Morphometric and taxonomic approach to describe *Heterospio variabilis* (Annelida, Longosomatidae), a new species with three size-dependent morphotypes, from the Gulf of California, Eastern Pacific (#91796)

First submission

Guidance from your Editor

Please submit by **21 Nov 2023** for the benefit of the authors (and your token reward) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Author notes
Have you read the author notes on the guidance page?

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files

Download and review all files from the materials page.

12 Figure file(s)
2 Table file(s)
1 Other file(s)

Custom checks

New species checks

- Have you checked our new species policies?
- Do you agree that it is a new species?
- Is it correctly described e.g. meets ICZN standard?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Morphometric and taxonomic approach to describe *Heterospio variabilis* (Annelida, Longosomatidae), a new species with three size-dependent morphotypes, from the Gulf of California, Eastern Pacific

Pablo Hernández Alcántara [1] , **Vivianne Solis-Weiss** [Corresp. 2]

[1] Departmento de Ecología Acuática y Biodiversidad., Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autonoma de México (UNAM), Mexico city, Tlalpan, D.F., Mexico
[2] Departmento de Sistemas Arrecifales, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Puerto Morelos, Quintana Roo, Mexico

Corresponding Author: Vivianne Solis-Weiss
Email address: solisw@cmarl.unam.mx

The Longosomatidae, a poorly known polychaete family, includes only 23 recognized species; in this study, based on morphometric and taxonomic analyses, we describe a new species with three morphotypes: *Heterospio variabilis* from the Gulf of California, Mexico. The specimens examined exhibit large morphological variations but were clearly separated from close species due to a unique combination of morphological characters: chaetiger 9 as the first elongated chaetiger, 4–8 branchial pairs; chaetae from chaetiger 10 forming rings in two rows, posterior row with thin and robust capillaries, anterior row with subuluncini, aristate spines, acicular spines and thick acicular spines. With the discriminant analysis, carried out on 11 morphometric characters, the presence of three morphological groups were recognized (Wilks' lambda= 0.093, p= 0.0001). However, the variables selected to discriminate the specimens (partial Wilks' lambda > 0.57) were correlated to their size: number of branchiae, body width, prostomium width, rate length CH9/CH1-CH8, length CH1-CH8 and length CH9 (r> 0.5). So, we concluded that they belong to a single species with three morphotypes: morpho A with 8 branchial pairs, morpho B with 5–6–7 pairs and morpho C with 4 pairs. No correlations between the distribution of the distinct morphotypes along the eastern gulf shelf and the environmental conditions where they settle were detected.

Morphometric and taxonomic approach to describe *Heterospio variabilis* (Annelida, Longosomatidae), a new species with three size-dependent morphotypes, from the Gulf of California, Eastern Pacific

Pablo Hernández-Alcántara[1], Vivianne Solís-Weiss[2]

[1] Departmento de Ecología Acuática y Biodiversidad. Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autonoma de México (UNAM). Mexico City 04510, Mexico.
[2] Departmento de Sistemas Arrecifales. Instituto de Ciencias del Mar y Limnología Universidad Nacional Autonoma de México I (UNAM). Puerto Morelos Quintana Roo, 77580, Mexico.

Corresponding Author:
Vivianne Solis-Weiss[2]
Prol. Av. Niños Héroes s/n Puerto Morelos, Quintana Roo, 77580, México
Email address: solisw@cmarl.unam.mx

Abstract

The Longosomatidae, a poorly known polychaete family, includes only 23 recognized species; in this study, based on morphometric and taxonomic analyses, we describe a new species with three morphotypes: *Heterospio variabilis* from the Gulf of California, Mexico. The specimens examined exhibit large morphological variations but were clearly separated from close species due to a unique combination of morphological characters: chaetiger 9 as the first elongated chaetiger, 4–8 branchial pairs; chaetae from chaetiger 10 forming rings in two rows, posterior row with thin and robust capillaries, anterior row with subuluncini, aristate spines, acicular spines and thick acicular spines. With the discriminant analysis, carried out on 11 morphometric characters, the presence of three morphological groups were recognized (Wilks' lambda= 0.093, *p*= 0.0001). However, the variables selected to discriminate the specimens (partial Wilks' lambda > 0.57) were correlated to their size: number of branchiae, body width, prostomium width, rate length CH9/CH1-CH8, length CH1-CH8 and length CH9 (*r*> 0.5). So, we concluded that they belong to a single species with three morphotypes: morpho A with 8 branchial pairs, morpho B with 5–6–7 pairs and morpho C with 4 pairs. No correlations between the distribution

of the distinct morphotypes along the eastern gulf shelf and the environmental conditions where they settle were detected.

Introduction

At present, the polychaetes comprise nearly 11,500 accepted nominal species (Pamungkas *et al*., 2019; Read, 2019), and are one of the most abundant and diverse groups of invertebrates in soft marine bottoms worldwide (Hutchings, 1998; Brooks *et al*., 2006; Pamungkas Glasby & Costello, 2019; Pamungkas *et al*., 2021). However, around 60–65 % of their species are still to be described according to Read (2019), and that limits the knowledge of their role on the ecology and evolutionary processes operating in benthic ecosystems (Martin *et al*., 2022). This is the case of the Longosomatidae Hartman, 1944, a marine benthic family poorly known worldwide, particularly in the Gulf of California, where only one valid species and an unnamed species had been reported (Hernández-Alcántara & Solís-Weiss, 2005).

The family Longosomatidae comprises small polychaetes with few segments, characterized by a short anterior region (usually named thorax) with 8–9 chaetigerous segments bearing 1–8 pairs of elongated branchial filaments; a middle-body region having very long segments with almost complete rings of chaetae around the body, which include thickened aristate, subuluncini-like capillaries, subuluncini and/or spines; and a posterior bulbous region with 3–5 short segments, a feature unknown in several species, because they are usually lost during the sampling process (Borowski, 1994; Parapar, Aguirrezabalaga & Moreira, 2014; Hernández-Alcántara & Solís-Weiss, 2021; Blake & Maciolek, 2023).

The first species, *Heterospio longissima*, was described from an incomplete specimen collected in 1869 during the "Porcupine" expedition in deep-sea of Irish waters, and its descriptor, Ehlers (1875), placed it in the family Spionidae (Blake & Maciolek, 2023). More than a hundred years later, Borowski (1994) described a new species and left undescribed an additional indeterminate specimen from the Peru Basin; also, studying the earlier publications, he recognized six species and summarized the knowledge on the taxonomy and biology of this family. He concluded that all longosomatids must be included in the genus *Heterospio* Ehlers, 1874, and as Petersen (1992) had previously proposed, the name Longosomatidae (originally Longosomidae) had priority and should prevail.

Recently, Blake and Maciolek (2019) reviewed the biology of longosomatids and Blake and Maciolek (2023) carried out an excellent and detailed study of specimens deposited in various biological collections coming from the North Atlantic, Gulf of Mexico, Caribbean Sea, off Brazil, off California, the Indian Ocean, New Zealand, Australia and South China. In their last publication, they described 13 new species and presented new descriptions and records of *H. catalinensis* (Hartman, 1944), *H. indica* Parapar *et al*., 2016 and *H. peruana* Borowski, 1994. They also examined the original material with which Hartman (1965) described *H. longissima* from the Atlantic Ocean; they found significant differences between the published description and the examined specimens, such as the number of branchiae, the prostomium shape, the origin of the chaetal fascicles or the chaetal types, concluding that the specimens belonged to two separate new species: *H. hartmanae* from abyssal depths off New England to Bermuda and *H. guiana* from the upper slope depths off Surinam. Thus, at present, the longosomatids comprise 23 valid species and four unnamed species, but it is likely that many species have still to be discovered.

In the Mexican Pacific, only *H. catalinensis* and *Heterospio* sp. 1 from the Gulf of California had been recorded (Hernández-Alcántara & Solís-Weiss, 2005), but when those individuals, together with other specimens collected in three oceanographic expeditions, were examined with more detail, we found that they belong to a new species. So, the aim of the present study is to describe this new species, supported with scanning electron microscopy (SEM) photographs and, to confirm its erection as a new taxon, we also assessed its interspecific variability with statistical multivariate analysis. This work was based on 11 selected quantitative morphometric characteristics, focusing on the likely relation between the morphological variability and the body size. The inclusion of quantitative characters to carry out morphometric analyses has been rarely applied in studies aimed at describing new polychaete species. Nevertheless, it has been observed that the addition of quantitative features, provides important additional information to support the discrimination between polychaete taxa, mainly when the examined specimens exhibit a wide morphological variability (Hernández-Alcántara & Solís-Weiss, 2014; Hernández-Alcántara, Mercado-Santiago & Solís-Weiss, 2017; Martin *et al*., 2022; Molina-Acevedo, Fernández-Rodríguez & Idris, 2022). Additionally, the relationships between the environmental variables and the distribution of the new species along the Gulf of California shelf were also examined to improve the knowledge about the poorly known ecology of the longosomatids.

Materials & Methods

Samples collection and morphological examination. The biological material was collected during three oceanographic expeditions carried out in the Gulf of California (Mazatlán Bay in May 1980; Cortes 2 in March 1985; Cortes 3 in August 1985) (Fig. 1, Table 1). All these expeditions were conducted on board the O/V "El Puma" of the Instituto de Ciencias del Mar y Limnología (ICML), Universidad Nacional Autónoma de México (UNAM). The stations were georeferenced on-board with a Global Positioning System (GPS) and depth was measured with a Kongsberf echosounder. In the Mazatlán Bay expedition, the material was collected with a Van Veen grab (0.063 m^2), the temperature was measured with a thermometer (± 0.05°C) and the salinity with a conductivity sensor Plessey (Model 6230). During the Cortes 2 and 3 expeditions, the samples were collected with a Smith-McIntyre grab (0.1 m^2) and, at each station, temperature and salinity were measured with a Niels Brown CTD, and the dissolved oxygen with the Winkler method (Strickland & Parsons, 1977). Additional sedimentary samples were taken to quantify the organic matter content by the Walkley and Black (1934) acid digestion method, and the sediment textural characteristics following the sieving method (Folk, 1980) (Table 1).

The biological samples were washed on-board through a 0.5 mm mesh and fixed with 4% formaldehyde in seawater solution. Later, in the laboratory, the material was washed again with freshwater to eliminate the formaldehyde and the specimens were separated under a stereoscope and preserved in 70% ethanol. The observations, drawings and measurements of the specimens and their morphological characteristics were made with stereoscope and compound microscopes. The abbreviations used on figures were: acsp, acicular spine; arsp, aristate spine; br, branchia; dCr, dorsal crest; dT, dorsal tentacle; dTs, dorsal tentacle scar; neL, neuropodial lamella; nuO, nuchal organ; per, peristomium; pr, prostomium; rca, robust capillary flattened in distal half; sub, subuluncini; tacsp, thick acicula spine; thca, thin capillary.

The methyl green staining pattern was determined by immersing the specimens for two minutes in a saturated solution of methyl green in 70% ethanol (Warren *et al*., 1994). Scanning Electron Microscopy (SEM) observations and micrographs were made using a JEOL JSM6360L microscope at the ICML, UNAM. Specimens were previously dehydrated via graded ethanol series, dried with liquid-CO_2 at critical point and coated with gold. The holotype, paratypes and additional material examined were catalogued and deposited in the Colección Nacional de Anélidos Poliquetos, ICML-UNAM. Additional paratypes were also deposited in the Natural History Museum of Los Angeles County (NHMLA).

The species description was based on the holotype, but the morphological variability associated to paratypes was also included in parentheses. A total of 56 specimens of the new species were examined and their occurrence along the Gulf of California is shown in the Material examined section. To standardize the description of the longosomatid species, in general we followed the formats suggested by Parapar, Aguirrezabalaga and Moreira (2014), and Parapar *et al*. (2016) to describe the morphology of the new species. The number of segments in the anterior body ("thoracic region") and the relative length of the following elongated segments ("mid-body region") are two significant characteristics to separate the longosomatid species. However, in this family, the intersegmental channels are usually not so evident, and therefore, the separation of segments and the transition between the anterior and the middle body region are difficult to distinguish. So, also following Parapar, Aguirrezabalaga and Moreira (2014), and Parapar *et al*. (2016), the limits between segments were established based on the position of the chaetae on the anterior edge of the segments and, therefore, the length of a segment is the distance from the chaetal bundle of one chaetiger to the chaetal bundle of the next.

Although the term "palps" has been usually used in the descriptions of the longosomatid species, following Blake and Maciolek (2023), here we used "dorsal tentacles" to name these grooved feeding structures, due to the new evidence showing that longosomatids are more closely related to cirratulids than spionids (Rouse, Pleijel & Tilic, 2022; Blake & Maciolek, 2023).

Despite the small number of described longosomatid species, their types of chaetae are highly variable. Although Borowski (1994) and Parapar, Aguirrezabalaga and Moreira (2014) discussed some aspects of their chaetal variability, it is necessary to examine in detail their shapes and to review the terminology used, since, as Borowski (1994) and Parapar *et al*. (2016) indicated, some chaetal types could be transitional stages of the same chaeta. Therefore, and due to the wide chaetal variety observed in the new species, we kept the terms currently used to characterize the chaetal types: capillary, stout capillary, subuluncini, aristate spines, acicular spines and hooks (Borowski, 1994, Parapar *et al*., 2016).

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:F3462F09-2330-42F3-BA76-C2ACDEE10504. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central SCIE and CLOCKSS.

Anatomical and morphometric comparisons. In order to examine the taxonomy and morphological variability of the new species, 11 characters were measured from the 56 specimens mentioned: number of branchiae (Bn); length (Pl) and width (Pw) of prostomium; length of chaetiger 1 to chaetiger 8 (CH1-l–CH8-l); width body (at chaetiger 5 without parapodia) (Aw); length of chaetiger 9, the first elongated segment (CH9-l); length of chaetiger 10 (CH10-l), 11 (CH11-1) and 12 (CH12-1); and a measure of the relative length of the first elongated chaetiger: the rate between the length of chaetiger 9 and length of anterior region (tip of prostomium to chaetal bundle of chaetiger 8) (RCH9/Al). For comparative purposes, and because all the specimens were incomplete and had suffered mechanical damage during the collection and sieving processes, and that 88% of the specimens had 12 or more chaetigers, the total size of individuals was standardized to the length back to the 12th chaetiger, naming it here: "total length" (Tl).

An estimation of the descriptive statistic parameters (mean, range, standard deviation (SD) and coefficient of variation (CV) was carried out to examine the variability of the characters measured. To determine whether the morphological variability was linked to the body size of the specimens, we examined the correlations between the total length (Tl) and each morphological variable with the Pearson correlation coefficient (r).

The morphological relations between the examined specimens, based on the 11 measured morphometric variables, were calculated by means of a Principal Component Analysis (PCA). The morphometric distinction between the specimens was made with a Discriminant Analysis using the forward stepwise method; the standard statistic Wilks' lambda (ranging from 1, no discriminatory power, to 0, perfect discriminatory power) was used to assess the characters that most significantly contributed to differentiate the specimens' groups. The partial Wilks' lambda index was used to evaluate the individual contribution of each measured character to discriminate the groups. The graphical representation for distinction among the specimen groups was performed with a canonical analysis (Stat Soft, 2007). The relationships between the environmental conditions and the distribution of the morphological groups in the Gulf of California shelf were estimated with a Principal Component Analysis (PCA). All morphometric analyses were carried out with the STATISTICA 7.0 software (Stat Soft, 2007) for Windows.

Results

Systematics

Family Longosomatidae Hartman, 1944

Genus *Heterospio* Ehlers, 1874

Heterospio variabilis **sp. nov**.

LSID: urn:lsid:zoobank.org:act:C8DF52A4-B1F1-4F9B-83C4-5A410414A9E7

(Figs. 2A-J, 3A-K, 4A-H, 5A-K, 6A-F, 7A-L, 8A-L)

Heterospio sp. 1.– Hernández-Alcántara & Solís-Weiss, 2005: 277.

Material examined. ***Type locality*.** MEXICO • Gulf of California, North Consag Rocks; 31°16.1'N, 114°21.7'W; 30.3 m. ***Holotype*:** MEXICO • 1 spec.; Gulf of California, North Consag Rocks, Sta. 37 Cortes 2; 31°16.1'N, 114°21.7'W; 30.3 m; 16 Mar. 1985; P. Hernández-Alcántara leg.; fine sand sediment; CNAP-ICML: POH–13–001. ***Paratypes:*** MEXICO • 5 specs.; Gulf of California; same collection data as for holotype; one specimen coated with gold for SEM studies; CNAP-ICML: POP–13–001 • 2 specs.; Gulf of California, El Fuerte River, Sta. 52 Cortes 2; 25°39.9'N, 109°28.6'W; 28.6 m; 20 Mar. 1985; P. Hernández-Alcántara leg.; fine sand sediment; CNAP-ICML: POP–13–002 • 4 specs.; Gulf of California, North Consag Rocks, Sta. 37 Cortes 2; 31°16.1'N, 114°21.7'W; 30.3 m; 16 Mar. 1985; P. Hernández-Alcántara leg.; fine sand sediment; LACM-AHF Poly.

Additional material. MEXICO • 20 specs.; Gulf of California, North Consag Rocks, Sta. 37 Cortes 2; 31°16.1'N, 114°21.7'W; 30.3 m; 16 Mar. 1985; P. Hernández-Alcántara leg.; fine sand sediment; CNAP-ICML: PO–13–004/2014-GCA-CS • 1 spec.; Gulf of California, El Fuerte River, Sta. 51 Cortes 2; 25°42.1'N, 109°30.6'W; 49.5 m; 20 Mar. 1985; P. Hernández-Alcántara leg.; fine sand sediment; CNAP-ICML: PO–13–004/2015-GCA-CS • 1 spec.; Gulf of California, Mita Point, Sta. 61 Cortes 2; 20°53.9'N, 105°27.5'W; 50.4 m; 23 Mar. 1985; P. Hernández-Alcántara leg.; fine sand sediment; CNAP-ICML: PO–13–004/2016-GCA-CS • 2 specs.; Gulf of California, North Consag Rocks, Sta. 38 Cortes 2; 31°08.3'N, 114°13.3'W; 71.9 m; 16 Mar. 1985; P. Hernández-Alcántara leg.; CNAP-ICML: PO–13–004/2017-GCA-CS • 10 specs.; Gulf of California, El Fuerte River, Sta. 50 Cortes 2; 25°46.8'N, 109°35.4'W; 97 m; 20 Mar. 1985; P. Hernández-Alcántara leg.; fine sand sediment; CNAP-ICML: PO–13–004/2018-GCA-CS • 1 spec.; Gulf of California, El Fuerte River, Sta. 52 Cortes 3; 25°43.6'N, 109°29.3'W; 22.1 m; 8 Aug. 1985; P. Hernández-Alcántara leg.; very fine sand sediment; CNAP-ICML: PO–13–004/2019-GCA-CS • 2 specs.; Gulf of California, Tepoca Cape, Sta. 44 Cortes 3; 30°00.5'N, 112°59.5'W; 106 m; 5 Aug. 1985; P. Hernández-Alcántara leg.; CNAP-ICML: PO–13–004/2020-GCA-CS • 3 specs.; Gulf of California, North Consag Rocks, Sta. 37 Cortes 3; 31°19.8'N, 114°23.2'W; 21.5 m; 4 Aug. 1985; P. Hernández-Alcántara leg.; very fine sand sediment; CNAP-ICML: PO–13–004/2021-GCA-CS • 2 specs.; Gulf of California, Arboleda Point, Sta. 15 Cortes 3; 26°53.2'N, 110°05.9'W; 39 m; 31 Jul. 1985; P. Hernández-Alcántara leg.; fine sand sediment; CNAP-ICML: PO–13–004/2022-GCA-CS • 1 spec.; Gulf of California, El Fuerte River, Sta. 50 Cortes 3; 25°49.5'N, 109°37.9'W; 80 m; 8 Aug. 1985; P. Hernández-Alcántara leg.; very fine sand sediment; CNAP-ICML: PO–13–004/2023-GCA-CS • 1 spec.; Gulf of California, Mazatlan Bay, Sta. C8–7; 235°14.2'N, 106°26.8'W; 7 m; 25 Jan. 1980; E. Arias-González leg.; CNAP-ICML: PO–13–004/2024-GCA-CS.

Diagnosis. Body elongated, threadlike. Anterior region with eight short chaetigers; median region with greatly elongate segments. Chaetiger 9 is the first elongated segment, about twice as long as chaetiger 8. Prostomium conical, anteriorly rounded, continuing as a dorsal crest over peristomium until chaetiger 1; a pair of lateral nuchal organs. Peristomium with two rings interrupted dorsally by the dorsal crest; one pair of grooved dorsal tentacles. Anterior region with 4–8 pairs of long, cirriform branchiae from chaetiger 2, their number can be related to size body. First nine chaetigers biramous with only simple capillaries, without neuropodial acicular spines. From chaetiger 10 with chaetae forming cinctures, arranged in two rows: posterior row with thin capillaries and robust capillaries flattened in distal half; anterior row with subuluncini with long distal end, aristate spines, acicular spines, and thick, curved acicular spines.

Description. Holotype incomplete with 13 chaetigers, 18.6 mm long, 3.7 mm width~~. The~~ 11 ~~selected~~ paratypes ~~are~~ anterior fragments with 12–19 chaetigers, 8.2–24.5 mm long, 2–3.8 mm width. Body elongated, threadlike (Figs~~.~~ 2A, 3A), pale in ethanol without special pigmentation. Prostomium conical, anteriorly rounded, slightly flattened dorsoventrally, continuing as a mid-dorsal crest over peristomium until chaetiger 1 (Fig. 3A-E); without eyes. Nuchal organs as deep grooves (Fig. 3D) with cilia (Fig. 3F). Peristomium with two rings, interrupted dorsally by the crest ridge (Fig. 3B, E). One pair of grooved dorsal tentacles easily deciduous; a paratype with a single dorsal tentacle arising from the right side of peristomium (Fig. 3E, G), lined with cilia (Fig. 3H, I). Everted pharynx not observed in any of the individuals examined.

Anterior body region slightly flattened dorsoventrally, with eight short chaetigers (CH1–CH8) (Figs~~.~~ 2A, 3A, B), more than twice as wide as long (Figs~~.~~ 2A, 3B). Chaetiger 9 (CH9) is the first elongated, longer than wide, about twice as long as CH8 (Fig~~.~~ 2A, 3B, J). First chaetiger (CH1) without branchiae (Figs~~.~~ 2A, B, 3B). With 8 pairs of filiform branchiae from CH2 to CH9, dorsal to notopodia (Figs. 2A, 3B) (7–8 pairs in paratypes, 4–8 pairs in additional material); most branchiae missing, the scars are difficult to observe. From CH10, segments strongly elongated (Fig. 3A), length progressively increasing towards posterior segments (Fig. 3J): CH10 3.5 times longer than CH9 (3.4 in paratypes); CH11 1.7 times longer than CH10 (1.7 in paratypes); CH12 2.2 times longer than CH11 (0.9 in paratypes).

Chaetigers 1 to 9 with biramous parapodia, as lateral pads (Figs~~.~~ 2B, C, 3~~A,~~ C, 4A-~~C~~, 5A); neuropodia with short, rounded to triangular, postchaetal lamella (Figs~~.~~ 2B, C, 4A, B, 5A); noto- and neuropodial chaetal bundles well separated, bearing fan-shaped fascicles with numerous simple capillaries arranged in several rows (Fig. 2B, C); those from posterior row longer (Figs~~.~~ 2B, C, 4B, C). No neuropodial acicular hooks in any anterior chaetiger. From CH10 backwards, all parapodia as elongated ridges forming nearly closed flange-like cinctures near anterior margin of segment (Figs~~.~~ 2D, ~~2D,~~ 3J, K). Chaetae from CH10 backwards arranged in two transversal rows (Fig. 4D-H); the posterior row presents thin capillaries (Figs~~.~~ 2E, 4D-G) and robust capillaries flattened in distal half (Figs~~.~~ 2E, 4E-G, 5I), both with long distal tips. Anterior row: CH10 with subuluncini (robust capillaries armed with long appendage) (Figs. 2F, 4D, 5B, C); from CH11 with subuluncini (Fig~~s~~. 5C), aristate spines (when they lack a distal appendage look like acicular spines) (Figs~~.~~ 2H, I, 4E, 5D-G), acicular spines (Figs~~.~~ 2J, 4F-H, 5H), some resembling aristate spines without distal appendage (Fig. 4F-H), and thick, slightly curved acicular spines (Figs. 2G, 4H, 5J, K). Posterior region unknown.

Methyl Green staining. Body stains uniformly, without a color pattern (Figs. 6A, B). The specimens with 6 (Fig. 6C, D) or 4 (Fig. 6E, F) branchial pairs do not exhibit either any methyl green staining pattern.

Variation. Specimens having 4 branchial pairs (Fig. 7A) were smaller (see morphological analyses section), but with conical prostomium anteriorly rounded, with posterolateral nuchal organs (Fig. 7B, C), and peristomium with two rings separated by deep dorsolateral grooves, interrupted dorsally by a dorsal crest from prostomium (Fig. 7C). They were similar to those observed in individuals bearing more branchiae ~~(Fig. 7C, D)~~. The CH9 was always the first elongated (Fig. 7E). From CH10 backwards, parapodia progressively more elongated, with chaetae arranged in two rows, forming nearly closed cinctures (Fig. 7E, F). However, the distribution of chaetal types along the elongate chaetigers exhibited some differences with that observed in specimens with more branchiae: the CH10 only had thin capillaries in the posterior row and slightly thicker in the anterior row (Fig. 7G-H). From CH11, posterior row with thin and robust capillaries (Fig. 7I, J, K), and anterior row with subuluncini (Fig. 7J), aristate spines and acicular spines with deciduous distal ends (Fig. 7J-L).

On the other hand, specimens with 5–6–7 branchial pairs were longer, but their prostomium and peristomium had similar characteristics to those observed in individuals with different number of branchial pairs (Fig. 8A, B, D). The CH9 was also the first elongated (Fig. 8A, E). In the middle region the chaetae showed the following patterns: CH10 with thin and robust capillaries in the posterior row, and subuluncini in the anterior row, some with no distal end. From CH11(Fig. 8F), anterior row with subuluncini and aristate spines, several of them lacking a distal appendage (Fig. 8G, J), acicular spines (Fig. 8K) and thick acicular spines, some curved (Fig. 8L).

Remarks. Considering the relative size of the anterior chaetigers, *Heterospio variabilis* sp. nov. belongs to the large group of 18 longosomatid species (75% of the valid species) having eight shorts anterior chaetigers (CH1–CH8) and chaetiger 9 as the first elongate segment (see Blake & Maciolek, 2023). Eight of them have chaetiger 9 longer, at least as long as the first 1–4 or as the 6–8 chaetigers combined. However, the other 10 species have chaetiger 9 only 2–3 times longer than chaetiger 8: *Heterospio variabilis* sp. nov., *H. indica* Parapar *et al*., 2016, *H. peruana*

Borowski, 1994~~; and~~ *H. africana*, *H. brunei*, *H. ehlersi*, *H. guiana*, *H. hartmanae*, *H. knoxi* and *H. paulolanai* described by Blake and Maciolek (2023) (Table 2).

In *H. variabilis* sp. nov., the number of branchiae is largely variable: most of the specimens had 8 (16 ind.), 7 (13 ind.) or 6 (19 ind.) branchial pairs, and less frequently 4 (5 ind.) or 5 (3 ind.) pairs. Nevertheless, the species can be separated from other longosomatids with CH 9 only 2–3 times longer than CH8, due to the shape and distribution of chaetae in its elongated segments, the shape of the neuropodial postchaetal lamellae or the prostomium and peristomium form, for example. Among the other species with 8 branchial pairs, in *H. indica* the prostomium is triangular, the peristomium has 1 ring, prominent neuropodial postchaetal lamellae, thin and thicker capillaries in CH10 and subuluncini and thin capillaries from CH11~~;~~ *Heterospio knoxi* has a triangular prostomium, with a dorsal crest extending to chaetiger 2, rounded neuropodial postchaetal lamellae as low flanges, CH10 bearing thin and thicker capillaries, and from CH11 with subuluncini, aristate chaetae and acicular spines (Blake & Maciolek, 2023) (Table 2). Conversely, *H. variabilis* sp. nov. has a conical prostomium, tapering to a rounded tip, with neuropodial postchaetal lamellae short and rounded, and from CH10 bears a large chaetal variety: subuluncini, aristate spines, blunt acicular spines and thick, curved acicular spines.

Heterospio variabilis sp. nov. can be also separated from those species having 6 or 7 branchial pairs: *H. guiana* which has a triangular prostomium, tapering to a narrow tip, and only bears capillaries in CH10 and aristate spines in CH11-CH12 (Table 2). On the other hand, *H paulolanai* has a pear-shaped prostomium with a narrow-rounded tip, neuropodial postchaetal lamellae as low ridges and bears capillaries in CH10, and from CH11, aristae spines, acicular spines and subuluncini. This last species was described from only one individual with branchiae on chaetigers 2–8 (7 pairs), but due to damage from an earlier dissection, the presence of branchiae on CH9 remains in doubt (Blake & Maciolek, 2023).

Heterospi~~u~~o variabilis sp. nov. also showed important morphological differences with those species having few branchiae: *Heterospio africana*, with 5 branchial pairs, has a wide and large dorsal crest on the peristomium, the neuropodial postchaetal lamellae are short, bear only capillaries in CH10 and CH11, and capillaries and subuluncini in CH12-CH13 (Table 2). Furthermore, the two species bearing 4 branchial pairs lack neuropodial postchaetal lamellae and the chaetae in elongated segments are distinct: *H. brunei* only bears acicular spines in CH10-

CH19, while *H. hartmanae*, with a pear-shaped prostomium, has acicular spines in CH10, and capillaries and acicular spines in Ch11-CH24 (Table 2).

Heterospio peruana was described bearing 4 branchial pairs, but two individuals bearing 5 pairs and small specimens with 1–3 pairs were also reported (Borowsky, 1994). This longosomatid has also a wide variety of chaetae on elongated segments: capillaries, subuluncini, aristate chaetae and acicular spines from chaetiger 10, although some paratypes lack subuluncini and aristate chaetae (Borowsky, 1994). Thus, besides the fact that most specimens of *H. variabilis* sp. nov. have 6–8 pairs and only 5 individuals with 4 pairs and 3 individuals with 5 pairs were found, it can be also separated from *H. peruana* by the presence of robust capillaries flattened in their distal half in the posterior row, and because thick curved acicular spines are also present in the anterior row of some elongated segments (Table 2).

Etymology. The term "*variabilis*" to name the new species was chosen to emphasize its high variability in the number of branchiae.

Habitat. At 7 to 106 m depth, in sediments with 49–94% of fine sand, temperatures of 13.2–30°C, 34.2–36.06 ups of salinity, 2.4–8.4% of organic matter and 1.03–5.4 ml/L of dissolved oxygen.

Distribution. Widely recorded in the eastern continental shelf of the Gulf of California.

Morphometric analyses. The ninth chaetiger was the first elongated segment, and is an invariant character in all examined individuals. The length and variability of the elongated segments gradually increased towards the posterior region: CH9 (mean= 0.41 mm; CV= 40.95), CH10 (mean= 1.62 mm; CV= 44.17), CH11 (mean= 2.72 mm; CV= 51.60) and CH12 (mean= 2.95 mm; CV= 55.84) (Table S1). The rate of length CH9/CH1-CH8 (mean= 0.13; CV= 38.95), the total length (mean= 10.91 mm; CV= 32.35), width of anterior region (mean= 0.54 mm; CV= 30.39) and the prostomium length (mean= 0.28 mm; CV= 27.66) exhibited an intermediate variability. Conversely, the prostomium width (mean= 0.32 mm; CV= 24.3), length of CH1-CH8 (mean= 2.92 mm; CV= 24.16) and the number of branchiae (mean= 6.57 mm; CV= 18.55) presented the lowest variability (Table S1).

The length of chaetigers 10 (r= 0.79), 11 (r= 0.86) and 12 (r= 0.8), the width of anterior region (r= 0.56), number of branchiae (r= 0.53) and length of chaetiger 9 (r= 0.48) exhibited the highest Pearson correlation with the total length of specimens (Tl). In general, the larger individuals were wider, with the chaetigers 9 to 12 longer and with more branchial pairs. Conversely, the length and width of prostomium, the length of CH1-CH8 and the rate of length CH9/CH1-CH8 exhibited the minor correlation with the specimen's length ($r < 0.45$). In particular, the number of branchiae was highly correlated to the width of the anterior region ($r = 0.81$), length of chaetiger 9 ($r = 0.68$), length of prostomium ($r = 0.68$), width of prostomium ($r = 0.57$), and the rate of length chaetiger 9/CH1-CH8 ($r = 0.53$) (Fig. 9, Table S2).

The first two PCA components explained the 70.3% of the total morphological variation (Fig. 10). The ~~PC1~~ described the highest variance of the model (53.8%), with the most important explicative variables being the width of the anterior region (-0.36) and the length of chaetiger 9 (-0.35); the ~~PC2~~ only accounted for 16.5% of variability, mainly linked to the length of chaetiger 12 (0.5) and the total length (0.43). The number of branchiae has been typically used as a diagnostic character to differentiate the species, but in this case, it only contributed with -0.33 (PC1) and -0.14 (PC2) to explain the total variation (Table S3).

The addition of specimens on the PCA plot, arranged according to their number of branchial pairs because, among the characters correlated with the body size, it presented the lowest variability (CV= 18.55), suggested the presence of different morphological groups. Significant differences were found between individuals with 8 or 4 branchial pairs ($R_{ANOSIM} = 0.83$, $p = 0.001$) and between specimens with 8 or 6 pairs ($R_{ANOSIM} = 0.41$, $p = 0.001$), but the individuals with 5 or 6 branchial pairs ($R_{ANOSIM} = 0.05$, $p = 0.583$) and those with 6 or 7 pairs ($R_{ANOSIM} = 0.04$, $p = 0.218$) integrated the same group (Fig. 10).

As a result, the individuals were classified in three morphological groups, distinguished by the presence of 8 (morphotype A), 5–6–7 (morphotype B) or 4 (morphotype C) branchial pairs (Fig. 10), whose differences were tested by a discriminant analysis. The Wilks' lambda value of 0.093 was highly significant ($F_{(12, 96)} = 18.2$, $p = 0.0001$), supporting the hypothesis that the examined individuals could be assembled in three morphological groups; the analysis also showed that the individuals were appropriately classified inside the corresponding group. The forward stepwise way removed five morphological variables from the discriminant function model (F*value* < 1), so, six variables remained to discriminate the groups (Table S4). Subsequently, the partial Wilks' lambda selected the number of branchiae (Bn) (0.57) as the most important variable to the discriminant function, followed by the width of the anterior body (Aw), the width of prostomium (Pw), the rate length CH9/CH1-CH8 (RCH9/Al), the length

CH1-CH8 (CH1-l–CH8-l) and the length of CH9 (Table S4). The first three variables exhibited tolerance values larger than 0.5 but, as initially reported, except for the prostomium width, they were highly correlated with the total length of specimens.

The plot of the discriminant functions confirmed the separation of the three suggested morphotypes, with the first canonical root explaining 94.8% of the variance (Fig. 11), which was mainly defined by variables associated with the body length and number of branchiae (Table S5). It clearly separated the specimens with 8 branchial pairs having the longer CH1-CH8 and higher rate of CH9/CH1-CH8, but a CH9 short in length, from those specimens with only 4 branchial pairs with shorter CH1-CH8 and rate CH9/CH1-CH8; the individuals with 5–6–7 branchial pairs presented intermediate values in these characters. The second canonical root only explained 5.2% of variance, was barely relevant to discriminate the morphotypes, but exhibited the great variability of each group (Fig. 11). The multivariate analysis showed that the number of branchiae and length of some chaetigers determined the presence of three morphological groups. However, their great variability and high correlation with the body length, together with the few differences found in the morphology of specimens with distinct number of branchiae, clearly showed that they could not be considered as separate taxa. Particularly, the number of branchiae has been regularly used to separate taxonomically the longosomatids but, until the importance of this character and other diagnostic characters to discriminate species are fully understood, as well as their variability with specimens' size, we cannot conclude that the examined specimens here belong to distinct species.

Distribution patterns. *Heterospio variabilis* sp. nov. was found along the eastern shelf of the Gulf of California, mostly in the winter-spring season (46 ind.), whereas in the summer-autumn only 9 individuals were collected. During the winter-spring, the highest abundances were found in Rocas Consag (Sta. 37= 30 ind.; Stat. 38= 2 ind.) in the northern gulf, and El Fuerte River (Stat. 50= 10 ind.; Sta. 52= 2 ind.; Stat. 51= 1 ind.) in the south, (Fig. 1, Table 1). In the summer-autumn, it was also collected in both localities, but clearly with lower abundance (Sta. 37= 3 ind.; Sta, 50= 1 ind.; Sta. 52= 1 ind.). In the far southern gulf, during the winter-spring season, only one individual in Mazatlán Bay (Sta. C8–7) and another in Punta Mita (Sta. 61) were found.

Morphotype B (5–6–7 branchial pairs) was the most abundant (35 ind.), followed by morphotype A (8 pairs) with 16 specimens. The higher abundances of both morphotypes were found in the same localities: Consag Rocks and in front of El Fuerte River. On the other hand, the five specimens of morphotype C (4 branchial pairs) were exclusively collected in front of El Fuerte River (Fig. 1, Table 1).

The PC1 explained 61.8% of the environmental variability where the new species was distributed, basically associated with dissolved oxygen (-0.49) and depth (0.484) changes. The PC2 accounted for 20.2% of variance, mainly related to temperature fluctuations (0.872) (Table S6). The highest abundance of morphotypes A and B was found at 30.3 m depth in the northern gulf, on well oxygenated bottoms (5.4 ml/L) and with low organic matter percentage (2.4%) (Fig. 12). However, these morphotypes were basically collected in the same sampling stations and no environmental differences were found where they dwell. ~~Conversely,~~ morphotype C was only collected in front of El Fuerte River, in the central gulf, at 97 m depth, with lower oxygen levels (1.47 ml/L) and higher organic matter concentrations (5.7%). Seasonally, the higher abundances were found in the winter-spring, where the lower temperatures were recorded (mean= 15.35°C against 24.94°C in summer-autumn) (Table 1).

Discussion

Taxonomy and morphology. Of the 23 species of the family Longosomatidae currently recognized, 13 of them (56.5%) were only recently described, in 2023, by Blake and Maciolek (2023). Consequently, the taxonomy and the importance of the diagnostic characters in this family, are in the process of being better understood. This is especially relevant as more intraspecific morphological variability is being observed in several taxa. In fact, four taxa where doubts persist on their taxonomy´s identity, remain catalogued as “sp”, and other species, including *H. longissima*, the type species of the genus, have presented problems during their identification (Blake & Maciolek, 2023). The scarce knowledge about the taxonomy of longosomatids can be perceived, for example, when the term palps is used to designate the grooved feeding structures, which, since the longosomatids are phylogenetically more related to cirratulids than to spionids (Rouse *et al*., 2022), must be named dorsal tentacles, as Blake and Maciolek (2023) appropriately proposed.

Indeed, the presence of dorsal tentacles (palps) has been described and/or drawn for several species (Hartman, 1965; Wu & Chen, 1966; Laubier, Picar & Ramos, 1972–1973; Borowski, 1994; Parapar *et al*., 2016; Blake & Maciolek, 2023). However, they are easily lost during the collecting and fixation processes and are usually missing in the specimens examined

(Parapar, Aguirrezabalaga & Moreira, 2014). This has caused uncertainties about the actual presence of their dorsal tentacles, and some authors as Uebelacker (1984), Parapar *et al*. (1994) or Bochert and Zettler (2009) have interpreted the deep grooves behind the prostomium as nuchal organs and not dorsal tentacles scars (Parapar *et al*., 2016). Parapar, Aguirrezabalaga & Moreira (2014), for example, in their description of *H. reducta*, indicated that the unique structure observed under the SEM, was the deep groove between the prostomium and peristomium, which did not look like a palp (dorsal tentacle) or scar, but rather like a nuchal organ. Notwithstanding, as Parapar *et al*. (2016) describing *H. indica* and Blake and Maciolek (2023) describing *H. bathyala* and *H. hartmanae* confirmed, here we also showed the presence of a dorsal tentacle in a SEM photo (Fig. 3A-E). In a paratype, a large dorsal tentacle is attached on the right side of the peristomium but also, posterolateral to the prostomium, the nuchal organs, as deep grooves with cilia, are shown too.

Although the presence of distinct types of modified chaetae and their distribution along the elongated segments in the examined specimens did not exhibit any clear trends, it showed some signals that, as Borowski (1994) and Parapar, Aguirrezabalaga & Moreira (2014) previously suggested, they could be transitional stages from capillaries to acicular spines, associated with the development state of individuals. However, it is necessary to examine in detail complete specimens with different sizes and to compare their variations with other longosomatid species, to detect some pattern and to validate its importance as a diagnostic character.

Morphometry. The identification of longosomatid species has been traditionally based on the number of branchial pairs, the number of short anterior segments, the location of the first elongated segment and its length relative to preceding and following segments, the presence of modified neurochaetae on chaetiger 1 and the chaetal types in abdominal segments (Wu & Chen, 1966; Borowski, 1994; Parapar, Aguirrezabalaga & Moreira, 2014; Parapar *et al*., 2016; Blake & Maciolek, 2023). So, the wide variability recorded in the examined individuals in some of their diagnostic characters, as the number of branchiae or the relative length of the first elongated segment, for example, could be interpreted as meaning that they belong to different species. However, some previous species descriptions have shown that their morphological variations could be related to their body size (Borowski, 1994; Parapar *et al*., 2016).

Although the morphological intraspecific variations in relation to body size have been poorly explored in longosomatids, Borowski (1994) observed a possible association between the number of branchiae and the individual size. He found that small specimens of *H. peruana* have 1–3 branchial pairs and that with the increase of the specimens' size, they reach 4 pairs, but the larger specimens do not necessarily bear more branchiae. These likely relationships with the body size were also observed in other species, such as *H. indica*, whose large specimens bear 7–8 branchial pairs, while those smaller have 4 pairs (Parapar *et al*., 2016).

In *H. variabilis* sp. nov. both large and small specimens have different numbers of branchiae, but the material examined provided new insights to recognize the relationships between the morphological variation and the body size in longosomatids. The examination of 11 characters by multivariate analyses showed that the observed differences between the suggested morphotypes are precisely related to the specimen length. In fact, the presence of three morphological groups, considering the number of branchiae, was confirmed by the discriminant analysis, but the main characters explaining their separation, number of branchial pairs and length of the first elongated segment (CH9), were related to the individual length. However, these characters presented many variations, so that they were not entirely size-correlated: for example, in smaller specimens (< 8 mm) between 4 and 7 branchial pairs are present, but larger individuals (> 12 mm) can bear between 6 and 8 pairs; also, chaetiger 9 was from 0.13 to 0.53 mm long in small individuals, but from 0.33 to 0.97 mm in larger individuals. Therefore, we propose that the three detected groups are morphotypes of a single species, *H. variabilis* sp. nov., whose morphological variability is largely depending on the body size. Unfortunately, no gametes were seen in the collected specimens to consider whether the morphotypes correspond to different ontogenetic stages, since among the longosomatids, the morphological variations associated with ontogeny or development have not been studied yet.

So, it would be necessary to examine more specimens, particularly short individuals bearing few branchial pairs, to corroborate the variations or homogeneity of the analyzed characters. In addition, due to dependence of several morphological characters to the individual size, in future descriptions of species, it will be necessary to analyze whether other morphological characters, such as length of the anterior region (CH1-CH8), length of the first elongated segment (CH9) or the rate of length CH9/CH1-CH8, could be also appropriate to differentiate the species in this family.

Ecology. The publication of new records of longosomatid species are very important, since their reports are very scarce, and the localities where they were collected are scattered around the world seas (Borowski, 1994). Although the interpretations about their distribution patterns are difficult to establish, six of the seven species having eight short anterior chaetigers and chaetiger 9 as the first elongate segment, have only been recorded in the Pacific Ocean. Among them, three species are so far exclusively distributed in the Eastern Pacific: *H. catalinensis* from southern California, in shelf and deep-sea (Hartman, 1944), *H. peruana* from abyssal depths of Peru Basin (Borowsky, 1994) and now *H. variabilis* sp. nov. from the Gulf of California shelf (Table 2).

The longosomatid species have been reported in marine regions too distant between them and in large depth intervals, but mainly in the continental slope and abyssal depths (Blake & Maciolek, 2023). Although they were mainly found in soft bottoms, and being potentially subsurface deposit feeders (Jumars, Dorgan & Lindsey, 2015), little is known about their biology and ecology (Blake & Maciolek, 2023). Although the distribution of *H. variabilis* sp. nov. is, so far, limited to the continental shelf, it lives on a large range of environmental conditions. Its highest abundances were recorded in the winter-spring season, linked to a decrease in temperature and organic matter percentage. However, no relationships were found between the environmental conditions and the occurrence of the three morphotypes found in the Gulf of California. The morphotypes B (5–6–7 branchial pairs) and A (8 pairs) were the most abundant, but they were practically collected in the same sampling stations, and no environmental preferences were detected. However, morphotype C (4 branchial pairs) was only found in the outer shelf, subject to low oxygen levels and higher organic matter percentage. Thus, it is necessary to get more information about the habitats where the species occur, to understand the effect of environmental factors on the settlement and development of the longosomatids.

Conclusions

We can conclude that *Heterospio variabilis*, the new species described here, does differ significantly from other close species of longosomatids. However, the morphological variability found in the examined individuals is remarkably high, although not sufficient to separate them into different species. Several of the 11 characters analyzed by multivariate techniques often overlap between the specimens with different number of branchiae, a character usually considered the strongest character to separate species in this family. The distribution and measured environmental conditions where the morphotypes with 8, 5–6–7 or 4 branchial pairs were found does not allow us to determine any pattern that would help to separate them either in different species. So, we believe that the new species has high morphological plasticity, and although such variability has seldom been found in other longosomatid species, until more studies are carried out with more class sizes to examine, we think that they all belong to only one species, *H. variabilis* sp. nov.

Acknowledgements

We would like to thank Michel Hendrickx, head of the institutional project "Cortes", for inviting both of us to participate in these oceanographic expeditions. We also acknowledge Píndaro Díaz Jaimes, Director of the ICML, UNAM, for his financial support.

References

Blake JA, Maciolek NJ. 2019. 7.3.1.9. Longosomatidae Hartman, 1944. In: Purschke G, Böggemann M, Westheide W, eds. *Handbook of Zoology. Volume 1. Annelida: Basal groups and Pleistoannelida, Sedentaria I.* Berlin: De Gruyter, 457–465. DOI 10.1515/9783110291582-007.

Blake JA, Maciolek NJ. 2023. New species and records of *Heterospio* (Annelida, Longosomatidae) from continental shelf, slope and abyssal depths of the Atlantic Ocean, Pacific Ocean, Indian Ocean, and adjacent seas. *Zootaxa* **5260**:1–74. DOI 10.11646/zootaxa.5260.1.1.

Bochert R, Zettler ML. 2009. A new species of *Heterospio* (Polychaeta, Longosomatidae) from offshore Angola. *Zoological Science* **26**:735–737. DOI 10.2108/zsj.26.735

Borowski C. 1994. New records of Longosomatidae (Heterospionidae) (Annelida, Polychaeta) from the abyssal Southeast Pacific, with the description of *Heterospio peruana* sp. n. and general remarks on the family. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* **92**:129–144.

Brooks RA, Purdy CN, Bell SS, Sulak KJ. 2006. The benthic community of the eastern US continental shelf: A literature synopsis of benthic faunal resources. *Continental Shelf Research* **6**:804–818.

Ehlers E. 1874. Annulata nova vel minus cognita in Expeditione "Porcupine" capta. *The Annals and Magazine of Natural history* **Series 4, 13**:292–298.

Ehlers E. 1875. Beiträge zur Kenntniss der Verticalverbreitung der Borstenwürmer im Meere. *Zeitschrift für wissenschaftliche Zoologie* **25**:1–102.

Folk RL. 1980. *Petrology of sedimentary rocks*. Austin: Hemphill Publishing Company.

Hartman O. 1944. Polychaetous annelids. Part VI. Paraonidae, Magelonidae, Longosomidae, Ctenodrilidae, and Sabellariidae. *Allan Hancock Pacific Expeditions* **10**:311–481, pls. 27–42.

Hartman O. 1965. Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic areas. *Allan Hancock Foundation Occasional Paper* **28**:1–378.

Hernández-Alcántara P, Mercado-Santiago AJ, Solís-Weiss V. 2017. Description, morphometric characterization and distribution pattern of *Paradiopatra multibranchiata*, a new onuphid (Annelida: Polychaeta) from the Tropical Eastern Pacific. *Marine Biology Research* **13**: 942–954.

Hernández-Alcántara P, Solís-Weiss V. 2005. Seasonal variations of the Spionida (Palpata: Canalipalpata) in the sublittoral zone of the Gulf of California. *Marine Ecology* **26**:273–285.

Hernández-Alcántara P, Solís-Weiss V. 2014. Anatomical and morphometric analysis of a new species of *Leitoscoloplos* (Annelida: Orbiniidae) with numerous stomach papillae, from the Gulf of California, Eastern Pacific. *Contributions to Zoology* **83**:133–150.

Hernández-Alcántara P, Solís-Weiss V. 2021. 26. Longosomatidae Hartman, 1944. In: De León-González JA, Bastida-Zavala JR, Carrera-Parra LF, García-Garza ME, Salazar-Vallejo SI, Solís-Weiss V, Tovar-Hernández MA. (Eds.). *Anélidos marinos de México y América Tropical*. México: Universidad Autónoma de Nuevo León, 361–366.

Hutchings P. 1998. Biodiversity and functioning of polychaetes in benthic sediments. *Biodiversity and Conservation* **7**:1133–1145.

Jumars PA, Dorgan KM, Lindsey SM. 2015. Diet of worms emended: an update of polychaete feeding guilds. *Annual Review of Marine Science* **7**:497–520. DOI 10.1016/j.dsr2.2007.07.007.

Laubier L, Picard C, Ramos J. 1974. Les Heterospionidae (Annélides Polychètes Sédentaires) de Méditerranee occidentale. *Vie et Milieu* **23**:243–254.

Martin M, Capa M, Martínez A, Costa AM. 2022. Taxonomic implications of describing a new species of *Loimia* (Annelida, Terebellidae) with two size-dependent morphotypes. *European Journal of Taxonomy* **833**:60–96. DOI 10.5852/ejt.2022.833.1887.

Molina-Acevedo IC, Fernández-Rodríguez V, Idris I. 2022. Morphometric approach to support distinction among three species of *Paucibranchia* Molina-Acevedo, 2018 (Annelida: Eunicidae). *Zootaxa* **5155**:347–370. DOI 10.11646/zootaxa.5155.3.3.

Pamungkas J, Glasby CJ, Read GB, Wilson SP, Costello MJ. 2019. Progress and perspectives in the discovery of polychaete worms (Annelida) of the world. *Helgoland Marine Research* **73**:1–10.

Pamungkas J, Glasby CJ, Costello MJ. 2021. Biogeography of polychaete worms (Annelida) of the world. *Marine Ecology Progress Series* **657**:147–159. DOI 10.3354/meps13531.

Parapar J, Aguirrezabalaga F, Moreira J. 2014. First record of Longosomatidae (Annelida: Polychaeta) from Iceland with a worldwide review of the family. *Journal of Natural History* **48**:983–998. DOI 10.1080/00222933.859316.

Parapar J, Vijapure T, Moreira J, Sukumaran S. 2016. A new species of *Heterospio* (Annelida, Longosomatidae) from the Indian Ocean. *European Journal of Taxonomy* **220**:1–17. DOI 10.5852/ejt.2016.220.

Petersen ME. 1992. Provisional review of the Cirratulidae from the Faroes, with comments on some other groups (Annelida, Polychaeta). *Norðurlandahúsið í Føroyum Årbok*, 1991–1992: 79–80.

Read GB. 2019. 1. A history of Annelida research. In: Purschke G, Böggemann M, Westheide W, eds. *Handbook of Zoology. Volume 1. Annelida: Basal groups and Pleistoannelida, Sedentaria I.* Berlin: De Gruyter, 3–36. DOI 10.1515/9783110291582-001.

Rouse GW, Pleijel F, Tilic E. 2022. *Annelida*. Oxford: Oxford University Press.

StatSoft Inc. 2007. *STATISTICA (data analysis software system)*. Version 7.0. www.statsoft.com.

Strickland JDH, Parsons TR. 1977. A Practical Handbook of Seawater Analysis. *Fisheries Research Board of Canada, Bulletin* **167**:1–293.

Uebelacker JM. 1984. Chpater 10. Family Heterospionidae Hartman, 1963. In: Uebelacker JM, Johnson PG, Vittor BA & Associates, eds. *Taxonomic Guide to the Polychaetes of the Northern Gulf of Mexico. Vol. II*. U.S. Louisiana: Department of the Interior, Minerals Management Service, Metairie, 10-1-10-6.

Walkley A, Black IA. 1934. An examination of the Degthareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* **27**:29–38.

Warren LM, Hutchings PA, Doyle S. 1994. A revision of the genus *Mediomastus* Hartman, 1944 (Polychaeta: Capitellidae). *Records of the Australian Museum* **46**:227–256.

Wu BL, Chen M. 1966. A new and interesting species of the genus *Heterospio* (Polychaeta, Heterospionidae). *Oceanology and Limnology Sinica* **8**:163–167.

Figure 1

Gulf of California including the sampling stations where *Heterospio variabilis* sp. nov. was collected.

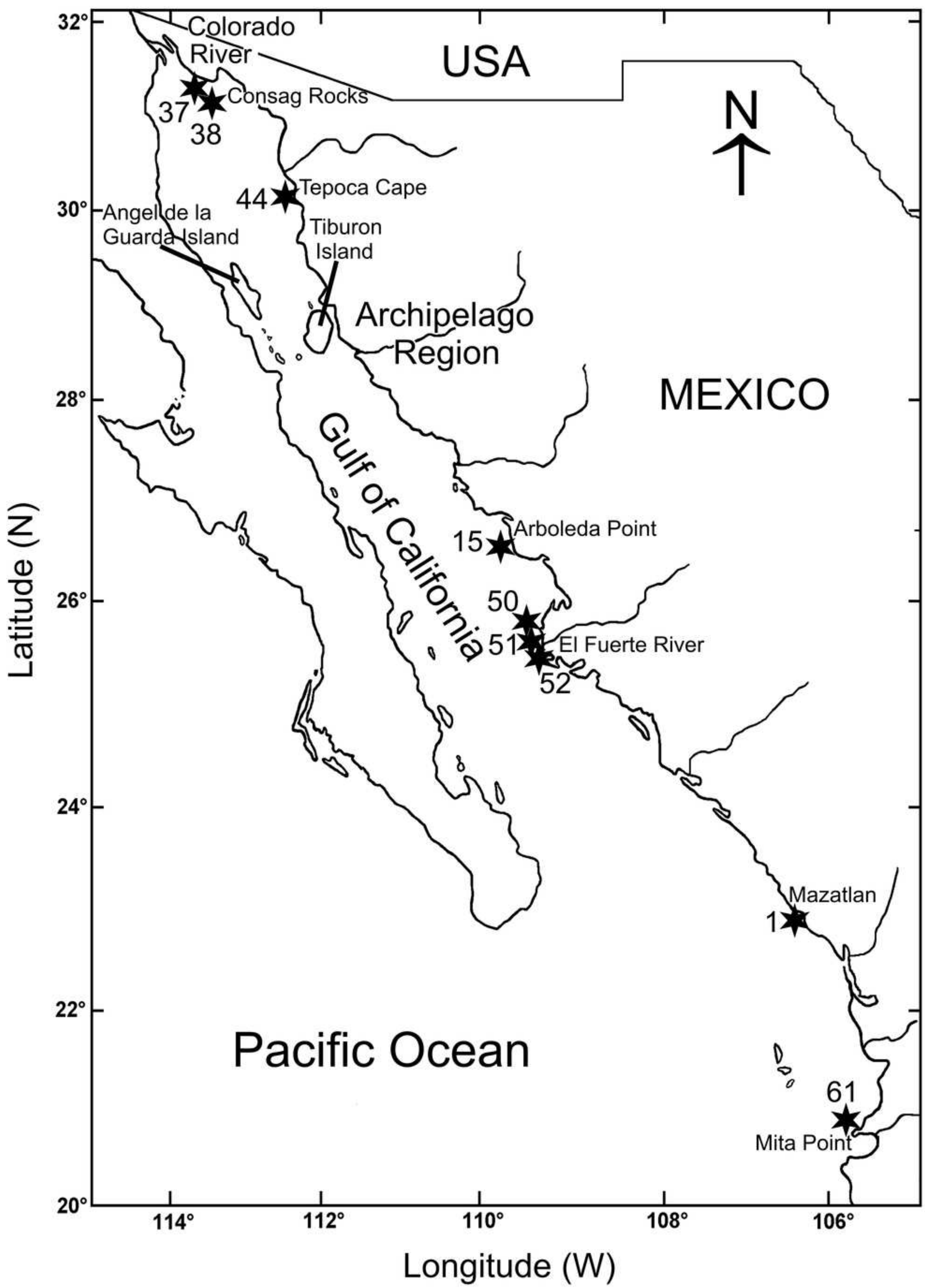
Colorado
River
USA
Consag Rocks
37
38
N
44
Tepoca Cape
Angel de la
Guarda Island
Tiburon
Island
Archipelago
Region
MEXICO
Gulf of California
Arboleda Point
15
50
51
El Fuerte River
52
Mazatlan
1
Pacific Ocean
61
Mita Point
32°
30°
28°
26°
24°
22°
20°
114°
112°
110°
108°
106°
Latitude (N)
Longitude (W)

Figure 2

Heterospio variabilis sp nov.

(A) Anterior and middle body, dorsal view. (B) Chaetiger 1. (C) Chaetiger 9. (D) Chaetiger 12, cross section. (E) Thin capillaries and robust capillaries flattened in middle half. (F) Subuluncini. (G) Thick acicular spines. (H) Aristate spines. (I) Detail of distal end of aristate spines, one with the aristate missing. (J) Acicular spines. Scale bars: A, D= 500 µm; B, C= 100 µm; E, F, J= 20 µm; G, H, I= 10 µm.

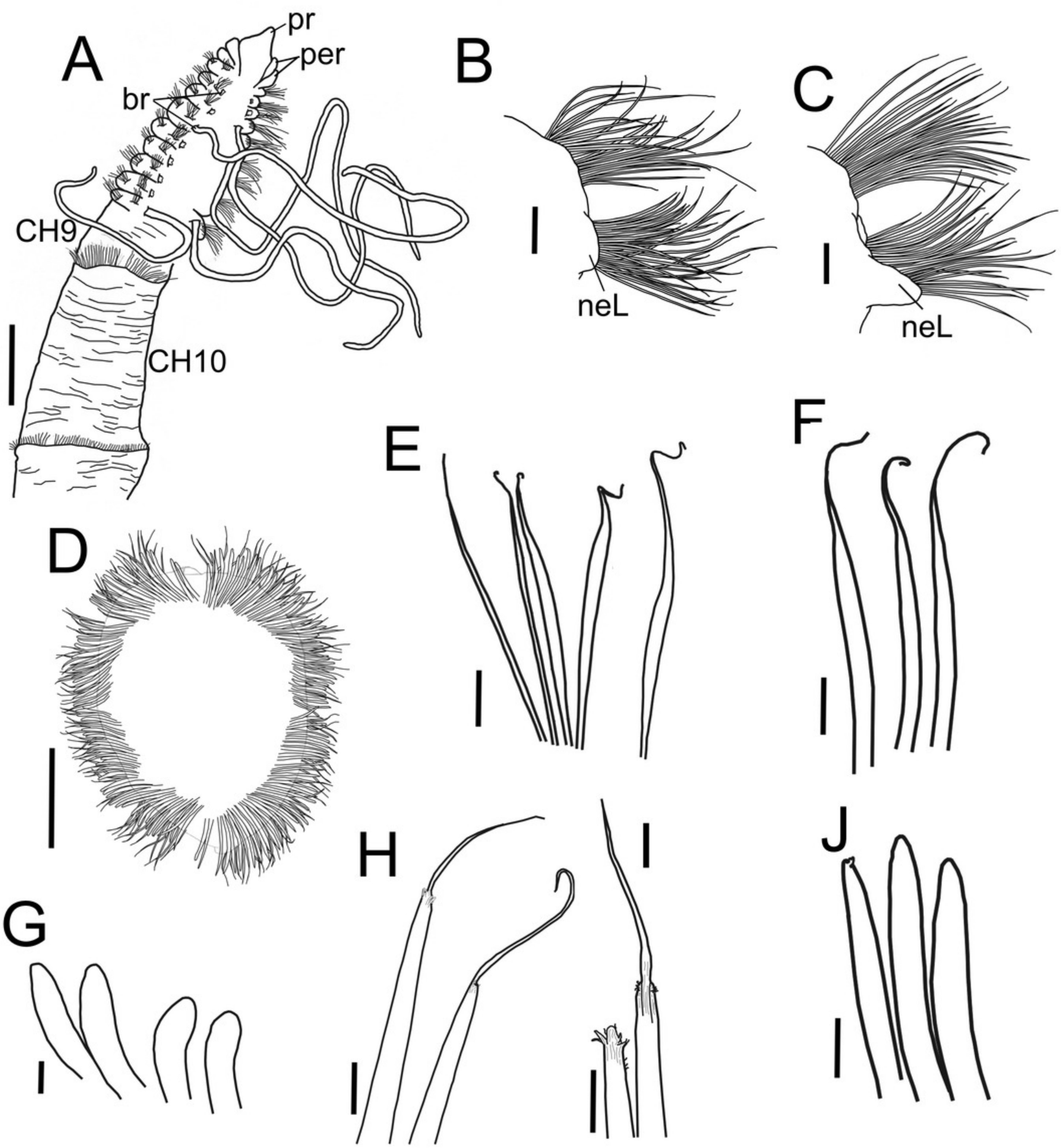
A
pr
per
br
CH9
CH10
B
neL
C
neL
D
E
F
G
H
I
J

Figure 3

Heterospio variabilis sp. nov.

A) Anterior and middle body, lateral view. (B) Anterior end, dorsal view. (C) Anterior end, ventral view. (D) Detail of prostomium, nuchal organ and palp scar, lateral view. (E) Detail of prostomium and palp. (F) Detail of nuchal organ opening. (G) Palp. (H) Detail of palp, middle region. (I) Detail of cilia on the middle palp. (J) Chaetigers 9-12. (K) Chaetiger 11. Scale bars: A, J= 500 µm; B= 200 µm; C, E, G, K= 100 µm; D= 50 µm; F, H= 10 µm; I= 1 µm.

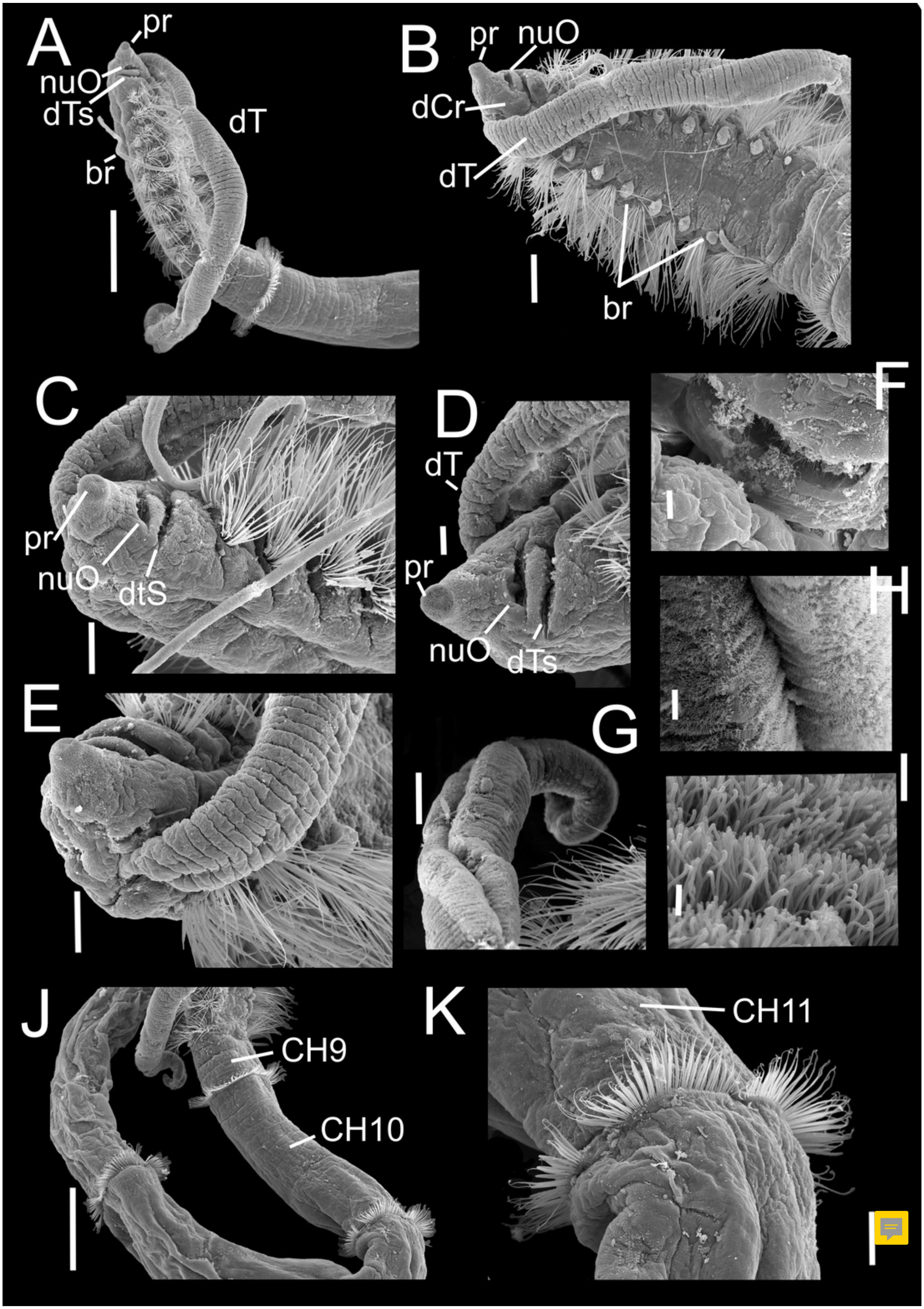
A
pr
nuO
dTs
br
dT
B
pr
nuO
dCr
dT
br
C
pr
nuO
dtS
D
dT
pr
nuO
dTs
F
H
E
G
J
CH9
CH10
K
CH11

Figure 4

Heterospio variabilis sp. nov.

(A) Chaetigers 7–8. (B) Chaetiger 9. (C) Notopodia chaetiger 9. (D) Chaetae of chaetiger 10. (E) Chaetae of chaetigers 11. (F) Chetae of chaetiger 12. (G) Chaetae of chaetiger 12. (H) Chaetae of chaetiger 14. Scale bars: A, B, C, F, H= 50 µm; D, E, G= 20 µm.

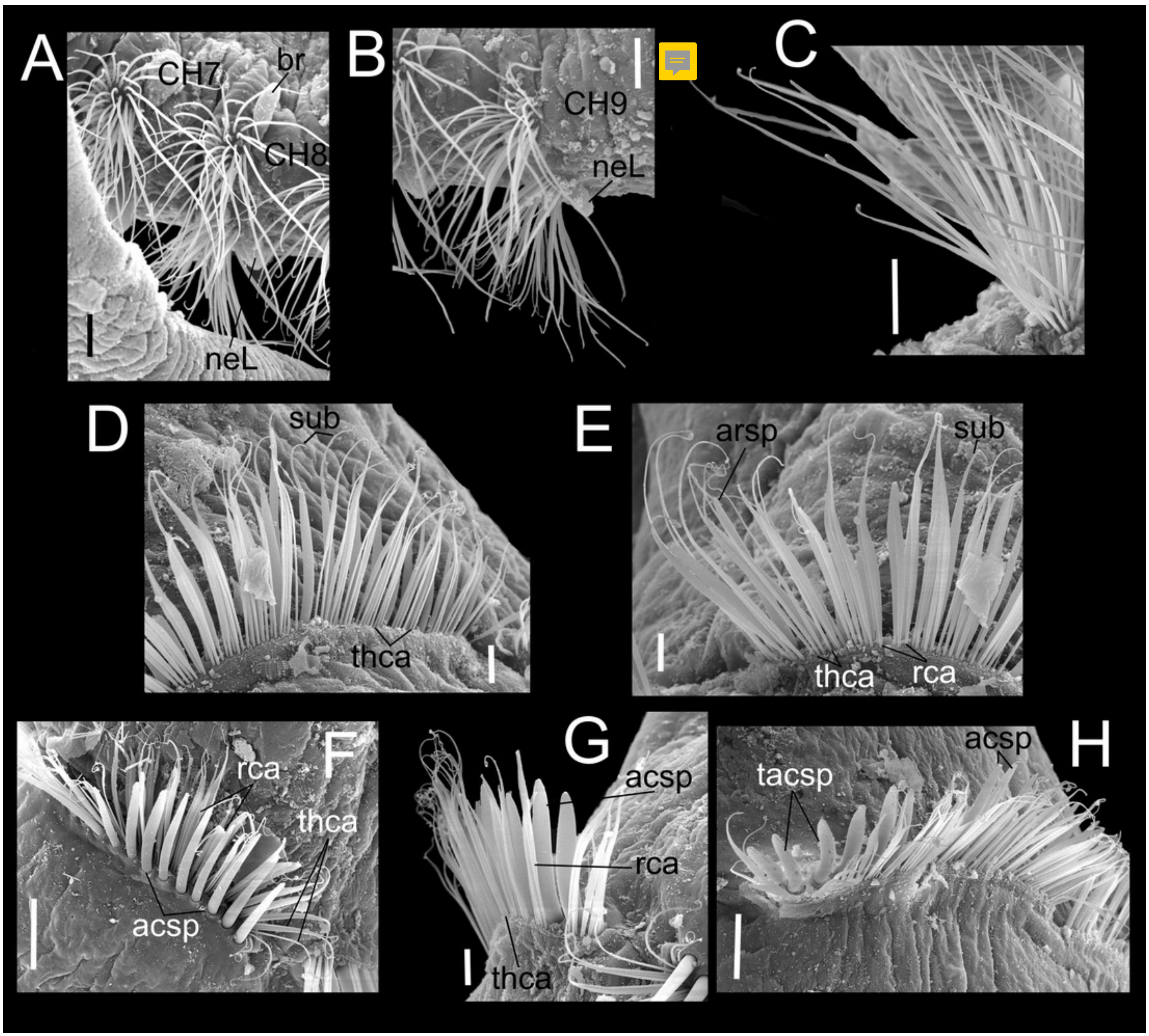

Figure 5

Heterospio variabilis sp. nov.

(A) Capillaries of chaetigers 8–9. (B) Subluncini, thin and robust capillary chaetae of chaetiger 10. (C) Subuluncini, thin and robust capillary chaetae of chaetiger 11. (D) Aristate spines without distal appendage of chaetiger 11. (E) Aristate spines of chaetiger 11. (F, G) Detail of distal end of aristate spines. (H) Acicular spine of chaetiger 14. (I) Robust capillaries flattened in the distal half, of chatiger 15. (J) Thick acicular spines of chaetiger 15. (K) Thick acicular spines and robust capillaries of chaetiger 16. Scale bars: A= 100 µm; B, E, F, I, J, K= 10 µm; C, D, H= 20 µm; G= 2 µm.

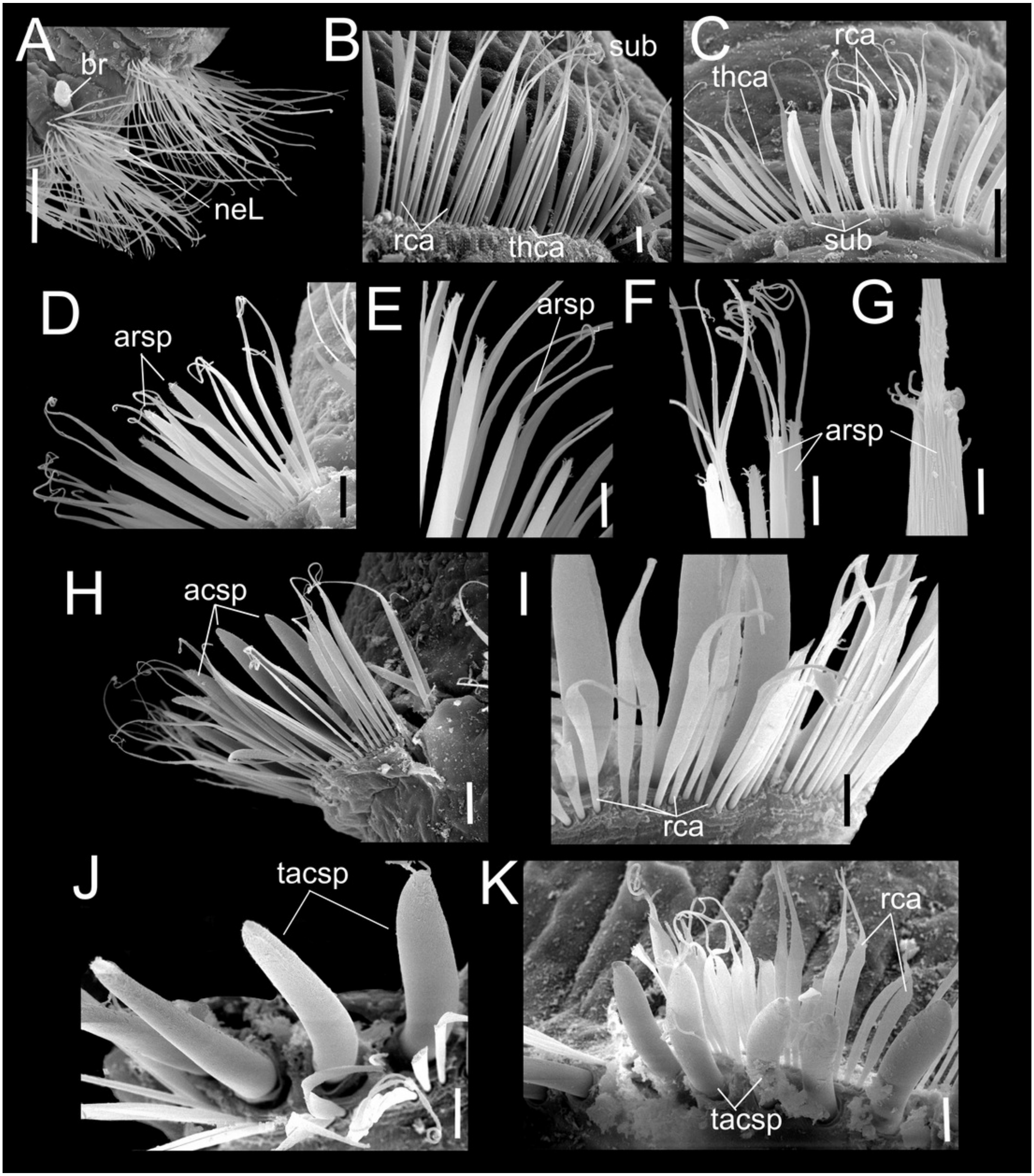
A
br
neL
B
sub
rca
thca
C
thca
rca
sub
D
arsp
E
arsp
F
arsp
G
H
acsp
I
rca
J
tacsp
K
rca
tacsp

Figure 6

Heterospio variabilis sp. nov., methyl green staining patterns.

Specimen with 8 branchial pairs, dorsal (A) and ventro-lateral (B) view. Specimen with 6 branchial pairs, dorsal (C) and ventro-lateral (D) view. Specimen with 4 branchial pairs, dorsal (E) and ventral (F) view. Scale bar: A, B= 500 µm; C, D= 250 µm; E, F= 250 µm.

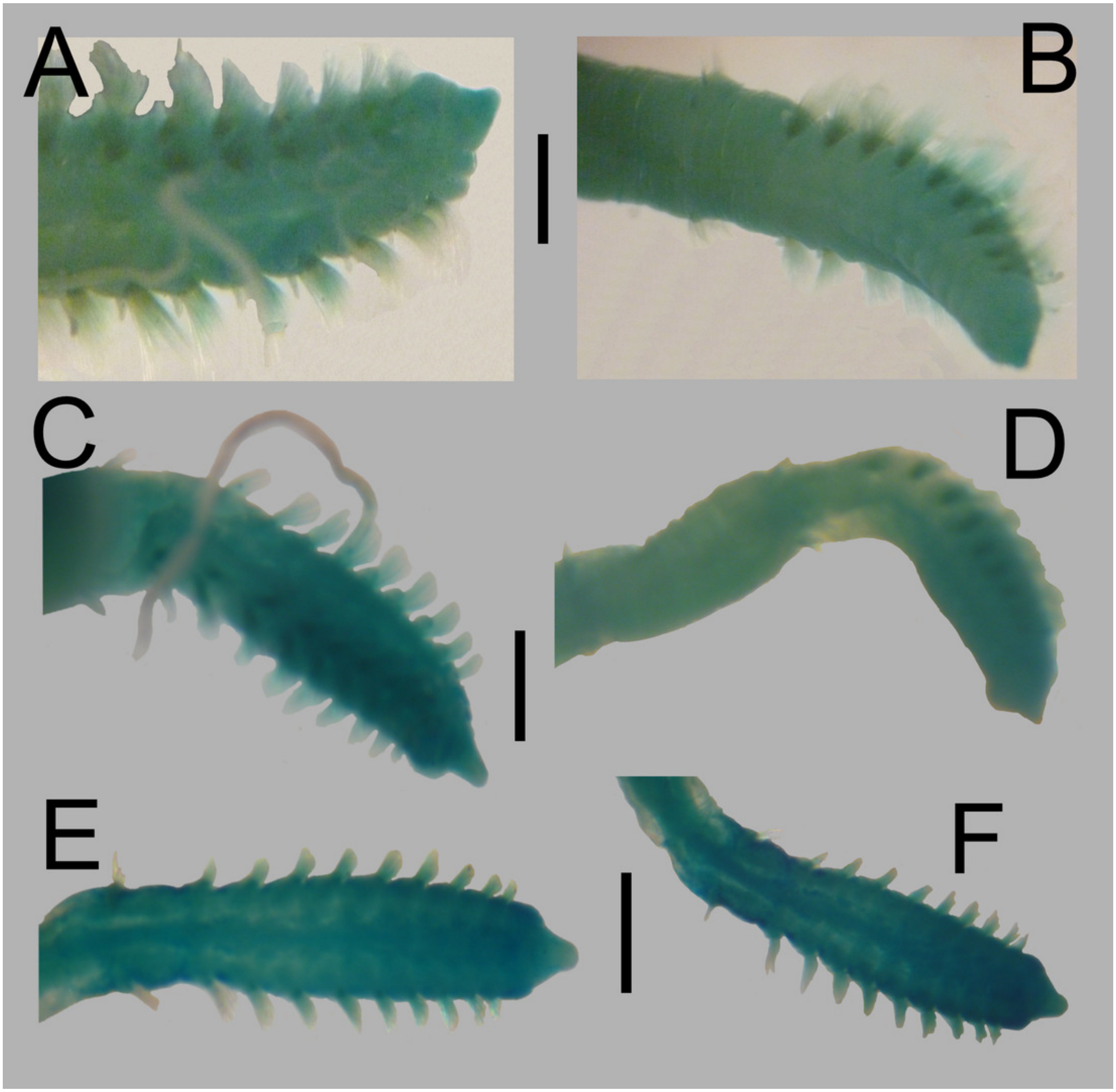
A
B
C
D
E
F

Figure 7

Heterospio variabilis sp. nov. with 4 branchial pairs.

A) Anterior and middle region, dorsal view. (B) Anterior region, lateral view. (C) Anterior region, dorsal view. (D) Chaetigers 2–6. (E) Chaetigers 7–9. (F) Chaetiger 10. (G, H) Dorsal and ventral capillaries of chaetiger 10. (I) Chaetiger 11. (J) Subuluncini and thin capillaries of chaetiger 12. (K) Capillaries, acicular and aristate spines of chaetiger 13. (L) Aristate and acicular spines of chaetiger 13. Scale bars: A, F= 200 µm; B, D, I= 50 µm; C, E= 100 µm; G, H, J, K= 20 µm; L=5 µm.

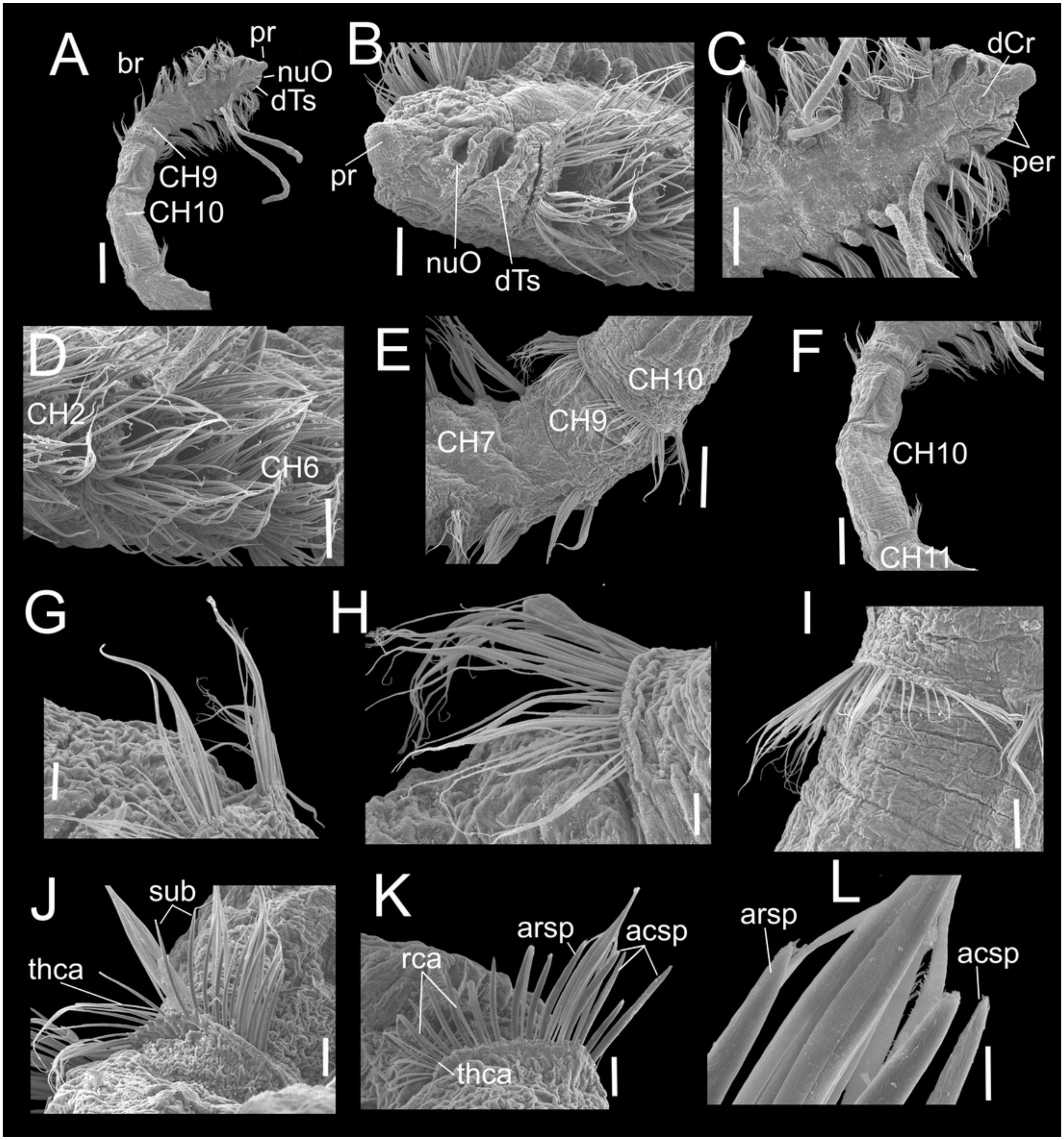
A
br
pr
nuO
dTs
CH9
CH10
B
pr
nuO
dTs
C
dCr
per
D
CH2
CH6
E
CH10
CH9
CH7
F
CH10
CH11
G
H
I
J
sub
thca
K
arsp
acsp
rca
thca
L
arsp
acsp

Figure 8

Heterospio variabilis sp. nov. with 6 branchial pairs.

A) Anterior and middle region, dorsal view. (B) Prostomium, dorsal view. (C) Chaetigers 2–4. (D) Anterior and branchial region, dorsal view. (E) Chaetigers 9–11. (F) Chaetiger 11. (G) Thin capillaries and subuluncini of chaetiger 11. (H) Robust capillaries flattened in middle half and acicular spines of chaetiger 12. (I) Chaetiger 13. (J) Robust capillaries and aristate spines of chaetiger 13. (K) Robust capillaries and acicular spines of chaetiger 14. (L) Thick acicular spines of chaetiger 14. Scale bars: A, E= 200 µm; B, C, D, F= 100 µm; G, H= 20 µm; I= 50 µm; J, K, L= 20 µm.

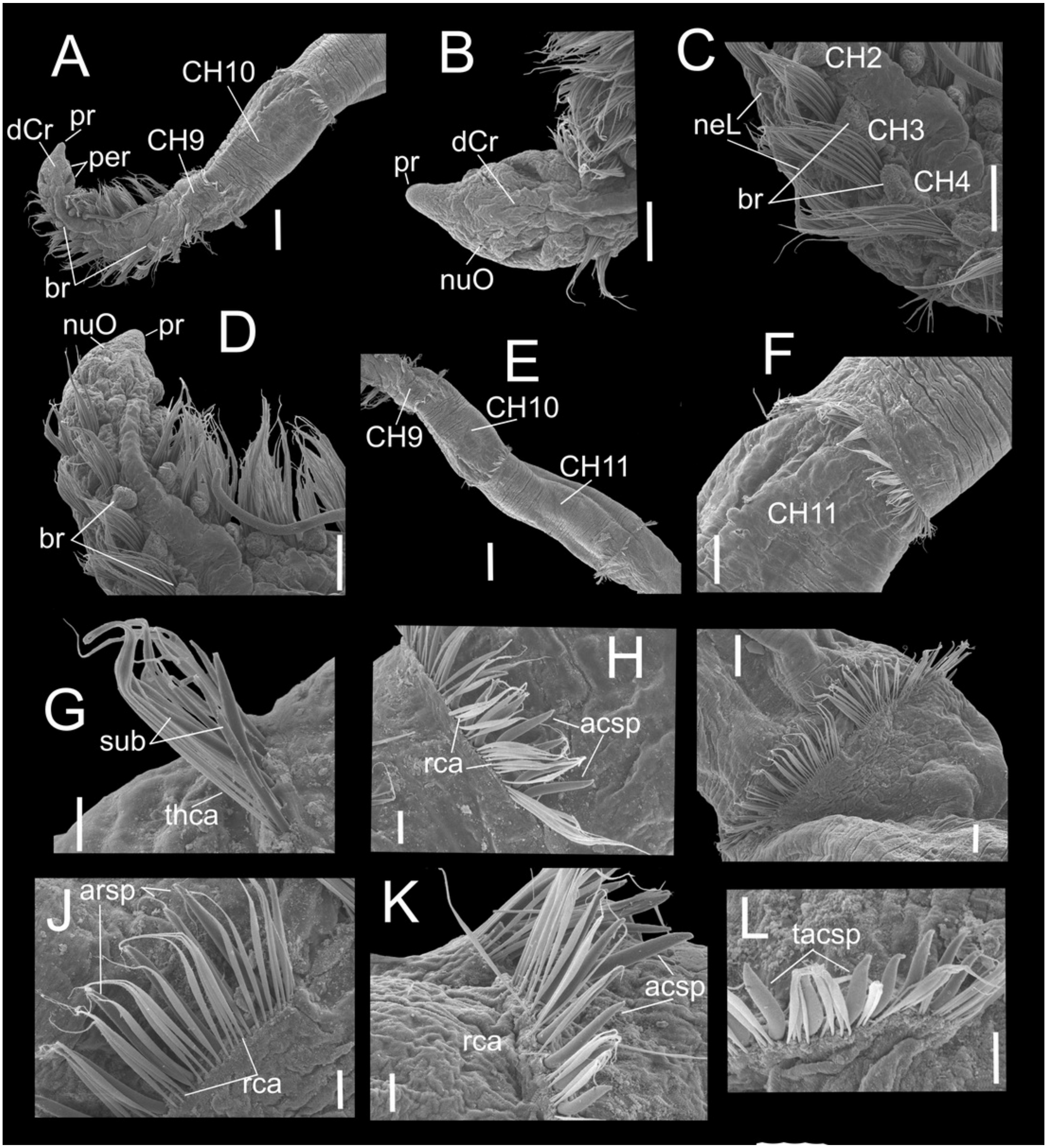
A
CH10
dCr
pr
per
CH9
br
B
dCr
pr
nuO
C
CH2
neL
CH3
CH4
br
D
nuO
pr
br
E
CH9
CH10
CH11
F
CH11
G
sub
thca
H
acsp
rca
I
J
arsp
rca
K
acsp
rca
L
tacsp

Figure 9

Pearson's correlation matrix among the total length and the 10 other examined morphological characters of *Heterospio variabilis* sp. nov.

Abbreviations are explained in the methodology section.

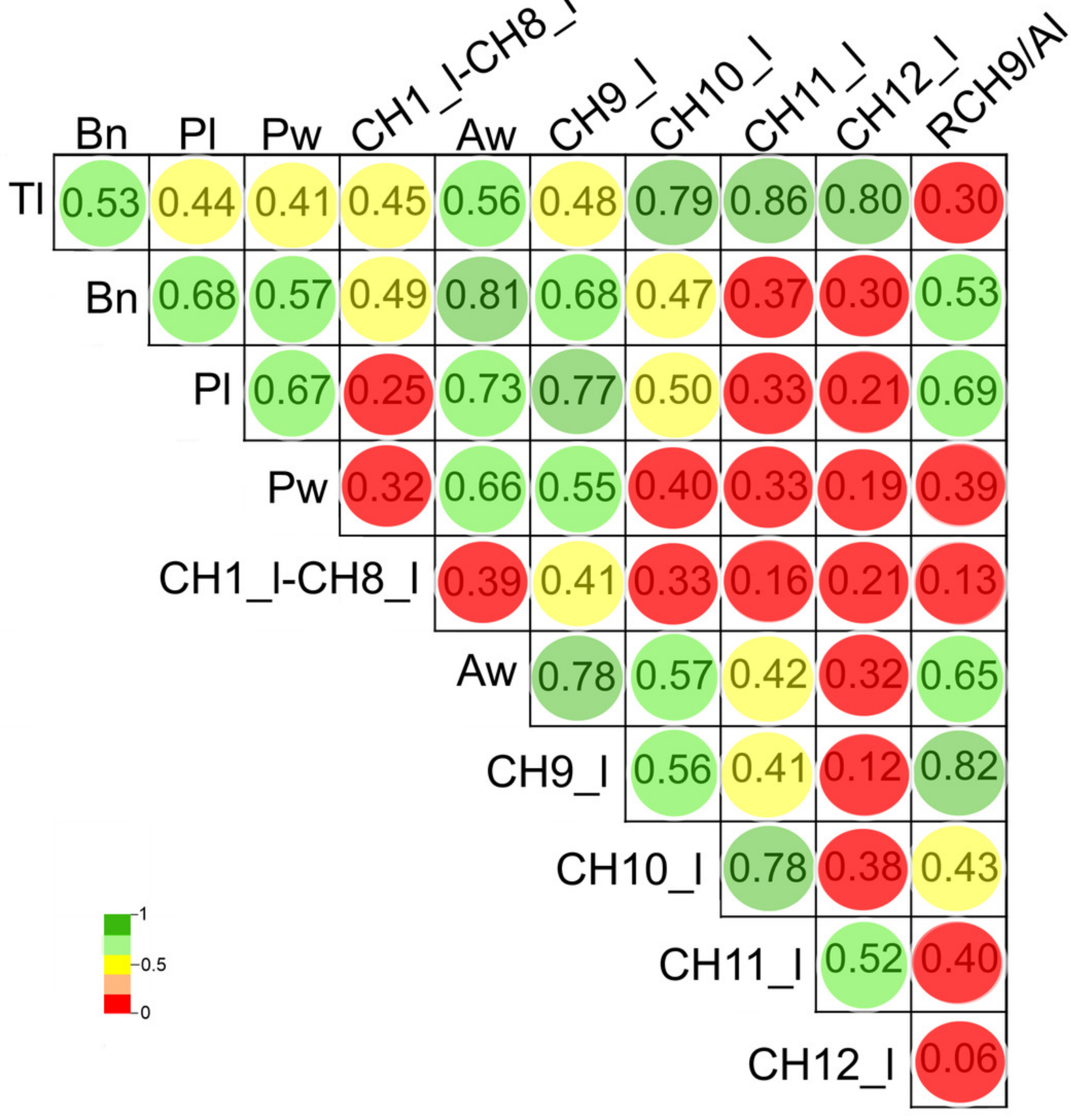
Bn Pl Pw CH1_l-CH8_l Aw CH9_l CH10_l CH11_l CH12_l RCH9/Al
Tl 0.53 0.44 0.41 0.45 0.56 0.48 0.79 0.86 0.80 0.30
Bn 0.68 0.57 0.49 0.81 0.68 0.47 0.37 0.30 0.53
Pl 0.67 0.25 0.73 0.77 0.50 0.33 0.21 0.69
Pw 0.32 0.66 0.55 0.40 0.33 0.19 0.39
CH1_l-CH8_l 0.39 0.41 0.33 0.16 0.21 0.13
Aw 0.78 0.57 0.42 0.32 0.65
CH9_l 0.56 0.41 0.12 0.82
CH10_l 0.78 0.38 0.43
CH11_l 0.52 0.40
CH12_l 0.06
1
0.5
0

Figure 10

PCA based on 11 morphological characters; specimens labeled according to their number of branchial pairs.

Shadow A= 8 branchial pairs; B= 5–6–7 pairs; C= 4 pairs; abbreviations are explained in the methodology section.

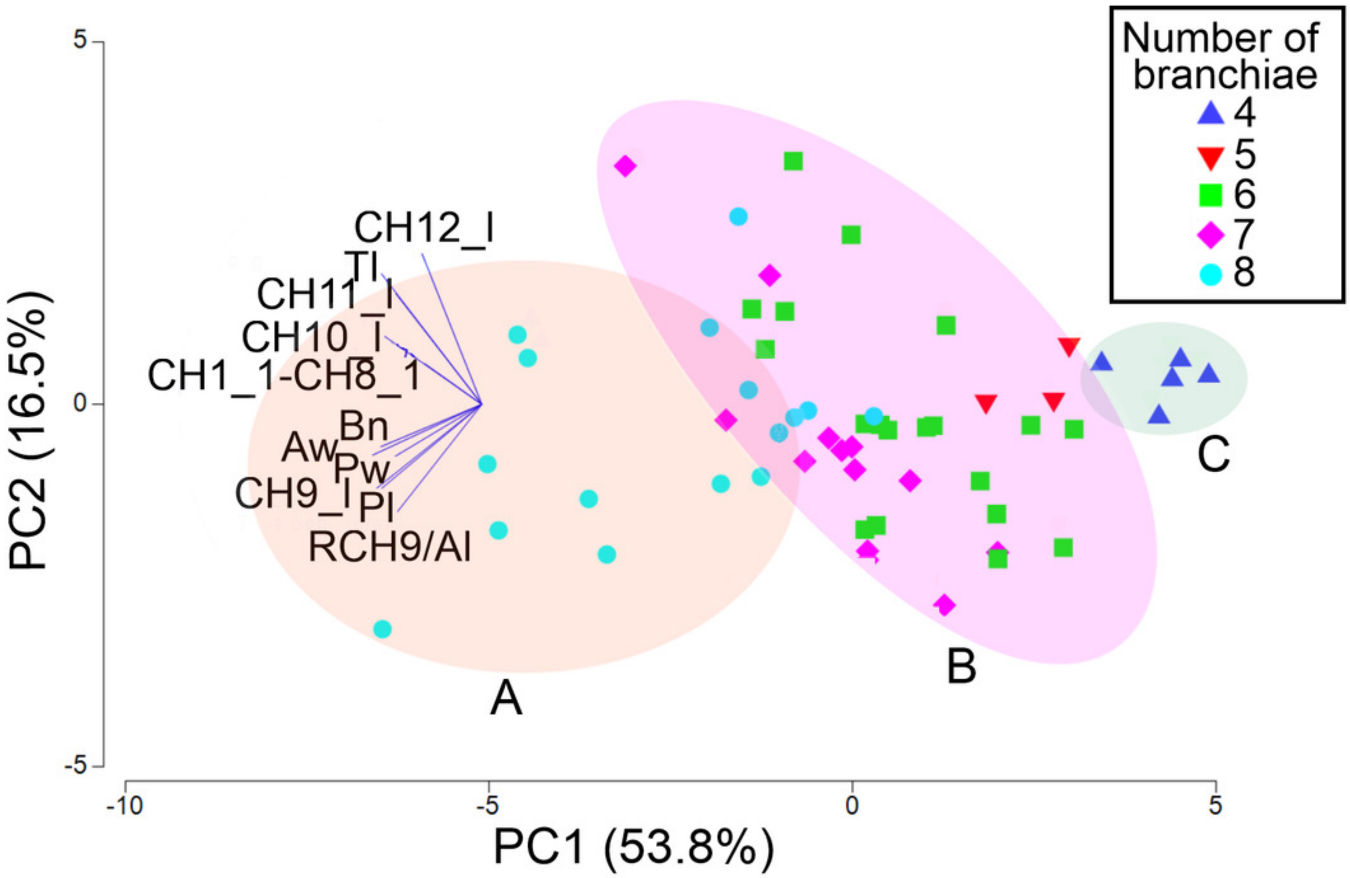

Figure 11

Canonical analysis based on the first and second discriminant functions.

A= morphotype A (4 branchial pairs); B= morphotype B (5–6–7 pairs); C= morphotype C (8 pairs).

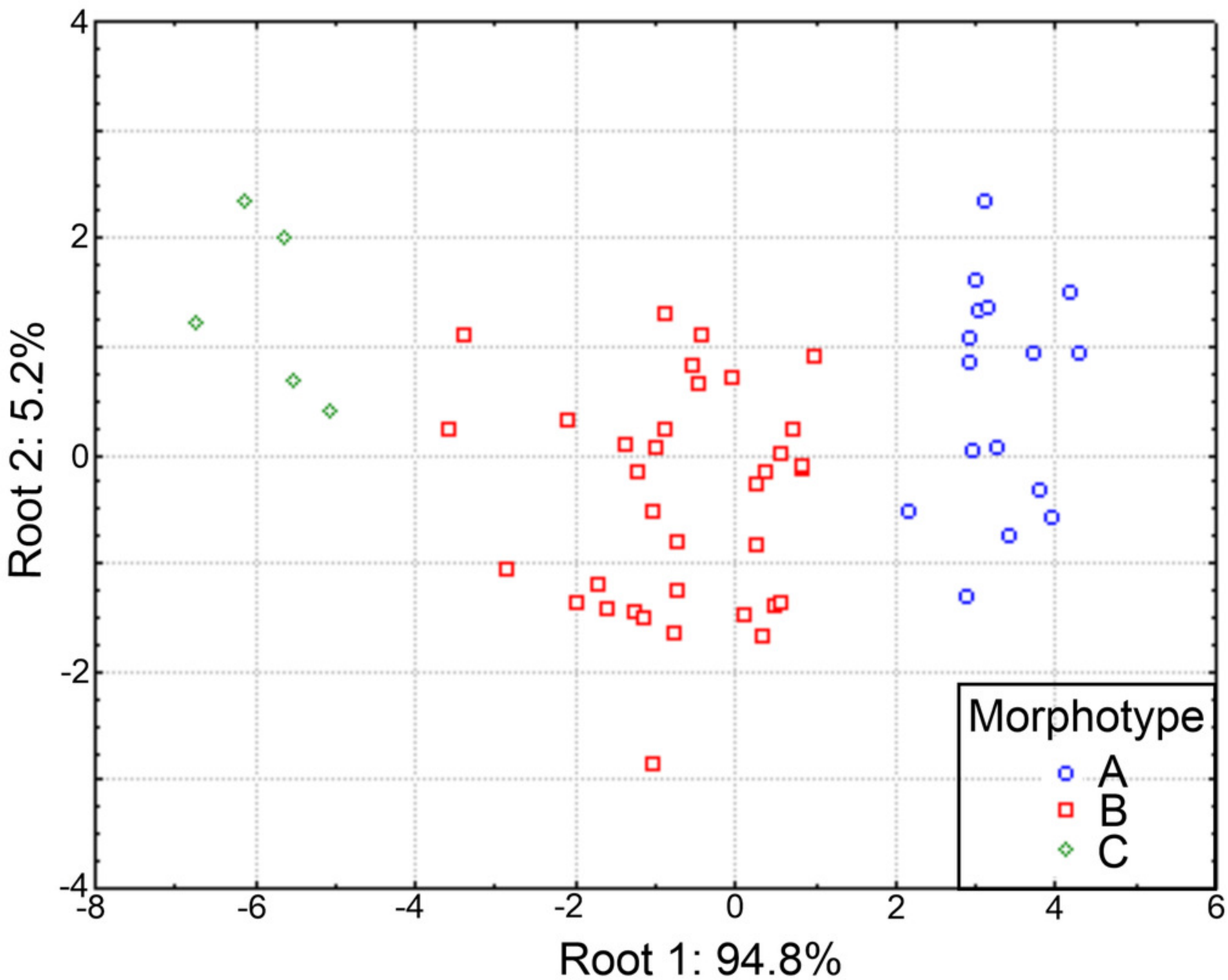

Figure 12

PCA for the first two components based on the environmental conditions where the three morphotypes (A, B, C) were found.

Stations were labeled according to their sampling season: W-S= winter-spring season; S-A= summer-autumn season.

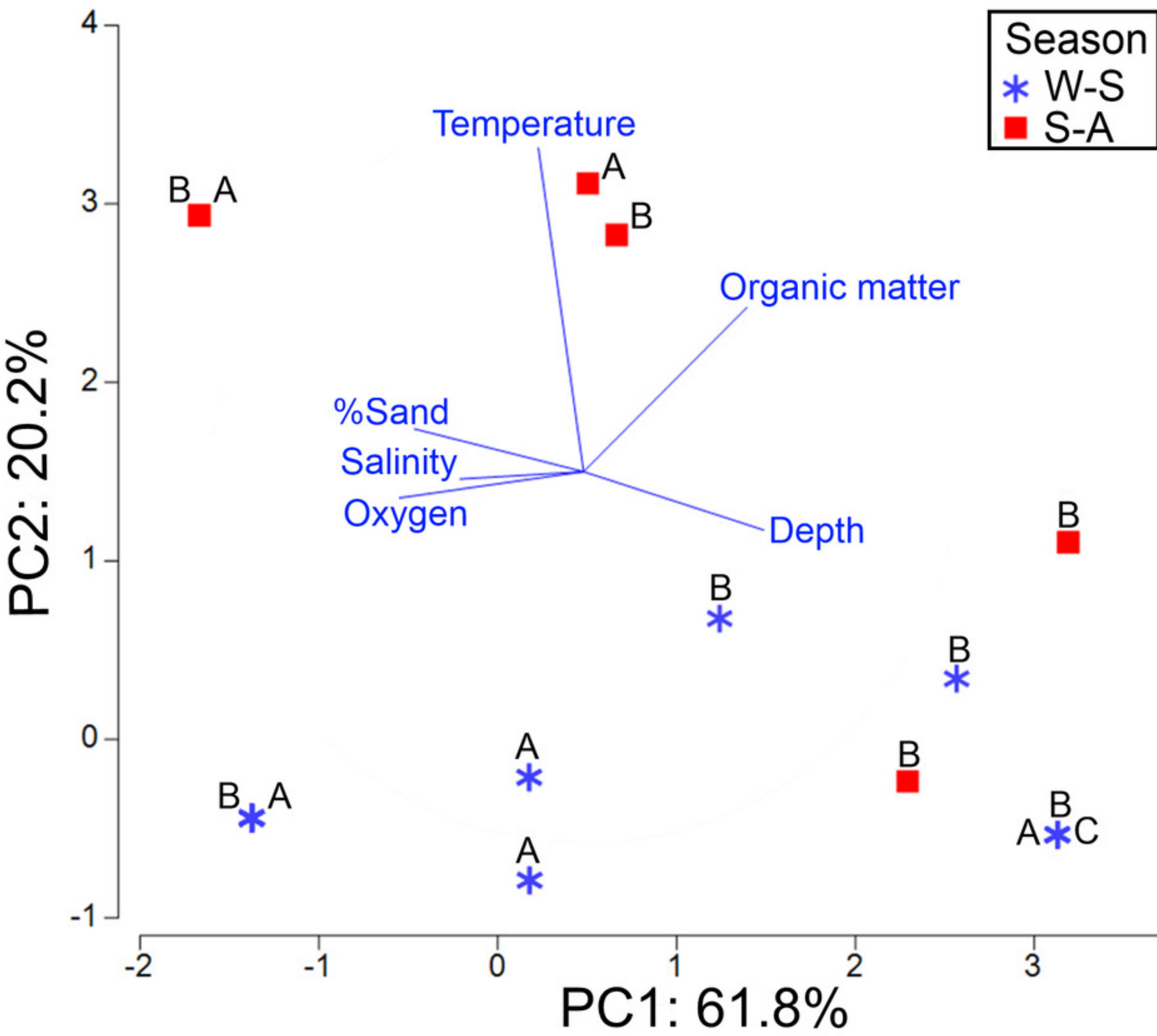

Table 1(on next page)

Location, environmental data and number of individuals of *Heterospio viariabilis* sp. nov. by sampling station in the Gulf of California.

Table 1:

Location, environmental data and number of individuals of *Heterospio viariabilis* sp. nov. by sampling station in the Gulf of California.

Station	Latitude (N)	Longitude (W)	Depth (m)	Salinity (psu)	Temperature (°C)	Dissolved oxygen (ml/L)	Organic matter (%)	Sand (%)	Sediment type	Number of specimens
37-Cortes 2	31°16.1'	114°21.7'	30.3	35.51	16.0	5.40	2.4	85	Fine sand	30
38-Cortes 2	31°08.3'	114°13.3'	71.9	35.45	14.5	3.17	-----	-----	-----	2
52-Cortes 2	25°39.9'	109°28.6'	28.6	35.19	16.8	5.40	3.6	58	Fine sand	2
51-Cortes 2	25°42.1'	109°30.6'	49.5	35.15	14.8	1.80	7.2	58	Fine sand	1
50-Cortes 2	25°46.8'	109°35.4'	97.0	34.99	13.2	1.47	5.7	62	Fine sand	10
61-Cortes 2	20°53.9'	105°27.5'	50.4	34.92	16.8	1.03	5.5	94	Fine sand	1
37-Cortes 3	31°19.8'	114°23.2'	21.5	36.06	29.6	4.26	5.00	91	Very fine sand	3
44-Cortes 3	30°00.5'	112°59.5'	106.0	35.63	19.4	2.56	8.40	52	Very fine sand	2
15-Cortes 3	26°53.2'	110°05.9'	39.0	34.80	28.1	3.83	6.10	81	Fine sand	2
52-Cortes 3	25°43.6'	109°29.3'	22.1	34.20	30.0	4.34	5.30	83	Very fine sand	1
50-Cortes 3	25°49.5'	109°37.9'	80.0	35.22	17.6	2.22	3.80	49	Very fine sand	1
C8-7	23°14.2'	106°26.8'	7.0	34.78	23.4	-----	-----	-----	-----	1

Table 2(on next page)

Summary of the morphological characters of *Heterospio* species without acicular hooks on neuropodia 1 and with chaetiger 9 (first elongated) only 2–3x longer than chaetiger 8.

Completed from Blake & Maciolek, 2023.

Table 2:

Summary of the morphological characters of *Heterospio* species without acicular hooks on neuropodia 1 and with chaetiger 9 (first elongated) only 2–3x longer than chaetiger 8. Completed from Blake & Maciolek, 2023.

Morphological characters / Species	*H. africana* Blake & Maciolek, 2023	*H. brunei* Blake & Maciolek, 2023	*H. ehlersi* Blake & Maciolek, 2023	*H. guiana* Blake & Maciolek, 2023	*H. hartmanae* Blake & Maciolek, 2023
Prostomium	Conical, rounded anteriorly	Conical, tapering anteriorly	Triangular, tapering anteriorly	Triangular, tapering anteriorly	Pear-shaped, tapering anteriorly
Peristomium	2 rings; large dorsal crest	2 rings; incomplete dorsally	1 ring; incomplete dorsally	2 rings	2 rings
First elongated chaetiger	9; ± 2.5x longer than CH8	9; ± 2x longer than CH8	9; ± 2.5x longer than CH8	9; ± 3x longer than CH8	9; ± 2.5x longer than CH8
Chaetigers with branchiae	CH2-CH6 (5 pairs)	CH2-CH5 (4 pairs)	CH2-CH4 (3 pairs)	CH2-CH7–8 (6–7 pairs)	CH2-CH5 (4 pairs)
Neuropodial postchaetal lobes	Short on CH1-CH6	Absent	Absent	Absent	Absent
Modified chaetae of elongated segments	Mostly encircling body from CH10. CH10-CH11: capillaries; CH12-CH13: subuluncini; from CH14 with acicular spines, rarely aristate spines	Forming cinctures from CH10. CH10: acicular spines; CH10-CH19: acicular spines and capillaries	Cinctures not present until chaetigers 20–23. CH10: acicular spines; CH11-CH23: acicular spines and capillaries	Entirely encircling body from CH10. CH10: capillaries; CH11-CH12: aristate spines and capillaries	Forming cinctures from CH10. CH10-CH24: acicula spines and capillaries
Posterior end	Bulbous posterior (4 chaetigers) end with curved hooks; large folds surrounding anus	Bulbous posterior end (4 chaetigers) with 1–2 acicular spines each	Bulbous posterior end (3 chaetigers) with 1–2 spines	Unknown	Bulbous posterior end (3 chaetigers) with 2 spines in each ramus
Depth	55 m	1,400–1,922 m	60–70 m	520–550 m	2,470–4,950 m
Habitat	Sand and mud sediment	Silty clay sediment with few grain size particles; 0.9–3.5% organic carbon	-----	-----	Silty clay sediment
Distribution	Off Mozambique, Eastern Africa	Off Borneo, Southern China	Gulf of Thailand, Southern China	Suriname, NE South America	NW Atlantic; Off Eastern North America
Source	Blake & Maciolek, 2023	Blake & Maciolek, 2023	Blake & Maciolek, 2023	Blake & Maciolek, 2023	Blake & Maciolek, 2023

5 **Table 2. Continue…**

Morphological characters / Species	*H. indica* Parapar et al., 2016	*H. knoxi* Blake & Maciolek, 2023	*H. paulolanai* Blake & Maciolek, 2023	*H. peruana* Borowski, 1994	*H. variabilis* sp. nov.
Prostomium	Triangular, rounded anteriorly	Triangular, rounded anteriorly	Pear-shaped, tapering to rounded anteriorly	Conical, rounded anteriorly	Conical, rounded anteriorly
Peristomium	1 ring, with dorsal crest	2rings, with dorsal crest	2 rings, incomplete dorsally	2 rings, incomplete dorsally	2 rings, incomplete dorsally, dorsal crest
First elongated chaetiger	9; ± 2x longer than CH8	9; ± 3x longer than CH8	9; ± 2.5x longer than CH8	9; ± 2–3.5x longer than CH8	9; ± 2x longer than CH8
Chaetigers with branchiae	CH2-CH8–9 (7–8 pairs)	CH2-CH8–9 (8 pairs)	CH2-CH8 (7 pairs)	CH2 to CH5 (CH6)	CH2-CH6–9 (4–8 pairs)
Neuropodial postchaetal lobes	Prominent on CH1-CH9	Low flanges on CH1-CH9	Low ridges	Absent	Short on CH4–5 to CH9
Modified chaetae of elongated segments	Forming cinctures. CH10: thin and robust capillaries, highly flattened in distal half; CH11 CH13: capillaries and subuluncini	Mostly encircling body. CH10: thin and thicker capillaries; CH11: subuluncini and acicular spines; CH12-CH13: aristate and acicular spines	Nearly surrounding body. CH10: capillaries; CH11: aristate spines and capillaries; CH12: acicular spines and subuluncini	Forming cinctures. CH10: subuluncini and capillaries; CH11-CH12: thickened, aristate spines, many lacking aristae, acicular spines and capillaries	Forming cinctures. CH10: subuluncini; CH11-CH13: aristate and acicular spines; from CH14 with thick acicular spines
Posterior end	Bulbous (5 chaetigers) with 2–4 acicular hooks each	Unknown	Unknown	Bulbous (5 chaetigers) with recurved hooks	Unknown
Depth	2.5–22 m	13–61 m	69 m	4,125–4,423 m	7–106 m
Habitat	Mostly clayey silt and sandy silt sediments	Grey sands to fine muddy sands; 13–13.3°C	-----	Manganese nodule area	49–94% fine sand; 13.2–30°C; 34.2–36.06 ups; 2.4–8.4% organic matter; 1.03–5.4 ml/L oxygen
Distribution	Malvan, Western India; Sudan, Arabian Sea	North Island, Hawke Bay, New Zealand	Off NE Brazil	Peru Basin; Peru-Chile Trench	Gulf of California
Source	Parapar et al. 2016; Blake & Maciolek, 2023	Knox, 1960; Blake & Maciolek, 2023	Blake & Maciolek, 2023	Borowski, 1994; Blake & Maciolek, 2023	This study