

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~~ three families: Aponchiidae Gerlach, 1963; ~~Molgolaimidae Jensen, 1978;~~ Monoposthiidae Filipjev, 1934 and Microlaimidae Micoletzky, 1922 (Leduc *et al.*

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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 intertidal 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 (Tchunov 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; Tchunov 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 cm² corer.

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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).

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Nematoda were counted (and removed) under a stereomicroscope using a Dolfus 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 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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work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: [urn:lsid:zoobank.org: pub: 414C399D-A60E-494E-9E36-C4866FBC9539](http://zoobank.org/pub:414C399D-A60E-494E-9E36-C4866FBC9539). The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

Results

SYSTEMATICS

Class CHROMADOREA Inglis, 1983

Subclass CHROMADORIA Pearse, 1942

~~Order Microlaimida Leduc, Verdon & Zhao, 2017~~ Desmodorida De Coninck, 1965

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~~Superfamily Subfamily~~ Microlaimoidea Micoletzky, 1922

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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

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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*. The gubernaculum of *Microlaimus paraundulatus* has a wave-shaped structure, similar to the gubernaculum of *Microlaimus undulatus* Gerlach, 1953.~~

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

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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-shaped~~wave shaped. Precloacal supplements absent. Three caudal glands. Tail conical, about 4.9 times the cloacal body diameter.

Paratype female. Similar to male. Body measuring 465 µm in length, with a maximum diameter of 18 µm. Cephalic sensilla equivalent to 43% of head diameter. Amphidial fovea, occupying 56% of corresponding body width and located 13 µm from anterior end. Buccal cavity, teeth and pharynx similar to that of the male. Basal bulb occupying 80% of the corresponding body diameter. Nerve ring situated at 59% of the pharynx length, from anterior end. Vulva located 244.5 µm from anterior end, at 53% of body length. Reproductive system didelphic-amphidelphic, with outstretched ovaries. Anterior ovary situated to the right side of intestine, posterior ovary to the left side of intestine. Tail conical, about 5.5 times the anal body diameter.

Diagnosis. *Microlaimus paraundulatus* **sp. n.** characterized by its body length (439–465 µm). Cuticle finely annulated. Head slightly set off. Four cephalic sensillae in the shape of thin setae (3 µm long), corresponding to 43% of head diameter. Amphidial fovea occupying 44% of the corresponding body diameter in the males and 56% in the female, located at about 1.6–1.9 times the head diameter. Buccal cavity with three small teeth, one dorsal and two ventrosublateral, the dorsal is slightly larger. Spicule arched and slender (1.8–2.3 times the cloacal body diameter) with a wave-shaped gubernaculum. Tail conical which corresponds to 4–6 cloacal or anal body diameter.

Differential diagnosis (Table 3). Firstly, it is important to establish that only males of each species mentioned in this section were used in the comparisons with males of the new species. More detailed information, such as some measurements and proportions, about females is absent in the original descriptions of some of the species in question. The measurements and proportions missing from the descriptions were obtained from available images.

Microlaimus paraundulatus **sp. n.** resembles *M. undulatus* Gerlach, 1953 mainly due to the peculiar shape of the gubernaculum (wave-shaped). Additionally, in both

species the amphidial fovea occupies a similar proportion of the corresponding body diameter (44% in *M. paraundulatus* **sp. n.** and 42% in *M. undulatus*) and the spicules are very similar in length (23–27 μm in *M. paraundulatus* **sp. n.** and 27 μm in *M. undulatus*). Although, the species differ in 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. zosteræ* Allg  n, 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. zosteræ* provided by Kovalyev & Tchesunov (2005), whose original description was based on females alone, *M. paraundulatus* **sp. n.** differs from the males of *M. zosteræ* 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. zosteræ*) and the precloacal supplements that are absent in *M. paraundulatus* **sp. n.** vs present in *M. zosteræ*.

Microlaimus modestus **sp. n.**

(Table 4; Figs 4–7)

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–short~~ body length. From Latin *modestus*: ~~restrained~~~~short in length~~.

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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 ~~the~~ 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~~are comparable with the ~~that of~~ male ~~oness~~. 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 *Paramicrolaimus* *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.

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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.

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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.

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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. *Microloaimus 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 *Microilaimus 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 species differs from *M. cyatholaimoides* in terms of the shape of the gubernaculum (funnel-shaped surrounding the spicules at the distal end in the new species 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.

Microilaimus 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 species 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 species vs lamellar in *M. porus*). Moreover, *M. nordestinus* **sp. n.** has three small teeth vs two visible teeth in *M. porus*.

Microilaimus 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 species 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*.

Microilaimus 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~~ eight to eleven

Commented [MC8]: Based on the introduction
(4Gerlach+1Ward+3Lima)

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