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Research article

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On species of *Asclerocheilus* Ashworth, 1901 (Annelida: Scalibregmatidae) from Brazil

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Abstract. Scalibregmatidae Malmgren, 1867 is a family of annelids, with only few taxa reported from Brazil. In this work, we describe two new species of *Asclerocheilus* Ashworth, 1901. The new species *A. geiseae* sp. nov. and *A. blakei* sp. nov. were collected during scientific expeditions to the Espírito Santo and Campos sedementary basins. These expeditions were coordinated by PETROBRAS/CENPES. The species *A. geiseae* sp. nov. is unique among its congeners by the presence of acicular spines with rounded and curved tips in two rows. The second species, *A. blakei* sp. nov., differs from its congeners by the combination of the presence of a trapezoidal prostomium, acicular spines on chaetigers 1–3, lyrate chaetae from chaetiger 3 on notopodia and 2 on neuropodia. Moreover, we report the species *A. tropicus* Blake, 1981 from the localities sampled on this study. We also provide an identification key to all species of *Asclerocheilus* of the world.

Keywords. South Atlantic, deep sea, Polychaetes, balloon worms, benthic macrofauna.

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Introduction

Scalibregmatidae Malmgren, 1867 is a small family of marine annelids, mainly found living on soft bottoms at great depths (Blake 2020, 2023; Parapar *et al.* 2021; Rouse *et al.* 2022). 81 species grouped in 14 genera are currently accepted (Blake 2020, 2023; Parapar *et al.* 2021; Rouse *et al.* 2022; Mendes *et al.* 2024a, 2024b). Of these, 25% are known to occur at abyssal depths (Blake 2020, 2023; Parapar *et al.* 2021; Rouse *et al.* 2022). Ecologically, most species are active burrowers and sub-surface deposit feeders, commonly found in low densities (Blake 2020, 2023; Parapar *et al.* 2021; Rouse *et al.* 2022). However, two studies mentioned benthic communities dominated by *Scalibregma australis* Blake, 2015 at the Antarctic Peninsula and *Scalibregma inflatum* Rathke, 1843 at Cape Hatteras, North Carolina, NW Atlantic (Blake & Hilbig 1994; Blake 2015, 2020; Parapar *et al.* 2021). Their reproduction and development are still poorly understood (Blake 2015, 2020, 2023; Parapar *et al.* 2021).

Morphologically, scalibregmatids are easily recognized by their T-shaped prostomium followed by an inflated anterior region transitioning abruptly to a narrow posterior region (Rouse 2001). Their body segments are secondarily annulated, with each annulus composed of a row of small pads, giving to most species a complex areolate appearance (Blake 2020). The prostomium may have a pair of horns, with different shapes. Parapodia may have branchiae, dorsal and ventral cirri. In the anterior region, short spinous chaetae, long acicular chaetae and lyrate chaetae are present, grouped in several rows including capillaries. In the middle and posterior regions of the body, only capillaries and lyrate chaetae are present. The pygidium often possesses pygidial cirri, long or short, emerging from the pygidial margin, which could be long or short, commonly with a crenulated margin. These characteristics are the most important ones to separate scalibregmatid genera, following Ashworth (1901) and the taxonomic arrangement of the family proposed by Kudenov & Blake (1978). However, most genera overlap their diagnostic characteristics (Parapar *et al.* 2021), causing identification problems, mainly related to the ontogenetic development of juveniles (Blake 2015).

The family's phylogenetic position is still debated, and results from morphology-based and molecular-based approaches conflict (Blake 2020; Parapar *et al.* 2021). Thus, a unified approach using both kinds of data, and the understanding of character evolution within the group are still needed (Parapar *et al.* 2021). Traditionally, Scalibregmatidae was considered as a clade of the suborder Opheliida (Fauchald 1977; Fauchald & Rouse 1997), within the superorder Scolecida (Rouse 2001). Subsequent studies based on molecular evidence proposed different topologies, separating the members of Opheliida and finding *Travisia* Johnston, 1840 nested within Scalibregmatidae (Bleidorn *et al.* 2003a, 2003b; Persson & Pleijel 2005; Martínez *et al.* 2013; Rouse *et al.* 2022). However, these studies indicated important methodological limitations, and flaws on vouchers identifications used to conduct the molecular analysis (Blake 2020; Parapar *et al.* 2021). For this reason, we will follow Blake & Maciolek (2016) in relation to the treatment of *Travisia* as a separate family.

Information on Brazilian Scalibregmatidae is still limited to southern and southeastern regions, with ten species reported so far (Amaral *et al.* 2013; Mendes *et al.* 2023, 2024a, 2024b). They are *Asclerocheilus tropicus* Blake, 1981, *Oligobregma nonatoi* Mendes, Paiva & Rizzo, 2024, *O. bakkeni* Mendes, Paiva & Rizzo, 2024, *O. cruzae* Mendes, Paiva & Rizzo, 2024, *Pseudoscalibregma ermindae* Mendes, Paiva & Rizzo, 2024, *P. parapari* Mendes, Paiva & Rizzo, 2024, *P. magalhaesi* Mendes, Paiva & Rizzo, 2024, *Scalibregma inflatum* Rathke, 1843, *S. lanai* Mendes, Paiva & Rizzo, 2023, and *S. cynthiae* Mendes, Paiva & Rizzo, 2023. The records related to *S. inflatum* are questionable, taking into consideration the observations made by Mackie (1991) and Bakken *et al.* (2014) related to the morphology and distribution of the species. Mendes *et al.* (2023) discussed the validity of these reports. Here, we present two new species of *Asclerocheilus* Ashworth, 1901 from the southeastern Brazilian coast, along with an identification key to the currently accepted species, according to Blake (2020, 2023), Parapar *et al.* (2021), and Mendes *et al* (2023, 2024a, 2024b).

Material and methods

This study was conceived under the scope of projects developed by PETROBRAS/CENPES from surveys in order to obtain environmental data for biomonitoring activities in the Espírito Santo and Campos sedimentary basins, respectively related to the two following projects: I) Ambes Project – Espírito Santo Basin Assessment Project, both coordinated by PETROBRAS (Bernardino *et al.* 2016), II) Habitats project – Campos Basin Regional Characterization Program (Ribeiro-Ferreira *et al.* 2017).

In the Ambes project, the continental slope of Espírito Santo and the northern region of the Campos Basin were sampled during two research campaigns in 1) December 2012, then 2) June and July 2013. The Habitats project was conducted in three campaigns in 1) May and July 2008, 2) January and March 2009, then 3) June and July 2009. Triplicate box core samples were collected from 42 stations along seven transects evenly distributed across the regions. On board, samples were fixed in borax-buffered 10% formaldehyde.

Sampling stations were positioned along eight transects (A–H), on 11 isobaths in the Ambes and Habitats projects, five of them positioned on the continental shelf at depths of 25, 50, 75, 100 and 150 m, and the six others were positioned on the slope at depths of 400, 700, 1000, 1300, 1900, 2400 and 3000 m. The sediment was collected in triplicate, with the aid of a modified van Veen grab (shelf) or a box-corer (slope), depending on the type of bottom. For macrofauna, the first 10 centimetres of the sediment were collected, and for the slope, the samples were stratified at depths of 0–2, 2–5 and 5–10 cm.

The specimens were separated from the sediment, identified, and deposited at the Rio de Janeiro State University (UERJ), Collection of Polychaeta (UERJ-Pol) at the Invertebrate Zoology Laboratory (LZI-UERJ). Specimens were prepared for Scanning Electron Microscopy (SEM) using a 99% solution hexamethyldisilazane (HMDS) following this process: 1) removal of water through ethanol (EtOH) in a series of 70%, 80%, 90%, then absolute EtOH concentration, for 15–20 minutes in each step; 2) replacement of ethanol using HMDS in a series of 1:2, 1:1, 2:1 ratios of absolute EtOH and HMDS each step for 15–20 minutes, and finally 3) total evaporation of HMDS during about 4–5 hours. Additionally, to improve the observation of morphological characters under optical microscopy, some specimens were stained with Shirlastain A.

Abbreviations for morphological terms

1stPd = ventral groove's first pad
As = acicular spines
Ch1 = chaetiger 1
Hor = prostomial horn
iA = intermediate annulation
Ly = lyrate chaeta
NeL = neuropodial lobe
NoL = notopodial lobe
Pe = peristomium
Pg = pygidium
Pgc = pygidial cirrus
plA = parapodial lobe's annuli
Pro = prostomium

Results

Taxonomy

Phylum Annelida Lamarck, 1802
Class Polychaeta Grube, 1850
Family Scalibregmatidae Malmgren, 1867

Genus ***Asclerocheilus*** Ashworth, 1901

Gwasitoa Chamberlin, 1919: 390–391.
Kebuita Chamberlin, 1919: 390–391.

Type species

Asclerocheilus intermedius (Saint-Joseph, 1894).

Diagnosis

Body elongate, arenicoliform. Prostomium T-shaped with frontal horns. Parapodia of posterior segments reduced; dorsal and ventral cirri absent; interramal papillae or cilia present or absent; postchaetal lamellae absent. Branchiae absent. Chaetae include capillaries, furcate chaetae, and large, conspicuous curved spines on setigers 1 to 4, sometimes accompanied by short spinous setae. Pygidium with long anal cirri.

Remarks

The genus was erected by Ashworth (1901), to include the species *A. intermedius* (Saint-Joseph, 1894), previously assigned to the genus *Lipobranchius* Cunningham & Ramage, 1888. In the same work, Ashworth (1901) differentiated *Asclerocheilus* from *Sclerocheilus* Grube, 1863 by the absence of parapodial cirri, positioning the genus within the family subgroup I, containing species with an "arenicoliform" body shape. Subsequently, several new species were described. Blake (1981) transferred *S. acirratus* Hartman, 1966 to *Asclerocheilus acirratus* (Hartman, 1966) and presented two new species to the genus. Hartmann-Schröder (1994) compared the known species, providing important information on their morphological differences. Blake (2000) referred *Kebuita glabra* (Ehlers, 1887) to *Asclerocheilus glabrus* (Ehlers, 1887). The author also followed Hartman's (1938) synonymization of *Gwasitoa* Chamberlin, 1919 with *Kebuita* Chamberlin, 1919, and synonymized *Kebuita* with *Asclerocheilus*. Moreover, Blake (2000) erected *A. victoriensis* Blake, 2000 as a new name to replace *A. heterochaetus* Kudenov & Blake, 1978. This was proposed because *Oncoscolex heterochaetus* is a synonym of *Kebuita glabra* (Ehlers, 1887), and *K. glabra* was transferred to *A. glabrus* within the same work. In this sense, *A. heterochaetus* consequently is a junior homonym of *O. heterochaetus*. Thus, Blake (2000) renamed the homonym *A. victoriensis* (Hartman 1938; Blake 2000, 2023). Finally, Blake (2023) published the most recent advances on the genus taxonomy and ecology, transferring *Oligobregma tasmania* to *A. tasmanius* and describing an abyssal species from Australia.

Currently, the genus *Asclerocheilus* counts 15 described species, they are *A. glabrus* (Ehlers, 1887), from the Caribbean Sea; *A. intermedius* (Saint-Joseph, 1894) from the eastern North Atlantic; *A. beringianus* Ushakov, 1955 from the Bering Sea; *A. capensis* Day, 1963 from South Africa; *A. californicus* Hartman, 1963 from Southern California; *A. acirratus* (Hartman, 1966) from California; *A. ashworthi* Blake, 1981 from the Southern Ocean in Sub-Antarctic waters; *A. tropicus* Blake, 1981 from Ecuador; *A. mexicanus* Kudenov, 1985 from the Gulf of Mexico; *A. shanei* Hartmann-Schröder, 1994 from Tasmania; *A. kudenovi* Blake, 2000 from Central California; *A. victoriensis* Blake, 2000 from Southeast Australia; *A. elisabethae* Eibye-Jacobsen, 2002 from the Andaman Sea, Thailand; *A. shanonae* Eibye-Jacobsen, 2002 from the Andaman Sea, Thailand and *A. abyssalis* Blake, 2023 from Eastern Australia.

Regarding the Brazilian coast, possible occurrences may be related to the species described in the following works: Day (1963, 1967), Blake (1981) and Kudenov (1985). *Asclerocheilus tropicus* is the only known species of *Asclerocheilus* found in Brazil, having been redescribed by Nogueira (2002). The material was sampled from Laje de Santos (24°19′ S, 46°11′ W), on 17 March 1996, and Ilha dos Alcatrazes (26°06′ S, 45°42′ W), on 4 December 1996; both localities are rocky shores with large colonies of cnidarians, from which the scalibregmatids were sorted (Nogueira 2002). Moreover, Nogueira (2002) provided an important account on the character variability within the species, such as the horns and prostomium shape, presence or absence of eyes as well as its colour and shape, the acicular spines shape, and the ratio of lyrate chaetae tynes. The latter account is important, because it provided important arguments regarding the validity of this character to separate species, firstly proposed by Blake (1981).

Asclerocheilus geiseae sp. nov.

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Figs 1–3

Diagnosis

Triangular prostomium, with two long horns, both thin and projected upwards. Eyes absent. Ventral groove present from chaetiger 1 with first pad asymmetrically rounded, contributing to mouth's lower lip formation. Acicular spines present on chaetigers 1–3 on notopodia, and 1–2 on neuropodia. Short spinous chaetae not observed. Lyrate chaetae present from chaetiger 2 on neuropodia and 3 on notopodia. Anterior chaetigers parapodial lobes larger, more prominent, present as broad blunted projection in both noto- and neuropodia. Pygidium present as short uniannulated segment, with crenulated margin.

Etymology

The specific epithet '*geiseae*' was chosen to honour the Brazilian mastozoologist Dr Lena Geise, from the Rio de Janeiro State University (UERJ), for her important contributions to Brazilian zoology and to celebrate our friendship.

Type material

Holotype

BRAZIL – **Espírito Santo, Espírito Santo Basin** • complete spec.; 19.959° S, 39.892° W; depth 11 m; Dec. 2010; van Veen grab; subtidal zone; UERJ, UERJ-8065.

Paratypes

BRAZIL – **Espírito Santo, Espírito Santo Basin** • 1 complete spec., 1 incomplete spec.; 19.960° S, 39.892° W; depth 11 m; Dec. 2010; van Veen grab; subtidal zone; UERJ, UERJ-8066 • 1 complete spec.; 19.9590° S, 39.893° W; depth 47 m; Jul. 2011; van Veen grab; subtidal zone; UERJ, UERJ-8067 • 1 complete spec.; 19.959° S, 39.892° W; depth 47 m; Dec. 2010; van Veen grab; subtidal zone; UERJ, UERJ-9223 • 1 complete spec.; 19.959° S, 39.892° W; depth 47 m; Dec. 2010; van Veen grab; subtidal zone; UERJ, UERJ-9224.

Description

Holotype complete, 10 mm long, 1 mm wide over expanded region, 0.3 mm wide on narrowest region for 57 chaetigers. Medium-sized species, paratypes measuring 4–5 mm long, 0.4–0.6 mm wide expanded on anterior region, 0.13–0.25 mm wide on narrowest posterior region, for 32–44 chaetigerous segments. Body arenicoliform, weakly expanded over chaetigers 2–19. Colour in alcohol pale tan. Body surface covered by secondarily annulated rings. Secondary annuli composed by rounded to rectangular small pads, basally fused to each other, in both anterior and posterior regions. Annuli's small pads on anterior region may be weaker in comparison to posterior region. Internal glands were not observed.

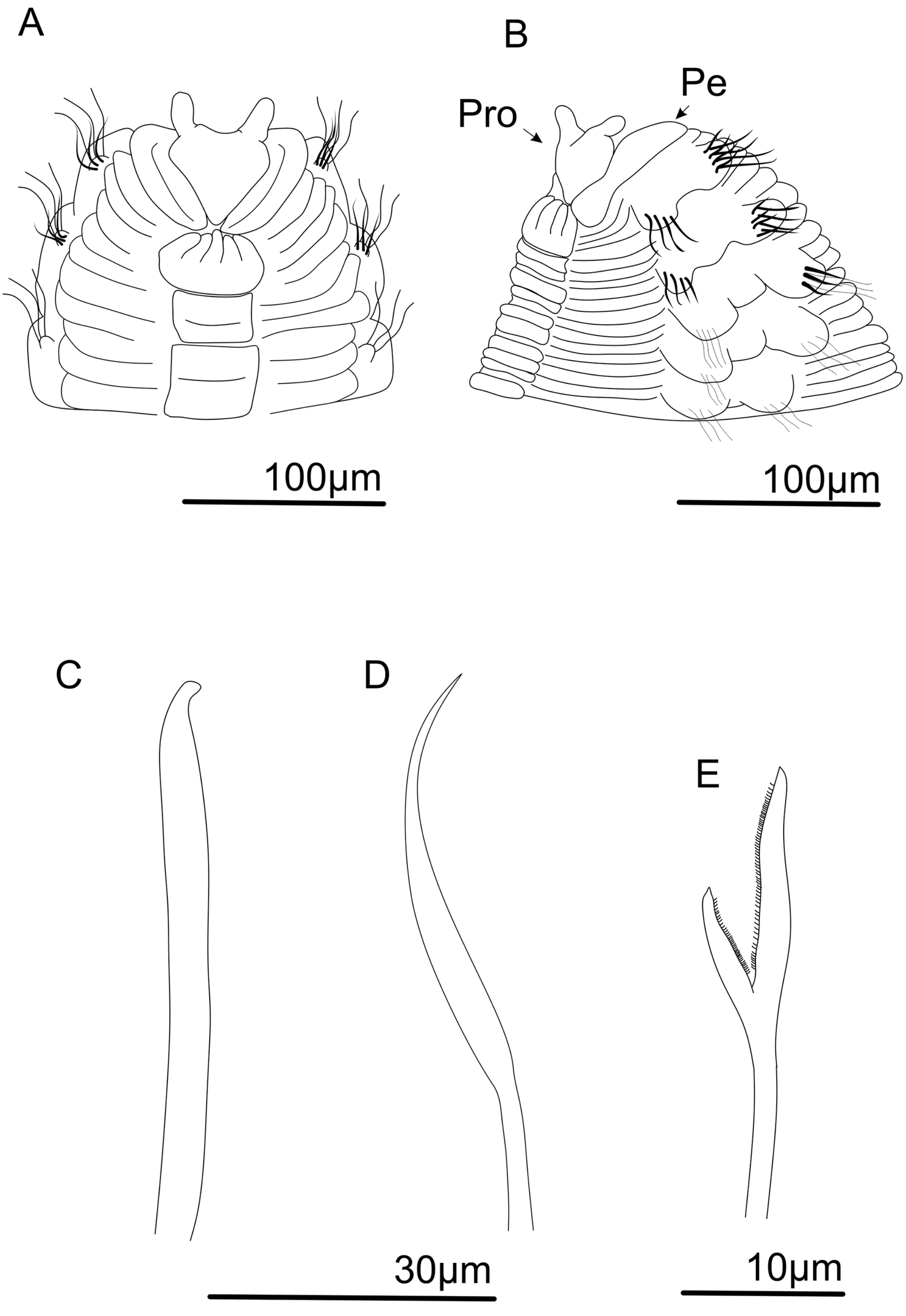

Fig. 1. *Asclerocheilus geiseae* sp. nov., holotype (UERJ-8065), schematic representation. **A**. Ventral view of anterior region. **B**. Lateral view of anterior region. **C**. Acicular spine with rounded tip present on chaetigers 1–2. **D**. Acicular spine with pointed tip present only on chaetiger 3. **E**. Lyrate chaeta. Abbreviations: see Material and methods.

Triangular prostomium, with two long horns, both thin and projected upwards (Figs 1A–B, 2B, 3A–B). Eyes absent. Nuchal organs not observed. Peristomium achaetous, uniannulated in holotype, but some paratypes presented a peristomium biannulated ventrally and uniannulated dorsally. Proboscis smooth. Mouth's lips presenting single row of paired lobes formed superiorly by peristomium and inferiorly by ventral groove first pad. Upper lip presents 5–6 lobules, lower lip presents 5–7 lobules. Ventral

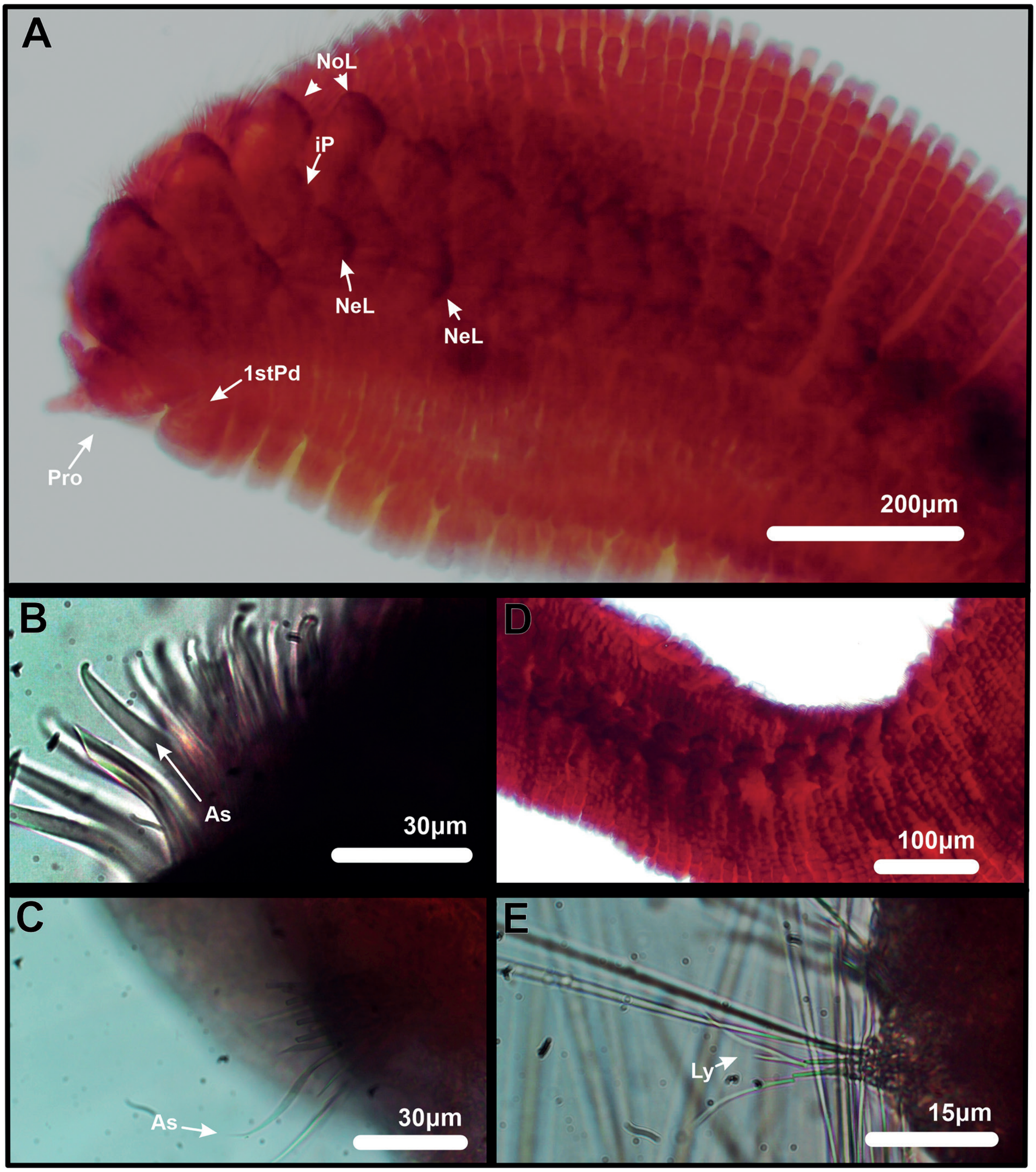

Fig. 2. *Asclerocheilus geiseae* sp. nov., paratype (UERJ-8066), under optical microscopy, specimens stained with Shirlastain A. **A**. Latero-ventral view of anterior body. **B**. Acicular spines with rounded tips. **C**. Acicular spine with pointed tip. **D**. Mid-body chaetigers in lateral view. **E**. Lyrate chaetae. Abbreviations: see Material and methods.

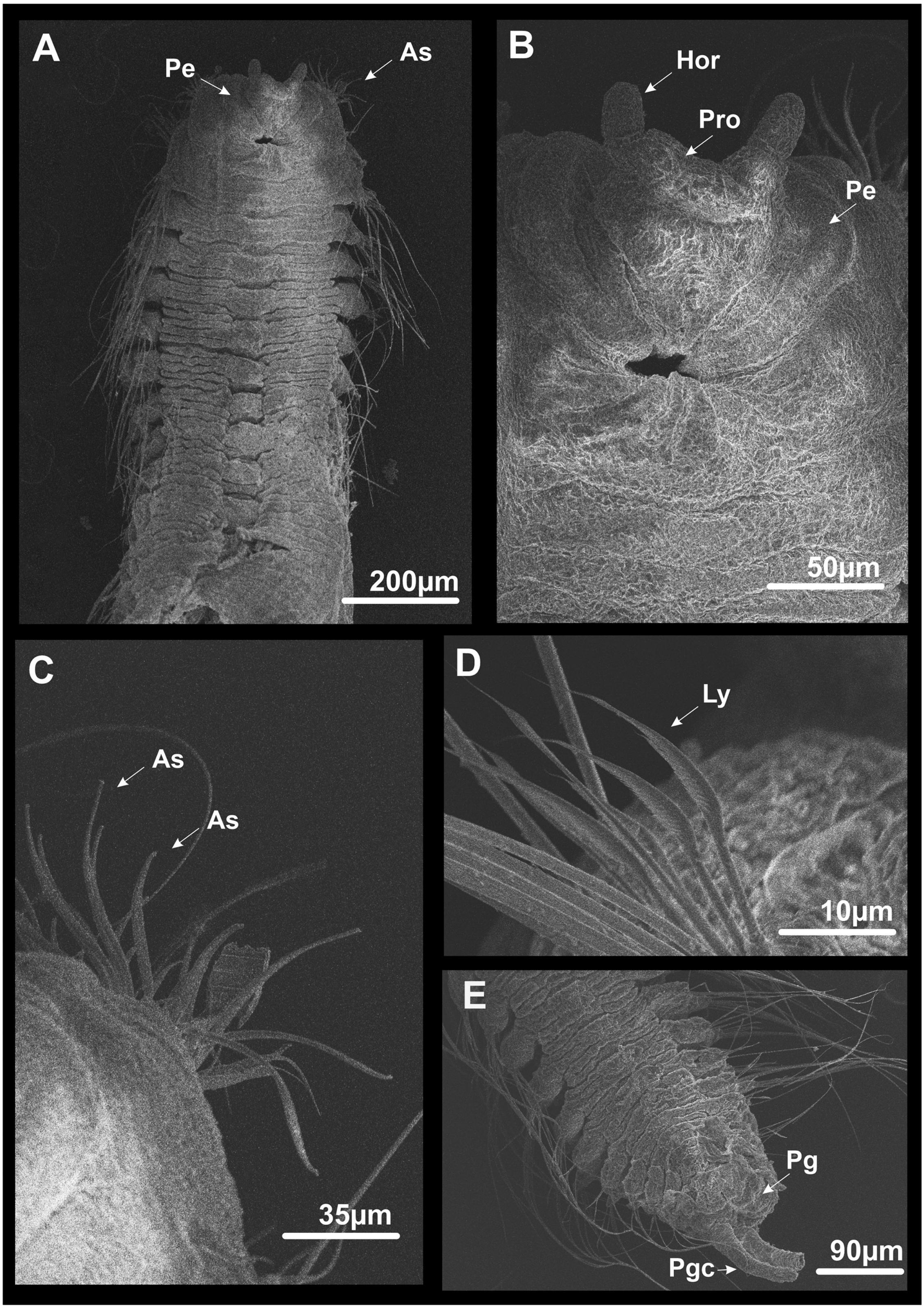

Fig. 3. *Asclerocheilus geiseae* sp. nov., under SEM. **A**. Ventral view of anterior body. **B**. Prostomium and peristomium in ventral view. **C**. Acicular spines with rounded tips. **D**. Lyrate chaetae. **E**. Ventral view of posterior most chaetigers and pygidium, only two cirri are present, the others were lost in this specimen. Abbreviations: see Material and methods.

groove present from chaetiger 1 with first pad asymmetrically rounded, contributing to mouth's lower lip formation on its superior margin (Figs 1A–B, 3A–B). Quadrangular bi- to triannulated pads present from chaetiger 2, forming ventral mid-ridge up to end of the body. Each pad is paired to a single chaetiger. Posterior pads thinner than anterior ones.

Dorsally, chaetiger 1 with two secondary annuli connected to parapodial lobe, plus an intermediate annulation between chaetigers (Figs 1A–B, 3A–B); following chaetigers with three secondary annuli connected to parapodial lobe, and an intermediate annulation between chaetigers (Fig. 2A, D). Ventrally, chaetigers 1–4 with two secondary annuli connected to parapodial lobes, plus an intermediate annulation between chaetigers (Figs 1A–B, 3A–B). Following chaetigers on anterior region with three secondary annuli connected to parapodial lobe, and also an intermediate annulation between chaetigers (Fig. 3A). First and second chaetigers often smooth, but this variation should be interpreted as artefact. Interramal papillae rounded, present from anterior chaetigers (Fig. 2A), smaller and sometimes absent in posterior chaetigers (Fig. 2D), internal content unknown.

Acicular spines present on chaetigers 1–3 on notopodia, and 1–2 on neuropodia (Figs 1C–D, 2B–C, 3C). Short spinous chaetae not observed. Lyrate chaetae present from chaetiger 2 on neuropodia and 3 on notopodia (Figs 1E, 2E, 3D), numbering 5–6 lyrate chaetae per fascicle, with unequal tynes (tynes ratio: 2.5 on anterior chaetigers to 1.8–2.22 on midbody to posterior chaetigers).

Notopodial acicular chaetae organized in two rows on chaetigers 1–2, numbering up to 9 per row, with rounded tips (Figs 1C, 2B, 3C). Notopodial acicular chaetae on chaetiger 3 organized in single row, numbering 5 transitional acicular spines, with pointed tips. Neuropodial acicular spines weaker than notopodial ones, organized in single row on chaetigers 1–2, all curved with pointed tips (Figs 1D, 2C), numbering 8 per fascicle, transitional on chaetiger 2. Capillaries organised in single row on chaetigers 1–2, then in two rows on chaetiger 4 up to middle of body. In posterior chaetigers, capillaries become organized in single row.

Anterior chaetigers parapodial lobes larger, more prominent, present as broad blunted projection in both noto- and neuropodia, more robust and distinct than on posterior chaetigers (Fig. 2A). Parapodial lobes becoming gradually thinner and smaller in midbody and posterior chaetigers, emerging as apically rounded projections (Fig. 2D). Pygidium present as short uniannulated segment, with crenulated margin, from which emerges 4 long anal cirri; two dorso-lateral and two ventro-lateral (Fig. 3E).

Remarks

This species is morphologically similar to *A. intermedius, A. shanei, A. abyssalis* and *A. blakei* sp. nov. by having acicular spines on notopodia of chaetigers 1–3. However, it is unique among them by the presence of acicular spines with rounded tips in two rows on notopodia of chaetigers 1–2, then replaced by one row of acicular spines with pointed tips on notopodia of chaetiger 3. Moreover, *A. abyssalis* is the only species of the genus with short spinous chaetae mentioned on its description.

Ecology

This species was found at the Rio Doce river mouth near the Campos Basin (Brazilian southern region), living on sandy sediment at depths of 11–50 m.

Asclerocheilus blakei sp. nov.

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Figs 4–6

Diagnosis

Trapezoidal prostomium, with two long horns, both thin and projected laterally with inconspicuous aperture at their basis. Ventral groove present from chaetiger 1 with first pad asymmetrically rounded, bearing up to 4 small lobules on its superior margin, contributing to mouth's lower lip formation. Acicular chaetae with pointed tips present in chaetigers 1–3 on notopodia and neuropodia. Lyrate chaetae from chaetiger 3 on notopodia and 2 on neuropodia, with unequal tynes. Pygidium present as short crenulated margin with up to 10 small lobes, from which emerges 6 long anal cirri.

Etymology

The specific epithet '*blakei*' was chosen to honour Dr James A. Blake, for his important contributions to polychaete taxonomy, ecology and evolution. Most of the taxonomical issues concerning the family Scalibregmatidae were solved from his contributions, especially on the genus *Asclerocheilus*, which was revised by him (Blake 2000). The author also contributed to the second attempt to delimitate the genera within the family (Kudenov & Blake 1978), in the first work on species from the South Atlantic (Blake 1981), and in the first work describing the ontogeny and development of a scalibregmatid species (Blake 2015).

Type material

Holotype

BRAZIL • complete spec.; Rio de Janeiro, Campos Basin; 23.654317° S, 41.310094° W; depth 693 m; Jun. 2008; box corer; continental slope; UERJ, UERJ-1133.

Paratypes

BRAZIL • 2 complete specs; Rio de Janeiro, Campos Basin; 21.186° S, 40.216° W; depth 683 m; Feb. 2009; box corer; continental slope; UERJ, UERJ-6297 • 1 complete spec.; Espírito Santo, Espírito Santo Basin; 19.970° S, 39.519° W; depth 1908 m; Jun. 2013; box corer; continental slope; UERJ, UERJ-9222.

Description

Holotype complete, 5 mm long, 1 mm wide over expanded region, 0.25 mm wide on narrowest region, for 36 chaetigerous segments. Moderate-sized species, paratypes measuring 6–13 mm long to 0.15–2 mm wide for 33–43 chaetigerous segments. Body arenicoliform, weakly expanded over chaetiger 8– 7. Colour in alcohol pale tan to yellowish. Body surface covered by secondarily annulated rings in well preserved specimens. Secondary annuli composed by rounded to quadrangular small pads in both anterior and posterior regions. Annuli's small pads on anterior region may be weaker in comparison to posterior region. Internal glands not observed.

Trapezoidal prostomium, with two long horns, both thin and projected laterally (Figs 4A–B, 5A, 6A). One pair of small eyespots on middle of prostomium in small specimens, but absent in adults. Nuchal organs not observed. Proboscis smooth. Peristomium achaetous, uniannulated dorsally (Figs 4A, 6A) and biannulated ventrally (Figs 4B, 5A), contributing to mouth's upper lip formation. Ventral groove present from chaetiger 1 (Fig. 4B) with first pad asymmetrically rounded, bearing up to 4 small lobules on its superior margin, contributing to mouth's lower lips formation. Rectangular triannulated pads present from chaetiger 2, forming ventral mid-ridge up to end of body. Each pad paired to single chaetiger. Posterior pads thinner than anterior ones.

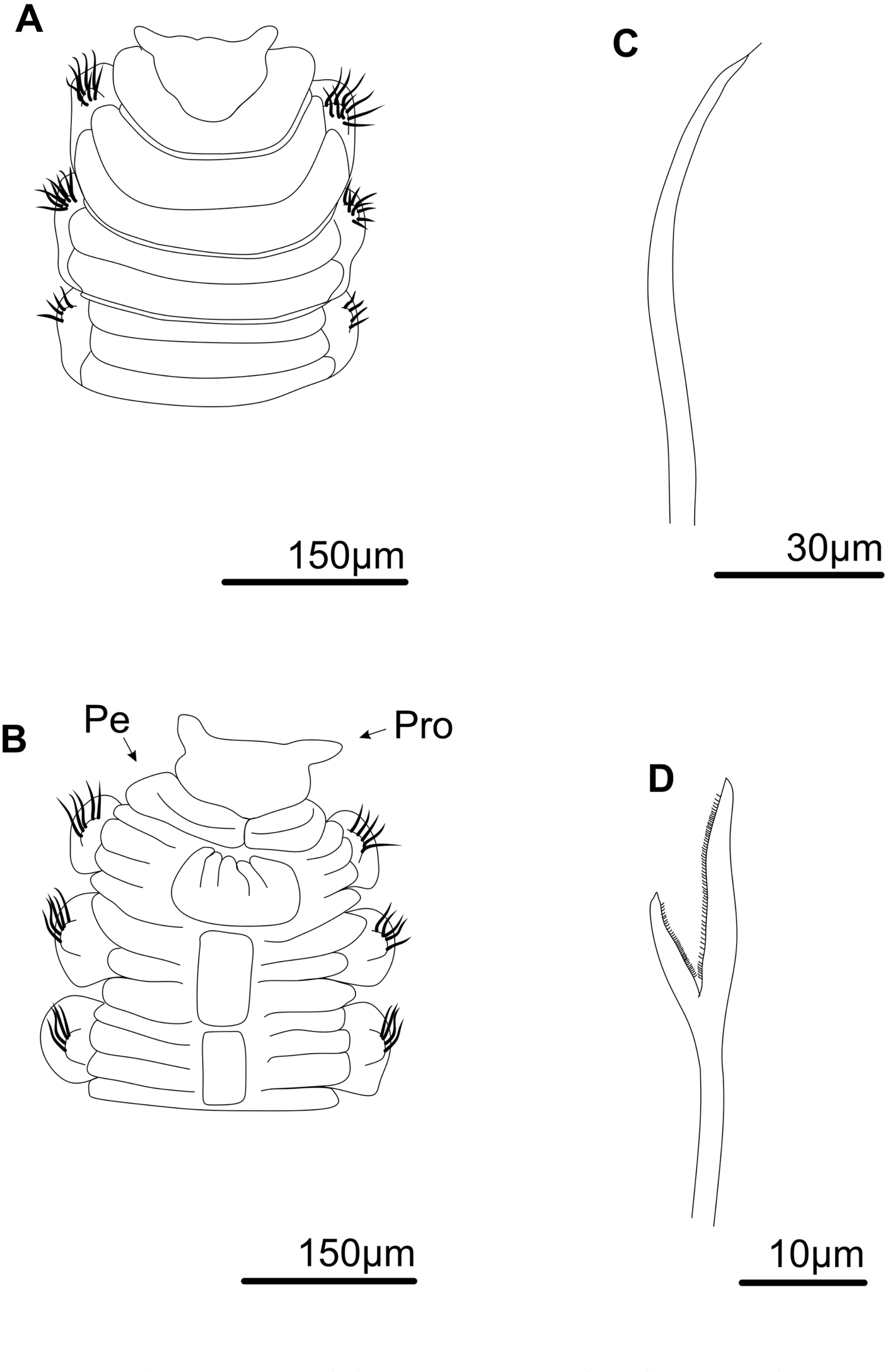

Fig. 4. *Asclerocheilus blakei* sp. nov., holotype (UERJ-1133), schematic representation. **A**. Dorsal view of anterior body. **B**. Ventral view of anterior body. **C**. Acicular spine. **D**. Lyrate chaeta. Abbreviations: see Material and methods.

Body chaetigers 1–2 with two secondary annuli connected to parapodial lobe, in addition intermediate annulation between chaetigers (Fig. 4A–B). From chaetiger 3, three secondary annuli connected directly to parapodial lobe, in addition intermediate annulation between chaetigers (Figs 4A–B, 5E). Interramal papillae present, but inconspicuous on anterior and midbody chaetigers, emerging as blunted projection between noto- and neuropodial lobes on posterior chaetigers (Fig. 5F).

Acicular chaetae with pointed tips present in chaetigers 1–3 on notopodia and neuropodia (Figs 4C, 5C–D, 6B–D). Lyrate chaetae with unequal tynes (tynes ratio 2.125), present in a single row from

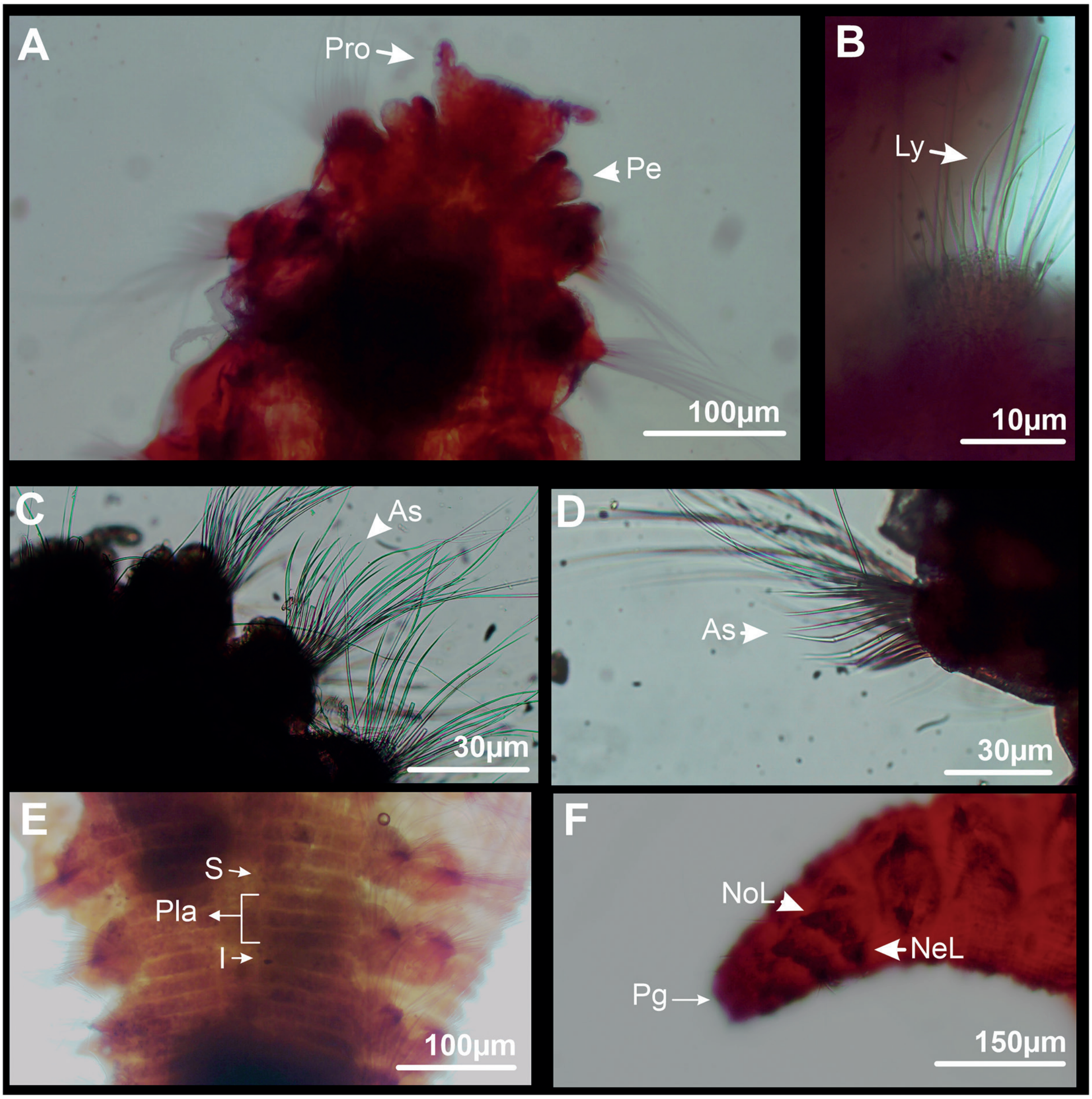

Fig. 5. *Asclerocheilus blakei* sp. nov., paratype (UERJ-6297), under optical microscope, specimens stained with Shirlastain A. **A**. Ventral view of anterior body (coll. code). **B**. Lyrate chaetae. **C**. Acicular spines on neuropodia of chaetigers 1–3. **D**. Acicular spines on notopodia of chaetiger 3 in detail. **E**. Ventral view of mid-body annulation pattern. **F**. Posterior most chaetigers in lateral view. Abbreviations: see Material and methods.

chaetiger 3 in notopodia and from chaetiger 2 in neuropodia (Figs 4D, 5B, 6C); numbering 3–4 chaetae in both rami on anterior chaetigers, up to 8 in midbody chaetigers, then 4–5 in posterior chaetigers. Short spinous chaetae absent.

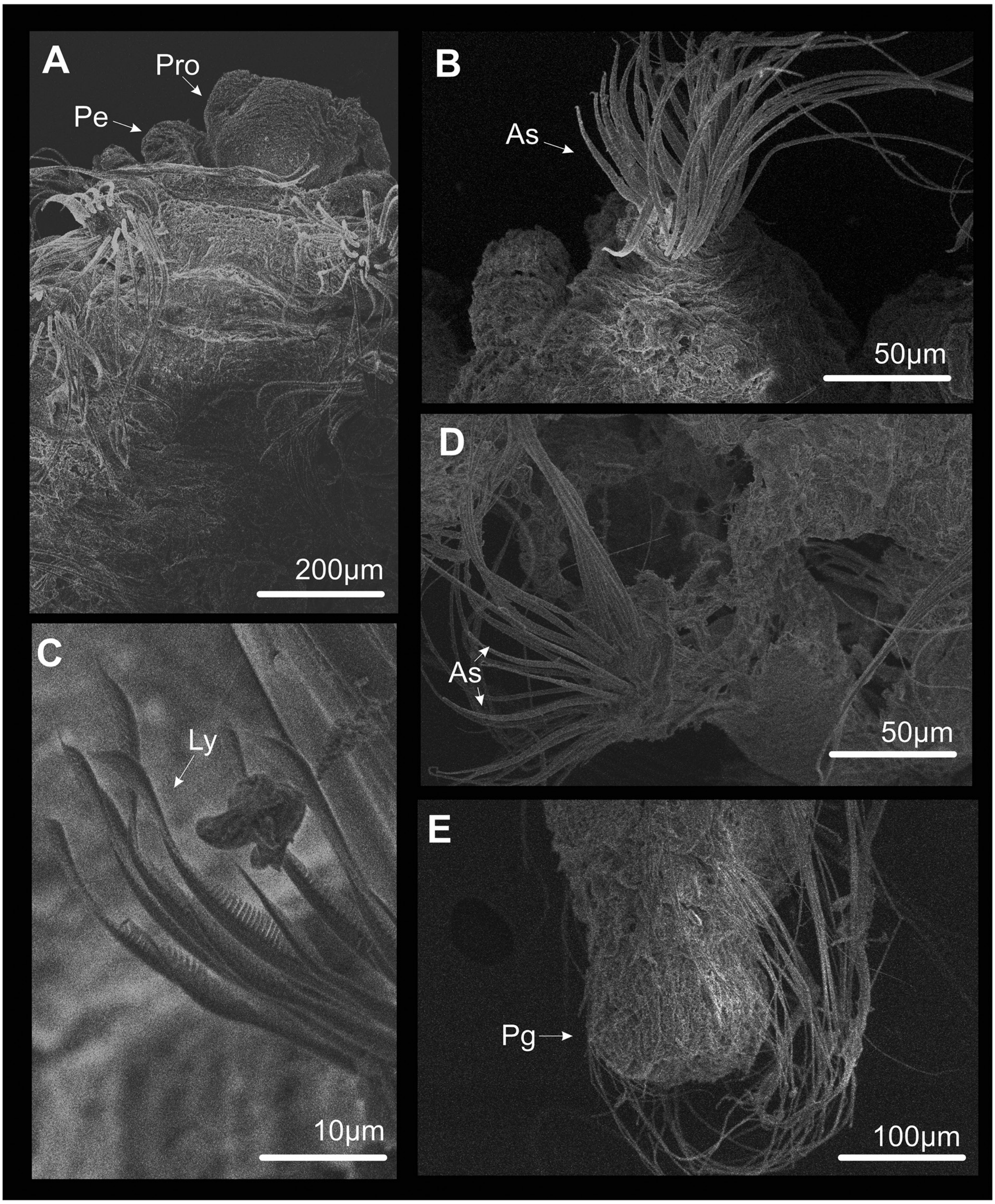

Fig. 6. *Asclerocheilus blakei* sp. nov., under SEM. **A**. Dorsal view of anterior body. **B**. Acicular spines with terminal arista in detail. **C**. Lyrate chaetae. **D**. Acicular spines with pointed tips from chaetiger 3 in detail. **E**. Pygidium in dorsal view. Abbreviations: see Material and methods.

Notopodial acicular spines organized in two rows on chaetigers 1–2, numbering 6–7 in anterior row and 8–9 in posterior row (Figs 4C, 5C–D, 6B). Notopodial acicular spines on chaetiger 3 organized in single row of 8 transitional acicular spines (Fig. 6D), with one row replaced by lyrate chaetae. Neuropodial acicular spines present on chaetigers 1–3, weaker than notopodial ones, organized in single row, numbering 5 per fascicle, being accompanied by anterior row of lyrate chaetae on chaetiger 2.

Notopodial capillaries present in one row on chaetigers 1–2; then in two rows on chaetiger 3, then in three rows on chaetiger 4. Neuropodial capillaries organized in two rows in chaetigers 1–3, then in three rows on chaetiger 4. Noto- and neuropodia on posterior chaetigers with two rows of capillaries, then in single row on last 5–10 chaetigers. Smaller specimens present more posterior chaetigers with single row of capillaries. Notopodial and neuropodial capillaries longer on anterior body, up to chaetiger 8, giving spinous appearance.

Parapodial lobes always asymmetrical, emerging as short and inconspicuous basis ending in rounded tip on anterior chaetigers, forming ‘hill-like’ structure; on midbody chaetigers, parapodial lobes inflate at their basis, transitioning abruptly to pointed tip; on posterior most chaetigers, parapodial lobes become smaller and thinner, emerging as thin basis transitioning smoothly to pointed tip (Fig. 5F). Pygidium present as short crenulated margin with up to 10 small lobes, from which emerges 6 long anal cirri (Figs 5F, 6E).

Remarks

This species is morphologically similar to *A. intermedius, A. shanei, A. abyssalis* and *A. geiseae* sp. nov. by having acicular spines on notopodia of chaetigers 1–3. However, it is set apart from *A. abyssalis* by the absence of short spinous chaetae on noto- and neuropodia of chaetiger 3. *Asclerocheilus shanei* differs from *A. blakei* sp. nov. by the absence of capillaries following the acicular spines on chaetigers 1–3. *Asclerocheilus intermedius* differs from *A. blakei* by presenting lyrate chaetae fron chaetiger 4. *Asclerocheilus blakei* presents a single type of acicular spine on its chaetigers; they are curved with pointed tips, differing from *A. geiseae* spines, that end in blunt tips. Finally, both species have different prostomial and peristomial morphologies, being triangular with horns directed upwards in *A. geiseae*, whereas in *A. blakei* the prostomium is rounded to trapezoidal and its horns are thinner and laterally directed.

Ecology

This species was found living on the continental shelf and slope of the Espírito Santo and Campos basins (Brazilian southern region), from a bathymetric range of 683–2900 m.

Asclerocheilus tropicus Blake, 1981

Fig. 7

Asclerocheilus tropicus Blake, 1981: 1133, figs 1a–c.

Material examined

BRAZIL – **Espírito Santo, Espírito Santo Basin** • 1 incomplete spec.; 19.765° S, 39.507° W; depth 150 m; Jan. 2012; box corer; continental slope; UERJ-9244 • 2 complete specs; 19.601° S, 39.176° W; depth 143 m; Jun. 2013; van Veen grab; continental slope; UERJ, UERJ-4622.

Description

Medium- to small-sized specimens; 3.5–5 mm long, 0.1–0.5 mm wide for 13–34 chaetigers. Prostomium with two large flaring lobes (Fig. 7A–B). When present, eyes as broad obliquely longitudinal lines, with orange coloration, positioned near posterior margin of prostomium. Peristomium uniannulated, smooth. First chaetigers biannulated; then tri- to quadriannulated from midbody to posterior chaetigers.

Acicular spines on notopodia of chaetiger 1, numbering 5–6, present in two rows (Fig. 7C). Short spinous chaetae absent. Lyrate chaetae from chaetiger 2 (Fig. 7D), with unequal tynes (tynes ratio = 1.8–2.1), present in single row anterior to capillaries, numbering 3–5. Capillaries organized in two rows on anterior to midbody chaetigers then in single row on posterior chaetigers. Two specimens lack posterior end, but one presented short pygidial margin, without cirri.

Remarks

The specimens collected during the research campaigns material agree in their morphological and ecological features those described by Blake (1981) and Nogueira (2002). Importantly, Nogueira (2002) confirmed his identifications comparing the Brazilian specimens with type material of *A. tropicus*. Here, we expand the distribution of the species to the Espírito Santo and Campos basins. Interestingly, the

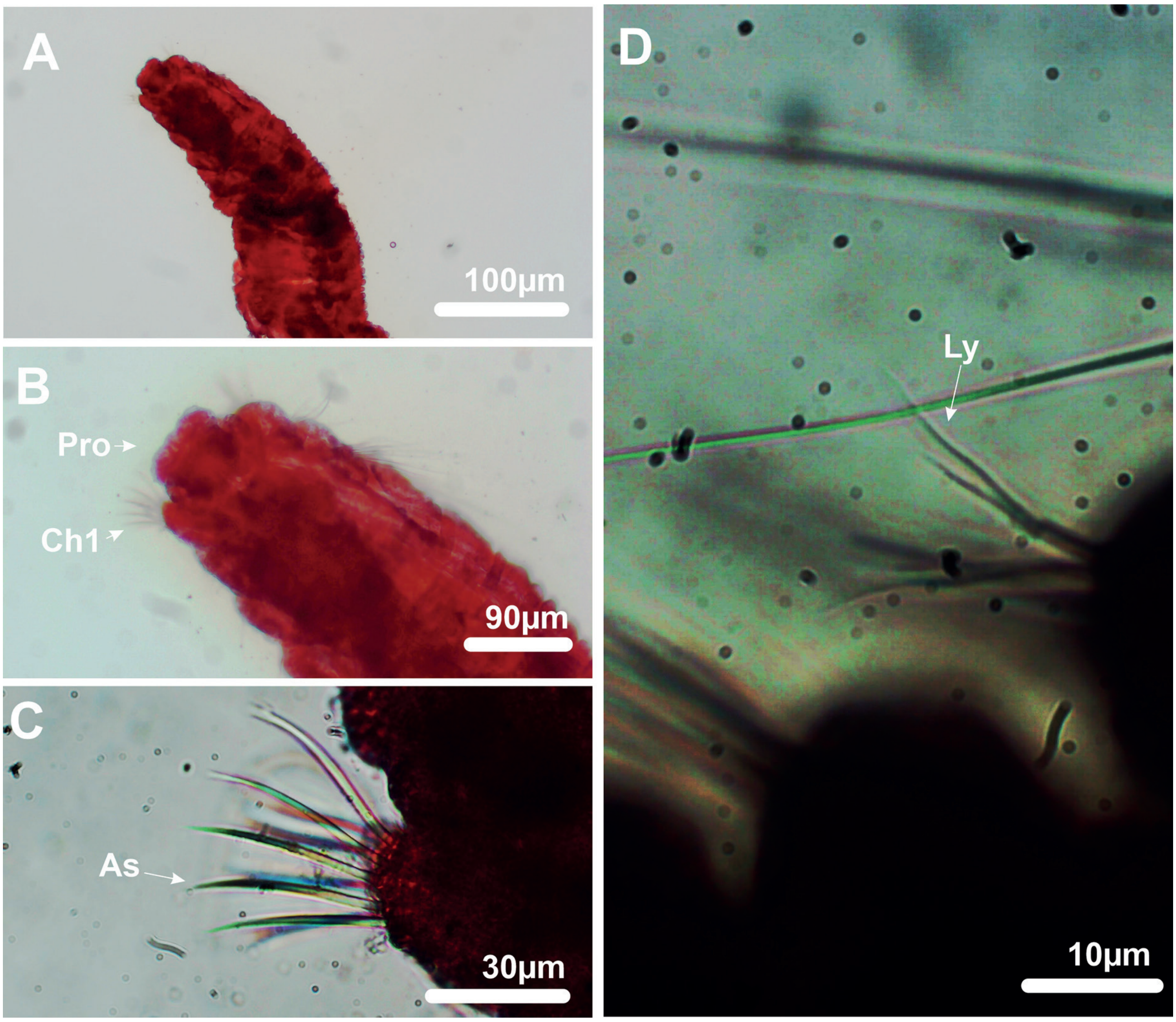

Fig. 7. *Asclerocheilus tropicus* Blake, 1981 (UERJ 9244), under optical microscopy, specimens stained with Shirlastain A. **A**. Anterior region in ventral view. **B**. Anterior region in ventral view, emphasizing the prostomium and first chaetiger. **C**. Acicular spines on chaetiger 1. **D**. Lyrate chaetae from a posterior chaetiger. Abbreviations: see Material and methods.

variation regarding the presence of a sulcus separating the prostomial lobes was also observed in some specimens, with the prostomium lacking a clear separation from them, even under staining (Fig. 7A–B). On the other hand, the number of acicular spines (Fig. 7C) and lyrate chaetae (Fig. 7D) did not reach the maximum number reported by Nogueira (2002) but agreed in overall shape. Regarding the body annulation, most specimens were in poor state, hampering a clear distinction of secondary annuli in most chaetigers. In the light of the abovementioned considerations, the specimens did not present enough variability to justify their separation from *A. tropicus* sensu Nogueira (2002). Further studies exploring the identity of this species using molecular data are needed, considering its Pacific and South Atlantic records, both confirmed by morphology.

Ecology

We found specimens living on the continental shelf of the Espírito Santo and Campos basins, in a bathymetric range up to 157 m deep. This find is relevant, because we expand its bathymetric distribution, considering the previous accounts based on records of specimens living in shallow subtidal waters (Blake 1981; Nogueira 2002).

Key to species of Asclerocheilus Ashworth, 1901

1. Acicular spines present on chaetiger 1, 1–2 or 1–3 2
– Acicular spines present on chaetigers 1–4 ....
.... ***A. victoriensis*** Blake, 2000; from Southeast Australia at shallow subtidal depths

2. Short spinous chaetae present on noto- and neuropodia of chaetigers 1–3 ....
.... ***A. abyssalis*** Blake, 2023; from Eastern Australia at depths of 3952–4280 m
– Short spinous chaetae absent on notopodia 3

3. Acicular spines only on chaetiger 1 4
– Acicular spines on chaetigers 1–2 or 1–3 8

4. Lyrate chaetae from chaetiger 2 or further on neuropodium 5
– Lyrate chaetae from chaetiger 1 on neuropodium 6

5. Short spinous chaetae present on chaetiger 1 neuropodia ....
..........***A. shanonae*** Eibye-Jacobsen, 2002; from the Andaman Sea, Thailand, at depths of 70–76 m
– Short spinous chaetae absent ***A. glabrus*** (Ehlers, 1887); from the Caribbean Sea at depths of 320 m

6. Prostomium with a pair of thin lateral horns ....
.... ***A. acirratus*** (Hartman, 1966); from California at shallow subtidal depths
– Prostomium with a pair of distally rounded lateral lobes 7

7. Prostomium T-shaped with a pair of stout lateral lobes ....
..................................***A. mexicanus*** Kudenov, 1985; from the Gulf of Mexico at depths of 2–75 m
– Prostomium heart-shaped, with a pair of flaring lobes; prostomium generally entire, but sometimes separated by a median sulcus in some specimens ....
....***A. tropicus*** Blake, 1981; from Ecuador at depths of 8–9 m

8. Acicular spines on chaetigers 1–2. 9
– Acicular spines on chaetigers 1–3 14

9. Lyrate chaetae from chaetiger 2 on notopodium ....
A. ashworthi Blake, 1981; from the Southern Ocean in Sub-Antarctic waters at depths of 200–400 m
– Lyrate chaetae from chaetiger 3 on notopodium 10

10. Notopodial and neuropodial lobes simple or reduced 11
– Notopodial and neuropodial lobes long and enlarged from chaetiger 6 .... ***A. californicus*** Hartman, 1963; from Southern California at depths of 542–890 m

11. Prostomium triangular to trapezoidal 12
– Rounded prostomium ..... ***A. kudenovi*** Blake, 2000; from Central California at depths of 90–120 m

12. Lyrate chaetae from chaetiger 1 on neuropodium 13
– Lyrate chaetae from chaetiger 2 on neuropodium ....***A. elisabethae*** Eibye-Jacobsen, 2002; from the Andaman Sea, Thailand, at depths of 0–80 m

13. Prostomium triangular, bearing two recessed horns directed upwards; chaetiger 1 uniannulated, chaetiger 2 triannulated, then quadriannulated from chaetiger 4 up to posterior end of the body ....***A. beringianus*** Ushakov, 1955; from the Bering Sea at depths of 986–2006 m
– Prostomium sub triangular, bearing two large rounded horns directed laterally; chaetigers 1–7 biannulated, then quadriannulated ....***A. capensis*** Day, 1963; from South Africa at depths of 9 –26 m

14. Acicular spines with capillaries on chaetigers 1–3 15
– Acicular spines without capillaries on chaetigers 1–3 ....***A. shanei*** Hartmann-Schröder, 1994; from Tasmania at depths of 125 m

15. Lyrate chaetae from chaetiger 2 in neuropodia 16
– Lyrate chaetae from chaetiger 4 ....***A. intermedius*** (Saint-Joseph, 1894); from the eastern North Atlantic at depths of 551 m

16. Acicular spines with rounded tips present ....***A. geiseae*** sp. nov.; from the Espírito Santo Basin at depths of 11–50 m
– Acicular spines with rounded tips absent ....***A. blakei*** sp. nov.; from the Espírito Santo and Campos basins at depths of 683–1908 m

Discussion

Taking into consideration the available information on the morphology of species of *Asclerocheilus*, it is possible to separate the genus in three large subgroups based on the presence of acicular spines on the first chaetigers of the body. The first group presents such spines only on chaetiger 1, the second group on chaetigers 1–2, the third group on chaetigers 1–3. Apart from them, *A. victoriensis* is unique to present acicular spines on chaetigers 1–4. This species was described by Blake (2000) from shallow waters in Australia. Moreover, only two species descriptions report the presence of short spinous chaetae, which are commonly present anterior to capillaries or long acicular chaetae in other genera, that is *A. abyssalis* from the abyssal depths of Australia (Blake 2023) and *A. shanonae* from the Andaman Sea, Thailand (Eibye-Jacobsen 2002).

In the genus taxonomy, the most important characters used to separate species are related to the chaetal configuration of chaetigers 1–4, the prostomium shape and body annulation. Although most species in the genus present tynes ratios varying from 1.5 to 2.5, several works discussed the validity of this feature as an additional criterion to distinguish species (Blake 1981, 2000, 2023; Nogueira 2002; Parapar *et al.* 2021). They conclude that this criterion is variable both: 1) within species and 2) on the body from anterior to posterior chaetigers. However, it is still useful, as demonstrated by Blake (2023) in the description of *A. abysallis*, mentioning the most dissimilar tynes ratio in comparison to its congeners. In relation to the Brazilian species: *A. tropicus*, *A. blakei* sp. nov. and *A. geiseae* sp. nov., they share similar values, ranging from 1.6 to 2.5 (Nogueira 2002).

Regarding the body annulation pattern, we argue that this criterion should be used with caution to distinguish species, based on the analysis of some specimens found in the present work. Commonly, specimens were found with irregular smooth surfaces over expanded chaetigers in the anterior region, sometimes in the posterior region as well. The presence of 'smooth chaetigers' also varied dorsally to ventrally with no distinguishable pattern. However, they agreed in all other characteristics, such as the prostomium shape, absence of eyes, parapodial lobe morphology and chaetal configuration. We interpreted the variation regarding body annulation as artefacts of sampling, fixation or post-mortem body contractions. Furthermore, the interesting annulation pattern, initially outlined by Bakken *et al.* (2014) for *Pseudoscalibregma parvum* (Hansen, 1879), and observed in select scalibregmatid species (Blake 2020, 2023; Mendes *et al.* 2024a, 2024b), is also documented in the new species described in this study (see Figs 3A, 5E). As described by Bakken *et al.* (2014), this pattern encompasses a variable number of secondary annuli directly linked to the parapodial lobes, along with an intermediate annulation occurring between the chaetigers anteriorly and posteriorly. This characteristic may cause confusion in the interpretation of the number of secondary annuli per chaetiger in poorly preserved specimens.

Ecologically, three groups of species may be defined based on the available information of their bathymetric range of reported occurrences. The first group groups ten species found in shallow waters of 8 to 200 m deep, including the Brazilian species *A. tropicus* and *A. geiseae* sp. nov. The second group contains species living in deeper waters at a bathymetric range from 500 to 2000 m deep, including *A. blakei* sp. nov. The third group contains only two species found at abyssal depths, they are A *beringianus* and *A. abyssalis*.

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