

Morphological and molecular systematic review of *Marphysa* Quatrefages, 1865 (Annelida: Eunicidae) species from South Africa (#48929)

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Morphological and molecular systematic review of *Marphysa* Quatrefages, 1865 (Annelida: Eunicidae) species from South Africa

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A vast polychaete fauna is hidden behind complexes of cryptic and pseudo-cryptic species, which has greatly hindered our understanding of species diversity in several regions worldwide. Among the eunicids, *Marphysa sanguinea* (Montagu, 1813) is a typical example, recorded in three oceans and with various species considered its junior synonyms. In South Africa, specimens previously misidentified as *M. sanguinea* are now known as *M. elityeni* Lewis & Karageorgopoulos, 2008. Of the six *Marphysa* Quatrefages, 1865 species recorded from the same region, three have their distributions restricted to South Africa while the others are considered to have worldwide distributions. Here, we evaluated the taxonomic status of the indigenous *M. elityeni* and investigated the presence of the widespread species *M. macintoshi* Crossland, 1903 and *Marphysa depressa* (Schmarda, 1861) in South Africa using morphological and molecular data. Our results reveal that *Marphysa elityeni* is a junior synonym of *M. haemasoma*, a species previously described from South Africa which is herein reinstated as a valid species. Both *Marphysa macintoshi* and *M. depressa* are not present in South Africa and their status as being distributed worldwide deserves further investigation. *Marphysa durbanensis* Day, 1934 and the new species described here, *M. sherlockae* **n. sp.**, had been, respectively, misidentified as *M. macintoshi* and *M. depressa*. Thus, the number of *Marphysa* species with distributions restricted to South Africa increased from three to five. This study reiterates the importance of implementing an integrated taxonomic framework to unravel local biodiversity.

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Abstract

A vast polychaete fauna is hidden behind complexes of cryptic and pseudo-cryptic species, which has greatly hindered our understanding of species diversity in several regions worldwide. Among the eunicids, *Marphysa sanguinea* (Montagu, 1813) is a typical example, recorded in three oceans and with various species considered its junior synonyms. In South Africa, specimens previously misidentified as *M. sanguinea* are now known as *M. elityeni* Lewis & Karageorgopoulos, 2008. Of the six *Marphysa* Quatrefages, 1865 species recorded from the same region, three have their distributions restricted to South Africa while the others are considered to have worldwide distributions. Here, we evaluated the taxonomic status of the indigenous *M. elityeni* and investigated the presence of the widespread species *M. macintoshi* Crossland, 1903 and *Marphysa depressa* (Schmarda, 1861) in South Africa using morphological and molecular data. Our results reveal that *Marphysa elityeni* is a junior synonym of *M. haemasoma*, a species previously described from South Africa which is herein reinstated as a valid species. Both *Marphysa macintoshi* and *M. depressa* are not present in South Africa and their status as being distributed worldwide deserves further investigation. *Marphysa durbanensis* Day, 1934 and the new species described here, *M. sherlockae* n. sp., had been, respectively, misidentified as *M. macintoshi* and *M. depressa*. Thus, the number of *Marphysa* species with distributions restricted to South Africa

increased from three to five. This study reiterates the importance of implementing an integrated taxonomic framework to unravel local biodiversity.

Keywords: COI sequences, distribution, morphology, new species, diversity.

Introduction

Studies implementing molecular and morphological tools in an integrated framework have found that a large portion of polychaete diversity has been hidden among complexes of cryptic and pseudo-cryptic species (Knowlton 1993; Nygren 2014; Hutchings & Kupriyanova 2018). Thus, unravelling these species complexes can uncover patterns of distribution, regional biodiversity, and areas of endemism of previously overlooked polychaete species, which could have management and conservation implications (Bickford et al. 2007; Nygren 2014).

Species belonging to *Marphysa* Quatrefages, 1865 (Quatrefages 1865a,b), which serve as important bait species around the world (Izuka 1912; Lewis & Karageorgopoulos 2008; Idris et al. 2014; Liu et al. 2017; Lavesque et al. 2017; Watson et al. 2017; Cole et al. 2018; Martin et al., 2020), are ideal candidates to investigate the incidence of complexes of pseudo-cryptic species. These complexes are frequently a consequence of very brief original species descriptions, as is *Marphysa sanguinea* (Montagu, 1813), type species of the genus (Hutchings & Karageorgopoulos, 2003). As a result of the brief species description, several morphologically similar species from far-flung places globally were considered junior synonyms of *M. sanguinea* (Hutchings & Karageorgopoulos 2003; Molina-Acevedo & Carrera-Parra 2015). As a consequence, its already broad distribution range was expanded, and it was reported to occur in Spain (Parapar et al. 1993), South Africa (Day 1967), Australia (Day 1967), Mexican Caribbean (Salazar-Vallejo & Carrera-Parra 1998) and Japan (Miura 1986) among others.

However, the detailed redescription of *M. sanguinea* and designation of the neotype (Hutchings & Karageorgopoulos 2003) resulted in the reinstatement of at least three junior synonyms as valid species, including *M. acicularum* Webster, 1884, *M. nobilis* Treadwell, 1917, and *M. viridis* Treadwell, 1917 (e.g., Molina-Acevedo & Carrera-Parra 2015; Molina-Acevedo & Idris, 2020). Furthermore, several new species with restricted distributions were described (e.g., Hutchings & Karageorgopoulos 2003; Glasby & Hutchings 2010; Zanol et al. 2016; Zanol et al. 2017; Liu et al. 2017; Martin et al. 2020), some of which had been erroneously identified as *M. sanguinea* (e.g., Hutchings & Karageorgopoulos 2003; Lewis & Karageorgopoulos 2008; Lavesque et al. 2017; Wang et al. 2018). Detailed observations of specimens demonstrated the variability in diagnostic characters, like branch distribution, parapodia shape, types of pectinate chaetae, coloration and shape of subacicular hooks, for *Marphysa* species that had previously been overlooked. The above-mentioned characters may apply to other species such as *M. teretiuscula* (Schmarda, 1861) and *M. macintoshi* Crossland, 1903, which also have suspiciously wide distribution ranges (Treadwell 1906, Read & Fauchald 2018).

Six valid species belonging to *Marphysa* are currently recognized as present in South Africa. Three have type localities in South Africa; *Marphysa capensis* (Schmarda, 1861), *Marphysa posteriobranchia* Day, 196, and *Marphysa elityeni* Lewis & Karageorgopoulos, 2008 (Day, 1967; Lewis & Karageorgopoulos 2008). The latter is commonly known as the “wonder worm” by local fishermen, and is part of the global *M. sanguinea* species complex (Day 1967; Lewis & Karageorgopoulos 2008; Simon et al. 2019). The remaining three *Marphysa* species

recorded for the region, namely *M. corallina* (Kinberg, 1865), *M. depressa* (Schmarda, 1861), and *M. macintoshi* Crossland, 1903 have type localities outside of South Africa and wide distributions (Day 1967). *Marphysa depressa* has a type locality in Auckland, New Zealand (Schmarda 1861), and has since been recorded in Hong Kong (Wang et al. 2018) and South African estuaries from Saldanha Bay to Durban Bay (Day 1953, 1967). *Marphysa macintoshi* was described from Zanzibar (Crossland 1903) and has since been recorded from several localities including Australia, South Africa, Caribbean Sea, Mozambique, Red Sea, Trinidad and Tobago and China (Read & Fauchald 2018). In South Africa, this species is supposedly present from Cape St. Francis to Durban Bay (Day, 1967). Interestingly, *M. durbanensis* Day, 1934 described from KwaZulu-Natal in South Africa, is considered a junior synonym of *M. macintoshi* (Day, 1967). Similarly, *M. haemasoma* Quatrefages, 1866 was described from Table Bay in South Africa and is currently considered a junior synonym of *M. sanguinea*. Thus, both species probably represent valid indigenous species that were incorrectly synonymized.

In this study, we investigated whether *M. depressa* and *M. macintoshi* occur in South Africa and examined the taxonomic validity of *M. haemasoma*. These were achieved by conducting thorough taxonomic revisions and where possible, molecular comparisons. We also provide redescrptions of *M. haemasoma*, *M. durbanensis*, and a description of *M. sherlockae* n. sp., a species new to science from South Africa.

Material and Methods

Examined material

Fresh *Marphysa depressa*-like specimens were collected from rock crevices in the fringing intertidal zones from Strand (-34.116108, 18.821698) (n = 4) (Fig. 1). Fresh specimens of *M. elityeni* were collected from the fringing intertidal zone at low tide from burrows in gravely-sand type sediment under boulders in Kommetjie (n = 5) (-34.159709, 18.327851) (Fig. 1). Full collection data for both species can be found in the respective species accounts in the results section. Live specimens were brought back to the laboratory where they were anesthetized with 7% MgCl₂ in distilled water, and photographed. Whole specimens from Strand were fixed in 96% ethanol. Posterior ends of the Kommetjie specimens were fixed in 96% ethanol, while the anterior ends were fixed in a 4% seawater-formalin solution. The collection of live material was approved by The Department of Agriculture, Forestry and Fisheries in South Africa under the permit number RES2019/49. Type and non-type material of *M. depressa*, *M. macintoshi*, *M. durbanensis*, *M. haemasoma* and *M. elityeni* deposited at the Natural History Museum (BMNH), Museum National d'Histoire Naturelle, Paris (MNHN) and the Natural History Museum, Vienna, Austria (NHM) and the Iziko South African Museum (SAM) were examined.

Morphological examination

Species descriptions were produced based on the type material, but a variation section with all specimens reviewed was also included.

The general structures such as the prostomium, peristomium, anterior region of the body, maxillary apparatus, branchiae, parapodia, chaetae, and pygidium were included in the descriptions. A dorsal incision was made in the specimen to extract and describe the maxillary apparatus, after which it was returned to its original position. The maxillary formula (MF) and

measurements were taken according to Molina-Acevedo & Carrera-Parra (2015, 2017). Six parapodia (three from the anterior region, two from the median, and one from the posterior region) were dissected to describe the morphology of the cirri and lobes, and simple and compound chaetae.

The chaetigers where branchiae and subacicular hooks start were indicated depending on the side where they began ('L' for Left, 'R' for Right) with the chaetiger number. In the region with the maximum number of branchial filaments, the long filaments are ≥ 4 times as long as dorsal cirri, whereas the short filaments are < 4 times as long as dorsal cirri. The terminology used for the descriptions of the pectinate chaetae is according to the classification proposed by Molina-Acevedo & Carrera-Parra (2015, 2017) and Zanol et al. (2016). Herein, thin and thick refers to the thickness of the pectinate shaft; wide and narrow refers to the width of the pectinate blade; and anodont and isodont refer to the relative length of external teeth in relation to each other and internal teeth, e.g., thin, wide isodont with long and slender teeth.

The length through chaetiger 10 (L10) and the width of chaetiger 10 excluding parapodia (W10) were measured in the specimens as standard measures when the specimens were collected incomplete. Likewise, the total length (TL) and variations of the total number of chaetigers (TChae) were recorded. All descriptions were illustrated with a series of photos taken with Canon EOS T6i. These were then stacked using Helicon Focus® 6 (Method A) software to improve the depth of field, and the final editing was performed in Adobe Photoshop® 2020.

To understand patterns of intraspecific variation, linear regression analyses were conducted to evaluate the possible relationships between size (length of specimens using L10 measurement) and morphological features such as the chaetigers where branchiae or the subacicular hooks begin, the number of branchial filaments. The degree of predictability of variation in morphological features following size variation is given by R^2 (e.g., $R^2 = 0.63$, $p = 0.05$, $n = 34$).

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Molecular methods

DNA extraction, amplification and sequencing

DNA was extracted from tissue samples using the ZR Genomic DNA Tissue MiniPrep Kit according to the standard manufacturer's protocol. The universal primer pair LCO1490 and HCO2198 (Folmer et al. 1994) was used to amplify a fragment of the mitochondrial gene cytochrome oxidase I (COI). PCR amplifications were carried out using 12.5 μ l of OneTaq Quick-Load Master Mix (New England BioLabs), 9.5 μ l of molecular biology grade water, 0.50 μ l of forward and reverse primer (10 μ M), 1 μ l of 1% bovine serum albumin (BSA) and 1 μ l of template

DNA to make up a total reaction volume of 25 μ l. Thermal cycling conditions were as follows for *M. elityeni* and *M. sherlockae* n. sp.: initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of 94 °C for 20 seconds, 45 °C for 30 seconds and 72 °C for 1 minute, followed by a final extension time at 72 °C for 5 minutes. Amplicons were Sanger sequenced at the Central Analytical Facility at Stellenbosch University using just the forward primer (LCO1490). Quality control was performed on sequences to check for any sequencing errors using BioEdit (v7.2.6) (Hall 1999).

Phylogenetic and species delimitation methods

The COI sequences were edited, trimmed, and aligned with ClustalW (Thompson et al. 1994) using multiple alignment methods in BioEdit (v7.2.6). Several species belonging to the *Marphysa* genus were included in the analysis for comparison together with seven other species from different genera within the Eunicidae and one species from Onuphidae as they were used as outgroups to root the tree (see Table 1). DnaSP v5 (Librado & Rozas 2009) was used to generate a nexus file for subsequent analysis. PAUP (Swofford 2003) and MrModelTest v2.3 (Nylander 2004) were used to calculate the best fit model of evolution for the data set using the Aikaike Information Criterion (AIC). Bayesian inference (BI) was used to reconstruct phylogenetic relationships using the best fit model SYM+G in MrBayes 3.1.2 (Ronquist et al. 2012). The trees were calculated using 4 Markov Chains of 5 million generations sampled simultaneously with every 1000th tree sampled. A 50% majority-rule consensus tree with posterior probability support was constructed by discarding the first 25% of trees as burn-in. Tracer v1.5 (Rambaut & Drummond 2009) was used to investigate the convergence of runs by analysing the average standard deviation of split frequencies (≤ 0.01). The mixing quality of all parameters was verified by analyzing the plot of likelihood versus the sampled trees and the effective sample sizes (ESS > 200), of which both criteria were satisfied. FigTree v1.4.4 (Rambaut 2013) was used to visualize trees. A Maximum Likelihood tree was computed in MEGA X (Kumar et al. 2018) and was run for 500 bootstrap replicates using the best-fit model of evolution, GTR, that was calculated in the same program.

A Newick formatted phylogenetic tree generated using FigTree v1.4.4 from the previous analysis was used as input for the Bayesian implementation of the Poisson tree process (bPTP) (Zhang et al. 2013) model for species delimitation using the online webserver <https://species-hits.org/>. The tree was rooted and run for 500,000 MCMC generations, with thinning set to 100 and burn-in and seed set to 0.1 and 123, respectively. The convergence of MCMC chains was visually checked on the maximum likelihood plot generated by the online server.

MEGA X was used to calculate the interspecific genetic distances between species using the Kimura 2-parameter (K2P) model with complete deletion of gaps and run for 500 bootstrap replicates.

Results

Thorough morphological comparisons indicate that *M. macintoshi* and *M. depressa* do not occur in South Africa. Instead, *M. durbanensis* (type locality: South Africa), which was previously synonymized with *M. macintoshi* (type locality: Tanzania/Zanzibar) (Day, 1967) has been found to differ from the latter species with regards to the shape of the prostomium, anterior postchaetal lobes, pectinate chaetae, and the shape and distribution of branchiae throughout the length of the body. As a result, we here consider *M. durbanensis* as a valid species.

Moreover, specimens initially identified as *M. depressa* (type locality: New Zealand) in South Africa were a misidentification and instead represents a new species to science, here named *M. sherlockae* n. sp.. Morphological comparisons reveal that *M. sherlockae* n. sp. differs from *M. depressa* in the shape and distribution of compound chaetae, the shape of postchaetal lobes, and the maximum number of branchial filaments. COI sequences of *M. depressa* were not available from its type locality and could not be compared with sequences of *M. sherlockae* n. sp. Nonetheless, *M. sherlockae* n. sp. forms an independent phylogenetic clade with high posterior probability and maximum likelihood support (Fig. 2) and genetically differs from other *Marphysa* species included in the phylogenetic analysis by 18-25%, confirming that it is a separate species. Additionally, results from the bPTP analysis supported *M. sherlockae* n. sp. as a single independent species (BS>0.95) (S1, supplementary information). *M. sherlockae* n. sp. is phylogenetically closest to *Marphysa californica* Moore, 1909, and *Marphysa brevitentaculata*, but the clade is poorly supported. Nonetheless, all three species genetically differ from each other by 18–20%.

Marphysa haemasoma is a valid species. The examination of type materials allowed us to confirm that *M. haemasoma* differs from *M. sanguinea* in the shape of the postchaetal lobe in anterior chaetigers and subacicular hooks, the maximum number of branchial filaments and in the distribution of the swollen base of ventral cirri. Furthermore, types of *M. elityeni* only differ from those of *M. haemasoma* in size-related features, such as the length of prostomial appendices, and where branchiae and ventral cirri with a swollen base start. For these reasons, and in view of the principle of priority (ICZN 1999, Arts. 23), we consider *Marphysa haemasoma* a senior synonym of *M. elityeni*. Furthermore, *M. haemasoma* forms a well-supported phylogenetic clade independent of the *M. sanguinea* clade (Fig. 2). The species are genetically different from each other by 20%, with results from the bPTP analysis (S1 supplementary information), confirming their separation as independent species (BS>0.95). Thus, these species are not synonymous.

Systematics

Order EUNICIDA Dales, 1962
Family EUNICIDAE Berthold, 1827
Genus *Marphysa* Quatrefages, 1865

Marphysa durbanensis Day, 1934

Figure 3, 4A, 5

Marphysa durbanensis Day, 1934:51–53, text-fig. 10.

Marphysa macintoshi – Day 1967:378 (*non* Crossland, 1903); Day 1974:59; Branch et al. 2016:68–69, Pl. 26, Fig. 26.6.

Material examined. *Type material:* Lectotype designated here BNHM 1934.1.19.166, Durban, South Africa, 1933, coll. JH. Day. One paralectotype BNHM 1934.1.19.166 designated here, same information as lectotype.

Comparative material examined. *Marphysa macintoshi*, syntypes, three specimens, BNHM 1924.3.1.22-3, slide BNHM.1924.3.1.22A, Zanzibar, Africa, 1901-1902, by digging in sand between intertidal on both east and west coasts of Zanzibar (syntype 1 incomplete specimen with

262 chaetigers, L10: 8.1 mm, W10: 2.7 mm; syntype 2 incomplete specimen with 106 chaetigers, L10: 5.3 mm, W10: 3 mm; syntype 3 incomplete specimen with 160 chaetigers, L10: 7.8 mm, W10: 3 mm).

Description. Lectotype complete, ventrally dissected from peristomium until chaetiger 9, with 380 chaetigers, L10= 14 mm, W10= 3.6 mm, TL= 305 mm. Last 48 chaetigers regenerating. Anterior region of body with convex dorsum and flat ventrum; body depressed from chaetiger 7, widest at chaetiger 24, tapering after chaetiger 37.

Prostomium bilobed, 1.7 mm long, 2.5 mm wide; lobes anteriorly rounded; median sulcus shallow dorsally (Fig. 3A), deep ventrally (Fig. 3B). Prostomial appendages in a semicircle, median antenna isolated by a gap. Palps reaching middle of first peristomial ring; lateral antennae reaching middle of second peristomial ring; median antenna broken, in paralectotype reaching middle of first chaetiger. Palpophores and ceratophores ring-shaped, short, thick; palpostyles and ceratostyles tapering, slender. Eyes not observed.

Peristomium (2.7 mm long, 3 mm wide) longer and wider than prostomium, first ring twice as long as second ring; separation between rings distinct on all sides (Fig. 3A–C). Ventral anterior edge of peristomium longer than dorsal, remaining features ventrally distorted by the dissection (Fig. 3B–C).

Maxillary apparatus with MF= 1+1, 5+6, 6+0, 4+8, 1+1 (Fig. 3D). MI 3.1 times longer than maxillary carriers. MI forceps-like, MI 4.6 times longer than closing system (Fig. 3D–E); ligament between MI and MII sclerotized. MII wider than rest of maxillae, with triangular teeth; MII 3.6 times longer than cavity opening oval (Fig. 3D–E); ligament present between MII–MIII and right MII–MIV slightly sclerotized (Fig. 3E). MIII with triangular teeth; with rectangular attachment lamella, situated in the centre of ventral edge of maxilla, slightly sclerotized (Fig. 3D–E). Left MIV with two left-most teeth big  attachment lamella semicircle, slender, better developed in central portion, situated 1/2 along anterior edge of maxilla. Right MIV with teeth of equal size; attachment lamella semicircle, slender, better developed in central portion, situated 2/3 along anterior edge of maxilla, sclerotized (Fig. 3D–E). MV square, with a short triangular tooth. Mandibles dark; missing calcareous cutting plates; sclerotized cutting plates brown, with 20 growth rings (Fig. 3F).

Branchiae pectinate with up to 11 long filaments at around 64–80% of the body, present from chaetigers 28L–29R to 370 (Fig. 3J–K). First pair and last 10 with one filament; reach the maximum 10 or 11 filaments in chaetigers 241L–307L (Fig. 4A). Branchial filaments longer than dorsal cirri except in first five and last seven branchiae.

First two parapodia smallest; best developed in chaetigers 6–26, following ones becoming gradually smaller. Notopodial cirri conical in anterior-median chaetigers, digitiform in posterior ones; longer than ventral cirri in anterior chaetigers, of similar length in posterior ones; best developed in chaetigers 3–30, following ones gradually smaller (Fig. 3G–K). Prechaetal lobes short, as transverse fold in all chaetigers (Fig. 3G–K). Chaetal lobes rounded in all chaetigers, shorter than postchaetal lobes in anterior region, longer than the other lobes in median-posterior region; with aciculae emerging dorsal to midline (Fig. 3G–K). Postchaetal lobes well developed in first 40 chaetigers; digitiform in first five chaetigers, rounded from chaetiger 6; progressively smaller from chaetiger 22; from chaetiger 41 inconspicuous (Fig. 3G–K). Ventral cirri bluntly conical in first five chaetigers; in chaetigers 6 to 355 with a short oval base and digitiform tip; conical from chaetiger 356, gradually reducing in size (Fig. 3G–K).

Aciculae blunt, reddish along most of their length, amber on the distal tip (Fig. 3G–K). First eight chaetigers with three aciculae; in chaetigers 9–18 with four aciculae; in chaetigers 19–44 with three or four aciculae; in chaetigers 45–124 with two aciculae; from chaetiger 125 with only one acicula.

Limbate chaetae of two lengths in same chaetiger, dorsalmost longer; reduced in number around chaetiger 30. Five types of pectinate chaetae, anterior chaetigers: thin, narrow isodont with long and slender teeth, 3–4 pectinate, with up to 14–15 teeth (Figs. 3L, 5A); median and posterior chaetigers: thin, wide isodont with short and slender teeth, 4–5 pectinate, with up to 23–24 teeth (Figs. 3M, 5B); thick, wide isodont with short and thick teeth, 1–2 pectinate, with up to 19 teeth (Figs. 3N, 5C); and thick wide anodont with short and slender teeth, 1–2 pectinate, with 12 teeth (Figs. 3O, 5D); posterior chaetigers: thick, wide anodont with long and thick teeth, 1–2 pectinate, with up to 17 teeth. Compound spinigers present in all chaetigers, in anterior-median chaetigers with blades of two lengths, shorter ones more abundant (Fig. 3P). Subacicular hooks unidentate, amber, present from chaetiger 46, one or two per chaetiger, with continuous distribution (Fig. 3Q).

Pygidium with dorsal pair of anal cirri as long as last eight chaetigers; ventral pair short, as long as last two chaetigers.

Variations. Material examined L10= 12–14 mm, W10= 3.6–4 mm, TChae= 322–380. Palps reaching middle of first or second peristomial ring; lateral antennae reaching middle of second peristomial ring or first chaetiger; median antenna reaching first chaetiger. The maxillary variations are MII 5–6+6–8, MIII 6, MIV 3–4+6–8. The proportion of maxillary apparatus varies as follows: MI are 3.1–3.2 times longer than maxillary carriers; MI are 4.6–5.3 times longer than closing system; MII are 3.5–3.6 times longer than length of cavity opening. Branchiae from chaetigers 28–32 to 10–13 chaetigers before pygidium. Maximum number of branchial filaments varied from 11 to 12. Postchaetal lobe well developed in the first 40 chaetigers. Ventral cirri with a swollen base from chaetigers 4–5 to 25 chaetigers before pygidium. Start of subacicular hooks in chaetigers 46–47.

Habitat. Day (1934) does not provide information about the specific substrate, although he did clarify that the collection was between the tidemarks in Durban Bay and Umkomaas.

Distribution. Day (1934) recorded this species from Durban Bay and Umkomaas in KwaZulu-Natal, South Africa.

Remarks. The original description of *Marphysa durbanensis* provides a variation of the two specimens collected that matches with the specimens deposited in the BMNH. Day (1934) described almost colorless eyes, but they were not observed in this study. Possibly the color has faded due to the long-term preservation of the specimens. The best-preserved specimen is herein selected as a lectotype to fix the species definition (ICZN 1999, Arts. 74.1, 74.7.3), whereas the other is considered a paralectotype (ICZN 1999, Art. 74F).

Day (1934) considered *M. durbanensis* different from morphologically similar species such as *M. simplex* Crossland, 1903 (= *M. teretiuscula*), and *M. acicularum* when he described the species. However, in his monograph of the polychaetes from South Africa, the author considered *M. durbanensis* a junior synonym of *M. macintoshi* without making any reference to this nomenclatural action (Day 1967, page 378). Herein, apparent differences were found between the species. *Marphysa durbanensis* (L10: 14 mm) has a bilobed prostomium, the branchiae are

pectinate and start from chaetigers 28–32, the postchaetal lobe is digitiform in first four chaetigers, and there are five types of pectinate chaetae; while in *M. macintoshi* (L10: 4.5 mm) the prostomium is unilobed with a shallow median sulcus at the anterior edge, the branchiae are palmate with a short button-shaped branchial stem and start from chaetiger 32–47, the postchaetal lobe is conical in the first four chaetigers, and there are only three types of pectinate chaetae. Due to these morphological differences, *M. durbanensis* is considered a valid species.

Marphysa durbanensis resembles *M. haemasoma* (see below) by the presence of compound spinigers distributed in all chaetigers; however, *M. durbanensis* has more teeth in MII (5–6+6–8), digitiform postchaetal lobes in first four chaetigers, five types of pectinate chaetae, and the subacicular hook with a continuous distribution even in smaller specimens. However, *M. haemasoma* has fewer teeth in MII (4+4). The postchaetal lobe is ovoid in the first four chaetigers. There are only four types of pectinate chaetae, and the subacicular hook has a discontinuous distribution in small specimens.

Marphysa durbanensis resembles *M. victori* Lavesque, Daffe, Bonifacio & Hutchings, 2017, *M. hongkongensa* Wang, Zhang & Qiu, 2018, *M. leidii* Quatrefages, 1866, *M. parishii* Baird, 1869 and *M. teretiuscula* by the presence of five types of pectinate chaetae; however, *M. durbanensis* has a digitiform postchaetal lobe in the first four chaetigers, and the subacicular hook is amber, while *M. teretiuscula* has an ovoid postchaetal lobe in the first four chaetigers, and the subacicular hook is reddish basally and translucent in the distal region. Also, *M. leidii* has a conical postchaetal lobe in the first chaetigers. Otherwise, *M. durbanensis* has long branchial filaments, and the branchiae are pectinate; while for *M. hongkongensa*, the branchial filaments are short, and the branchiae are pectinate and palmate with a short button-shaped branchial stem in some regions of the body. On the other hand, in *M. durbanensis* (L10: 14 mm), the eyes are present, and the branchiae start in chaetigers 28–32; while *M. victori* (L10: 6.3–7.9 mm) lacks eyes, and the branchiae start in chaetiger 36. Finally, *M. durbanensis* has up to 11–12 branchial filaments while *M. leidii* (L10: 10.7–17 mm) and *M. parishii* (L10: 17.2 mm) only have 4 to 6 filaments.

Marphysa haemasoma Quatrefages, 1866

Figure 3, 6–7

Marphysa haemasoma Quatrefages, 1866:334–335; Grube 1870:299.

Marphysa sanguinea – Marenzeller 1888:11, Fauvel 1902:61; Day 1967:378 (*non* Montagu, 1813); Day 1974:59.

Marphysa sanguinea haemasoma Willey, 1904:263, Pl.13, Fig.15

Marphysa elityeni Lewis & Karageorgopoulos, 2008:279–281, Figs. 1–2, Table 1, 2, 3; Branch et al. 2016:68–69, Pl. 26, Fig. 26.5.

Material examined. *Type material:* Holotype *Marphysa haemasoma* MNHN type 613, Cape of Good Hope, South Africa. *Additional material:* Five incomplete specimens SAM-A090272, SAM-A090273, SAM-A090274, SAM-A090275, SAM-A090317, Kommetjie, South Africa from sand burrows under boulders at fringing intertidal zone, coll. A.N. du Toit, 10 Mar 2017, 18°19'40.7"E 34°09'33.0"S.

Comparative material examined. Holotype *Marphysa elityeni* SAM-A21478, Cape of Good Hope, South Africa. Eight paratypes of *Marphysa elityeni* BNHM 2007.69, SAM-A21479, SAM-A21480, SAM-A21481, Buffels Bay in the Cape of Good Hope, South Africa, 15 Sep 2004,

18°29'27" E 34°21'6" S. Neotype *Marphysa sanguinea* Benth. 1867.1.7.24, Polperro, Cornwall, in mud and gravel at low water mark, coll. Laughrin, Reddell. Hutchings (2 specimens from this lot), Desig. P. Hutchings (Neotype complete specimen with 286 chaetigers, L10: 16.7 mm, W10: 10 mm; topotype complete specimen with 239 chaetigers, L10: 20.4 mm, W10: 7.2 mm).

Description. Holotype complete, gravid female, with 322 chaetigers, L10= 12.3 mm, W10= 7 mm TL= 309 mm. Anterior region of the body with convex dorsum and flat venter; body depressed from chaetiger 5, widest at chaetiger 25, tapering after chaetiger 41.

Prostomium bilobed, 2.8 mm long, 4 mm wide; lobes anteriorly rounded; median sulcus dorsally shallow (Fig. 6A), ventrally deep (Fig. 6B). Prostomial appendages in a semicircle, median antenna isolated by a gap. Palps reaching first chaetiger; lateral and median antennae reaching second chaetiger. Palpophores and ceratophores ring-shaped, short, thick; palpostyles and ceratostyles tapering, slender. Eyes colourless, as a scar between palps and lateral antennae.

Peristomium (2.8 mm long, 6.3 mm wide) wider than prostomium; first ring three times as long as second ring, separation between rings distinct only dorsally and ventrally (Fig. 6A–C). Ventral region of the first ring with a slight central depression in anterior edge (Fig. 6B).

Maxillary apparatus with MF= 1+1, 4+4, 5+0, 3+7, 1+1 (Fig. 6D). MI 3 times longer than maxillary carriers. MI forceps-like, MI 4 times longer than closing system (Fig. 6D–E); ligament between MI and MII, sclerotized. MII with triangular teeth, right anterior teeth broken; MII 3.6 times longer than cavity opening (Fig. 6D–E); ligament present between MII–MIII and right MII–MIV slightly sclerotized (Fig. 6E). MIII with triangular teeth; with rectangular attachment lamella, situated only in the centre of right edge of maxilla, slightly sclerotized (Fig. 6D–E). Left MIV with all teeth of similar size; attachment lamella semicircle, wide, better developed in right portion, situated 2/3 of anterior edge of maxilla. Right MIV with lateral larger teeth; attachment lamella semicircle, wide, better developed in central portion, situated 2/3 of anterior edge of maxilla, sclerotized (Fig. 6D–E). MV square, with a short triangular tooth. Mandibles dark; with calcareous cutting plates present and sclerotized cutting plates brown, with nine growth rings (Fig. 6F).

Branchiae pectinate with up to six long filaments for around 20–54% of the body, present from chaetigers 26L–27R to 308L–311R (Fig. 6I–J). First two and last 13 pairs with one filament; with six filaments in chaetigers 79L to 173L (Fig. 4B). Branchial filaments longer than dorsal cirri except in first two and last branchiae.

First two parapodia smallest; best developed in chaetigers 7–40, following ones gradually becoming smaller. Notopodial cirri conical in all chaetigers; of similar length as ventral cirri in anterior and posterior chaetigers, shorter than ventral cirri in median chaetigers; best developed in chaetigers 4–37, following ones gradually smaller (Fig. 6G–K). Prechaetal lobes short, as transverse folds in all chaetigers (Fig. 6G–K). Chaetal lobes in first 37 chaetigers rounded, shorter than postchaetal lobe in anterior region, with aciculae emerging dorsal to midline; from chaetiger 38 triangular, longer than other lobes in median-posterior chaetigers (Fig. 6G–K). Postchaetal lobes well developed in first 60 chaetigers; ovoid in first six chaetigers, rounded in chaetigers 7–9, auricular from chaetiger 10, progressively smaller from chaetiger 35; from chaetiger 61 inconspicuous (Fig. 6G–K). Ventral cirri digitiform in first three chaetigers; in chaetiger four to last chaetiger with a short oval base and digitiform tip (Fig. 6G–K).

Aciculae blunt, reddish along most of their length, amber on distal tip (Fig. 6G–K). First 10 chaetigers with three aciculae; in chaetigers 11–77 with three or four; in chaetigers 78–161 with three; in chaetigers 162–322 with two or three.

Limbate chaetae of two lengths in same chaetiger, dorsalmost longer, reduced in number around chaetiger 24. Four types of pectinate chaetae; in anterior chaetigers: thin, narrow isodont with long and slender teeth, with 2–3 pectinate, with up to 17 teeth (Fig. 6L); median-posterior chaetigers: thick, wide isodont with short and slender teeth, with 6–7 pectinate, with up to 17 teeth (Fig. 6M); posterior chaetigers: thick, wide anodont with short and slender teeth, with 6–7 pectinate, with up to 13–14 teeth (Fig. 6N), and thick, wide anodont with long and thick teeth, with 1–2 pectinate, with up to 10 teeth (Fig. 6O). Compound spinigers present in all chaetigers, with blades of two sizes in the same chaetiger (Fig. 6P), shorter slightly more abundant than longer blade. Subacicular hooks absent; in paratype of *M. elityeni* (L10= 9.3 mm) subacicular hook bidentate, translucent, present only in regenerating chaetigers, one per chaetiger; with triangular teeth, distal tooth smaller than proximal, directed upward; proximal tooth triangular, directed laterally (Fig. 6Q).

Pygidium with dorsal pair of anal cirri broken; ventral pair as long as last chaetiger.

Variations. Material examined L10= 9.3–20.1 mm, W10= 6.2–14.5 mm, TChae= 194–486. Palps reaching second peristomial ring or first chaetiger; lateral antennae reaching first or second chaetiger; median antenna reaching first or middle of second chaetiger. The maxillary variations are MII 4+4, MIII 3–5, MIV 3–4+6–7. The proportion of maxillary apparatus varies as follows: MI are 2.6–3 times longer than maxillary carriers; MI are 4.1–4.6 times longer than closing system; MII are 4–4.3 times longer than cavity opening. Branchiae from chaetigers 26–37 to 10 chaetigers before pygidium. Maximum number of branchial filaments varied from six to 10. Postchaetal lobe well developed in first 57–60 chaetigers. Ventral cirri with a swollen base from chaetigers 3–6 to last chaetigers.

DNA barcode. Type locality: Kommetjie, Western Cape, South Africa (MB-A090272) (GenBank accession number: MN067877) (Simon et al. *unpublished data*). 577 bp fragment isolated with universal mitochondrial cytochrome oxidase subunit 1 gene, primer pair: LCO1490, HCO2198 (Folmer et al. 1994).

Habitat. Very common in the boulder fields at the lower intertidal zones of sheltered bays, and in rock pools. Worms can be found under rocks in sand burrows up to 1 m deep.

Distribution. Table Bay to Buffels Bay, Cape Point, Western Cape South Africa (Quatrefages 1866; Lewis & Karageorgopoulos 2008). Branch et al. (2016) recorded this species to occur from Namibia in southwest Africa to East London in South Africa. Simon et al. (*unpublished data*) recorded this species from Melkbosstrand to Knysna in the Western Cape and therefore falls within the currently accepted distribution range of this species according to Branch et al. (2016). However, the records from Namibia have not been verified and may also represent an overlooked indigenous species of that region and therefore should be revised.

Remarks. Specimens of *M. haemasoma* were previously redescribed by Grube (1870) and then identified as *M. sanguinea* after von Marenzeller (1888) synonymized *M. haemasoma* with *M. sanguinea* due to similarities in morphology and habitat observed in the specimens from the Cape of Good Hope. Later, Lewis & Karageorgopoulos (2008) realized that specimens from this region had been misidentified as *M. sanguinea*, which led to the description of *Marphysa elityeni* Lewis

& Karageorgopoulos, 2008. However, Lewis & Karageorgopoulos (2008) overlooked *M. haemasoma*.

After the comparison between the type material of *M. haemasoma* and *M. elityeni* we found stable similarities in the shape of the prostomium (Figs. 6A, 7A), the proportions of maxillary apparatus, the number of teeth per maxilla and the shape of the maxillary apparatus (Figs. 6D–E, 7B–C), the form of the branchiae in median-posterior chaetigers (Figs. 6J, 7F), the shape of the dorsal cirri, ventral cirri, and postchaetal lobe in anterior chaetigers (Figs. 6G–H, 7D–E, G–H), as well as, the presence of the same type of pectinate chaetae (Figs. 6M–N, 7I–J) and compound chaetae, and the form and coloration of subacicular hook (Figs. 6Q, 7K). Some differences were related to the size dependence of characters, like the beginning of the branchiae, the number of filaments, and the development of the postchaetal lobe (*M. elityeni* material L10: 9.3–18.5 mm, branchiae from chaetiger 27–37, number of filaments from 6–10, ending of the postchaetal lobe from chaetiger 33–82; *M. haemasoma* material L10: 12.3 mm, branchiae from chaetiger 26, number of filaments reached 6, ending of the postchaetal lobe in chaetiger 60).

Marphysa haemasoma (L10: 9.3–18.5 mm) is considered a different species from *M. sanguinea* (L10: 11.5–20.4) because the former has up to 10 branchial filaments, and ovoid postchaetal lobes in anterior chaetigers; whereas the latter has 9–18 branchial filaments, and digitiform postchaetal lobes in anterior chaetigers. Moreover, in *M. haemasoma* the swollen base of the ventral cirri continues until the last chaetigers, and the subacicular hook is translucent; while in *M. sanguinea* the swollen base of the ventral cirri ends between 8–18 chaetigers before the pygidium, and the subacicular hook is reddish basally and translucent distally.

Marphysa haemasoma resembles *M. aegypti* Elgetany, El-Ghobashy, Ghoneim & Struck, 2018, *M. fauchaldi* Glasby & Hutchings, 2010, *M. gravelyi* Southern, 1921, *M. nobilis* Treadwell, 1917, *M. teretiuscula* (Schmarda, 1861) and *M. tripectinata* Liu, Hutchings & Sun, 2017 by the presence of the ovoid postchaetal lobes; however, *M. haemasoma* has subacicular hooks that are completely translucent, while *M. nobilis*, *M. teretiuscula*, and *M. tripectinata* have subacicular hooks that are reddish at the base and translucent in the distal region. Furthermore, *M. haemasoma* has four types of pectinate chaetae, while *M. fauchaldi* and *M. gravelyi* have only three types. Additionally, when present in *M. haemasoma*, subacicular hooks (in regenerating chaetigers) are bidentate, while *M. aegypti* bears unidentate subacicular hooks (Martin et al. 2020). Moreover, *M. haemasoma* has fewer teeth in MII and MIII (4+4, 4–5+0), while *M. gravelyi* has more teeth in the same plates (MI 8+7, MII 8+0). Finally, *M. haemasoma* has long branchial filaments, while in *M. fauchaldi*, the branchial filaments are short.

Type material of *M. elityeni* was collected from Buffels Bay, Cape Peninsula (Lewis & Karageorgopoulos 2008), which is ~58.4 km away from Table Bay where type material of *M. haemasoma* was collected (Fig. 1). Additionally, Kommetjie, where the fresh materials examined and sequenced in this study were collected, is near both Buffels Bay (~29.4 km) and Table Bay (~43 km). Thus, all these collections fall within the type region of the original collected material from Table Bay (Fig. 1).

Marphysa sherlockae n. sp.

urn:lsid:zoobank.org:act:2D2AC893-C074-46CC-B731-F0D632C66836

Figure C, 8–10

Marphysa depressa – Day 1953:434, text-figs. 5 n, p; 1967:395–396, Figs. 17.5 n–t (non Schmarda, 1861); Day 1974:59; Branch et al. 2016:68–69, Pl. 26, Fig. 26.8.

Material examined. *Type material:* Holotype BNHM 1963.1.84, Langebaan Lagoon, South Africa, coll. J.H. Day. Paratype, one specimen BNHM 1952.5.10.7. Paratype, two specimens SAMC-A089089 and SAMC-A089090), Strand, False Bay, South Africa, 34°06'57.9" S, 18°49'18.1" E, coll. J. Kara, 20 March 2019, det. J. Kara. *Additional material:* two specimens BNHM XXXX, same data as holotype. One incomplete specimen SAMC-A20578, Langebaan lagoon, South Africa, coll. UCT ecological survey, 24 April 1949, det. J.H. Day. One complete specimen SAMC-A60425, Langebaan Lagoon, South Africa, coll. UCT ecological survey, 24 April 1949, det. D. Clarke. Two complete specimens, SAMC-A089091 and SAMC-A089092), Strand, False Bay, South Africa, 34°06'57.9" S, 18°49'18.1" E, coll. J. Kara, 20 March 2019, det. J. Kara.

Comparative material examined. Syntypes, two specimens, *Marphysa depressa* NHM XXX, New Zealand, Port of Auckland, coll. Schmarda (syntype 1 complete specimen with 328 chaetigers, L10: 9.5, W10: 4 mm; syntype 2 complete specimen with 132 chaetigers, L10: 9.5 mm, W10: 4.8 mm).

Description. Holotype complete, with 208 chaetigers, L10= 6.6 mm, W10= 1.7 mm, TL= 67 mm. Anterior region of body with convex dorsum and flat venter, body depressed from chaetiger 6, widest at chaetiger 38, tapering after chaetiger 112.

Prostomium bilobed, 1 mm long, 1.1 mm wide; lobes frontally oval; with median sulcus dorsally shallow (Fig. 8A), ventrally sulcus deep (Fig. 8B). Prostomial appendages in a semicircle, median antenna isolated by a gap. Palps reaching first chaetiger; lateral antennae reaching second chaetiger; median antenna reaching middle of second chaetiger. Palpophores and ceratophores ring-shaped, short, thick; palpostyles and ceratostyles tapering, slender. Eyes as a brown line, between palps and lateral antennae.

Peristomium (1.1 mm long, 3.2 mm wide) wider than prostomium, first ring twice as long as second, separation between rings distinct on all sides (Fig. 8A–C). Ventral region of the first ring with a slight central depression in anterior edge (Fig. 8B).

Maxillary apparatus with MF= 1+1, 3+5, 5+0, 4+8, 1+1 (Fig. 6D). MI 2.3 times longer than maxillary carriers. MI forceps-like, MI 4.3 times longer than the closing system; ligament between MI and MII, slightly sclerotized (Fig. 8D–E). MII with recurved teeth; MII 5 times longer than cavity opening oval (Fig. 8D–E); ligament present between MII and MIII and right MIV slightly sclerotized (Fig. 8E). MIII with blunt teeth; with rectangular attachment lamella, situated in the anterior of right edge of maxilla, slightly sclerotized (Fig. 8D–E). Left MIV with left-most tooth larger; attachment lamella semicircle, wide, better developed in right portion, situated along anterior edge of maxilla (Fig. 8D–E). Right MIV with right-most tooth larger; attachment lamella semicircle, wide, better developed in central portion, situated along anterior edge of maxilla (Fig. 8D–E). MV square, with a short-rounded tooth. Mandibles dark; missing calcareous cutting plates, sclerotized cutting plates brown, with 10 growth rings (Fig. 8F).

Branchiae palmate with a short button-shaped branchial stem, with up to two long filaments, present from chaetigers 28R–37L to 195L–196R (Fig. 8J–K). One filament in chaetigers 28L and 31L–45L; without filament in chaetigers 29L–30L; two filaments in chaetigers 46L–170L; one filament in chaetigers 171L–196L (Fig. 4C). Branchial filaments longer than dorsal cirri.

First two parapodia smallest; best developed in chaetigers 6–42, following ones becoming gradually smaller. Notopodial cirri conical in all chaetigers; longer than ventral cirri in anterior chaetigers, shorter in median chaetigers, of similar size in posterior ones; best developed in chaetigers 3–41, following ones gradually decreasing in size (Fig. 8G–K). Prechaetal lobes short. Chaetal lobes in first 29 chaetigers rounded, shorter than postchaetal lobe, with aciculae emerging dorsal to midline; from chaetiger 30 triangular, longer than other lobes (Fig. 8G–K). Postchaetal lobes slightly developed in first 24 chaetigers; triangular first 5 chaetigers, following ones auricular, progressively smaller from chaetiger eight; from chaetiger 25 inconspicuous (Fig. 8G–K). Ventral cirri conical in first six chaetigers; from chaetigers 7 to 138 with a short oval base and digitiform tip; conical from chaetiger 139, gradually smaller (Fig. 8G–K).

Aciculae blunt, reddish from base to most of its length, translucent on the distal tip (Fig. 8G–K). First five chaetigers with 2 aciculae; in chaetiger 6–10 with three aciculae; in chaetigers 11–73 with two aciculae; from chaetiger 74 with only one acicula.

Limbate chaetae of two lengths in same chaetiger, dorsal-most longer, reduced in number around chaetiger 13. Two types of pectinate chaetae; in anterior chaetigers: thin, narrow isodont with long and slender teeth, 1–2 per parapodium and up to 10–11 teeth (Figs. 8L, 9A–C); in median-posterior chaetigers, thick, wide isodont with long and thick teeth, 4–5 per parapodium and up to 14 teeth (Figs. 8M, 9D–E); anodont pectinate not observed. Compound spiniger chaetae present in all chaetigers, with blades of similar size in the same chaetiger (Fig. 8N), longer blades in median-posterior chaetigers. Compound falciger chaetae in anterior-median chaetigers, more abundant than compound spiniger in first 26 chaetigers; in anterior region blades of similar length (56 μ m, Fig. 8O), with triangular teeth, both of similar size, proximal tooth directed laterally, distal directed upward; in median chaetigers with blades shorter (38.5 μ m) with teeth of similar shape. Subacicular hooks bidentate, reddish from base to most of its length, with translucent tip, starting from chaetigers 41R–42L, one per chaetiger, with continuous distribution; with blunt teeth, distal tooth smaller than proximal, both teeth directed upward (Fig. 8P); some chaetigers with subacicular hook unidentate with hoods.

Pygidium with dorsal pair of anal cirri as long as last seven chaetigers; ventral pair short, as long as the last chaetiger.

Variations. Material examined varied in the following features: L10= 3–6.6 mm, W10= 1.3–2.1 mm. Palps reaching second peristomial ring or first chaetiger; lateral antennae reaching middle of first or second chaetiger; median antenna reaching third or fourth chaetiger. Maxillary formula varies as follows: MII 3–4+4–5, MIII 5–6, MIV 3–4+7–8. The proportion of maxillary apparatus varies as follows: MI are 2.4–2.7 times longer than maxillary carriers; MI are 4.3–5 times longer than closing system; MII are 3–3.3 times longer than cavity opening. Branchiae from chaetigers 25–34. The maximum number of branchial filaments 2. Postchaetal lobe well developed in first 17–91 chaetigers. Ventral cirri with a swollen base from chaetigers 3–7 to 70 chaetigers before pygidium. Falcigers present up to last chaetiger (L10= 3–6 mm) or median region (L10= 6.1–66 mm). Start of subacicular hooks in chaetigers 28–43.

Regression analyses indicated that there are no correlations between the start of the branchiae ($R^2 = 0.0702$, $p = 0.26$, $n = 11$, Fig. 10), the maximum number of branchial filaments ($R^2 = 0.000$, $p = 0.00$, $n = 11$, Fig. 10) or the start of the subacicular hooks ($R^2 = 0.1307$, $p = 0.35$, $n = 11$, Fig. 10) with the length to chaetiger 10 for this species. The chaetiger where the branchiae start does not follow a pattern regarding their growth but starts to emerge from chaetiger 20 to 30 (Fig. 8, blue points). This same situation is repeated with emergence of subacicular hooks, starts

between chaetiger 30 and 40 (Fig. 10, orange points). However, the number of filaments (2 filaments) seems to be fixed regardless of the size of the organism, a contrasting pattern with other *Marphysa* species in which the number of filaments appears to increase with the length of the specimen.

On the other hand, *M. sherlockae* n. sp. has similar characteristics to other species of *Marphysa* where the presence of compound chaetae is size-dependent (Aiyar 1931; Pillai 1958; Salazar-Vallejo & Carrera-Parra 1998; Molina-Acevedo & Carrera-Parra 2017; Molina-Acevedo 2018). *Marphysa sherlockae* n. sp. specimens with $L10 \leq 6\text{mm}$ possess compound falcigers to the last chaetiger. In this group of individuals, the number of falcigers per chaetiger decreased from median to posterior region, which was more noticeable in specimens with $L10$ close to 6 mm. Additionally, specimens with $L10 > 6\text{ mm}$ do not have falcigers in the posterior region. This condition indicates that in the largest specimens of *M. sherlockae* n. sp. falcigers will be lost, and only compound spinigers will be observed, as demonstrated in *M. gravelyi* Southern, 1921, *M. borradailei* Pillai, 1958 and *M. brevitentaculata* Treadwell, 1921.

Etymology: The species is named after Emma Sherlock, in recognition of her valuable work on the polychaete collections of BHNM.

DNA barcode: Type region: Strand, False Bay, Western Cape, South Africa (Museum number: SAMC-A089090) (GenBank accession number: MT840249). 577 bp fragment isolated with universal mitochondrial cytochrome oxidase subunit 1 gene, primer pair: LCO1490, HCO2198 (Folmer et al. 1994).

Habitat. Fringing rocky zones at low tide in sheltered bays. Worms can be found in rock crevices.

Type locality. Langebaan Lagoon, South Africa.

Distribution. Day (1953; 1967) and Branch et al. (2016) recorded this species to occur in rocky coasts and estuaries from Saldanha Bay in the Western Cape to Durban in KwaZulu-Natal, South Africa.

Remarks. Day (1953) studied the material collected by himself and other members of the Zoology Department at the University of Cape Town during ecological surveys of the rocky coasts and estuaries in South Africa. The author identified some specimens as *Marphysa depressa* collected from localities such as East London, Bushman's Estuary, Still Bay, Cape Agulhas, and Langebaan Lagoon due to the presence of compound spinigers and falcigers in the same chaetiger which is similar to the New Zealand species. As a result, this was the first record of the species in South Africa. Additionally, Day compared his material with a specimen collected from New Zealand by Ehlers (1904), most likely to confirm his identification. However, thorough taxonomic revisions revealed marked differences between the material from South African and New Zealand and led us to conclude that the South African specimens belong to a new species named herein as *Marphysa sherlockae* n. sp..

Marphysa sherlockae n. sp. differs from *M. depressa* in the chaetal distribution. For example, the former has compound spinigers in all chaetigers, and compound falcigers restricted to the median and posterior chaetigers; whereas in *M. depressa*, the compound falciger is present in all chaetigers, but the spinigers are only present in the anterior region. Also, *M. sherlockae* n.

sp. has a triangular postchaetal lobe, while *M. depressa* has a digitiform postchaetal lobe. Furthermore, *M. sherlockae* **n. sp.** (L10: 5.7–6.6 mm) has only two branchial filaments, while *M. depressa* (L10: 9.5 mm) has up to four filaments.

Marphysa sherlockae **n. sp.** resembles *M. durbanensis* and *M. haemasoma* by having compound spinigers. However, *M. sherlockae* **n. sp.** (L10: 5.7–6.6 mm) has two branchial filaments, triangular postchaetal lobe in anterior chaetigers, and ventral cirri with a swollen base ending 70 chaetigers before pygidium; whereas *M. durbanensis* (holotype, L10: 14 mm) has 11–12 branchial filaments, digitiform postchaetal lobes, and ventral cirri with a swollen base ending 25 chaetigers before pygidium. Further, *M. haemasoma* (L10: 9.3–18.5 mm) has 6–10 branchial filaments, ovoid postchaetal lobe, and ventral cirri with a swollen base until the last chaetiger.

Marphysa sherlockae **n. sp.** resembles *M. angelensis* Fauchald, 1970, *M. brevitentaculata* Treadwell, 1921, *M. digitibranchia* Hoagland, 1920, *M. emiliae* Molina-Acevedo & Carrera-Parra, 2017, *M. formosa* Steiner & Amaral, 2000, *M. mangeri* Augener, 1918, *M. orensanzi* Carrera-Parra & Salazar-Vallejo, 1998 and *M. sebastiana* Steiner & Amaral, 2000 by having compound falcigers and spinigers present; however, *M. brevitentaculata*, *M. digitibranchia*, and *M. mangeri* have limbate capillaries in the subacicular position from the middle to the posterior region of the body, while in *M. sherlockae* **n. sp.** these simple chaetae are absent. Furthermore, *M. angelensis* and *M. emiliae* have a digitiform postchaetal lobe in first four chaetigers, while in *M. sherlockae* **n. sp.** the postchaetal lobe is triangular at the same first chaetigers. Also, in *M. emiliae* (L10: 3.5–5.4 mm) branchiae begin in chaetigers 8–12; while in *M. sherlockae* **n. sp.** (L10: 3–6.6 mm) branchiae begin from 25–34. On the other hand, *M. formosa* has pectinate branchiae, while *M. sherlockae* **n. sp.** have palmate branchiae with a short button-shaped branchial stem. Furthermore, *M. formosa* (TL: 55 mm), *M. orensanzi* (TL: 12 mm), and *M. sebastiana* (LT: 120 mm) have up to 4–6 branchial filaments while *M. sherlockae* **n. sp.** (TL: 67 mm) only has two filaments. Finally, *M. sebastiana* and *M. angelensis* have short branchial filaments, while the filaments in *M. sherlockae* **n. sp.** are long.

Discussion

This study revealed that *M. macintoshi* and *M. depressa* recorded for the region actually represent (1) an incorrectly synonymized species, i.e., *M. durbanensis* that was reinstated herein, and (2) a new indigenous species that was previously overlooked and herein described, i.e., *M. sherlockae* **n. sp.**, respectively. We also confirm the notion addressed by Lewis & Karageorgopoulos (2008), that *M. sanguinea* is not present along the South African coast. However, the local species should be named *M. haemasoma* Quatrefages, 1866 and not *M. elityeni* Lewis & Karageorgopoulos, 2008, since the latter is a junior synonym of the former.

Marphysa depressa and *M. macintoshi* were first recorded along the South African coast by Day (1953, 1967) with summary descriptions and general illustrations. The recurrent identification of *M. macintoshi* and *M. depressa* along the South African coast (e.g., Branch et al. 2016) reflects the overlooking of detailed characteristics and the use of traditional and conspicuous diagnostic features considered enough to define *Marphysa* species, such as the color and shape of the subacicular hook, distribution of compound chaetae throughout the body, the shape and distribution of branchiae, and the number of branchial filaments (Quatrefages 1866, Grube 1878, McIntosh 1910, Hartman 1944, Fauchald 1970, among others). The sole use of distinctive conspicuous features in the identification may lead to spurious records of cosmopolitanism in

species (Hutchings & Kupriyanova 2018), and also to the proliferation of misleading species records and synonymization.

The detailed study of the traditional conspicuous features, the discovery of new unique characters as well as the examination of type specimens, as carried out here, has improved the morphological delimitation of *Marphysa* species, and the understanding of the diversity within the genus (e.g., Glasby & Hutchings 2010; Molina-Acevedo & Carrera-Parra 2015, 2017). Therefore, recent studies on *Marphysa* have focused on detecting unique characters or in the re-assessment of those forgotten features, such as the shapes of dorsal cirri, postchaetal lobes, and pectinate chaetae, and the first appearance of the ventral cirrus with a swollen base. For instance, Miura (1986) and Molina-Acevedo & Carrera-Parra (2015) have shown that the distribution of the number of filaments and the region where the maximum number is reached can be informative in species delimitation. Here, the distribution of branchial filaments is different in each analyzed species (Fig. 4). Thus, whenever possible, it should be incorporated in future descriptions of *Marphysa* species. The main challenge of using “new” features in taxonomic investigations is the lack of this information in older descriptions preventing comparison. Thus, the examination of type material deposited in museums or examining newly collected material from the type locality in cases where no types were deposited previously is an essential step towards improving the taxonomy and recognition of new or inappropriate synonyms as in the case of *M. haemasoma*.

Molecular data provide an additional source of information that improves our knowledge on species boundaries and aids in recognition of intraspecific variation (e.g., Lewis & Karageorgopoulos 2008; Zanol et al. 2016, 2017, Lavesque et al. 2017, Elgetany et al. 2018, Lavesque et al. 2019, Glasby et al. 2019, Abe et al. 2019, Martin et al. 2020). The phylogenetic tree revealed two distinct South African monophyletic clades, belonging to the new species *M. sherlockae* n. sp., and the other to *M. haemasoma*. The molecular analyses reinforced the re-establishment of *M. haemasoma* as a valid species by confirming its distinction from *M. sanguinea*, which concurs with previous findings from the region (Lewis & Karageorgopoulos 2008). Furthermore, for the first time, this study provided COI sequences of *M. haemasoma*, from South Africa.

A total of nine *Marphysa* species have been newly proposed or redescribed under an integrative taxonomic framework since 2003 (Zanol et al. 2016; Zanol et al. 2017; Lavesque et al. 2017; Elgetany et al. 2018; Lavesque et al. 2019; Glasby et al. 2019; Abe et al. 2019; Martin et al. 2020; present study), thus, increasing the number of publicly available sequences of *Marphysa* species globally. This framework, in turn, provides a starting point from which other studies can address more complex hypotheses, such as resolving the phylogenetic placements of species within the genus.

This study has confirmed that the indigenous diversity of *Marphysa* in South Africa was indeed previously underestimated and thus increases the number of described indigenous species from three to five (Day 1967; Lewis & Karageorgopoulos 2008) and reduces the number of putative cosmopolitan species to one (i.e., *Marphysa corallina*). Similarly, studies by Lewis & Karageorgopoulos (2008); Clarke et al. (2010); Kara et al. (2018) and Simon et al. (2019) provide additional evidence that many cosmopolitan species reported in the Day (1967) polychaete monograph for this region are actually incorrect assignments. Undoubtedly, the polychaete monograph by Day (1967) is an invaluable resource for polychaete descriptions and distributions. However, it is widely used by researchers from many disciplines, including those working outside of the region (Hutchings & Kupriyanova 2018). Thus, biologists locally and internationally should

take cognizance of this fact and use the monograph with caution, especially concerning species considered “cosmopolitan”.

Using information from Day (1967), Awad et al. (2002) determined that only 20% of polychaete species in South Africa are endemic to the region. Thus, if only half the remaining 80% prove to be misidentifications of indigenous species, our understanding of diversity, biogeography, and endemism of polychaete worms in South Africa has been severely underestimated, and priority conservation areas may need to be reviewed. Furthermore, the resolution of taxonomically confusing species, such as those belonging to *Marphysa*, and development of realistic diversity estimates will be improved if voucher specimens are deposited in museums for taxonomic and molecular investigations.

Conclusion

Marphysa in South Africa is represented by six species, namely, *M. capensis*, *M. corallina*, *M. durbanensis*, *M. haemasoma*, *M. posteriobranchia*, and *M. sherlockae* n. sp. Although the number of species is similar to previous identifications, the resurrection of *M. haemasoma*, synonymization of *M. elityeni* with *M. haemasoma*, reinstatement of *M. durbanensis* from *M. macintoshi* and redescription of *M. sherlockae* n. sp. from *M. depressa* has changed the composition of endemic and cosmopolitan species. As such, gaining a better understanding of our true local biodiversity may help us to understand the extent of biodiversity loss in the face of climate change and make better decisions regarding the designation of marine protected areas.

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Figure 1

Sampling localities of *M. depressa* (Langebaan, Strand), *M. macintoshi* (Durban Bay), *M. haemasoma* (Table Bay) and *M. elityeni* (Buffels Bay and Kommetjie) from South Africa.

Triangles represent museum material examined and circles represent samples collected in 2017 (*M. elityeni* - Kommetjie) and 2019 (*M. depressa* - Strand).

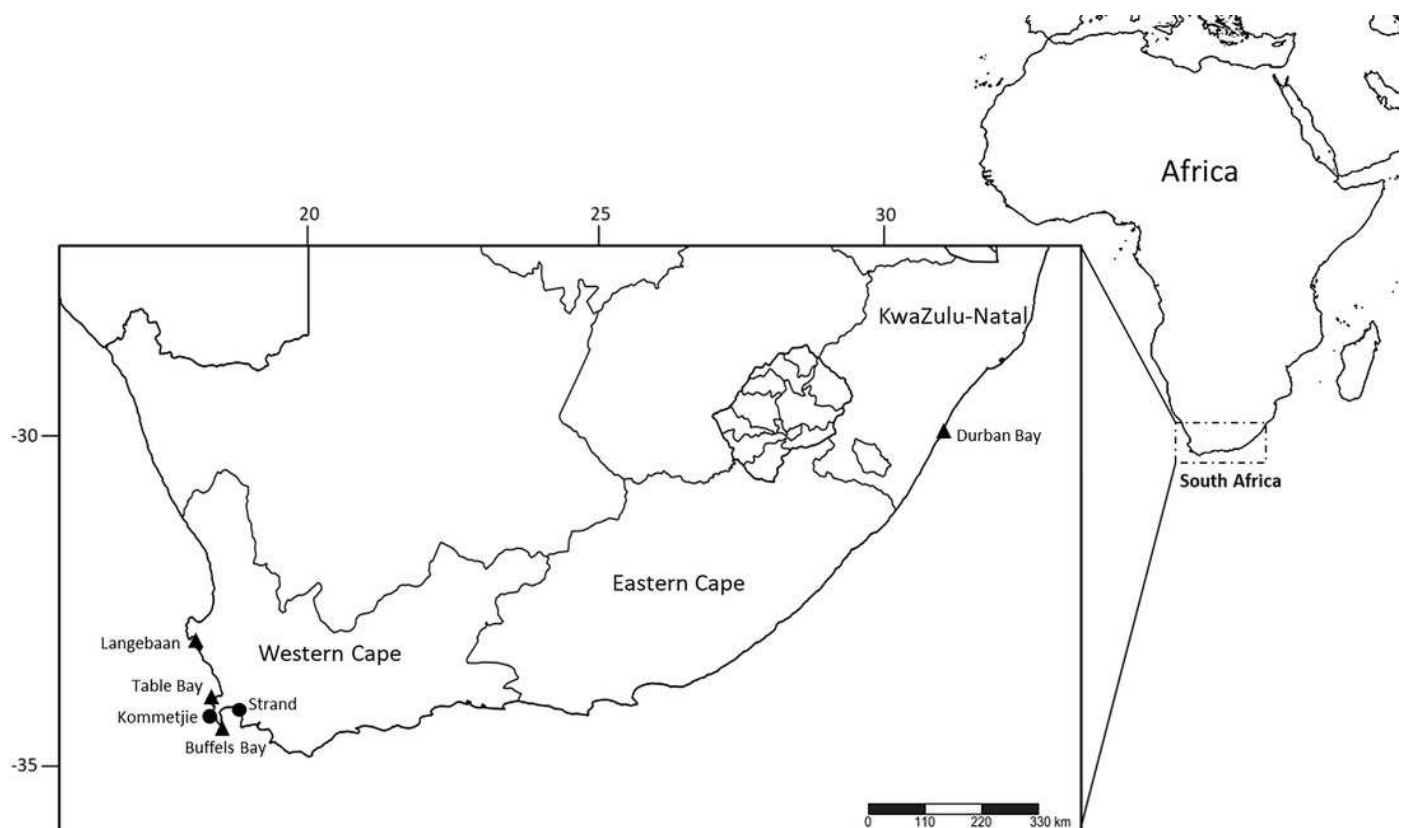


Figure 2

Phylogenetic tree based on the mitochondrial cytochrome c oxidase subunit 1 alignment of *Marphysa* spp.

Bayesian posterior probabilities and maximum likelihood bootstrap values >95% are represented by an * at each node with the former on the left side of forward slash and latter on the right side of the forward slash. The hyphens, -, represent nodes that had maximum likelihood values <90%. Purple clade - the reinstated *M. haemasoma*. Blue clade - newly described *M. sherlockae* **n. sp.**

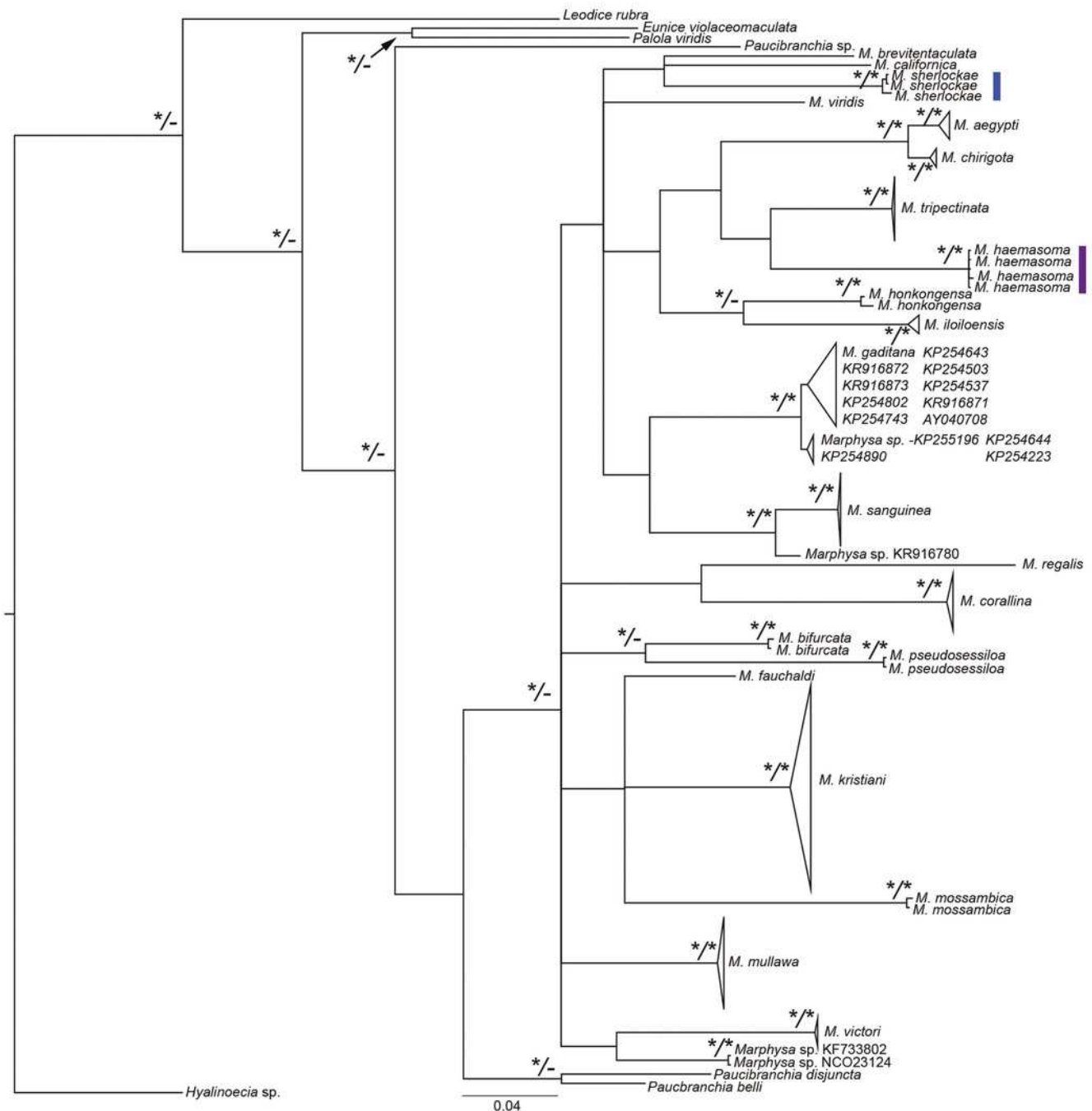


Figure 3

Marphysa durbanensis Day, 1934.

A. Anterior end, dorsal view; B. Anterior end, ventral view; C. Anterior view, lateral view; D. Maxillary apparatus, dorsal view; E. Left MI-II-III-IV-V, lateral view; F. Mandible; G. Parapodium 3; H. Parapodium 8; I. Parapodium 12; J. Parapodium 69; K. Parapodium 217; L. Thin narrow isodont pectinate with long and slender teeth, chaetiger 3; M. Thin wide isodont with short and slender teeth, chaetiger 69; N. Thick wide isodont pectinate with short and thick teeth, chaetiger 140; O. Thick wide anodont with short and slender teeth, chaetiger 140; P. Compound spinigers, chaetiger 3; Q. Subacicular hook, chaetiger 278. A-C, G-P from Lectotype BNHM 1934.1.19.166; D-F, Q from paralectotype BNHM 1934.1.19.166. All chaetigers in anterior view; LMI-II: Ligament between MI and MII; LMII-III: Ligament between MII and MIII. Scale bars: A-C, 3.5 mm; D-E, 0.9 mm; F, 0.8 mm; G-K, 0.2 mm; L-O, Q 30 µm; P, 0.1 mm.

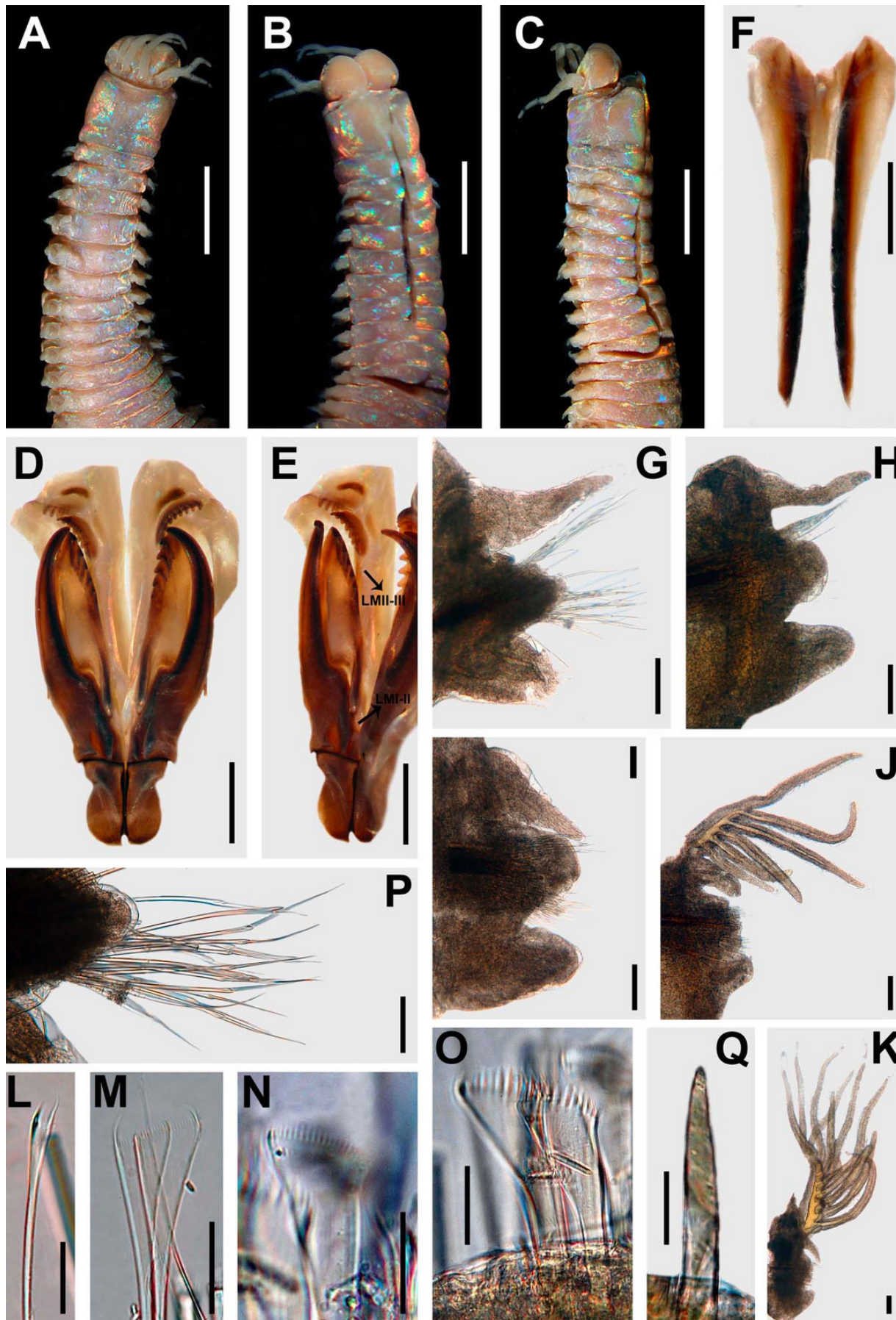


Figure 4

Distribution of branchial filaments throughout the body in

A. *Marphysa durbanensis* Day, 1934 (L10: 14 mm, 380 chaetigers); B. *Marphysa haemasoma* Quatrefages, 1865 (L10: 123 mm, 322 chaetigers); C. *Marphysa sherlockae* **n. sp.** (L10: 6.6 mm, 208 chaetigers).

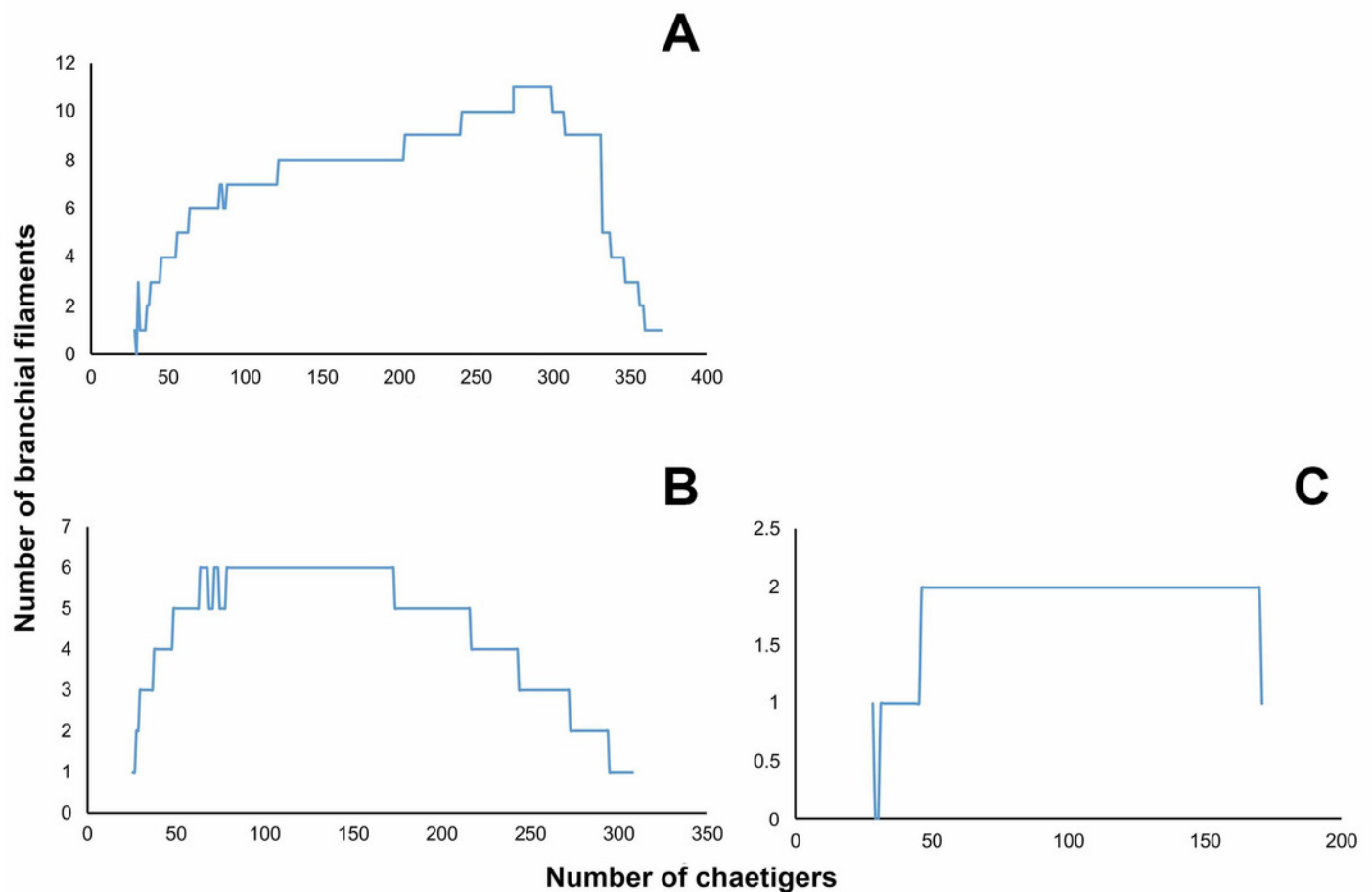


Figure 5

Marphysa durbanensis Day, 1934, lectotype BNHM 1934.1.19.166.

A. Thin narrow isodont pectinate with long and slender teeth, chaetiger 3; B. Thin wide isodont with short and slender teeth, chaetiger 69; C. Thick wide isodont pectinate with short and thick teeth, chaetiger 140; D. Thick wide anodont with short and slender teeth, chaetiger 140. Scale bars: A-D, 30 μ m.

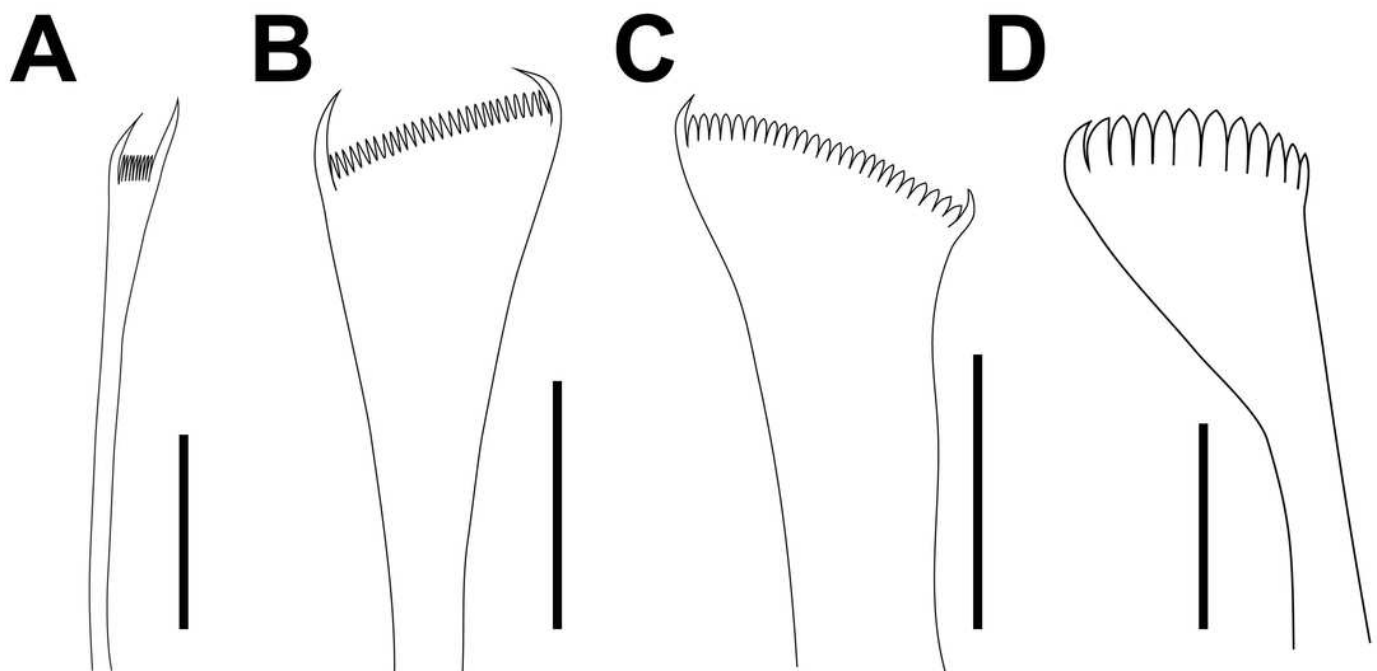


Figure 6

Marphysa haemasoma Quatrefages, 1866

A. Anterior end, dorsal view; B. Anterior end, ventral view; C. Anterior view, lateral view; D. Maxillary apparatus, dorsal view; E. Left MI-II-III-IV-V, lateral view; F. Mandible; G. Parapodium 3; H. Parapodium 12; I. Parapodium 30; J. Parapodium 154; K. Parapodium 307; L. Thin narrow isodont with long and slender teeth, chaetiger 3; M. Thick wide isodont with short and slender teeth, chaetiger 251; N. Thick wide anodont with short and slender teeth, chaetiger 307; O. Thick wide anodont with long and thick teeth, chaetiger 251; P. Compound spinigers, chaetiger 3; Q. Subacicular hook, chaetiger 209. A-B, D-E, G-L, N, P from Holotype *M. haemasoma* MNHN type 613; F, M, O, Q from Paratype *Marphysa elityeni* BNHM 2007.69. All chaetigers in anterior view; al-MIII: attachment lamella MIII; al-MIV: attachment lamella MIV; LMI-II: Ligament between MI and MII; LMII-III: Ligament between MII and MIII. Scale bars: A-B, 3.1 mm; C, 3.8mm; D-E, 1.2 mm; F, 1.7 mm; G-K, 0.2 mm; L-O, Q, 30 µm; P, 0.1 mm.

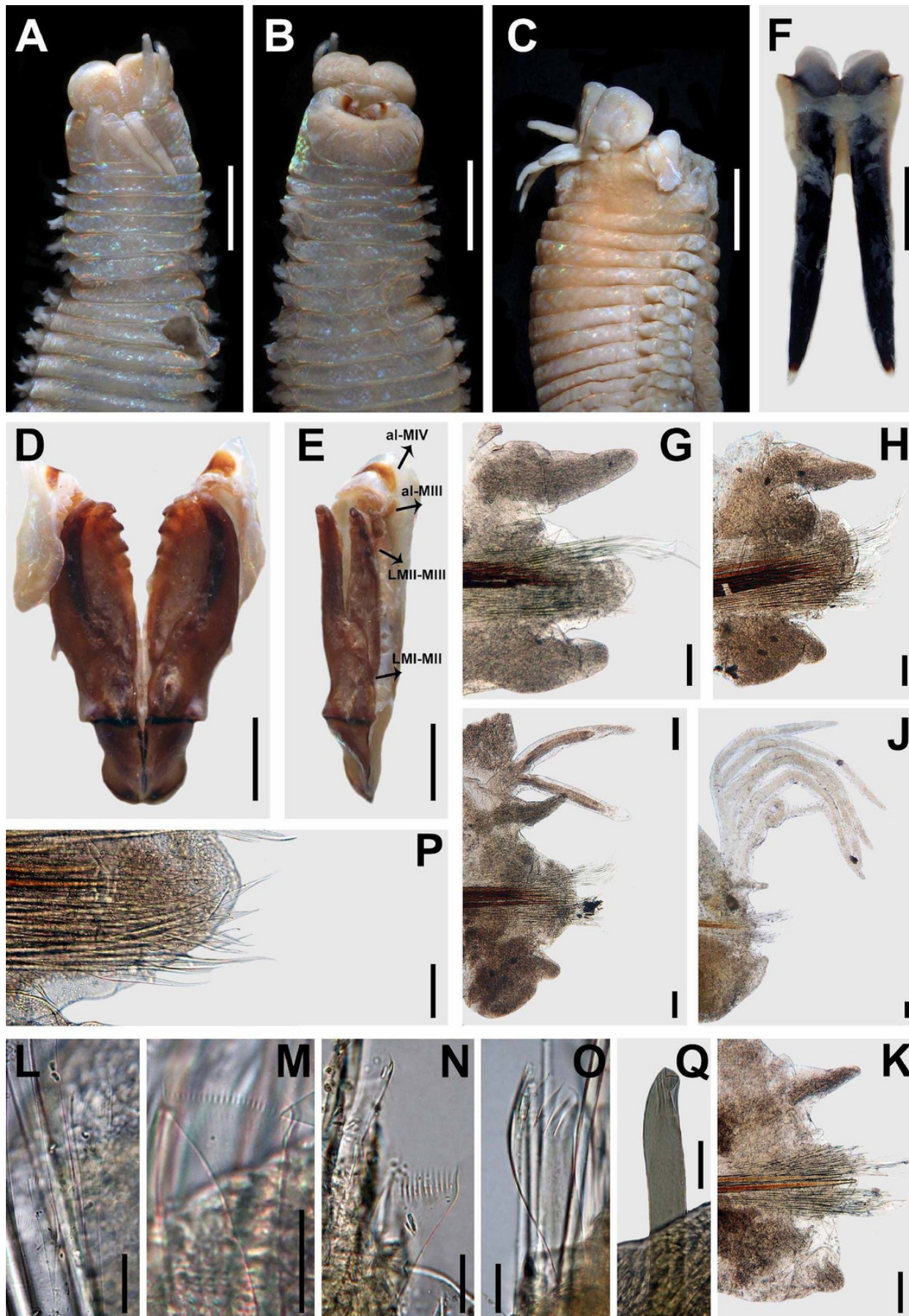


Figure 7

Marphysa haemasoma Quatrefages, 1866. Type and additional material from *Marphysa elityeni* Lewis & Karageorgopoulos, 2008.

A. Anterior end, dorsal view; B. Left MI-II-III-IV-V, lateral view; C. Right MI-II-IV-V, lateral view; D. Parapodium 3; E. Parapodium 13; F. Parapodium 208; G. Parapodium 3; H. Parapodium 12; I. Thick wide anodont with short and slender teeth, chaetiger 209; J. Thick wide isodont with short and slender teeth, chaetiger 209; K. Subacicular hook, chaetiger 209. A-F, from paratype *Marphysa elityeni* BNHM 2007.69; G-H from holotype *M. haemasoma* MNHN type 613; I-K, from topotype *Marphysa elityeni* BN  237. Chaetigers D-E, G-H in posterior view, chaetiger F in anterior view; al-MIII: attachment lamella MIII; al-MIV: attachment lamella MIV; LMI-II: Ligament between MI and MII; LMII-III: Ligament between MII and MIII; LMII-IV: Ligament between MII and MIV; PL: Postchaetal lobe. Scale bars: A, 4.6 mm; B-C, 1.8 mm; D-E, 0.4 mm; G-H, 0.2 mm; I-J, 30 µm.

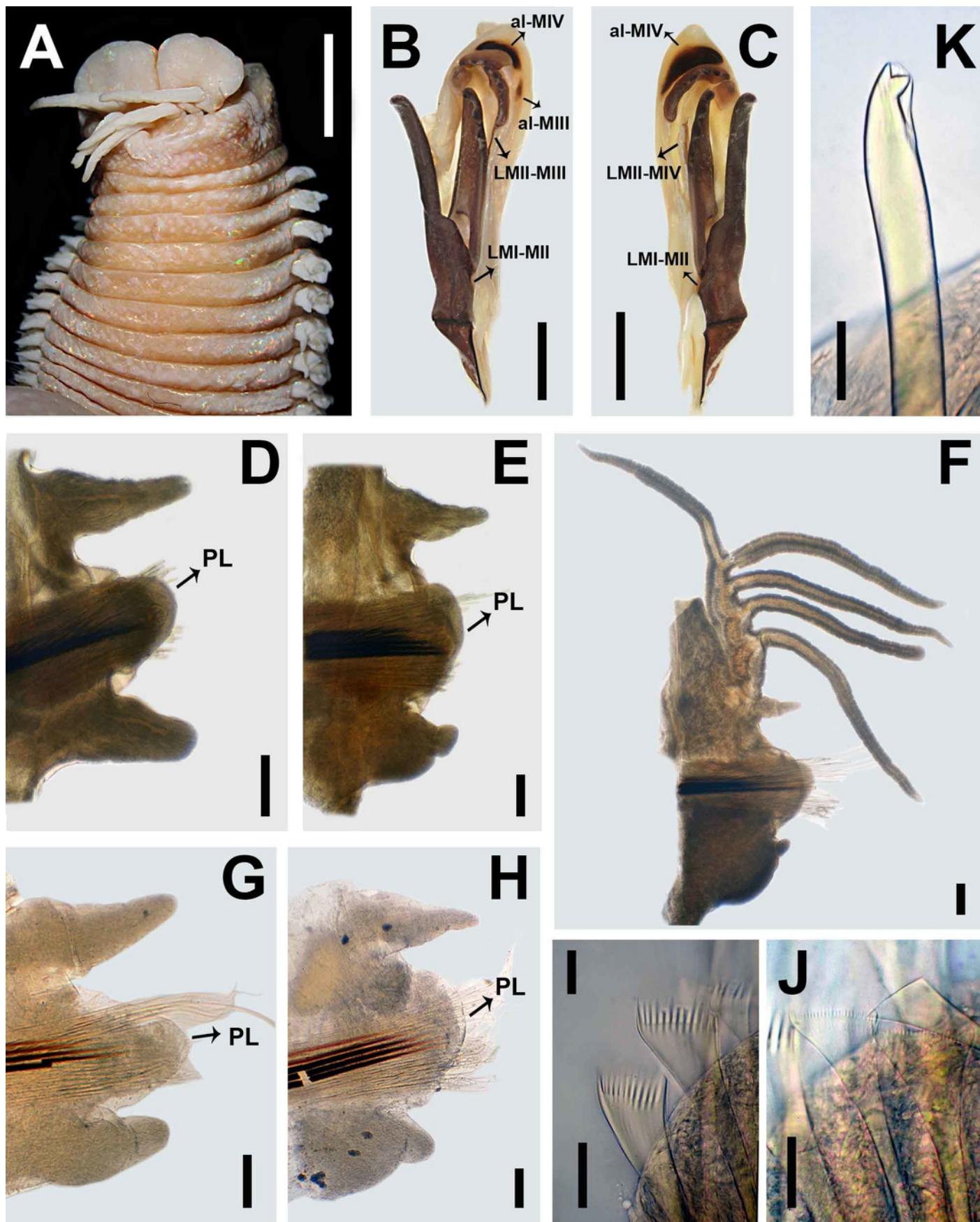


Figure 8

Marphysa sherlockae n. sp. Holotype BNHM 1963.1.84.

A. Anterior end, dorsal view; B. Anterior end, ventral view; C. Anterior end, ventral view; D. Maxillary apparatus, dorsal view; E. Left MI-II-III-IV-V, lateral view; F. Mandible; G. Parapodium 3; H. Parapodium 6; I. Parapodium 14; J. Parapodium 114; K. Parapodium 185 L. Thin narrow isodont with long and slender teeth, chaetiger 3; M. Thick wide isodont with long and thick teeth, chaetiger 185; N. Compound spinigers, chaetiger 3; O. Compound falcigers, chaetiger 3; P. Subacicular hook, chaetiger 49. All chaetigers in anterior view; al-MIII: attachment lamella MIII; al-MIV: attachment lamella MIV; LMI-II: Ligament between MI and MII; LMII-III: Ligament between MII and MIII. Scale bars: A-C, 1.7 mm; D-E, 0.6 mm; F, 0.4 mm; G-K, 0.1 mm; N-P, 30 μ m.

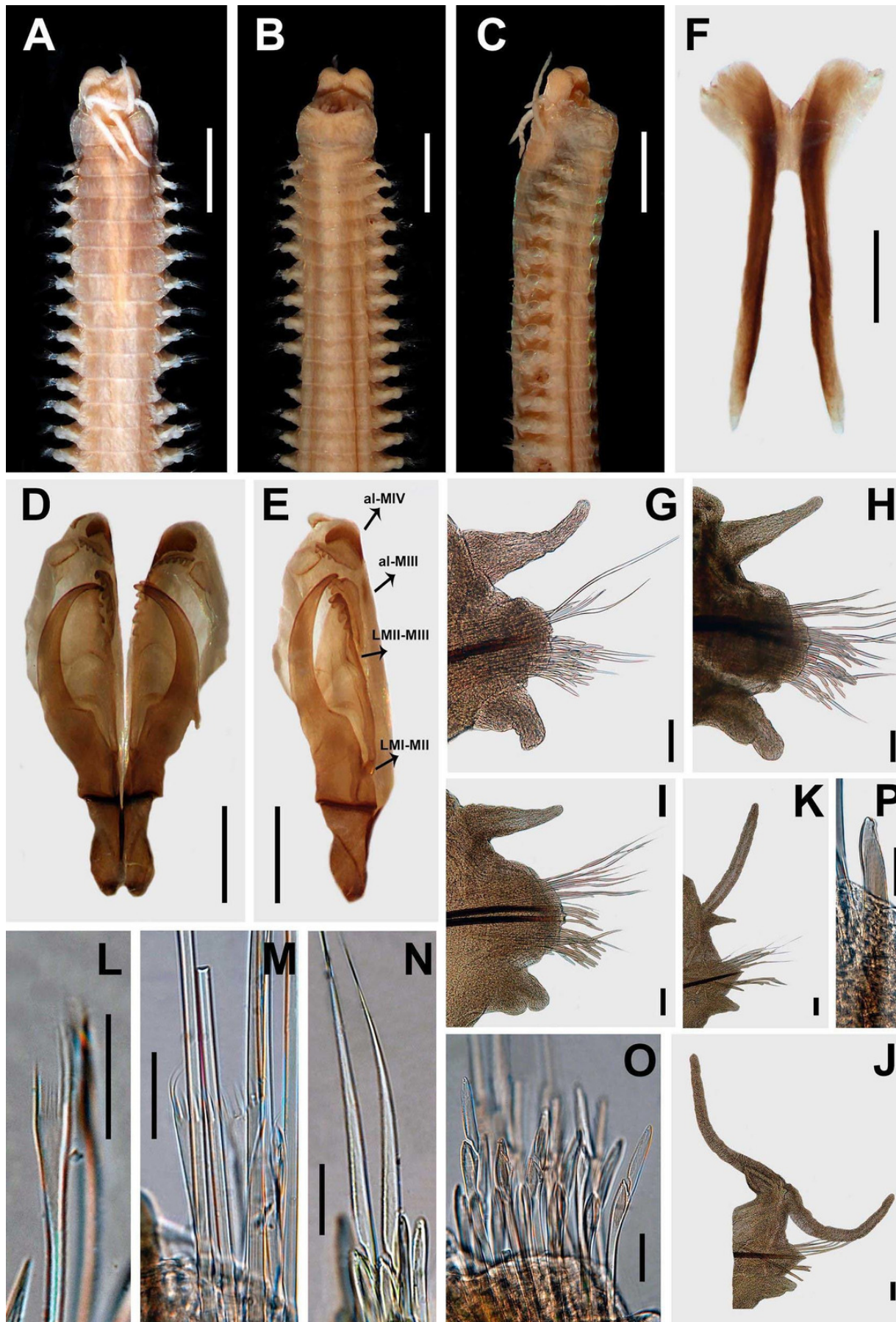


Figure 9

Marphysa sherlockae n. sp.

A. Thin narrow isodont pectinate chaetae with long and slender teeth, anterior chaetiger; B. Thin narrow isodont pectinate chaetae with long and slender teeth, anterior chaetiger; C. Thick narrow isodont pectinate chaetae with long and slender teeth, chaetiger 32; D. Thick wide isodont pectinate chaetae wide with long and thick teeth, posterior chaetiger; E. Thick wide isodont pectinate chaetae with long and thick teeth, posterior chaetiger. A, B, C from SAMC-A20578; D, E SAMC-A089089 Scale bars: A-E, 0.05 mm.

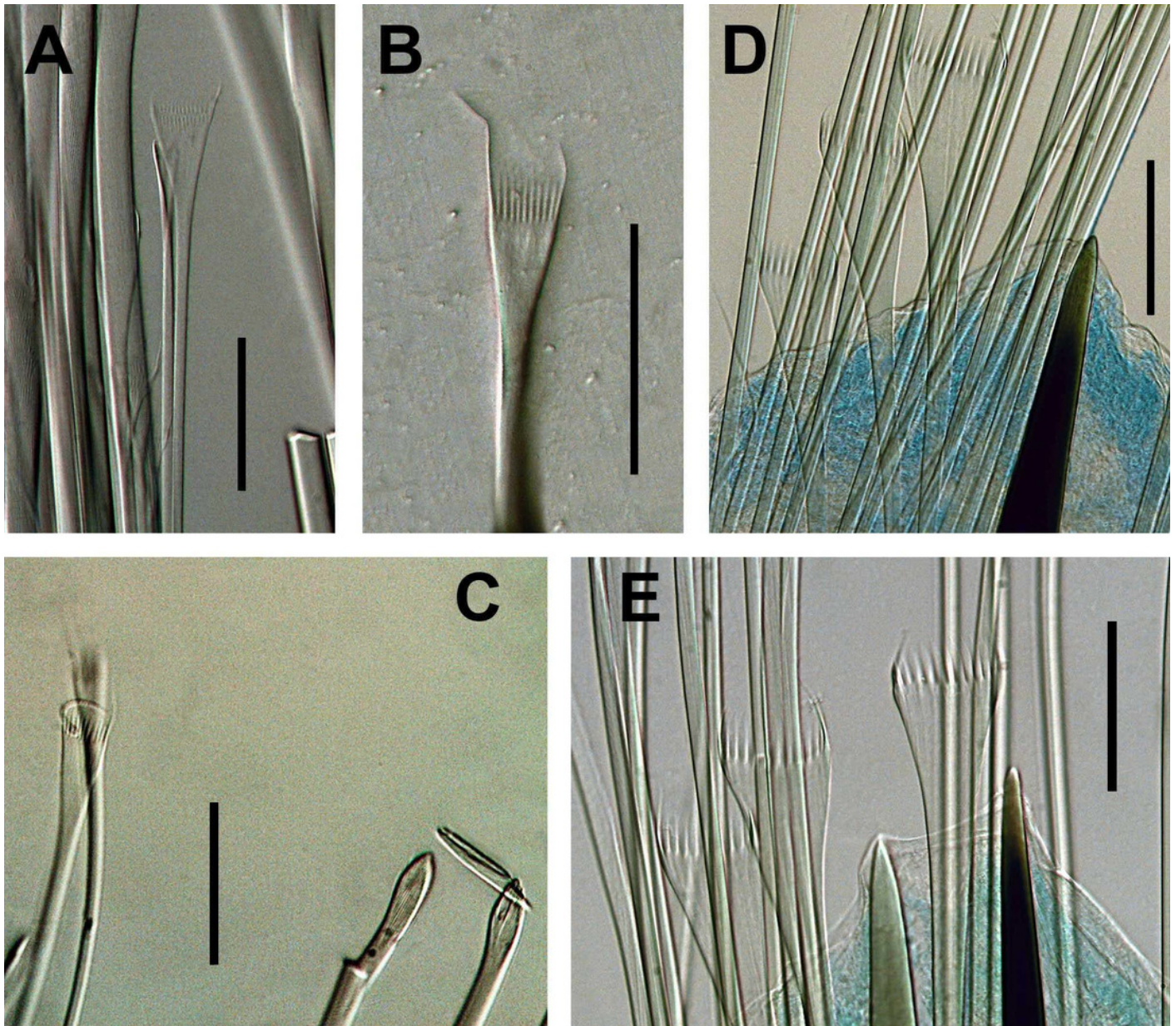


Figure 10

Length-dependent variation of some morphological features in *Marphyssa sherlockae* n. sp.

Orange point: Chaetiger where subacicular hook start ($p=0.35$, $n=11$). Blue points: First chaetiger with branchia ($p=0.26$, $n=11$); Green points: Maximum number of branchial filaments ($p=0.00$, $n=11$).

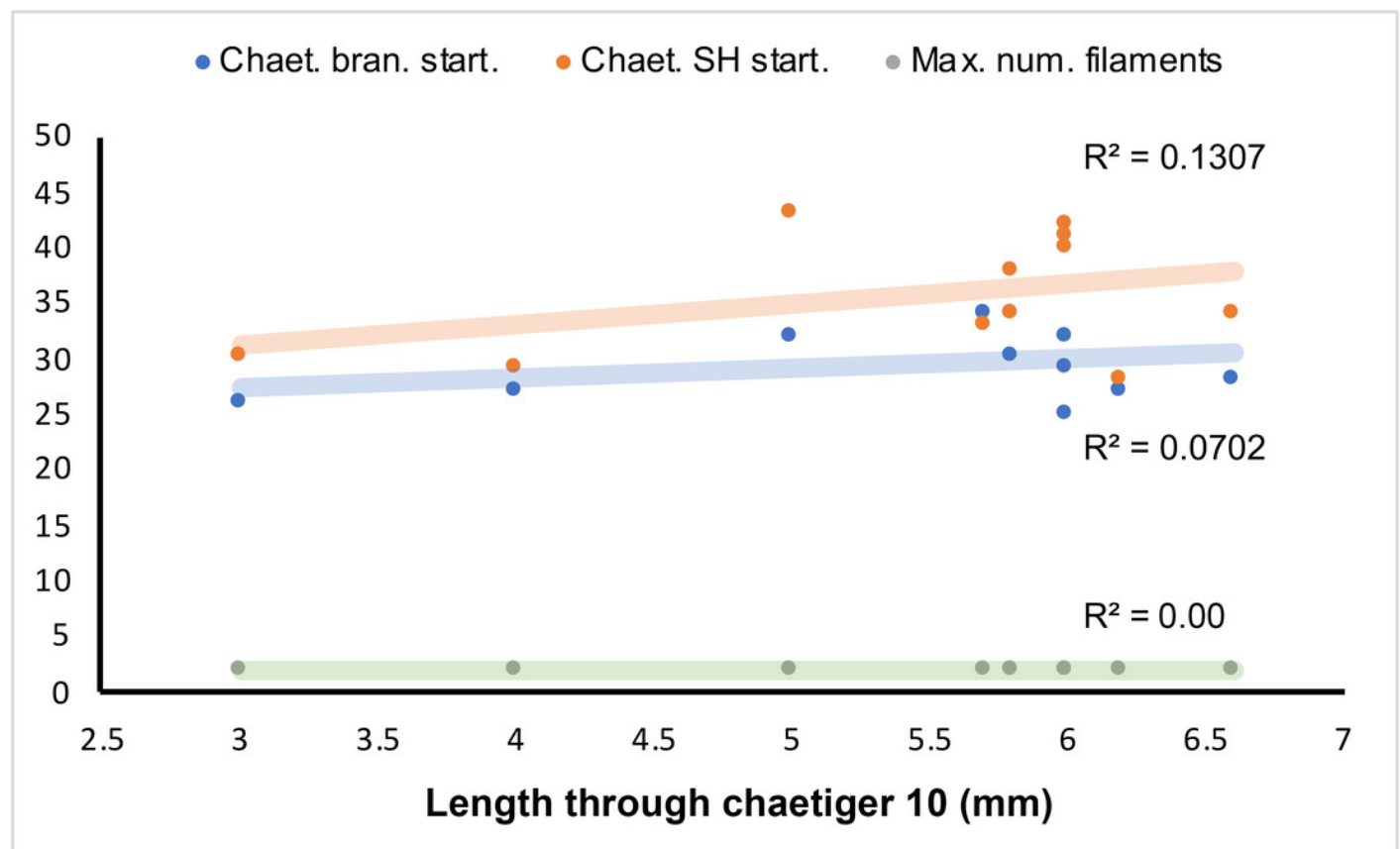


Table 1(on next page)

COI sequences of *Marphysa* taxa used in the phylogenetic analysis

COI sequences of *Marphysa* taxa used in the phylogenetic analysis, with GenBank accession numbers, museum voucher numbers, type locality of species, location of specimens from which sequences were obtained and references to the respective studies.

Table 1. COI sequences of *Marphysa* taxa used in the phylogenetic analysis, with GenBank accession numbers, museum voucher numbers, type locality of species, location of specimens from which sequences were obtained and references to the respective studies.

Species	Genbank accession number	Type locality (TL)	Collection locality	Reference
<i>Marphysa haemasoma</i>	MN067877	Cape of Good Hope, South Africa	Kommetjie, South Africa	Simon et al. <i>unpublished data</i> .
<i>Marphysa sherlockae</i> n. sp.	MT840349 – MT840351	Durban, South Africa	Strand, South Africa	This study
<i>Marphysa aegypti</i>	MF196971, MF196969, MF196970, MF196968	Suez Canal, Egypt	Suez Canal, Egypt	Elgetany et al. 2018
<i>Marphysa chirigota</i>	MN816441, MN816442, MN816443	Cádiz Bay, SW Iberian Peninsula	Cádiz Bay, SW Iberian Peninsula	Martin et al. 2020
<i>Marphysa bifurcata</i>	KX172177, KX172178	Sheltered North Reef at Poin Peron, Western Australia	Australia	Zanol et al. 2016
<i>Marphysa brevitentaculata</i>	GQ497548	Scarborough, Tobago, Trinidad and Tobago	Mexico	Zanol et al. 2010
<i>Marphysa californica</i>	GQ497552	San Diego County, California	California	Zanol et al. 2010
<i>Marphysa corallina</i>	KT823271, KT823300, KT823306, KT823343, KT823371, KT823389, KT823410	Hawaii	Multiple localities in KwaZulu-Natal and Eastern Cape, South Africa	Kara et al. <i>unpublished</i>
<i>Marphysa fauchaldi</i>	KX172165	off Elizabeth River, Darwin region, Australia	Australia	Zanol et al. 2016
<i>Marphysa</i>	MN816444,	Cádiz Bay, SW	Cádiz Bay,	Martin et al.

Species	Genbank accession number	Type locality (TL)	Collection locality	Reference
<i>gaditana</i>	KR916870, AY040708, KR916871, KR916872, KR91687, KP254503, KP254537, KP254643, KP254743, KP254802	Iberian Peninsula	SW Iberian Peninsula Portugal, France, Virginia (USA)	2020, Lobo et al. 2016, Siddal et al. 2001, Leray et al. 2015
<i>Marphysa honkongensa</i>	MH598526	Tolo Harbour, Hong Kong	China	Wang et al. 2018
<i>Marphysa iloiloensis</i>	MN133418, MN106279, MN106280, MN106281	Tigbauan, Iloilo Province	Philippines	Glasby et al. 2019
<i>Marphysa kristiani</i>	KX172141, KX172142, KX172143, KX172144, KX172145, KX172146, KX172147, KX172148, KX172149, KX172150, KX172151, KX172155, KX172152, KX172153, KX172154, KX172156, KX172157, KX172158, KX172159, KX172160, KX172161, KX172162, KX172163	Stingray Bay, New South Wales	Australia	Zanol et al. 2016
<i>Marphysa mossambica</i>	JX559751, KX172164	Mossambique	Philippines, Australia	Zanol et al. 2010, Zanol et al. 2016
<i>Marphysa</i>	KX172166,		Australia	Zanol et al.

Species	Genbank accession number	Type locality (TL)	Collection locality	Reference
<i>mullawa</i>	KX172167, KX172168, KX172169, KX172170, KX172171, KX172172, KX172173, KX172174, KX172175, KX172176	Moreton Bay, Fisherman's Island, Queensland		2016
<i>Marphysa pseudosessilaa</i>	KY605405, KY605406	Careel Bay, New South Gales	Australia	Zanol et al. 2017
<i>Marphysa regalis</i>	GQ497562	Bermuda	Brazil	Zanol et al. 2016
<i>Marphysa victori</i>	MG384996, MG384999, MG384997, MG384998	Arcachon Bay	France	Lavesque et al. 2017
<i>Marphysa viridis</i>	GQ497553	Boca Grande Key, Florida	Brazil	Zanol et al. 2010
<i>Marphysa sanguinea</i>	GQ497547, MK541904, MK950851, MK950852, MK950853, MK967470, MN106282, MN106283, MN106284	Polperro, Cornwall	Cornwall (UK), France	Zanol et al. 2010, Lavesque et al. 2019, Glasby et al. 2019
<i>Marphysa tripectinata</i>	MN106271, MN10622, MN1062723, MN106274, MN106275, MN106276, MN106277, MN106278	Beihai, China	China	Liu et al. 2017
<i>Marphysa sp.</i>	KP255196, KP254890,	-	Florida (USA),	Leray et al.

Species	Genbank accession number	Type locality (TL)	Collection locality	Reference
	KP254644, KP254223, NC023124, KF733802		China	2015, Li et al. 2016
<i>Paucibranchia bellii</i>	KT307661	Chausey Island, France	Spain	Aylagas et al. 2016
<i>Paucibranchia disjuncta</i>	GQ497549	Los Angeles County, California	California, USA	Zanol et al. 2010
<i>Paucibranchia</i> sp.	JX559753		Phillipines	Zanol et al. 2014
<i>Palola viridis</i>	GQ497556	Samoa	Micronesia	Zanol et al. 2010
<i>Eunice cf. violaceomaculata</i>	GQ497542	-	Belize	Zanol et al. 2010
<i>Leodice rubra</i>	GQ497528	-	Brazil	Zanol et al. 2010
<i>Hyalinoecia</i> sp.	GQ497524	-	Massachusetts, USA	Zanol et al. 2010

