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1 **Insights into the symbiotic relationship between scale worms and carnivorous sponges**
2 **(Cladorhizidae, *Chondrocladia*)**

3

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26 **Abstract**

27 Symbiotic associations between polynoid scale worms and other marine invertebrates are
28 common, but sometimes poorly understood. Compounding this problem is the fact that
29 polynoid systematics is largely unresolved. Here, we transfer the species originally described
30 as *Nemidia antillicola chondrocladiae* Fauvel, 1943, and currently synonymized with
31 *Neopolynoe acanellae* (Verrill, 1881), to the species *Neopolynoe chondrocladiae* n. comb.
32 This species is characterized by living in association with the carnivorous sponges
33 *Chondrocladia robertballardi* Cristobo, Ríos, Pomponi & Xavier, 2015 and *Chondrocladia*
34 *virgata* Thomson, 1873. The presence of specialized chaetae in *N. chondrocladiae* n. comb.
35 and the occurrence of open galleries in the sponge, derived from a gradual overgrowth of the
36 sponge to accommodate the worm, suggest an obligate symbiotic relationship between worm
37 and sponge. The presence of a gravid female with relatively small oocytes (maximum
38 diameter $56.94 \pm 14.89 \mu\text{m}$) suggests that *N. chondrocladiae* n. comb. is a gonochoristic
39 broadcaster with a planktotrophic larva, a means of reproduction that would maximize the
40 chances of this species finding new suitable hosts to colonize. We also provide a
41 phylogenetic placement, using four genetic markers (*18S*, *28S*, *16S* and *COI*), for *N.*
42 *chondrocladiae* n. comb. and *N. acanellae*, which confirms they are two different species. In
43 addition, we also report here the occurrence of another deep-water polynoid species in
44 association with the carnivorous sponge *Chondrocladia verticillata* Topsent, 1920, from the
45 Gulf of Mexico, and place it in a phylogeny.

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48 **Keywords:** Mutualism; Polynoidae; *Neopolynoe*; Adaptation; Phylogeny; Reproduction

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51 Introduction

52 Symbiotic relationships between annelid polychaetes and other marine invertebrates are not
53 unusual. A recently updated review on symbiotic polychaetes (Martín & Britayev 2018),
54 identified more than 600 species involved in symbiotic relationships, a number that has
55 almost doubled since the last review on the topic 20 years ago (Martín & Britayev 1998). In
56 their review, Martin & Britayev (2018) also reported that, out of the 33 polychaete families
57 with commensal representatives, the scale worm family Polynoidae Kinberg, 1856 has the
58 greatest number of examples (ca. 45% of all known commensal species belong to this
59 family). Polynoidae is currently represented by ca. 900 valid species (Pamungkas *et al.*
60 2019), of which more than 200 species (ca. 25% of the total) are known to be involved in ca.
61 600 symbiotic associations with other marine organisms (Martín & Britayev 2018), denoting
62 the importance of this family in the context of symbiosis in marine invertebrates. Among the
63 hosts of polynoids, cnidarians, decapods and other polychaetes appear to be the most
64 common ones (Martín & Britayev 1998; Molodtsova *et al.* 2016; Assis *et al.* 2019). Very few
65 examples of scale worms living in association with, for instance, sponges are known (e.g.
66 Martín *et al.* 1992), which may be due to sponges being poor hosts for polynoids or an
67 artefact of sampling effort (Martín & Britayev 2018). As for host specificity, in polynoids
68 there are examples of monoxenous (*i.e.* associated to one host) and polyxenous species (*i.e.*
69 associated to many different hosts), the former being more frequent for polychaete hosts
70 (mainly from the families Hesionidae Grube, 1850 and Polynoidae) and the latter being more
71 common in the case of cnidarians (see Martin & Britayev 1998, 2018).

72 Symbiotic and free-living polynoids are not considered taxonomically differentiated,
73 since commonly, genera with commensal representatives also include free-living species. A
74 clear example of that is the genus *Harmothoe* Kinberg, 1856, with a handful of species being
75 classed as commensal out of the hundreds of species currently accepted (see Martin &

76 Britayev 1998). Other genera, however, such as *Australaugeneria* Pettibone 1969 or
77 *Gorgoniapolynoe* Pettibone, 1991, could be considered as wholly commensal (Pettibone
78 1969a; b; Molodtsova *et al.* 2016; Ravara & Cunha 2016). Interestingly, the presence of
79 neuropodial hooks in the first chaetigers, which might be an adaptation to living in symbiosis
80 with their host (see Molodtsova *et al.* 2016), has been reported in all species of the genus
81 *Australaugeneria* described to date. In turn, the presence of polynoids may also cause their
82 host to adapt, and a clear example of this are the so-called ‘worm runs’, hollow tube-like
83 reticulated structures built by antipatharian corals to accommodate their symbiotic polynoids
84 (Molodtsova & Budaeva 2007).

85 Within Polynoidae, there are 12 or 13 subfamilies currently accepted, with the
86 majority of the extant species belonging to Lepidonotinae Willey, 1902, Macellicephalinae
87 Hartmann-Schröder, 1971, and Polynoinae Kinberg, 1856 (Read & Fauchald 2019). To date,
88 no study has attempted to resolve the phylogenetic relationships within Polynoidae using a
89 molecular approach, due to the relatively small number of species, from limited number of
90 genera, with available molecular data. However, recently published phylogenetic studies
91 point to some of the subfamilies in Polynoidae being poly- and paraphyletic (Gonzalez *et al.*
92 2017; Bonifácio & Menot 2018), as previously suggested based on morphological characters
93 (Rouse & Pleijel 2001). Interestingly, a recent study by Serpetti *et al.* (2017) attempted to
94 assess the implications of symbiosis in the phylogeny of the family, and the potential role of
95 hosts in speciation processes. They concluded that most basal species of the subfamily
96 Polynoinae were obligate symbionts displaying specific morphological adaptations, and that
97 obligate and facultative commensal species evolved several times (Serpetti *et al.* 2017).
98 Additionally, Serpetti *et al.* (2017), also found that obligate coral commensal species
99 appeared to be monophyletic, but pointed out that this was probably due to limited taxon
100 sampling. Amongst Polynoinae, some genera are known to be entirely comprised of

101 commensal species, either obligate or facultative, including the genus *Gorgoniapolynoe*,
102 which occurs in association with a number of alcyonaceans and hydroids (see Molodtsova *et*
103 *al.* 2016), and the genus *Neopolynoe* Loshamn, 1981, known to occur in a variety of hosts
104 including alcyonaceans, hydroids, tube-building polychaetes, and even carnivorous sponges
105 (Loshamn 1981; Kirkegaard 2001; Bock *et al.* 2010; Barnich *et al.* 2012).

106 The first mention of a symbiotic association between a polynoid and a carnivorous
107 sponge was in Fauvel's 1943 description of *Nemidia antillicola chondrocladiae* Fauvel, 1943.
108 Later, *Neopolynoe africana* Kirkegaard, 2001, recently synonymized with *Neopolynoe*
109 *acanellae* (Verrill, 1881) (Bock *et al.* 2010), was also reported in association with
110 carnivorous sponges of the genus *Chondrocladia* Thomson, 1873. More recently, an
111 unnamed polynoid was reported by Cristobo *et al.* (2015) in association with *Chondrocladia*
112 *robertballardi* Cristobo, Ríos, Pomponi & Xavier, 2015. *Chondrocladia* sponges are part of
113 the mostly deep-sea family Cladorhizidae Dendy, 1922, where the main feeding habit
114 consists of carnivory, with the sponges preying mainly on small crustaceans and polychaetes
115 (Hestetun *et al.* 2016b; a). Members of the genus *Chondrocladia* capture their prey in
116 spicule-rich, water inflated spheres, where prey appendages get trapped in the hook-like
117 spicules, and the prey are then engulfed and digested by the sponge's tissues and
118 microsymbionts (Vacelet & Boury-Esnault 1995; Vacelet & Duport 2004; Lee *et al.* 2012).
119 The fact that *Neopolynoe* species live in association with *Chondrocladia* spp., a potential
120 predator of the worm, makes this symbiotic association one of real note.

121 Here, using a combined morphological and molecular approach, we transfer the
122 species originally described as *N. antillicola chondrocladiae*, and later synonymized with *N.*
123 *acanellae*, to the species *Neopolynoe chondrocladiae* n. comb. We also report on the
124 occurrence of another deep-water polynoid species in association with the carnivorous
125 sponge *Chondrocladia verticillata* Topsent, 1920 from the Gulf of Mexico.

126 **Materials and Methods**127 *Samples*

128 *Chondrocladia robertballardi* specimens, with their associated polynoid worms, were
129 collected from the Cantabric Sea on board the Instituto Español de Oceanografía (IEO)
130 research vessels B/O *Vizconde de Eza*, in October 2003, as part of the ECOMARG project,
131 and on board the B/O *Ángeles Alvariño*, in June 2017, as part of the SponGES project.
132 Samples from the Goringe Bank were collected on board the Ocean Exploration Trust
133 research vessel E/V *Nautilus*, in October 2011, as part of the NAO17 expedition (Fig. 1;
134 Table 1).

135 Additional polynoid specimens used for both morphological and molecular analyses
136 include *Neopolynoe acanellae* from the Cantabric Sea, living in association with the
137 cnidarian *Acanella arbuscula* (Johnson, 1862), and *Robertianella synophthalma* McIntosh,
138 1885, living in association with the hexactinellid sponge *Pheronema carpenteri* (Thomson,
139 1869) (Fig. 1; Table 1).

140 Samples collected from the Cantabric Sea were preserved in 96% ethanol and kept at
141 -20°C (for molecular and morphological studies), or in 10% formalin buffered in seawater,
142 transferred to 70% ethanol and kept at room temperature (for morphological studies);
143 samples from the Goringe Bank were preserved in 70% ethanol and kept at room
144 temperature (Fig. 1; Table 1).

145 Samples of polynoids used for morphological comparisons were chosen based on the
146 voucher material used by Bock et al. (2010), including voucher material for all currently
147 recognized synonyms of *N. acanellae*, obtained from the Canadian Museum of Nature,
148 Ottawa, Ontario, Canada (CMNA), the Museum of Comparative Zoology, Harvard
149 University, Cambridge, Massachusetts, USA (MCZ), the Natural History Museum, London,
150 United Kingdom (NHMUK), the Muséum National d'Histoire Naturelle, Paris, France

151 (MNHN), the Musée Océanographique de Monaco, Monaco (MOM), the Peabody Museum
152 of Natural History, Yale University, New Haven, Connecticut, USA (YPM), and the
153 Smithsonian National Museum of Natural History, Washington, DC, USA (USNM) (see
154 Table 1). All voucher material was also compared to their published descriptions (Kinberg
155 1857; Storm 1881; Verrill 1885; Storm 1888; Augener 1906; Fauvel 1913, 1914; Ditlevsen
156 1917; Hartman 1942; Fauvel 1943; Pettibone 1963; Kirkegaard 2001). In addition, sponge
157 specimens of *C. virgata* deposited in the NHMUK, and the type material of *Chondrocladia*
158 *michaelsarsi* Arnesen, 1920 (ZMBN 25639–25641), recently synonymized with *C. virgata*
159 (Hestetun et al., 2017), were also examined for the presence of polynoids.

160 *DNA extraction, amplification and sequencing*

161 DNA extraction of polynoid samples was performed using DNeasy® Blood and Tissue kit
162 (QIAGEN, Germany), following the manufacturer's protocol. DNA concentration of the
163 eluted samples was quantified using NanoDrop™ 8000 (Thermo Fisher Scientific, USA).
164 Extracted DNA was amplified for gene fragments of cytochrome *c* oxidase subunit I (*COI*),
165 *16S* rRNA (*16S*), *18S* rRNA (*18S*) and *28S* rRNA (*28S*). Primer pairs and PCR programmes
166 used to amplify polynoid genetic markers were as follows: (i) for *COI* the primer pair
167 consisted of LCO 1490 and HCO 2198 (Folmer *et al.* 1994), and the PCR programme was
168 95°C/5 min – (95°C/1 min – 58°C/1 min – 72°C/1 min) x 38 cycles – 72°C/10 min; (ii) *16S*
169 was amplified using the 16S arL/brH primer pair (Palumbi 1996), and the PCR programme
170 was 94°C/5 min – (94°C/1 min, 55°C/45 s, 68°C/45 s) x 38 cycles – 68°C/10 min; (iii) *18S*
171 was amplified using three overlapping fragments, with the primer pairs 1F/5R, 4F/7R and
172 a2.0/9R (Whiting *et al.* 1997; Giribet *et al.* 2002), and the programme was at 94°C/5 min –
173 (94°C/1 min, 52°C/1 min, 72°C/1 min) x 38 cycles – 72°C/10 min; (iv) and *28S* was amplified
174 using the overlapping primer pairs a/rD5b, C1/C2 and F63.2/PO28R4 (Le *et al.* 1993;
175 Whiting *et al.* 1997; Whiting 2002; Struck *et al.* 2006), and the following PCR programmes:

176 for the first two primer pairs, 94°C/5 min – (94°C/1 min, 55°C/1 min, 72°C/1 min) x 30 cycles
177 – 72°C/10 min; for the last primer pair the programme consisted of 94°C/5 min – (94°C/30 s,
178 55°C/30 s, 72°C/1.5 min) x 30 cycles – 72°C/10 min.

179 All DNA markers were amplified in 12.5 µL reactions using 10.5 µL of VWR Red
180 Taq DNA Polymerase 1.1x Master Mix (VWR International bvba/sprl, Belgium), 0.5 µL of
181 the forward and reverse primers, and 1 µL of DNA template. PCR products, stained with
182 GelRed® (Biotium, USA), were visualized in a 2.5% agarose gel electrophoresis, run at 90 V
183 for 30 min. Sequencing was conducted on an ABI 3730XL DNA Analyser (Applied
184 Biosystems, USA) at the Molecular Core Labs (Sequencing Facility) of the NHMUK, using
185 the forward and reverse primers mentioned above.

186 *Morphological analyses*

187 Macroscopic morphological features of all collected samples and voucher specimens were
188 photographed in a Leica MZ6 stereomicroscope (Leica Microsystems, Germany), with an
189 Olympus UC50 camera and cellSens Standard interface v.1.16 (Olympus Corporation,
190 Japan). When possible, we removed and photographed parapodia from the 1–2, middle, and
191 posterior chaetigers, and also elytra from the middle dorsum region of the polynoids.
192 Maximum width was measured including parapodia.

193 A specimen of *N. chondrocladiae* n. comb. preserved in formalin was prepared for
194 histological study. A portion of 3 to 4 mid-body segments of the specimen was embedded in
195 paraffin, cut into 5 µm sections, stained with haematoxylin-eosin, and mounted with DPX.
196 All histological preparations were photographed in an Olympus BX43 compound microscope
197 (Olympus Corporation, Japan), with the same camera used to take macroscopic pictures. The
198 maximum diameter of oocytes were obtained with ImageJ v.1.50i (Schneider *et al.* 2012),
199 distinguishing between internal and external (*i.e.* near the epidermis, close to being released)
200 oocytes; differences in the maximum diameter of these oocytes were tested for statistically

201 significant differences in their maximum diameter using a two-tailed unpaired Student's t-
202 test, in R v.3.4.2 (R Core Team 2017).

203 For scanning electron microscopy (SEM) observations, three *N. chondrocladiae* n.
204 comb. specimens were dehydrated in an ascending ethanol series, critical-point-dried,
205 mounted on pins and coated with gold. SEM images were performed in a FEI QANTA FEG
206 650 (FEI, USA) at the NHMUK Imaging Facilities.

207 *Phylogenetic analyses*

208 Overlapping sequence fragments were assembled and trimmed into consensus sequences
209 using the software Geneious v.10.1.3 (<http://www.geneious.com>, Kearse *et al.* 2012).
210 Consensus sequences were checked for contamination using BLAST (Altschul *et al.* 1990),
211 and aligned with the inbuilt MAFFT v.7.309 (Katoh & Standley 2013), using the Q-INS-I
212 option.

213 For the phylogenetic analyses, the sequences of *N. chondrocladiae* n. comb., *N.*
214 *acanellae*, and one *R. synophthalma* were aligned with a selection of polynoid sequences
215 used by Gonzalez *et al.* (2017) and Serpetti *et al.* (2017) (Table 2). The sequences were
216 aligned with MAFFT for each genetic marker and Gblocks v.0.91b (Castresana 2000) was
217 run for the non-coding genes (*16S*, *18S*, and *28S*) with “minimum number of sequences for a
218 flank position” set to $n/2 + 1$, where n is the total number of sequences in the alignment,
219 “maximum number of contiguous non-conserved positions” set to 10, “minimum length of a
220 block” to 5, and “allowed gap positions” set to “with half”. The resulting alignments were
221 manually trimmed in Geneious, resulting in the following alignment lengths: 1682 bp for
222 *18S*, 895 bp for *28S*, 429 bp for *16S*, and 574 bp for *COI*. These alignments were
223 concatenated for the phylogenetic analyses using Geneious v.10.1.3.

224 Nucleotide substitution models were fitted using jModelTest v.2.1.7 (Darriba *et al.*
225 2012), with the number of substitution schemes set to 3, given the limited number of models

226 that can be implemented in RAxML (Stamatakis 2014). Based on the Akaike Information
227 Criterion (AIC) (Akaike 1998), the best fit model for all partitions was GTR+I+G.
228 Phylogenetic analyses were run using model-based approaches using the evolutionary models
229 inferred in jModeltest, with partitions distinguishing between coding (*COI*) and non-coding
230 genes (*16S*, *18S*, and *28S*). Maximum Likelihood (ML) analyses were implemented using
231 RAxML v.8.2.10 (Stamatakis 2014) on XSEDE in the CIPRES Science Gateway (Miller *et*
232 *al.* 2010), while Bayesian inference (BI) analyses were run in MrBayes v.3.2.2 (Ronquist *et*
233 *al.* 2011) in the NHMUK cluster. The ML analyses consisted of a multiple tree search with
234 1000 alternative runs, without bootstrap, and a multiparametric bootstrap analysis with 1000
235 iterations, the results of which were combined. BI analyses consisted of two chains run for 10
236 million generations, sampled every 1000 generations, and a 25% burn-in. Convergence
237 between chains, mixing within chains (i.e. ESS values) and the number of burn-in
238 generations were monitored with the program TRACER 1.6 (Rambaut *et al.* 2015). The
239 resulting trees for both ML and BI analyses were visualized and edited in FigTree v.1.4.2
240 (Rambaut 2014).

241

242 **Results**

243 *Systematics*

244 Family POLYNOIDAE Kinberg, 1856

245 Genus *Neopolynoe* Loshamn, 1981

246 *Neopolynoe chondrocladiae* (Fauvel, 1943) n. comb. Figs. 2–4, 6.

247 *Polynoe antillicola chondrocladiae* Fauvel, 1943: 200, Fig.1a-d.

248 *Neopolynoe africana* Kirkegaard, 2001: 392, Fig. 1.

249 Not *Polynoe (Eunoe) acanellae* Verrill, 1881: pl. 6 Figs. 5, 5a; Verrill (1885a): 424; Verrill
250 (1885b): 525, pl. 39 Fig. 172.

- 251 Not *Polynoe acanellae*: Hartman (1942): 27, Figs. 27-31.
- 252 Not *Harmothoe acanellae*: Ditlevsen (1917): 27, pl. 1 Figs. 6, 8, 9, 13; pl. 2 Fig. 4.
- 253 Not *Harmothoe (Hermadion) acanellae*: Pettibone (1963): 35-36, Fig. 6l-m.
- 254 Not *Neopolynoe acanellae*: Bock et al. (2010): 56-58, Fig. 3.
- 255 Not *Nemidia antillicola* Augener, 1906: 126, pl. 3 Figs. 53-59.
- 256 Not *Polynoe antillicola*: Fauvel (1913): 24; Fauvel (1914): 68.
- 257
- 258 *Material examined. Polynoe antillicola chondrocladiae*: holotype from Cape Ortegal, Spain
- 259 (MNHN-IA-TYPE0822). *Neopolynoe africana*: holotype from Northeast of Canary Islands,
- 260 29°23.8'N 12°12.3'W (NHMUK 2003.848); about 13 specimens from Northeast of Canary
- 261 Islands, 29°23.8'N 12°12.3'W (NHMUK 2003.849-858); 1 specimen inside *Chondrocladia*
- 262 sponge from Northeast Canary Islands, 29°23.8'N 12°12.3'W (NHMUK 2003.859). *Polynoe*
- 263 (*Eunoe*) *acanellae*: lectotype from South of Cape Cod, USA, 39°48.5'N 70°54'W (YPM
- 264 2741); 3 paralectotypes from South of Cape Cod, USA, 39°53'N 70°58.583'W (YPM 2742); 2
- 265 syntypes from off Martha's Vineyard, USA, 39°53'N 70°58.583'W (NHMUK 1976.947-948).
- 266 *Neopolynoe acanellae*: 1 specimen from Orphan Basin, Canada, 50°04.05'N 50°08.067'W
- 267 (CMNA 2014-0001). *Nemidia antillicola*: 1 syntype from Southeast of Martha's Vineyard,
- 268 USA, 40°11.667'N 68°22'W (MCZ.ANNb-2120); 1 syntype from off Georges Bank, USA,
- 269 41°29.75'N 65°47.167'W (MCZ.ANNb-2105). *Polynoe antillicola*: 1 specimen from the
- 270 Azores, 37°40'N 26°26.25'W (MOM 18 0492). *Neopolynoe antarctica*: 1 specimen from East
- 271 Falkland (NHMUK 2015.524); 2 specimens from the Patagonian Shelf, 51°34.5'S 67°18.5'W
- 272 (NHMUK 2015.528-529). *Neopolynoe paradoxa*: 1 specimen from South of Ireland,
- 273 48°45.642'N 10°27.648'W (NHMUK 2016.290); 1 specimen from South of Ireland,
- 274 48°45.642'N 10°27.648'W (NHMUK 2016.292); 1 specimen from South of Ireland,
- 275 48°45.642'N 10°27.648'W (NHMUK 2016.294). Unidentified polychaetes never reported: 1

276 specimen in association with *C. virgata* (NHMUK 1882.7.28.97); 2 specimens in association
277 with *C. virgata* (NHMUK 1890.4.10.6); 1 specimen in association with *C. virgata* (NHMUK
278 1898.5.7.37); 2 specimens in association with *C. virgata* (ZMBN 25639); 2 specimens in
279 association with *C. virgata* (ZMBN 25640); 2 specimens in association with *C. virgata*
280 (ZMBN 25641). This study: 6 specimens in association with *C. robertballardi*, 43°58.717'N
281 6°28.980'W (SponGES0617-BT5 St.); 24 specimens in association with *C. robertballardi*,
282 43°58.866'N 6°28.622'W, (SponGES0617-BT6 St.); 3 specimens in association with *C.*
283 *robertballardi*, 43°58.884'N 5°49.484'W (ECOMARG St.); 2 specimens in association with
284 *C. robertballardi*, 36°38.9713'N 11°03.232'W (NAO17 St.).

285

286 Description (based on holotype *Polynoe antillicola chondrocladiae* MNHN-IA-TYPE0822).
287 Complete individual, broken in two pieces, accounting for 121 chaetigers, 60 mm long, 4 mm
288 wide (including parapodia); body tapering posteriorly (Fig. 2A). Prostomium bilobed, with
289 two small cephalic peaks and four large eyes: anterior pair at broadest part of prostomium
290 and posterior pair near the hind margin of prostomium (Fig. 2B). Median antenna with large
291 ceratophore, style lost; two lateral antennae inserted ventrally, with large ceratophores and
292 short styles (Fig. 2B). Palps lost. Tentacular cirri long (Fig. 2B). Pharynx everted completely,
293 showing two chitinous brown jaws, each with two teeth; pharynx is smooth, distally with 24
294 digitated papillae (Fig. 2B).

295 Fifteen pairs of elytra, on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29 and 32.
296 Elytra oval, not covering the posterior part of the animal. Elytral surface covered by
297 numerous conical microtubercles with numerous digitated papillae intermingled; digitated
298 papillae at outer lateral and posterior margin of elytra (Fig. 2C–D).

299 Parapodia biramous. Notopodium rounded with acicular lobe, neuropodium also rounded
300 with acicular lobe and a short supra-acicular process. Notochaetae all unidentate, with

301 estriations at the widest part of the chaetae, stouter than neurochaetae in bundles of 20–25
302 and 40–45, respectively (Fig. 2E). Neurochaetae all unidentate, ending in a curved tip. Dorsal
303 cirri as long as tentacular cirri, thinner posteriorly. Ventral cirri short, never extending
304 beyond parapodia (Fig. 2E).

305 *Additional morphological information from other specimens* (*N. africana*: NHMUK
306 2003.848–858; polychaetes in association with *C. virgata*: NHMUK 1882.7.28.97, NHMUK
307 1890.4.10.6, NHMUK 1898.5.7.37, and ZMBN 25639-25641; This study: SponGES0617-
308 BT5 St., SponGES0617-BT6 St., ECOMARG St., and NAO17 St.). Due to the limited access
309 granted to study the holotype of the species, some of its key morphological features could not
310 be described in detail. In order to complement this description, we provide information based
311 on a number of other specimens (see Table 1) and also on *in situ* observations of live
312 specimens (Fig. 3A–B). Other complete specimens of this species had between 56 and 94
313 chaetigers, 11–48 mm in length, and 1–5 mm in width (Table 3). Eyes in live specimens are
314 black and prostomium is pink (Fig. 3B). Same number, arrangement and shape of elytra as in
315 the holotype. Elytra surface and margin as in holotype (Fig. 3F–H).

316 Palps are robust and long, up to 4–5 times the length of prostomium (Fig. 3C–D). Bundles of
317 both notochaetae and neurochaetae emerging from several rows (up to 7 or 8). Notochaetae
318 unidentate, spade-like, with fine striations present at the widest part of the chaetae (Fig. 4A–
319 B). Neurochaetae at the first parapodium of two groups: straight numerous chaetae with a
320 median line appearing as the chaetae widens, with numerous horizontal striations (Fig. 4C–
321 D); curved short chaetae appearing occasionally, present at the base of the bundle of chaetae,
322 also presenting numerous striations (Fig. 4E). Notochaetae of mid-body parapodia similar to
323 those in anterior segments. Neurochaetae in mid-body parapodia of four types: distally-
324 hooked chaetae with striations on the widest part located ventrally (Fig. 4F); longer spatula-
325 like chaetae with engrossment at the posterior end with striations located dorsally (Fig. 4G),

326 probably the result of the growing of the distally-hooked chaetae; curved short chaetae
327 appearing occasionally with striations at the ventralmost part of the parapodia (Fig. 4H); and
328 straight and slightly bent chaetae, probably the result of the growing hook-like chaetae
329 located dorsally (Fig. 4I). Two individuals of relatively small size (BT6-602-B2 and 15-11B-
330 1) showed a similar pattern in the neurochaetae: distally-hooked short chaetae running from
331 15/16–20 chaetiger; transition of short distally-hooked to long spatula-like chaetae between
332 20–21 chaetiger; and long spatula-like are found from the anterior chaetigers to chaetiger 15
333 or 16 and from chaetiger 22 to the posterior end. A bigger individual (BT6-602-B1), though,
334 did not display any region with short distally-hooked chaetae between 15–20 chaetigers.

335 *Remarks.* Bock *et al.* (2010) established a new combination for *N. acanellae*, including
336 species originally described as *Polynoe (Eunoe) acanellae* Verrill, 1881, *Polynoe acanellae*
337 (Verrill, 1885), *Harmothoe acanellae* (Verrill, 1885), *Harmothoe (Hermadion) acanellae*
338 Pettibone, 1963, *Nemidia antillicola* Augener, 1906, *Polynoe antillicola* Fauvel, 1913,
339 *Polynoe antillicola chondrocladiae* Fauvel, 1943, and *Neopolynoe africana* Kirkegaard,
340 2001. In their study, Bock *et al.* (2010) examined: (i) the type material of specimens of
341 *Polynoe (Eunoe) acanellae* collected by Verrill (1881, 1885a, 1885b), found in association
342 with the cnidarian alcyonacean *Acanella normanni* [= *Acanella arbuscula* (Johnson, 1862)] in
343 the NE coast of USA; (ii) the type material of *N. africana* described by Kirkegaard (2001) in
344 association with the carnivorous sponge *Chondrocladia* sp., collected in the NE of the Canary
345 Islands; (iii) and specimens in association with the cnidarian alcyonacean *Anthomastus* sp.
346 collected off Newfoundland, Canada. In their paper, Bock *et al.* (2010) highlighted that,
347 amongst other features, *N. acanellae* was characterized by having a long tapering ventral cirri
348 always reaching beyond the tip of the neuropodium and also by having a digitiform supra-
349 acicular neuropodial process (see Fig. 3D in Bock *et al.* 2010). Our re-examination of the
350 material studied by Bock *et al.* (2010), along with other material (see Table 3), confirmed

351 that there exists consistent morphological differences in the length of ventral cirri and the
352 supra-acicular neuropodial process in specimens living in association with cnidarians and the
353 ones found in association with *Chondrocladia* species. In the latter specimens, including
354 newly collected material from the Cantabric Sea and type material for *N. africana* (NHMUK
355 2003.848), *N. antillicola chondrocladiae* (MNHN-IA-TYPE0822), and *C. virgata* (NHMUK
356 1882.7.28.97, 2 specimens living inside galleries never reported in the original description of
357 the sponge), ventral cirri were always shorter than the neuropodium and the supra-acicular
358 neuropodial process was short and blunt (Fig. 2E, 3E). Other morphological differences were
359 revealed between those specimens living in association with *Chondrocladia* and the rest of
360 specimens studied by Bock *et al.* (2010). In terms of gross morphology, the specimens living
361 in *Chondrocladia* are slenderer than the others, and the tapering at the posterior end is less
362 pronounced (Fig. 2A, 3C–D, 5A–B). All examined material living in association with
363 *Chondrocladia* was white in color, including fresh material, while other specimens tended to
364 be reddish-brown (Fig. 2A, 3C–D, 5A), except for one white specimen (CMNA 2014-0001).
365 The chaetae of specimens associated with cnidarians were consistently bronze coloured, and
366 more robust than those of in association with the carnivorous sponge, while notochaetae
367 bundles of specimens associated to cnidarians had fewer chaetae (ca. 5) than the specimens
368 associated to carnivorous sponges (>15) (Fig. 2E, 3E, 5C). Also, while elytra in the
369 specimens living in association with the carnivorous sponge do not cover the dorsum, elytra
370 in the specimens living in association with cnidarian are more robust and do cover the entire
371 dorsum (Fig. 3D, 5A–B). Additionally, SEM revealed the presence of short, stout
372 neurochaetae, with pronounced hooked tips, in some middle parapodia of the specimens
373 associated to *Chondrocladia* (Fig. 4F). All morphological differences consistently found
374 between specimens of *N. acanellae*, along with the phylogenetic results we obtained (see
375 Phylogenetic results below), made us take the following taxonomical decision: the *N.*

376 *acanellae* specimens found in association with different species of cnidarians, including
377 *Acanella arbuscula* (Verrill 1877; Ditlevsen 1917), *Acanthogorgia armata* Verrill, 1878
378 (Verrill 1881), *Pseudoanthomastus agaricus* (Studer, 1890) (Ditlevsen 1917; Molodtsova
379 2013), and *Pennatula grandis* Ehrenberg, 1834 (Pettibone 1963; Hamel *et al.* 2015) should
380 keep the name *Neopolynoe acanellae* (Verrill, 1881); the *N. acanellae* specimens found in
381 association with *C. virgata* and *C. robertallardi* should be named *Neopolynoe*
382 *chondrocladiae* n. comb. (Fauvel, 1943), since Fauvel was the first (principle of priority,
383 ICZN Code) to describe this species as *Polynoë (Nemidia) antillicola chondrocladiae* after
384 examining a carnivorous sponge identified as *Chondrocladia* sp. off the Galician coast of the
385 Iberian Peninsula. Thus, *Polynoë (Nemidia) antillicola chondrocladiae* deposited at the
386 MNHN should be considered as the type species of *Neopolynoe chondrocladiae* n. comb.,
387 while *N. africana* should be considered as its junior synonym.

388 *Neopolynoe chondrocladiae* n. comb. also clearly differs from two congeneric
389 *Neopolynoe* (Table 3). As opposed to *Neopolynoe chondrocladiae* n. comb., elytra in
390 *Neopolynoe antarctica* (Kinberg, 1858) cover the entire dorsum and lack lateral papillae. In
391 addition, *N. antarctica* has bidentated neurochaetae as opposed to the rest of its congeneric
392 species. Also, *Neopolynoe paradoxa* (Anon, 1888) appears to have papillae in both dorsal
393 and ventral cirri, while *N. chondrocladiae* n. comb. has smooth cirri.

394

395 *Biology.* Always in association with the carnivorous sponges *C. robertballardi* and *C.*
396 *virgata*, laying on open galleries formed by the sponge in its axis. All the galleries appeared
397 not to be excavated by the polynoid but seemed to be produced naturally by the sponge
398 overgrowing on top of the area where the worm lays. These open galleries were normally not
399 connected among each other and had just one worm per gallery, with some galleries
400 appearing to be empty.

401 Maximum diameter of oocytes in the histological sections of *N. chondrocladiae* n.
402 comb. (Fig. 6A, B) yielded a mean oocyte diameter of $56.94 \pm 14.89 \mu\text{m}$, with no significant
403 difference between the diameters of internal and external oocytes (internal oocytes:
404 $57.04 \pm 15.68 \mu\text{m}$; external oocytes = $56.35 \pm 9.26 \mu\text{m}$; p -value = 0.81) (supplementary material
405 3). Oocytes appeared to be extruded laterally at the base of the parapodia.

406 Except for a few occasions, the exact number of specimens of *N. chondrocladiae* n.
407 comb. per sponge host was not obtained, since most *C. robertballardi* specimens were
408 collected using trawling gears. In the two specimens of *C. robertballardi* that were collected
409 intact using ROV, the number of symbionts per sponge was two and three.

410 *Distribution.* Northeastern Atlantic Ocean from the Canary Islands to the Porcupine Abyssal
411 Plain, in 735 m to 2,487 m.

412

413 Polynoidae sp. 1

414 *Material examined.* Two specimens from the Gulf of Mexico (USNM-1482940 and USNM-
415 1482941) in association with *Chondrocladia verticillata* (USNM-1482939), $24^{\circ}38'60''\text{N}$
416 $83^{\circ}54'36''\text{W}$, 735 m.

417 *Description.* Incomplete specimens lacking approximately half of their posterior part. When
418 complete accounting for 34 and 42 chaetigers, ca. 30 mm long, ca. 10 mm wide (estimated
419 from photographs of live complete specimens). Prostomium bilobed, with two small cephalic
420 peaks and four large eyes: anterior pair at broadest part of prostomium and posterior pair near
421 the hind margin of prostomium (Fig. 5D–E). Median antenna with large ceratophore, style
422 lost; two lateral antennae inserted ventrally, with short styles (Fig. 5E). Palps robust and long,
423 up to 3 times the length of the prostomium. Pharynx everted completely, showing two
424 chitinous brown jaws, each with two teeth; pharynx is smooth, with 24 digitated papillae in
425 rim.

426 Number of elytra impossible to determine but probably 16–18 (estimated from
427 photographs of live complete specimens). Elytra oval, covering the posterior part of the
428 animal. Elytral surface covered by numerous conical microtubercles with numerous club-
429 shaped papillae intermingled; club-shaped papillae covering almost the entire elytra
430 perimeter (Fig. 5G–I).

431 Parapodia biramous. Notopodium triangular with acicular lobe, neuropodium also
432 triangular with a long acicular lobe without supra-acicular process. Notochaetae and
433 neurochaetae stout and striated in numerous bundles of ca. 30 and ca. 45, respectively (Fig.
434 5F). Neurochaetae all unidentate. Dorsal cirri long and slender, thinner posteriorly; ventral
435 cirri very short, never extending past the mid length of neuropodium (Fig. 5F).

436 *Biology.* In association with the carnivorous sponge *C. verticillata*, laying on the sponge axis.
437 No galleries observed in the sponge.

438

439 *Phylogenetic analyses*

440 Both BI and ML phylogenetic analyses recovered Polynoidae as monophyletic with high
441 support (BI: 0.99 posterior probability (PP), ML: 92 bootstrap proportion (BP)), while the
442 subfamily Polynoinae was recovered as polyphyletic, with a large a clade containing most
443 species (PP=1.00, BP=98) (Fig. 7), with the exception of *Paradyte crinoidicola* (Potts, 1910)
444 and *Paralepidonotus ampulliferus* (Grube, 1878): the former as sister species of the two
445 members of the family Arctonoinae Hanley, 1989 included in the analyses (PP=1.00,
446 BP=100), and the latter as the most basal taxa within Polynoidae (PP=0.99, BP=92).
447 Additionally, *Lepidonotus sublevis* Verrill, 1873, member of the subfamily Lepidonotinae,
448 appeared nested within the genus *Gattyana* McIntosh, 1897 of the subfamily Polynoinae in
449 our phylogenetic analyses (PP=1.00, BP=95) (Fig. 7), making Polynoinae paraphyletic. The
450 only three subfamilies of Polynoidae recovered as monophyletic were Arctonoinae (PP=0.98,

451 BP=80), Lepidastheniinae Pettibone, 1989 (recovered with low support), and
452 Macellicephalinae (PP=1.00, BP=100) (Fig. 7). Members of the genus *Neopolynoe* were
453 recovered as polyphyletic, with *N. chondrocladiae* n. comb. recovered as the sister species to
454 *N. paradoxa*, the type species of the genus, with high posterior probability (PP=0.99,
455 BP=66), and formed a clade with *Melaenis loveni* Malmgren, 1866 and *Malmgreniella*
456 *mcintoshii* (Tebble & Chambers, 1982) (PP=0.93, BS=31). *Neopolynoe acanellae* was
457 recovered as the sister species to a clade including *Antarctinoe ferox* (Baird, 1865),
458 *Polyeunoa laevis* McIntosh, 1885, and *Eunoe* sp. (PP=0.99, BS=54). The clade containing *N.*
459 *acanellae* was recovered as sister group to a clade including species of the genera
460 *Gorgoniapolynoe* Pettibone, 1991, *Antipathipolyeunoa* Pettibone, 1991, *Robertianella*
461 McIntosh, 1885, *Brychionoe* Hanley & Burke, 1991, and *Harmothoe* Kinberg, 1856
462 (PP=1.00, BP=71) (Fig. 7). As for Polynoid sp. 2, the species collected in association with *C.*
463 *verticillata* in the Gulf of Mexico, it clustered together with *Hermenia verruculosa* Grube,
464 1856 (PP=0.93, BP=66), outside of the subfamily Polynoinae (Fig. 7).

465 **Discussion**466 *Taxonomy and systematics of symbiotic polynoids of the genus Neopolynoe*

467 Members of the genus *Neopolynoe* are relatively common organisms in the deep-water
468 Atlantic, generally living in association with other marine invertebrates (see Table 3). Based
469 on a combined morphological and molecular approach, here we establish a new combination
470 for the genus with the species *Neopolynoe chondrocladiae* n. comb., thus raising the number
471 of *Neopolynoe* species to four (Table 3). This species, to our knowledge only found in
472 association with the carnivorous sponges *C. robertbatllardi* and *C. virgata*, was originally
473 described by Fauvel (1943) as *Nemidia antillicola chondrocladiae* in a very concise manner,
474 based on several individuals collected from a single *Chondrocladia* specimen off Cap
475 Ortegal, Spain (NW Iberian Peninsula). This description was, however, overlooked by
476 Kirkegaard (2001), who described *Neopolynoe africana* based on specimens collected in
477 association with a *Chondrocladia* species east of the Canary Islands, adding important
478 information on the arrangement of parapodia and the length of the ventral cirri. Years later,
479 Bock *et al.* (2010) synonymized *N. africana* with *Neopolynoe acanellae* without a clear
480 justification and neglected the clear distinct characters of *N. chondrocladiae* n. comb.
481 described by Kirkegaard (2001), and corroborated by our study. Our thorough morphological
482 review of type material and newly collected specimens unequivocally differentiates *N.*
483 *chondrocladiae* n. comb. and *N. acanellae* as two clearly separate entities. This
484 morphological differentiation is mainly based on the external appearance (short and relatively
485 robust body in *N. acanellae*; elongated and slender body in *N. chondrocladiae* n. comb.), the
486 length of the ventral cirri (long in *N. acanellae* always extending past the neurochaetae; short
487 in *N. chondrocladiae* n. comb., never extending past the neurochaetae), the length and shape
488 of the neuropodial supra-acicular process, the general appearance of the chaetae, and the
489 number of chaetae present in the notochaetae bundles, among other characters. Similarly, the

490 other two congeneric species of *Neopolynoe* already described, namely *N. antarctica* and *N.*
491 *paradoxa*, also clearly differ from *N. chondrocladiae* n. comb. (see Table 3 and Remarks in
492 the Results section).

493 Morphological differences observed in the two previously synonymized species
494 appear to match with the hosts where these organisms are normally found: while *N. acanellae*
495 appears to live in association with alcyonarian and pennatulacean corals, including *Acanella*
496 *arbuscula*, *Acanthogorgia armata*, *Pseudomastus agaricus* and *Pennatula grandis* (Hamel *et*
497 *al.* 2015; see Molodtsova *et al.* 2016), to our knowledge *N. chondrocladiae* n. comb. has only
498 been reported in carnivorous sponges of the genus *Chondrocladia* from the NW Atlantic
499 (Fauvel 1943; Kirkegaard 2001; Cristobo *et al.* 2015; this study). Surprisingly, *N.*
500 *chondrocladiae* n. comb. was never reported by Thompson (1873) in the original description
501 of *C. virgata*, despite being obviously present in the lectotype deposited in the NHMUK
502 (1882.7.28.97), which we revisited. Given the locations and depths where *N. chondrocladiae*
503 n. comb. studied by Fauvel (1943) and Kirkegaard (2001) were collected, it is very likely that
504 they occurred in association with either *C. virgata* or *C. robertballardi* (they were never
505 identified to species level in the original works), since these are the only known
506 *Chondrocladia* species present in this area (Cristobo *et al.* 2015; Hestetun *et al.* 2017). As for
507 the other two congeneric *Neopolynoe* species, *Neopolynoe antarctica* (Kinberg, 1858) has
508 been reported in association with tube-building polychaetes and a cnidarian of the *Thuiaria*
509 Fleming, 1828 genus (Martín & Britayev 1998; Barnich *et al.* 2012), while *Neopolynoe*
510 *paradoxa* (Anon, 1888) has been reported in association with the coral *Lophelia pertusa*
511 (Linnaeus, 1758) (Jensen & Frederiksen 1992).

512 The clear morphological differences and host preferences observed between *N.*
513 *acanellae* and *N. chondrocladiae* n. comb. were corroborated in our phylogenetic analysis,
514 where both species were recovered in two different clades, with *N. chondrocladiae* n. comb.

515 having *N. paradoxa* as sister species (Fig. 7). Given the marked morphological differences
516 reported above, the host preference we observed, and the phylogenetic results we obtained,
517 we conclude that *N. chondrocladiae* n. comb. should be reinstated as a valid species.
518 Furthermore, the recovery of *N. acanellae* in a different clade to the other two *Neopolynoe*
519 species used in the analysis (including the type species of the genus, *N. paradoxa*), suggests
520 that the genus *Neopolynoe* should be revised. Interestingly, three of the four species we
521 sequenced in our study (*N. chondrocladiae* n. comb., *N. acanellae* and *Robertianella*
522 *synophtalma*) were recovered in a clade made up of symbiotic polynoids, as already pointed
523 by Serpetti *et al.* (2017). As for Polynoid sp. 2, the species collected in association with *C.*
524 *verticillata* in the Gulf of Mexico, it clustered with *Hermeneia verruculosa* (Fig. 7). The
525 limited morphological observations we were able to carry out on the two specimens of
526 Polynoid sp. 2 investigated, indicate that it is most likely a member of the genus *Harmothoe*
527 or *Eunoe* Malmgren, 1865. Thus, if Polynoid sp. 2 was established as a symbiont of *C.*
528 *verticillata*, then closely related species in the genus *Chondrocladia* (namely *C. verticillata*
529 and *C. robertballardi/C. virgata*) appear to have established independent symbiotic
530 relationships with annelid polynoids of distinct origin.

531 *Adaptive morphological modifications to a symbiotic life*

532 Some of the morphological features present in symbiotic polychaetes have been defined as
533 adaptive modifications, resulting from co-evolution with the host (see Pettibone 1969a;
534 Martin & Britayev 1998, 2018; Molodtsova *et al.* 2016). One of the most obvious adaptations,
535 especially for organisms inhabiting the host's surface, is cryptic coloration (see Martin &
536 Britayev 1998). Unlike *N. acanellae*, *N. chondrocladiae* n. comb. presents a similar
537 coloration to its host, making it very difficult to spot the worms in their galleries (Fig. 3A–B).
538 This color mimicry has already been reported in other polynoids, which also inhabit galleries
539 and tunnels, such as the deep-sea *Gorgoniapolynoe caeciliae* (Fauvel, 1913), which display a

540 very similar whitish color to its cnidarians hosts (Britayev *et al.* 2014). In this case, similar to
541 *N. chondrocladiae* n. comb., the combination of a similar body color to the host and the
542 partial cover of the galleries/tunnels inhabited by the polynoid, provide the worm with a
543 cryptic appearance that may confer a clear advantage against visually-oriented predators.
544 Another remarkable case of mimicry in a shallow-water polynoid is the one between
545 *Medioantenna variopinta* Di Camillo, Martin & Britayev, 2011 and its cnidarian host
546 *Solanderia secunda* (Inaba, 1892), where different parts of the body of the polynoid mimic
547 colors of the different parts of its host (Di Camillo *et al.* 2011). Color mimicry, though, is not
548 restricted to symbiotic relationships involving polynoids, and equally striking examples can
549 be found in other polychaete families such as syllids (see Martin & Britayev 1998).

550 Open galleries where *N. chondrocladiae* n. comb. rest were briefly reported by
551 Cristobo *et al.* (2015) in their original description of *C. robertballardi* and also noticed in our
552 examination in deposited and newly collected material. Similar induced galleries have been
553 reported in octocorals with symbiotic polynoids, such as *G. caeciliae* living inside highly
554 modified sclerites of the gorgonian (Britayev *et al.* 2014), and also in other polynoids
555 associated to hexacoral antipatharians, that build the so-called ‘worm runs’, defined as
556 hollow tube-like reticulated structures (Molodtsova & Budaeva 2007). The healthy
557 appearance of the colonies where these ‘worm runs’ were reported, made Molodtsova &
558 Budaeva (2007) suggest that these tube-like structures were indeed the result of the
559 physiological reaction of antipatharians to the presence of the polynoid symbionts. Similarly,
560 as for the antipatharians and their polynoid symbionts, we suggest that the open galleries
561 described in *C. virgata* and *C. robertballardi*, where *N. chondrocladiae* n. comb. are
562 normally found, are not the result of the polynoid excavating or boring, but derive from a
563 gradual overgrow of the sponge to accommodate the worm on its body. Still, many questions
564 remain unsolved about these galleries, such as at which stage of the development do the

565 worms start to modify these structures, what are the physical and/or chemical mechanisms
566 behind the genesis of these galleries, or how they are modified (if they are) after a worm dies
567 or abandons the sponge.

568 The presence of specialized hooked chaetae in some chaetigers also seems to be an
569 adaptation of polynoids and other polychaetes to the symbiotic life in their hosts. These
570 chaetae have been hypothesized to facilitate attachment to the host (Pettibone 1969a; Martín
571 & Britayev 1998; Molodtsova *et al.* 2016). For instance, Pettibone (1969a) reported the
572 presence of neuropodial hooks in the first to third chaetigers of three polynoid species of the
573 genus *Australaugeneria*, all symbiotic with alcyonarian corals (see Molodtsova *et al.* 2016),
574 and also reported the occurrence of stout neurochaetae with slightly hooked tips in the
575 median segments. More recently, Ravara & Cunha (2016) also described neuropodial hooks
576 in the first parapodium of *Australaugeneria iberica* Ravara & Cunha, 2016, which is also an
577 alcyonarian symbiont. Interestingly, we observed similar hooked neurochaetae in the *N.*
578 *chondrocladiae* n. comb., which were overlooked and never reported in the descriptions of
579 the species (Fauvel 1943; Kirkegaard 2001; Bock *et al.* 2010). Given the branching
580 morphology of *Chondrocladia* sponges, we propose that the distally-hooked neurochaetae
581 (Fig. 4F), may help *N. chondrocladiae* n. comb. navigate its host's branches in order to reach
582 the spicule-rich spheres where the sponge traps its prey. We hypothesize that the worm might
583 be feeding on the prey that the sponge traps in their spheres. However, it is not clear yet
584 whether the relationship between the carnivorous sponge and the polynoid should be
585 considered as kleptoparasitic (in the case that the worm is stealing food from the sponge and
586 the sponge is not obtaining any benefit in return) or mutualistic (if the worm is providing a
587 benefit to the sponge by cleaning its surface and/or dissuading potential predators; see
588 Mortensen 2001). Further studies are certainly needed to establish the true nature of

589 symbiotic relationship between *C. virgata* and *C. robertballardi* with *N. chondrocladiae* n.
590 comb.

591 Symbiosis between annelids and carnivorous sponges appears not to be restricted to
592 the example between *N. chondrocladiae* n. comb. and *C. robertballardi* and *C. virgata*. Here
593 we report for the first time the occurrence of two individuals of an unidentified polynoid
594 living in association with *C. verticillata*. Previous investigations on preserved material of *C.*
595 *verticillata* never reported the presence of worms in association with the carnivorous sponge
596 (J.H. personal observation). Also, unlike in the case of *C. robertballardi* and *C. virgata*, no
597 open galleries to accommodate the worms were observed in *C. verticillata*. All this may
598 indicate that the relationship between *C. verticillata* and the unidentified polynoid might not
599 be as close as the one reported for *N. chondrocladiae* n. comb. and its hosts.

600 *Reproductive mode in N. chondrocladiae* n. comb.

601 Annelid polychaetes are probably one of the groups with the most diverse reproductive
602 modes in the animal kingdom, and have been intensively studied in shallow-water species
603 (see Giangrande 1997). In contrast, very scarce information exists about reproductive modes
604 and life cycles in commensal polynoids, which is especially true for deep-water species (see
605 Eckelbarger *et al.* 2005). Except for the polynoid *Branchipolynoe seepensis* Pettibone, 1986,
606 a commensal to hydrothermal vent mussels in the Mid-Atlantic Ridge, with inferred
607 lecithotrophic larva, or direct development, due to its relatively large (395–400 μm maximum
608 diameter) mature oocytes (Jollivet *et al.* 2000), most commensal polynoids, whose
609 reproduction has been investigated, have been inferred to be broadcasters with planktotrophic
610 larvae (Eckelbarger *et al.* 2005). Given the sizes of the oocytes we reported for *N.*
611 *chondrocladiae* n. comb., being relatively small (18–104 μm maximum diameter), we
612 suggest that this species might be a gonochoristic broadcaster with a planktotrophic larva,
613 since the figures we reported fall within the ranges reported for other polynoids with this type

614 of development (Eckelbarger *et al.* 2005). Reproduction via a planktotrophic larva in *N.*
615 *chondrocladiae* n. comb. would maximize chances of this species to find new suitable hosts
616 to colonize, since individuals of *C. virgata* and *C. robertballardi* are normally sparsely
617 distributed in the habitats where they occur (authors' personal observation). Although we
618 were only able to determine the sex of a single organism (female) in our study, the
619 occurrence of various adult individuals of *N. chondrocladiae* n. comb. on a single host might
620 allow for synchronized spawning, thus enhancing the fertilization rates for the species.

Journal Pre-proof

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- 814

815 **Figure Legends.**

816 **Figure 1.** Map showing all the location of the material examined in this study. Insert map
817 corresponds to the area of the Cantabric Sea surveyed in the present study.

818 **Figure 2.** Holotype of *Neopolynoe chondrocladiae* n. comb. (MNHN-IA-TYPE0822). **A.**
819 General view of the complete specimen divided in two parts. **B.** Detail of the anterior part
820 showing the pharynx completely everted. **C.** Mid-body elytrum. **D.** Detail of the elytrum
821 showing microtubercles and digitated papillae. **E.** Detail of mid-anterior parapodium showing
822 the chaetal arrangement, aciculae, and ventral cirrus.

823 **Figure 3.** *Neopolynoe chondrocladiae* n. comb. **A.** Two live specimens inside the galleries of
824 *Chondrocladia robertballardi* (ECOMARG St.). Lolly-pop structures are spheres used by the
825 sponge to trap their preys. **B.** Live specimen broken in two parts inside the gallery of *C.*
826 *robertballardi* (SponGES0617-BT6 St.). Notice the pink prostomium. **C.** Preserved specimen
827 with everted pharynx, showing jaws (SponGES0617-BT6 St.). **D.** Preserved specimen with
828 some elytra still attached (NHMUK 1882.7.28.97). **E.** Detail of parapodium from chaetiger
829 24 showing the chaetal arrangement, aciculae, and ventral cirrus (SponGES0617-BT6 St.). **F.**
830 Mid-body elytrum (SponGES0617-BT6 St.). **G.** Detail of the margin of the elytrum showing
831 microtubercles and digitated papillae (SponGES0617-BT6 St.). **H.** Detail of the mid part of
832 the elytrum showing microtubercles and intermingled digitated papillae (SponGES0617-BT6
833 St.).

834 **Figure 4.** *Neopolynoe chondrocladiae* n. comb. SEM micrographs (SponGES0617-BT6 St.).
835 **A.** Notochaetae of parapodia 1. **B.** Detail of notochaeta of parapodia 1. **C.** Neurochaetae of
836 parapodia 1. **D.** Detail of neurochaetae of parapodia 1. **E.** Curved neurochaetae of parapodia
837 1. **F.** Distally-hooked neurochaetae of midbody parapodia. **G.** Spatula-like neurochaetae of
838 midbody parapodia. **H.** Curved neurochaeta of midbody parapodia. **I.** Staright neurochaeta of
839 midbody parapodia.

840 **Figure 5.** **A.** Preserved specimen of *Neopolynoe acanellae* (SponGES0617-BT6 St.). **B.**
841 Preserved specimen of *Neopolynoe acanellae* (YPM 2741). **C.** Detail of midbody
842 parapodium showing the chaetal arrangement and aciculae (SponGES0617-BT6 St.). **D.**
843 Preserved specimen of Polynoidae sp. 1 (USNM-1482941). **E.** Detail of the anterior part of
844 Polynoidae sp. 1 (USNM-1482941). **F.** Detail of midbody parapodia of Polynoidae sp. 1
845 (USNM-1482941) showing chaetal arrangement, aciculae, and ventral cirrus. **G.** Midbody
846 elytrum. **H.** Detail of the elytrum showing microtubercles and club-shaped papillae. **I.**
847 Detail of the club-shaped papillae.

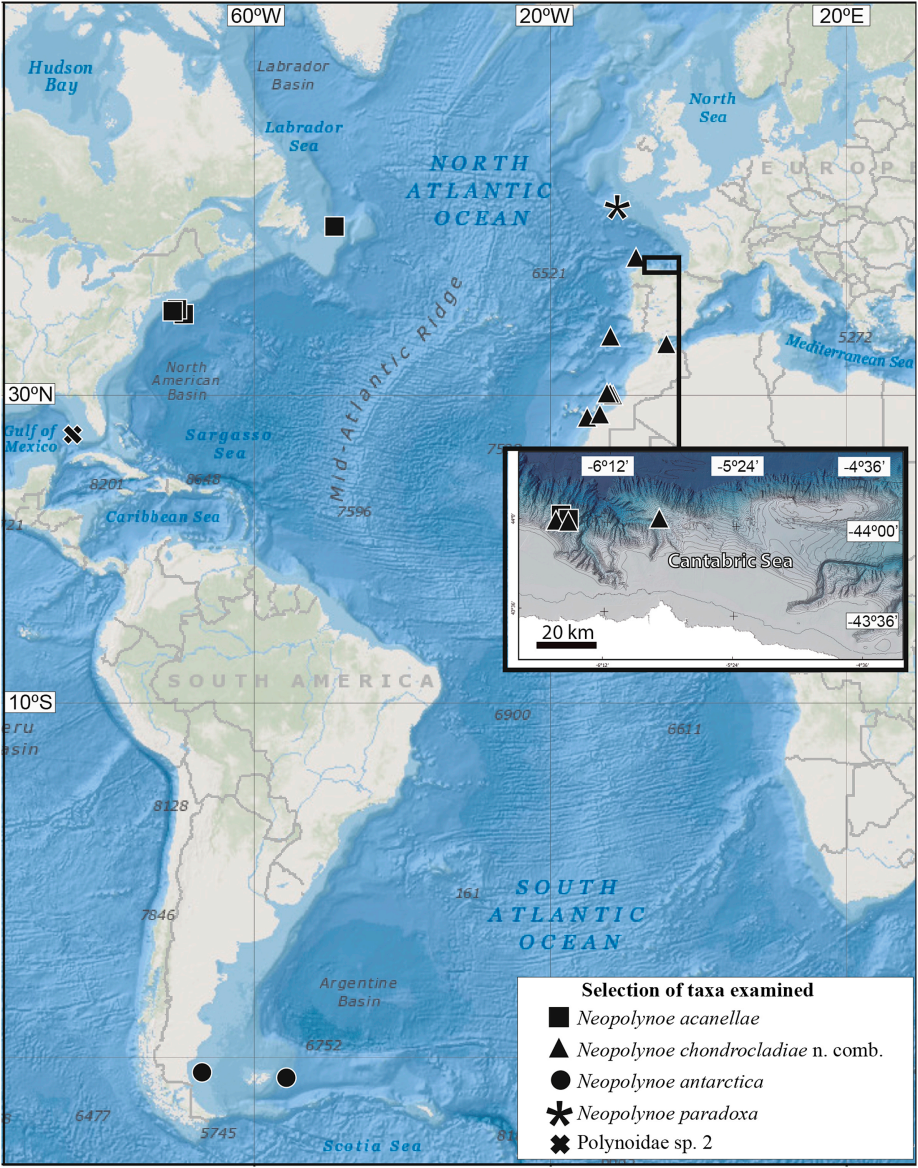
848 **Figure 6.** *Neopolynoe chondrocladiae* n. comb. histological sections. **A.** Midbody chaetiger
849 showing dorsal longitudinal muscles (DLM), ventral longitudinal muscles (VLM), gut (G),
850 and base of the acicula (A). Square delimiting the area with oocytes. **B.** Detail of the enlarged
851 area from A, showing oocytes (arrowed) both in the inner and the outer part of the body.

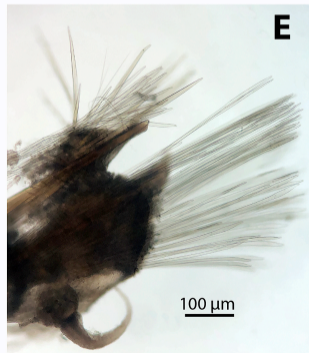
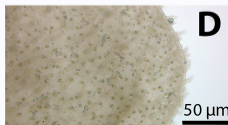
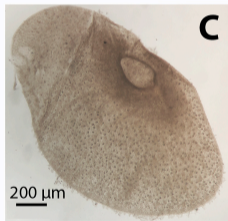
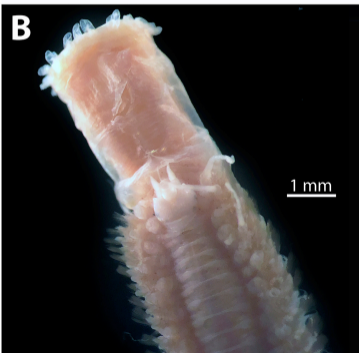
852 **Figure 7.** Phylogenetic tree of Aphroditiformia based on the concatenated analysis
853 (Gblocked dataset) of *COI*, *16S*, *18S*, and *28S*. Tree topology based on the Bayesian
854 inference (BI) analysis. Numbers on nodes correspond to Posterior Probability (PP) –left–
855 and Bootstrap Support (BS) –right– values. Only values of PP > 0.90 were considered.
856 subfamilies within Polynoidae are colour coded. In bold and with a red dot in the terminal,
857 new taxa sequenced in this study.

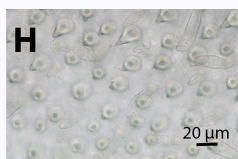
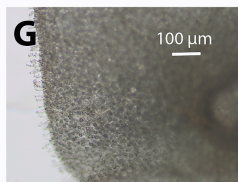
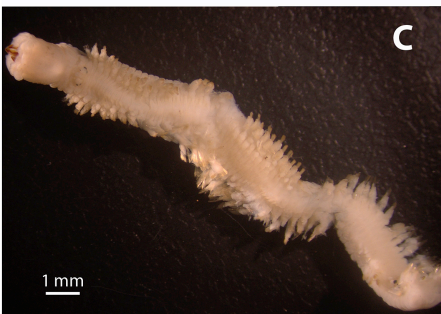
Highlights

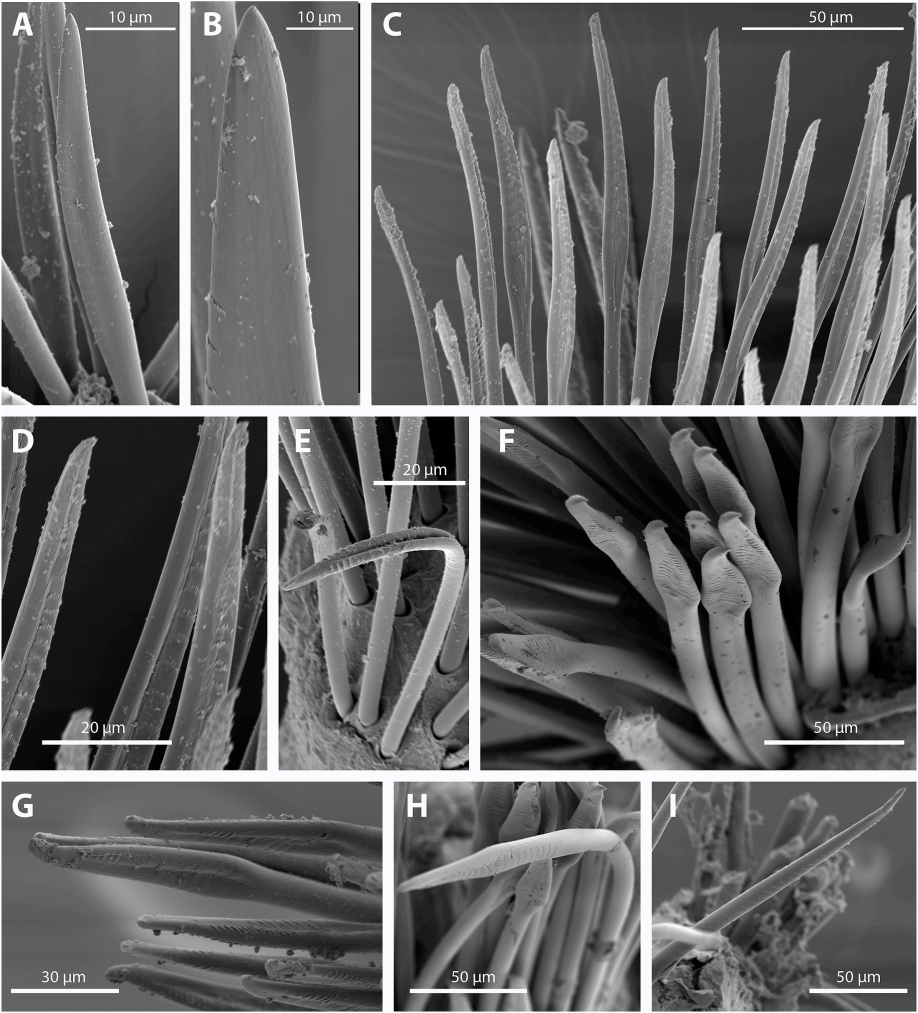
- Two polynoid species reported to live with two *Chondrocladia* carnivorous sponges
- A new combination of a species of the genus *Neopolynoe* (Annelida, Polynoidae)
- We provide an updated phylogenetic framework for symbiotic polynoids
- Morphological adaptations on both carnivorous sponge and polynoids are highlighted
- We suggest *Neopolynoe chondrocladiae* n. comb. is gonochoristic and broadcaster

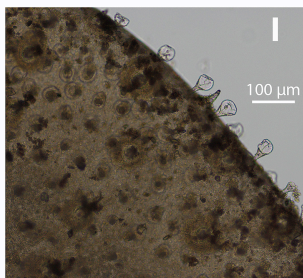
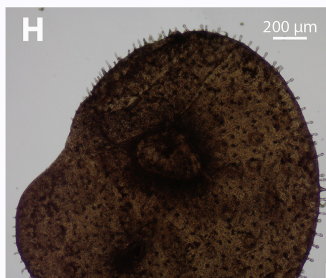
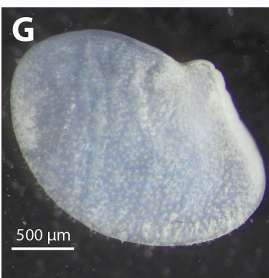
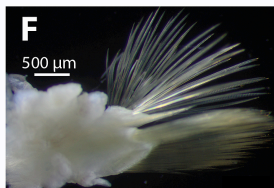
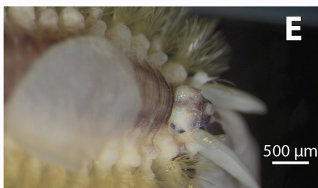
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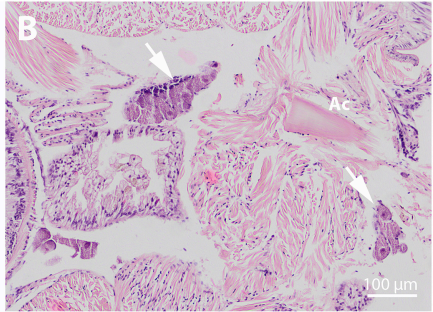






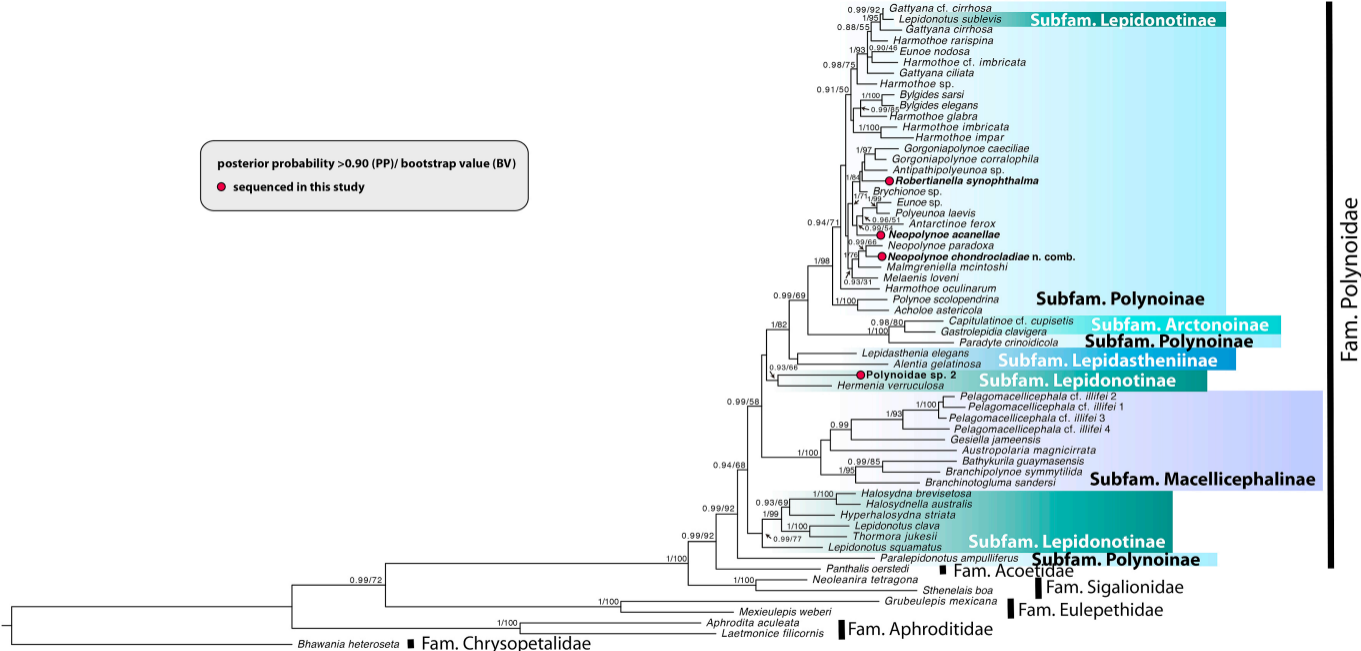






posterior probability >0.90 (PP)/ bootstrap value (BV)

● sequenced in this study



0.1

Table 1. List of voucher specimens and newly collected specimens used in this study. ^a Accepted names in WoRMS for the polychaetes. See Figure 1 for the location in the map of *Neopolynoe* spp. and Polynoidae sp. 2. ^b Number of *N. chondrocladiae* n. comb. specimens never reported in association with the correspondent *Chondrocladia* specimen.

Species identification	Accepted name (polychaetes) ^a	N	Area	Latitude	Longitude	Depth (m)	Type status	Accession number
Annelida								
<i>Neopolynoe acanellae</i>	<i>Neopolynoe acanellae</i>	1	Orphan Basin (Canada)	50°04.05'N	50°08.067'W	760	---	CMNA 2014-0001
<i>Nemidia antillicola</i>	<i>Nemidia antillicola</i>	1	off Georges Bank (USA)	41°29.75'N	65°47.167'W	1792	Syntype	MCZ.ANNb-2105
<i>Nemidia antillicola</i>	<i>Nemidia antillicola</i>	1	SE Martha's Vineyard (USA)	40°11.667'N	68°22'W	556	Syntype	MCZ.ANNb-2120
<i>Polynoe (Eunoa) acanellae</i>	<i>Neopolynoe acanellae</i>	2	off Martha's Vineyard (USA)	39°53'N	70°58.583'W	668	Syntype	NHMUK 1976.947-948
<i>Neopolynoe africana</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	1	NE Canary Islands (Spain)	29°23.8'N	12°12.3'W	1600	Holotype	NHMUK 2003.848
<i>Neopolynoe africana</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	11	NE Canary Islands (Spain)	29°24'N	12°12'W	1600	---	NHMUK 2003.849-859
<i>Neopolynoe antarctica</i>	<i>Neopolynoe antarctica</i>	1	East Falkland	---	---	---	---	NHMUK 2015.524
<i>Neopolynoe antarctica</i>	<i>Neopolynoe antarctica</i>	2	Patagonian Shelf	51°34.5'S	67°18.5'W	92–106	---	NHMUK 2015.528-529
<i>Neopolynoe paradoxa</i>	<i>Neopolynoe paradoxa</i>	3	S Ireland	48°45.642'N	10°27.648'W	750	---	NHMUK 2016.290,,292,.294
<i>Nemidia antillicola chondrocladiae</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	1	Cap Ortegal, Galicia (Spain)	---	---	735	Holotype	MNHN-IA-TYPE0822
<i>Polynoe (Nemidia) antillicola</i>	---	1	Azores (Portugal)	37°40'N	26°26.25'W	---	---	MOM 18 0492
<i>Polynoe acanellae</i>	<i>Neopolynoe acanellae</i>	1	S Cape Cod (USA)	39°48.5'N	70°54'W	462	Lectotype	YPM 2741
<i>Polynoe acanellae</i>	<i>Neopolynoe acanellae</i>	1	S Cape Cod (USA)	39°53'N	70°58.583'W	668	Paralectotype	YPM 2742
Polynoidae sp.	Polynoidae sp.	1	Gulf of Mexico	24°39'N	03°54.6'W	735	---	USNM-1482940
Polynoidae sp.	Polynoidae sp.	1	Gulf of Mexico	24°39'N	03°54.6'W	735	---	USNM-1482941
<i>Neopolynoe acanellae</i>	<i>Neopolynoe acanellae</i>	17	Cantabric Sea (Spain), SponGES0617-BT5 St.	43°58.717'N	6°28.980'W	1510	---	This study
<i>Neopolynoe acanellae</i>	<i>Neopolynoe acanellae</i>	18	Cantabric Sea (Spain), SponGES0617-BT6 St.	43°58.866'N	6°28.622'W	1525	---	This study

<i>Neopolynoe chondrocladiae</i> n. comb.	<i>Neopolynoe chondrocladiae</i> n. comb.	6	Cantabric Sea (Spain), SponGES0617-BT5 St.	43°58.717'N	6°28.980'W	1510	---	This study
<i>Neopolynoe chondrocladiae</i> n. comb.	<i>Neopolynoe chondrocladiae</i> n. comb.	24	Cantabric Sea (Spain), SponGES0617-BT6 St.	43°58.866'N	6°28.622'W	1525	---	This study
<i>Neopolynoe chondrocladiae</i> n. comb.	<i>Neopolynoe chondrocladiae</i> n. comb.	3	Cantabric Sea (Spain), ECOMARG St.	43°58.884'N	5°49.484'W	1167	---	This study
<i>Neopolynoe chondrocladiae</i> n. comb.	<i>Neopolynoe chondrocladiae</i> n. comb.	2	Gorringe Bank (Portugal), NAO17 St.	36°38.9713'N	11°03.232'W	1738	---	This study
<i>Robertianella synophthalma</i>	<i>Robertianella synophthalma</i>	7	Cantabric Sea (Spain), SponGES0617-BT12 St.	43°57.300'N	4°58.288'W	890	---	This study
Porifera								
<i>Chondrocladia</i> sp.	<i>Neopolynoe chondrocladiae</i> n. comb.	1 ^b	NE Canary Islands (Spain)	29°24'N	12°12'W	1600	---	
<i>Chondrocladia virgata</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	2 ^b	---	---	---	872	Lectotype	NHMUK 1882.7.28.97
<i>Chondrocladia virgata</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	1 ^b	---	---	---	---	---	NHMUK 1890.4.10.6
<i>Chondrocladia virgata</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	1 ^b	Porcupine Abyssal Plain?	---	---	2487	---	NHMUK 1898.5.7.37
<i>Chondrocladia virgata</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	2 ^b	Spanish Bay	35°32'N	007°07'W	1215	Holotype	ZMBN25639
<i>Chondrocladia virgata</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	2 ^b	Off Cape Bojador, Canary Islands (Spain)	27°27'N	014°52'W	2603	---	ZMBN25640
<i>Chondrocladia virgata</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	2 ^b	Off Cape Bojador, Canary Islands (Spain)	28°08'N	013°35'W	1365	---	ZMBN25641

Table 2. List of all taxa included in the phylogenetic analysis including the NCBI accession number. In bold, new taxa sequenced in this study.

Family	Subfamily	Taxon	18S	28S	16S	COI
Chrysopetalidae		<i>Bhawania heteroseta</i>	EU555035	EU555025	EU555044	EU555053
Acoetidae		<i>Panthalis oerstedii</i>	AY839572	JN852845	JN852881	AY839584
Aphroditidae		<i>Aphrodita aculeata</i>	AY176281	JN852846	–	AY839578
		<i>Laetmonice filicornis</i>	JN852816	JN852847	JN852883	JN852919
Eulepethidae		<i>Grubeulepis mexicana</i>	JN852817	JN852848	JN852884	–
		<i>Mexieulepis weberi</i>	JN852818	–	JN852885	JN852920
Polynoidae	Polynoinae	<i>Acholoe astericola</i>	AY839567	JN852850	JN852888	AY839576
	Lepidastheniinae	<i>Alentia gelatinosa</i>	AY839566	–	–	AY839577
	Polynoinae	<i>Antarctinoe ferox</i>	KF713423	–	KF713463	KF713373
	Polynoinae	<i>Antipathipolyeunoa</i> sp.	KU738169	KU738184	KU738149	KU738202
	Macellicephalinae	<i>Austropolaria magnicirrata</i>	JX863895	–	JX863896	–
	Macellicephalinae	<i>Bathykurila guaymasensis</i>	DQ074765	–	–	DQ074766
	Macellicephalinae	<i>Branchinotogluma sandersi</i>	JN852821	JN852851	JN852889	JN852923
	Macellicephalinae	<i>Branchipolynoe symmytilida</i>	–	–	AF315055	AY646021
	Polynoinae	<i>Brychionoe</i> sp.	KU738182	KU738200	KU738167	–
	Polynoinae	<i>Bylgides elegans</i>	JN852822	JN852852	JN852890	JN852924
	Polynoinae	<i>Bylgides sarsi</i>	JN852823	JN852853	JN852891	JN852925
	Arctonoinae	<i>Capitulatinoe</i> cf. <i>cupisetis</i>	KF919301	KF919302	KF919303	–
	Polynoinae	<i>Eunoe nodosa</i>	JN852824	JN852854	JN852892	JN852926
	Polynoinae	<i>Eunoe</i> sp.	KU738183	KU738201	KU738168	KU738214
	Arctonoinae	<i>Gastrolepidia clavigera</i>	JN852825	JN852855	JN852893	JN852927
	Polynoinae	<i>Gattyana</i> cf. <i>cirrrosa</i>	KY823462	KY823462	KY823479	–
	Polynoinae	<i>Gattyana ciliata</i>	AY894297	DQ790035	–	AY894312
	Polynoinae	<i>Gattyana cirrhosa</i>	JN852826	JN852856	JN852894	JN852928
	Macellicephalinae	<i>Gesiella jameensis</i>	Ky454403	Ky823476	Ky454412	Ky454429

Polynoinae	<i>Gorgoniapolynoe caeciliae</i>	KU738170	KU738185	KU738150	KU738203
Polynoinae	<i>Gorgoniapolynoe corralophila</i>	KU738173	KU738189	KU738154	KU738206
Lepidonotinae	<i>Halosydna brevisetosa</i>	JN852827	JN852857	JN852895	AY894313
Lepidonotinae	<i>Halosydnella australis</i>	KY823449	KY823463	KY823480	KY823495
Polynoinae	<i>Harmothoe cf. imbricata</i>	KY823450	KY823464	KY823481	KY823496
Polynoinae	<i>Harmothoe glabra</i>	JN852828	JN852858	JN852896	JN852929
Polynoinae	<i>Harmothoe imbricata</i>	AY340434	AY340400	AY340463	AY839580
Polynoinae	<i>Harmothoe impar</i>	JN852829	JN852859	JN852897	JN852930
Polynoinae	<i>Harmothoe oculinarum</i>	AY894299	JN852860	JN852898	AY894314
Polynoinae	<i>Harmothoe rarispina</i>	KY657611	KY657624	KY657641	KY657659
Polynoinae	<i>Harmothoe sp.</i>	KU738178	KU738196	KU738163	–
Lepidonotinae	<i>Hermenia verruculosa</i>	JN852830	JN852861	JN852899	JN852931
Lepidonotinae	<i>Hyperhalosydna striata</i>	JN852831	JN852862	JN852900	JN852932
Lepidastheniinae	<i>Lepidasthenia elegans</i>	JN852832	JN852863	JN852901	JN852933
Lepidonotinae	<i>Lepidonotus clava</i>	JN852833	JN852864	JN852902	JN852934
Lepidonotinae	<i>Lepidonotus squamatus</i>	AY894300	JN852865	JN852903	AY894316
Lepidonotinae	<i>Lepidonotus sublevis</i>	AY894301	DQ790039	–	AY894317
Polynoinae	<i>Malmgreniella mcintoshii</i>	JN852834	JN852866	JN852904	JN852935
Polynoinae	<i>Melaenis loveni</i>	JN852835	JN852867	JN852905	JN852936
Polynoinae	<i>Neopolynoe acanellae</i>	MN653050	MN653123	MN653064	MN656076
Polynoinae	<i>Neopolynoe chondrocladiae n. comb.</i>	MN653051	MN653124	MN653092	MN656104
Polynoinae	<i>Neopolynoe paradoxa</i>	JN852836	JN852868	JN852906	JN852937
Polynoinae	<i>Paradyte crinoidicola</i>	JN852837	JN852869	JN852907	JN852938
Polynoinae	<i>Paralepidonotus ampulliferus</i>	JN852838	AF185164	JN852908	JN852939
Macellicephalinae	<i>Pelagomacellicephalo cf. Illifei 1</i>	–	–	KY454424	KY454440
Macellicephalinae	<i>Pelagomacellicephalo cf. Illifei 2</i>	KY454408	KY823474	KY454420	KY454435
Macellicephalinae	<i>Pelagomacellicephalo cf. Illifei 3</i>	KY454411	KY823475	KY454428	KY454443
Macellicephalinae	<i>Pelagomacellicephalo cf. Illifei 4</i>	KY454405	–	KY454416	KY454431

	Polynoinae	<i>Polyeunoa laevis</i>	KU738177	KU738194	KU738161	KU738213
	Polynoinae	<i>Polynoe scolopendrina</i>	JN852839	JN852870	JN852909	JN852940
	Polynoinae	<i>Robertianella synophthalma</i>	MN653053	MN653126	MN653122	MN656132
	Lepidonotinae	<i>Thormora jukesii</i>	JN852840	JN852871	JN852910	JN852941
	–	Polynoidae sp. 2	MN653052	MN653125	MN653121	MN656131
Sigalionidae		<i>Neoleanira tetragona</i>	AY839570	JN852872	JN852911	AY839582
		<i>Pholoe pallida</i>	AY894302	JN852874	JN852913	AY894318
		<i>Sthenelais boa</i>	DQ779672	DQ779711	DQ779635	—

Table 3. Comparative list of characters for the species of the genus *Neopolynoe*

Species	<i>Neopolynoe acanellae</i>	<i>Neopolynoe antarctica</i>	<i>Neopolynoe chondrocladiae</i> n. comb.	<i>Neopolynoe paradoxa</i>
Host	corals of the genus <i>Anthomastus</i> and <i>Acanella</i>	tube-building polychaetes, hydroids of the genus <i>Thuiaria</i>	<i>Chondrocladia robertballardi</i> , <i>Chondrocladia virgata</i>	corals
Distribution	N Atlantic. 48–2250 m	SW Atlantic, Magellan region. 0–300 m	NE Atlantic. 735–2487 m	Norway, Iceland. 70–957 m
Size	25–55 mm long; 6–9 mm wide	20–50 mm long; 4–6 mm wide	11–60 mm long; 1– mm wide	8–80 mm long; 3–11 mm wide
N segments	23–54	40–80	<100	40–58
Elytra shape	oval, covering dorsum	oval to subreniform, covering dorsum	oval, not covering dorsum	oval to subreniform, not covering dorsum
Elytra	few scattered papillae on lateral border, surface covered by microtubercles	smooth, except for scattered microtubercles near anterior margin	papillae on lateral border, conical microtubercles and papillae on surface	papillae on lateral border, conical microtubercles on surface
Supra-acicular process	digitiform	digitiform	short	thick, stout
Dorsal cirrus	smooth	smooth	smooth	with numerous papillae
Ventral cirri	longer than neuropodia	not reaching tip of neuropodia	not reaching tip of neuropodia	not reaching half parapodia, with papillae
Notochaetae	bundles of 4–5, stouter than neurochaetae	few, stouter than neurochaetae	bundles of 15–16, stouter than neurochaetae	bundles of >20, stouter than neurochaetae
Neurochaetae	unidentate with falcate tip	bidentate	unidentate, several types	unidentate, curved tip
References	Bock et al. (2010); This study	Barnich et al. (2012); This study	Kirkegaard (2001); This study	Loshman (1981); This study



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Declaration of interest

We declare that the article “Insights into the symbiotic relationship between scale worms and carnivorous sponges (Cladorhizidae, Chondrocladia)” (DSR1_2019_152) by Sergi Taboada, Ana Serra Silva, Lenka Neal, Javier Cristobo, Pilar Ríos, Patricia Álvarez-Campos, Jon Thomassen Hestetun, Vassiliki Koutsouveli, Emma Sherlock and Ana Riesgo, has not been published previously.

Sincerely yours,



Sergi Taboada (on behalf of the other authors).