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# Loimia ramzega sp nov., a new giant species of Terebellidae (Polychaeta) from French waters (Brittany, English Channel)

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# Abstract :

A new species of Terebellidae, *Loimia ramzega* sp. nov., has been identified from North Brittany beaches (English Channel). This new species is characterized by its gigantic size (max 650 mm, live), two pairs of lateral lappets on segments 1 and 3; first pair more ventral, second pair more developed and lateral but oblique, with wavy edge. Nine ventral pads from segment 2 (fused on segments 2 and 3), first three pads swollen, next ones subsequently decreasing in size. Three types of notochaetae, asymmetrically bilimbate, symmetrically bilimbate and capillary. Uncini pectinate with 6 teeth (some with 5 teeth) slightly decreasing in size, in one vertical row. Pygidium with about 14 long conical marginal papillae surrounding anus.

Keywords : Terebellinae, morphology, molecular, taxonomy, DNA COI 16S, North-east Atlantic, France

# 39 INTRODUCTION

Terebellids belong to a very species-rich group of sedentary polychaetes, widely distributed in 40 41 most of marine benthic substrates, from shallow waters to deep-sea environments (Rouse & 42 Pleijel, 2001). Terebellidae Johnston, 1846 is currently subdivided into 3 subfamilies: Polycirrinae Malmgren, 1867, Terebellinae Johnston, 1846 (frequently referred to as 43 Amphitritinae) and Thelepodinae Hessle, 1917 (as Thelepinae) (Londoño-Mesa & Carrera-44 Parra, 2005). However, Nogueira et al. (2013) carried out a large phylogenetic analysis which 45 resulted in splitting the family Terebellidae into four families: Polycirridae, Terebellidae (= 46 47 previous Terebellinae), Telothelepodidae, and Thelepodidae (Hutchings et al., in press). Herein, the former proposal will be followed (Read, 2016). 48

49 According to Carrerette & Nogueira (2015), this family includes around 300 species 50 belonging to 50 genera. Among the subfamily Terebellinae, the genus Loimia Malmgren, 1865 currently comprises 28 valid species, of which 7 have been recently described from 51 52 Brazilian and Australian coasts (Carrerette & Nogueira, 2015; Nogueira et al., 2015). This genus can be found worldwide, especially occurring in tropical waters (Read & Bellan, 2011), 53 with only two species known from European waters. Loimia arborea Moore, 1903a was 54 recorded from the Mediterranean Sea (RESOMAR - French marine stations and observatories 55 network - database, http://resomar.cnrs.fr/bases/index.php; Faulwetter, 2010) and from Irish 56 57 Sea (Guiry & Guiry, 2011). However, these records are doubtful since this species originated from Japan. According to World Register of Marine Species, validation of these occurrences 58 59 and species presence beyond Japanese waters is needed (WoRMS, 2008). Loimia medusa 60 (Savigny in Lamarck, 1818) was previously believed to occur from the Mediterranean to 61 Norway but this species was redescribed by Hutchings & Glasby (1995) who suggested that 62 its distribution may restricted to the Arabian Sea Region. Due to the lack of accurate 63 literature, many European studies have identified their specimen as Loimia sp. (e.g. Mackie et al., 1995 in the southeastern Irish Sea; RESOMAR database along the French coasts). Since 64 2011, one of the authors (JG) regularly found specimens of a giant species of Loimia within 65 Northern Brittany beaches that could not be related to any described species. The present 66 paper provides the description of this new species based on morphological characters and 67 68 supplemented by molecular data. The geographical origin of the species, perhaps not 69 originating from Europe, is discussed.

70

#### 71 MATERIALS AND METHODS

# 72 Sampling and morphological analyses

73 The first specimens of this new *Loimia* species were sampled in 2011 from intertidal sandy 74 beaches of the English Channel (Brittany, France) (Figure 1) after notification of strange large 75 annelids tubes (Figure 2A) by Michel Glémarec (senior French benthic ecologist from Brest University). Specimens examined in this study were collected in 2011, 2012 and 2016 by 76 77 hand, using a shovel-fork and a technique called "finger tube tracking" allowing the 78 monitoring of the tube position and changes in direction at any moment (Figure 2B). Live 79 specimens were anaesthetized with 7% magnesium chloride (MgCl<sub>2</sub>) and photographed using a Canon EOS 600D Camera. A small piece of body was removed from several specimens and 80 fixed in 96% ethanol for molecular studies. The main material was fixed in 4% formaldehyde 81 seawater solution, then transferred to ethanol 70% solution for morphological analysis. 82 Preserved specimens were examined under a Nikon SMZ25 stereomicroscope and a Nikon 83 Eclipse E400 microscope, and photographed with a Nikon DS-Ri 2 camera. Total length, 84 length of thorax and width of thorax (10<sup>th</sup> chaetiger) were measured with the NIS-Elements 85 Analysis software. Drawings were made from pictures using Inkscape software and Wacom 86 87 Intuos 5 tablet. Holotype and most paratypes were deposited at the Muséum National d'Histoire Naturelle, Paris (MNHN), other paratypes were deposited in the National Museum 88 89 Wales, Cardiff (NMW-Z) and in Colección Estuarina y Marina, Universidad de Antioquia 90 (CEMUA) in Medellín, Colombia. Additional material was lodged in collections of Arcachon 91 and Brest Marine Stations, in France.

92

# 93 DNA isolation, amplification and sequencing

Sub-samples for DNA analysis were removed from live specimens, placed in ethanol 96% 94 95 and frozen at -20°C. Extraction of DNA was done with NucleoSpin Tissue (Macherey-Nagel) kit following protocol supplied by the manufacturers. About 450 bp of 16S and 700 bp of COI 96 97 (cytochrome c oxidase subunit I) genes were amplified using primers Ann16SF and 16SbrH for 16S (Palumbi, 1996; Sjölin et al., 2005), and polyLCO and polyHCO for COI (Carr et al., 98 99 2011). The PCR (Polymerase Chain Reaction), with 25  $\mu$ L mixtures contained: 5 $\mu$ L of Green 100 GoTaq® Flexi Buffer (final concentration of 1X), 2.5 µL of MgCl2 solution (final 101 concentration of 2.5mM), 0.5 µL of PCR nucleotide mix (final concentration of 0.2 mM each 102 dNTP), 9.875  $\mu$ L of nuclease-free water, 2.5  $\mu$ l of each primer (final concentration of 1 $\mu$ M), 2

103	µl template DNA and 0.125 of GoTaq® G2 Flexi DNA Polymerase (Promega). The
104	temperature profile was as follows: 95°C/240s - (94°C/30s-52°C/60s-72°C/75s) *35 cycles
105	(for 16S) or *40 cycles (for COI) - 72°C/480s - 4°C. PCR-products, which produced light
106	bands after electrophoresis on 1% agarose gel, were sent to the MacroGen Europe Laboratory
107	in Amsterdam (Netherlands) to obtain sequences, using same set of primers as used for the
108	PCR. Overlapping sequence (forward and reverse) fragments were merged into consensus
109	sequences using Geneious Pro 8.1.7 2005-2015 (Biomatters Ltd.) and aligned using the
110	plugins: MAAFT (Katoh et al., 2002) for 16S and MUSCLE (Edgar, 2004) for COI. For COI,
111	the sequences were translated into amino acid alignment and checked for stop codons to avoid
112	pseudogenes. The minimum length coverage was around 450bp for 16S and 610 bp for COI.
113	All sequences obtained in this study have been deposited in GenBank
114	(http://www.ncbi.nlm.nih.gov/genbank/).

115	
116	RESULTS
117	SYSTEMATICS
118	Family TEREBELLIDAE Johnston, 1846
119	Subfamily TEREBELLINAE Johnston, 1846
120	Genus Loimia Malmgren, 1865
121	
122	Type species: Terebella medusa Savigny in Lamarck, 1818
123	GENERIC DIAGNOSIS
124	Malmgren (1865: 380). Hessle (1917: 170). Hutchings and Glasby (1995: 149-150).
125	Branchiae on segments 2-4; lateral lappets on segments 1 and 3, 1 and 2/3 (in combination of
126	segments 2 and 3) or 1, 3 and 4; ventral shields from segment 2 or 3; nephridial papillae on
127	segments 3-4 and 6-8; 17 pairs of thoracic notopodia from segment 4; chaetae alimbate,
128	unilimbate, symmetrical or asymmetrically bilimbate, smooth tipped; neuropodia from

segment 5, uncini avicular or pectinate with a single vertical series of teeth, arranged in single 

- rows on segment 5-10, in double rows, back to back, up to segment 20, and in single rows
- along the abdomen; pygidium sometimes with anal cirri or papillae.

133	Loimia ramzega sp. nov.
134	(Figures 2-5)
135	
136	TYPE MATERIAL
137	Holotype: anterior fragment, 139 segments, 254.2 mm long; thorax 58.6 mm long and 15.84
138	mm wide (MNHN-IA-TYPE 1788); France, English Channel, Brittany, Plouguerneau, Lilia
139	beach (48°37'37.2" N, 4°34'08.5" W), intertidal, 23 February 2016.
140	Measured Paratypes: complete specimen, broken, 165 segments, 241.13 mm long; thorax
141	65.56 mm long and 13.23 mm wide (MNHN-IA-TYPE 1789), anterior fragment, 41
142	segments, 68.5 mm long; thorax 63.29 mm long and 13.11 mm wide (MNHN-IA-TYPE
143	1790); France, location and sampling as for holotype. Anterior fragment, 47 segments, 154.37
144	mm long; thorax 74.64 mm long and 13.22 mm wide (MNHN-IA-TYPE 1791), anterior
145	fragment, 46 segments, 130.91 mm long; thorax 64.52 mm long and 13.44 mm wide (MNHN-
146	IA-TYPE 1792); France, English Channel, Brittany, Landéda beach, (48°36'37.7" N,
147	04°36'24.5" W), intertidal, 25 January 2012.
148	Other Paratypes: 6 anterior fragments, 6 abdominal fragments, 3 posterior fragments
149	(NMW.Z.2017.002.0001); France, English Channel, Brittany, Landéda beach, (48°36'37.7"
150	N, 04°36'24.5" W), intertidal, 27 December 2011; one complete specimen
151	(NMW.Z.2017.002.0002), one anterior fragment (CEMUA-POLY-TERE-0100); France,
152	English Channel, Brittany, Landéda beach, (48°36'37.7" N, 04°36'24.5" W), intertidal, 25
153	January 2012.
154	January 2012.

## ADDITIONAL MATERIAL

Four anterior fragments and 2 posterior fragments (Arcachon Marine Station Collection);
France, English Channel, Brittany, Landéda Beach (48°36'37.7" N, 04°36'24.5" W), intertidal,
January 2012. One anterior fragment (Arcachon Marine Station Collection), 2 anterior
fragments (Brest Marine Station Collection); France, English Channel, Brittany,
Plouguerneau, Lilia Beach (48°37'37.2" N, 4°34'08.5" W), intertidal, 23 February 2016.

162 DIAGNOSIS

Gigantic size (max 650 mm, live), two pairs of lateral lappets on segments 1 and 3; first pair more ventral, second pair more developed and lateral but oblique, with wavy edge. Nine ventral pads from segment 2 (fused on segments 2 and 3), first three pads swollen, next ones decreasing in size. Uncini pectinate with 6 teeth (some with 5 teeth) slightly decreasing in size, in one vertical row. Pygidium with about 14 long conical marginal papillae surrounding anus.

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170 DESCRIPTION (BASED ON HOLOTYPE AND PARATYPES, MNHN)

171 Tube long and only 17 mm in width, made of shells fragments and small gravels, emerging 172 end with fragments of macroalgae attached, inner surface with strong smooth membrane 173 allowing tube to maintain a hard consistency (Figures 2A, C). In life, body colour pinkish to light green (Figures 2C, D); buccal tentacles translucent; upper lip light pink; first pair of 174 175 lateral lappets white with blood red margins (Figure 2C); second pair blood red; branchiae 176 blood red; first ventral pad whitish, second one whitish anteriorly and blood red posteriorly, ventral pads from segment 5 to 11 (S5 to S11) blood red and with tiny whitish lateral bands, 177 178 shields from S12 to end of thorax dark red (Figure 2C). Dorsal posterior part of abdomen with dark spots. Formalin fixed body with brownish lappets, ventral shields from S4 to S11 light 179 180 brown, others dark brown, dorsal-posterior margin of thoracic segment light orange. In 181 alcohol, body whitish.

Tentacles abundant and long, reaching end of thorax when projecting backward on live 182 183 specimens (Figure 3), without ridges, with a deep groove. Eyespots absent from very developed tentacular membrane. Upper lip rounded, with free edge, projecting forward. 184 Lower lip small, completely covered ventrally by membrane connecting lappets of segment 1. 185 186 Lateral lappets discontinuous, 2 pairs on S1 and S3. First pair large, oval, projecting forwards, merged ventrally (originating ventrally), covering upper lip (Figures 3, 4A). Second pair of 187 188 lateral lappets the largest, laterally concealing S2, originating ventro-laterally and connected 189 to first ventral shields, anterior margin wrinkled or wavy, dorsolaterally ear-shaped, covering the base of the first and second pairs of branchiae (Figure 3). 190

Three pairs of arborescent branchiae, long, starting from S2, first pair longest, projecting forward, third pair smallest. Branchiae with thick stalks, and many dendritic branches arranged in five levels (Figure 3). Nephridial papillae, tube-like, from S3-4 and S6-8. Whitish glandular patches, gradually decreasing in size, surrounding the first eleven notopodia (Figure

4B). Sixteen ventral shields from S2, fused on S2-S3 (Figures 2C, 4C), not subdivided,
progressively narrower and more indented posteriorly by neuropodia from S12 to S17.
Ventral shields more evident in live specimens, appearing as blood red pigmentation (Figure 2C). Abdomen ventrally smooth.

199 Notopodia from S4, extending through S20. Notopodial lobes well developed (Figure 4B), 200 first notopodia button-like. Notochaetae of three types within a fascicle: long serrated, asymetrically bilimbate; long bilimbate, serrated, with narrow limbs; and slightly shorter 201 202 alimbate capillaries few in number, around 1:10 relative to long chaetae (Figure 5A). Thoracic 203 neuropodia as a ventrolateral belts of uncini, decreasing in width toward the posterior thoracic 204 region. Abdominal neuropodia long, projected posteriorly, with narrow belt of uncini along posterior margins. Thoracic (n=280 to 350) and abdominal (n=90 to 110) uncini pectinate, 205 206 arranged in double rows from segment 11, similar in size and shape, with 6 teeth in single 207 vertical row, some uncini with 5 teeth (Figures 5B, C). Uncini long with curved occipitium, 208 posterior process absent, basis concave, anterior process absent, anterior filament long, 209 projected downwards, long and narrow subrostrum, subrostral process absent. Pygidium with 210 terminal rounded anus, surrounded by 14 long, conical papillae (Figure 5D).

211

### 212 VARIATION

One paratype (MNHN-IA-TYPE 1790) with the first pair of lateral lappets showing a deep anterodorsal notch. One posterior part of additional material with only 12 pygidial papillae, but probably two lost. Remaining characters strictly similar to holotype.

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217 DNA

COI and 16S genes were successfully sequenced and published at NCBI GenBank for:
holotype MNHN-IA-TYPE 1788 accession number 16S: KY555058 and COI: KY555061;
paratypes MNHN-IA-TYPE 1789 accession number 16S: KY555059 and COI: KY555062
and MNHN-IA-TYPE 1790 accession number 16S: KY555060 and COI: KY555063.

222

223 ETYMOLOGY

224 The species name *ramzega* refers to the gigantic size of this species. In Breton "ramzeg"

225 means titanic, giant.

226

227 DISTRIBUTION

Only known from France, English Channel, North Brittany (along 60 km) and from Molèneisland; on sandy beaches, intertidal (Figure 1)

230

## 231 DISCUSSION

According to Holthe (1986), the genus Loimia had 16 valid and described species, one 232 233 subspecies and 4 synonymized species. However, Read & Bellan (2011) stating recent newly 234 described species, reported 28 valid species and one nomen dubium. This genus shows a 235 tropical distribution, with only few subtropical species, and no arctic species. Loimia medusa, the type species described from the Red Sea, has long been erroneously identified in many 236 237 distant localities, hiding possible new species identifications (Hutchings & Glasby, 1995). 238 Thus, true cosmopolitan species are now a topical subject for debate by marine taxonomists (Hutchings, 2016). Recently, new species of polychaetes have been identified from supposed 239 240 well studied localities (Londoño-Mesa, 2009; Carrerete & Nogueira, 2015; Nogueira et al., 2015). Concerning European waters, no species belonging to the genus Loimia has been 241 242 officially described, although some specimens originating from the area may have been erroneously deposited in museums under the name L. medusa. Thus, the present description of 243 244 L. ramzega sp. nov. may offer a local solution for those misidentified species but given the 245 presence of *Loimia* sp. in the south-eastern Irish Sea (Mackie *et al.*, 1995), there are probably 246 more undescribed *Loimia* species in Europe (especially small species) and all specimens should be observed carefully. Indeed, a similar exercise has been done by Londoño-Mesa & 247 Carrera-Parra (2005) with specimens initially identified as L. medusa from the Caribbean. 248 249 These authors found that L. medusa does not occur in the western tropical Atlantic, and that 250 the material identified as L. medusa from the region already belonged to three species; one 251 incorrectly hidden by synonymy, Loimia minuta Treadwell, 1929, and one new for science, 252 Loimia salazari Londoño-Mesa & Carrera-Parra, 2005.

We report *L. ramzega* sp. nov. for the first time from one of the best known marine ecosystems of the world: The western English Channel. We believe that a species of such size and from an easily accessible habitat could not have been overlooked through 150 years of marine biological study in the area. Indeed, Roscoff and Concarneau Biological stations both founded during the XIXth century, and later the Biological Oceanography laboratory of the 258 University of Western Brittany since the 1960s, have lead hundreds of benthic surveys in the 259 area. It is thus very unlikely that the species would not have been noticed before 2011. The 260 main hypothesis for lack of record previous to the 2010s is that L. ramzega sp. nov. could be a 261 non-European indigenous species recently introduced to Brittany. The fact that it has been 262 first identified within two areas with extensive oyster farming of the Pacific Oyster 263 Crassostrea gigas (Thunberg, 1793) (some spat and juveniles being directly imported from marine areas outside France) would support such hypothesis. The question of its area of origin 264 then arises. Another way could be an immigration from southern Europe or Africa. Warming 265 266 seas with climate change could have brought the species further north. This hypothesis seems 267 unlikely as "tropicalization" of the English Channel is not yet documented, especially in the area (Treguer et al., 2014), at the opposite of Bay of Biscay (Arias & Crocetta, 2016). Indeed, 268 269 thermal and haline structures in the Iroise Sea hinder dispersal between the Bay of Biscay and 270 English Channel and numerous benthic fauna species find their limit of distribution in 271 Brittany (Le Garrec et al., 2016, Gallon et al., in revision). However, because it is so difficult to catch, we cannot exclude the fact that the species may be native from Europe, and when (if) 272 sampled, would have been misidentified under the name L. medusa. 273

*Loimia ramzega* sp. nov. is easily identifiable by its very large size, the pigmentation on the
ventral shields, and the development of its lateral lappets. Before this record, the largest
species within the genus was *L. salazari* from the Mexican Caribbean, at 221 mm length, but
it differs from *L. ramzega* by having avicular instead of pectinate uncini.

We do not believe that the very large size of *L. ramzega* represents a reliable example of gigantism. Indeed gigantism is rather known from polar or abyssal environments (Moran & Woods, 2002) and not from intertidal temperate waters. Gigantism that would result from a consequence of a pollution is also dismissed. Indeed, sampling sites are not particularly affected by anthropogenic perturbations. Moreover, no other benthic invertebrate species from these highly diversified benthic communities are oversized in comparison with other sites or adjacent waters.

Species belonging to *Loimia* having pectinate uncini, and from geographically close regions are scarce. *Loimia viridis* Moore, 1903b, from Massachussets (United states), has similar shape on first ventral shields, but it differs from *L. ramzega* sp. nov, by having pectinate uncini with 7-8 teeth, only one type of notochaeta, being symmetrically bilimbate, and with the second pair of lateral lappets transverse to the body axis. Other *Loimia* species with pectinate uncini differ by combinations of morphological characters (disposition and number of lateral lappets and number of uncinal teeth) as well as by their geographical distribution. 292 For example, *L. arborea* (from Japan) has lateral lappets on segments 1 and 2/3, and thoracic 293 uncini with 5 (rarely 6) teeth, and abdominal ones with 6 (rarely 7) teeth. Loimia batilla 294 Hutchings & Glasby, 1988 (from Queensland, Australia) has lateral lappets on segments 1 and 295 2/3, and thoracic uncini with 6 teeth, and abdominal ones with 7 teeth. Loimia bermudensis 296 Verrill, 1900 (from Bermuda) has lateral lappets on segments 1 and 3, and uncini with 5 teeth (rarely 6 very reduced). Loimia decora Pillai, 1961 (from Sri Lanka) has lateral lappets on 297 segments 1 and 2/3, and uncini having 5 teeth. Loimia grubei Holthe, 1986 (from Philippines) 298 has lateral lappets on segments 1 and 2/3, and thoracic uncini with 5 teeth and abdominal 299 300 uncini with 6-7 teeth. Loimia triloba Hutchings & Glasby, 1988 (from Queensland) has three pairs of lateral lappets on segments 1, 3 and 4, and uncini with 5 (rarely 6) teeth. Finally, 301 302 Loimia vertucosa Caullery, 1944 (from Indonesia) has lateral lappets on segments 1 and 3, 303 and uncini with 7 teeth. Thus, none of the species having pectinate uncini have been described 304 from Europe, and L. ramzega sp. nov. is the first such species being described from the 305 region.

Finally, a revision of the genus is ongoing, examining the phylogenetic relationship of the species within the genus based on morphology.

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## 422 Figure legends

- 423 Fig. 1. Sampling sites of *Loimia ramzega* sp. nov. on the Brittany coasts (English Channel,
- 424 Western France). Dots: presence; star: locality type.
- 425 Fig. 2. Loimia ramzega sp. nov. A. Tube opening; B. Trench required to collect a single
- 426 specimen; C. Live specimen in its tube, antero-ventral view; D. Entire live specimen. Scale
- 427 bars: A, 2 cm; B, 50 cm; D, 10 cm.
- Fig. 3. *Loimia ramzega* sp. nov. anterior part, lateral view (holotype MNHN-IA-TYPE 1788).
  Scale bar: 1cm.
- 430 Fig. 4. Loimia ramzega sp. nov. A. Anterior part, lateral view (holotype MNHN-IA-TYPE
- 431 1788); B. Anterior part, dorsal view (paratype MNHN-IA-TYPE 1792); C. Anterior part,
- 432 ventral view (holotype MNHN-IA-TYPE 1788). Numbers refer to segments; LL1 = first
- lateral lappet; LL2 = second lateral lappet; p = peristomium; ul = upper lip; br1, br2, br3 refer
- to the three pairs of branchiae. Scale bars: 5mm.
- 435 Fig. 5. Loimia ramzega sp. nov. A. Notochaetae from chaetiger 11 (paratype MNHN-IA-
- 436 TYPE 1790); **B.** Thoracic uncini from segment 12, lateral views (paratype MNHN-IA-TYPE
- 437 1791); C. Abdominal uncini from segment 25, lateral views (paratype MNHN-IA-TYPE
- 438 1791); **D**. Pygidium lateral view (paratype MNHN-IA-TYPE 1789). Scale bars: A 0.1mm; B,
- 439 C 25μm; D 0.5mm.



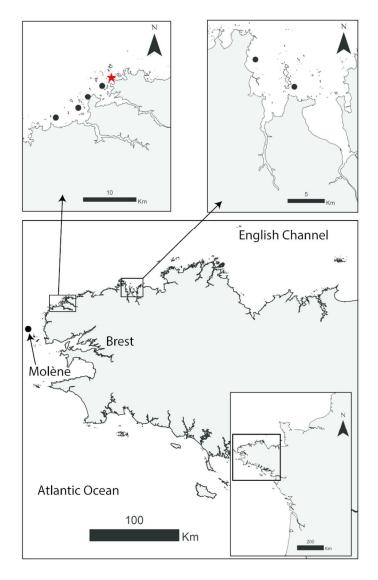


Figure 1 82x126mm (300 x 300 DPI)



Figure 2

185x155mm (300 x 300 DPI)

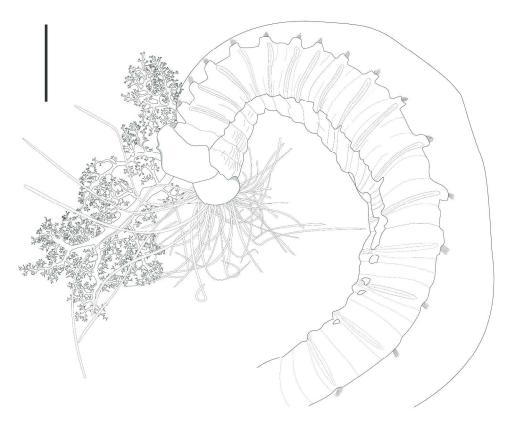


Figure 3

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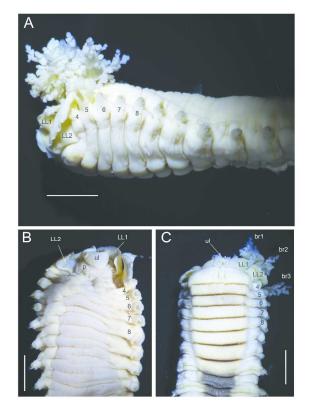


Figure 4 401x593mm (300 x 300 DPI)

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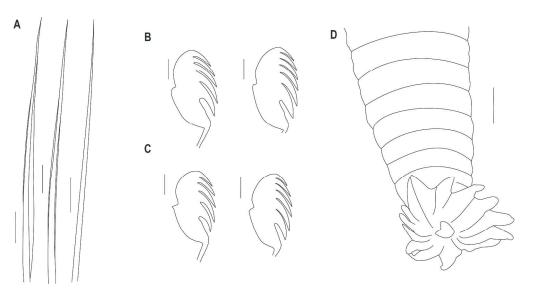


Figure 5

389x208mm (300 x 300 DPI)