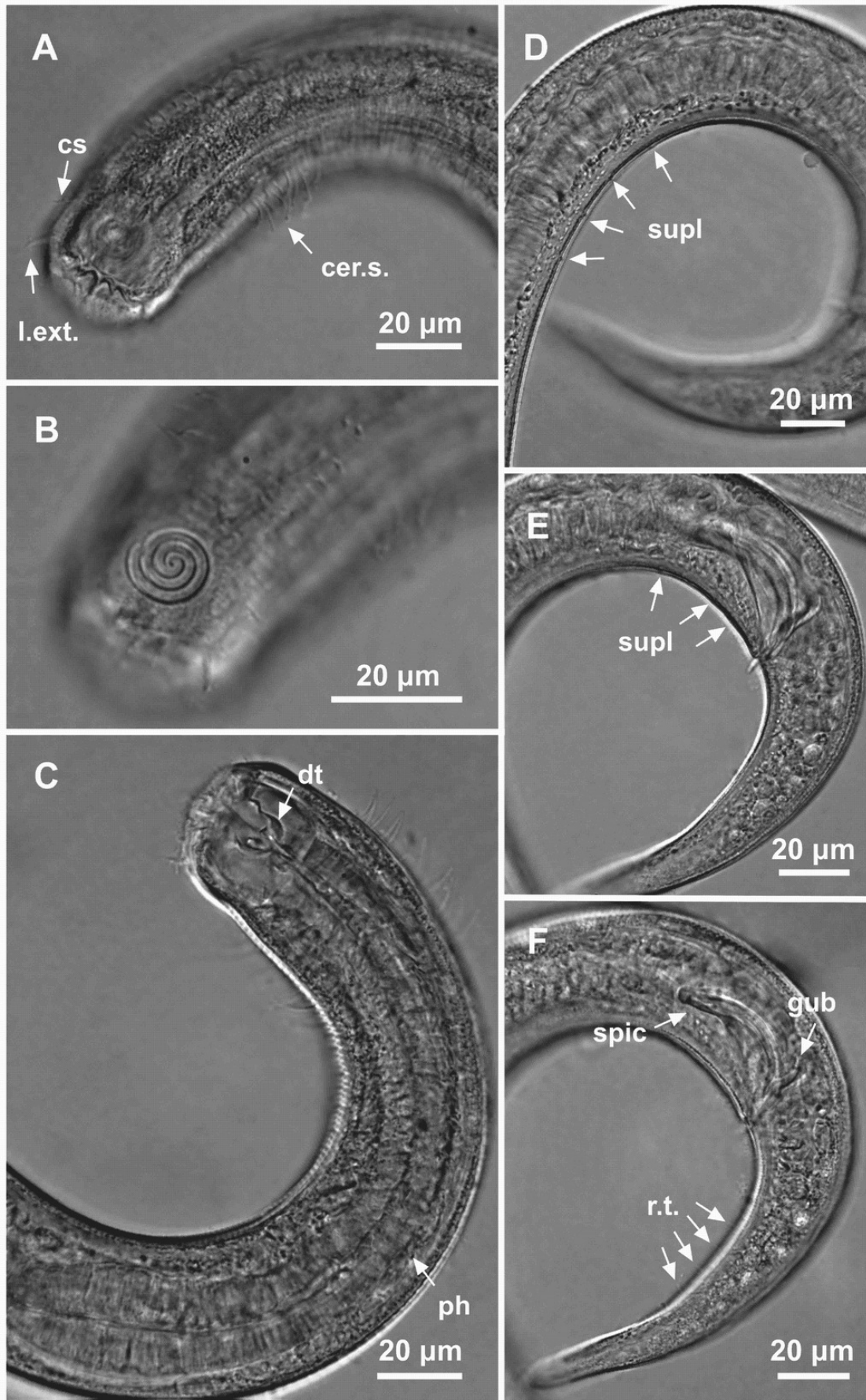


Figure 4

Spirobolbolaimus pernambucanus sp. n. paratype female.

Paratype female: (A) anterior region: arrows indicating outer labial setae (l. ext.), cephalic setae (cs) and cervical setae (cer. s.); (B) anterior region (amphidial fovea); (C) anterior end: arrow indicating the dorsal tooth (dt); (D) tail; (E) vulva region: arrows indicating the vulva (V) and sperm (sper).

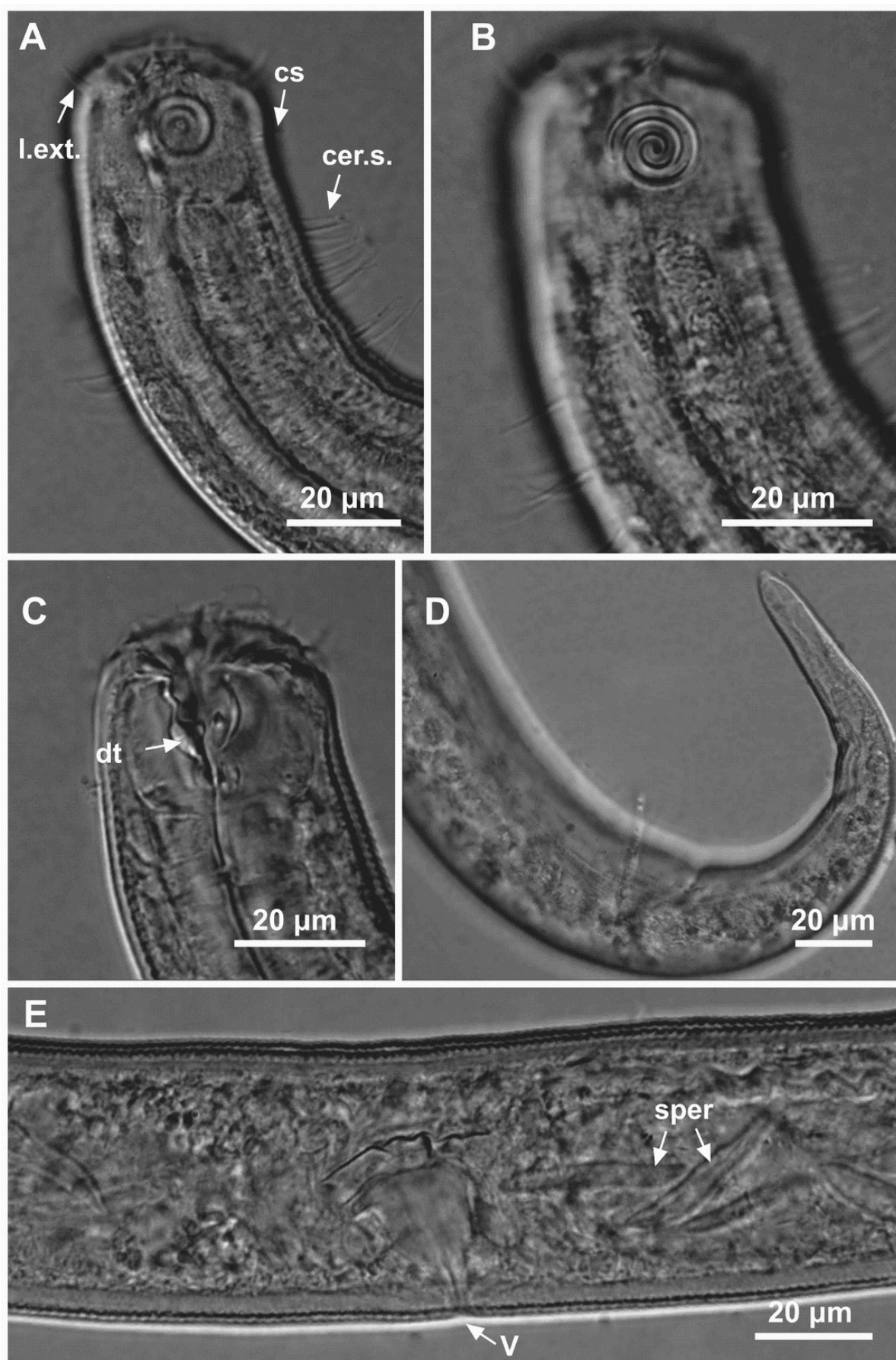


Table 1(on next page)

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

Station	Latitude	Longitude	Depth
6	S 07°25'15.48"	W 34°29'16.56"	47 m
8	S 08°16'17.10"	W 34°39'34.80"	52 m
12	S 09°39'14.52"	W 35°15'21.66"	50 m

1

Table 2 (on next page)

Morphometric data of *Spirobolbolaimus pernambucanus* 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

<i>Spirobolbolaimus pernambucanus</i> sp. n.	Holotype male	Male paratype	Females paratypes
Body length	1716	1848	1686–1800
Inner labial papillae length	2	-	2
Outer labial setae length	7	7	7
Cephalic setae length	7	7	5.5–7
Head diameter at level of the cephalic setae	34	34	35–36
Cephalic setae in relation to head diameter (%)	21%	21%	15–19%
Distance from anterior end to amphidial fovea	12	13	8–10.5
Distance from anterior end to amphidial fovea in relation to head diameter	0.4	0.4	0.2–0.3
Amphidial fovea diameter (maximum width)	12	12	11–12
Body diameter at level of the amphidial fovea	34	34	34.5–37
% of the amphidial fovea diameter in relation to corresponding body diameter	36%	36%	31–35%
Pharynx length	220	223	226.5–241.5
Position of nerve ring from anterior end	120	133.5	125–138
Nerve ring position in relation to pharynx length (%)	55%	60%	55–56%
Pharyngeal bulb diameter	26	30	28.5–31
Body diameter at level of the pharyngeal bulb	38	40	44–46.5
% of basal bulb diameter in relation to corresponding body diameter	67%	75%	64–66%
Maximum body diameter	40	44.5	46.5–48
Anal or cloacal body diameter	31	36	32–33
Tail length	120	114	117–131
Length of spicule along arc	53	55	*
Length of spicule along cord	40	42	*
Length of gubernaculum	19.5	19	*
Length of gubernaculum in relation to length of spicule along arc	37%	35%	*
Length of spicule along arc in relation to cloacal body diameter	1.7	1.5	*
Distance from anterior end to vulva	*	*	867–984
Position of vulva from anterior end (%)	*	*	51–55%
Body diameter in vulva region	*	*	46.5–48
Anterior ovary length	*	*	354–408
Posterior ovary length	*	*	252–288
Reproductive system length	927	1104	642–666
% of reproductive system in relation to body length	54%	60%	36–39%
a	43	42	35–39
b	8	8	7–7.5
c	14	16	13–14
c'	4	3	4

2

3

Table 3 (on next page)

Morphometric data of *Ixonema gracielea* sp. n. The measurements are expressed in micrometers, or if noted, as a percentage or ratio. Not applicable (*); a, b, c, c' = de Man's ratios (1880).

1

<i>Ixonema gracielea</i> sp. n.	Holotype male	Males paratypes	Females paratypes
Body length	586.5	562.5–630	553.5–667.5
Outer labial setae length	<2	<2	<2
Cephalic setae length	6	5–7	6–7
Head diameter at level of the cephalic setae	8	8–9	8–9
Cephalic setae in relation to head diameter (%)	74%	67–82%	71–88%
Distance from anterior end to amphidial fovea	31	31.5–34	29–32.5
Distance from anterior end to amphidial fovea in relation to head diameter	4	3.7–4.1	3.2–3.9
Amphidial fovea diameter (maximum width)	3	3	2.5–3
Body diameter at level of the amphidial fovea	13	14	13–14
% of the amphidial fovea diameter in relation to corresponding body diameter	25%	21–22%	19%–23%
Pharynx length	104	99–104	96–102
Position of nerve ring from anterior end	63	68–71	64–67
Nerve ring position in relation to pharynx length (%)	60%	68%	65–67%
Pharyngeal bulb diameter	18	17.5–19	19–19.5
Body diameter at level of the pharyngeal bulb	26	24–26	25–26
% of basal bulb diameter in relation to corresponding body diameter	68%	70–78%	74–76%
Maximum body diameter	32	31–32	35–36
Anal or cloacal body diameter	21	20	20–20.5
Tail length	73	65.5–69	64–67
Length of spicule along arc	39	33–41	*
Length of spicule along cord	33	30–32	*
Length of gubernaculum	11.5	13–14.5	*
Length of gubernaculum in relation to length of spicule along arc	29%	35–38%	*
Length of spicule along arc in relation to cloacal body diameter	1.9	1.7–2	*
Distance from anterior end to vulva	*	*	261–411
Position of vulva from anterior end (%)	*	*	47%–62%
Body diameter in vulva region	*	*	35–36
Anterior ovary length	*	*	52–69
Posterior ovary length	*	*	42–69
Reproductive system length	431	425–479.5	105–138
% of reproductive system in relation to body length	73%	67%–82%	16–25%
a	18	18–20	15–19
b	6	6	5–7
c	8	9	8–10
c'	3.5	3–3.5	3

2

Table 4(on next page)

Comparison between the main features of valid species of *Spirobolbolaimus* Soetaert & Vincx, 1988. The original descriptions were used to construct the table (Adult specimens only).

The measurements are expressed in micrometers, or if noted, as a percentage or ratio.

Absent (-); a, b, c, c' = de Man's ratios (1880); length of cephalic setae in relation to length of outer labial setae (cs. length/ols. length %); distance of amphidial fovea from anterior end in relation to head diameter (Amph/hd); length of spicule along arc in relation to cloacal body diameter (spic/c.b.d.); length of gubernaculum in relation to length of spicule along arc (gub/spic %).

1

	<i>Spirobolbolaimus bathyalis</i>	<i>Spirobolbolaimus boucherorum</i>	<i>Spirobolbolaimus pernambucanus</i> sp. n.	<i>Spirobolbolaimus undulatus</i>
L	595–755	1460–1870	1686–1848	2035–2558
a	22.2–30.2	38.4–50	35–43	47–58
b	5–6.2	7.3–9.8	7–8	9–10.2
c	7.7–10.2	12.2–17.4	13–16	14–16.8
c'	3.5–4.5	2.9–3.6	3–4	3.8–4.4
Outer labial setae	non-jointed	non-jointed	non-jointed	jointed
cs. length/ols. length %	25–30%	75%	79–100%	53–80%
Amph/hd	0.3	0.25–0.3	0.2–0.4	0.3
Number of turns of amphids	4 ³ / ₄ turns	2 ³ / ₄ turns	2 ³ / ₄ –3 turns	3 turns
amph%	55–65%	29–35%	31–36%	39–43%
Rows of cervical setae	6	8	8	8
Precloacal supplements	-	7 pore-like	12 pore-like	18–19 elevations with pores on tops
spic/c.b.d.	1.5	1.7	1.5–1.7	1.3–1.5
gub/spic %	36–42%	43%	35–37%	48–56%

2

Table 5 (on next page)

Comparison between the main features of valid species of *Ixonema* Lorenzen, 1971. The original descriptions were used to construct the table.

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); *corpus gelatum* (cg.); distance of amphidial fovea from anterior end in relation to head diameter (Amph/hd); length of spicule along arc in relation to cloacal body diameter (spic/c.b.d.); length of gubernaculum in relation to length of spicule along arc (gub/spic %); not applicable (x).

1

	<i>Ixonema deleyi</i>	<i>Ixonema gracielea</i> sp. n.	<i>Ixonema powelli</i>	<i>Ixonema sordidum</i>
L	245–405	553.5–667.5	640–900	530
a	12.3–22.5	15–19	13–23	16–20
b	3.6–4.9	5–7	5.9–7	5.1–5.3
c	7.3–9.2	8–10	7.3–10.6	8.2–8.8
c'	2.5–4.5	3–3.5	3.5	3
Amphids	circular (rod-like <i>cg.</i>)	circular (<i>cg.</i> not seen or absent)	pocket-shaped (<i>cg.</i> not seen or absent)	circular (rod-like <i>cg.</i>)
Amph/hd	4.8–6.2	3.2–4	4–5	3.6–3.8
Somatic setae	stout	on peduncles	jointed	stout
Supplements	1 non-jointed precloacal setae	4 jointed setae* (1 just behind pharynx +3 precloacal)	3 jointed setae (1 just behind pharynx +2 precloacal)	-
spic/c.b.d.	2	1.7–2	1.4–1.8	1.4
Gubernaculum	-	+	+	+
gub/spic %	x	29–38%	14%	44%

2 * Precloacal seta furthest from the cloaca apparently non-jointed using light microscopy in
3 *Ixonema gracielea* **sp. n.**

4

Figure 5

Ixonema gracielea sp. n. holotype male.

Setae on peduncles distributed along the body.

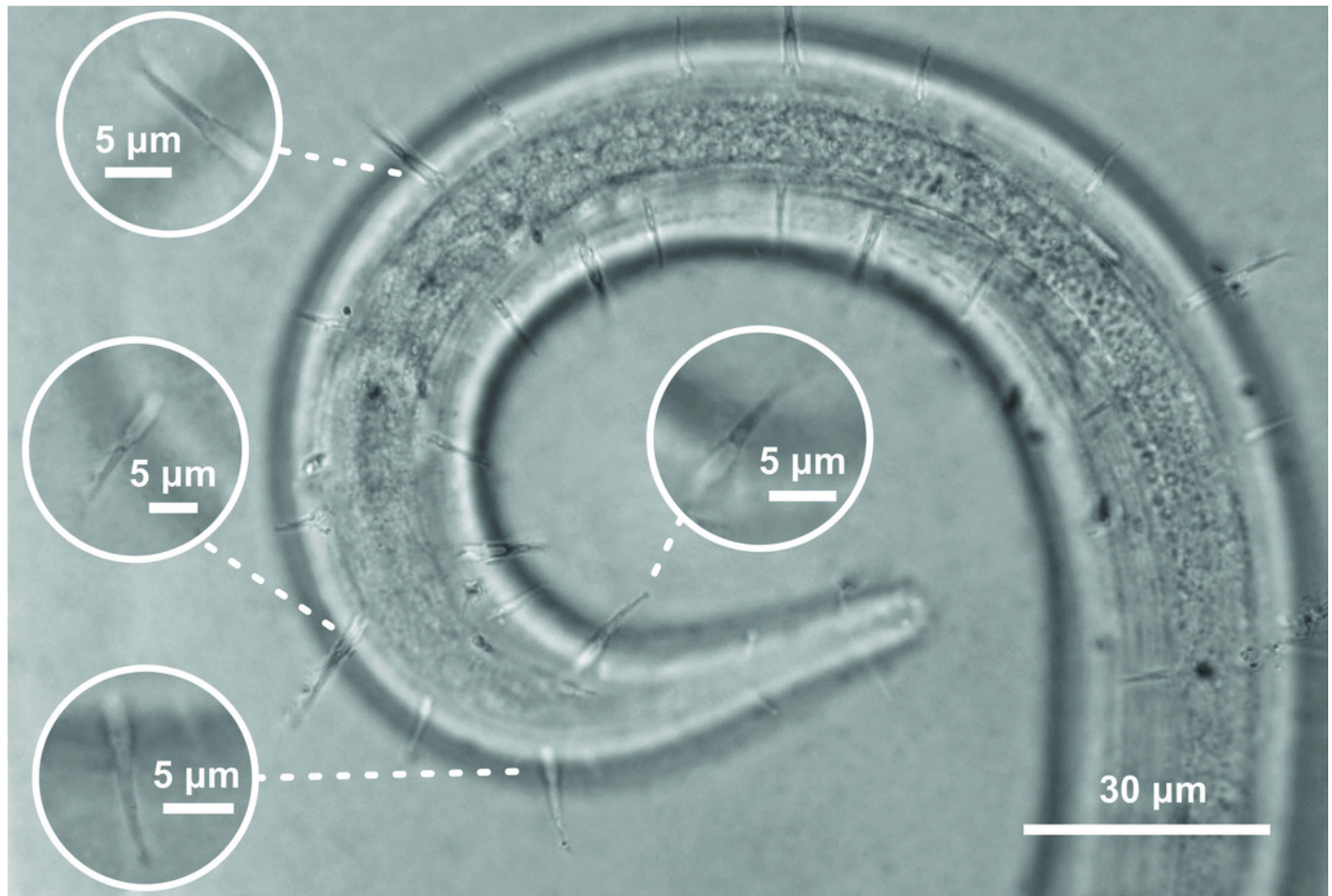


Figure 6

Ixonema gracielea sp. n. holotype male.

Holotype male: (A) overview; (B) anterior region; (C) anterior end; (D) posterior region.

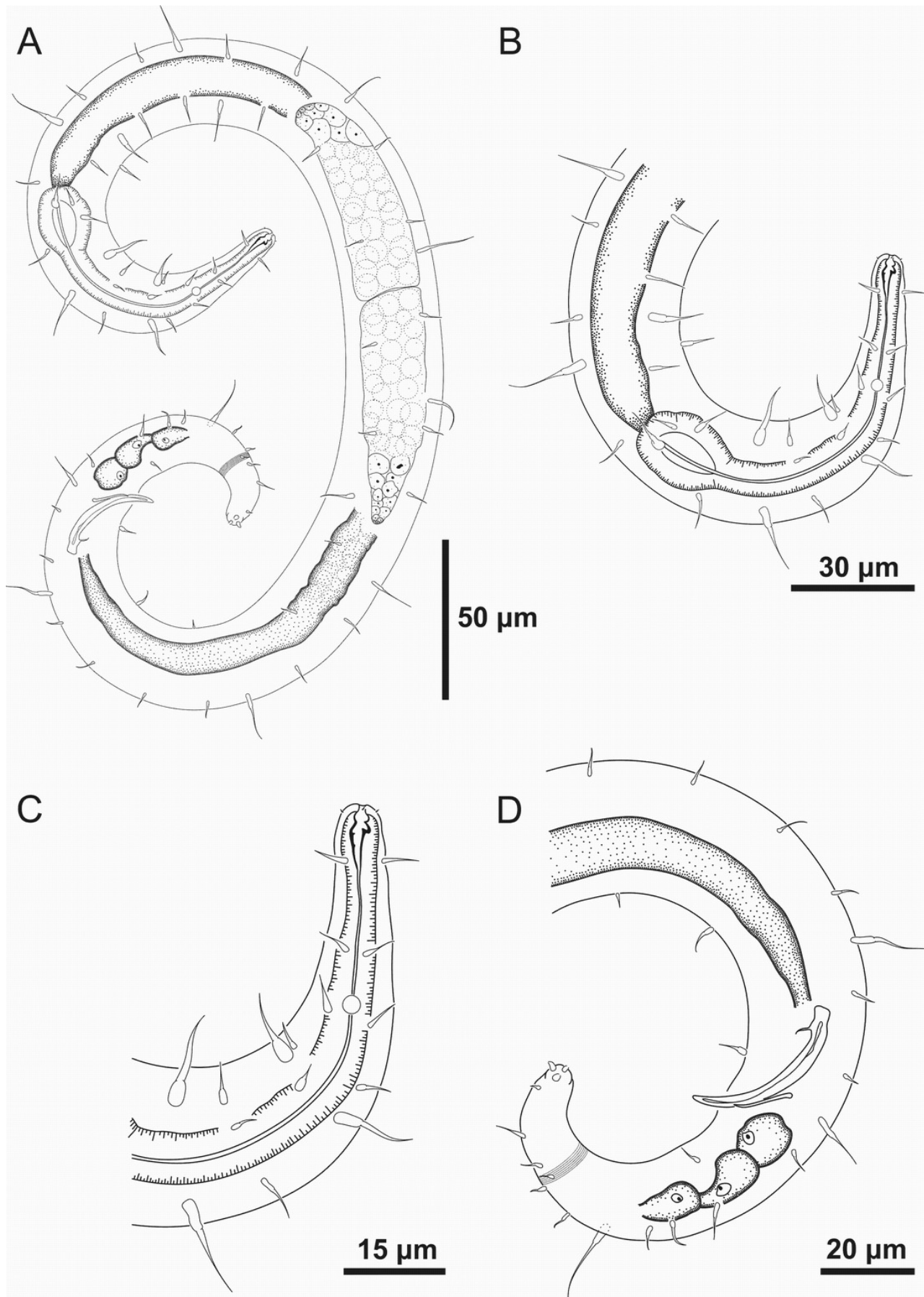


Figure 7

FIGURE 7. *Ixonema gracielea* sp. n.: paratype female.

Paratype female: (A) overview; (B) anterior region; (C) anterior end; (D) tail.

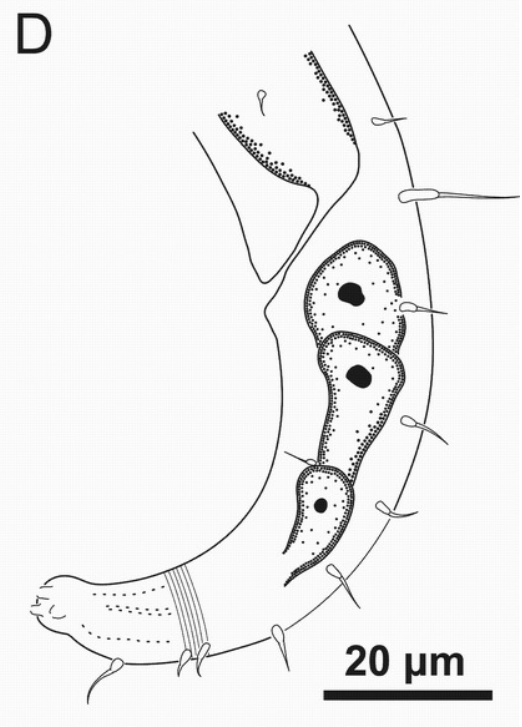
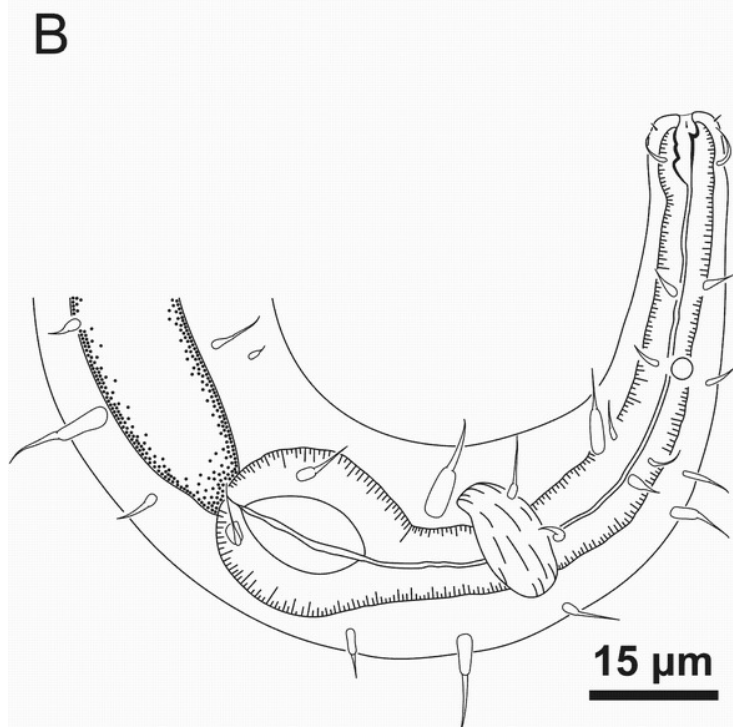
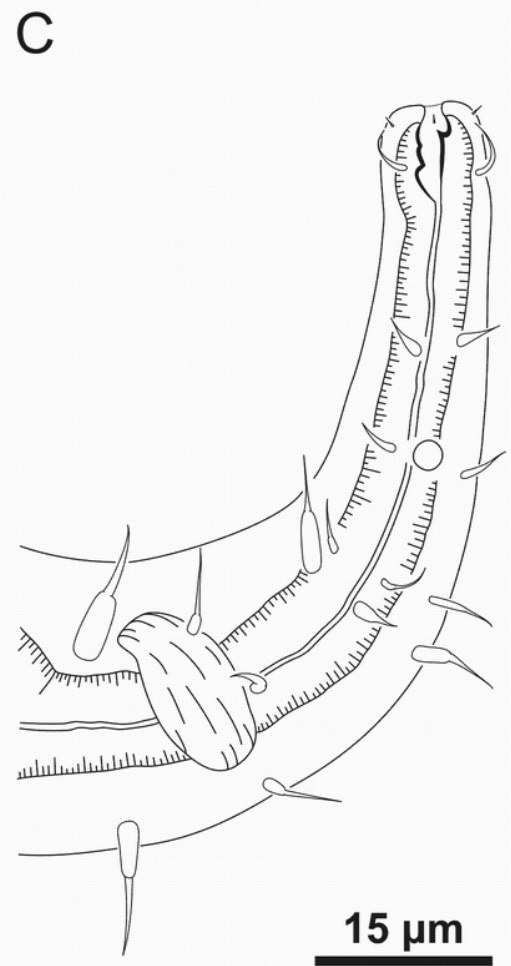
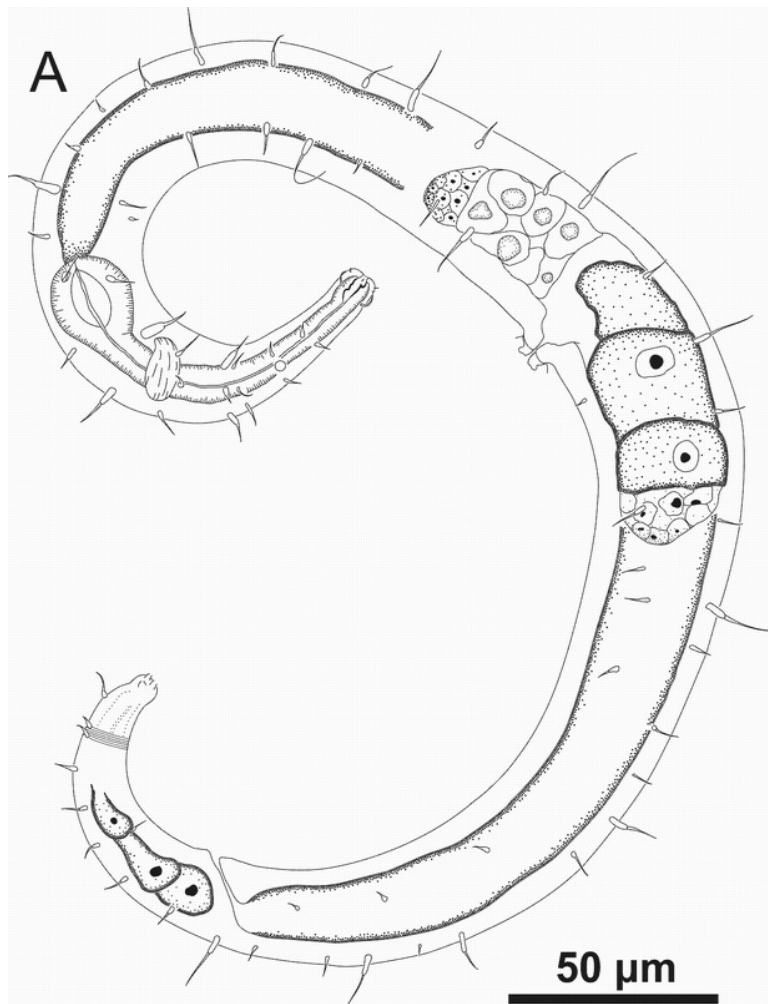


Figure 8

FIGURE 8. *Ixonema gracielea* sp. n. holotype male.

Holotype male: (A) anterior region: arrows indicating cephalic setae (cs), a single ventral seta in the anterior region (vs) and pharyngeal bulb (bulb); (B) anterior region: arrows indicating the dorsal tooth (dt) and amphidial fovea (amph); (C) anterior end (somatic setae); (D) tail (arrow indicating three separate tail tip outlets); (E) posterior end: arrows indicating precloacal supplements (supl); (F) posterior end: arrows indicating precloacal supplement (supl), spicule (spic) and gubernaculum (gub).

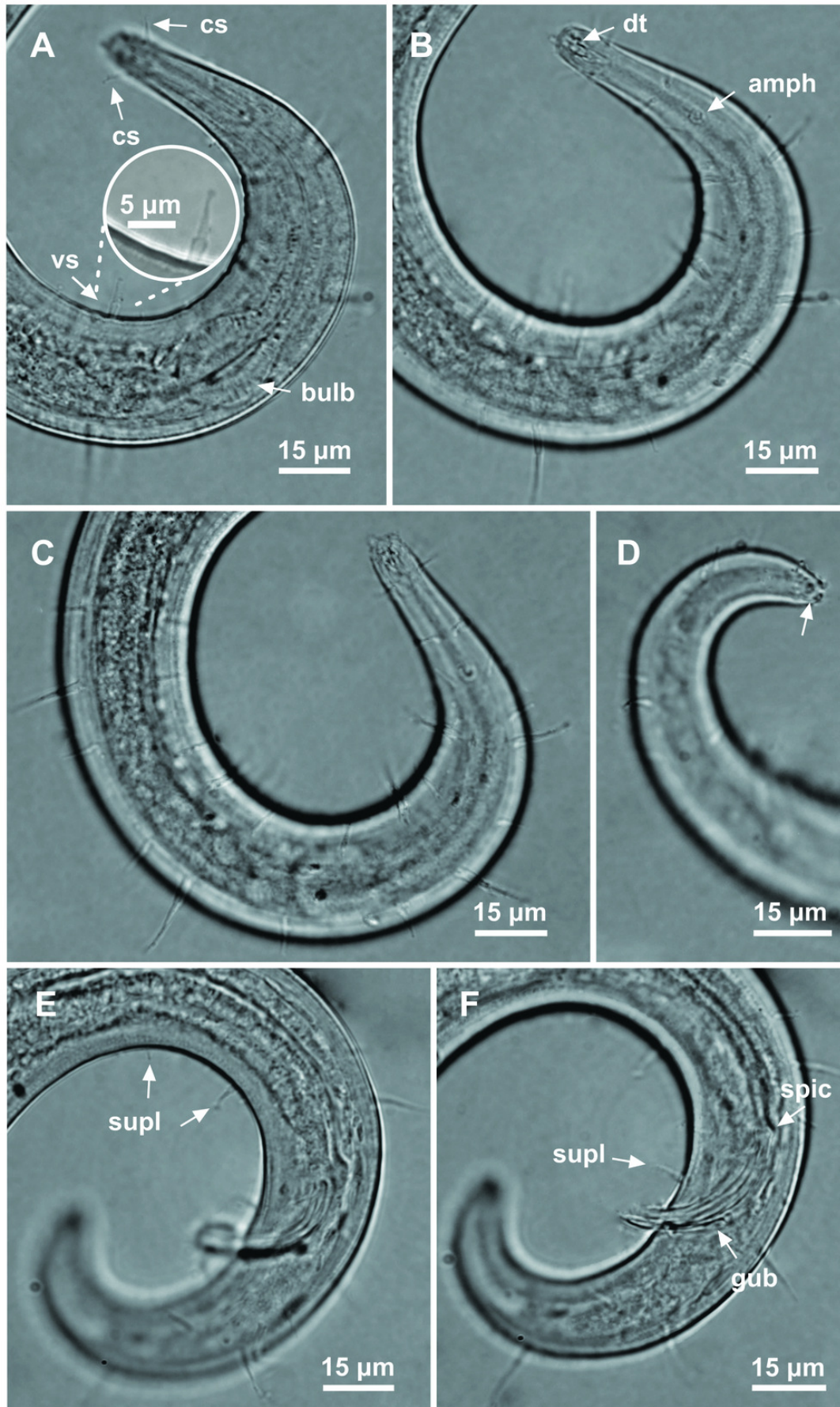


Figure 9

FIGURE 9. *Ixonema gracielea* sp. n. paratype female 1 and paratype female 2.

Paratype female 1: (A) anterior region: arrows indicating cephalic setae (cs) and the dorsal tooth (dt); (B) anterior region: arrows indicating amphidial fovea (amph) and pharyngeal bulb (bulb); (C) anterior end (somatic setae); (D) reproductive system: arrows indicating the anterior ovary (ov.a.), posterior ovary (ov.p.) and the vulva (V). Paratype female 2: (E) tail (arrow indicating three separate tail tip outlets).

