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Long distance dispersal and pseudo-cryptic species in Gastrotricha: first description of a new species (Chaetonotida, Chaetonotidae, *Polymerurus*) from an oceanic island with volcanic rocks

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Abstract. The majority of meiofaunal organisms have limited abilities to disperse over long distances, yet they may still have disjointed distributions. Many studies have found evidence of long distance meiofauna dispersal due to passive transport by wind and/or animals that serve as vectors for these widespread distributions. Our research on an archipelago in northeast Brazil uncovered a species of freshwater gastrotrich that at first sight appeared to be a 'cosmopolitan' species that had surpassed the connectivity constraint to occupy an island more than 350 km from the mainland. However, through an integrative approach using molecular sequences and morphology, we have uncovered evidence of a pseudo-cryptic species in this freshwater gastrotrich. *Polymerurus insularis* sp. nov. closely resembles its congeners and can easily be mistaken for similar species such as *P. nodicaudus*, a cosmopolitan gastrotrich. Unique to *P. insularis* sp. nov. are (1) a cuticular armature composed of simple spined scales with polygonal shape (*Type 1* scales), (2) a single, spineless dorsal scale with a triangular shape located terminally next to the furca base (*Type 2* scale), (3) a spineless zone composed by a patch lacking cuticular ornamentation and flat, rounded or polygonal scales without spines (*Type 4* scales) (4) particular sets of terminal spined or keeled scales located both dorsally and ventrally around the furca base (*Types 3*, *5*, *6*, *7* and *8* scales). The presence of this species on a volcanic island is discussed, as is the relationship between pseudocryptism and dispersal in gastrotrichs and other meiofauna.

Keywords. Dispersion, meiofauna paradox, ubiquity, cosmopolitanism, integrative taxonomy.

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Introduction

The spatial distributions of freshwater organisms are usually restricted by discrete boundaries, as they are surrounded by land patches without obvious connections. However, these organisms can display a much wider geographic distribution, which might be correlated to their dispersal – that is, the movement of individuals or propagules – between discrete habitats (Bilton *et al.* 2001; Bohonak & Jenkins 2003). This dislocation allows organisms to reach and occupy new places beyond their original area, thus expanding the spatial distribution of their populations.

Although several mechanisms of dispersion are known, it can roughly occur in two major ways: actively or passively (Bilton *et al.* 2001; Bohonak & Jenkins 2003; Coughlan *et al.* 2017). Active dispersion occurs when the organism itself is responsible for its own displacement (e.g., areal flight), while passive dispersion consists in movements that are generated by external agents (e.g., vectors, wind, or water currents) (Bilton *et al.* 2001).

Regarding meiofaunal organisms – i.e., individuals that are able to pass through a 500 µm sieve, but are retained by a 42 µm (Giere 2009) – active dispersion is very restricted, due to their small size, absence of a larval phase and a low capacity of movement, making it harder to cover great distances (Giere 2009; Cerca *et al.* 2018).

Gastrotricha Metschnikoff, 1865 is a meiobenthic metazoan taxon with individuals that range from 50 µm up to 3500 µm in total body length (Balsamo *et al.* 2014; Kieneke & Schmidt-Rhaesa 2015). Gastrotrichs are commonly found in the benthos of marine, estuarine and freshwater environments across the globe. They are easily recognizable by the presence of adhesive tubes and a characteristic locomotory ciliation that is restricted to their ventral body surface, after which the phylum is named – from the Greek *gaster*: gut/stomach + *thrix*: cilia/hair (Balsamo *et al.* 2014; Kånneby & Hochberg 2015; Kieneke & Schmidt-Rhaesa 2015). The phylum comprises nearly 880 valid species, which are classified into two orders (Balsamo *et al.* 2015; Kieneke & Schmidt-Rhaesa 2015): Macrodasyida Remane, 1925 (Rao & Clausen 1970) and Chaetonotida Remane, 1925 (Rao & Clausen 1970). The first order currently contains 384 nominal species, mostly marine, with a vermiform body plan and many adhesive tubes distributed along the length of the body (Todaro *et al.* 2019); only four freshwater species are known (Ruttner-Kolisko 1955; Kisielewski 1987; Todaro *et al*. 2012; Kånneby & Kirk 2017; Garraffoni *et al.* 2019a). The second order, Chaetonotida, is composed of approximately 494 species of tenpin-like organisms, most with only one pair of posterior adhesive tubes (Balsamo *et al.* 2014; Kieneke & Schmidt-Rhaesa 2015). Approximately two-thirds of the species are described from continental freshwaters (Balsamo *et al*. 2008; Balsamo *et al.* 2020), while the remainder are marine or estuarine.

The family Chaetonotidae Gosse, 1864, is the most specious family within Chaetonotida and indeed also Gastrotricha, with 410 species distributed in two subfamilies and 14 genera (Kolicka *et al*. 2016; Garraffoni *et al*. 2017). Chaetonotids show a wide diversity of morphologies, especially regarding body shape, size, and cuticular ornamentation (Balsamo *et al.* 2014; Kieneke & Schmidt-Rhaesa 2015; Kånneby 2016). The genus *Polymerurus* Remane, 1927 contains exclusively freshwater species and has the largest representatives among chaetonotids, with total body lengths reaching 770 µm (Kisielewski 1991; Hochberg 2005). Besides their elongated bodies, other typical features of this genus include a cephalion (cephalic plate) with lateral expansions (pleurae), adhesive tubes strongly reduced and furcal rami showing an apparent segmentation pattern (Kisielewski 1991; Kieneke & Schmidt-Rhaesa 2015). These characteristics can vary a lot among species (Kisielewski 1991; Hochberg 2005) and the existence of intraspecific variability has also been observed (Kisielewski 1979, 1991).

Most of the diversity documented in *Polymerurus* comes from species described in the Northern Hemisphere (Europe: Remane 1927; Kisielewski 1979, 1981; Balsamo 1983; Martin 1990; Schwank 1990; Nesteruk 2004; Grilli *et al.* 2008, 2010; Kånneby 2011; Kieneke & Hochberg 2012; Balsamo *et al.* 2015; Middle East: Kisielewski 1999; North America: Brunson 1950; Packard 1956, 1959; Schwank & Kånneby 2014; Asia: Saito 1937; Lee & Chang 2000). Only a few species are documented from the southern hemisphere, including both 'cosmopolitan' species and newly described taxa from specific geographic regions (South America: Grosso & Drahg 1986; Kisielewski 1991; India: Sharma & Sharma 1990; Australia: Hochberg 2005).

In spite of the worldwide distribution of the taxon *Polymerurus*, current knowledge on its diversity is scarce and poorly established. As far as the number of species included in this genus is concerned, there is still no consensus in the literature (see Supp. file 1). To date, there may be 15 to 18 species in the genus (Balsamo *et al.* 2009; Kieneke & Schmidt-Rhaesa 2015; Todaro 2019; WoRMS 2019). This variation arises due to disagreement among the authors about the taxonomic status of some species, either by recognizing (or not) species inquirenda or by debating the validation of synonymous species (see Supp. file 1). This lack of agreement has created confusion within the taxon and can impede our knowledge about its diversity as well as its biogeography. It is therefore important to consider how such difficulties reported above have affected present knowledge about a species' distribution. For example, the two most commonly reported species, *P. nodicaudus* (Voigt, 1901) and *P. rhomboides* (Stokes, 1887), are currently acknowledged as cosmopolitan and have been reported in inland waters of Australia, across the Middle East, Asia, Europe, North and South America (e.g., Kisielewski 1979, 1991, 1999; Lee & Chang 2000; Hochberg 2005; Kånneby 2011; Schwank & Kånneby 2014). However, several of these reports provide descriptions that are insufficient to distinguish *P. nodicaudus* and *P. rhomboides* from other species of *Polymerurus*, suggesting that this apparent cosmopolitanism could be the result of the methodological limitations of past studies.

Recent descriptions of new species of gastrotrichs have been increasingly employing updated techniques to describe and better understand their morphological variation, such as optical microscopy with Differential Interference Contrast (DIC), Scanning (SEM) and Transmission (TEM) Electron Microscopy, and Confocal Laser Scanning Microscopy (CLSM) (Todaro *et al.* 2015; Kieneke & Nikoukar 2017; Garraffoni *et al.* 2019a; Bosco *et al.* 2020). The use of these tools allows an enhanced quality of observation of species-specific characteristics, which was not accessible in the past (Balsamo *et al.* 2008, 2014).

The present study aims at describing a new species of the genus *Polymerurus* that was discovered in freshwater systems of an oceanic volcanic island located in northeast Brazil. The Fernando de Noronha archipelago is situated more than 350 km from the American Continent (Teixeira *et al.* 2003) and was formed around 8 to 12 Ma ago by volcanic activity (Cordani *et al.* 2004). Prior to our current study, no gastrotrichs have been described from the archipelago, whose distance from the continent likely poses a dispersion challenge to tiny, freshwater invertebrates such as the representatives of *Polymerurus*. The new species described herein is documented using an integrated approach combining both optical microscopy (DIC and SEM) and molecular sequencing (rDNA nuclear genes 18S and 28S sequencing) in order to differentiate it from the 'cosmopolitan' species that have caused confusion in this taxon.

Material and methods

Sampling area

Fernando de Noronha is an archipelago formed by volcanic activity (with soils that belong to the classes: Cambisoils, Vertisoils and Neosoils) during the Upper Miocene, around 8 to 12 Ma ago

(Marques *et al.* 2014). This territory encompasses an 18 island complex situated at approximately 365 km from the brazilian coast, with a total area of 18.4 km², 90% of which is occupied by the main island (Silva e Silva & Olmos 2006). The archipelago has a tropical climate with oceanic domain of AW type, according to Koppen's classification (hot and humid with summer-autumn rains), with two well defined seasons: a dry season from August to January and a rainy season from February to July (Alvares *et al*. 2014). Average annual temperature is about 25°C (ranged ± 4°C), while average annual rain precipitation is of 1300 mm, although showing great interannual variation. The archipelago shows predominantly deciduous vegetation, resembling what is found in Northeast Brazil's subregion Agreste (Teixeira *et al*. 2003). Samples from the uppermost sediment layer of the Xaréu reservoir (3°85′ S–32°42′ W – Fig. 1) were collected in August 2015, stored in plastic containers and gastrotrichs were extracted at the Laboratory of Evolutionary Meiofaunal Organisms, University of Campinas (Campinas, Brazil).

Differential Interference Contrast Microscopy (DIC)

Sorting and extraction of living organisms from the sediment were carried in the laboratory, following protocol as reported by Balsamo *et al.* (2014). Small amounts of sediment were filtered in a 42 µm mesh sieve, poured into a Petri dish and sorted under a Zeiss Stemi 2000 stereo microscope. Living animals were isolated and singly mounted in glass slides, narcotized with Magnesium Chloride 2% and observed using a Zeiss Axio Imager M2 light microscope equipped with Differential Interference Contrast (DIC) and AxioCam MRC5 digital video camera.

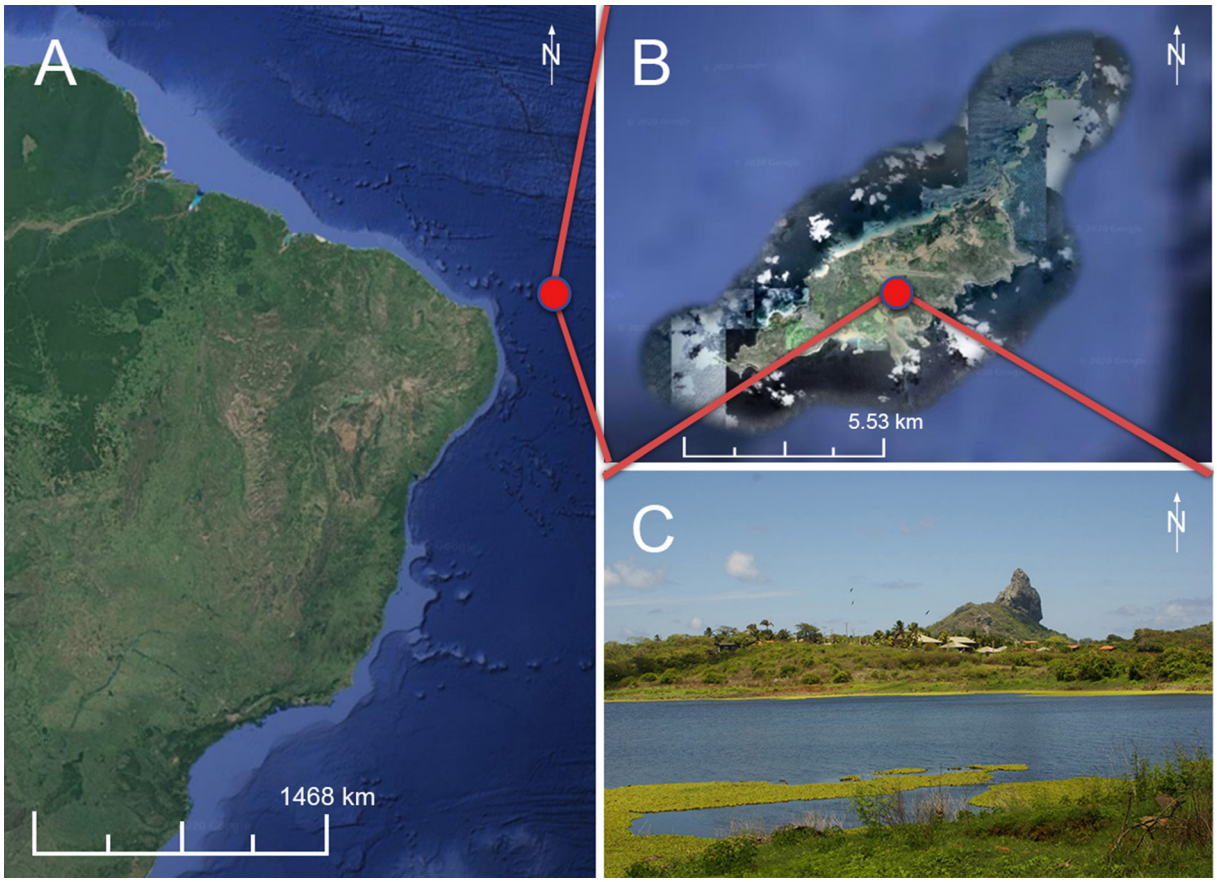

Fig. 1. Sampling location, at the state of Pernambuco, Fernando de Noronha archipelago, Brazil **A**. Brazil. **B**. Fernando de Noronha Archipelago. **C**. Xaréu açude. Images provided by Google Earth (A–B) and Prof Dr Felipe Toledo, University of Campinas (C).

According to Hummon *et al.* (1992), the measuring parameter U was employed to elaborate the description of the new species, which varies from 1–100 and generically corresponds to a size proportion, in which the anterior-most portion of the head corresponds to the value 1, and the posterior-most end is set as 100.

Scanning Electron Microscopy (SEM)

Specimens extracted under stereo microscope were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) at room temperature and stored within fixative for a few months at 4°C. The organisms were dehydrated through a series of ethanol solutions with increasing concentrations (20%, 30%, 40%, 50%, 60%, 70%, 95%, 100%), dehydrated using hexamethyldisilazane (HMDS) (Hochberg & Litvaitis 2000), mounted onto aluminum stubs, and coated with gold-palladium using sputter coating (Baltec SCD 050). They were observed under a JSM 5800LV scanning electron microscope at an accelerating voltage of 10 kV. The images were recorded with Semafore software (ver. 5.2) at the Laboratory of Electron Microscopy of the Biology Institute, University of Campinas.

DNA extraction and amplification

In order to obtain the coding sequences of ribosomal subunits 18S and 28S, nuclear DNA was extracted separately from each of the three specimens of the new species using a QIAamp DNA Micro Kit (Qiagen), according to the manufacturer's instructions. Amplification via PCR was carried using a reaction mix with 3 µL of genomic DNA, 12.5 µL of Taq PCR Master Mix (Qiagen), 8.7 µL of nuclease-free water and 0.4 µL (4 pmol) of specific primers. Primer sequences and PCR protocols were implemented by Garraffoni *et al.* (2019a). Amplification products were analyzed through agarose gel electrophoresis, using a 1% gel with SYBR® Green (Life Technologies). DNA fragments were sequenced via BigDye Terminator reaction in a 3730XL (Applied Biosystems) DNA analyzer at the Central Laboratory of High Performance Technologies – LaCTAD (Campinas, Brazil). The obtained sequences for 18S rDNA and 28S rDNA were deposited in GenBank under the following access numbers: MT711236–MT711237 (18S) and MW300430 (28S).

Phylogenetic analyses

Coding sequences of subunits 18S and 28S of two specimens of the new species along with 31 sequences of 29 Gastrotricha species (Table 1), were aligned using MAFFT ver. 7 on the online server (strategy G-INS-i) (Katoh *et al.* 2019). Alignments were visually inspected for misaligned regions and manually corrected using AliView ver. 2018 (Larsson 2014). The 28S subunit coding sequence for the second specimen of the new species was excluded due to high levels of misalignment and uncertain nucleotides. Both DNA alignments were concatenated on Sequence Matrix (Vaidya *et al.* 2011) and maximum likelihood phylogenetic trees were generated with W-IQ-TREE multicore ver. 1.6.11 (Trifinopoulos *et al.* 2016). In order to evaluate branch support of the consensus trees, Ultrafast Bootstrap Analysis was performed with 10 000 bootstrap replicates, 1000 maximum iterations and a minimum correlation coefficient of 0.99. The following single branch tests were conducted to access the support for Maximum Likelihood Trees: SH-aLRT branch test, set for 5000 replicates, and Approximate Bayes test, to maximize the confidence of the tree. Default setting was maintained for the remaining available parameters and the best-fit model based on ModelFinder analysis was GTR+F+I+G4 (GTR = General Time Reversible with unequal rates and unequal base frequencies (Tavaré 1986) + F = Empirical codon frequencies counted from the data + I = allowing for a proportion of invariable sites + G4 = discrete Gamma model (Yang 1994) with default 4 rate categories).

Table 1. Taxa included in this study, with GenBank accession numbers of 18S and 28S rDNA sequences.

Species	18s	28S	Reference
Family Chaetonotidae			
Arenotus strixinoi Kisielewski, 1987	JQ798537	JQ798608	Kånneby *et al*. (2013)
Aspidiophorus polystictos Balsamo & Todaro, 1987	JQ798598	JQ798665	Kånneby *et al*. (2013)
Aspidiophorus tetrachaetus Kisielewski, 1986	JN185505	JN185540	Kånneby *et al*. (2012)
Bifidochaetus arcticus Kolicka & Kisielewski, 2016	KP713403	KP713404	Kolicka *et al*. (2016)
Chaetonotus acanthodes Stockes, 1887	JQ798585	JQ798624	Kånneby *et al*. (2013)
Chaetonotus aemilianus Balsamo, 1978	JQ798556	JQ798626	Kånneby *et al*. (2013)
Chaetonotus daphnes Balsamo & Todaro, 1995	JQ798549	JQ798621	Kånneby *et al*. (2013)
Cephalionotus kisielewskii Garraffoni *et al*., 2017	KX159486	–	Garraffoni *et al*. (2017)
Halichaetonotus aculifer (Gerlach, 1953)	JQ798550	JQ798622	Kånneby *et al*. (2013)
Halichaetonotus paradoxus (Remane, 1927)	JQ798599	JQ798666	Kånneby *et al*. (2013)
Heterolepidoderma acidophilum Kånneby *et al*., 2012	JN185462	JN185521	Kånneby *et al*. (2012)
Heterolepidoderma macrops Kisielewski, 1981	JN185469	JN185515	Kånneby *et al*. (2012)
Ichthydium squamigerum Balsamo & Fregni, 1995	JQ798607	JQ798674	Kånneby *et al*. (2013)
Ichthydium skandicum Kånneby *et al*., 2009	JQ798573	JQ798673	Kånneby *et al*. (2013)
Lepidochaetus brasilense Kisielewski, 1991	JN185495	JQ798658	Kånneby *et al*. (2013)
Lepidochaetus zelinkai (Grünspan, 1908)	JN185486	JQ798643	Kånneby *et al*. (2013)
Lepidodermella intermedia Kånneby *et al*., 2012	JN185468	JN185514	Kånneby *et al*. (2012)
Lepidodermella squamata (Dujardin, 1841)	JN185479	KC193103	Kånneby *et al*. (2012)
Polymerurus nodicaudus (Voigt, 1901) - TK205	JN185490	JN185531	Kånneby *et al*. (2012)
Polymerurus nodicaudus (Voigt, 1901) - TK78	JN185502	JN185537	Kånneby *et al*. (2012)
Polymerurus rhomboides (Stokes, 1887) - TK207	JQ798584	JQ798715	Kånneby *et al*. (2012)
Polymerurus rhomboides (Stokes, 1887) - TK217	JN185493	JN185533	Kånneby *et al*. (2012)
Family Dasydytidae			
Dasydytes carvalhoae Kisielewski, 1991	JQ798570	JQ798639	Kånneby *et al*. (2013)
Dasydytes elongatus Kisielewski, 1991	JQ798568	JQ798656	Kånneby *et al*. (2013)
Ornamentula paraënsis Kisielewski, 1991	JQ798562	JQ798632	Kånneby *et al*. (2013)
Stylochaeta fusiformis (Spencer, 1890)	JN185471	JN185517	Kånneby *et al*. (2012)
Stylochaeta scirtetica Brunson, 1950	JN185492	JN185532	Kånneby *et al*. (2012)
Family Neogosseidae			
Kijanebalola devestiva Todaro *et al*., 2013	KR822112	KR822118	Kånneby & Todaro (2015)
Neogossea acanthocolla Kisielewski, 1991	KR822114	KR822119	Kånneby & Todaro (2015)
Family Xenotrichulidae			
Draculiciteria tesselata (Renaud-Mornant, 1968)	JN185470	JN185516	Kånneby *et al*. (2012)
Xenotrichula intermedia Remane, 1934	JF357664	JF357712	Todaro *et al*. (2011)

Results

Taxonomic description

Phylum Gastrotricha Metschnikoff, 1865
Order Chaetonotida Remane, 1925 (Rao & Clausen 1970)
Suborder Paucitubulatina d'Hondt, 1971
Family Chaetonotidae Gosse, 1864 (sensu Garraffoni, Araújo, Lourenço, Guidi & Balsamo, 2017)
Genus *Polymerurus* Remane, 1927

Polymerurus insularis sp. nov.
urn:lsid:zoobank.org:pub:54D43422-15B7-4EC9-ACC9-E764D3A7BF6D
Figs 2–8; Tables 2–3

Diagnosis

Elongated body with head separated from the trunk by slight neck constrictions. Total body length of 215–408 μm; furcal rami 93 μm long with 19–21 segments per side. Three-lobed, rounded head with a well-developed cephalion showing two lateral projections; one rugous pair of lateral pleurae and a ventral hypostomium present. Body mostly covered by pentagonal or hexagonal spined scales (*Type 1* scales) of various sizes (3.5–11 μm) increasing in length progressively from anterior (3.5–8.5 μm) to posterior body (4.5–11 μm) ends and from ventral (3.5–7 μm) to dorsal surface (3.5–11 μm). Overall, the shape of the *Type 1* scales is very distinct, with 5–6 corners and an anterior elevation that forms the spine base and opens towards a middle groove and a posterior cleavage. A triangular dorsal scale is found immediately anterior to the furca base, highly lifted and spineless (*Type 2* scale). Dorsally on the furca base there is a spineless zone composed by a patch lacking cuticular ornamentation and flat, rounded or polygonal scales without spines measuring 2.5–5 μm (*Type 4* scales). The posterior end of the ventral field is covered by a pair of keeled oval scales (*Type 6* scales) and two pairs of small, elongated (*Type 7* scales) or rounded scales (*Type 8* scales), both bearing straight and long spines.

Etymology

From the Latin *insularis* meaning 'belonging to an island', in reference to the type locality.

Material estudied

Holotype
BRAZIL • adult (photographs, the specimen was destroyed); State of Pernambuco, Fernando de Noronha archipelago, Xaréu Reservoir; ZUEC GCH 55.

Specimen was examined while still alive under a compound microscope however, due to the fragility of the body, it was destroyed and is no longer available (Garraffoni *et al*. 2019b). Photographs of the specimen are available at the Museum of Zoology of the University of Campinas under the access number ZUEC GCH 55 (ICZN 2017: Article 73, Recommendation 73G, Statement 45). The holotype is shown in Figs 2A–C, 3A–I and 4B, D.

Paratypes
BRAZIL • 3 adults specs (micrographs, the specimens were destroyed); same collection data as for holotype; ZUEC GCH 56 to GCH 58 • 2 specs (prepared for SEM); same collection datas as for holotype; ZUEC GCH 59, GCH 60.

Specimens were examined while still alive under a compound microscope, however, due to the fragility of their bodies, they were destroyed and are no longer available (Garraffoni *et al.* 2019b). Photographs of the specimens are available at the Museum of Zoology of the University of Campinas, under the

access numbers ZUEC GCH 56 to 58 (ICZN 2017: Article 73, Recommendation 73G, Statement 45). An example of paratypes are shown in Fig. 4A, C.

Other material

BRAZIL • 8 specs (examined while still alive under a compound microscope and, among those, three were prepared for DNA sequencing (no longer available)); same collection datas as for holotype.

Description

The description is based on both the holotype and 5 paratypes (Figs 2–8; Table 2).

Habitus. Specimens have a slender body with total length ranging from 215 µm to 408 µm, showing a slight neck constriction, represented by a small variation in width between the head and the neck. The cylindrical trunk is 31–55 µm wide at middle body length, showing little variation in width throughout its length, except for the set apart furcal rami, which are preceded by a pronounced constriction (Fig. 2). Body widths at head, medium length and furca base are, respectively, 24–42 µm, 31–55 µm and 23–25 µm.

Head. The three-lobed head is 24–42 µm wide and has three sets of distinct plates. The cephalion (U1–U6) is 19–25 µm long and 20–36 µm wide, with a free (detached from the cuticle) posterior portion and short paired lateral projections (lappets), each 10–18 µm long, posteriorly detached from

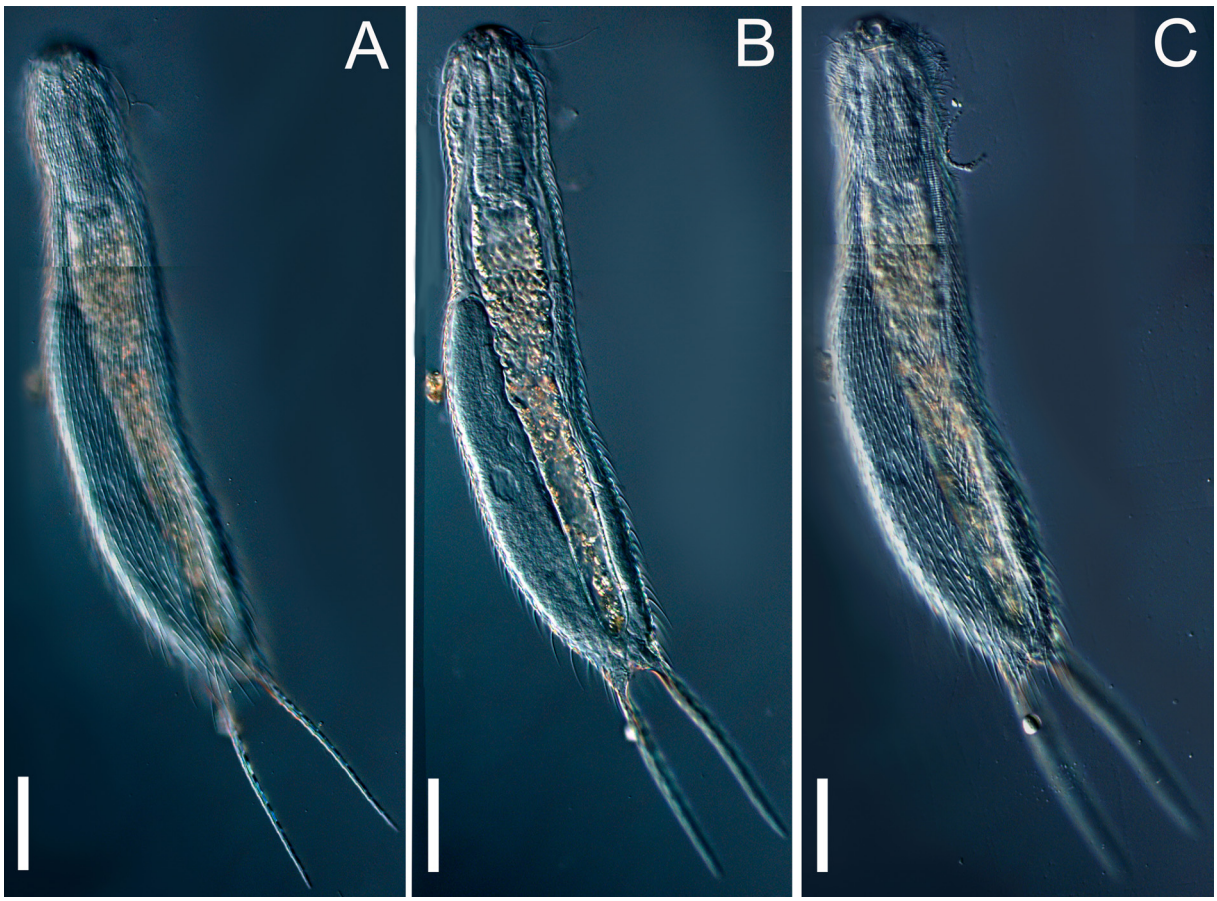

Fig. 2. Light microscopy – DIC. *Polymerurus insularis* sp. nov., holotype (ZUEC GCH 55). Full body view. **A**. Dorsal view. **B**. Internal view. **C**. Ventral view. Scale bars = 30 µm.

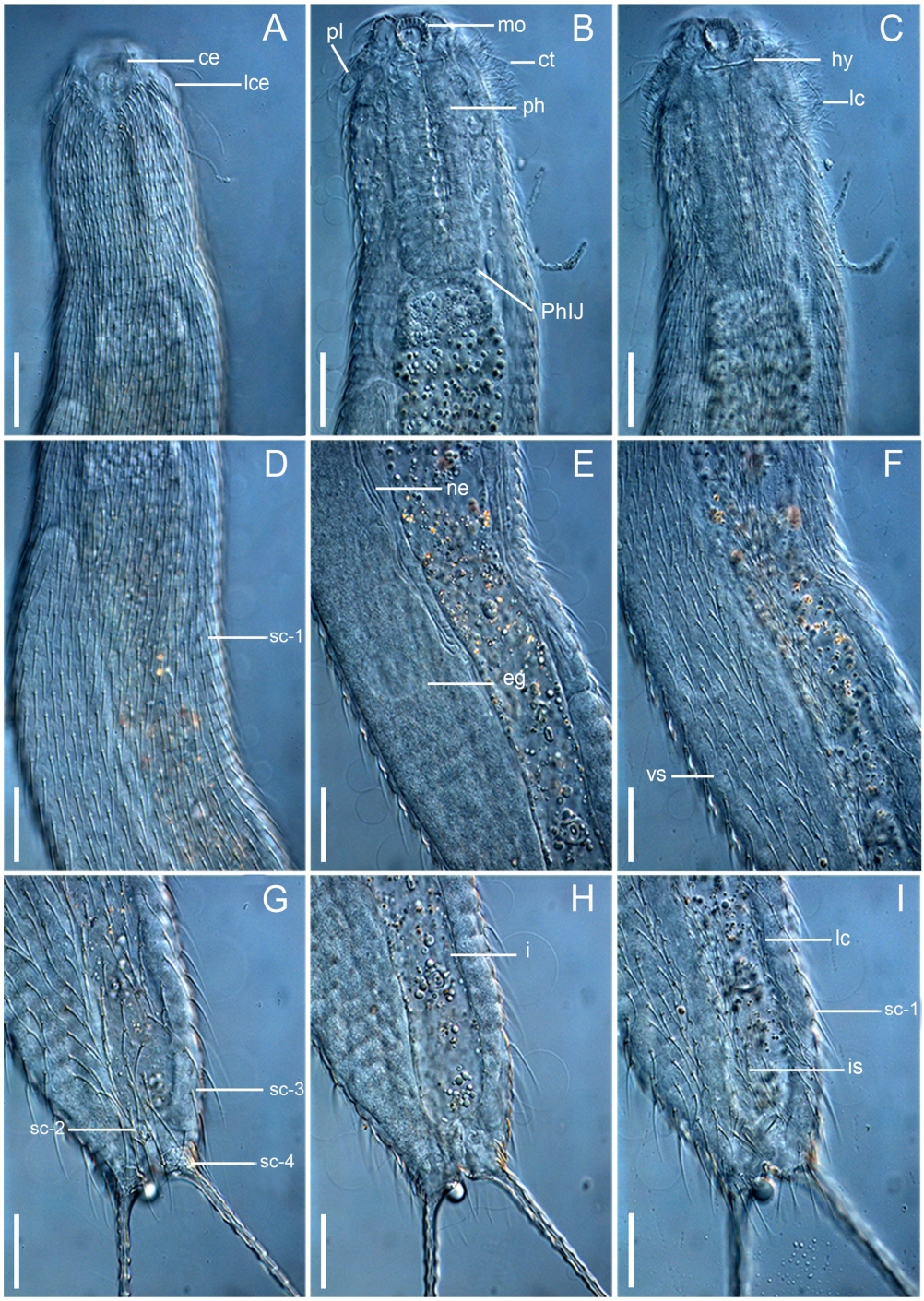

Fig. 3. Light microscopy – DIC. *Polymerurus insularis* sp. nov., holotype (ZUEC GCH 55). **A–C**. Correspond to the most anterior third of the specimen. **A**. Anterior dorsal region. **B**. Anterior internal region. **C**. anterior ventral region. **D–F**. Correspond to the trunk. **D**. Dorsal trunk. **E**. Internal trunk. **F**. Ventral trunk. **G–I**. Correspond to the posterior third of the specimen. **G**. Dorsal posterior third. **H**. Internal posterior third. **I**. Ventral posterior third. Abbreviations: ce = cephalion; ct = cephalic bristles; eg = egg; hy = hypostomium; i = intestine; is = interciliary spines; lc = locomotory cilia; lce = lateral cephalic expansions; mo = mouth; ne = nephridia; pl = pleurae; ph = pharynx; PhIJ = pharyngeal-intestinal junction; sc-1 = *Type 1* scales; sc-2 = *Type 2* scale; sc-3 = *Type 3* scales; sc-4 = *Type 4* scales; vs = *Type 1* ventral scale. Scale bars = 20 µm.

Table 2 (continued on next page). Morphometric features of *Polymerurus insularis* sp. nov. All scale lengths for which the scale type is not specified are from *Type 1* scales. Abbrevation: N = total number of measured adult specimens.

Features	Range (µm)	Mean (µm)	N
Total body length	215–408	322	5
Body length (furcal rami excluded)	139–308	222	5
Furcal rami length	91.5–94.5	93	2
Furcal rami + adhesive tubes length	109–117	113	2
Adhesive tubes length	16–23	19.5	2
Furca base width	26.5–27.5	27	1
Number of furcal rami segments per side	19–21	20	1
Length of ornamentation bristles on the furcal rami segments	4–8	6	3
Body width at head	24–42	32	4
Body width at medium length	31–55	40	4
Body width at furca base	23–25	24	4
Diameter of mouth ring	8–13	10	3
Number of cephalic lobes	3	3	1
Number of head cilia tufts	4	4	3
Length of first cilia tuft	9–12	10.5	1
Length of second cilia tuft	11–19.5	14	1
Cephalion length	19–25	23	3
Cephalion width	20–36	28	2
Lateral projections (lappets) length	10–18	14	3
Pleurae length	12–15	14	3
Hypostomium length	13–17	15	2
Pharynx length	74	74	1
Pharynx width at upper length	19	19	1
Pharynx width at medium length	25	25	1
Pharynx width at pharingeointestinal junction	27	27	1
Egg width	30	30	1
Protonephridia length	105	105	1
Length of locomotory cilia	5–8.5	7	1
Total number of longitudinal columns of scales	42	42	1
Number of scales in a single longitudinal column	55	55	1
Total number of horizontal rows of scales	64	64	1

Table 2 (continued).

Features	Range (µm)	Mean (µm)	N
Number of scales in a single horizontal row	10	10	1
Length of neck dorsolateral scales	3.5–8.5	6	4
Length of middle trunk dorsal scales	5.5–9	8.5	2
Length of middle trunk lateral scales	6.5–11	8.5	2
Length of rear dorsolateral scales	7.5–9	6	2
Length of rear triangular scale (*Type 2*)	5–7	6	3
Length of small rounded scales with posterior spines (*Type 3*)	4.5	4.5	1
Length of rear dorsal small scales (*Type 4*)	2.5–5	3	1
Length of rear indented scales (*Type 5*)	7–8	7.5	1
Length of neck ventral scales	3.5–5	4	2
Length of middle trunk ventral scales	4.5	4.5	1
Length of rear trunk ventral scales	4–7	5	1
Length of keeled ventral scales (*Type 6*)	9.5–10	10	1
Length of small ventral elongated scales (*Type 7*)	3.5–4	3.5	1
Length of small ventral round scales (*Type 8*)	4.5–7	6	1
Length of head and neck dorsolateral spines	2–13	7.5	2
Length of upper trunk dorsolateral spines	8–15	11.5	2
Length of middle trunk dorsolateral spines	8.5–22.5	15.5	5
Length of rear trunk and furca base dorsolateral spines	18–33	25.5	5
Length of neck ventral spines	5.5–7.5	6.5	1
Length of trunk ventral spines	5.8–9.8	8	2
Length of rear ventral spines	5.7–15	10	1
Length of keels on *Type 6* scales	6–7	6.5	1

the head and slightly lifted (Figs 3A, 7A–C). There is a pair of pleurae (U2–U6), each 12–15 µm long and presenting a rough texture with small parallel grooves, contrasting with the smooth surface of the cephalion (Figs 3B, 7C). The ventral hypostomion is a well-marked transversal bar, at U4, 13–17 µm long and shaped like an arch (i.e., decreasing in thickness towards the lateral extremities) with a middle concavity (Fig. 3C). Two tufts of cilia (cephalic sensory bristles) are present on each side of the head. The first tuft is shorter, approximately 10.5 µm long and located below the cephalion projections (U1), while the second is situated between the cephalion and the pleurae (U2), bearing longer cilia with an average length of 14 µm (Fig. 3B). No dorsal sensory bristles were observed. Mouth is subterminal, with 8–13 µm of diameter and surrounded by a ring that is segmented with longitudinal ridges (Fig. 3A–C). The pharynx is relatively long, reaching up to one-quarter body length (adhesive tubes excluded) and situated between U2 and U20. It is 74 µm long, 25 µm wide at mid-length and divided in 2 regions, without any distinct constrictions. Pharynx becomes wider towards its inferior portion at the pharyngeal

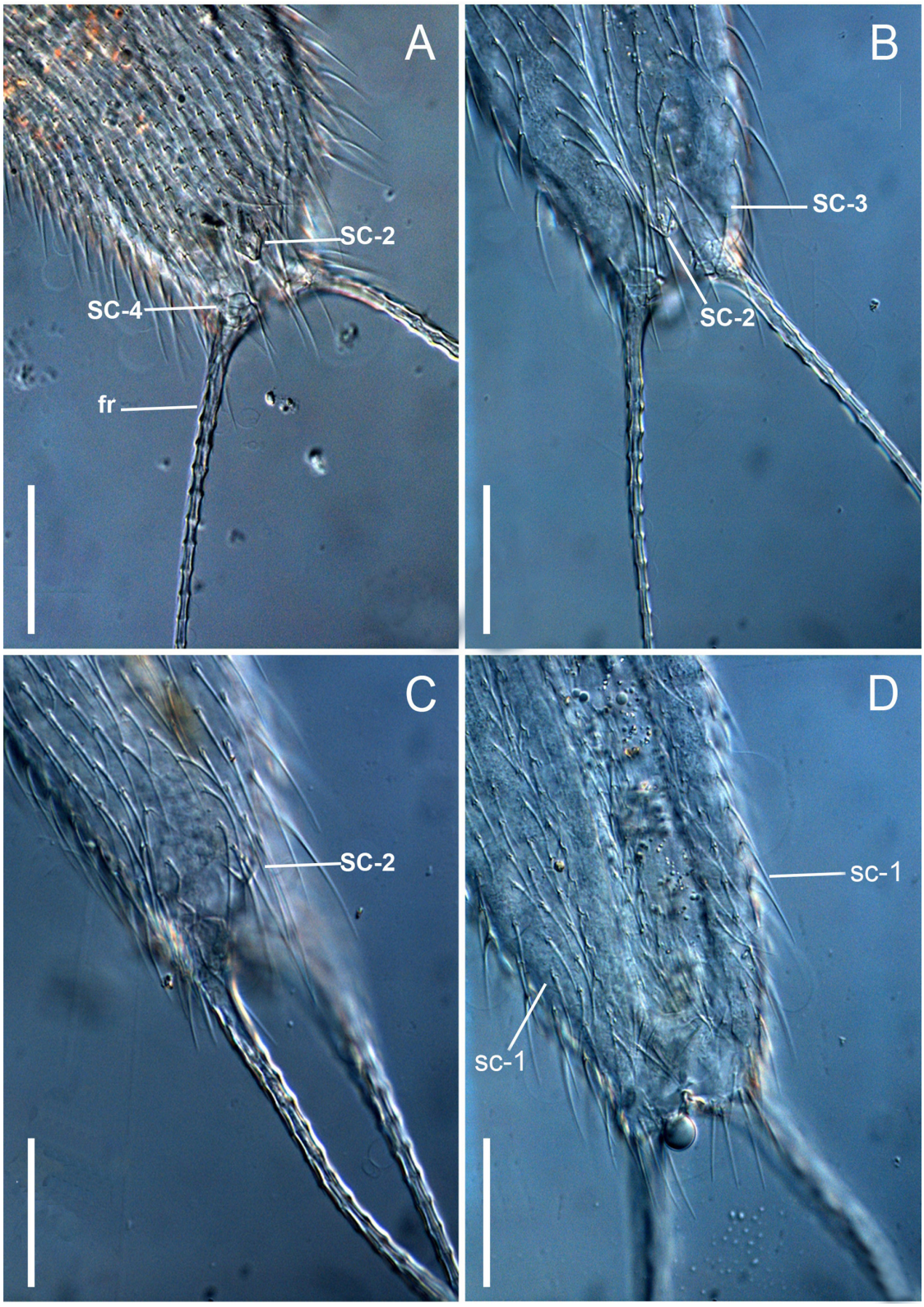

Fig. 4. Light microscopy – DIC. *Polymerurus insularis* sp. nov. Posterior region of the body. **A**. Paratype (ZUEC GCH 56). **B, D**. Holotype (ZUEC GCH 55). **C**. Paratype (ZUEC GCH 57). **A–B**. Posterior dorsal view. **C**. Posterior dorsolateral view. **D**. Posterior ventral view. Abbreviations: fr = furcal rami; sc-1 = *Type 1* scales; sc-2 = *Type 2* scale; sc-3 = *Type 3* scales; sc-4 = *Type 4* scales. Scale bars = 40 µm.

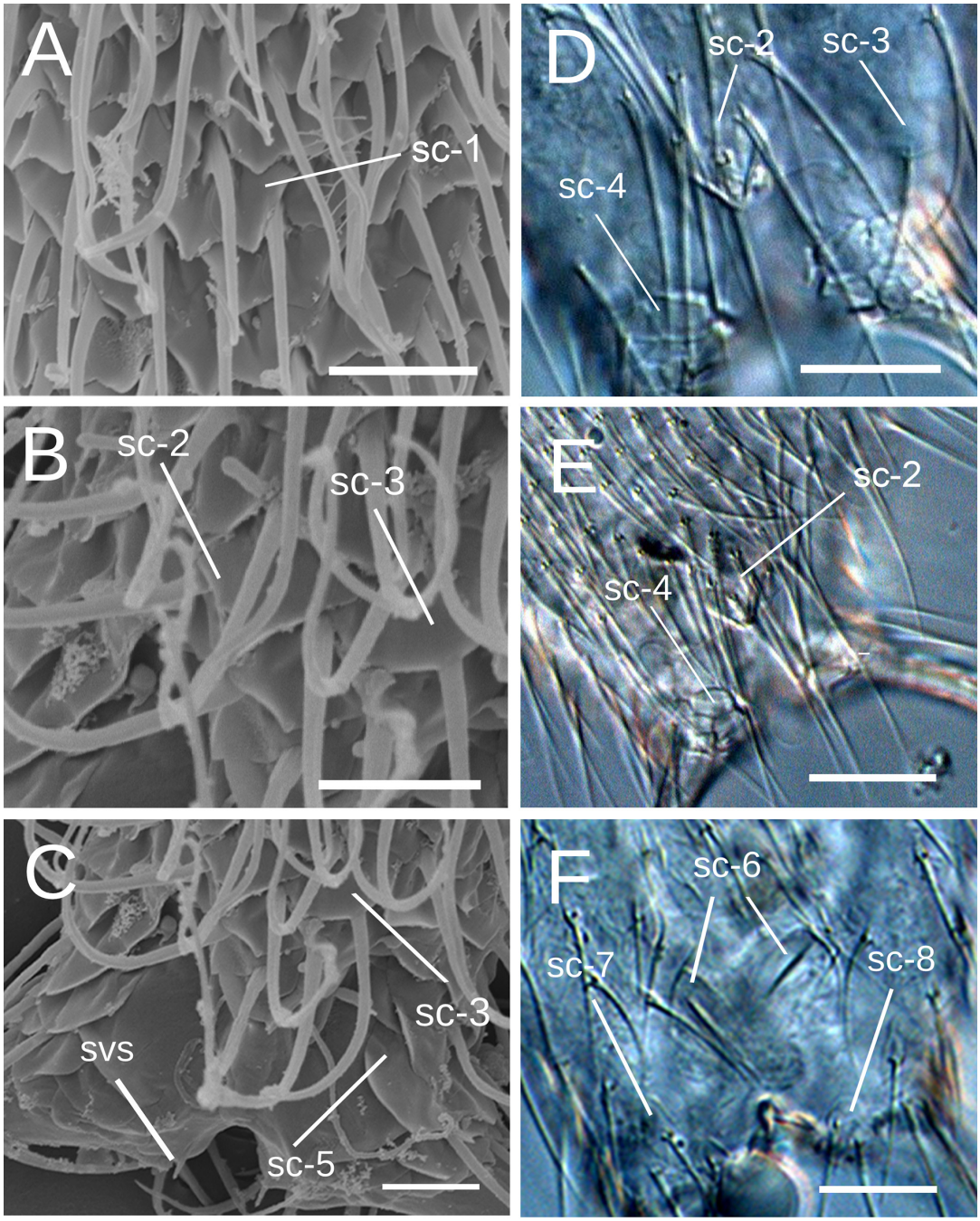

Fig. 5. Close-up of the different types of scales described for *Polymerurus insularis* sp. nov. **A–C**. Paratype (ZUEC GCH 59). **D, F**. Holotype (ZUEC GCH 55). **E**. Paratype (ZUEC GCH 56). **A–C**. Scanning electron microscopy. **D–F**. Light microscopy – DIC. **A**. Detail of a section of the dorsal middle trunk, showing the most common type of scale, *Type 1*, with emphasis on its characteristic shape. **B**. Detail of a section of the dorsal posterior trunk, showing *Type 2* and *Type 3* scales. **C–E**. Detail of the transition between the dorsal posterior trunk and the dorsal furca base, showing the particular scale covering of this region. **F**. Detail of the transition between the ventral posterior trunk and the ventral furca base, showing the particular scale covering this region. Abbreviations: sc-1 = *Type 1* scales; sc-2 = *Type 2* scale; sc-3 = *Type 3* scales; sc-4 = *Type 4* scales; sc-5 = *Type 5* scales; sc-6 = *Type 6* scales; sc-7 = *Type 7* scales; sc-8 = *Type 8* scales; svs = small ventral pair of spines. Scale bars: A–C = 5 µm; D = 10 µm; E = 15 µm; F = 10 µm.

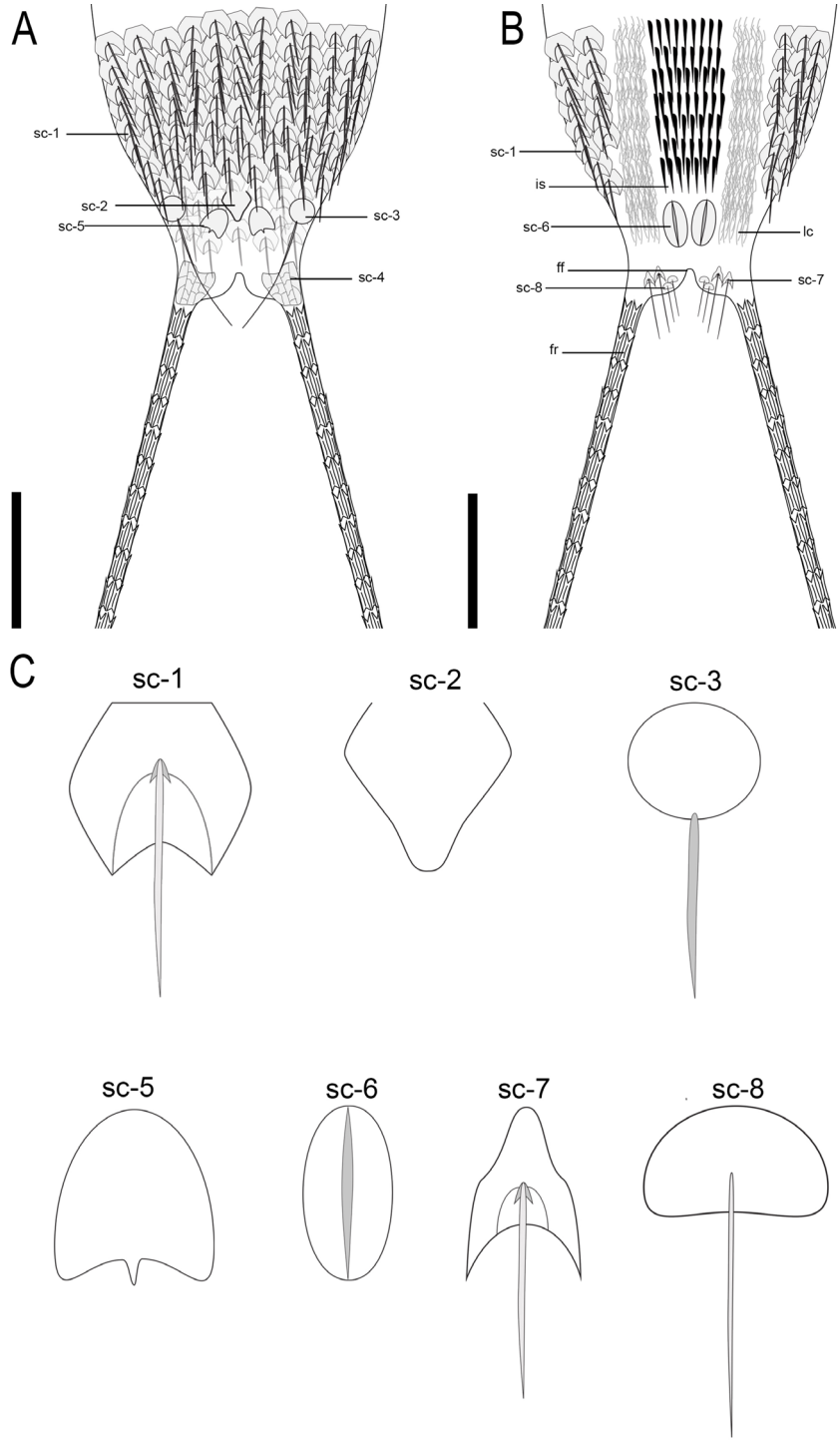

Fig. 6. Schematic illustration of dorsal and ventral posterior regions and type scales of *Polymerurus insularis* sp. nov. **A–B**. Paratype (ZUEC GCH 56). **A**. Dorsal view of the posterior end. Some *Type 1* scales are faded for a better visualization of *Types 2*, *3* and *5* scales. **B**. Ventral view of the posterior end. **C**. Each type of scale, individually depicted (not to scale). Abbreviations: ff = furcal furrow; fr = furcal rami; lc = locomotory cilia; is = interciliary spines; sc-1 = *Type 1* scales; sc-2 = *Type 2* scale; sc-3 = *Type 3* scales; sc-4 = *Type 4* scales; sc-5 = *Type 5* scales; sc-6 = *Type 6* scales; sc-7 = *Type 7* scales; sc-8 = *Type 8* scales. Scale bars = 40 μm.

intestinal junction (from 19 µm to 27 µm) while at its superior end a distinct, although small, furrow is formed at the junction of the internal regions (Fig. 3B).

Internal anatomy. Internally, a pair of protonephridia (105 µm long) are present, lateral to the intestine and posterior to the pharyngeointestinal junction (U29 – U75) (Fig. 3E).

Furca. Is 109–117 µm long (¼ of the body) and furca base is 27 µm wide, showing a V-shaped gap with a large and distinct U-shaped middle furrow ("helmet like" shape, as described by Roszczak 1969). Presence of a single pair of very small ventral spines, one per side of the furrow (Fig. 5C). Furcal rami are 93 µm long (around 1/5 of body length) and appear to be composed of 19–21 segments (Figs 4A, 6B, 7A, 8C). Segments of the furcal rami show well marked ornamentations in both DIC and SEM images, with well defined edges and covered at both sides – sometimes almost entirely – with short, straight or slightly bent up spines (bristles) measuring around 4–8 µm (Figs 4A–C, 8D–E). These ornamentations decrease in thickness and length towards the posterior end of the furca, which seems to be partially due to the decrease in the number and length of the spines (bristles). In fact, thickness, number and length of the spines on the ornamentation are variable among the specimens (Figs 2A, 4A–C, 8D–E). The inner portion of the first 10th of the furca length, which precedes segmentation, is smooth, while the outer portion is covered by the aforementioned bristles.

Ventral ciliature. Composed of two longitudinal bands of locomotory cilia with approximately 5–8.5 µm of length, starting at U1, immediately below the mouth line, and ending at U71, right before the location of the *Type 6* scales (Figs 3C, I, 6B, 7C). Ventral ciliary bands are separated by the ventral interciliary field covered by small spined scales (Figs 3I, 6B). Although cilia are not well visible in the pictures and were not very well oriented for measurements, due to the position and preservation state of the individuals, they are depicted in the illustrated schematics (Fig. 6B).

Dorsal scales. Most of the cuticular armature of the body is composed of simple spined scales arranged in approximately 42 longitudinal columns, each column bearing 55 scales, and approximately 64 horizontal, alternate rows, with around 10 scales per row (Fig. 2A). Dorsolateral scales covering anterior, middle and most of the posterior body surface are roughly polygonal in shape (most presenting pentagonal or hexagonal form), with an elevated anterior portion, a distal incision and bearing a single spine (*Type 1* scales – Figs 3D, 4D, 5A, 6A–C, 7D–E). These polygonal scales are the most common type found on the specimens, covering the majority of the body, both dorsolaterally and ventrally. They are outlined by five to six corners, with two distinctive elements (Figs 5A, 6A–C, 7D–E): (a) an elevated anterior portion formed by the spine's curvature – as it arises from the scale's surface at middle range, from semi triangular keels – and (b) a longitudinal concavity delimited by two sloping edges that extend from the spine's insertion to the posterior end of the scale, often ending in a gap, where the two most distal corners meet forming a wide angle. SEM images (Figs 7A, D–E, 8A–B) reveal that these scales are considerably overlapped (overlapping usually hides their anterior half). Dorsal scales arranged in the first five transversal rows are rounded, very small and rather close to each other, measuring 3.5–5.5 µm (scale type unidentified) (Fig. 3A). From the fifth transversal row, dorsal scales become predominantly polygonal (*Type 1* scales), reaching 8.5 µm at the pharygeointestinal junction (U20 – Fig. 3A–B, D). At the middle trunk (from the pharingeointestinal junction to the proximities of furca base, at U73–U75) they range from 5.5 to 9 µm, while at the lateral sides they become slightly larger, measuring 6.5–11 µm (Figs 3D, I, 4D). All dorsal scales are mostly parallel to the transverse axis of the body, with the exception of one distinct elevated spineless scale immediately anterior to the furca base (U71), which is 5–7 µm long and shaped as a triangle with well-marked edges (*Type 2* scale – Figs 3G, 4A–C, 5B, D–E, 6A, C). Laterally and below the *Type 2* scale, at U72, there is a pair of small (4.5 µm) rounded scales, one at each side of the caudal field, bearing long spines (16–21 µm) which arise directly from the scale's most posterior end, instead of from the middle, as it normally occurs (*Type 3* scales – Figs 3G,

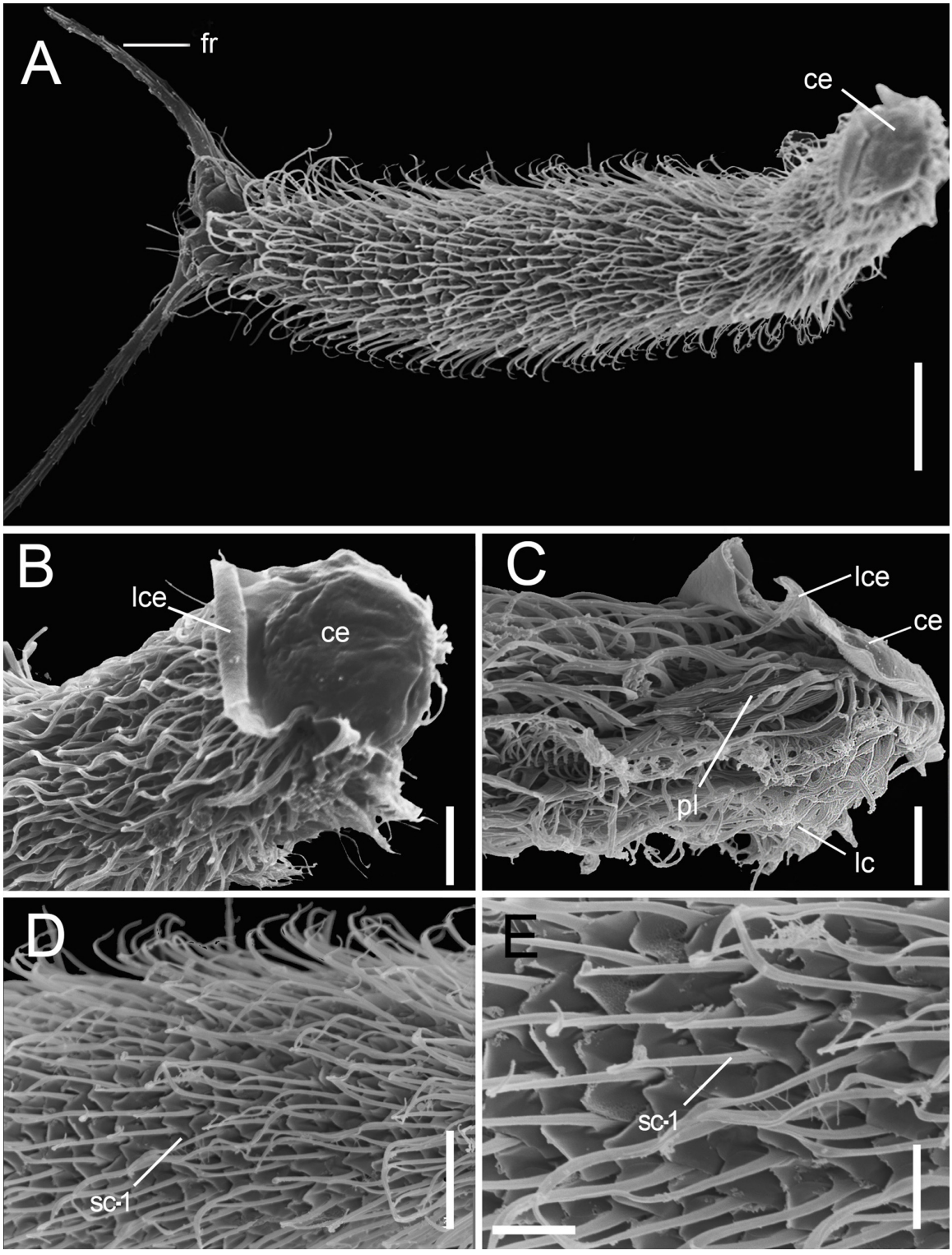

Fig. 7. Scanning electron microscopy. *Polymerurus insularis* sp. nov. **A–B, D–E**. Paratype (ZUEC GCH 59). **C**. Paratype (ZUEC GCH 60). **A**. Dorsal view of the body. **B**. Dorsolateral view of the head, showing the cephalion and its lateral projections. **C**. Lateral view of the head, highlighting the lateral pleurae. **D–E**. Dorsal trunk view, highlighting scale *Type 1*. Abbreviations: ce = cephalion; fr = furcal rami; lc = locomotory cilia; lce = lateral cephalic expansions; pl = pleurae; sc-1 = *Type 1* scales. Scale bars: A = 40 µm; B–C = 5 µm; D = 20 µm; E = 10 µm.

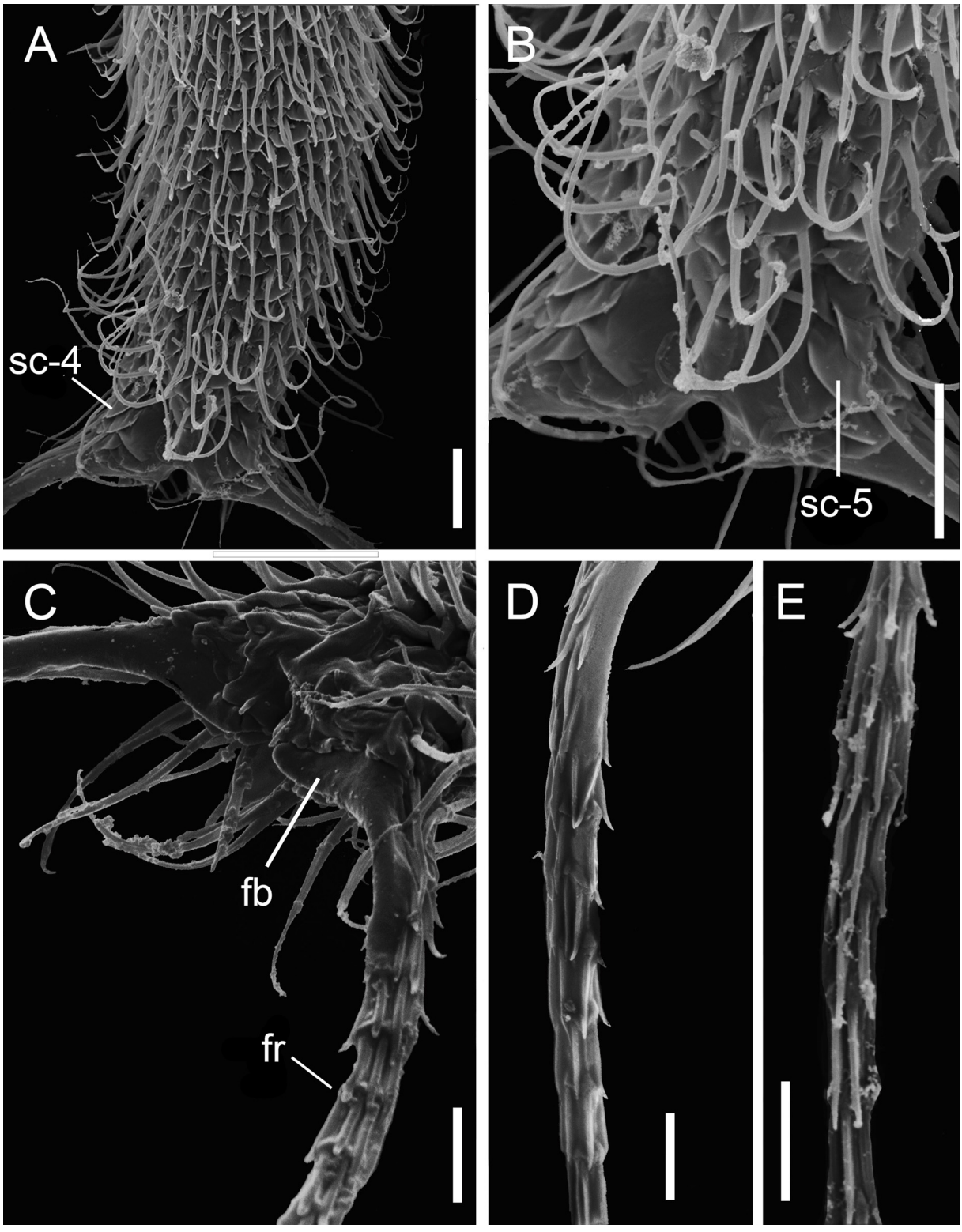

Fig. 8. Scanning electron microscopy. *Polymerurus insularis* sp. nov. **A–B, D**. Paratype (ZUEC GCH 59). **C, E**. Paratype (ZUEC GCH 60). **A–B**. Posterior dorsal view. **C**. Detail of the furca base in posterior dorsolateral view. **D–E**. Details of the furcal rami. Abbreviations: fb = furca base; fr = furcal rami; sc-4 = *Type 4* scales; sc-5 = *Type 5* scales. Scale bars = 10 µm.

4B, 5B–D, 6A, C). Immediately following this region (at U62 in the paratype photographed with SEM and between U71–75 in the holotype), there is a rather noticeable spineless field covered by: (a) a patch lacking cuticular ornamentation (situated medially from the surroundings of the furca base until the uppermost portion of the adhesive tubes), and (b) a complex of small, flat, rounded or polygonal and spineless scales (U73–U75), 2.5–5 µm long, covering the initial portion of each furcal rami (*Type 4* scales) (Figs 3G, 4A, 5D–E, 6A, 8A). Below *Type 2* scales there is a pair of spineless, wide and rounded scales, 7.5 µm long, presenting small indentations at their posterior ends (*Type 5* scales – Figs 5C, 6A, C, 8B).

Dorsal spines. Dorsolateral spines covering the head and neck (U1–U20) are rather short, ranging from 2–13 µm. The first two rows of spines, disposed immediately around and below the cephalic plates are much shorter (2–7 µm) and slightly curved, while the remaining rows along the neck contain longer and straighter spines. Through the upper and middle trunk (U21–U44), the dorsal spines increase progressively in length, with an average length of 11.5 µm and 15.5 µm, respectively, reaching their largest dimensions at the rear trunk (U45–U51), with an average range of 25.5 µm. At rear trunk, towards the furca base (U75), dorsolateral spines become much longer (18–33 µm) and straighter, grouping into two pairs pair of small, tight clusters at the lateral edges of the body surrounding the anterior-most portion of the furca base (Figs 2A, 3G, 4A–B). Most spines arise at a medial point on a scale's surface, under a small ridge-shaped elevation that is formed by the junction of: (a) the spine's anterior end, as it rises from the scale, and (b) the superposition between the scale's anterior portion and the posterior ends of the two scales in the former row (Figs 6A–C, 7E).

Ventral interciliary scales. Are small in the head and neck region (3.5–5 µm) and increase in size towards the middle trunk (4.5 µm) and rear trunk (4–7 µm), similarly to the dorsal and lateral scales. At the posterior end of the ventral surface, the scales are rounded or polygonal and flat (unidentified type). Among these scales is situated a particular and very distinct pair of oval scales at the middle of the posterior interciliary ventral field (U71); each scale bears a keel that emerges from the anterior portion of the scale and extends across its length (*Type 6* scales – Figs 5F, 6B–C). In the holotype, the largest scale of the pair is 10 µm long, with a keel 6 µm long, while the smaller scale is 9.5 µm long, showing a 7 µm long keel. However, such asymmetrical pattern might not be ubiquitous; it was only reported for the holotype because it was not possible to visualize and measure these structures in the paratypes. The most distal extremity of the ventral furca base (U75) is covered by two columns of small (3.5–7 µm long) scales (*Types 7* and *8* scales) bearing spines that are very long and straight, measuring from 11 up to 20 µm. These scales are elongated antero-posteriorly and laterally narrowed, showing a middle furrow and lateral projections (*Type 7* scales), or rounded and antero-posteriorly narrowed (*Type 8* scales) (Figs 5F, 6B–C).

Ventral interciliary spines. Are shorter than the dorsal spines. They range in size from 5.5–15 µm, and become longer and thicker from anterior to posterior region and from ventral to ventrolateral sides.

Egg. A single egg was present in the holotype (Fig. 3E); it was 30 µm wide and situated between the cuticle and the intestine, extending from the upper to rear trunk (U21–U51). No sperm and reproductive organs were observed.

Taxonomic remarks

Specimens of the genus *Polymerurus*, when compared with the remaining members of Chaetonotida, are easily recognized due to their large size (some species are the longest known chaetonotidans), the presence of ring-like ornamentations – usually called segmentations – on the furcal rami, and a cephalion with prominent lateral expansions. Although all species of *Polymerurus* share these characteristics, it is possible to recognize among them very distinct morphotypes, generally based on their cuticular

Table 3 (continued on next page). Comparison of several morphological structures among different species of *Polymerurus* Remane, 1927 belonging to the 'spined scales' morphotype.

Characteristics	*P. insularis* sp. nov.	*P. nodicaudus*	*P. entzii*	*P. nodifurca*	*P. paraelongatum*	*P. ringueleti*
Body shape	Elongated	Elongated	Elongated	Elongated	Elongated	Elongated
Total length	215–408 µm	330–544 µm	350–420 µm	395 µm	504 µm	290 µm
Pharynx constriction	Slightly evident	Slightly evident	Slightly evident	Slightly evident	Slightly evident	Slightly evident
Furca base constriction	Accentuated	Accentuated	Accentuated	Accentuated	Slightly accentuated	Accentuated
Pharynx	Cylindrical shape, gradative enlargement towards the rear end	Cylindrical	No description	Cylindrical	No description	Slightly evident posterior enlargement
General structure of scales	Spined, no peduncle	Spined, no peduncle	Spined, no peduncle	Spined, no peduncle	Spined, no peduncle	Spined, no peduncle
General shape of scales	Pentagonal, anteriorly curved as a dome and distally incised, or all-rounded and smaller	Anteriorly rounded, posterior end shaped as an U	Anterior and lateral extremities with concave shape	No description	Anteriorly rounded, posterior extremity V-shaped	No description
Spines	Present	Present	Present	Present	Present	Present
Shape of dorsal spines	Straight, usually long, shorter near the head	Short and simple, larger posteriorly	No description	Short, simple	Simple	No description

Table 3 (continued).

Characteristics	*P. insularis* sp. nov.	*P. nodicaudus*	*P. entzii*	*P. nodifurca*	*P. paraelongatum*	*P. ringueleti*
Furca	Shaped as a U, middle furrow V-shaped	V-shaped, no furrow	Shaped as a wider U, middle furrow present	No description	V-shaped	U-shaped
Ventral scales at furca base	Polygonal or rounded, no peduncle nor superposition	No description	No description	No description	No description	No description
Dorsal scales at furca base	Spineless, mostly pentagonal, a few visibly by SEM oval or rounded, larger and flat	Not described	Not described	Not described	Not described	Not described
Internal pair of small spines at ventral furca base	Present	Not described	Not described	Absent	Not described	Not described

ornamentation and body outline. Regarding the latest, it is possible to distinguish completely straight outlines with absent body constrictions, as in *P. serraticaudus* (Voigt, 1901) from more tenpin-like shapes as occurs in *P. rhomboides*. Furthermore, the caudal portion varies in length, thickness and segmentation type (partial or complete). In what accounts for cuticular coverage, species may present spined scales that lack a peduncle (column-like base) or pedunculated scales (stalked scales). *Polymerurus insularis* sp. nov. bears spined scales that lack a peduncle, and in terms of cuticular coverage resembles six other species: *P. nodicaudus*, *P. serraticaudus*, *P. entzii* (Daday, 1882), *P. nodifurca* (Marcolongo, 1910), *P. paraelongatus* (Grosso & Drahg, 1986) and *P. ringueleti* (Grosso, 1975). However, the new species has a specific set of characteristics that distinguishes it from these congeners: (1) a cuticular armature composed of simple spined scales with polygonal shape (*Type 1* scales), (2) a single, spineless dorsal scale with a triangular shape located terminally next to the furca base (*Type 2* scale), (3) a spineless zone composed by a patch lacking cuticular ornamentation and flat, rounded or polygonal scales without spines (*Type 4* scales) (4) particular sets of terminal spined or keeled scales located both dorsally and ventrally around the furca base (*Types 3*, *5*, *6*, *7* and *8* scales). In addition to these characteristics, the new species can be differentiated from *P. nodicaudus* and *P. paraelongatus* by the presence of a U-shaped furca instead of a V-shaped furca, and from *P. entzii* by the absence of long spines in each of the ring-like ornamentations (segments) of the furca rami. Additionally, the new species contrasts with *P. nodifurca* by long and straight spines instead of short, curved spines, as found in the aforementioned species. Finally, spined-scales are present along the dorsolateral, lateral and ventrolateral regions in *Polymerurus insularis* sp. nov., which distinguishes it from *P. ringueleti*; while the long, narrow and segmented furcal rami of the new species are very different from the short, thick and unornamented furca present in *P. serraticaudus*.

Phylogenetic relationships

The final alignments of 18S rDNA and 28S rDNA yielded 1761 and 4141 positions, respectively, while the concatenated alignment had 5902 positions. The phylogenetic reconstruction based on a multigene approach supported the currently recognized monophyly of the genus *Polymerurus* with a very high bayesian (1–0.98) and bootstrap (99–98) support for the phylogenetic signal on both internal and external nodes of the Maximum Likelihood (Fig. 9) and Consensus trees (Supp. file 2). Accordingly, the two specimens of *Polymerurus insularis* sp. nov. were grouped together and nested within *Polymerurus*, with branch supports of 1 and 99. As expected, *P. nodicaudus* was recovered as sister clade to *P. insularis* sp. nov., reflecting the morphological similarities between the two species, such as spined scales with no peduncles, in contrast with the pedunculated scales of *P. rhomboides*.

Discussion

Dispersal through long distances

Freshwater gastrotrichs have previously been reported from oceanic islands (e.g., Balsamo 1982; Fregni *et al*. 1998; Balsamo *et al*. 1994; Hochberg 2005); however, *Polymerurus insularis* sp. nov. is the first new species to be found on an oceanic island of volcanic origin. This geological origin, together with the long geographic distance from the Brazilian coast means that the islands were never in contact with the continental lands (Silva e Silva & Olmos 2006; Marques *et al.* 2014). The early physical isolation from the continents and the thousands of square kilometers of ocean surrounding the island forms a nearly unsurmountable barrier for the active dispersal of freshwater gastrotrichs (e.g., given hostile conditions such as salinity and oxygen levels). Thus, this scenario results in a very unlikely environment to be reached/accessed by a tiny freshwater invertebrate such as a gastrotrich. It is important to take into account some crucial characteristics of the gastrotrichs and other meiofaunal organisms that also play a role as a barrier to their dispersion: their microscopic body sizes, short life cycles (restrained to a few weeks), and limited swimming capacity (Higgins & Thiel 1988; Boeckner *et al*. 2009; Giere 2009).

Such evidence raises the question: how was it possible to find a freshwater gastrotrich on an oceanic island without any – present or historical – contact with the continent? A first hypothesis for this question is related to passive dispersal events, in which adult/larval individuals or their propagules are carried long distances by external agents (Cerca *et al.* 2018). Considering freshwater invertebrates, passive dispersal events can happen through wind gusts termed anemochory (Greek: *anemo*, wind, *choro*, dance), or via an animal vector (termed zoochory, Greek, *zoo*, animal) attached to feet, feathers, and fur (Bilton *et al*. 2001). In this case, the transported organisms are frequently at a specific stage of their life cycle in the form of a drying resistant propagule, such as a diapause egg or annhydrobiotic stage (e.g., Balsamo & Todaro 2002; Nkem *et al*. 2006; Rivas Jr. *et al*. 2019).

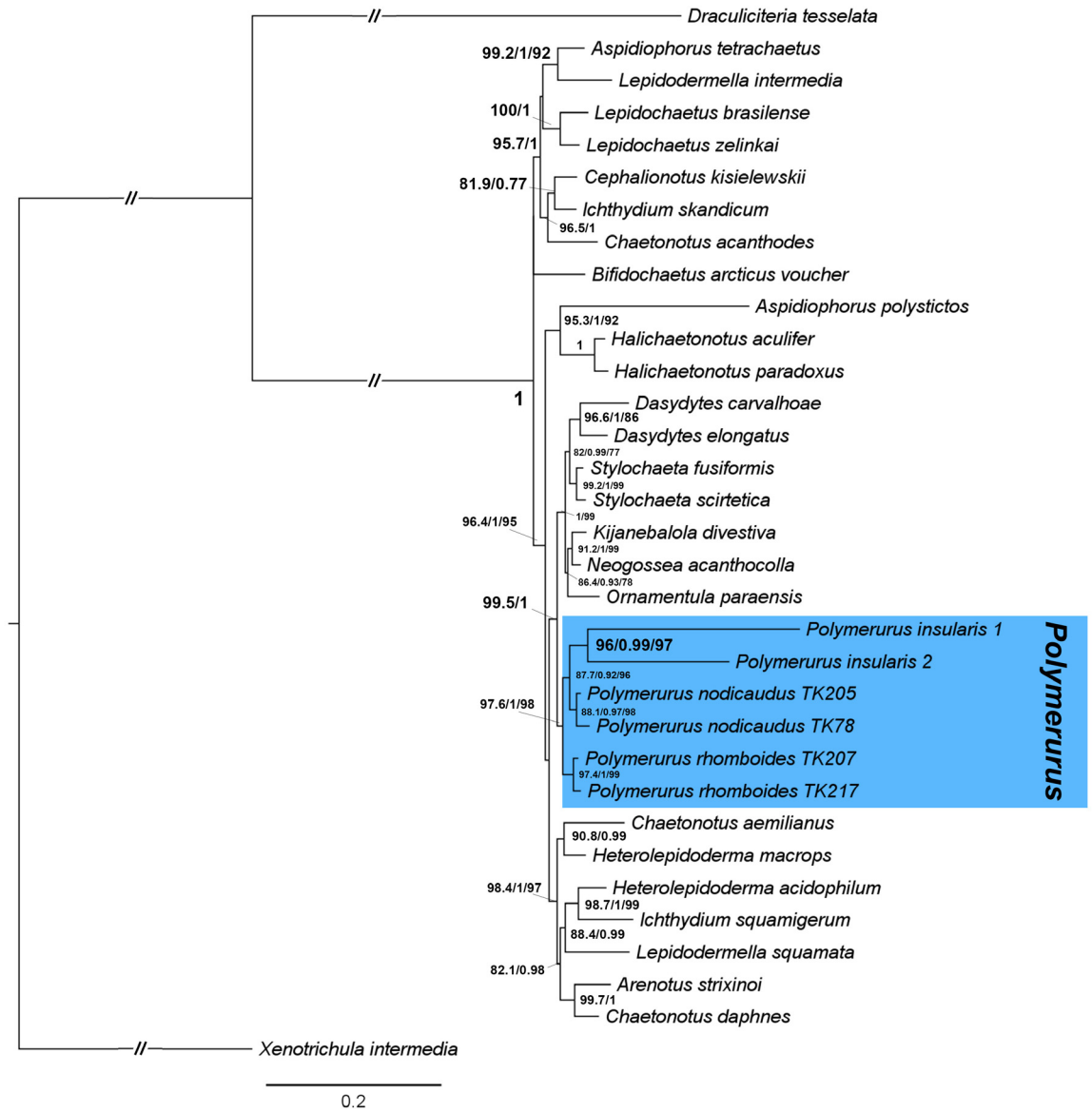

Fig. 9. Maximum Likelihood tree based on multigene approach with 18S and 28S sequences. Highlighted branches correspond to the *Polymerurus* Remane, 1927 species sequences. Values on the branches correspond, respectively, to: SH-aLRT support (%) / aBayes support / ultrafast bootstrap support (%).

Regarding freshwater meiofaunal organisms, Hochberg (2005) suggested that the presence of Gastrotricha in isolated water bodies may be facilitated by parthenogenetic reproduction (in some species), which allows a single individual to establish a population in a new habitat (Incagnone *et al.* 2015), as well as the eggs' high dispersal capacity through the wind. Conversely, several other studies point to birds as important dispersal vectors of limnic organisms such as copepods (Halse *et al*. 2000; Green & Figuerola 2005; Frisch *et al*. 2007; Dimante-Deimantovica *et al*. 2018), cladocerans (Halse *et al*. 2000; Frisch *et al*. 2007; Dimante-Deimantovica *et al*. 2018) or limnic-terrestrial species, such as tardigrades (Mogle *et al*. 2018). In this scenario, birds that constantly move between two suitable habitats, e.g., a temporary pond and a stream, might transport Gastrotricha propagules attached to their feathers or feet, through epizoochory (Greek: *epi*, upon), or ingested, through endozoochory (Greek, *endo*, inside) (Incagnone *et al.* 2015).

The Fernando de Noronha archipelago, specifically, is well known as a stopping-point for many migratory birds (Silva e Silva & Olmos 2006; Silva e Silva 2008; Ferreira *et al.* 2019). The islands are visited by approximately 75 species of birds (Silva e Silva & Olmos 2006), from which at least 14 were effectively observed within the extension of the Xaréu Reservoir (Silva e Silva & Olmos 2006; Ferreira *et al.* 2019). It is important to highlight that, besides a few endemic bird species, the majority of birds observed in Fernando de Noronha archipelago are migratory or stray seabirds knowingly capable of undertaking long migration routes and coming from several different areas, such as North America (five species), Central America (three species), South America (five species), Africa (three species), Europe (seven species) and the Artic Polar Circle (one species) (Silva e Silva & Olmos 2006; Piacentini *et al*. 2015; Ferreira *et al.* 2019).

Aside from passive dispersal through other animals as external vectors, anthropic actions cannot be excluded as a dispersal factor allowing freshwater gastrotrichs to reach Fernando de Noronha archipelago. The archipelago has been visited by humans for more than 500 years, and, along with that, various exotic animals have been constantly introduced to this environment and may have acted as vectors for passive dispersal: reptiles such as the black-and-white tegu *Salvator merianae* (Duméril & Bibron, 1839), house geckos *Hemidactylus mabouia* (Moreau De Jonnès, 1818), mammals as the Rock Cavy *Kerodon rupestris* (Wied-Neuwied, 1820) and two species of amphibians, the Venezuela Snouted Treefrog *Scinax x-signatus* (Spix, 1824) and the Jimi's toad *Rhinella jimi* (Stevaux, 2002) (Santos 2011).

Within Chaetonotidae, the taxon *Polymerurus* can be considered an exception, as it is a strictly freshwater and a highly supported monophyletic group (Kånneby *et al*. 2013; Kolicka *et al*. 2020). Thus, in order to further investigate the evolutionary origins and phylogenetic relationships of *Polymerurus insularis* sp. nov. it is imperative to verify the existence of coastal specimens of the new species. In case such specimens are found, comparing them with the island specimens would contribute to the understanding of how and when *P. insularis* has reached the Fernando de Noronha Archipelago. Given the aforementioned condition of some descriptions of Gastrotricha and the overall resemblance of *P. nodicaudus* with *P. insularis*, it is not possible to discard that some representatives of *P. insularis* might have been described as *P. nodicaudus*.

New species definition and pseudocryptism within Gastrotricha

The definition of a new species can be especially challenging when the study of systematics and phylogeny are still emergent, as in the case within Gastrotricha. The morphological heterogeneity among gastrotrichs, in particular among the genus *Polymerurus*, had been almost fully undercovered due to the questionable cosmopolitan distribution of *P. nodicaudus* and *P. rhomboides* (see discussion below).

It is noteworthy that many species descriptions of gastrotrichs remain unstable for a series of reasons: i) the fragility of the specimens and the extensive morphological diversity among groups (Balsamo

et al. 2008; Garraffoni *et al.* 2019b); ii) many descriptions date from decades/years ago and are brief and too generic (Garraffoni & Melchior 2015; Kieneke & Nikoukar 2017); iii) the use of rudimentary optical equipment for visualizing the specimens leading to schematic drawings that are too simplified and poorly illustrated; iv) the majority of the species do not have type series deposited in a zoological museum (Garraffoni *et al.* 2019b); v) the lack of a standardized method for the description of new species causing great disparity between species descriptions of the same genus from distinct authors (Visvesvara 1963; Kisielewski 1979, 1991; Balsamo *et al.* 2008; Grilli *et al.* 2010; Kånneby 2011).

Currently, such scenario raises important taxonomic problems, since, whenever scientists must consult past descriptions for a new study, they are often unable to discriminate among different species. It is very likely that overlooked morphological characters (or briefly mentioned and not effectively characterized features) might have impaired the identification of new species and/or species delimitation within *Polymerurus* (also in other taxa). This phenomenon of lumping distinct species into a single type can either create artificially cosmopolitan morphospecies (Klautau *et al.* 1999) or represent complexes of cryptic or pseudo-cryptic species.

Cryptic species are the morphotypes recognized as morphologically identical although genetically distinct (Lundholm *et al*. 2012). The definition and identification of cosmopolitan gastrotrichs became increasingly common in the past, however, reports of cryptic species, even though appearing few times, may lead to questioning the real cosmopolitanism of some Gastrotricha (Todaro *et al*. 1996; Leasi & Todaro 2009; Kieneke *et al*. 2012; Kieneke & Nikoukar 2017). On the other hand, when representatives of different populations (sympatric or allopatric) that were a priori recognized as morphologically very similar become independent species after the evidence of new characteristics, it is said that pseudocryptism has occurred (Bickford *et al.* 2007). Oftentimes, the acknowledgment of such characteristics is possible due to the improvement of the research methods and/or technologies that allow a more detailed analysis of those species' morphological structures (Saéz & Lozano 2005; Lundholm *et al*. 2012, Kawauchi & Giribet 2014).

Regarding Gastrotricha, even though the actual term pseudocryptism was never mentioned, it is possible that a few studies have detected this phenomenon before (e.g., Schwank 1990; Garraffoni & Melchior 2015; Kieneke & Nikoukar 2017). In this sense, Schwank (1990) revealed the existence of several new species, especially in South America and Africa, by reconsidering the status of some identifications presented in taxonomic surveys that happened in the early 20th century (e.g., Daday 1905). Another example of pseudocryptism in Gastrotricha emerged from successive confocal analyses of the musculature of *Xenotrichula intermedia* Remane, 1934, a classic example of a widely distributed gastrotrich. Leasi & Todaro (2009) reported differences in the muscular architecture between specimens classically determined as *Xenotrichula intermedia* found in Italy, United States and Kuwait. More recently, Münter & Kieneke (2017) described a new type of muscular architecture for representatives of that species in Germany, while Araújo *et al*. (in prep.) have found two other muscular arrangements in sympatric populations on the east coast of the United States.

In the present paper, we observe that the newly proposed species was rather similar to representatives of *Polymerurus nodicaudus* regarding overall morphology, size and general features of the cuticular armature, and the differences diagnosed between the two species were only detected through detailed analysis using varied techniques. The presence of a triangular scale at the rear dorsal trunk (*Type 2* scales), for example, the small spineless scales (*Type 4* scales) at the base of the furca and the morphological details of *Type 1* scales were observed with the support of DIC and SEM microscopy. Specimens of *Polymerurus nodicaudus* have been widely reported around the world for Palearctic (Voigt 1901; Kisielewski 1999; Leasi *et al*. 2006; Kånneby 2011; Kieneke & Hochberg 2012), Neotropical (Kisielewski 1991), Australian (Hochberg 2005) and Oriental (Saito 1937; Sharma & Sharma 1990)

regions; however, a minor fraction of those works employed a DIC equipped microscope (Hochberg 2005; Kånneby 2011), and almost none used SEM in order to further examine finer details of the specimens structure (Hochberg 2005). In that sense, it is reasonable to say that, without the use of integrative techniques together with a careful morphological analysis, *Polymerurus insularis* sp. nov. could have been easily confused with *P. nodicaudus*. In fact, the same could have happened to other specimens in the past, including species described by former studies that detected *P. nodicaudus* worldwide. A review of *P. nodicaudus* is therefore necessary to investigate the possible presence of pseudocryptism within the described representatives of this species.

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Supplementary files

Supp. file 1: List of valid species of the genus *Polymerurus* Remane, 1927 according to different versions in the literature. https://doi.org/10.5852/ejt.2021.746.1319.4025

Supp. file 2: Ultrafast bootstrap consensus tree based on multigene approach with 18S and 28S sequences. Highlighted branches correspond to the *Polymerurus* Remane, 1927 species sequences. Values on the branches correspond to ultrafast bootstrap branch support.
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