

Distribution patterns of polychaetes associated to artificially hydrocarbon-enriched bottoms, with the description of *Sigambra nkossa* (Annelida, Pilargidae) (#68662)

1

First submission

Guidance from your Editor

Please submit by **24 Feb 2022** for the benefit of the authors (and your \$200 publishing discount) .



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.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files

Download and review all files from the [materials page](#).

8 Figure file(s)

5 Table file(s)

2 Raw data file(s)

! Custom checks

Field study



Have you checked the authors [field study permits](#)?



Are the field study permits appropriate?

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.



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.

Distribution patterns of polychaetes associated to artificially hydrocarbon-enriched bottoms, with the description of *Sigambra nkossa* (Annelida, Pilargidae)

Daniel Martin^{Corresp., 1}, João Gil², Claude-Henri Chaîneau³, Sébastien Thorin⁴, Romain Le Gall⁴, Éric Dutrieux⁵

¹ Center for Advanced Studies of Blanes, Consejo Superior de Investigaciones Científicas, Blanes, Catalunya, Spain

² Centre of Marine Sciences, CCMAR, University of Algarve, Faro, Portugal

³ Department of HSE/EP/Environment, TotalEnergies, Paris, La Défense Cedex, France

⁴ Creoccean, Montpellier, France

⁵ Creoccean, Montpellier, France

Corresponding Author: Daniel Martin
Email address: dani@ceab.csic.es

The monitoring of the N’Kossa offshore oil and gas fields (Republic of Congo) allowed us to assess the ecological traits of two polychaete species belonging to *Sigambra* (Annelida, Pilargidae): *Sigambra parva*, occurring in very low densities at all bottoms except the most impacted, and an undescribed species that reached $>4,000$ ind. m^{-2} in hydrocarbon-enriched sediments. Their distribution patterns are compared with those of other polychaetes showing a range of affinities for hydrocarbon-enriched sediments in the N’Kossa region. Our results suggest that *S. parva* would be a representative of the original local fauna, while the species associated to artificial hydrocarbon-enriched sediments could be natively associated with natural hydrocarbon-enriched sediments, using the former as alternative habitats and as dispersal stepping stones. This ecological segregation, together with a careful morphological and morphometric analyses led us to described the later as a new species, namely *Sigambra nkossa* sp. nov. Moreover, the morphometric analysis allowed us to discuss on the taxonomic robustness of the key morphological characters of *S. nkossa* sp. nov., as well as to emend the generic diagnosis of *Sigambra* to allow including the new species.

Distribution patterns of polychaetes associated to artificially hydrocarbon-enriched bottoms, with the description of *Sigambra nkossa* (Annelida, Pilargidae)

Daniel Martin^{1,*}, João Gil², Claude-Henri Chaîneau³, Sébastien Thorin⁴, Romain Le Gall⁴, Éric Dutrieux⁴

¹ Department of Marine Ecology, Centre d'Estudis Avançats de Blanes (CEAB – CSIC), Blanes (Girona), Catalunya (Spain).

² Centre of Marine Sciences, CCMAR, University of Algarve, Faro, Portugal.

³ Department of HSE/EP/Environment, Total Energies, Paris la Défense Cedex, France

⁴ Creocan, Montpellier, France.

Corresponding Author:

Daniel Martin

Carrer d'accés a la Cala Sant Francesc 14 Blanes, Blanes (Girona), Catalunya, 17300, Spain

Email address: dani@ceab.csic.es

Abstract

The monitoring of the N'Kossa offshore oil and gas fields (Republic of Congo) allowed us to assess the ecological traits of two polychaete species belonging to *Sigambra* (Annelida, Pilargidae): *Sigambra parva*, occurring in very low densities at all bottoms except the most impacted, and an undescribed species that reached >4,000 ind. m⁻² in hydrocarbon-enriched sediments. Their distribution patterns are compared with those of other polychaetes showing a range of affinities for hydrocarbon-enriched sediments in the N'Kossa region. Our results suggest that *S. parva* would be a representative of the original local fauna, while the species associated to artificial hydrocarbon-enriched sediments could be natively associated with natural hydrocarbon-enriched sediments, using the former as alternative habitats and as dispersal stepping stones. This ecological segregation, together with a careful morphological and morphometric analyses led us to described the later as a new species, namely *Sigambra nkossa* sp. nov. Moreover, the morphometric analysis allowed us to discuss on the taxonomic robustness of the key morphological characters of *S. nkossa* sp. nov., as well as to emend the generic diagnosis of *Sigambra* to allow including the new species.

Introduction

The structure of the meio- and macroinfaunal assemblages has been often used as a bio-indicator to assess the environmental impact of both crude oil spills (e.g., Gómez Gesteira & Dauvin 2005; Washburn et al. 2016) and offshore hydrocarbon (HYD) extraction activities (e.g., ~~Carroll et al. 2000~~; Montagna & Harper 1996). The drill mud disposal in offshore oil and gas fields off the Congolese coasts of the Gulf of Guinea (Western Africa) is not an exception (see Denoyelle et

al. 2010, and references therein). HYDs are an important component of drilling muds that often accumulate in the bottoms surrounding offshore oil and gas platforms, leading to the presence of highly modified macroinfaunal assemblages.

Alterations caused by HYD accumulation may occur both at assemblage and individual levels and may include decreasing abundances and diversities or reduced growth and reproductive rates, respectively (e.g., Olsen et al. 2007; Washburn et al. 2016 and references herein). The macroinfaunal patterns in the vicinity of drilling platforms acting as a local sources of HYD enriched sediments (HES) often did not differ from those related to other sources of organic enrichment. It is thus possible to find the typical succession mirroring an organic enrichment gradient, ranging from scarce, more diverse, sensitive species to highly abundant, opportunistic ones, with the most enriched (i.e. polluted) areas being devoid of infauna in extreme cases (Pearson & Rosenberg 1978). The main difference in the succession patterns affects the indicator species associated to the source of organic enrichment, which in this case, are obviously HYD tolerant (Dalmazzone et al. 2004; Denoyelle et al. 2010; ~~Montagna & Harper 1996~~).

Private companies are virtually always responsible of the worldwide activities related with offshore HYD routine exploration and production. Thus, access to these highly interesting macrofaunal and environmental data is often only possible through environmental assessment or monitoring projects. These projects are frequently a legal imperative to companies exploiting natural resources in the sea and habitually include benthic monitoring. Thus, they regularly generate large macrobenthic datasets (sometimes including reference invertebrate collections), from often previously poorly or non-studied areas. This is the case of the surveys carried out in association to the oil and gas activities of Total Energies (TE) in the Atlantic coasts of the Gulf of Guinea facing the Republic of Congo. The datasets of benthic macrofauna generated have been partly managed through the long-term collaboration between the scientists of the Centre d'Estudis Avançats de Blanes (CEAB-CSIC) and the French company CREOCEAN. The in-deep analysis of these datasets proved to have a high scientific and management interest. Among the currently existing outputs, there are studies on sediment toxicity and recolonization (Dalmazzone et al. 2004) or on the contrasted role as indicators of foraminifers vs. macrofauna (Denoyelle et al. 2010).

Framed within the studies sponsored by TE around the N'Kossa field, we are here analysing the influence of the environmental characteristics of the area on the distribution of several polychaete species showing a range of affinities for HES, which were found during the monitoring surveys carried out by CREOCEAN and the CEAB-CSIC. These include *Capitella* sp. (Capitellidae), *Raricirrus* sp. (Ctenodrilidae), *Paramphinome trionyx* Intes and Le Loeuff, 1975 (Amphinomidae), *Oxydromus berrisfordi* (Day, 1967) (Hesionidae), *Lindaspio sebastiena* Bellan, Dauvin and Laubier, 2003 (Spionidae), a unidentified species of Ampharetidae (referred to as Ampharetidae sp.) and two species of *Sigambra* (Pilargidae), *Sigambra parva* (Day 1963) and an undescribed species (Bellan et al. 2003; Day 1963; Day 1967; Intès & Le Loeuff 1975). The implications of the presence of HES-associated species in artificially enriched, off-shore, shallow-water soft bottoms are discussed in light of the currently available knowledge on the ecological relationships between polychaetes and HYD.

We would also like to stress the importance of the studies focusing on taxonomy, which constitute an excellent example of the potential contribution of monitoring surveys to increase the current knowledge on marine biodiversity. Accordingly, several new species have been described from areas of the Congolese region exploited by TE. These include the vesicomid

bivalve *Isorropodon bigoti* Cosel and Salas, 2001, and the two polychaete annelids *Lindaspio sebastiena* Bellan, Dauvin and Laubier, 2003 (Spionidae) and *Anotochaetoneo michelbhaudi* Britayev and Martin, 2006 (Polynoidae) (Bellan et al. 2003; Britayev & Martin 2006; Cosel et al. 2001). To date, the first two species are known only from their type localities, which are the HES bottoms just below the extraction platforms of the N’Kossa field, while the third one has also been recently reported from muddy bottoms in the Gulf of Cádiz, in an area rich in mud volcanoes and pockmarks (Ravara et al. 2017).

In the present paper, we are reporting the third new polychaete for the N’Kossa field, a species of *Sigambra* (Pilargidae) that we are here naming as *Sigambra nkossa* sp. nov. Together with its formal description, we are comparing *S. nkossa* sp. nov. with all previously known species of *Sigambra*, we are performing a morphometric analysis of the relationships between size and several taxonomically relevant features, which allows us to discuss on its taxonomic robustness, and we emended the generic diagnosis of *Sigambra* to include the new species.

Materials & Methods

Sample collection, treatment and statistical analyses

Samples were collected in the N’Kossa oil and gas field, at the area surrounding the NKF I and NKF II platforms (off the Republic of Congo, Gulf of Guinea, Atlantic coasts of western Africa) (Fig. 1). Sediments were sampled at around 180 m depth using a Van Veen grab (35 x 42 x 90 cm, about 0.1 m² per grab) in November 2000, March 2002 and April 2003. Stations 2, 3, 4 and 6 were sampled during the three surveys; stations 1, 5 and 7–10 only in 2000; stations 13 and 16 in 2002 and 2003; station 15 only in 2002; and stations 17–20 only in 2003.

Three grabs were collected at each station. Each grab contents were mixed in a sufficiently large container and then sieved out on board by pouring the contents through a 1 mm mesh sieve. The retained sediment was then transferred to a plastic bag, fixed with a 4% formaldehyde/seawater solution, stained with Rose Bengal and stored until sorting. The macrofaunal taxa were preliminarily sorted and identified under a stereomicroscope (Zeiss Stemi 2000-C), and preserved in 70% ethanol. All specimens of the eight target polychaete species were counted to express their densities as number of individuals per m² (Supplementary data 1).

The stations were characterized by the distance from drilling point (DDP) expressed in meters (m), as well as by a series of sediment descriptors (Supplementary data 1). Laser granulometry (% volume) was performed on dry sediments after sifting through a 0.8 mm mesh sieve using a laser coulter LS 230. Sediments were characterized by the percentage of silt and clay (S&C, diameter < 63 µm) and coarse sand (CS, 0.2 mm < diameter < 2 mm). Pore water content (PW, % in volume) was measured according to the European experimental AFNOR standard NF ISO 11465. Concentration of total organic matter (OM, % dry weight) was calculated by steam drying at 105°C, according to the AFNOR standard Pr EN 12879. Contents of Nitrogen (N) and phosphorous (P), two nutrients that are known to be highly affected by the presence of HYS spill in marine systems (Al-Hawash et al. 2018), were estimated as % in volume, according to the AFNOR standards NF ISO 11261 and NF EN 1189, respectively. Concentrations of HYD in the sediment were determined by the infrared method according to the AFNOR standard XP T 90–114 and expressed as dry weight mg/kg. Barium (Ba) contents, a metal commonly used as a tracer of the dispersal of drill cuttings on the sea floor (Kennicutt et al. 1982), were estimated after mineralization by total attack in acid medium according to the ICP protocol and expressed as mg/kg of sediment. All physical-chemical analyses were performed by the Laboratoire Municipal et Régional de la Ville de Rouen, France. The methods

and results of these analyses are fully described in Dalmazzone et al. (2004).

The possible existence of temporal and spatial differences in sediment descriptors, as well as in the presence of the target species was assessed by parametric multidimensional analysis (PMDA), based on the Mahalanobis distance and applying the Bonferroni correction to prevent Type I errors (Bland & Altman 1995). The influence of sediment descriptors on the studied samples was analyzed by Principal Component Analysis (PCA). Both types of data, sediment descriptors and polychaete densities, were plotted on the obtained axis to graphically visualize their distributions. The relationships between species densities and sediment descriptors were assessed by Pearson correlation. The significance of the differences between the sample groups obtained in the PCA was assessed by Analysis of Similarity (ANOSIM) and confirmed by PMDA (same procedure as above), both for sediment descriptors and target species. The significance of the differences for each sediment descriptor and target species was then assessed by independent one-way Analysis of Variance (one-way ANOVA), and the group(s) responsible for the observed differences were assessed by a post hoc Tukey test. All analyses were based on log-transformed data to meet with the assumptions of normality and homoscedasticity required for parametric analyses (Zar 1984) and were performed with the XLSTAT software, version 2016.02.27390 (copyright Addinsoft 1995–2016), except the PCA and ANOSIM, which were performed with the respective routines of the Primer 6 software, version 6.1.11, and Permanova, version 1.0.1 (copyright Primer-e Ltd. 2008).

Taxonomy

Light micrographs of preserved specimens were made with a Zeiss Axioplan (body) and a Zeiss Stemi 2000–c (chaetae) stereomicroscopes equipped with a CMEX5 digital camera (Euromex). For Scanning Electron Microscope (SEM) observations, the worms were washed three times in distilled water (30 min each), run through a series of increasing ethanol concentrations, and stored in 70% ethanol until observation. Immediately prior to viewing in a Hitachi S-3500N (SEM Service of the Centre Mediterrani d'Investigacions Marines i Ambientals of Barcelona, Spain, CMIMA–CSIC), they were run through a series of increasing ethanol concentrations ending with 100%, critical point dried, attached to a stub, and coated with gold. All images were captured and stored in digital format using Printerface System hardware and software (GW Electronics & K.E. Development Ltd.).

Type and non-type specimens of the new species are deposited in the collections of the Centre d'Estudis Avançats de Blanes (CEAB), the Museo Nacional de Ciencias Naturales (MNCN, Madrid, Spain), Museu Nacional de História Natural e da Ciência (MB, Lisboa, Portugal), and Senckenberg Research Institute and Natural History Museum (SMF, Frankfurt, Germany). The specimens of *S. parva* are deposited in the CEAB and the MNCN.

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (International Commission of Zoological Nomenclature 1999), 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:0FCB04BD-5ACA-4808-8091-67F7EF106021. The

online version of this work is archived and available from the following digital repositories:
PeerJ, PubMed Central SCIE and CLOCKSS.

Morphometry

The number of chaetigers was used as surrogate of size. All relevant morphological features susceptible to be size-dependent were identified and counted or measured in a selected subset of 21 entire individuals of *S. nkossa* sp. nov. (Supplementary data 2). Dry weight was estimated after drying (60 °C) for 24 h. The relationships between size and morphological features were assessed by Pearson correlation, using the same procedures and software as for the ecological analyses.

Results

Ecology

Neither the most relevant environmental descriptors, DDP and HYD (Table 1A), nor the populations of the targeted polychaetes show significant differences during the three surveys (Table 1B). Thus, for the purposes of this study, all surveys are considered together.

When analyzing the distributions of the target species with respect to the sediment descriptors (Table 2C), the most common pattern is a significant increase with rising levels of Ba, occurring for all species except *S. parva* and *P. trionyx*. Only *Sigambra nkossa* sp. nov., *L. sebastiena*, *Raricirrus* sp. and Ampharetidae sp. also show significant increasing densities with rising levels of HYD. Among them, the first two show the same trend in relation with OM, but the density of *L. sebastiena* decreases with the rise of PW. Finally, both the densities of *Capitella* sp. and Ampharetidae sp. increase with rising levels of N and decrease with increasing levels of CS and P, with those of *Capitella* sp. also increasing with S&C and those of Ampharetidae sp. significantly decreasing with DDP.

The first axis of the PCA (Eigenvalue 15.5, 84.4% of the explained variation) is defined by the opposite trend of Ba and, especially HYD, on the negative (left) sector and DDP on the positive (right) sector (Fig. 2). Axis two (Eigenvalue 2.15, 11.7% of the explained variation) is defined by the opposite trend of S&C on the negative (left) sector and, specially CS, on the positive (right) sector (Fig. 2). The densities of *S. nkossa* sp. nov. and *S. parva* plotted on the PCA axis clearly show the disjunct distributions of these two species, highlighting the particularly high densities of the new species in the stations with HES (Fig. 2).

The sampling stations group in three significantly different groups (ANOSIM: Global R = 0.846, significance level = 0.1%) according to the PCA, which coincide with markedly different HYD ranges (Fig. 2). The significance of the differences is confirmed by PMDA (Table 2B). Group I includes the stations 5 and 6 (HYD > 20000 mg/kg DW of), group II includes the stations 4, 7, 13, and 18 (HYD: 20000–100 mg/kg DW) and group III includes the stations 1–3, 8–11, and 15–16 (HYD < 100 mg/kg DW). The most significant differences occur always when group III is involved (Table 2A). Group I is very homogeneous but, within groups II and III, two subgroups can be respectively distinguished. Characteristically, the smallest subgroups including stations 4, 13 and 18 (from 2003) and 13 (from 2002) in group II, and 2, 13, and 19 (all from 2003) in group III, are mainly isolated from the other stations due to the extremely low CS (<2%). The differences between these two subgroups and the remaining stations of each group prove to be also significant (ANOSIM: Global R = 0.944 / 0.630, significance level = 2.9% / 1.5%, respectively).

When analyzed individually, DDP, HYD, and Ba show highly significant differences between the PCA groups, but also S&C and P slightly differ (Table 2D, Fig. 3). The pairwise analyses (Table 3) show: in group III, DDP is always significantly higher and HYD and Ba are always significantly lower; neither DDP, nor Ba differ between groups I and II, while HYD is significantly much higher in the former; S&C is only slightly lower in group I than in group II, while none of them differ from group III, and there is no clear pattern for P (Fig. 3). Finally, CS, PW, OM and N do not show significant differences between the PCA groups (Table 3, Fig. 3).

The density of the target benthic species also shows significant differences in the PCA groups (Table 2C), with all species having also clearly different distribution patterns (Table 3, Fig. 2). *Sigambra nkossa* sp. nov., *Capitella* sp. and *O. berrisfordi* have non-significantly different densities between groups I and II, which are significantly higher than those in group III. In fact, *O. berrisfordi* is completely absent from group III, as they are *L. sebastiena* and *S. parva*. *Raricirrus* sp. shows significantly higher densities in group I than in group III, while those in group II do not differ from those in groups I and III. *Paramphionome trionyx* shows non-significant differences in density between group I and groups II and III, while the density is significantly higher in group II than in group III. Finally, Ampharetidae sp. shows significantly higher densities in group II than in groups I and III, which, do not differ from each other.

Taxonomic account

Family Pilargidae Saint-Joseph, 1899

Sigambra Müller, 1858

Sigambra. Müller (1858): 214 (original description); Pettibone (1966): 179 (genus reinstated with key to species); Licher & Westheide (1997): 2 (key to species and synoptic table with characters of all species); Nishi et al. (2007): 65 (synoptic table with characters of all species); Salazar-Vallejo et al. (2019): 24 (diagnosis), 44–46 (key to species).

Type species. *Sigambra grubii* Müller, 1858, by monotypy.

Diagnosis. Emended from Salazar-Vallejo et al. (2019). Pilarginae with body depressed, usually obconic. Prostomium with three antennae, longer than palps; palps biarticulate. Tentacular cirri as long as half width of tentacular segment. Parapodia biramous. Dorsal and ventral cirri foliose to tapered, dorsal ones usually longer than ventral ones. Notopodium with one strongly recurved dorsal hook, or with one strongly recurved dorsal hook and one slightly bent spine along many segments, sometimes with protruding tips of notoacaculae. Neuropodia with shorter pectinate capillaries, medium-sized denticulate capillaries, and longer finely denticulate capillaries, often twisted distally.

Sigambra nkossa sp. nov.

LISID: urn:lsid:zoobank.org:act:9015B0CC-4C6B-4DFF-A9AE-F403F512AE2B.

Figures 4–5

Material examined: Holotype: CEAB-A.P. 913A, entire specimen, N’Kossa oil and gas field, Republic of Congo, Gulf of Guinea, Atlantic coasts of western Africa (approximately 05°16’S, 11°34’E), collected by DM in 2003, on soft sediments at around 180 m depth, fixed in a 4%

formaldehyde/seawater solution, preserved in 70% ethanol. Paratypes: CEAB-A.P. 913B, 1 specimen in two fragments, anterior end dissected to reveal the pharynx, other data as in holotype; CEAB-A.P. 913C, 1 entire specimen, prepared for SEM, other data as in holotype; CEAB-A.P. 913D to 913J, several hundreds of specimens collected by ED and DM, in 2000, 2002 and 2003, other data as in holotype; MNCN 16.01/19138, 10 specimens, data as in holotype; MB29-000359 – MB29-000368, 10 specimens, data as in holotype; SMF 31812 – 31821, 10 specimens, data as in holotype.

Diagnosis. Prostomium bilobed, with biarticulate palps; median antenna longer than lateral ones; proboscis with fourteen pointed distal and numerous subdistal papillae; peristomium with a transverse row of epidermal papillae; dorsal tentacular cirrus longer than ventral one, dorsal parapodial cirrus much longer than ventral cirrus (which is absent from chaetiger 2); pointed neurochaetae serrated and pectinated; notopodial hook from chaetiger 5 to body end, notopodial spines from chaetiger 9 to body end; tip of notoacacula protruding from acicular lobe from mid-body parapodia to body end.

Description. Body measuring 2.8–26 mm long, 0.6–1.9 mm wide for 23–134 segments. Body dorsoventrally flattened, with faint blackish dorsal and ventral pigmentation on prostomium, peristomium, antennae and tentacular cirri, almost disappearing in preserved specimens (Fig. 4A–C). Eyes lacking (Fig. 4A, B). Prostomium bilobed; palps biarticulated, with large palpophores and small palpostyles; interpalpal area distinct, anteriorly depressed, widely expanded posteriorly (Fig. 4B, C). Three antennae slender, tapering distally, on posterior half of prostomium; median antenna 1.5 to 2.0 (1.7 on average) times as long as lateral ones; laterals clearly surpassing palp tips, median antenna reaching chaetiger 2 (Fig. 4A, B); lateral antennal furrow distinct, slightly divergent (Fig. 5A).

Tentacular segment three times wider than longer, with two pairs of slender and subequal tentacular cirri, slightly longer than lateral antennae, and two small anteriorly projected lobes between lateral antennae and tentacular cirri and a central anteriorly projected lobe ventrally (Figs. 4B, C and 5A, D). Small epidermal papillae, 5–9 µm in diameter, near posterior prostomial margin, in a single row laterally, in several middle rows, between bases of lateral antennae (Fig. 5A, B). Rows of similar epidermal papillae on posterior margin of each parapodium, dorso-laterally near bases, 11–16 papillae on each side.

Proboscis with fourteen distal conical papillae, with long pointed ends in two lateral groups of four papillae, usually bigger than dorsal (three papillae) and ventral (three papillae) groups (Figs. 4F and 5C, D); two central longitudinal rows with numerous randomly distributed small papillae on both sides dorsally (Fig. 4E), numerous randomly distributed small papillae ventrally (Fig. 4F) and five bigger papillae on a transversal row and several randomly distributed and progressively smaller papillae laterally (Fig. 4G, 5D); all papillae pointed, with cusps directed backwards in totally everted proboscis.

Parapodia sesquiramous; dorsal and ventral cirri tapering distally, first dorsal cirri 1.7 longer than central antenna, 2.5 longer than remaining ones, being progressively longer and slender in middle and posterior segments; dorsal cirri 1.5 longer than ventral in anterior segments, to up to three times longer in posterior most segments (Fig. 4H, I, J). Notopodia with dorsal cirrus and one or two pointed aciculae, one with curved end protruding from acicular lobe in posterior segments and one straight, non-protruding; one emergent hook from chaetiger 5 (5–6), one emergent spine from chaetiger 9 (9–12), both present until posterior-most chaetigers, more

exposed posteriorly (Figs. 4I–L and 8C, D). First chaetiger with dorsal cirri longer than tentacular cirri and following dorsal cirri (Fig. 4A–C); second chaetiger with dorsal cirri shorter than following ones, lacking ventral cirrus (Figs. 4A–C, H, I, J). Neuropodia well-developed; ventral cirri similarly shaped but shorter than dorsal ones, extending far beyond neuropodial lobe tips, longer posteriorly (Fig. 4A, B, D); acicula straight, pointed, sometimes slightly protruding outside acicular lobe; parapodial lobe blunt, almost square; about 10 short pectinate supraacicular chaetae with long spinulation and filiform tips; about same number of slightly longer, distally pointed pectinate chaetae; numerous minutely serrated, distally pointed, capillary chaetae of variable length but much longer than the other neurochaetae; spinulation always directed upwards, thicker in shorter chaetae and in proximal part of longer chaetae (Fig. 5E, F).

Pygidium with two slender anal cirri, pointed, of about same length as first dorsal cirri (Fig. 4A).

Etymology. The specific epithet “*nkossa*” is considered as a substantive in apposition, referring to the N’kossa oil and gas field, off the Republic of Congo, the type locality of the species.

Distribution. Known only from the N’Kossa oil and gas field (approximately 05°16’S, 11°34’E), western Atlantic Ocean, about 60 km off the coasts of the Republic of Congo (Gulf of Guinea); associated to HES (Fig. 1).

Sigambra parva (Day, 1963)
Figures 6–7

Material examined. CEAB-A.P. 922A – 922M, 36 specimens, N’Kossa oil and gas field, Republic of Congo, Gulf of Guinea, Atlantic coasts of western Africa (approximately 05°16’S, 11°34’E), collected by DM in 2000, 2002 and 2003, on soft sediments at around 180 m depth, fixed in a 4% formaldehyde/seawater solution, preserved in 70% ethanol; MNCN 16.01/19139, 4 specimens collected in 2003, other data as in specimens at CEAB-CSIC.

Extended diagnosis. Body dorsoventrally flattened, tapering toward pygidium. Prostomium bilobed, with palps fused at basis, biarticulate, with large palpophores and small palpostyles; with three slender antennae on posterior prostomium region, tapering distally; median antenna about 1.5 times as long as lateral ones; four subdermal eyes in trapezoidal arrangement, sometimes non-visible. Peristomium with two pairs of slender tentacular cirri, subequal or with dorsal one slightly longer, and longer than lateral antennae; two small anteriorly projected lobes between lateral antennae and tentacular cirri. Small epidermal papillae dorsally on posterior prostomium margin, and on posterior dorsolateral margin of each segment and near parapodial bases. Fourteen distal proboscideal papillae, conical with round tips (laterally one pair on each side, big; two groups of five small papillae, one ventral and one dorsal); two subdistal rows of oval, non-pointed papillae; three triangular, pointed papillae, one median and two on each lateral behind subdistal rows, with cusp pointing backwards. Parapodia sesquiramous. Notopodia with 1–2 aciculae and pointed, slender dorsal cirrus. First chaetiger with dorsal cirri longer than tentacular cirri and following dorsal cirri; second chaetiger with much shorter dorsal cirri; hooks from chaetiger 4–5, more strongly recurved and emergent posteriorly. Neuropodia well-developed, with conical acicular lobes, one straight acicula and ventral cirri similar to dorsal ones but shorter, extending beyond tip of acicular lobes and absent in chaetiger 2; numerous

pointed, minutely serrated simple chaetae; 1–6 short pointed pectinate supracircular chaetae on posterior chaetigers. Anal cirri slender, as long as dorsal tentacular cirri.

Morphometric analysis of *S. nkossa* sp. nov.

Most morphological characters measured are size-dependent (Pearson correlation > 0.7), particularly dry weight, width of chaetiger 15 (with parapodia), length of median antennae, and length of the first dorsal and ventral cirri (Pearson correlation > 0.9) (Table 3, Fig. 8). Conversely, only a few characters do not vary with size, most of them being ratios between different anterior appendages (antennae and peristomial dorsal and ventral cirri) and only three (i.e., the starting segment of notopodial hooks and spines, and the absence of ventral cirri on segment 2) refer to characters previously considered as species-specific (Supplementary data 3).

Discussion

Ecology

The first benthic samples here analyzed were collected one year after the impact on the sediments caused by oily drill cuttings. Drilling muds were mostly deposited in the surrounding bottoms just below the two N’Kossa platforms and their amount was clearly decreasing with the increasing DDP, as previously reported by Denoyelle et al. (2010). This pattern was highly consistent during all three surveys, to the extent that, with a few exceptions, the environmental parameters analyzed did not differ with time, in a similar way as it happened with the density distribution patterns of the population of the polychaete species targeted in this paper.

As a result, the stations located at a greater distance from the platforms (PCA group III) were clearly isolated from the impacted ones (PCA groups I and II), as proven by the significant presence in the latter of Ba. In all three surveys, the stations were grouped in one or another of the two impacted groups independently, to some extent, from DDP. This was likely because the locally dominant currents in the area generated different levels of accumulation whose distribution depended not only on DDP, but also of location around the platforms. In turn, our statistical analyses showed very small differences in granulometry, except for the slight gradient observed in the second PCA axis (which is likely a residual effect of the drilling deposits causing the presence of slightly bigger particles in the most HYD enriched stations). This clearly supported that, before the impact, the analyzed stations had all the same type of sedimentary bottoms. Nevertheless, the drilling disposal caused the HYD concentration to be much higher in group I than in group II stations. Therefore, the main reason explaining the differences in the distribution patterns observed for the polychaete species here targeted was the presence of HYD, as it also occurred with those of the descriptors of the whole macrofaunal community (Denoyelle et al. 2010).

Slope and shelf seeps represent organic-rich areas in the overall poor deep-sea bottoms (Washburn et al. 2018), where they may function as local, ephemeral disturbances supporting infaunal species pre-adapted to organic-rich, reducing environments (Levin et al. 2000). Natural HYD seeps provide unique habitats to many different species, although macrofaunal organisms often receive less attention than megafauna and microorganisms (Levin 2005). However, it has been suggested that, at higher taxonomic levels, there was no specific infauna that may be considered as seepage indicators (Washburn et al. 2018). Conversely, at the species level, there are numerous well-known inhabitants of these particular environments, such as siboglinid

tubeworms (Bright & Lallier 2010; Karaseva et al. 2020; Schulze & Halanych 2003) and other polychaete species belonging to Orbiniidae (Blake 2000), Nautiliniellidae (Aguado et al. 2013) and Dorvilleidae (Ravara et al. 2021) or Hesionidae (Desbruyères & Toulmond 1998). Among hesionids, for instance, *Hesiocaeca methanicola* Desbruyères and Toulmond, 1998 was originally described from methane seeps (Desbruyères & Toulmond 1998) and later found also inhabiting fossil fuel reserves (Fisher et al. 2000).

Sigambra nkossa sp. nov. responded positively to the presence of Ba in the sediments, indicating a connection with drilling cuttings, but the relationship was stronger with respect to the effective presence of HES. This pattern was completely different to that of *S. parva*, which was already present in the 1994 survey in sediments devoid of HYD, and just survived in some HES (but not in the most impacted ones). Conversely, *S. nkossa* sp. nov. was not present in 1994. From 2000 to 2003, however, both species showed enormous differences in density, with *S. parva* having a maximum of 50 ind. · m⁻² and *S. nkossa* sp. nov. reaching from 1,000 ind. · m⁻² (2000) to more than 4,000 ind. · m⁻² (2003). We thus suggest that the presence of *S. parva* obeyed to the typical composition of the local benthic assemblages in non-disturbed bottoms of the area and, although it seemed to tolerate HYD to some extent, it did not proliferate in HES. In turn, *S. nkossa* sp. nov. certainly appeared in the study area in connection with the presence of HES, with its bloom (and thus its close relationship with HYD) being certainly unique among the currently known species of Pilargidae.

Similar to *S. nkossa* sp. nov., all other polychaete species targeted in this paper were present in HES and virtually absent (or showed very low densities) in non-impacted sediments. The distribution patterns of the whole macrofaunal assemblage were suggested to be equivalent to those traditionally associated to eutrophication phenomena in areas impacted by waste disposals (Denoyelle et al. 2010). However, only *S. nkossa* sp. nov. and *L. sebastiena* responded positively to an increase in OM, and only *Capitella* sp. showed increasing densities in correlation with increasing S&C. In turn, all studied species responded positively to Ba (except *P. trionyx* and, obviously, *S. parva*), while *S. nkossa* sp. nov., *Raricirrus* sp., *L. sebastiena*, and Ampharetidae sp. also responded specifically to HYD.

Among the described species of *Raricirrus*, *R. maculatus* Hartman, 1961 was found as living in highly organically enriched sediments off California, while *R. beryli* Petersen & George, 1991 was locally abundant in sediments from northern North Sea oil fields (Hartman 1961; Petersen & George 1991). *Lindaspio sebastiena* is, at present, known only from the N’Kossa field. Curiously enough, despite being relatively highly abundant in 2000 and 2002, it almost disappeared in 2003, in parallel with the enormous increase in abundance of *S. nkossa* sp. nov. exactly in the same bottoms. Therefore, as suggested by Dalmazzone (2004), we cannot discard a cause relationship between, with a possible competence with the pilargiid (which was effectively able to reproduce in the area), explaining the rapid the decrease in abundance of the spionid (which apparently did not reproduce in the area).

The relationship with HES in the N’Kossa field did not seem to be restricted to polychaetes. The large vesicomyid bivalve *Isorropodon bigoti* was originally described from the HES sediments around the N’Kossa platforms and later found also in cold seeps and methane hydrate areas off Gabon and in the Congo basin (Rodrigues et al. 2012). This species was likely misidentified as either *Anodontia* cf. *edentula* or *Loripes* cf. *contrarius* by Denoyelle et al. (2010). It was very abundant (> 500 ind. m⁻²) in the same HES sediments inhabited by *S. nkossa* sp. nov. and the other HES associated polychaetes studied here. Similarly, the bivalve was completely absent from bottoms devoid of HYD. The depth in the N’Kossa region (i.e. around

150 m depth) is unusually shallow for vesicomyids and its presence was initially explained as a result of the high OM concentration caused by the Congo River discharges (Cosel et al. 2001). The low salinities associated to the Congo River plume were detectable on the water surface even at 200 km offshore (Vangriesheim et al. 2009), which included the waters surrounding the N’Kossa platforms (i.e., about 60 nautical miles offshore). This was also perceptible by the recurrent presence of small “floating islands” formed by living fragments of riverine vegetation, sometimes with animals on them (D. Martin, personal observations). However, the riverine influence was not specifically concentrated in the immediate vicinity of the platforms. Moreover, according to our data, the unusually high OM concentrations underneath the platforms were only related to drilling mud disposals and, thus, to the presence of HES. Taking into account that Vesicomyidae include well-known inhabitants of chemosynthetic environments like hydrocarbon seeps (Audzijonyte et al. 2012), we strongly suggest that the occurrence of *I. bigoti* below the N’kossa platforms, rather than due to a supposed riverine influence, was instead following the presence of HES, as reported for other vesicomyids in the region (Cosel & Olu 2009).

Deep-sea hydrocarbon seeps occur all around the world oceans (Levin 2005; Sibuet & Olu 1998) and are particularly abundant in the West African oceanic region (e.g., Jatiault et al. 2018; Olu et al. 2009; Warén & Bouchet 2009). Therefore, we suggest that some of the species here studied and, particularly, the polychaetes *S. nkossa* sp. nov., *L. sebastiena* and *Raricirrus* sp., and the bivalve *I. bigoti*, may be native inhabitants of natural HES. We also suggest that these species colonized alternatively the unusual shallow depths around the N’Kossa platforms thanks to the HYD artificial source provided by the accumulation of drilling muds. Consequently, better than an opportunistic response associated to an anthropogenic disturbance, its presence in the artificial HES around these platforms could be an expression of its normal mode of life, with the human driven offshore HYD routine exploration and production activities acting as stepping stones that could facilitate dispersal and colonization of these somewhat atypical shallow water bottoms to species natively associated with deeper environments having natural HES.

Taxonomy

Virtually all morphological features characterizing *Sigambra* have been well defined in recent literature, particularly in Salazar-Vallejo et al. (2019). However, our observations on *S. nkossa* sp. nov., complemented with the original descriptions and figures of other species of the genus (Supplementary data 3), lead us to emend the generic diagnosis in what concerns the so called “capillary chaetae” associated with the notopodial hooks/spines. In fact, these supposed “capillary chaetae” revealed to be the tips of the notoaciculæ protruding from the acicular lobes. Accordingly, the notopodial character “sometimes with accessory capillaries” has been replaced by “sometimes with protruding tips of notoaciculæ”.

On the other hand, *S. nkossa* sp. nov. perfectly fits with all diagnostic characters of the genus except for the presence of spines in addition to hooks in most notopodia along the body, a peculiar feature also described for *Sigambra robusta* (Ehlers, 1908), *Sigambra bassi* (Hartman, 1947) and *Sigambra healyae* (Gagaev, 2008) (Ehlers 1908; Gagaev 2008; Hartman 1947) that allows distinguishing them well from all other species of the genus (Supplementary data 3). The presence or absence of stout emergent notochaetae and, when present, whether they are hooked or straight, are considered to be diagnostic characters at generic level within Pilargidae (Blake 1997; Gil 2011; Pettibone 1966). However, we are here considering that the presence of stout emergent notopodial chaetae prevails over the fact that these may be represented by both hooks and spines in at least part of the notopodia along the body and, thus, we have decided to emend

the diagnosis of the genus accordingly. The origin of these spines and their relationships with the notopodial hooks and aciculae needs to be further investigated, but their singularity seems to indicate an apomorphic character.

Sigambra robusta was collected in *Valdivia* stations 76 (type locality) and 77, (Licher & Westheide 1997). While the depth of these stations was not mentioned in the original description of *S. robusta*, it was mentioned as being “ca. 14 m” for station 76, in the collection data of *Nereis lucipeta* Ehlers, 1908. However, this last species was based on epitokous males collected in mass on the surface of the water during the night, attracted by lights, as mentioned by Ehlers (1908) (p. 71): “Die beigelegte Etikette enthält die Angabe: ‘In Masse an der Oberfläche des Wassers, unter den Lampen’). Thus, associated station depth seemed to be an estimate, as it was not mentioned elsewhere in the publication. Still, Both the official contemporary charts of the bay (Comissão de Cartographia 1896; Hydrographic Office 1915) and the account of the expedition (Chun 1903) reported the overall maximum depth at the collection area, in the northern region of Tigres Bay, as being around 20 m depth. The species was posteriorly recorded from other regions of Angola (including Cabinda, just 70 km away from N’Kossa) and Namibia, between 20-150 m depth (Kirkegaard 1981; Licher & Westheide 1997), and there is a possible report from Senegal at 23 m (Augener 1918). Indeed, although the upper bathymetric limit of *S. robusta* seemed to reach the depth of the bottoms around the N’Kossa oil and gas platforms, its overall habitat appeared to be around ten times shallower than that of *S. nkossa* sp. nov. Moreover, the new species showed pigmentation in the anterior end (absent in *S. robusta*), lacked the second ventral cirri (present in *S. robusta*) and had notopodial hooks from chaetiger 5–6 (instead of 43–70 in *S. robusta*).

Sigambra nkossa sp. nov. differs from *S. healyae* in having faint blackish dorsal and ventral pigmentation on prostomium, peristomium, antennae and tentacular cirri (instead of red spots on prostomium in *S. healyae*), notopodial hooks starting from chaetiger 5–6 (instead of 4 in *S. healyae*), notopodial spines from chaetiger 9–11 (instead of 12–15 in *S. healyae*) and neuropodial capillaries (absent in *S. healyae*), as well as in geographical location (off Congo at 180 m depth vs. Canadian Basin at 1,800 m depth).

Sigambra bassi (Hartman, 1947) was originally described from Florida (Gulf of Mexico) and reported repeatedly since then as having notopodial hooks together with emergent spines (Blake 1997; Licher & Westheide 1997; Moreira & Parapar 2002; Pettibone 1966; Wolf 1984) as having hooks and emergent spines. However, in her extended description of the species, Hartman (1947) clearly distinguishes the acicula from the notopodial spines, while discussing the presence of structures such as “notoacicular spines [whose] free end is strongly curved”, “dorsal acicular spines”, “recurved acicular spines” or “projecting hooks”. Moreover, she illustrated a parapodium with an emerging straight spine that seemed to be the prolongation of the acicula, being thus difficult to ascertain if spines were really present along with hooks, or if she was referring to the emerging tips of the acicula. This question was clarified by Pettibone (1966), who revised type and non-type material from Florida and stated “stout notopodial hooked notoseta beginning about setiger 14 (11–15); occasionally additional single emergent notoseta, straight or slightly curved (called an aciculum by Hartman)”, while representing the notopodial hooks and spines as structures clearly independent from the notoacicula, originating from a different and much more superficial region (Pettibone 1966, Fig. 16), just as it occurred in *S. nkossa* sp. nov.

With just a few exceptions (e.g., Pettibone 1966; Wolf 1984), most available descriptions of *S. bassi*, including the original one, comprehended material from both Atlantic and Pacific

USA coasts (e.g., Hartman 1947; Licher & Westheide 1997) or just from the Pacific coasts (Blake 1997). This mixture of different populations, representing very likely different taxa, explain certainly the wide range of variation of characters such as the starting of notopodial hooks between chaetigers 3–25 (Licher & Westheide 1997). This drawback was previously referred by other authors, and work is now under progress to solve it, likely by erecting a new taxa (see Salazar-Vallejo et al. 2019 and references herein). This lead us to compare *S. nkossa* sp. nov. only with descriptions of Gulf of Mexico specimens, discarding those based on Pacific specimens referred above, but also those of American Atlantic populations recorded outside the Gulf of Mexico (e.g., Gardiner 1976). Accordingly, *N. nkossa* sp. nov. can be clearly and easily distinguished from *S. bassi* by the presence of notopodial hooks from chaetigers 4–6 (instead of 10–15 in *S. bassi*), and by having a median antenna reaching only chaetiger 2 when bent backwards (instead of 12 in *S. bassi*).

The morphology of the specimens of *S. parva* found in the N’Kossa oil and gas field, agrees overall with that of the types redescribed by Moreira & Parapar (2002) from Cape Province (South Africa), except in the number of supracicular pectinate neurochaetae, which may be up to 6 in the Congolese specimens and were reported as being 1–2 in the type specimens. Some Congolese specimens showed four subdermal eyes, which were not visible in others, while the apparent lack of eyes in the South African specimens may be attributed either to the fading effect of their long storage in preservation fluid or to the hiding by a thicker epidermal layer. There seem to be some slight differences (mainly concerning the distribution of the proboscideal papillae) between the Congolese specimens and both the types of *S. parva* and the specimens from the Iberian Peninsula (Columbretes, NW Mediterranean; Baiona, NE Atlantic) identified as *S. parva* by Moreira & Parapar (2002). However, we have not been able to either confirm or reject this statement in light of our own observations, which were inconclusive. Considering the great distances separating the different populations, it is plausible to think that further work based on larger sets of specimens and, ideally, combining additional morphological observations with morphometric and molecular analyses, would help in assessing whether these and other populations considered as belonging to *S. parva* from areas far from the type location probably represent different taxa.

Morphometry

In addition to the rise of modern molecular techniques, morphometric analyses have also shown to be a valid and trustful method to assess morphological discrimination between cryptic polychaete species (Ford & Hutchings 2005; Glasby & Glasby 2006; Koh & Bhaud 2001; Koh & Bhaud 2003; Lattig et al. 2007; Martin et al. 2017). Morphometry is particularly useful when some of the relevant characters routinely used to define the species within a genus are quantitative and may be, to some extent, size-related. In traditional approaches, ranges are often provided, but this usually depends on the author, so that they are not available for all affected species and relevant characters, as can easily be perceived for the known species of *Sigambra* (Supplementary data 3).

Moreover, morphometric observations may be also used to determine whether a single measurement from a single individual may or may not be taxonomically relevant by assessing the variability at the population level. Based on 21 specimens representative of the whole size range of the population of *S. nkossa* sp. nov., we demonstrate that most quantitative characters commonly used to define the species of *Sigambra* are strongly size-dependent and show a highly significant positive correlation with size. This correlation may explain more than 70% of their

variability, or even more than 90% in the case of dry weight, width of chaetiger 15 (with parapodia), length of median antennae, and length of the first dorsal and ventral cirri. Therefore, these characters should be avoided when diagnosing a species, unless their variability at the population level can be reported and, if possible, statistically compared to those of the closely related species.

Conversely, a robust taxonomic relevancy can be attributed to size-independent characters. These include the length ratios for the main anterior appendages (i.e., the dorsal tentacular cirri being much longer than the ventral ones, and the dorsal parapodial cirri being much longer than ventral ones), as well as the only three discrete or binary features referring to specific morphologic characters (i.e. the starting of the notopodial hooks at segment 5–6, the starting of the notopodial spines at segment 9–12, and the absence of the 2nd ventral cirri), which are consistently constant all along the size range of the studied population. Thus, these characters can be considered unambiguous at the species level and species-specific at the individual level.

Care should also be taken when attributing size-independence to a given character, as this may also vary according to the species. In the particular case of the stating segment for the presence of hooks, for instance, it has been recently stated that this character may be invariable (or vary for a very few segments, more or less at random) or significantly change along the ontogeny, with the presence of hooks starting more posteriorly in larger specimens (Salazar-Vallejo et al. 2019).

Conclusions

Our results allowed us to suggest that native inhabitants of natural deep-sea HES may alternatively colonize unusual shallow depths around oil and gas platforms thanks to the HYD artificial sources provided by drilling mud deposits. Off the Republic of Congo, in the N’kossa oil and gas field, the polychaetes *L. sebastiena*, *Raricirrus* sp. and *S. nkossa* sp. nov., together with the bivalve *I. bigoti*, may be examples of this circumstance. Therefore, their association with artificial HES would be more an expression of their normal mode of life, rather than just an anthropogenically triggered opportunistic response.

Accordingly, the offshore oil and gas routine exploration and extraction activities could act as stepping stones for the dispersal of benthic inhabitants native from different natural deep-sea environments with HES.

The current knowledge on the species of Pilargidae and particularly on those belonging to *Sigambra*, revealed to be incorrect in what concerns to the presence of the “capillary chaetae” accompanying the notopodial hooks and spines. According to our observations, these structures represent, in fact, the protruding tips of the notoacacula. Thus, we have emended the generic diagnosis to reflect this finding, by replacing the notopodial character “sometimes with accessory capillaries” by “sometimes with protruding tips of notoacaculae”.

Moreover, generic diagnostic characters such as “presence/absence of stout emergent notochaetae” or “stout notochaetae either as hooks or as spines” seem to be in conflict with the definition of some of the current known genera of Pilargidae, as well as with most of the identification keys available for the family (e.g., Blake 1997; Gil 2011; Pettibone 1966). If these identification keys were followed strictly, species having both types of stout notochaetae (straight and hooked) on the same notopodial bundle (such as *S. robusta*, *S. bassi*, *S. helyae* and *S. nkossa* sp. nov.) would be keyed as probably belonging to a new genus. While further studies (including molecular analyses) will certainly help to clarify the taxonomic weight of the presence and type of these notopodial spines, we have considered the first character (i.e., presence of stout

emergent notochaetae) as prevailing over the second (i.e., stout notochaetae either as hooks or as spines). Therefore, we have modified the diagnosis of *Sigambra* to include the character “notopodium with one strongly recurved dorsal hook or with one strongly recurved dorsal hook and one slightly bent spine along many segments”. As a consequence, In addition, the identification keys of the family must replace the step generally represented as “*stout emergent notochaetae straight, not hooked*” vs. “*stout emergent notochaetae hooked*” by “*stout emergent notochaetae straight, not hooked*” vs. “*stout emergent notochaetae also including hooked chaetae*”.

Finally, our morphometric analyses allowed us to demonstrate that most quantitative characters commonly used to define the species of *Sigambra* are strongly size-dependent, proving that they must be avoided for species definition, except when it will be possible to define statistically their interpopulation variability to be compared with those of the most closely related species. Conversely, size-independent characters must be considered as taxonomically robust and unambiguous at the species level, as well as species-specific at the individual level.

Acknowledgements

We would like to thank TotalEnergies for partly sponsoring the field surveys and for their help with the sampling campaigns, the staff of the N’Kossa platform for their kind welcome and help during our visits, the staff of the Laboratoire Municipal et Régional de la Ville de Rouen (France) for the analyses of the sediment characteristics, and José Manuel Fortuño from the SEM Service of the CMIMA-CSIC (Spain), for his help with SEM micrographs.

References

- Aguado MT, Nygren A, and Rouse GW. 2013. Two apparently unrelated groups of symbiotic annelids, Nautiliniellidae and Calamyzidae (Phyllodocida, Annelida), are a clade of derived chrysopetalid polychaetes. *Cladistics* 29:610-628. <https://doi.org/10.1111/cla.12011>
- Al-Hawash AB, Dragh MA, Li S, Alhujaily A, Abbood HA, Zhang X, and Ma F. 2018. Principles of microbial degradation of petroleum hydrocarbons in the environment. *The Egyptian Journal of Aquatic Research* 44:71-76. <https://doi.org/10.1016/j.ejar.2018.06.001>
- Audzijonyte A, Krylova EM, Sahling H, and Vrijenhoek RC. 2012. Molecular taxonomy reveals broad trans-oceanic distributions and high species diversity of deep-sea clams (Bivalvia: Vesicomyidae: Pliocardiinae) in chemosynthetic environments. *Systematics and Biodiversity* 10:403-415. <https://doi.org/10.1080/14772000.2012.744112>
- Augener H. 1918. Polychaeta. In: Michaelsen W, ed. *Beiträge zur Kenntnis der Meeresfauna Westafrikas*. Hamburg, 67-625.
- Bellan G, Dauvin JC, and Laubier L. 2003. The genus *Lindaspio* (Annelida: Polychaeta: Spionidae), and a new species from an oil field off Congo, western Africa. *Journal of Natural History* 37:2413-2424.
- Blake JA. 1997. Family Pilargidae Saint Joseph, 1899. In: Blake JA, Hilbig B, and Scott PH, eds. *Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and the Western Santa Barbara Channel Volume 4 -The Annelida Part 1 Oligochaeta and Polychaeta: Phyllodocida (Phyllodocidae to Paralacydoniidae) Revised edition*. Santa Barbara: Santa Barbara Museum of Natural History, 261-284.

- Blake JA. 2000. A new genus and species of polychaete worm (Family Orbiniidae) from methane seeps in the Gulf of Mexico, with a review of the systematics and phylogenetic interrelationships of the genera of Orbiniidae. *Cahiers de Biologie Marine* 41:435-449.
- Bland JM, and Altman DG. 1995. Multiple significance tests: the Bonferroni method. *BMJ* 310:170. [10.1136/bmj.310.6973.170](https://doi.org/10.1136/bmj.310.6973.170)
- Bright M, and Lallier F. 2010. The biology of Vestimentiferan tubeworms. *Oceanography and Marine Biology An Annual Review* 48:213-265. <https://doi.org/10.1201/EBK1439821169-c4>
- Britayev TA, and Martin D. 2006. Scale-worms (Polychaeta, Polynoidae) associated with chaetopterid worms (Polychaeta, Chaetopteridae), with description of a new genus and species. *Journal of Natural History* 39:4081-4099. <https://doi.org/10.1080/00222930600556229>
- Carroll ML, Pearson T, Dragsund E, and Gabrielsen KL. 2000. Environmental status of the norwegian offshore sector based on the petroleum regional monitoring programme, 1996–1998. *The Norwegian oil industry association report APN* 411:1-33.
- Chun C. 1903. *Aus den Tiefen des Weltmeeres. Schilderungen von der Deutschen Tiefsee-Expedition*. Jena: Gustav Fischer.
- Comissão de Cartographia. 1896. Costa Occidental d'Africa. Plano da Bahia dos Tigres. [map]. 1:120,000. -Lisboa: Companhia Nacional Editora.
- Cosel R, and Olu K. 2009. Large Vesicomidae (Mollusca: Bivalvia) from cold seeps in the Gulf of Guinea off the coasts of Gabon, Congo and northern Angola. *Deep Sea Research Part II: Topical Studies in Oceanography* 56:2350-2379. <https://doi.org/10.1016/j.dsr2.2009.04.016>
- Cosel R, Salas C, and Høisæter T. 2001. Vesicomidae (Mollusca: Bivalvia) of the genera *Vesicomya*, *Waisiuconcha*, *Isorropodon* and *Callogonia* in the eastern Atlantic and the Mediterranean. *Sarsia* 86:333-366. <https://doi.org/10.1080/00364827.2001.10425523>
- Dalmazzone C, Blanchet D, Lamoureux S, Dutrieux É, Durrieu J, Camps R, and Galgani F. 2004. Impact of drilling activities in warm sea: Recolonization capacities of seabed. *Oil & Gas Science and Technology - Revue IFP* 59:625-647. <https://doi.org/10.2516/ogst.2004045>
- Day JH. 1963. The polychaete fauna of South Africa Part 8: New species and records from grab samples and dredgings. *Bulletin of the British Museum of Natural History* 10:381-445.
- Day JH. 1967. *A monograph on the Polychaetes of Southern Africa. Part 1. Errantia*. London: Trustees of the British Museum (Natural History).
- Denoyelle M, Jorissen FJ, Martin D, Galgani F, and Miné J. 2010. Comparison of benthic foraminifera and macrofaunal indicators of the impact of oil-based drill mud disposal. *Marine Pollution Bulletin* 60:2007-2021. <https://doi.org/10.1016/j.marpolbul.2010.07.024>
- Desbruyères D, and Toulmond A. 1998. A new species of hesionid worm, *Hesiocaeca methanicola* sp. nov. (Polychaeta: Hesionidae), living in ice-like methane hydrates in the deep Gulf of Mexico. *Cahiers de Biologie Marine* 39:93-98.
- Ehlers E. 1908. Die bodensässigen Anneliden aus den Sammlungen der deutschen Tiefsee-Expedition. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898-1899* 16:1-168. <https://doi.org/10.5962/bhl.title.2171>
- Fisher CR, MacDonald IR, Sassen R, Young CM, Macko SA, Hourdez S, Carney RS, Joye S, and McMullin E. 2000. Methane Ice Worms: *Hesiocaeca methanicola* colonizing fossil fuel reserves. *Naturwissenschaften* 87:184-187.
- Ford E, and Hutchings P. 2005. An analysis of the morphological characteristics of *Owenia* useful to distinguish species: description of three new species of *Owenia* (Oweniidae: Polychaeta) from Australian waters. *PSZN I: Marine Ecology* 26:181-196.

- Gagaev S. 2008. *Sigambra healyae* sp. n., a new species of polychaete (Polychaeta: Pilargidae) from the Canadian Basin of the Arctic ocean. *Russian Journal of Marine Biology* 34:73-75.
- Gardiner SL. 1976. Errant Polychaete Annelids from North Carolina. *The Journal of the Elisha Mitchell Scientific Society* 91:77-220.
- Gil J. 2011. The European Fauna of Annelida Polychaeta PhD. Faculdade de Ciências, Universidade de Lisboa.
- Glasby CJ, and Glasby TM. 2006. Two types of uncini in *Polycirrus* (Polychaeta: Terebellidae: Polycirrinae) revealed using geometric morphometrics. *Journal of Natural History* 40:237-253. <https://doi.org/10.1080/00222930600627137>
- Gómez Gesteira JL, and Dauvin JC. 2005. Impact of the Aegean Sea oil spill on the subtidal fine sand macrobenthic community of the Ares-Betanzos Ria (Northwest Spain). *Marine Environmental Research* 60:289–316. <https://doi.org/10.1016/j.marenvres.2004.11.001>
- Hartman O. 1947. Polychaetous annelids. Part VIII. Pilargidae. *Allan Hancock Pacific Expeditions* 10:483-512.
- Hartman O. 1961. Polychaetous annelids from California. *Allan Hancock Pacific Expeditions* 25:1-226.
- Hydrographic Office. 1915. Africa - West Coast. St. Paul de Loanda to Great Fish Bay. Compiled chiefly from sketch surveys by Captain W.F.W. Owen, H.M.S. 'Leven', 1825, with additions from various British and Foreign works to 1881. [map]. Scale not given [handwritten scale as 1:1,100,000]. London: Admiralty.
- International Commission of Zoological Nomenclature. 1999. *The International Code of Zoological Nomenclature. 4th Edition*. London: International Trust for Zoological Nomenclature.
- Intès A, and Le Loeuff P. 1975. Les annélides polychètes de cote d'Ivoire. I.-Polychètes errantes-compte rendu systématique. *Cahiers ORSTOM, séries Océanographie* 13:267-321.
- Jatiaux R, Dhont D, Loncke L, de Madron XD, Dubucq D, Channelliere C, and Bourrin F. 2018. Deflection of natural oil droplets through the water column in deep-water environments: The case of the Lower Congo Basin. *Deep Sea Research Part I: Oceanographic Research Papers* 136:44-61. <https://doi.org/10.1016/j.dsr.2018.04.009>
- Karaseva N, Gantsevich M, Obzhairov A, Shakirov R, Starovoitov A, Smirnov R, and Malakhov V. 2020. Correlation of the siboglinid (Annelida: Siboglinidae) distribution to higher concentrations of hydrocarbons in the Sea of Okhotsk. *Marine Pollution Bulletin* 158:111448.
- Kennicutt MC, Keeney-Kennicutt WL, Bresley BJ, and Fenner F. 1982. The use of pyrolysis and barium distributions to assess the areal extent of drilling fluids in surficial marine sediments. *Environmental Geology* 4:239–249. <https://doi.org/10.1007/BF02380517>
- Kirkegaard JB. 1981. The Polychaeta of West Africa. Part II. Errant species. 1. Aphroditidae to Nereididae. *Atlantide Report* 13:181-240.
- Koh BS, and Bhaud M. 2001. Description of *Owenia gomsoni* n. sp. (Oweniidae, Annelida Polychaeta) from the Yellow Sea and evidence that *Owenia fusiformis* is not a cosmopolitan species. *Vie et Milieu* 51:77-86.
- Koh BS, and Bhaud M. 2003. Identification of new criteria for differentiating between populations of *Owenia fusiformis* (Annelida, Polycheta) from different origins; rehabilitation of old species and erection of two new species. *Vie et Milieu* 53:65-96.
- Lattig P, San Martín G, and Martín D. 2007. Taxonomic and morphometric analyses of the *Haplosyllis spongicola* complex (Polychaeta: Syllidae: Syllinae) from Spanish seas, with re-

- description of the type species and descriptions of two new species. *Scientia Marina* 71:551-570. <https://doi.org/10.3989/scimar.2007.71n3551>
- Levin LA. 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. *Oceanography and Marine Biology an Annual Review* 43:11-56.
- Levin LA, James DW, Martin CM, Rathburn AE, Harris LH, and Michener RH. 2000. Do methane seeps support distinct macrofaunal assemblages? Observations on community structure and nutrition from the northern California slope and shelf. *Marine Ecology Progress Series* 208:21-39. <https://doi.org/10.3354/meps208021>
- Licher F, and Westheide W. 1997. Review of the genus *Sigambra* (Polychaeta: Hesionidae), redescription of *S. bassi* (Hartman, 1947), and descriptions of two new species from Thailand and China. *Steenstrupia* 23:1-20.
- Martin D, Meca MA, Gil J, Drake P, and Nygren A. 2017. Another brick in the wall: population dynamics of a symbiotic *Oxydromus* (Annelida, Hesionidae), described as a new species based on morphometry. *Contributions to Zoology* 86:181-211. <https://doi.org/10.1163/18759866-08603001>
- Montagna P, and Harper JDE. 1996. Benthic infaunal long-term response to offshore production platforms in the Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Sciences* 53:2567-2588. <https://doi.org/10.1139/f96-215>
- Moreira J, and Parapar J. 2002. Redescription of *Sigambra tentaculata* and re-establishment of *S. parva* (Polychaeta, Pilargidae) based upon type material. *Cahiers de Biologie Marine* 43:99-109.
- Müller F. 1858. Einiges über die Annelidenfauna der Insel Santa Catharina an der brasilianischen Küste. *Archiv für Naturgeschichte* 24:211-220.
- Nishi E, Tanaka K, Fujioka Y, and Sato M. 2007. Reinstatement of *Sigambra hanaokai* (Kitamori, 1960) (Polychaeta, Pilargidae), with an overview of the literature on the genus. *Zootaxa* 1653:57-68. <https://doi.org/10.11646/zootaxa.1653.1.4>
- Olsen G, Carroll M, Renaud PE, Ambrose WG, Olsson R, and Carroll JL. 2007. Benthic community response to petroleum-associated components in Arctic versus temperate marine sediments. *Marine Biology* 151:2167-2176. <https://doi.org/10.1007/s00227-007-0650-z>
- Olu K, Caprais JC, Galéron J, Causse R, Cosel R, Budzinski H, Ménach KL, Roux CL, Levaché D, Khrifounoff A, and Sibuet M. 2009. Influence of seep emission on the non-symbiont-bearing fauna and vagrant species at an active giant pockmark in the Gulf of Guinea (Congo–Angola margin). *Deep Sea Research Part II: Topical Studies in Oceanography* 56:2380-2393. <https://doi.org/10.1016/j.dsr2.2009.04.017>
- Pearson TH, and Rosenberg R. 1978. Macrobenthic succession in relation to organic enrichment and pollution in marine environment. *Oceanography and Marine Biology: An Annual Review* 16:229-311.
- Petersen ME, and George JD. 1991. A new species of *Raricirrus* from northern Europe, with notes on its biology and a discussion of the affinities of the genus (Polychaeta: Ctenodrilidae). *Ophelia* Suppl. 5:185-208.
- Pettibone MH. 1966. Revision of the Pilargidae (Annelida: Polychaeta), including descriptions of new species, and redescription of the pelagic *Podarmus ploa* Chamberlin (Polynoidae). *Proceedings of the United States National Museum* 118:155-208.
- Ravara A, Ramos D, Teixeira MAL, Costa FO, and Cunha MR. 2017. Taxonomy, distribution and ecology of the order Phyllodocida (Annelida, Polychaeta) in deep-sea habitats around the

- Iberian margin. *Deep Sea Research Part II: Topical Studies in Oceanography* 137:207-231.
<https://doi.org/10.1016/j.dsr2.2016.08.008>
- Ravara A, Wiklund H, and Cunha MR. 2021. Four new species and further records of
Dorvilleidae (Annelida, Polychaeta) from deep-sea organic substrata, NE Atlantic. *European
Journal of Taxonomy* 736:44-81. <https://doi.org/10.5852/ejt.2021.736.1251>
- Rodrigues CF, Cunha MR, Olu K, and Duperron S. 2012. The smaller vesicomysid bivalves in the
genus *Isorropodon* (Bivalvia, Vesicomysidae, Pliocardiinae) also harbour chemoautotrophic
symbionts. *Symbiosis* 56:129-137. <https://doi.org/10.1007/s13199-012-0168-0>
- Salazar-Vallejo SI, Rizzo AE, de León-González JÁ, and Brauco KM. 2019. Four new
Caribbean *Sigambra* species (Annelida, Pilargidae), and clarifications of three other *Sigambra*
species. *ZooKeys* 893:21-50. <https://doi.org/10.3897/zookeys.893.39594>
- Schulze A, and Halanych KM. 2003. Siboglinid evolution shaped by habitat preference and
sulfide tolerance. *Hydrobiologia* 496:199-205. <https://doi.org/10.1023/a:1026192715095>
- Sibuet M, and Olu K. 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-
seep communities at active and passive margins. *Deep Sea Research Part II: Topical Studies
in Oceanography* 45:517-567. [https://doi.org/10.1016/S0967-0645\(97\)00074-X](https://doi.org/10.1016/S0967-0645(97)00074-X)
- Vangriesheim A, Pierre C, Aminot A, Metzl N, Baurand F, and Caprais J-C. 2009. The influence
of Congo River discharges in the surface and deep layers of the Gulf of Guinea. *Deep Sea
Research Part II: Topical Studies in Oceanography* 56:2183-2196.
<https://doi.org/10.1016/j.dsr2.2009.04.002>
- Warén A, and Bouchet P. 2009. New gastropods from deep-sea hydrocarbon seeps off West
Africa. *Deep Sea Research Part II: Topical Studies in Oceanography* 56:2326-2349.
<https://doi.org/10.1016/j.dsr2.2009.04.013>
- Washburn TW, Demopoulos AWJ, and Montagna PA. 2018. Macrobenthic infaunal
communities associated with deep-sea hydrocarbon seeps in the northern Gulf of Mexico.
Marine Ecology 39:e12508. <https://doi.org/10.1111/maec.12508>
- Washburn TW, Rhodes ACE, and Montagna PA. 2016. Benthic taxa as potential indicators of a
deep-sea oil spill. *Ecological Indicators* 71:587-597.
<https://doi.org/10.1016/j.ecolind.2016.07.045>
- Wolf PS. 1984. Family Pilargidae Saint-Joseph, 1899. In: Uebelacker JM, and Johnston PG, eds.
Taxonomic Guide to the Polychaetes of the Northern Gulf of Mexico, Volume 4. Mobile,
Alabama: Barry A. Vittor & Associates, Inc., 29.21-29.41.
- Zar JH. 1984. *Biostatistical Analyses*. New Jersey: Prentice Hall International.

Figure 1

Geographical location of the study site and sampling stations

(A) Map of Africa showing the location of the Republic of Congo. (B) Map of the Republic of Congo showing the location of the N’Kossa field. (C) Location of the drilling points at NKF 1 and NKF2 and sampling stations in the N’Kossa field.

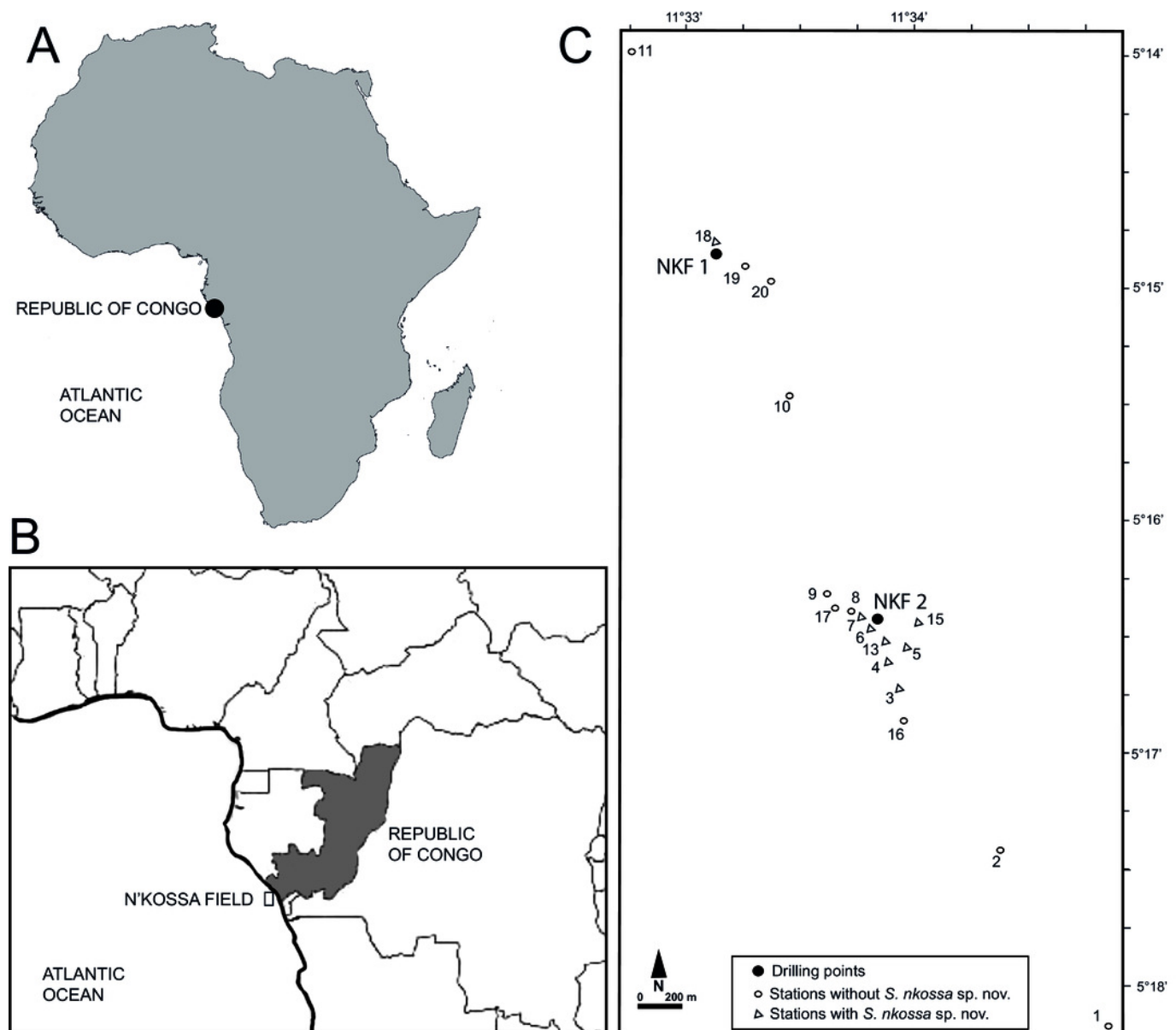
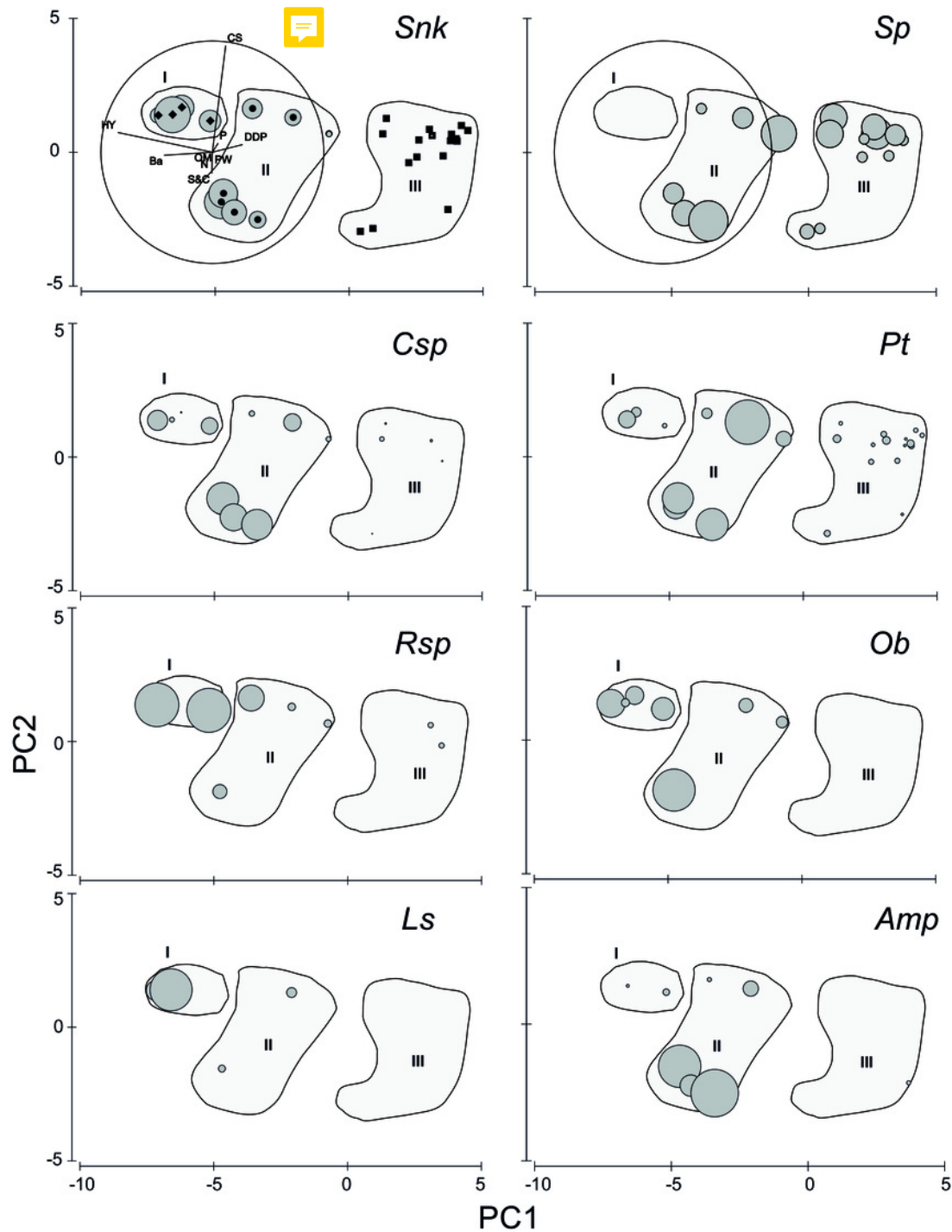


Figure 2

Results of the PCA based on environmental parameters

Samples grouped according to groups I, II and III; species densities (ind.·m⁻²) plotted as grey circles. DDP: distance from drilling point; HYD: Total hydrocarbons; Ba: Barium; S&C: silt and clay; CS: coarse sand; OM: organic matter; PW: pore water; N: Nitrogen; P: Phosphorous; *Snk*: *Sigambra nkossa* sp. nov.; *Sp*: *S. parva*; *Cap*: *Capitella* sp. *Pt*: *Paramphimetryonix*. *Rsp*: *Raricirrus* sp. *Ob*: *Oxydromus berrisfordi*. *Ls*: *Lindaspio sebastiena*. *Amp*: Ampharetidae sp.



	<i>Snk</i>	<i>Sp</i>	<i>Csp</i>	<i>Pt</i>	<i>Rsp</i>	<i>Ob</i>	<i>Ls</i>	<i>Amp</i>	Hydrocarbon
●	500	5	400	100	30	9	20	90	■ <100
●	2000	20	1600	400	120	36	80	360	● <20000
●	3500	35	2800	700	210	63	140	630	◆ >20000
●	5000	50	4000	1000	300	90	200	900	

Figure 3

Environment descriptors in the N’Kossa field, plotted against the groups obtained in the PCA

DDP: Distance from drilling points (m); HYD: Total hydrocarbons (as kg/kg of sediment). Ba: Barium (mg/kg of sediment). S&C: Silt and clay (%). CS: Coarse sand (%). PW: Pore water (%). OM: Organic matter (%). N: Nitrogen (%). P: Phosphorous (%)

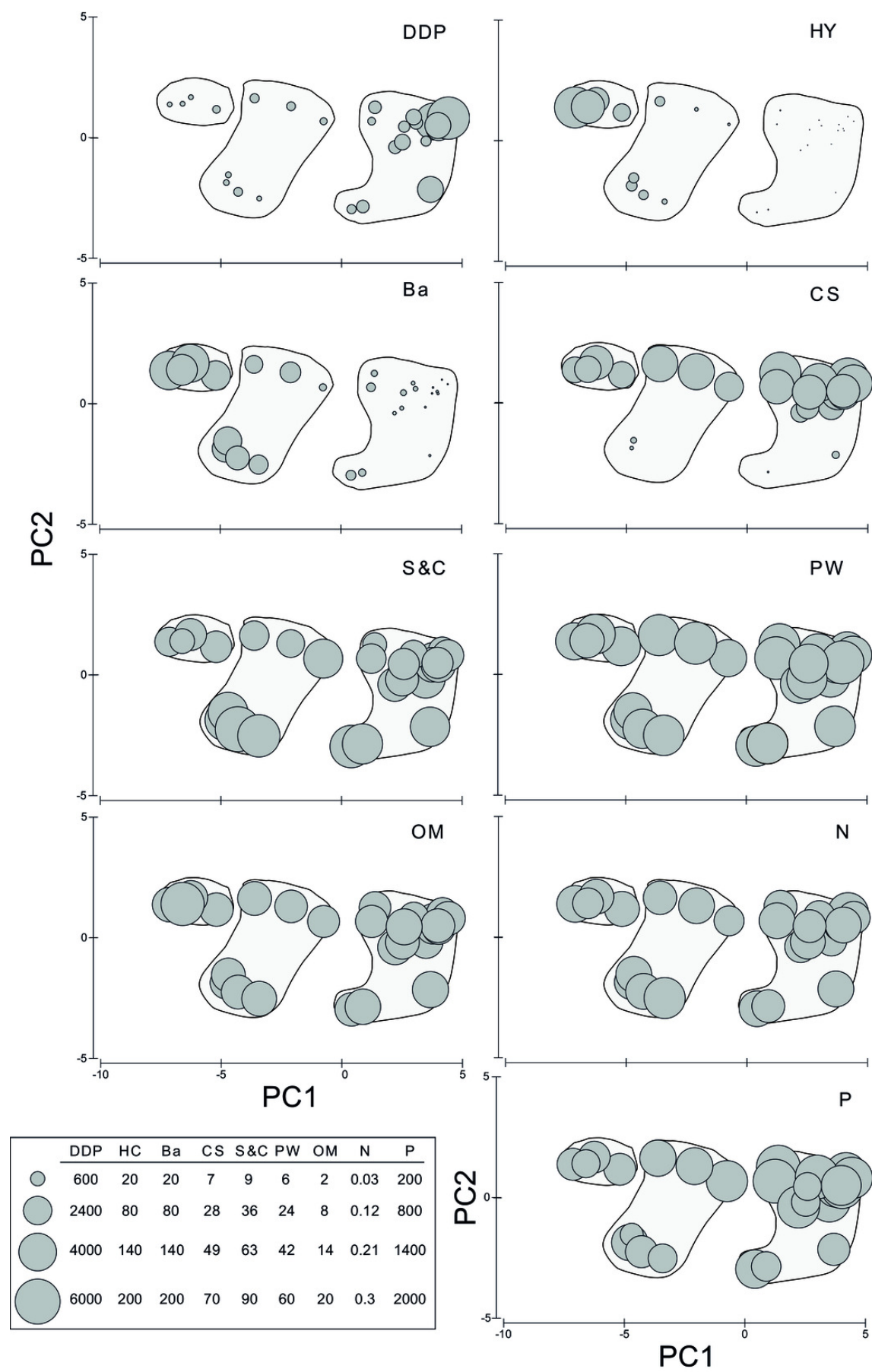


Figure 4

Sigambra nkossa sp. nov. light microscopy images

(A) Whole body, dorsal view. (B) Anterior end, dorsal view. (C) Anterior end, ventral view. (D) Detail of chaetigers 2 (lacking ventral cirrus) and 3. (E) Everted proboscis, dorsal view. (F) Everted proboscis, ventral view. (G) Everted proboscis, lateral view. (H) Parapodium 3. (I) Midbody parapodium. (J) Detail of hook and spine from I. (K) Posterior-most parapodium. (L) Detail of hook and spine from K

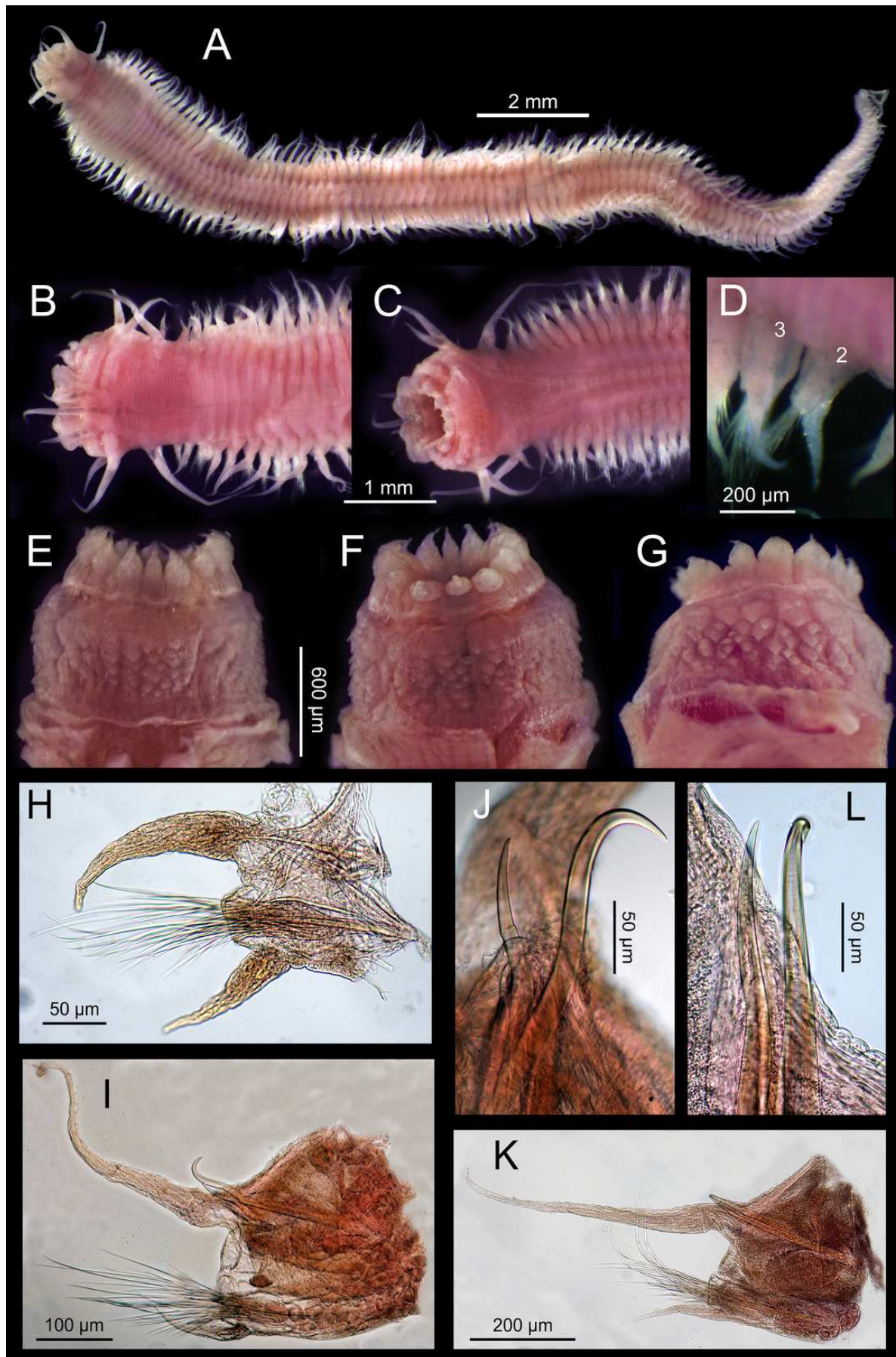


Figure 5

Sigambra nkossa sp. nov. SEM images

(A) Anterior end, dorsal view. (B) Detail of the anterior end, dorsal view. (C) Anterior end with everted proboscis, frontal view. (D) Anterior end with everted proboscis, lateral view. (E, F) Neuropodial chaetae from mid-body. (G) First segments with notopodial hook and spine (white arrows pointing on the first two spines). (H) Notopodial hook and spine from mid-body (white arrow pointing on protruding tip of notoacacula)

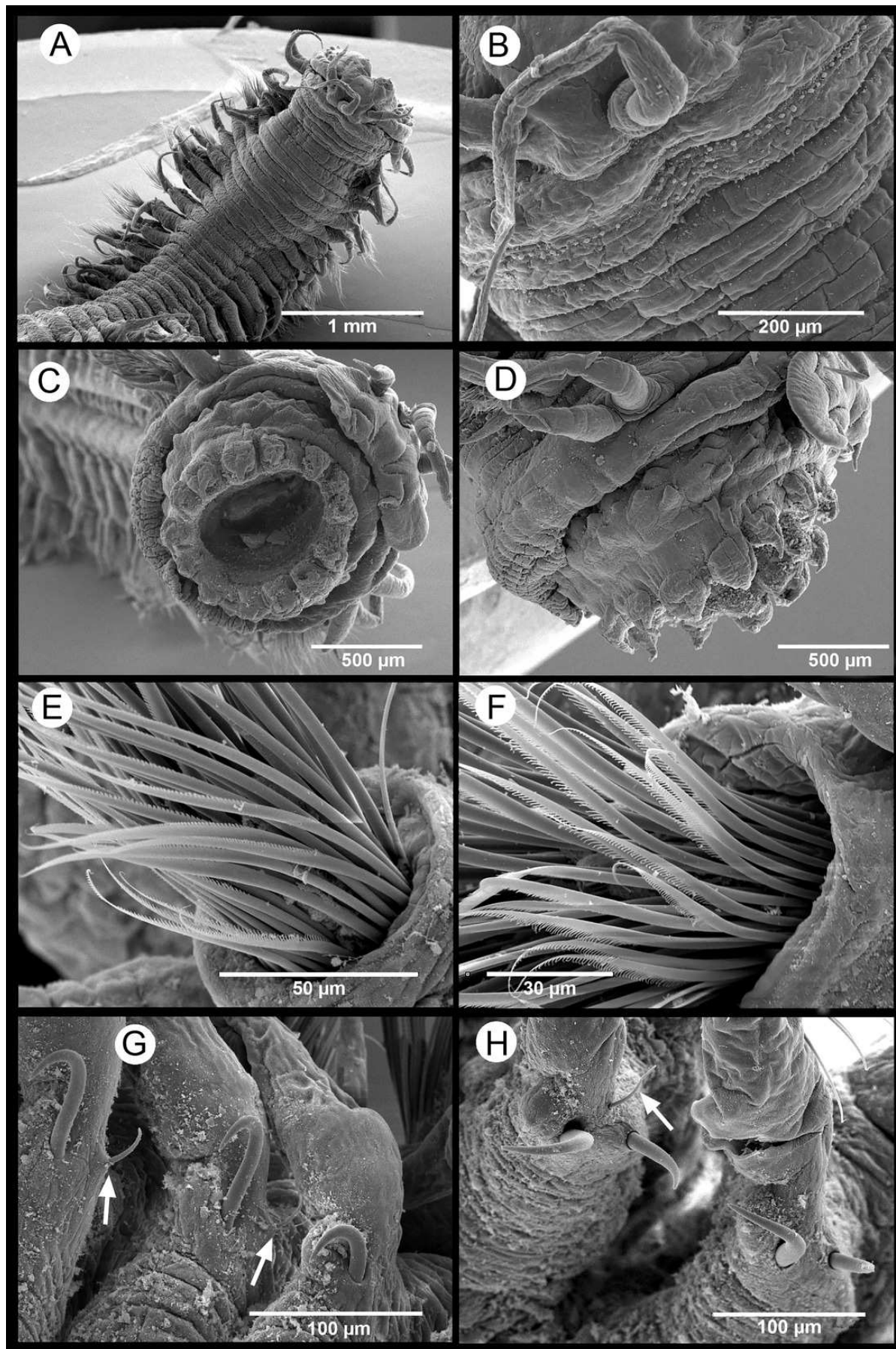


Figure 6

Sigambra parva. SEM images

(A) Anterior end, lateral view. (B) Anterior end, ventral view. (C) Detail of anterior end, frontal view. (D) Detail of anterior end, dorsal view. (E) Anterior end with everted proboscis, lateral view. (F) Posterior end

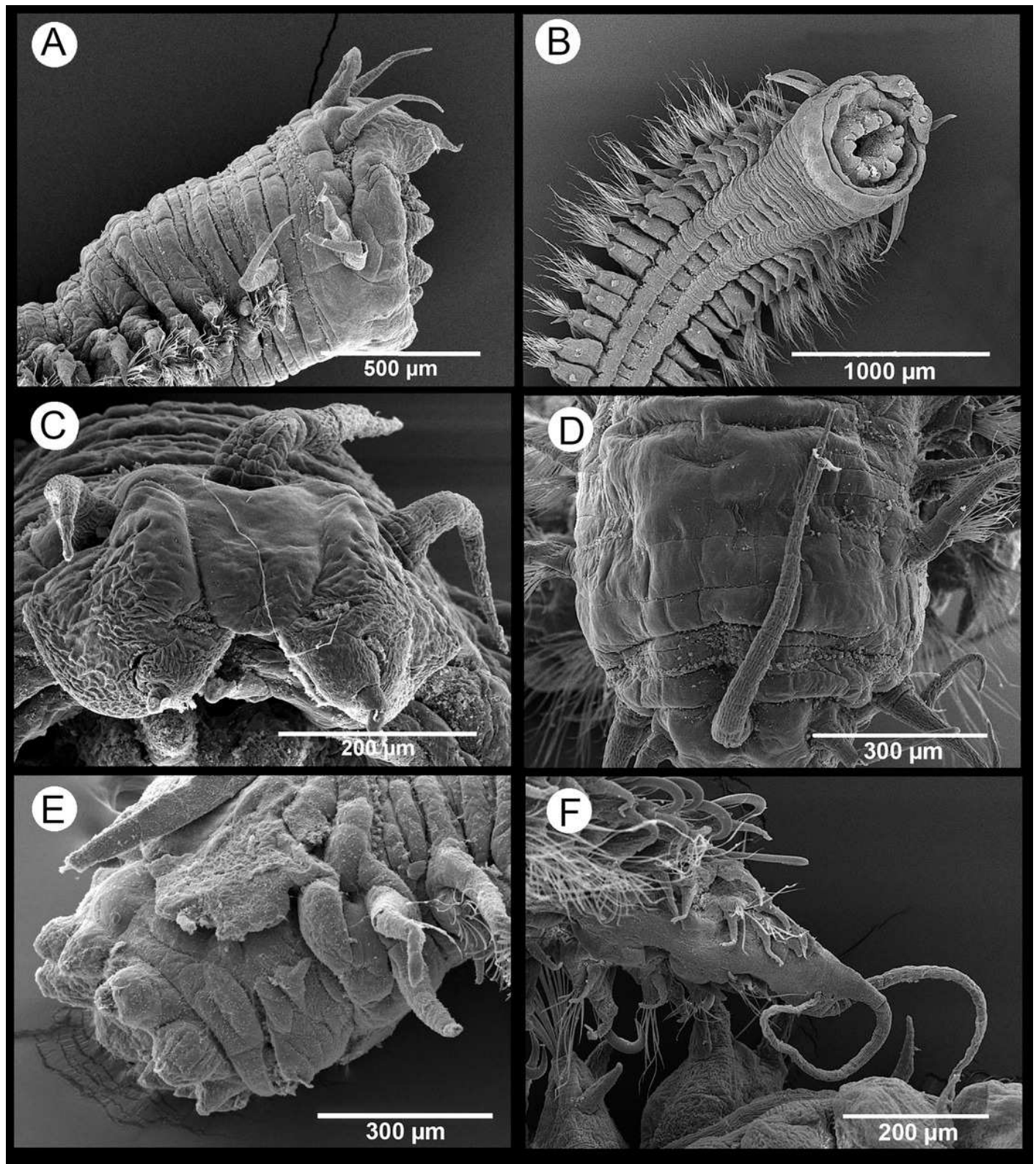


Figure 7

Sigambra parva. SEM images

(A) Mid-body parapodia. (B) Detail of hooks from mid-body parapodia. (C, D) Neuropodial chaetae from mid-body

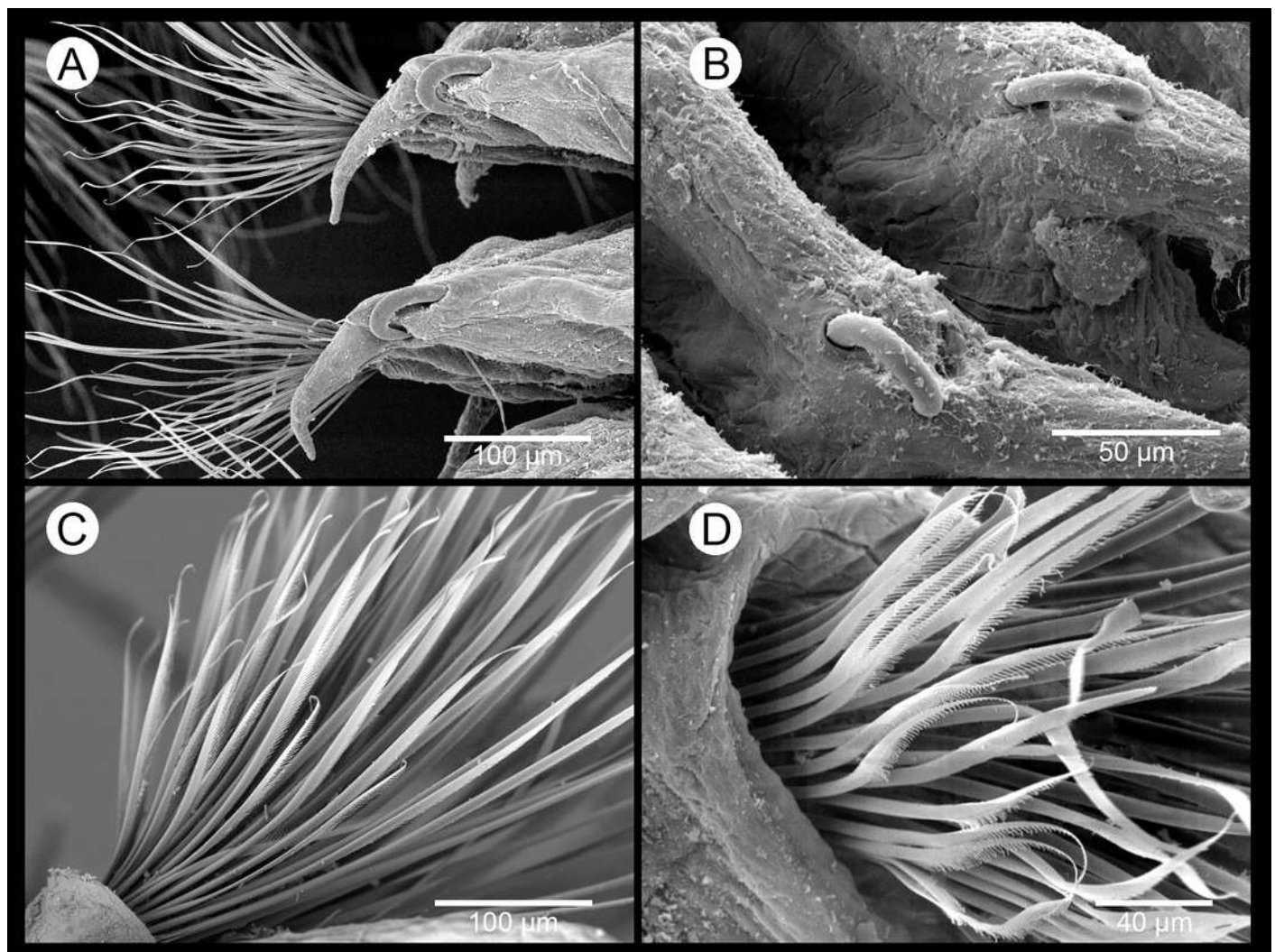


Figure 8

Sigambra nkossa sp. nov. selected morphological features and their respective relationships with size, expressed as number of chaetigers

(A) Total length (μm). (B) Dry weight (mg). (C) Width of chaetiger 15 (with parapodia, μm). (D) Length of median antennae (μm). (E) Length of first dorsal cirri (μm). (F) Starting segment for the dorsal capillary chaetae. (G) Length ratio for the antennae (middle / lateral). (H) Length ratio for the peristomial cirri (dorsal / ventral). (I) Starting segment for the notopodial hook. A-F: size-dependent characters; H-I: size-independent characters

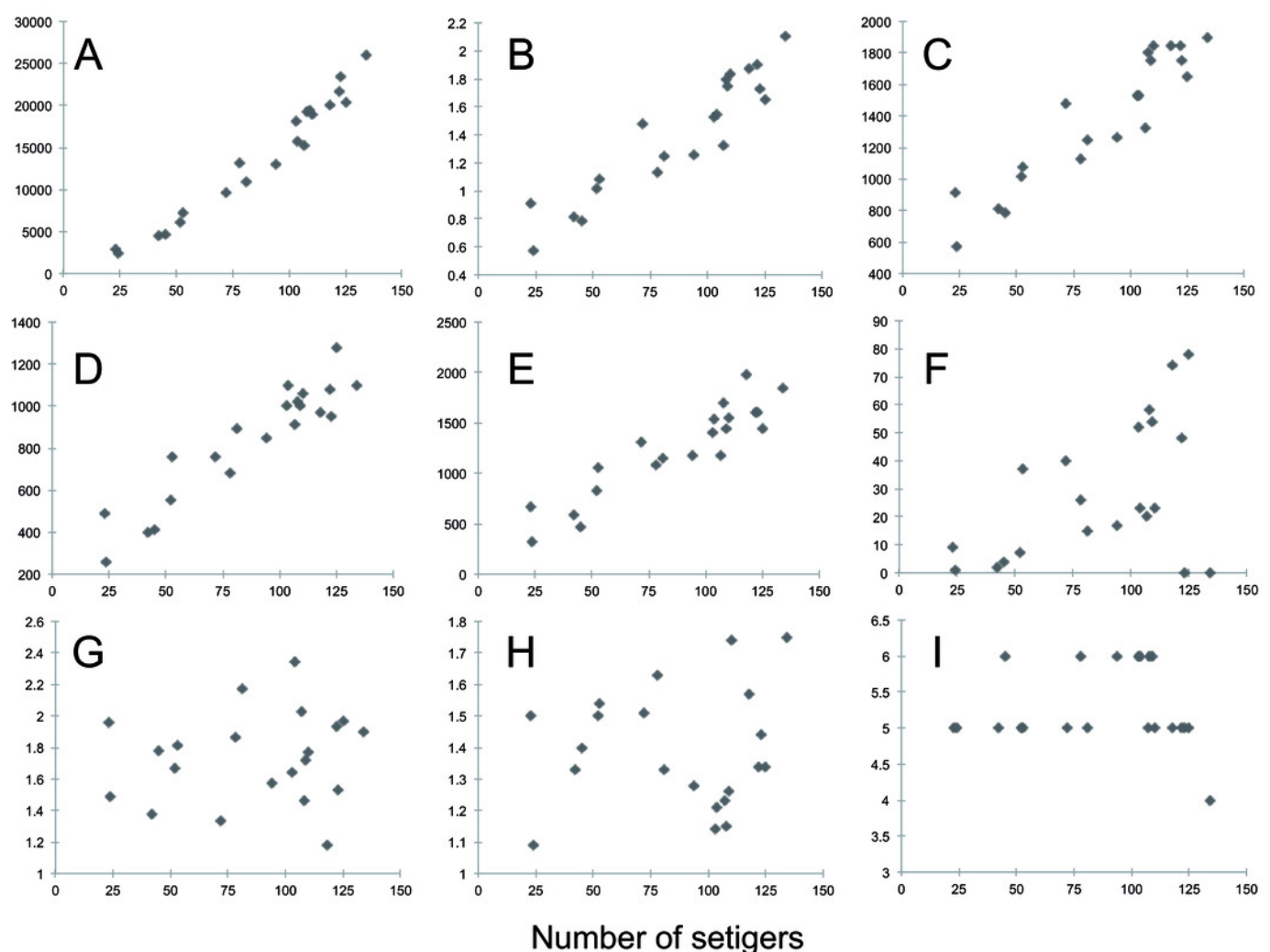


Table 1(on next page)

Relationships between species abundance and environment descriptors.

(A) Results of the Parametric Multidimensional analysis for the environment descriptors. (B) Results of the Parametric Multidimensional analysis for the species abundance according to the sampling years. (C) Results of the Pearson correlation analysis between species abundance and environment descriptors. Significant differences/correlations are highlighted in bold. MD: Mahalanobis distance; Pearson: correlation coefficient; p: significance level. 

A	2000		2001	
	MD	p	MD	p
	2001	0.669	0.272	-
2002	0.709	0.190	0.004	0.992

B	2000		2001	
	MD	p	MD	p
	2001	3.165	0.343	-
2002	2.944	0.267	5.763	0.086

C	Correlation	DDP	HYD	Ba	CS	S&C	PW	OM	N	P
<i>S. nkossa</i> sp. nov.	Pearson	-0.363	0.469	0.730	-0.350	0.125	-0.303	0.432	0.142	-0.541
	p	0.058	0.05	<0.0001	0.068	0.525	0.117	0.022	0.470	0.003
<i>S. parva</i>	Pearson	-0.285	-0.261	-0.182	-0.019	0.194	0.171	-0.268	0.245	0.165
	p	0.142	0.179	0.355	0.925	0.322	0.386	0.168	0.209	0.402
<i>Capitella</i> sp.	Pearson	-0.295	0.139	0.439	-0.505	0.456	-0.071	-0.020	0.457	-0.537
	p	0.128	0.481	0.020	0.006	0.015	0.781	0.920	0.015	0.003
<i>P. trionyx</i>	Pearson	-0.242	-0.052	0.223	-0.137	0.082	0.163	-0.039	0.301	-0.274
	p	0.214	0.792	0.255	0.487	0.677	0.406	0.845	0.120	0.159
<i>Raricirrus</i> sp.	Pearson	-0.200	0.596	0.558	-0.035	-0.195	-0.170	-0.028	0.156	-0.220
	p	0.307	0.001	0.002	0.860	0.320	0.387	0.889	0.428	0.260
<i>O. berrisfordi</i>	Pearson	-0.226	0.368	0.540	-0.244	0.147	-0.035	-0.114	0.138	-0.148
	p	0.247	0.054	0.003	0.211	0.456	0.859	0.562	0.483	0.452
<i>L. sebastiana</i>	Pearson	-0.159	0.665	0.422	-0.047	-0.315	-0.506	0.811	-0.093	-0.303
	p	0.420	0.000	0.025	0.814	0.102	0.006	< 0.0001	0.639	0.117
Ampharetidae sp.	Pearson	-0.387	0.431	0.497	-0.498	0.344	0.041	0.058	0.426	-0.554
	p	0.042	0.022	0.007	0.007	0.073	0.835	0.769	0.024	0.002

Table 2 (on next page)

Results of the analyses for the environment descriptors and species abundances according to the PCA station groups.

(A) ANOSIM pairwise tests (environmental descriptors). (B) Results of the Parametric Multidimensional Analysis for the sediment descriptors. (C) Results of the Parametric Multidimensional Analysis for the demsity of target species. (D) Results of the independent one-way ANOVAs for each sediment descriptor and target species. (E) Pairwise post-hock comparisons (Tuckey test). Bold: Significant differences. NS: Non significant; R: R statistic; PP: Possible permutations; AP: Actual permutations; N. Number $\geq$ Observed; DDP: Distance from drilling point; HYD: Total hydrocarbons; Ba: Barium; S&C: Silt and clay; CS: Coarse sand; PW: Pore water; N: Nitrogen; P: Phosphorous; DF: degrees of freedom; SS: Sum of Squares; MS: Mean squares; F: Fisher's test; p: significance level.

A	R	p	PP	AP	N
I vs. II	0.444	0.012	330	330	4
I vs. III	0.989	0.001	5985	999	0
II vs. III	0.863	0.001	346104	999	0

B	III		II		C	III		II	
	MD	p	MD	p		MD	p	MD	p
II	9.621	0.011	-	-	II	25.818	<0.0001	-	-
I	67.821	<0.0001	37.222	0.0003	II	35.059	<0.0001	19.637	0.0039

D	DF	SS	MS	F	p
DDP	2	4.655	2.328	17.329	<0.0001
HYD	2	53.722	26.861	177.048	<0.0001
Ba	2	12.821	6.411	63.591	<0.0001
S&C	2	0.120	0.060	3.423	0.049
CS	2	1.880	0.940	2.513	0.101
PW	2	0.009	0.004	2.675	0.089
OM	2	0.011	0.006	2.368	0.114
N	2	0.000	0.000	1.180	0.324
P	2	0.167	0.083	4.339	0.024
<i>S. nkossa</i> sp. nov.	2	43.195	21.597	179.281	<0.0001
<i>Capitella</i> sp.	2	25.918	12.959	39.718	<0.0001
<i>P. trionyx</i>	2	5.760	2.880	9.744	0.0007
<i>O. berrisfordi</i>	2	2.239	1.119	13.053	0.0001
<i>Raricirrus</i> sp.	2	2.897	1.448	6.879	0.004
<i>L. sebastiena</i>	2	1.335	0.668	8.175	0.002
Ampharetidae sp.	2	6.830	3.415	12.030	0.0002
<i>S. parva</i>	2	1.246	0.623	5.004	0.016

Table 3 (on next page)

Results of the Tuckey HSD pairwise comparisons for the environmental descriptors and the taget species densities.

D: difference; SD: Standard difference; CV: Critical value; P: probability; DDP: distance from drilling point; HYD: Total hydrocarbons; Ba: Barium; S&C: silt and clay; CS: coarse sand; OM: organic matter; PW: pore water; N: Nitrogen; P: Phosphorous; *Snk*: *Sigambra nkossa* sp. nov.; *Sp*: *S. parva*; *Cap*: *Capitella* sp. *Pt*: *Paramphinometryonix*. *Rsp*: *Raricirrus* sp. *Ob*: *Oxydromus berrisfordi*. *Ls*: *Lindaspio sebastiena*. *Amp*: Ampharetidae sp.

Table 3. Comparison of lateral lappets and number of teeth (including the main fang and the upper teeth) in thoracic and abdominal uncini of the species of *Loimia*; r: reduced.

Species	Lateral lappets	Thoracic uncini	Abdominal uncini	Location	Original description
<i>L. annulifilis</i>					Grube (1872)
<i>L. arborea</i>	1 and 2/3	5(6)	6(7)	Japan	Moore (1903)
<i>L. armata</i>					Carrerette & Nogueira (2015)
<i>L. bandera</i>					Hutchings (1990)
<i>L. batilla</i>	1 and 2/3	6	7	Australia	Hutchings & Glasby (1988)
<i>L. bermudensis</i>	1 and 3	5–6(6r)	5–6(6r)	Bermuda	Verrill (1900)
<i>L. borealis</i>					Wang <i>et al.</i> (2020)
<i>L. brasiliensis</i>					Carrerette & Nogueira (2015)
<i>L. contorta</i>					Ehlers (1908)
<i>L. crassifilis</i>					Grube (1878)
<i>L. davidi</i> sp. nov. (larger)	1 and 3	6(6r)	6(6r)	Açores Archipelago	This paper
<i>L. davidi</i> sp. nov. (smaller)	1 and 3	4	5	Açores Archipelago	This paper
<i>L. decora</i>	1 and 2/3	5	5	Sri Lanka	Pillai (1961)
<i>L. grubei</i>	1 and 2/3	5	5–6	Philippines	Holthe (1986)
<i>L. ingens</i>					Grube (1878)
<i>L. juani</i>					Nogueira Hutchings & Carrerette (2015)
<i>L. keablei</i>					Nogueira Hutchings & Carrerette (2015)
<i>L. macrobranchia</i>					Wang <i>et al.</i> (2020)
<i>L. medusa</i>	1 and 3	4–5	4–5	Persian Gulf / Red Sea	Savigny (1822)
<i>L. medusa angustescutata</i>					Willey (1905)
<i>L. megaoculata</i>					Carrerette & Nogueira (2015)
<i>L. minuta</i>	1 and 3	5	6	Caribbean (Mexico)	Treadwell (1929)
<i>L. nigrifilis</i>					Caullery (1944)
<i>L. ochracea</i>					Grube (1877)
<i>L. pseudotriloba</i>					Nogueira <i>et al.</i> (2015)
<i>L. ramzega</i>	1 and 3	6(5)	6(5)	Atlantic (France)	Lavesque <i>et al.</i> (2017)
<i>L. salazari</i>	1 and 3	4	4	Caribbean (Venezuela)	Londoño-Mesa & Carrera-Parra (2005)
<i>L. savignyi</i>					McIntosh (1885)
<i>L. savignyi</i>					Annenkova (1925)

trussanica

<i>L. triloba</i>	1 (3 and 4	5(6)	5(6)	Australia	Hutchings & Glasby (1988)
<i>L. tuberculata</i>					Nogueira <i>et al.</i> (2015)
<i>L. turgida</i>					Andrews (1891
<i>L. variegata</i>					Grube (1869)
<i>L. verrucosa</i>	1 and 3	7	7	Indonesia	Caullery (1944)
<i>L. viridis</i>	1 and 3	7–8	7–8	Massachusetts (USA)	Moore (1903)

4

5

Table 4(on next page)

Summary of the measurements (N = 21) and size correlations for the main morphological characters of *Sigambra nkossa* sp. nov.

(A) Size-dependent characters. (B) Size-independent characters; AVG: average; STD: standard deviation; Min: minimum; Max: maximum; Pearson: correlation coefficient; p: significance level; D: dorsal; V: ventral.

A	AVG ± STD	Min	Max	Pearson	P
Number chaetigers	87.000 ± 34.756	23	134	–	–
Total length (µm)	13941.429 ± 7243.994	2400	26000	0.979	<0.0001
Width chaetiger 15 with parapodia (µm)	1384.524 ± 411.321	570	1900	0.930	<0.0001
Width chaetiger 15 without parapodia (µm)	575.714 ± 186.167	190	850	0.896	<0.0001
Dry weight (mg)	1.396 ± 0.428	1	2	0.981	<0.0001
Length of prostomium (µm)	365.714 ± 102.887	175	550	0.789	<0.0001
Width of prostomium (µm)	553.810 ± 147.088	240	770	0.897	<0.0001
Length median antenna (µm)	834.524 ± 275.218	260	1280	0.932	<0.0001
Length lateral antennae (µm)	486.905 ± 169.422	175	825	0.861	<0.0001
D peristomial cirri (µm)	745.714 ± 287.269	185	1280	0.888	<0.0001
V peristomial cirri (µm)	536.429 ± 202.214	170	780	0.894	<0.0001
Length 1st D cirri (µm)	1237.143 ± 454.328	320	1985	0.918	<0.0001
Length 2nd D cirri (µm)	269.286 ± 111.100	85	450	0.880	<0.0001
Length 3rd D cirri (µm)	376.905 ± 160.113	120	650	0.816	<0.0001
Length 1st V cirri (µm)	229.048 ± 97.206	70	380	0.911	<0.0001
Length anal cirri (µm)	1240.476 ± 492.887	440	2460	0.801	<0.0001
Starting segment for notopodial capillaries	33.905 ± 31.868	0	108	0.780	<0.0001
B					
Starting segment for notopodial hooks	5.143 ± 0.359	5	6	-0.038	0.868
Starting segment for notopodial spines	9.380 ± 0.805	9	12	0.177	0.476
Length ratio for antennae (middle / lateral)	1.738 ± 0.288	1.18	2.34	0.122	0.599
Length ratio for peristomial cirri (D / V)	1.394 ± 0.189	1.09	1.75	0.086	0.709
Length ratio for cirri (D peristomial / 1st D)	1.679 ± 0.164	1.41	1.97	-0.063	0.787
Second V cirri (µm)	–	–	–	–	–
Length ratio for cirri (1st D / 1st V)	4.709 ± 0.730	3.75	6.24	-0.120	0.605

1

2