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Research article

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A new species of *Heterospio* (Annelida, Longosomatidae) from the Indian Ocean

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Abstract. Knowledge about the taxonomy and distribution of the monogeneric polychaete family Longosomatidae Hartman, 1944 is limited in all parts of the world. A new species of the genus *Heterospio* Ehlers, 1874 (Annelida, Longosomatidae) is described from the Indian Ocean. *Heterospio indica* sp. nov. is mainly characterised by the conspicuous flattening of the distal half of capillary chaetae located in anterior elongated segments. Several body characters of high taxonomic relevance in the genus are examined under the SEM and discussed. The 18S SSU rDNA and COI genes were sequenced and represent the first sequencing of a species of *Heterospio*, and the sequences have been deposited in GenBank.

Keywords. Polychaeta, *Heterospio indica* new species, taxonomy, SEM, DNA barcodes.

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Introduction

The family Longosomatidae Hartman, 1944 comprises a single genus, *Heterospio* Ehlers, 1874, that includes eight described species, namely *H. longissima* Ehlers, 1874, *H. longissima* Ehlers *sensu* Hartman 1965, *H. catalinensis* Hartman, 1944, *H. mediterranea* Laubier, Picard & Ramos, 1973, *H. reducta* Laubier, Picard & Ramos, 1973, *H. peruana* Borowski, 1994, *H. sinica* Wu & Chen, 1996 and *H. angolana* Bochert & Zettler, 2009 (see Parapar *et al.* 2014), as well as two described, but still

Table 1. Details of sampling locations off the west coast of India where specimens of *Heterospio indica* sp. nov. were found.

Area	Study period	Station code (sampling date)	Location	Depth (m)	Sediment
Malvan	Premonsoon	M2 (2 Apr. 2013)	16°02'41.7" N, 73°26'16.8" E	15	Clayey silt
	Postmonsoon	M4 (29 Jan. 2014)	16°01'37.1" N, 73°25'29.7" E	20	Clayey silt
	Monsoon	M4 (22 Sep. 2014)			
	Premonsoon	M3 (12 Mar. 2015)	16°02'23.9" N, 73°26'39.4" E	15	Clayey silt
Ratnagiri	Premonsoon	R1 (7 Apr. 2013)	17°00'27.7" N, 73°16'30.0" E	8	Silty sand
		R3 (8 Apr. 2013)	17°00'14.5" N, 73°15'03.0" E	16	Sandy silt
		R4 (8 Apr. 2013)	17°00'09.1" N, 73°13'50.6" E	20	Clayey silt
	Postmonsoon	R4 (3 Feb. 2014)	17°00'09.1" N, 73°13'50.6" E	20	Clayey silt
	Monsoon	R4 (27 Sep. 2014)			
Mumbai	Premonsoon	MY2 (15 Mar. 2013)	18°51'19.0" N, 72°50'37.6" E	10	Clayey silt
	Monsoon	MY4 (24 Sep. 2014)	18°49'36.7" N, 72°47'34.1" E	18	Sandy silt
Veraval	Premonsoon	V4 (19 Mar. 2013)	20°51'03.4" N, 70°22'36.3" E	22	Sandy silt
	Monsoon	V2 (21 Sep. 2014)	20°53'59.9" N, 70°22'28.8" E	2.5	Sandy silt

unnamed species (*Heterospio* sp. *A sensu* Uebelacker 1984; *Heterospio* sp. 1 *sensu* Borowski 1994) and one undescribed (Knox 1960). Members of this family are mostly reported from muddy sediments on continental shelves and slopes worldwide (Wilson 2000). According to Rouse (2001) their elongated body is characterized by an anterior region composed by 7–9 short segments and provided with 3–8 pairs of branchial filaments, a post-branchial region composed of elongate segments and a bulbous posterior region with terminal pygidium. The length of the first elongated segments, number of pairs of branchiae and chaetal types are the main taxonomic characters used to differentiate between species (e.g., Wu & Chen 1966; Laubier *et al.* 1973; Wilson 2000; Bochert & Zettler 2009; Parapar *et al.* 2014).

The type species of the genus, *Heterospio longissima* Ehlers, 1874, was described from specimens collected off the coast of Ireland, NE Atlantic Ocean (Ehlers 1874). This species was subsequently reported in numerous localities in the same ocean and in the Sea of Japan (see fig. 8 in Parapar *et al.* 2014). In the Indian Ocean the only records of the genus correspond to one specimen of *H. longissima* off the coast of Sudan, Red Sea (Rosenfeldt 1989; Türkay 1996; Wehe & Fiege 2002), and two specimens off the Indian and Pakistani coast of the Arabian Sea (Hartman 1974; Wehe & Fiege 2002; Rao 2005; Kazmi & Naushaba 2013) (Fig. 1).

During a benthic investigation in shallow waters off the west coast of India, several specimens of the genus *Heterospio* were collected; they belong to a new species which is described herein, both morphologically (including SEM study) and genetically (including 18S SSU rDNA and COI sequences); information about its habitat is also provided. The distribution of the genus in nearby areas of the Indian Ocean is also reviewed.

Table 2. List of PCR and sequencing primers.

Gene	Primer		Reference
18S	18SA	AYCTGGTTGATCCTGCCAGT	Medlin <i>et al.</i> (1988)
	584R	ACGCTATTGGAGCTGGAAT	Persson (pers. comm.); Norlinder <i>et al.</i> (2012)
COI	HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> (1994)
	LCO 1490	GGTCAACAAATCATAAAGATATTGG	

Material and methods

This study is based on material collected by several sampling campaigns off the central west coast of India. Specimens were collected in different seasons across subtidal areas off Malvan, Ratnagiri, Mumbai and Veraval during 2013–2015 (Table 1). Samples were taken with a van Veen grab with a 0.04 m² sampling area. Most of the specimens were fixed in 5% formaldehyde in seawater solution, and then preserved in 70% ethanol for morphological identification. Some specimens were put directly into 96% ethanol for molecular studies. Samples were sorted at the CSIR-National Institute of Oceanography (CSIR-NIO), Regional Centre, Mumbai, India. All studied specimens were deposited in the collections of the Museo Nacional de Ciencias Naturales de Madrid, Spain (MNCN), Senckenberg Research Institute and Natural History Museum, Frankfurt (SMF) and CSIR-National Institute of Oceanography (CSIR-NIO), Regional Centre, Mumbai.

Observations, drawings and measurements of specimens were made with an Olympus BX51 compound microscope equipped with a drawing tube. Specimens were stained with methylene blue for examination of body and parapodia under the light microscope. For examination with scanning electron microscopy (SEM), specimens were dehydrated in a graded ethanol series, critical-point dried using CO₂, mounted on aluminium stubs, covered with gold in a BAL-TEC SCD 004 evaporator, and examined and photographed under a JEOL JSM-6400 scanning electron microscope at the Servicios de Apoyo á Investigación (SAI), University of A Coruña (UDC), Spain.

The description is based on the holotype; features of the posterior end of the body and chaetal fine structure (SEM micrographs) are from some paratypes. Intraspecific variation is also reported whenever recorded (e.g., segment size, branchiae arrangement). For the delimitation of body segments we follow Parapar *et al.* (2014); these authors propose that chaetae are located on the anterior border of segments and therefore the length of a segment should be considered as the distance from the chaetal bundle (or ring) to the chaetal bundle of the next chaetiger. For comparative purposes, one specimen collected by Rosenfeldt (1989) off the Sudanese coast in the Red Sea was also studied.

Molecular analysis was carried out at CSIR-NIO, Regional Centre, Mumbai. Genomic DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen), as per manufacturer protocol. 18S SSU rDNA was amplified using primers 18SA and 584R resulting in 540–545 bp sequence-lengths. 687 bp of COI was amplified with HCO2198 and LCO1490 primers (for primer references see Table 2). PCR mixtures for both genes contained 5 µl of Qiagen PCR buffer, 1 µl dNTPs, 38.8 µl ddH₂O, 2 µl of each forward and reverse primer (10 mM), 1 µl template DNA and 0.2 µl Taq polymerase, making a total volume of 50 µl. Amplifications were carried under the following thermal conditions: 94°C for 3.0 min, 30 cycles of 94°C for 1 min, 48°C (COI) or 50°C (18S) for 1 min and 72°C for 2 mins. A final elongation at 72°C was carried out for 10 mins followed by cooling the reaction at 4°C. Amplified products were tested on a 0.8% agarose gel. PCR products were purified with the PCR Purification kit (Qiagen) and sequenced bidirectionally by the ABi 3730XL Genetic Analyzer: 96 capillary sequencer. The final sequences have been deposited in Genbank.

Results

Phylum Annelida Lamarck, 1809
Family Longosomatidae Hartman, 1944

Genus *Heterospio* Ehlers, 1874

Type species

Heterospio longissima Ehlers, 1874.

Diagnosis (modified from Borowski 1994)

Body elongated, linear, divided in three regions. Anterior region with 6–8 short chaetigers; median region with greatly elongated segments; posterior region with few short, inflated segments. Posterior part of prostomium with a pair of lateral nuchal organs. Proboscis eversible, epithelial pouch. One pair of grooved peristomial palps, easily lost, leaving scars on lateral surfaces. Anterior region with 3–8 pairs of cirriform branchiae, usually very long, one pair per segment, arising above notopodia, beginning from chaetiger 2. Anterior region with biramous parapodia provided with simple capillaries, with or without acicular spines; elongated segments with chaetae usually forming a cincture near anterior margin; chaetae simple capillaries, may be accompanied by subuluncini, aristae chaetae, and acicular spines; posteriormost inflated chaetigers provided with strong acicular hooks.

Remarks

Following Parapar *et al.* (2014), the terms “thorax” and “abdomen” are not used in the diagnosis due to the lack of a clear criterion about their limits. The presence of nuchal organs on the prostomium was included in the diagnosis. The range of 7–9 short anterior chaetigers provided by Borowski (1994) is modified to 6–8, following Parapar *et al.* (2014). Peristomial palps were qualified as “easily deciduous” because they are not reported in most descriptions. More details are provided about the number (range) and size of branchiae. The term “parapodia biramous” is used here only for anterior segments; it is also highlighted that in elongated segments there may be a change in the arrangement of chaetae, i.e., forming cinctures, although those seem not be present in *H. longissima* Ehlers, 1874 *sensu stricto*, *H. reducta* Laubier, Picard & Ramos, 1973 or *H. angolana* Bochert & Zettler, 2009.

Heterospio indica sp. nov.

[urn:lsid:zoobank.org:act:8C7B939E-777E-44C6-80F5-1877A49539A4](https://doi.org/10.21203/rs.3.rs-1000000)

Figs 1–9; Tables 1–2

Heterospio longissima – Rosenfeldt 1989: 231, fig. 7 (not Ehlers, 1874).

?*Heterospio longissima* – Hartman 1974: 232 (?not Ehlers, 1874).

Diagnosis

Eight anterior chaetigers short; chaetiger 9 (CH9) first elongated segment. Eight pairs of branchiae in fully developed individuals. Chaetae forming cinctures from CH10, provided with both thin and robust capillary chaetae arranged in two rows; both types of chaetae highly flattened in distal half; from CH14 robust capillary chaetae without fine distal end, subuluncini-like, thicker than on previous chaetigers. Aristate chaetae and acicular hooks not observed on elongated segments.

Etymology

The species is named after the Indian Ocean, where the specimens of the type series were collected.

Type material

Twenty two incomplete specimens and one posterior end were collected in 13 samples along the West coast of India (Table 1; Fig. 1).

Holotype

INDIA: Malvan, postmonsoon, M4, 29 Jan. 2014, MNCN 16.01/16996.

Paratypes

INDIA: 1 spec., Malvan, premonsoon, M2, MNCN 16.01/16997; 2 spec., Ratnagiri, premonsoon, R3, NIO/MUM/4/2013/1; 3 spec., Ratnagiri, premonsoon, R4, NIO/MUM/4/2013/2; 1 spec., Mumbai, premonsoon, MY2, NIO/MUM/3/2013/3; 1 spec., Veraval, premonsoon, V4, NIO/MUM/3/2013/4; 1 posterior end, Malvan, premonsoon, M3, MNCN 16.01/16998; 3 spec., Ratnagiri, premonsoon, R1, MNCN 16.01/16999; 3 spec. on SEM stub, Ratnagiri, premonsoon, R1, MNCN 16.01/16700; 1 spec., Ratnagiri, postmonsoon, R4, SMF 24097; 1 spec., Malvan, monsoon, M4, NIO/MUM/9/2014/5; 1 spec., Ratnagiri, monsoon, R4, NIO/MUM/9/2014/6; 1 spec., Mumbai, monsoon, MY4, NIO/MUM/9/2014/7; 1 spec., Veraval, monsoon, V2, NIO/MUM/9/2014/8; 1 spec. (sequenced), Ratnagiri, postmonsoon, R4, NIO/MUM/2/2014/R-H; 1 spec. (sequenced), Malvan, postmonsoon, M4, NIO/MUM/1/2014/M-H.

Type locality

W INDIA: Malvan, 16°01'37.1" N, 73°25'29.7" E, 20 m, clayey silt (Table 1).

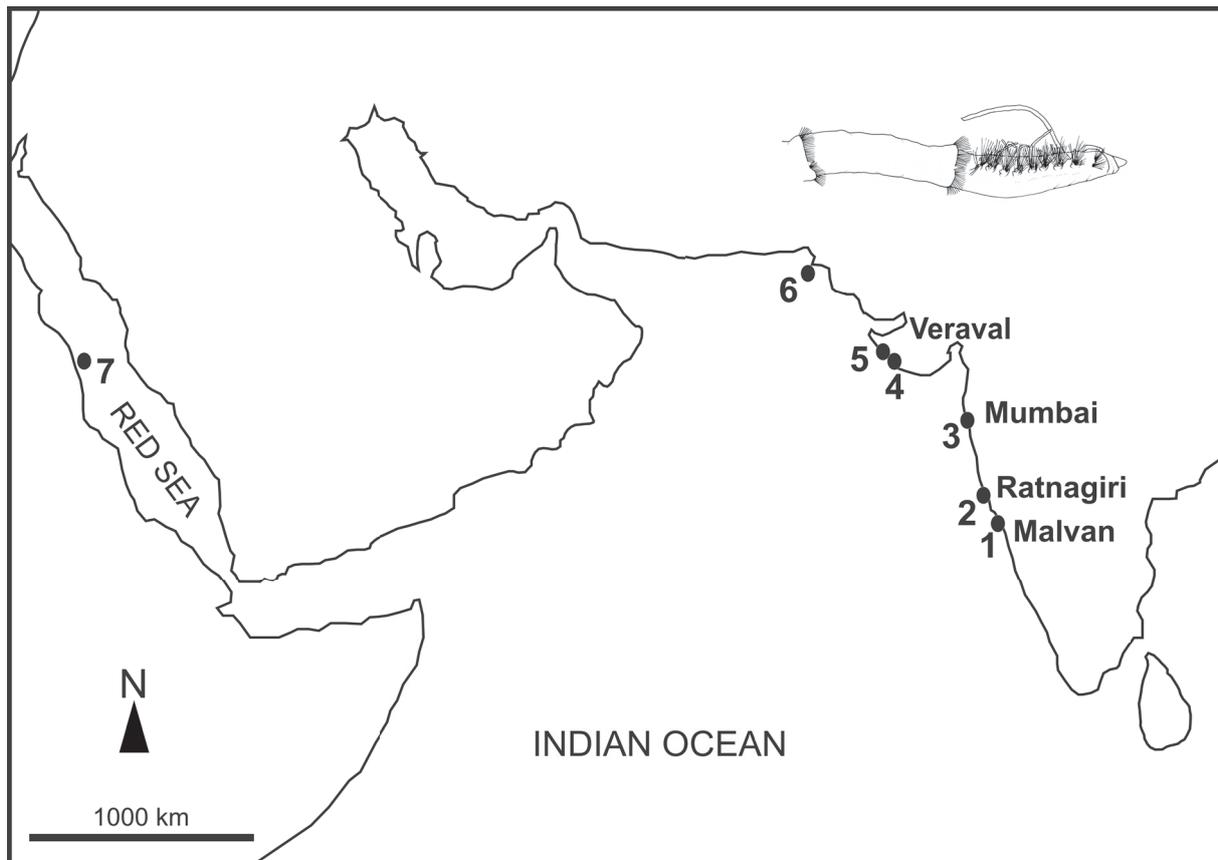


Fig. 1. Distribution of records of *Heterospio* around the Indian Ocean: *Heterospio indica* sp. nov. (present study) (1–4), *H. longissima* Ehlers, 1874 (data from Hartman 1974) (5–6) and *H. longissima* Ehlers, 1874 (data from Rosenfeldt 1989) (7).

Molecular identification

COI and 18S nucleotide sequences of *Heterospio indica* sp. nov were submitted to GenBank under the accession numbers: COI-KT259053, KU221229 and 18S-KT259051, KT259052.

Comparative material

One specimen collected by Rosenfeldt (1989) off the coast of Sudan, Red Sea (SMF 3795; labelled as *H. longissima*).

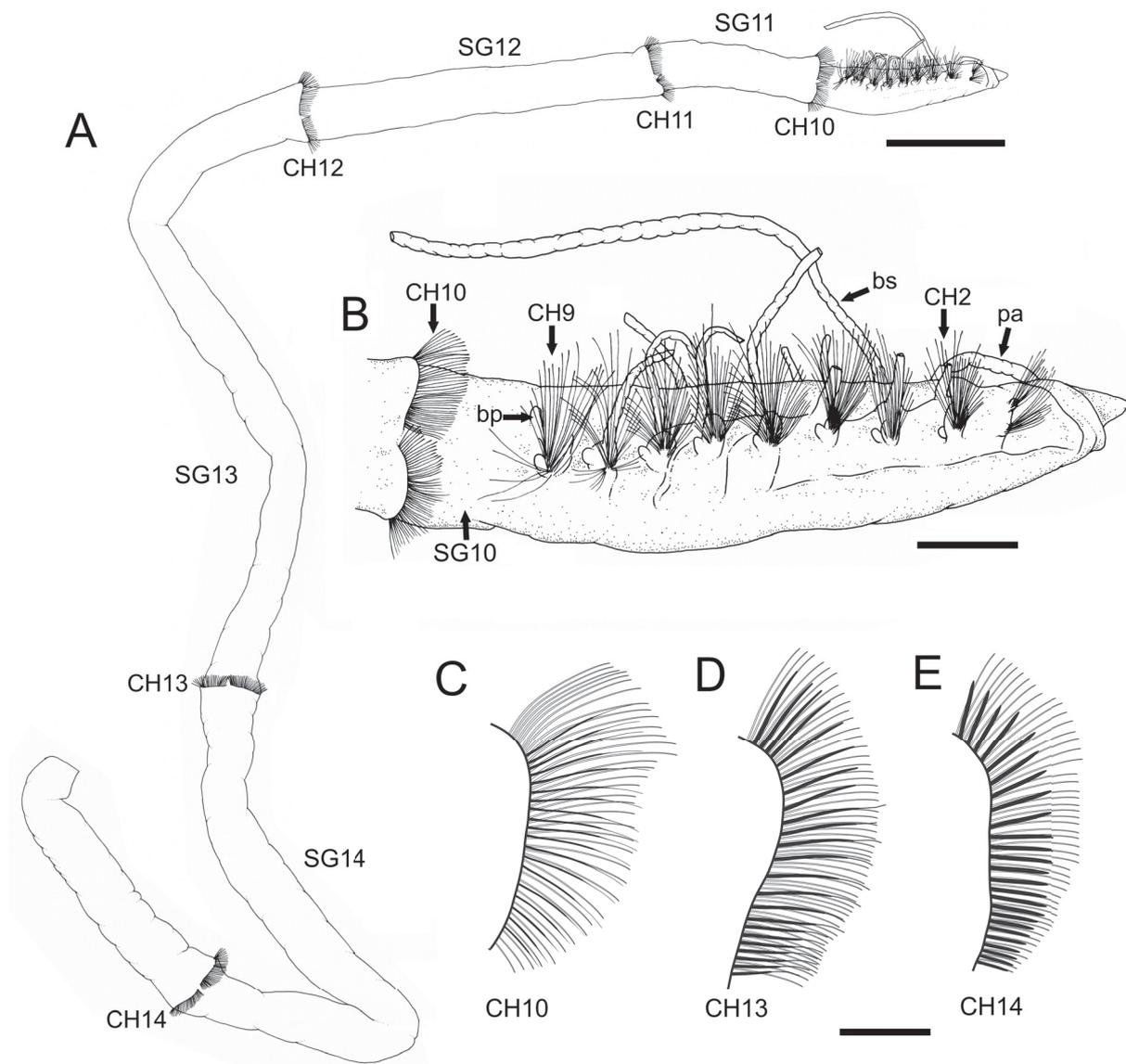


Fig. 2. *Heterospio indica* sp. nov., holotype (MNCN 16.01/16996). **A.** General view (scale bar = 2 mm). **B.** Anterior end, lateral view (scale bar = 400 µm). **C–E.** Chaetal arrangement (scale bar = 200 µm), details. **C.** CH10. **D.** CH13. **E.** CH14. Abbreviations: bp = branchiophore; bs = branchiostyle; CH = chaetiger; pa = palp; SG = segment.

Description of holotype (SEM images from paratypes)

Longest specimen available, incomplete, 39 mm long and 0.95 mm wide, with 14 chaetigers; body threadlike (Fig. 2A). Prostomium conical, anteriorly rounded (Figs 2B, 4A, 6C, 8C), slightly flattened dorso-ventrally. Eyes absent. Palp present on right side, reaching level of CH2 (Fig. 2B). Nuchal organs as deep grooves postero-lateral to prostomium, followed by deep peristomial (SG1) palp scars (Figs 4A, 6C–E). Pharynx sac-like, eversible and unarmed (Fig. 8C).

Anterior body region slightly flattened dorso-ventrally (Fig. 8B). First eight chaetigers (CH1–CH8; SG2–SG9) short, somewhat more than twice as wide as long. Chaetigers progressively longer from CH9 (SG10) onwards. CH9 first elongated chaetiger (FECH), longer than wide, about twice as long as CH8 (Fig. 2B) (length as distance from chaetal bundle to chaetal cincture of CH10). First chaetiger (CH1) abranchiate. Eight pairs of filiform branchiae from CH2 to CH9, dorsal to notopodia in almost all chaetigers and usually composed by short branchiophores and long branchiostyles (Figs 2B, 4B, 6B, F). From CH10 (SG11) segments strongly elongated and cylindrical in cross section (Figs 6A, 8A); length increasing in posterior segments; CH10 about five times longer than CH9; CH11 (SG12) about 2.5 times longer than CH10; CH12 (SG13) more than two times longer than CH11; CH13 (SG14) slightly shorter than CH12; CH14 incomplete.

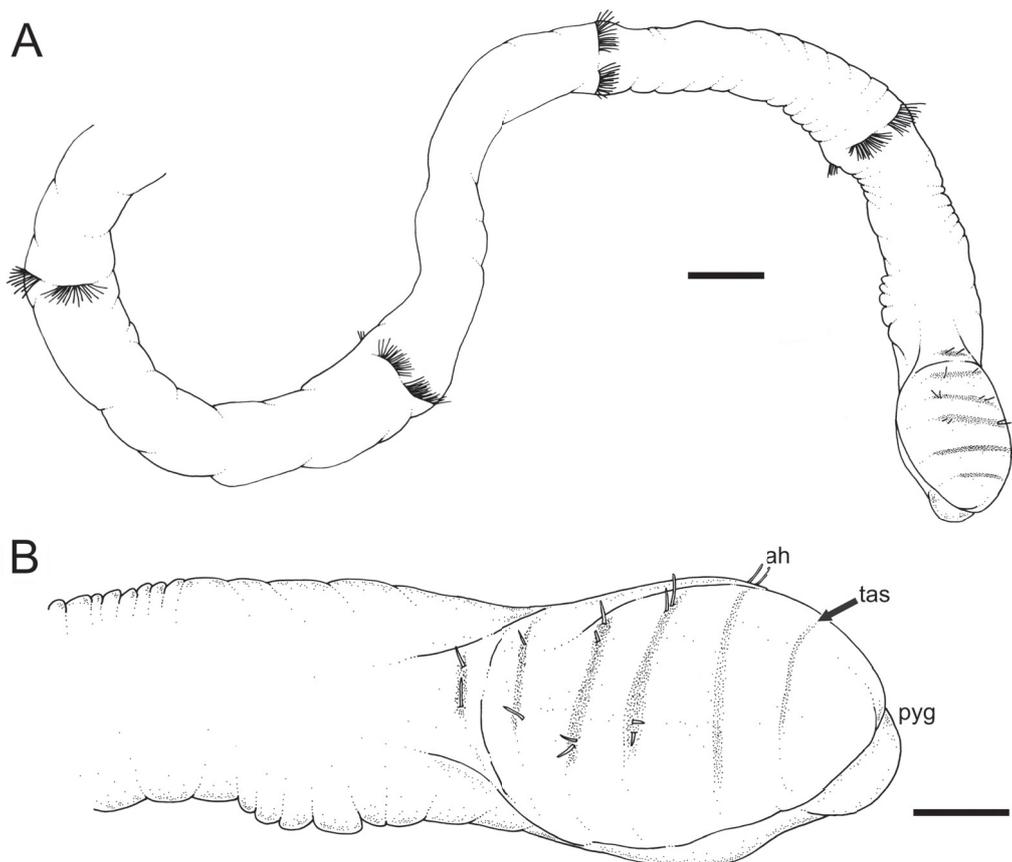


Fig. 3. *Heterospio indica* sp. nov., paratype (MNCN 16.01/16998). **A.** Posterior end (scale bar = 200 μ m). **B.** Detail of bulbous region of posterior end (scale bar = 100 μ m). Abbreviations: ah = acicular hook; pyg = pygidium; tas = terminal achaetigerous segment.

CH1–CH9 provided with biramous parapodia; noto- and neuropodial chaetal fascicles well separated (Figs 2B, 5A, D, 7A, 8D, F). Parapodia from CH10 backwards as elongated ridges forming a nearly closed flange-like cincture near anterior margin of segment (Figs 2A, 5D, 8B). Chaetae of CH1–CH9 simple capillaries, in fan-shaped fascicles (Figs 2B, 4C–D). Neuropodial hooks not observed. Chaetae of CH10 backwards shorter than those of anterior chaetigers, arranged in two rows (Figs 2C, 5B, 7C); anterior row of robust capillary chaetae and posterior row of fine capillary chaetae (Fig. 5E–F). Both types of chaetae circular in cross section at base but quickly flattening outwards and becoming much thinner at distal end (Figs 5C, F, 7B, D, 8E). Distal ends of both types of chaetae very similar in length and width, and provided with an opening at the beginning of the terminal flattening (Figs 5C, 7B, 8E). CH12–CH13 (Fig. 2D) also with some robust capillary chaetae lacking flat distal end (i.e., subuluncini-like chaetae); CH14 (Fig. 2E) with all robust capillary chaetae of subuluncini-like type. No aristate chaetae or acicular hooks observed.

For a description of the posterior end, see below.

Fixed specimens creamy white in colour.

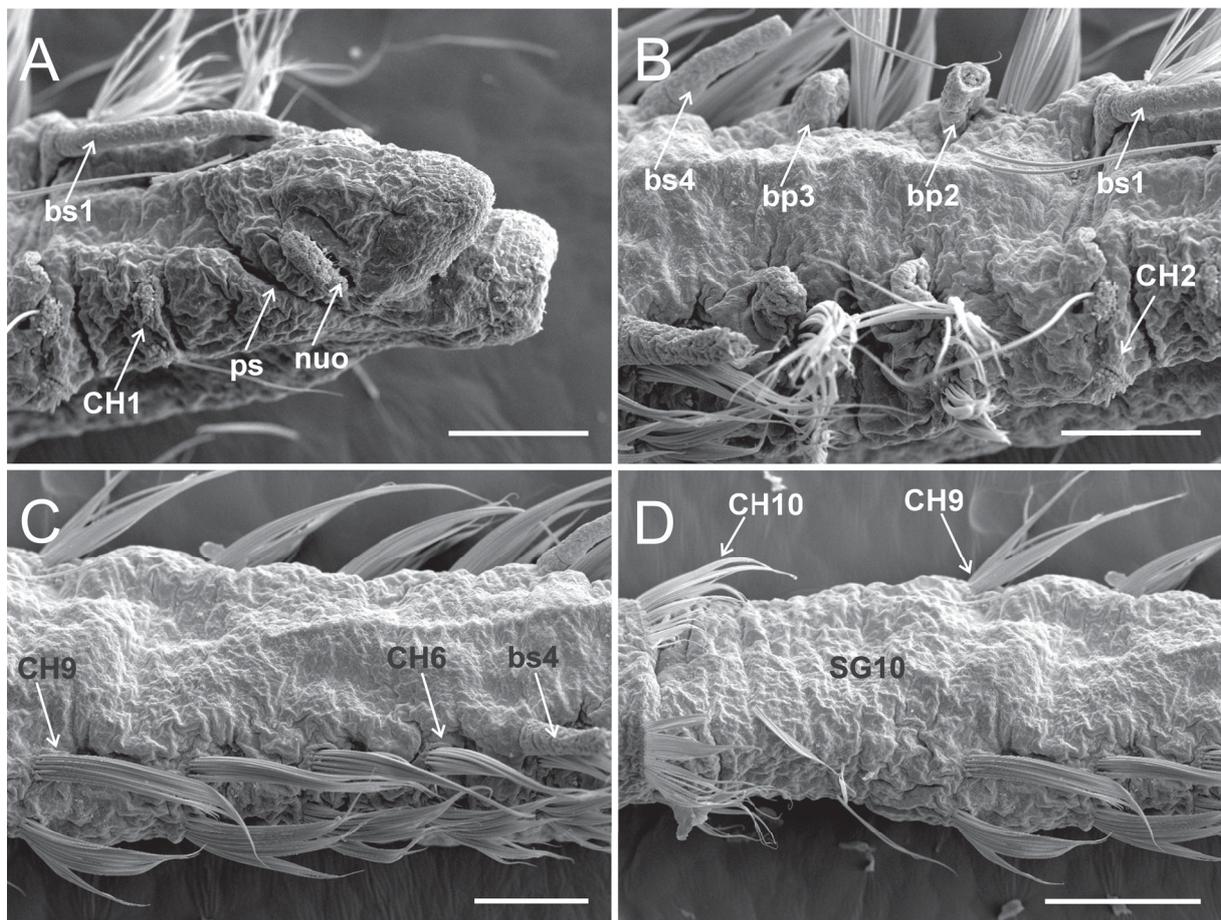


Fig. 4. *Heterospio indica* sp. nov., paratype (MNCN 16.01/17000), SEM micrographs. **A.** Anterior end, dorsolateral view (scale bar = 100 μ m). **B.** CH2–CH5, dorsal view (scale bar = 100 μ m). **C.** CH5–CH9, dorsal view (scale bar = 100 μ m). **D.** CH8–CH10, dorsal view (scale bar = 15 μ m). Abbreviations: bp = branchiophore; bs = branchiostyle; CH = chaetiger; nuo = nuchal organ; ps = palp scar; SG = segment.

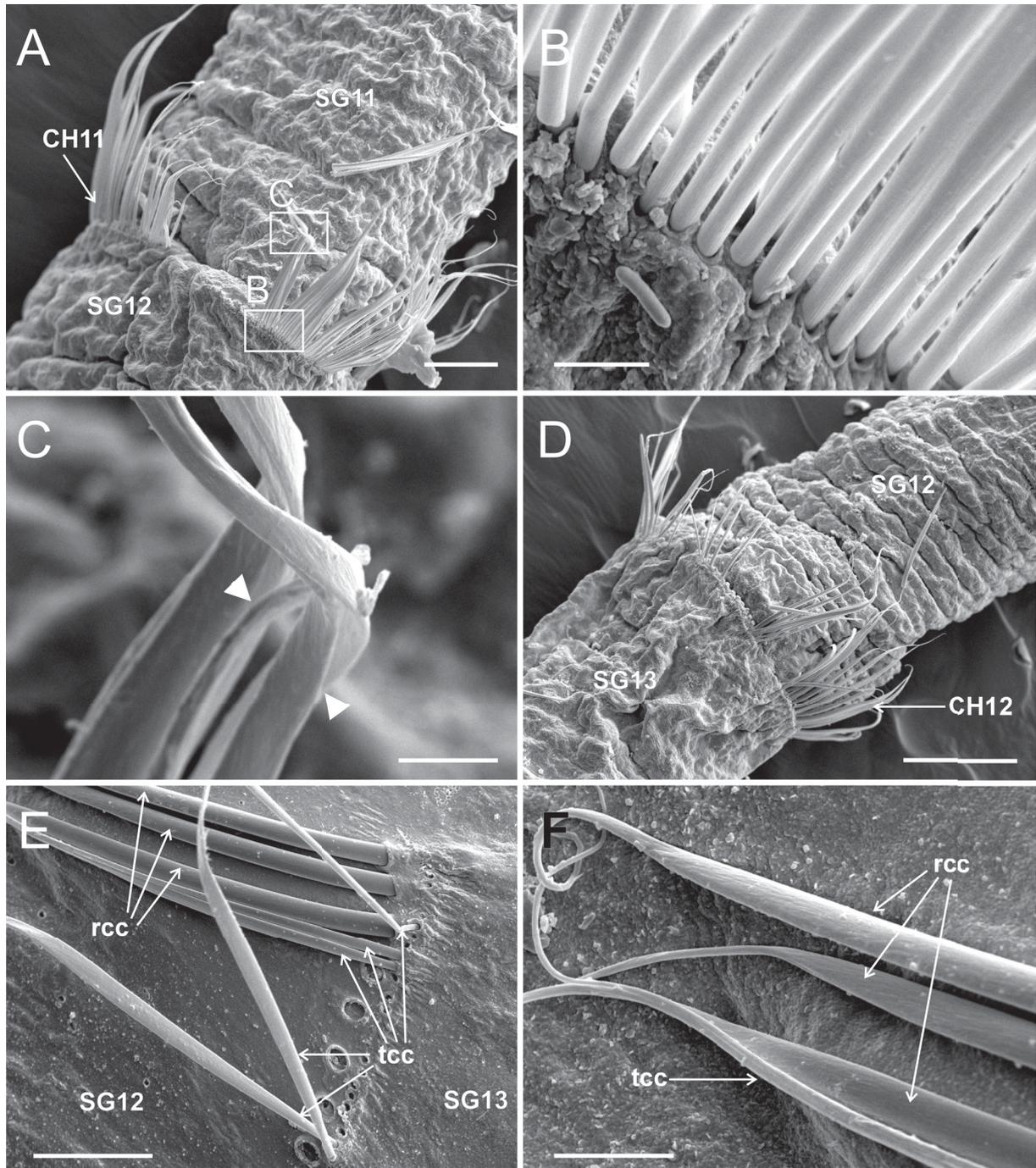


Fig. 5. *Heterospio indica* sp. nov., paratype (MNCN 16.01/17000), SEM micrographs. **A.** SG11–SG12, dorsal view, framed areas showing chaetal details in (B) and (C) (scale bar = 50 μ m). **B.** Basal part of CH11 chaetae (scale bar = 5 μ m). **C.** Distal part of CH11 capillary chaetae, arrowheads showing start of distal opening (scale bar = 5 μ m). **D.** SG12–SG13, dorsal view (scale bar = 100 μ m). **E.** Basal part of CH12 chaetae (scale bar = 25 μ m). **F.** Distal part of CH12 chaetae (scale bar = 10 μ m). Abbreviations: CH = chaetiger; rcc = robust capillary chaetae; SG = segment; tcc = thin capillary chaetae.

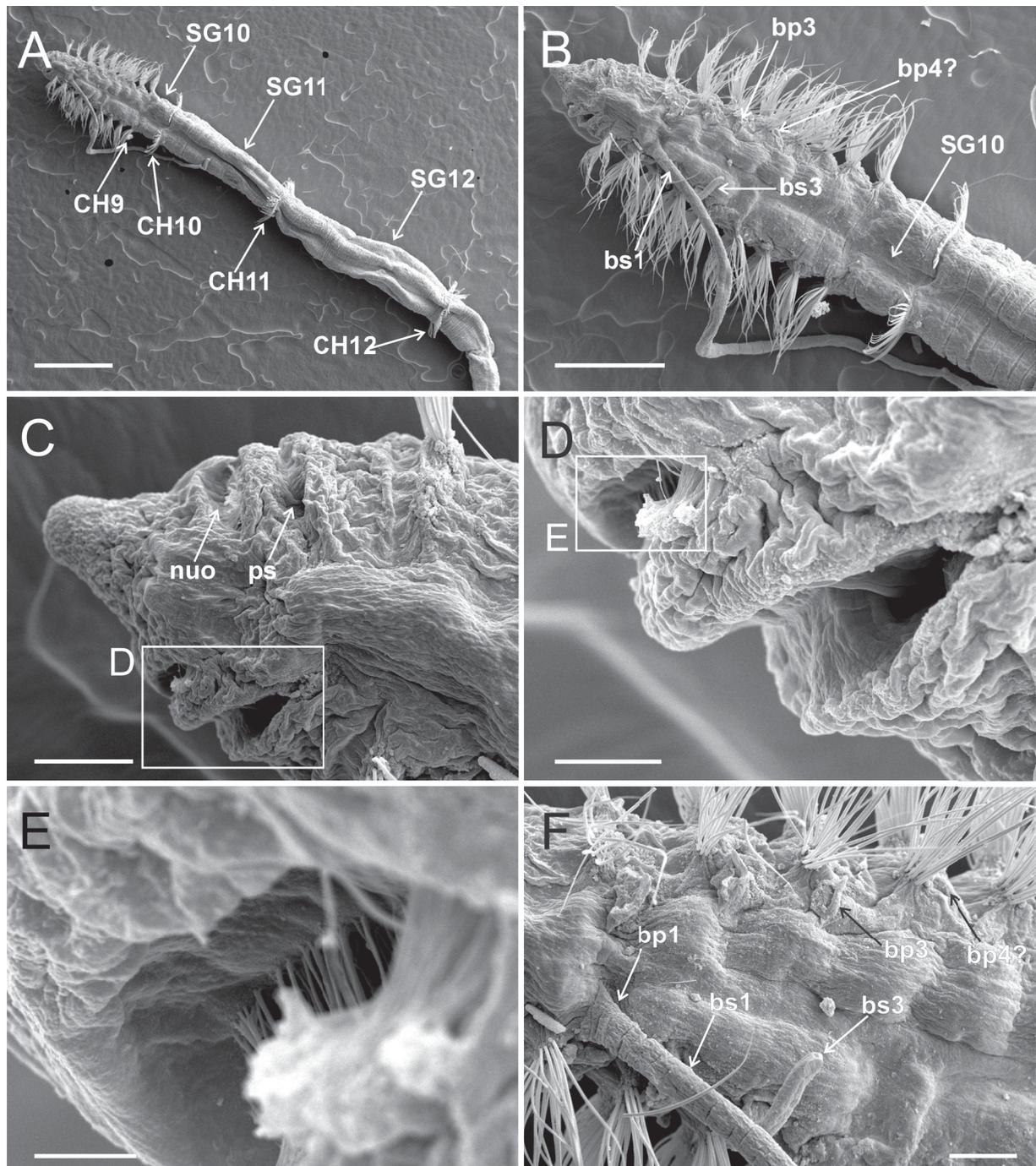


Fig. 6. *Heterospio indica* sp. nov., paratype (MNCN 16.01/17000), SEM micrographs. **A.** Anterior end, dorsal view (scale bar = 500 μ m). **B.** Detail of anterior short segments (SG1–SG9) and first elongated segment (SG10) (scale bar = 250 μ m). **C.** Anterior end, dorsal view, showing nuchal organ and palp scars, framed area shown in (D) (scale bar = 50 μ m). **D.** Detail of nuchal organ and palp scar, framed area shown in (E) (scale bar = 20 μ m). **E.** Detail of nuchal organ opening (scale bar = 5 μ m). **F.** Detail of branchial area, dorsal view (scale bar = 50 μ m). Abbreviations: bp = branchiophore; bs = branchiostyle; CH = chaetiger; nuo = nuchal organ; ps = palp scar; SG = segment.

Variations

The first body segments show a certain degree of variation for two relevant taxonomic characters: relative size of first elongated segments and number/size of branchiae. The size of the first elongated segment (CH9) seems constant, about 2.5–3 longer than any short anterior segment (CH1–CH8); the relative size of following elongated segments is somewhat variable: CH10 is slightly shorter or longer than the anterior body region (i.e., from tip of prostomium to posterior end of CH9). This variability increases for CH11, which is longer than CH10 and may be longer than all preceding segments and prostomium together (cf. Fig. 6A vs 8A). Variability in sizes might be dependent on the degree of contraction of the specimen, but it may still be used as a taxonomic character (see Parapar *et al.* 2014); however, relative sizes of segments should be reviewed across the genus, because this character has not been properly assessed in descriptions of other species. On the contrary, variability in number of branchiae is likely to be size-dependent. Specimens show a wide range of number and sizes of branchiae that seem to be correlated with the size of individuals (i.e., width at level of anterior non-elongated segments; see Fig. 9). The presence and number of branchial pairs increases from anterior to posterior segments; thus, small specimens (<0.4 mm wide) bear four pairs while larger individuals (0.7–0.95 mm)

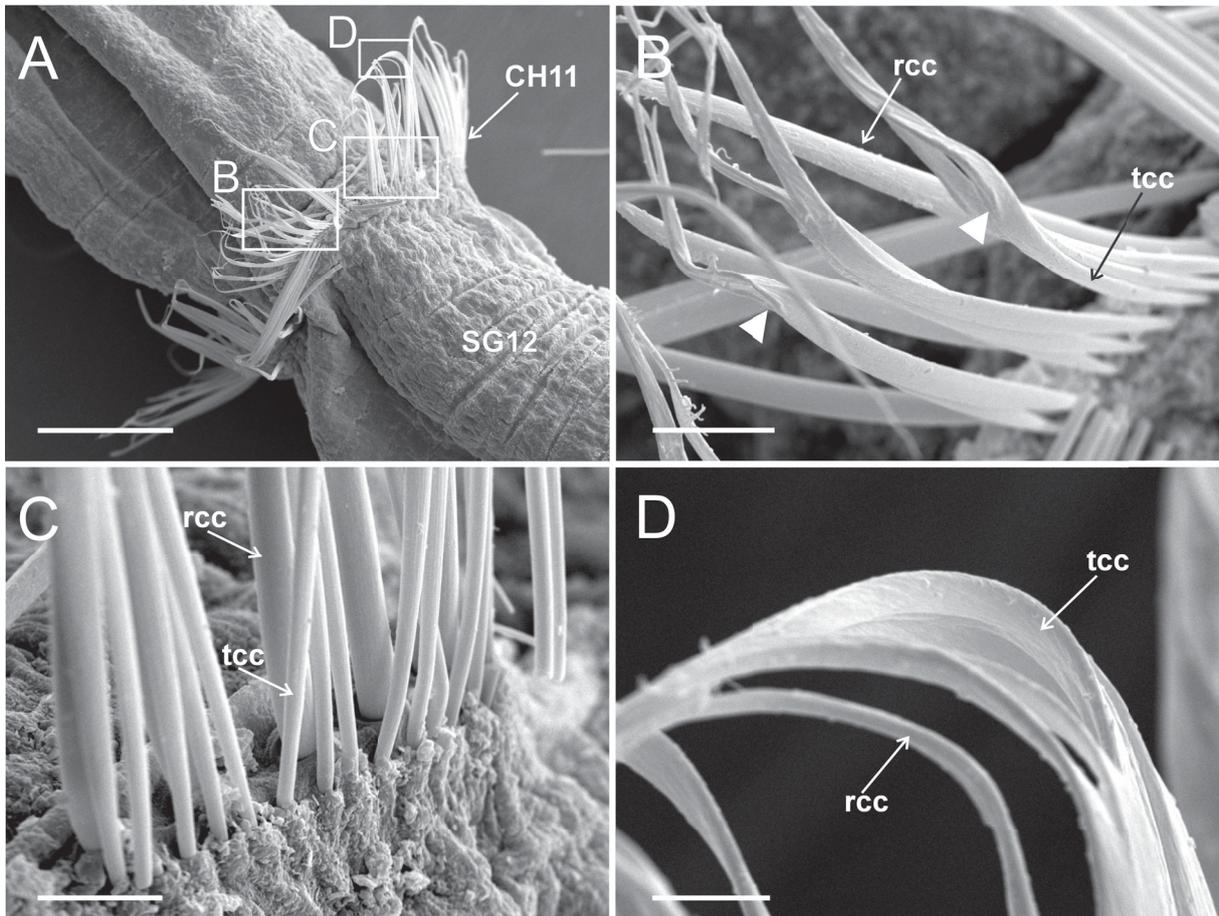


Fig. 7. *Heterospio indica* sp. nov., paratype (MNCN 16.01/17000), SEM micrographs. **A.** Elongated SG12, CH11 and flange-like neuropod, framed areas showing chaetal details in (B), (C) and (D) (scale bar = 100 µm). **B.** Detail of CH11 chaetae, arrowheads showing start of opening end in thin capillary chaetae (scale bar = 10 µm). **C.** Detail of proximal part of both types of CH11 capillary chaetae, anterior body end at left (scale bar = 10 µm). **D.** Detail of distal part of capillary chaetae (scale bar = 5 µm). Abbreviations: CH = chaetiger; rcc = robust capillary chaetae; SG = segment; tcc = thin capillary chaetae.

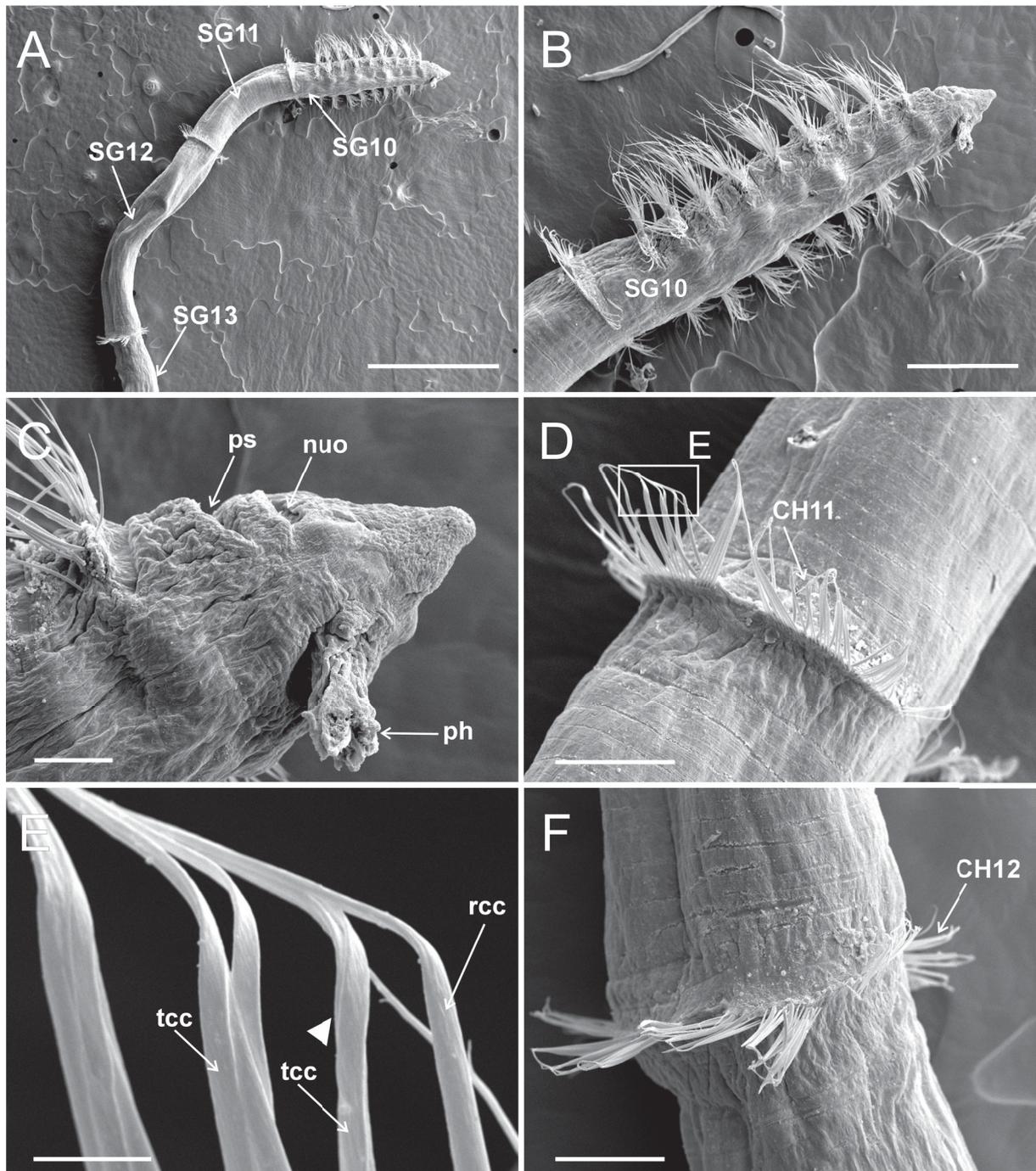


Fig. 8. *Heterospio indica* sp. nov., paratype (MNCN 16.01/17000), SEM micrographs. **A.** Specimen showing anterior short segments and first elongated segments, ventro-lateral view (scale bar = 1 mm). **B.** Detail of anterior region, ventro-lateral view (scale bar = 300 μ m). **C.** Detail of anterior end, ventral view (scale bar = 50 μ m). **D.** Flange-like chaetae of CH11, framed area showing chaetal detail in (E) (scale bar = 100 μ m). **E.** Detail of distal end of both types of capillary chaetae, arrowheads showing start of opening end in thin capillary chaetae (scale bar = 10 μ m). **F.** Flange-like chaetae of CH12 (scale bar = 100 μ m). Abbreviations: CH = chaetiger; nuo = nuchal organ; ph = pharynx; ps = palp scar; rcc = robust capillary chaetae; SG = segment; tcc = thin capillary chaetae.

show up to 7–8 pairs. The presence and size of branchiophores and branchiostyles also shows certain variability, e.g., in the same specimen there are large and thin branchiostyles indistinctly on large and small branchiophores.

All specimens are broken at the level of the anterior elongated segments (CH12 to CH15) but a posterior region could be examined from a posterior end found in a Malvan sample in March 2015. Only one posterior region was found, composed of 7 elongated segments and a bulb-like inflated posterior end provided with 5 chaetigers, a terminal achaetigerous segment and pygidium (Fig. 3A). The last 5 chaetigers are provided with 2–4 acicular hooks (Fig. 3B); the anteriormost elongated chaetiger bears chaetae similar to those of CH10 of the holotype, with flattened distal ends; the chaetae of the other four elongated chaetigers are similar to those of CH12–14 of the holotype, i.e., of capillary and subuluncini type.

Specimen from Sudan

The specimen collected by Rosenfeldt (1989) off the coast of Sudan, which was identified as *H. longissima* (SMF 3795), has also been examined. It is in poor condition, i.e., incomplete, broken in two pieces,

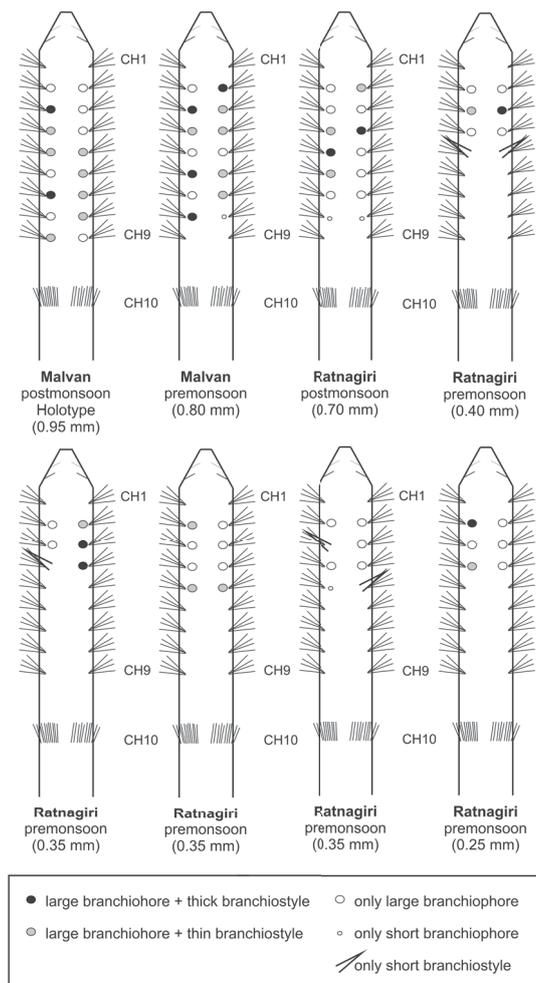


Fig. 9. Schematic drawings (not to scale) in dorsal view of some paratypes (MNCN and NIO) of *Heterospio indica* sp. nov., showing diversity of branchial arrangement in relation to size. Chaetigers numbered from CH1 (biramous) to CH10 (first flange-like). Width of anterior region indicated.

twisted and flattened dorsoventrally. According to its width (0.6 mm), this specimen fits within the range of large *H. indica* sp. nov. and it bears eight pairs of branchiae. Rosenfeldt (1989) highlighted the presence of palps which are now lacking. However, the relevant features of this specimen agree well with the description provided above of *H. indica* sp. nov.

Distribution and ecology

Heterospio indica sp. nov. was found off the west coast of India (Fig. 1) in shallow water (2.5 to 22 m depth) in mostly clayey silt and sandy silt sediments (Table 1). Hartman (1974) reported *H. longissima* based on two specimens from off the NW coast of India and Pakistan. In this article, Hartman (1974) only mentioned the papers by Ehlers (1895) and Hartman (1965) and did not provide any comparison with *H. mediterranea* or *H. reducta*, the two species described earlier by Laubier *et al.* (1972-73) from the Mediterranean Sea. Rosenfeldt (1989) reported *H. longissima* from Sudan (see above) based on a single specimen. She illustrated chaetae of the second elongated segment but did not provide any comparison with other species of *Heterospio*. Türkay (1996), Wehe & Fiege (2002), Rao (2005) and Kazmi & Naushaba (2013) mentioned these two records without any discussion of the identification of the reported material. We did not examine the material reported by Hartman (1974) but assume that it may belong to *H. indica* sp. nov. because of its geographic proximity to our material. The finding of the specimen originally reported as *H. longissima* by Rosenfeldt (1989) and identified here as *H. indica* sp. nov. (see above) extends the distribution of the new species to the Red Sea.

Key to world species of *Heterospio*

The key presented below is based on the provided by Bochert & Zettler (2009) but here the position and relative length of the first elongated chaetiger (FECH) and the number of pairs of branchiae have prevalence over chaetal types. The position of FECH in the species follows Parapar *et al.* (2014).

- | | | |
|----|--|--|
| 1 | FECH is CH9 | 2 |
| – | FECH is CH7 or CH8 | 7 |
| 2. | Acicular chaetae in neuropodium of CH1 | <i>H. catalinensis</i> Hartman, 1944 |
| – | No acicular chaetae in neuropodium of CH1 | 3 |
| 3. | Eight pairs of branchiae in adults | 4 |
| – | Four pairs of branchiae in adults..... | <i>H. peruana</i> Borowski, 1994 |
| 4. | FECH of almost same length as all anterior chaetigers together | <i>H. longissima</i> Ehlers, 1874 |
| – | FECH clearly shorter than all anterior chaetigers together | 5 |
| 5. | Capillary chaetae of elongated segments (ES) with flattened distal end | <i>H. indica</i> sp. nov. |
| – | Capillary chaetae of ES not flattened | 6 |
| 6. | Aristate chaetae present on elongated chaetigers | <i>H. sinica</i> Wu & Chen, 1996 |
| – | Aristate chaetae absent on elongated chaetigers | <i>H. longissima sensu</i> Hartman 1965 |
| 7. | FECH is CH 8 | 8 |
| – | FECH is CH7 | 9 |
| 8. | Five (or six) pairs of branchiae | <i>Heterospio</i> sp. A <i>sensu</i> Uebelacker 1984 |
| – | Seven pairs of branchiae | <i>H. mediterranea</i> Laubier, Picard & Ramos, 1973 |
| 9. | One pair of branchiae | <i>Heterospio</i> sp. 1 <i>sensu</i> Borowski 1994 |
| – | Three pairs of branchiae | 10 |

10. Length of CH8 about ½ that of all anterior chaetigers together; very long chaetae in CH8
 *H. reducta* Laubier, Picard & Ramos, 1973
 – Length of CH8 about ⅓ that of all anterior chaetigers together; chaetae in CH8 similar to preceding
 *H. angolana* Bochert & Zettler, 2009

Remarks

According to the relative size of anterior segments, *Heterospio indica* sp. nov. most closely resembles *H. longissima* (both the nominal species and *H. longissima sensu* Hartman 1965), *H. catalinensis*, *H. peruana* and *H. sinica*. In these species, the first elongated chaetiger is CH9 while in *H. mediterranea*, *H. reducta*, *H. angolana*, *Heterospio* sp. A *sensu* Uebelacker 1984 and *Heterospio* sp. 1 *sensu* Borowski 1994, it corresponds to either CH7 or CH8 (see figs 5 and 6 in Parapar *et al.* 2014). In *Heterospio indica* sp. nov., *H. longissima sensu* Hartman 1965, *H. peruana* and *H. sinica*, CH9 is about three times longer than any anterior segment (CH1–CH8). By contrast, CH9 is about as long as all anterior segments altogether in *H. longissima* Ehlers, 1874; *H. catalinensis* has a CH9 which is slightly longer than CH1–CH8 and bears acicular chaetae in the neuropodium of CH1. *Heterospio peruana* only bears four pairs of branchiae while *H. indica* sp. nov., *H. longissima sensu* Hartman 1965 and *H. sinica* bear eight pairs instead.

The flattened distal end of the capillary chaetae in the elongated segments in *H. indica* sp. nov. clearly distinguishes the new species from *H. longissima sensu* Hartman 1965 and *H. sinica*; in the former, both type of capillary chaetae are greatly different in size and shape while *H. sinica* bears aristate chaetae which are not found in the new species.

The COI and 18S SSU rDNA sequences from *H. indica* sp. nov. are publically available on GenBank and can be used as a reference for future molecular taxonomic and phylogenetic studies. They are the first DNA sequences published for any species of Longosomatidae.

Discussion

The main taxonomic characters of the genus *Heterospio* are the number and length of anterior elongated segments, the presence or absence of palps and/or nuchal organs, the number of pairs of branchiae, and chaetal morphology (see Parapar *et al.* 2014). The study of specimens of *H. indica* sp. nov., however, allowed us to amend some comments given by Parapar *et al.* (2014). For instance, the presence of palps in *Heterospio* has been described and/or illustrated in several previous works (e.g., Hartman 1965; Wu & Chen 1966; Laubier *et al.* 1973; Borowski 1994); other authors, however, questioned their very presence (Uebelacker 1984; Bochert & Zettler 2009; Parapar *et al.* 2014) by interpreting the deep grooves behind the prostomium as nuchal organs and therefore not as palp scars. Examination of specimens of *H. indica* sp. nov. has clearly demonstrated that both palps and nuchal organs are indeed present.

As was previously proposed by Parapar *et al.* (2014), we consider that the number of short anterior segments and the relative length of the first elongated segments are two key characters in longosomatid taxonomy; nevertheless, they should be evaluated carefully because some variation was observed (see above).

Chaetal terminology in Longosomatidae is surprisingly variable given the small number of described species. The following terminology has been used for chaetae: aristate, capillary, stout capillary, subuluncini, transitional subuluncini capillary-like, spines and acicular hooks. A review of this nomenclature, in the frame of an eventual revision of the genus, seems necessary since some types might simply be transitional stages of the same type of chaetae, as has already been discussed by Borowski (1994).

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