

1 **Revealing the diversity of the green *Eulalia* (Annelida, Phyllodocidae) species complex along the**
2 **European coast, with description of three new species**

3

4 Marcos A.L. Teixeira^{1,2*}, Pedro E. Vieira^{1,2}, Joachim Langeneck³, Fredrik Pleijel⁴, Bruno R. Sampieri⁵,
5 José Carlos Hernandez⁶, Ascensão Ravara⁷, Filipe O. Costa^{1,2}, Arne Nygren⁴

6

7 ¹ Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho,
8 Campus de Gualtar, 4710-057, Braga, Portugal

9 ² Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus de
10 Gualtar, 4710-057, Braga, Portugal

11 ³ Dipartimento di Biologia, Università di Pisa, via Derna 1, 56126 Pisa, Italy

12 ⁴ Institutionen for marina vetenskaper, Göteborgs Universitet, Tjärnö, Strömstad, Sweden

13 ⁵ Museu de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas – IB/UNICAMP, Rua
14 Charles Darwin, Bloco N, Cidade Universitária, Campinas, SP, Brasil

15 ⁶ Biología Animal, Edafología y Geología, Universidad de La Laguna, Tenerife, Spain

16 ⁷ Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro,
17 Campus de Santiago, 3810-193 Aveiro, Portugal.

18

19 **Corresponding author*

20 Mail: mark-us_teixeira@hotmail.com

21

22 **Running title:** Cryptic diversity in *Eulalia*

23

24 **ORCID**

25 Marcos A.L. Teixeira: 0000-0002-2228-2673

26 Pedro E. Vieira: 0000-0003-4880-3323

27 Fredrik Pleijel: none

28 Joachim Langeneck: 0000-0003-3665-8683

29 Bruno R. Sampieri: 0000-0002-1675-1090

30 José Carlos Hernandez: 0000-0002-1539-1783

31 Ascensão Ravara: 0000-0002-1689-2985

32 Filipe O. Costa: 0000-0001-5398-3942

33 Arne Nygren: 0000-0001-5761-8803

34

35

36 All authors contributed to the study conception and design. Material preparation, data collection and
37 analysis were performed by Marcos A.L. Teixeira, Pedro E. Vieira, Joachim Langeneck and Arne Nygren.

38 The first draft of the manuscript was written by Marcos A.L. Teixeira and all authors commented on
39 previous versions of the manuscript. All authors read and approved the final manuscript

40

41 **Abstract**

42 The green phyllodocids *Eulalia clavigera* and *E. viridis* are a known European pseudo-cryptic complex,
43 but questions about its distribution and evidence of additional lineages in previous studies call for an
44 investigation of the real diversity within the complex.

45 The analysis of DNA sequence data (mtCOI, ITS and 28S rRNA) of populations of the apparent *E.*
46 *clavigera* morphotype from intertidal and subtidal marine waters along the North East Atlantic,
47 Mediterranean Sea and the Macaronesia islands (Madeira, Savage islands, Azores and Canaries) provided
48 compelling evidence for the existence of six additional divergent evolutionary lineages. Three of the most
49 abundant lineages are described here as new species: *Eulalia feliciae* sp. nov., intertidal and unique to the
50 west Mediterranean, *Eulalia madeirensis* sp. nov., a subtidal variant unique to the Madeira island
51 (Portugal), and *Eulalia xanthomucosa* sp. nov., occurring mostly in subtidal habitats of the British Isles and
52 southern France. The molecular data was complemented with morphometric methodologies and compared
53 against the parent morphospecies (*E. clavigera* s.s.). *Eulalia feliciae* sp. nov. and *E. madeirensis* sp. nov.
54 formed two independent morphometric clusters, while measurements for *E. xanthomucosa* sp. nov. often
55 overlapped with *E. clavigera*. However, the latter new species presents an unique yellow coloration
56 produced by the worm's mucus and has larger parapodial cirri on median segments in relation to its body
57 size.

58 Recent biotechnological findings using *E. clavigera* highlights the importance of formally describing
59 cryptic complexes, since their chemistry might be unique to each lineage and can have a range of distinct
60 effects and applications.

61

62

63 **Keywords:** *Eulalia clavigera*; Annelida; integrative taxonomy; cryptic species; morphometry;
64 mitochondrial DNA; nuclear DNA

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81 Introduction

82 Biodiversity comprises three levels of variation: genetic, species and ecosystem. Molecular tools
83 have been enabling the in-depth appraisal of the true diversity present in animals, namely by detecting
84 cryptic or pseudo-cryptic species. The latter constitute a substantial fraction of biodiversity, and appear to
85 be a frequent phenomenon among marine benthic invertebrates (Miglietta et al., 2011; Nygren, 2014), in
86 well-known taxa and studied areas (e.g. Bleidorn et al., 2006; Carr et al., 2011; Grosse et al., 2021; Jolly et
87 al. 2006; Leite et al. 2020). Despite the increasing evidence for extensive occurrence of cryptic species, the
88 lack of formal taxonomic description (Fernandez-Triana, 2022) hinders accurate estimates of their
89 contribution to biodiversity (Delić et al., 2017; Fišer et al., 2018; Hutchings and Kupriyanova, 2018),
90 therefore limiting our understanding of their evolutionary and ecological significance, as well as their
91 recognition in large scale biomonitoring programs using high throughput sequence technologies.

92 The homogeneously green phyllodocid, *Eulalia viridis* (Linnaeus, 1767), has been reported from
93 throughout the northern hemisphere (Eibye Jacobsen, 1991; Eibye-Jacobsen, 1993) and is common in
94 intertidal and subtidal coastal areas and marinas, at depths until 50 m (Ushakov, 1972). This species usually
95 lives on rocky reefs in crevices, among algae, mussel beds, *Balanus spp.* blocks, *Dendropoma* reefs,
96 *Posidonia oceanica* meadows and coralligenous formations (Bonser et al., 1996; Viéitez et al., 2004; Çınar,
97 2005). However, it does not occur in the *Sabellaria alveolata* (Linnaeus, 1767) reefs from the
98 Mediterranean, where it is replaced by *Eulalia ornata* Saint-Joseph 1888, another greenish species
99 morphologically highly similar to *E. viridis* except for the pigmentation pattern (Schimmenti et al., 2016).
100 In a study from 1996, Bonse and colleagues, using isoelectric focusing and morphological data, found a
101 correlation between exclusive isoenzymes and protein patterns, the morphology and size of the midbody
102 dorsal cirri, and the size of the proboscideal papillae, that allowed to discriminate between two distinct
103 groups of *Eulalia* populations. One morphotype, sampled in the North Sea and Scandinavia coast, with
104 smaller papillae and slender dorsal cirri, corresponded to *E. viridis*, while the other one, occurring in France
105 and England, showing larger papillae and significantly thicker dorsal cirri, was attributed to *Eulalia*
106 *clavigera* (Audouin & Milne Edwards, 1833), hitherto considered synonymous with *E. viridis*. The
107 reproductive biology of these species in particular, and of phyllodocids in general, is poorly known. These
108 species have a planktonic larval stage and reproduce once a year (Meyer, 1938), but local populations along
109 the coasts of Northern Europe also differ in the time of reproduction with reproductive cycle starting 4 to 6
110 weeks earlier in Swedish specimens compared to the ones from the English and French coasts (Olive 1975,
111 Pleijel, 1993). Molecular studies based on the mitochondrial cytochrome c oxidase subunit I (COI) locus
112 (Hardy et al., 2011; Lobo et al., 2016) also allowed the separation of populations identified as *Eulalia viridis*
113 from Kandalaksha Bay (Russia) and Portugal, respectively with more than 20% Kimura's two parameter
114 (K2P) genetic divergence. The highly similar morphology and the large number of genetic markers
115 discriminating between this eastern and the western group implies the existence of a pseudo-cryptic species
116 complex. Given the high number of species already found within complexes from other phyllodocids such
117 as *Notophyllum* (Nygren et al., 2010) or *Eumida* (Nygren and Pleijel, 2011; Teixeira et al., 2022), and even
118 in other polychaete families (Sampieri et al., 2021, Lobo et al., 2016), the actual diversity and distribution
119 of the *Eulalia viridis/clavigera* species group in Europe is questioned. Langeneck et al. (2019) collected a
120 large amount of *E. clavigera* specimens from Nuevo Gulf, Patagonia (South-western Atlantic Ocean) and

121 using the mitochondrial COI marker detected no genetic structure between the north-eastern and south-
122 western Atlantic, supporting a non-native origin of the Patagonian population. However, a distinct
123 Mediterranean lineage was found when compared against the Patagonia and the NE Atlantic clade.

124 In this study we use a multi-locus approach and morphometric data to investigate the possible
125 occurrence of additional diagnosable species within the *Eulalia viridis/clavigera* complex, comparing the
126 *E. clavigera* species reported in Europe, from the United Kingdom to Portugal, the Macaronesia islands
127 (Azores, Madeira and Canaries) and the Western and Eastern Mediterranean Sea.

128

129 **Methods**

130 *Taxon sampling and molecular data retrieval*

131 We sampled a total of 134 *Eulalia* specimens presumably belonging to the *Eulalia*
132 *clavigera/viridis* complex and one *Phyllodoce* species distributed along the European coasts and
133 Macaronesia Islands (Fig. 1). Worms were collected at low tide in rocky beaches among the algae and
134 mussels, marinas or subtidal areas up to 34 meters in depth. The specimens were fixed in 96% ethanol.
135 Samples were harvested in continental Portugal (Canto Marinho, Leixoes, Aveiro, Nazaré) as well as in
136 Santa Maria and Madeira islands, Spain (Coruna, Tenerife, Gran Canaria and La Palma), France (Roscoff,
137 Morgat, Banyuls and Corsica), Great Britain (Plymouth and Cornwall), Norway (Espevaer, Grimstad,
138 Bergen, Trondheim and Finmark), Sweden (Koster), Italy (Livorno, Ischia island and Taranto) and Croatia
139 (Istria). Sample sites and abbreviations can be found in Table 1.

140 We sequenced a partial segment of the 5' end of the mitochondrial cytochrome oxidase subunit I
141 (mtCOI-5P) from 119 specimens, and a representative number per location for the ITS-regions (i.e. ITS1,
142 5.8S rRNA, and ITS2) and 28S rRNA. Mitochondrial sequences (COI) belonging to *Eulalia cf. clavigera*
143 sampled in the Mediterranean Sea (Capraia island and port of Stintino, Italy) from Langeneck et al. (2019)
144 were mined from GenBank for comparison purposes. Molecular data of *Eulalia aurea* Gravier, 1896 and
145 *Phyllodoce* sp. Lamarck, 1818 were used as outgroups for all the analysed loci to comprise the final dataset.
146 DNA was extracted, amplified, sequenced, and assembled as described in Nygren et al. (2010) or Lobo et
147 al. (2016). PCR conditions and primers are detailed in Table 2. As only a few parapodia or a small portion
148 of the posterior end were used for the extraction, the majority of the specimens included in this study have
149 been deposited in the Research Collection of Marine Invertebrates of the Department of Biology of the
150 University of Aveiro (COBI at DBUA) and are available for further morphological and molecular study.
151 Two specimens from Corsica were deposited in Muséum national d'Histoire naturelle (MNHN) and the
152 French Mediterranean specimen BI-2014/15-077 was donated to SCRIPPS Oceanography. Additionally,
153 the following specimens are stored in Arne's Nygren private collection and were assigned only with the
154 Process ID from the BOLD systems (<http://v4.boldsystems.org/>): MTE040-20, MTE042-20, MTE052-20,
155 MTE053-20, MTE054-20, MTE055-20, MTE057-20, MTE079-20, MTE080-20, MTE081-20 and
156 MTE088-20.

157 The full dataset and its metadata can be accessed at BOLD Systems under the project "*Eulalia*
158 *Species Complex (DS-MTE)*" and in the following link: (doi: *upon paper acceptance*), except for the 4
159 COI sequences from Langeneck et al. (2019), which cannot be found in BOLD. GenBank accession

160 numbers: xxx-xxx (COI), xxx-xxx (ITS2), and xxx-xxx (28S) (*upon paper acceptance*). Sampling
161 locations, GenBank accession numbers per specimen, and voucher data are detailed in Table S1.

162

163 *Phylogenetic analysis and genetic distances*

164 Maximum likelihood (ML) and Bayesian inference (BI) were used to perform the phylogenetic
165 analyses of the different loci. The nuclear markers (ITS-regions and 28S) and the mitochondrial COI locus
166 were concatenated with MEGA 10.0.05 (Kumar et al., 2018) and aligned with MAFFT online
167 (<https://mafft.cbrc.jp/alignment/server/>, Katoh and Standley, 2013). The sequence lengths for the different
168 markers are included in Table 2. Highly variable regions, extensive gaps and poorly aligned positions,
169 which were mainly present in the ITS-regions, were eliminated using Gblocks 0.91b
170 (http://molevol.cmima.csic.es/castresana/Gblocks_server.html; Castresana 2000), allowing all the options
171 for a less stringent selection and not allowing many contiguous non-conserved positions so that it becomes
172 more suitable for phylogenetic analysis. We used MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) to
173 conduct the Bayesian analysis. Best-fit models were selected using the Akaike Information Criterion in the
174 JModeltest software (Darriba et al., 2012; Guindon and Gascuel, 2003). For COI we applied the Hasegawa-
175 Kishino-Yano gamma distributed rates across sites (HKY +G) for the first two positions and the HKY
176 model with equal rates across sites for the third position. Regarding the concatenated ITS with 28S, we
177 applied the General Time Reversible model with equal rates across sites (GTR). Number of generations
178 was set to 10 000 000, and sample frequency to 500. Twenty-five percent of the samples were discarded as
179 burn-in (burninfrac = 0.25). The resulting tree files were checked for convergence in the effective sampling
180 sizes (ESSs >200) with Tracer 1.6 software (Rambaut et al., 2018) and then analysed in Figtree 1.4.3
181 (<http://tree.bio.ed.ac.uk/software/figtree/>). The final version of the concatenated tree was edited with the
182 software Inkscape 0.92.3 (<https://www.inkscape.org>). Maximum Likelihood phylogenies were performed
183 in MEGA 10.0.05 with 1000 bootstrap runs with the GTR model with equal rates across sites for the
184 concatenated dataset. Only the BI tree was displayed in the results and if a similar topology is found, with
185 the addition of the ML support values. The alignments (FASTA and NEXUS format) for each individual
186 marker and the concatenated ones are all publicly available online at Figshare (doi: *upon paper acceptance*).

187 The mean genetic distances (K2P) within and between molecular operational taxonomic units
188 (MOTUs) were calculated in MEGA 10.0.05 using the same GBlock alignment from above for the nuclear
189 loci.

190

191 *MOTU clustering*

192 To depict MOTUs, we applied three delineation methods to both the concatenated mitochondrial
193 and nuclear alignments except for COI where we also applied the Barcode Index Number (BIN),
194 implemented in BOLD (Ratnasingham and Hebert, 2013), which is exclusive to this locus. The Automatic
195 Barcode Gap Discovery (ABGD, Puillandre et al., 2012) was implemented on a web interface
196 (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>) with default settings using the K2P distance
197 matrix. The Generalized Mixed Yule Coalescent (GYMC) single threshold model (Fujisawa and
198 Barraclough, 2013), as well the Poisson Tree Processes (bPTP, Zhang et al., 2013) were applied, with both
199 analyses performed on a web interface (<https://species.h-its.org/>). BEAST 2.4.6 (Bouckaert et al., 2014)

200 was used to generate the Bayesian ultrametric tree for the GYMC with the appropriate best model (based
201 on AIC criteria; GTR equal rates), and four independent runs for 50 000 000 MCMC generations, sampled
202 every 5 000 generations. Tracer 1.6 software was used to estimate convergence ESSs > 200 for all
203 parameters. The consensus tree was obtained using TreeAnnotator 2.4.6 (Bouckaert et al., 2014) and loaded
204 into the Figtree software. ML phylogenies obtained above in the “phylogenetic analysis” section
205 contributed for the bPTP results. A final consensus MOTU was chosen using the majority rule (i.e. most
206 common number of MOTUs).

207

208 *Genetic diversity and structure*

209 Haplotype networks were made through the PopART software (Leigh and Bryant, 2015) using the
210 method of Templeton, Crandall and Sing (TCS, Clement et al., 2002) to evaluate the relationship between
211 the haplotypes and their geographical distribution. No GBLOCKS were applied in this analysis to avoid
212 underestimating the number of nuclear haplotypes. Indices of genetic diversity, namely number of
213 haplotypes (h), haplotype diversity (hd), polymorphic sites (S), nucleotide diversity (π), Fu & Li D and
214 Tajima D statistical tests, were estimated based on COI for each MOTU using DNASP 5.10 (Librado and
215 Rozas, 2009).

216

217 *Morphometric analysis*

218 Specimens from four *Eulalia* lineages were used for morphometric analysis and compared against
219 each other to complement the molecular data. The remaining lineages had less than three available
220 specimens with a very small size (therefore unsuitable for morphometric studies) and were not named or
221 used in this analysis. A minimum of 5 specimens with optimal conditions (i.e. specimens with the presence
222 of the proposed morphological characters for this study and whenever possible, similar in size) per
223 population were chosen.

224 The following characters were selected and measured (Fig. 2): the number of segments (NS); the
225 length (mm) of the worm (WL), chaetigerous lobes (CLL), terminal antennae (AL), palps (PL), middle
226 antenna (MAL), dorsal and ventral tentacular cirrus from the second segment (DTL, VTL, respectively),
227 dorsal and ventral cirri (DCL, VCL) and head (HL); the width (mm) of the worm with parapodia (WWP)
228 and without parapodia (WW), head (HW) and dorsal and ventral cirri (DCW, VCW); and the distance
229 between the eyes (DE) as well the height (mm) of the chaetigerous lobes (CLH). WW, WWP and the
230 different parapodia structures were measured from the worm’s widest part. The distance between the eyes
231 was measured from the center of the eyespots to avoid possible different individual responses to fixation as
232 is the case of hesionids in Martin et al. (2017).

233 To minimize bias based on size variability, measurements taken to analyse the inter-lineage
234 differences were converted to ratios and used to create scatter plots between relevant morphological
235 characters found in Phyllodocids similar to previous studies (Teixeira et al., 2020; 2022). All remaining
236 analyses were conducted using Microsoft Excel (Office 365 ProPlus). Measurements were done with a
237 LEICA MC170 HD stereo microscope, with an incorporated measurement software. Supplementary Table
238 S2 shows detailed morphometric values for each specimen.

239

240 Results

241 *Phylogenetic reconstruction*

242 Without any variation in the different delineation methods, nine MOTUs are retrieved from the
243 concatenated Bayesian phylogenetic tree (Fig. 3a), belonging to monophyletic clades with low divergence
244 (<3%). Apart from the previously described *E. clavigera* s.s. (MOTU 4) and *E. viridis* (MOTU 7),
245 molecular evidence for six new *Eulalia* species can be found with the addition of MOTU GB1 from
246 Langeneck et al. (2019). Major clade A englobes four MOTUs which are genetically close to *E. clavigera*
247 s.s. with high bootstrap support. This is composed of MOTU 1, unique to the western Mediterranean,
248 MOTU 2, unique to the subtidal habitats from the island of Madeira (Portugal) the unnamed *Eulalia* KRO53
249 (MOTU 3) occurring in the Eastern Mediterranean Sea and lastly, the Mediterranean *Eulalia* cf. *clavigera*
250 (MOTU GB1).

251 MOTUs 5 and 6 are within major clade B, are sister to each other and genetically closer to *E.*
252 *viridis* and the outgroup *E. aurea* instead. MOTU 5 is present both in the British Isles and Western
253 Mediterranean, while the subtidal samples from MOTU 6, together with MOTUs 3 and 8 have few and
254 very small specimens in relatively poor conditions or exhausted in the DNA analysis, and thus were not
255 named or used in the morphometric analysis.

256

257 *Genetic distances*

258 The Global intra- and interspecific mean distances for the nine MOTUs and two outgroups for
259 each marker are provided in Table 3. Apart from the outgroups and *Eulalia* IT2-1 (MOTU 8), the mean
260 intraspecific distance for COI is 0.93 (0.0 – 3.3)%, while the average congeneric distance is 17.9 (7.1 –
261 25.5)%. For the ITS-region it ranges between 1.4 (0.0 – 3.9)% and 17.2 (4.4 – 32.6)% for intra- and
262 interspecific divergence, respectively, while for 28S the corresponding distances are 0.04 (0 – 0.4)% and
263 2.7 (0 – 5.9)%, respectively. The populations between the continental Europe and the Macaronesia islands
264 from *E. clavigera* s.s. (MOTU 4) only have COI maximum distances up to 3.3% and no significant
265 divergence (<1%) in the nuclear markers. *Eulalia* IT2-1 has a particularly high interspecific distance in the
266 nuclear markers, reaching values higher than 60% for the ITS region and 12% for the 28S locus, similar to
267 the ones found in the *Phyllodoce* sp. (Outgroup). This lineage belongs to a very small specimen which at
268 first, seemed to fit the *E. viridis* morphotype based on the small size but pointed midbody dorsal cirri and
269 bright red eyes, however molecular data is very divergent, showing evidence of an entirely new *Eulalia*
270 group yet to be described.

271

272 *Haplotype networks*

273 Only the 28S network (Fig. 4b) fail to discriminate all the identified MOTUs from the concatenated
274 dataset and is characterized by a star-shape phylogeny, with most of the unique haplotypes closely related
275 to the common central haplotype which is composed by MOTUs 1, 2 and 4. However, MOTU 1 also has a
276 distinct haplotype, with a similar number of mutations apart as the outgroup *E. aurea*, from the common
277 one. The ancestral central haplotype might suggest the possibility of vicariance-driven speciation through
278 a single colonization event and subsequent diversification.

279 The COI (Fig. 3b) and ITS (Fig. 4a) networks reveal geographically structured populations within
280 MOTU 4, between continental Europe and the Macaronesia archipelagos (Azores, Canary and Savage
281 islands). This correspond to the two distinct clades found in the BI tree, but have not enough divergence to
282 be divided into two separate MOTUs. Other biogeographical signals, where certain haplotypes or parts of
283 the haplotype network can be correlated with a specific biogeographic region, can be found in the Madeira
284 island (MOTU 2), Scandinavia (MOTU 7), eastern Mediterranean (MOTU 3) and south of France (MOTU
285 1).

286 The COI haplotype diversity is relatively high ($H_d > 0.89$ to 0.985, Table 4) and in some cases it
287 can be extreme, with almost all specimens having an unique haplotype as seen in MOTU 1 (8 haplotypes
288 in 10 specimens) and MOTU 2 (11 haplotypes in 12 specimens). None of the MOTUs have a significant
289 Tajima D and Fu and Li's D tests, with the neutral model of nucleotide substitutions being accepted for all
290 the lineages.

291

292 *Morphometric measurements*

293 The most significative morphometric proportions can be seen in the scatter plots in Figs. 5a-f,
294 displaying considerable visible differences, with the formation of independent clusters among the analysed
295 species. The exception is represented by specimens of MOTU 5 (Fig. 6b) and *E. clavigera s.s.* (MOTU 4)
296 which often overlap the same morphometric cluster. However, specimens from the latter species are
297 considerably larger (both in number of segments and the worm's length and width) than the ones from
298 MOTU 5. Despite the worm's size difference (Number of segments, Worm's length and width), MOTU 5
299 is characterized by the presence of large morphological structures such as the dorsal and ventral cirri, head,
300 dorsal cirri on segment 2 and the antennae.

301 No considerable differences are found in *E. clavigera s.s.* between the populations from
302 continental Europe and the Canary islands. Nevertheless, partial morphometric clusters between the number
303 of segments compared to the worm width and the ratio between the middle antenna with the head length
304 are the only morphometric proportions able to partially differentiate between these two populations (Fig.
305 5g, h)

306 The use of morphometric proportions between the head length against either the head width, the
307 length of the antennae or dorsal cirri on segment; and between the length of the ventral cirri against either
308 the length of the chaetigerous lobe, dorsal cirri and width of the ventral cirri seems to be effective in
309 distinguishing MOTU 2 (Fig. 6c), MOTU 1 (Fig. 6a) and *E. clavigera s.s.* from each other. MOTU 2 has
310 smaller proportions when compared to the remaining analysed species, with MOTU 1 appearing in the
311 middle clusters (main morphometric findings, coloration, depth and geographic distribution summarized in
312 Table 5).

313

314 *Taxonomic section*

315

316 *Eulalia clavigera* (Audouin & Milne Edwards, 1833)

317 (Fig. 1; Fig. 3a)

318 *Phyllodoce clavigera* Audouin and Milne-Edwards 1833: 226–228, PL. XVI, fig. 9-13

319 *Eulalia clavigera*: Bonse et al. 1996: 40–45, Fig. 14 (redescr., syn.); Alós 2004: 193–196, Fig. 69 (SEM
320 photographs)

321 ? *Eulalia viridis*: Morgado and Amaral 1984: 51 (non Linnaeus, 1767)

322

323 Material examined

324 **Portugal: Aveiro**, 6 spms, DBUA0002468.01-06, 40°33'32.4"N - 8°46'19.2"W, low tide, among
325 rocks with algae and mussels, collected by Marcos AL Teixeira and Ascensão Ravara, 27-07-2018; **Canto**
326 **Marinho**, 7 spms, DBUA0002469.04-10, 41°44'13.2"N to 8°52'33.6"W, low tide, among rocks with algae
327 and mussels, collected by Marcos AL Teixeira, 20-05-2019; **Areosa**, 3 spms, DBUA0002469.01-03,
328 41°42'36.0"N - 8°52'12.0"W, low tide, among rocks with algae and mussels, collected by Marcos AL
329 Teixeira, 20-03-2018; **Leixões**, 1 spm, DBUA0002470.01, 41°10'58.8"N - 8°42'18.0"W, marina in pontoon
330 scrapplings, collected by Sofia Duarte, 23-06-2020; **Nazaré**, 3 spms, DBUA0002493.01-03, 39°36'13.0"N
331 - 9°04'44.0"W, among rocks, collected by Ascensão Ravara, 26-07-2021. **Santa Maria (Azores)**, 2 spm,
332 DBUA0002477.01-02, 36°57'03.6"N - 25°01'04.8"W, low tide, among rocks with algae and mussels,
333 collected by Ana Costa, 07-05-2019; **Savage islands**, 1 spm, MB29-000385, 30°08'23.9"N - 15°51'57.6"W,
334 low tide, among rocks with algae, kindly provided by the National Museum of Science and Natural History
335 (Portugal), collected in 22-06-2010. **Spain: Ferrol lagoon**, 5 spms, DBUA0002473.01-05, 43°30'07.2"N -
336 8°09'32.4"W, low tide, among rocks with algae and mussels, collected by Julio Parapar, 03-02-2015;
337 **Tenerife (Canary islands)**, 11 spms, DBUA0002476.01-11, 28°34'15.6"N - 16°20'02.4"W, low tide,
338 among rocks with algae, collected by Marcos AL Teixeira and Pedro E Vieira, 05-04-2019; **La Palma**
339 **(Canary islands)**, 10 spms, DBUA0002476.12-21, 28°48'18.0"N - 17°45'43.2"W, low tide, among rocks
340 with algae, collected by Marcos AL Teixeira and Pedro E Vieira, 09-04-2019; **Gran Canaria (Canary**
341 **islands)**, 5 spms, DBUA0002476.22-26, 27°59'06.0"N - 15°22'33.6"W, low tide, among rocks with algae,
342 collected by Marcos AL Teixeira and Pedro E Vieira, 06-04-2019. **France: Roscoff**, 8 spm,
343 DBUA0002471.01-08, 48°43'33.6"N - 3°58'40.8"W, low tide, among rocks with algae and mussels,
344 collected by Arne Nygren, 20-03-2018; **Morgat**, 2 spms, DBUA0002472.01-02, low tide, among rocks
345 with algae, 48°13'20.3"N - 4°29'42.5"W, collected by Nicolas Lavesque, 16-06-2018. **Great Britain:**
346 **Plymouth**, 12 spms, DBUA0002474.01-12, 50°21'25.2"N - 4°07'40.8"W, low tide, among rocks with algae
347 and mussels, collected by Arne Nygren and Fredrik Pleijel, 18-03-2006. **Italy: Livorno**, 3 spms,
348 DBUA0002475.01-03, 43°32'24.0"N - 10°18'00.0"E, marina in pontoon scrapplings, collected by Joachim
349 Langeneck, 20-09-2019.

350

351 Diagnosis (Updated from Pleijel, 1993)

352 Body anteriorly stout and posteriorly tapered. Complete specimens with up to 275 segments and
353 68 mm total length, up to 2 mm maximum width if parapodia included (smallest specimens: 30 mm long,
354 1.6 mm wide, 155 chaetigers). Living specimens are deep green, once preserved the pigment fades off into
355 a greenish hue and can turn into brownish once aged. Prostomium rounded triangular, wider than long.
356 Eyes medium-sized, rounded and occasionally partly covered by segment I. Distance between the eyes
357 about the same length of the head. Median antenna f similar size as the terminal ones situated well in front
358 of the eyes. Palps about the same size as antennae. Proboscis widest distally, densely covered with rounded

359 to conical papillae. Terminal ring with varying number of papillae. Tentacular cirri shorter than the body
360 width. Tentacular cirri of segment 1 reaching about segment 3 and half the size of the largest tentacular
361 cirri found in segment 2. Dorsal tentacular cirri of segment 2 and tentacular cirri from segment 3 reaching
362 about segment 7. Ventral tentacular cirri from segment 2 reaching about segment 3-4, often thick and
363 slightly flattened. Dorsal cirri of median segments asymmetrically lanceolate, about twice longer than
364 wider. Ventral cirri rounded slightly longer than wider and smaller than the chaetigerous lobes. Chaetae
365 usually present from segment 3, occasionally one or two setae arising from anterior side of ventral
366 cirrophores of segment 2.

367

368 Molecular data

369 COI, ITS and 28S sequences as in specimens DBUA0002468.01-06, DBUA0002469.01-08,
370 DBUA0002470.01, DBUA0002493.01-03, DBUA0002471.01-05, DBUA0002472.01-02,
371 DBUA0002473.01-05, DBUA0002474.01-10, DBUA0002475.01-03, DBUA0002476.01-05,
372 DBUA0002476.12-18, DBUA0002476.22-26, DBUA0002477.01-02 and MB29-000385 (Table S1).
373 Phylogenetic relationship as in Fig. 3a, where *E. clavigera* s.s. is clearly distinct from the remaining species
374 of the complex, grouping in MOTU 4. Interspecific COI mean distances to the closest and distant neighbour
375 are 7.5% (K2P, *Eulalia* KRO53) and 23.3% (K2P, *E. viridis*) respectively. DOI for the species' Barcode
376 Index Number (BIN): *upon paper acceptance*.

377

378 Distribution and habitat

379 From the NE Atlantic Ocean (United Kingdom, France, Iberian Peninsula) to the western
380 Mediterranean (western Italy). Present as well in the archipelagos of the Canaries, Azores and Savage
381 islands. Type locality: Brittany, France. It was also recorded as an introduced species in the South-western
382 Atlantic Ocean (Langeneck et al. 2019).

383 Usually present in intertidal rocky areas surrounded by algae, mussels and associated with
384 *Sabellaria* reefs. Also present in marinas among the algae attached to the pontoons.

385

386 Reproduction

387 The reproductive biology of this species is poorly known and available data most likely represent
388 different lineages, corresponding to *E. viridis* from Scandinavian samples and *E. clavigera* from the English
389 and French coasts. This species have a planktonic larval stage and reproduce once a year (Meyer, 1938),
390 but local populations along the coasts of Northern Europe also differ in the time of reproduction with
391 reproductive cycle starting 4 to 6 weeks earlier in Swedish specimens compared to the ones from the English
392 and French coasts (Olive, 1975; Pleijel, 1993)

393

394 Remarks

395 Bonse et al. (1996) redescribed *E. viridis* and reinstated *E. clavigera* (Audouin & Milne-Edwards,
396 1834) which have been previously synonymised by McIntosh (1908). These two species have slight
397 differences in prostomial, parapodial and pharynx papillation features that allow their distinction.
398 According to Bonse et al. (1996), the length-to-width ratio of dorsal cirri is the most useful character to

399 distinguish between *E. viridis* and *E. clavigera*. Smaller papillae and slender dorsal cirri, corresponded to
400 *E. viridis*, while *E. clavigera* have larger papillae and significantly thicker dorsal cirri. *Eulalia viridis* is
401 also unique to the Scandinavia and Northern Sea and seems to be a northern boreal and sub-arctic species
402 both in intertidal or subtidal waters. *Eulalia clavigera* is a temperate species mostly found in intertidal rocky
403 beaches, ranging from Great Britain to the western Mediterranean Sea, being present as well in the Azores,
404 Savage islands and widespread in the Canary islands.

405 Audouin & Milne Edwards (1833) erected the species *Phyllodoce gervillei* from Granville
406 (France), stating that it is identical to *P. clavigera*, with the exception of the missing median antenna and
407 smaller tentacular cirri. McIntosh (1908) synonymised both species with *E. viridis*, considering that the
408 absence of antennae in *P. gervillei* may have been accidental. However, given the type locality of *P.*
409 *gervillei*, that species is most probably a synonym of *E. clavigera*.

410 Specimens from the type locality of *E. clavigera* (Brittany, France) were collected for this study
411 and grouped in MOTU 4 (Fig. 3a). The number of segments compared to the worm width and the ratio
412 between the middle antenna with the head length were the only morphometric proportions able to better
413 separate the continental European populations from the ones found in the Canary islands (Fig. 5g, h). This
414 lack of variation is also reflected in the molecular data where these two populations, although present in
415 two distinct clades and with unique COI and ITS haplotypes, only diverge up to 3.3% (COI) between each
416 other, grouping in the same MOTU. *Eulalia clavigera* usually possess larger proportions in most of the
417 diagnostic characters when compared against the other three species from the complex described here,
418 especially the ratio between the length of the dorsal and ventral cirri, between the length of the chaetigerous
419 lobe and ventral cirri, the length to width ratio in the ventral cirri, as well as the ratio between the length of
420 the head against either the length of the dorsal cirri on segment 2, antennae or the width of the head. The
421 exception to this can sometimes be found against specimens from *E. xanthomucosa* sp. nov. (described
422 below), which can often share the same cluster measurements, however, analysed specimens from *E.*
423 *clavigera* were considerable larger in size (number of segments; worm's length and width).

424

425

426 *Eulalia madeirensis* Teixeira, Ravara, Langeneck & Nygren **sp. nov.**

427 (Fig. 6c)

428 urn:lsid:zoobank.org:act: upon paper acceptance

429

430 Material examined

431 *Type material.* **Portugal: Madeira (Funchal)**, 1 spm, holotype and hologenophore,
432 DBUA0002479.02, 32°38'09.6"N - 16°55'51.6"W, subtidal, 11 m depth, collected by Arne Nygren, 21-09-
433 2009; 4 spms, paratypes and paragenophores, DBUA0002479.01, DBUA0002479.03, DBUA0002479.05-
434 07, 32°38'09.6"N - 16°55'51.6"W, subtidal, 11 m depth, collected by Arne Nygren, 21-09-2009.

435 *Other material.* **Portugal: Madeira (Funchal)**, 1 spm, MTE052-20, 32°38'09.6"N -
436 16°55'51.6"W, subtidal, 11 meters depth, collected by Arne Nygren, 21-09-2009; **Madeira (Porto Moniz)**,
437 4 spms, DBUA0002479.04, MTE053-20, MTE055-20 and MTE057-20, 32°51'38.6"N 17°09'06.3"W,
438 subtidal, 11 meters depth, collected by Arne Nygren, 30/09/2009.

439

440 Diagnosis

441 Small worms both in width, length and number of segments; complete specimens with up to 115
442 segments and 10 mm total length and 0.4 mm maximum width if parapodia included (smallest specimen: 4
443 mm long, 0.3 mm wide, 52 chaetigers). Holotype lacking posterior end, 10 mm in length, 0.4 mm in width
444 and 115 chaetigers. Living specimens are yellowish to light green (Fig. 6c), once preserved the pigment
445 fades off into greenish brown. Prostomium rounded triangular, wider than long. Eyes medium-sized,
446 rounded and occasionally partly covered by segment I. Distance between the eyes about the same length of
447 the head. Median antenna of similar size as the terminal ones, situated well in front of the eyes. Palps
448 slightly larger than the antennae. Proboscis widest distally, densely covered with rounded to conical
449 papillae. Tentacular cirri of segment 1 reaching segment 3-4. Dorsal tentacular cirri of segment 2 usually
450 1.7 times the size of the ventral tentacular from the same segment. Ventral tentacular cirri from segment 2
451 often thick and slightly flattened, reaching segment 4-5. Dorsal tentacular cirri of segment 2 and 3 reaching
452 about segment 8. Dorsal cirri of median segments asymmetrically lanceolate, about twice longer than wider.
453 Ventral cirri of median segments rounded slightly longer than wider and half the length of the chaetigerous
454 lobes, especially in the posterior half of the worm. Chaetae usually present from segment 3, occasionally
455 one or two setae arising from anterior side of ventral cirrophores of segment 2.

456

457 Molecular data

458 COI, ITS and 28S sequences as in specimens DBUA0002479.01-07, MTE052-20- MTE055-20
459 and MTE057-20 (Table S1). Phylogenetic relationship as in Fig. 3a, where *E. madeirensis* sp. nov. is clearly
460 distinct from the remaining species of the complex, grouping in MOTU 2. Interspecific COI mean distances
461 to the closest and distant neighbour are 11.4% (K2P, *E. clavigera* s.s.) and 23.3% (K2P, *E. viridis*)
462 respectively. DOI for the species' Barcode Index Number (BIN): *upon paper acceptance*.

463

464 Etymology

465 The new species is named after the main Madeira island, the unique remote location where this
466 species can be found so far.

467

468 Distribution and habitat

469 Atlantic ocean: Exclusive to the Madeira island (Portugal), in subtidal environments up to 11
470 meters depth.

471

472 Remarks

473 Member of the *Eulalia clavigera* species complex, subtidal variant and mostly morphological
474 similar to *E. clavigera*. Besides the molecular data and its geographical distribution unique to the Madeira
475 island (Portugal), *E. madeirensis* sp. nov. can be distinguished from *E. clavigera* and the remaining species
476 of the complex mainly by the yellowish light green coloration of live specimens and smaller worm size
477 (Table 5). It also shows smaller morphometric proportions in most of the diagnostic characters when
478 compared against the other three species from the complex, especially the ratio between the length of the

479 dorsal and ventral cirri, between the length of the chaetigerous lobe and ventral cirri, the length to width
480 ratio in the ventral cirri, as well the ratio between the length of the head against either the length of the
481 dorsal cirri on segment 2, antennae or the width of the head.

482

483

484 *Eulalia feliciae* Teixeira, Ravara, Langeneck & Nygren **sp. nov.**

485 (Fig. 6a)

486 urn:lsid:zoobank.org:act: *upon paper acceptance*

487

488 Material examined

489 *Type material.* **France: Banyuls**, 1 spm, holotype and hologenophore, DBUA0002478.05,
490 42°28'48.0"N - 3°08'06.0"E, near shore at 0.5–1 m depth, rocky beach, collected by Arne Nygren and
491 Fredrik Pleijel, 22-04-2001; 5 spms, paratype and paragenophores, DBUA0002478.01-04 and
492 DBUA0002468.06, 42°28'48.0"N - 3°08'06.0"E, near shore at 0.5–1 m depth, rocky beach, collected by
493 Arne Nygren and Fredrik Pleijel, 22-04-2001.

494 *Other material.* **France: Banyuls**, 2 spms, DBUA0002478.07 and MTE040-20, 42°28'48.0"N -
495 3°08'06.0"E, subtidal at 10 m depth, among algae, rocks and mussels, collected by Arne Nygren and Fredrik
496 Pleijel, 02-04-2009; 2 spms, DBUA0002478.08 and MTE042-20, 42°28'48.0"N - 3°08'06.0"E, subtidal at
497 10 m depth, among rocks with hydroids, collected by Arne Nygren and Fredrik Pleijel, 05-04-2009.

498

499 Diagnosis

500 Small worm both in width, length and number of segments; complete specimens with up to 135
501 segments and 14 mm total length and 0.6 mm maximum width if parapodia included (smallest: 9 mm long,
502 0.5 mm wide, 93 chaetigers). Holotype lacking posterior end, 14 mm in length, 0.6 mm in width and 135
503 chaetigers. Living specimens are deep emerald green (Fig. 6a) once preserved the pigment fades off into a
504 greenish hue and can retain this color once aged. Prostomium rounded triangular, wider than long. Eyes
505 medium-sized, rounded and occasionally partly covered by segment I. Distance between the eyes shorter
506 than the length of the head. Median antenna of similar size as the terminal ones, situated well in front of
507 the eyes. Palps larger than the antennae. Proboscis widest distally, densely covered with rounded to conical
508 papillae. Cirri of segment 1 reaching segment 3-4. Dorsal tentacular cirri of segment 2 usually 1.8 times
509 the size of the ventral tentacular cirri from the same segment. Ventral tentacular cirri from segment 2 often
510 thick and slightly flattened, reaching segment 4. Dorsal tentacular cirri of segment 2 and 3 reaching about
511 segment 6-7. Dorsal cirri of median segments asymmetrically lanceolate, about 2.4 times longer than wider.
512 Ventral cirri of median segments twice as long as wide. Ventral cirri slightly shorter than the chaetigerous
513 lobes. Chaetae usually present from segment 3, occasionally one or two setae arising from anterior side of
514 ventral cirrophores of segment 2.

515

516 Molecular data

517 COI, ITS and 28S sequences as in specimens DBUA00024678.01-08, MTE040-20 and MTE042-
518 20 (Table S1). Phylogenetic relationship as in Fig. 3a, where *E. feliciae* sp. nov. is clearly distinct from the

519 remaining seven species of the complex, grouping in MOTU 1. Interspecific COI mean distances to the
520 closest and distant neighbour are 13.9% (K2P, *E. clavigera* s.s.) and 22% (K2P, *E. viridis*) respectively.
521 DOI for the species' Barcode Index Number (BIN): *upon paper acceptance*.

522

523 Etymology

524 The new species is named after Felicia Ulltin, a master student under the supervision of the last
525 author of this study, whose enthusiasm and love for polychaetes is unmatched and an inspiration for future
526 marine researchers.

527

528 Distribution

529 Mediterranean Sea: South of France. Usually present in intertidal or subtidal rocky areas among
530 algae, hydroids and mussels.

531

532 Remarks

533 Member of the *Eulalia clavigera* species complex and morphological similar to *E. clavigera*.
534 Besides the molecular data and its geographical distribution unique to the western Mediterranean Sea, *E.*
535 *feliciae* sp. nov. can be distinguished from *E. clavigera* and the remaining species from the complex mostly
536 by the deep emerald green coloration of the live specimens and the small to medium sized morphometric
537 proportions. It shows larger morphometric proportions in most of the diagnostic characters when compared
538 to *E. madeirensis* sp. nov. but smaller against *E. clavigera* and *E. xanthomucosa* sp. nov. (described below).
539 The most significant proportions are the ratio between the length of the dorsal and ventral cirri, between
540 the length of the chaetigerous lobe and ventral cirri, the length to width ratio in the ventral cirri, as well the
541 ratio between the length of the head against either the length of the dorsal cirri on segment 2, antennae or
542 the width of the head.

543

544 *Eulalia xanthomucosa* Teixeira, Ravara, Langeneck & Nygren **sp. nov.**

545 (Fig. 6b).

546 urn:lsid:zoobank.org:act: *upon paper acceptance*

547

548 Material examined

549 *Type material.* **United Kingdom: Cornwall (Newlyn Marina)**, 1 spm, holotype and
550 hologenophore, DBUA0002480.07, 50°06'10.8"N - 5°32'49.2"W, subtidal at 25 m depth, among
551 coralligenous samples, collected by David Fenwicki, 02-06-2016; 3 spms, paratypes and paragenophores,
552 DBUA0002480.01-03, 50°06'10.8"N - 5°32'49.2"W, lowshore in a rock crevice, collected by David
553 Fenwicki, 02-07-2016; 3 spms, paratypes and paragenophores, DBUA0002480.04-06, 50°06'10.8"N -
554 5°32'49.2"W, subtidal at 25 m depth, in rock crevices at Laminaria zones and among coralligenous,
555 collected by David Fenwicki, 22-08-2017.

556 *Other material.* **France: Banyuls**, 1 spm, BI-2014/15-077, 42°28'48.0"N - 3°08'06.0"E, subtidal
557 at 25 m depth, among algae and boulders, collected by Fredrik Pleijel, 07-04-2009; 1 spm,
558 DBUA0002481.01, 42°50'37.0"N - 3°14'12.0"E, subtidal at 25 m depth, among coralligenous, collected by

559 Felicia Ultin, 15-09-2020. **France: Corsica island**, 2 spms, MNHN-IA-2021-654 and MNHN-IA-2021-
560 655, 41°26,8'N - 008°54'E, subtidal at 34 m depth, collected by the CORSICABENTHOS expeditions, 23-
561 10-2020.

562

563 Diagnosis

564 Complete specimens with up to 230 segments and 104 mm total length and 2.378 mm maximum
565 width if parapodia included (smallest specimen: 12 mm long, 0.397 mm wide, 89 chaetigers). Holotype
566 lacking the posterior end, 26 mm in length, 1.2 mm in width and 128 chaetigers. Living specimens present
567 a yellow coloration provided by the worm's mucus (Fig. 6b), once preserved the pigment fades off into a
568 brownish color. Prostomium rounded triangular, wider than long. Eyes small to medium-sized, rounded
569 and occasionally partly covered by segment I. Distance between the eyes shorter than the length of the head.
570 Median antenna of similar size as the terminal ones, situated well in front of the eyes. Palps about the same
571 size as antennae. Proboscis not examined. Cirri of segment 1 reaching segment 4-5. Dorsal tentacular cirri
572 of segment 2 usually 1.8 times the size of the ventral tentacular cirri from the same segment. Ventral
573 tentacular cirri from segment 2 often thick and slightly flattened, reaching segment 5-6. Dorsal tentacular
574 cirri of segment 2 and 3 reaching about segment 8-9. Dorsal cirri of median segments asymmetrically
575 lanceolate, about 2.3 times longer than wider. Ventral cirri of median segments 1.5 times longer than wide.
576 Ventral cirri slightly shorter than the chaetigerous lobes. Chaetae usually present from segment 3,
577 occasionally one or two setae arising from anterior side of ventral cirrophores of segment 2.

578

579 Molecular data

580 COI, ITS and 28S sequences as in specimens DBUA0002480.01-07, DBUA0002481.01, BI-
581 2014/15-077, MNHN-IA-2021-654 and MNHN-IA-2021-655 (Table S1). Phylogenetic relationship as in
582 Fig. 3a, where *E. xanthomucosa* sp. nov. is clearly distinct from the remaining *Eulalia* species, grouping in
583 MOTU 5. Interspecific COI mean distances to the closest and distant neighbour are 12.1% (K2P, *Eulalia*
584 IS-BA) and 20.4% (K2P, *E. feliciae* sp. nov.) respectively. DOI for the species' Barcode Index Number
585 (BIN): *upon paper acceptance*.

586

587 Etymology

588 The new species is named based on its unique bright yellow ("xantho" from ancient Greek)
589 coloration produced by the worm's mucus.

590

591 Distribution and habitat

592 Atlantic Ocean: United Kingdom, Cornwall; Mediterranean Sea: France, Banyuls. Occasional
593 lower intertidal but typically shallow sublittoral in rock crevices at *Laminaria* zones, among coralligenous
594 material in marinas.

595

596 Remarks

597 This species was registered at the Natural History Museum as *Eulalia* sp. "Emits Yellow Mucus
598 A" (tvk NHMSYS0021180023, https://www.aphotomarine.com/worm_eulalia_species_28-09-11.html).

599 The species can easily be distinguished from *E. clavigera* using the live coloration (yellow instead of green),
600 but may be confused with *E. aurea* due to similar yellowish coloration. However, the unusually large dorsal
601 cirri of median segments in relation to the worm size is very distinct compared to both *E. clavigera* and *E.*
602 *aurea*. Based on our observations, *E. clavigera* and *E. xanthomucosa* sp. nov. can generally be found
603 together in marinas, but so far only confirmed at Newlyn Marina (Cornwall, United Kingdom). Usually, *E.*
604 *clavigera* occurs higher on the shore than *E. xanthomucosa* sp. nov..

605 *Eulalia xanthomucosa* sp. nov. presents larger morphometric proportions in most of the diagnostic
606 characters when compared against *E. feliciae* sp. nov., and *E. madeirensis* sp. nov., especially the ratio
607 between the length of the dorsal and ventral cirri; between the length of the chaetigerous lobe and ventral
608 cirri; the length to width ratio in the ventral cirri; as well the ratio between the length of the head against
609 either the length of the dorsal cirri on segment 2, antennae or the width of the head. The exception to this
610 can sometimes be found against specimens from *E. clavigera*, which can often share the same cluster
611 measurements, however, the analysed specimens from *E. clavigera* were considerably larger in size
612 (number of segments; worm's length and width). Similar ratio between the antennae and palps is also shared
613 with *E. clavigera*. Some specimens from *E. xanthomucosa* sp. nov. can reach similar worm sizes compared
614 to *E. clavigera*, as seen in the specimen DBUA0002481.01, up to 230 segments and 104 mm total length
615 and 2.378 mm maximum width if parapodia included).

616

617 **Discussion**

618 With the use of molecular tools, we were able to unravel hidden diversity in the *Eulalia* genus.
619 We have found compelling evidence for six additional European MOTUs within the *E. clavigera* and *E.*
620 *viridis* pseudo-cryptic complex. Based on the combination of different approaches (molecular,
621 morphometric, coloration and geographical distribution data), three of these lineages, are here described as
622 new species. Mean COI distances (17.9%) between lineages are within fit the range usually reported in
623 other annelids (Nygren et al., 2018; Ravara et al., 2017; Sampieri et al., 2021), including other Phyllodocids
624 (Teixeira et al., 2022) and the MOTU delineation was congruent among all the delineation methods
625 employed.

626 There is a clear geographic structure for most of the retrieved European MOTUs. In this study, *E.*
627 *viridis* (MOTU 7) is unique to the Scandinavia and Northern Sea and seems to be a northern boreal and
628 sub-arctic species, both in intertidal or subtidal waters, in agreement with previous works (Bonse et al.,
629 1996; Kato et al., 2001). *Eulalia clavigera* s.s. (MOTU 4) is a temperate species mostly found in intertidal
630 rocky shores, ranging from Great Britain to the western Mediterranean Sea, being present as well in the
631 Azores, Savage islands and widespread in the Canary islands. Its presence was also confirmed in Argentina
632 (Langeneck et al., 2019). Based on our sampling campaigns and personal observations, this species seems
633 to be one of the most dominant taxa present in the rocky beaches from the island of Tenerife and can even
634 be found in very large quantities close to artificial pools in tourist zones, despite the heavy human presence
635 in these areas. It should be noted that Langeneck et al. (2019) reported the occurrence of individuals
636 morphologically similar to *E. clavigera* collected in Brazil, although it was not possible to obtain molecular
637 data. It is also possible that specimens identified as *E. viridis* from southern Brazil (Morgado and Amaral
638 1983) might actually belong to *E. clavigera* instead.

639 Langeneck et al. (2019) suggested the possibility of *E. clavigera* being a relict species in the
640 Mediterranean Sea, while the majority of the Mediterranean shallow-water green *Eulalia* probably belong
641 to one or more different species. The new species, *E. feliciae* sp. nov. (MOTU 1) seems to co-exist in
642 sympatry with *E. clavigera* (MOTU 4) and *E. xanthomucosa* sp. nov. (MOTU 5) in the western
643 Mediterranean Sea. Together with a specimen of *E. clavigera* reported in Langeneck et al. (2019), these 3
644 MOTUs were collected in Banyuls-sur-Mer and can be found in the intertidal zone. However, as far as we
645 know, *E. xanthomucosa* sp. nov. seems to be more abundant in subtidal regions (mainly from recreational
646 marinas), it is also present in Great Britain and possesses an characteristic coloration (yellowish instead of
647 the characteristic green) similar to the outgroup *E. aurea*. Live coloration is one of the most important
648 features in the taxonomy of this genus, as most of the different *Eulalia* species are almost impossible to
649 distinguish based solely on morphologic features of the discoloured preserved specimens (Schimmenti et
650 al., 2016). *Eulalia xanthomucosa* sp. nov. was indeed the most divergent MOTU found in the complex and,
651 besides coloration, displayed some other visible phenotypic features comparable to the *E. clavigera*
652 morphotype. In particular, parapodia showed a larger size of the dorsal and ventral cirri compared to the
653 worm size. These morphological differences appear to parallel the molecular divergence data, e.g. the
654 interspecific nuclear genetic distances tripled when compared to the distances found between MOTUs
655 within the major “*clavigera*” clade (clade A, Fig. 3a). This clade, with the exception of the population from
656 Madeira, also shared 28S haplotypes, but this seems to be a common occurrence in other closely related
657 marine species (Borges et al., 2012; Vieira et al., 2019). Ribosomal nuclear loci (due to the lower
658 evolutionary rates) are not suitable to species-level discrimination in invertebrates (e.g. Jörger et al., 2012)
659 being more efficient in reconstructing deeper phylogenies instead (e.g. Weitschek et al., 2014).

660 The unnamed lineage from Croatia (MOTU 3) is genetically close to *E. clavigera* (COI, 7.5%;
661 ITS, 4.8%; no 28S variation), which suggests that the speciation might be recent and unlikely to be driven
662 by the Messinian salinity crisis (from 6 to 5.33 MY, e.g. Hupało et al., 2019). This important event is usually
663 referred to explain the emergence of geographic barriers preventing gene flow not only between the NE
664 Atlantic and the Mediterranean, but also between the Western and Eastern part of this Sea. However,
665 selection associated with the environmental features of the different habitats, which promoted local
666 adaptation (Peijnenburg et al., 2004), might also explain this apparently recent speciation. The small-size
667 morphotype and the type locality of MOTU 3 is close to *Eulalia virens* Ehlers, 1868, currently considered
668 a junior synonym (Read and Fauchald, 2022) of *E. viridis* described for the Adriatic sea, mainly
669 characterized by the low number of segments (54) and small size (length, 7mm; width, 0.5 mm). Further
670 sampling and examination of *Eulalia* specimens from this locality might elucidate if both designations
671 belong to the same morphotype.

672 At least four different *Eulalia* MOTUs seem to be exclusive to the Mediterranean Sea (Fig. 3), a
673 known biodiversity hotspot (Bianchi and Morri, 2000), including for hidden cryptic species (Calvo et al.,
674 2009; Langeneck et al., 2020; Taboada et al., 2017) and exotic species (Galil, 2009; Zenetos et al., 2008).
675 The role of the alternating glacial and interglacial stages has been often suggested as one of the reasons
676 reason for the high number of species in this Sea. Under the conditions of a characteristic interglacial
677 period, the Mediterranean region had a warm and arid climate and a deficient water balance, where the
678 input of Atlantic surface water into the Mediterranean through the Strait of Gibraltar plays an important

679 role. This may allow the possible introduction and maintenance of (sub)tropical littoral biota in this period
680 (Bianchi et al., 2012), with boreal species from the NE Atlantic introduced to Mediterranean refugia areas
681 during glacial periods (Gómez and Lunt., 2007; Maggs et al., 2008; Schmitt et al., 2021). The survival of
682 part of this fauna despite the water temperature fluctuation and different environmental and depth
683 conditions over time, sustains the hypothesis of the Mediterranean "biodiversity pump", a possible outcome
684 of the climatic events of the Quaternary (Bianchi and Morri, 2000).

685 In spite of the recent indication of high incidence of marine invertebrate endemisms in the
686 Macaronesia archipelagos (Desiderato et al., 2019; Vieira et al., 2019) no additional intertidal MOTUs were
687 recorded in the Azores and Canary islands. These volcanic islands never had contact with the mainland
688 continent, were formed at different times, are hundreds of kilometres apart, possess a range of unique
689 geological and climatic conditions, and their biota is the result of dispersal from distant geographical
690 sources and *in situ* evolution and diversification (Fernández-Palacios et al., 2011). However, no appreciable
691 differentiation was observed when compared to the continental populations, apart from two partial
692 morphometric markers and completely sorted COI and ITS haplotypes (Figs. 3b; 4a; 5g, h). Only intertidal
693 samples were collected in these islands, contrasting to the new lineage found in the subtidal populations
694 from Madeira (MOTU 2, *E. madeirensis* sp. nov.). Evidence of cryptic species among lineages inhabiting
695 at different depths has been found, as for example, for the species *Phyllodoce madeirensis* Langerhans,
696 1880 where three different MOTUs were reported, each corresponding to different sampling depths (Martin
697 et al., 2021). Additional sampling efforts in the subtidal habitats of the Canary or the Azores archipelagos
698 may reveal new *Eulalia* species yet to be discovered. Intertidal *Eulalia* populations from the South Eastern
699 Atlantic (Patagonia, Argentina) also failed to display any molecular or morphological divergence from the
700 European *E. clavigera* (Langeneck et al. 2019). This may suggest a recent colonization by anthropogenic
701 activities for both the Canary islands and the South American populations. Indeed, as reported by J. M.
702 Orensanz in a personal communication to the authors from the previously mentioned study, neither *E.*
703 *clavigera* or *E. viridis* were recorded during the intensive surveys done in the 70's, unlike the abundant
704 populations observed recently in Puerto Madryn, Argentina. Furthermore, according to the Biodiversity
705 Data Bank of the Canary islands (BDBC, <https://www.biodiversidadcanarias.es/biota/?lang=en>), the first
706 records of the *E. clavigera* in the Spanish archipelago date at least from 1976 (Sosa et al., 1976; Núñez et
707 al., 2005). However, unlike the Patagonia populations, the specimens from the Macaronesia islands do not
708 share COI or ITS haplotypes with mainland Europe (Fig. 3b, 4a, respectively), suggesting instead, an older
709 non-anthropogenic driven colonization compared to the South East populations. Schwindt et al. (2014)
710 hypothesized a recent unintentional introduction of *E. clavigera* due to shipping activities, either with
711 ballast waters or in fouling communities. Other studies also show evidence of many small benthic marine
712 fishes, chordate species or small-sized invertebrates and plankton, introduced as eggs, larvae or juveniles,
713 being first recorded from regions with major commercial ports and international shipping as the most
714 probable vector (Cuesta et al., 2016; Lockett and Gomon, 2001; Wonham et al., 2000).

715 Additional unsampled European MOTUs of *Eulalia* might still be uncovered. For example,
716 Audouin & Milne Edwards (1833) erected the species *Phyllodoce gervillei* from Granville (France), stating
717 that it is identical to *P. clavigera*, with the exception of the missing median antenna and smaller tentacular
718 cirri. McIntosh (1908) synonymised both species with *E. viridis*, considering that the absence of antennae

719 in *P. gervillei* may have been accidental. However, given the type locality of *P. gervillei*, that species is
720 most probably a synonym of *E. clavigera*. Furthermore, the species *Eulalia (Eumida) microceros*
721 Claparède, 1868, also a current synonym of *E. viridis*, is described for the Gulf of Naples and is
722 characterized by its large size (Length, 5cm; width, 3mm; number of segments, 300). This far surpasses
723 any of the analysed green *Eulalia* specimens from continental Europe in this study (Table 5, Table S2),
724 suggesting that this is either a larger specimen belonging to *E. clavigera* based on type locality and figures
725 from the original description (PL. XVI, fig.4), or another large species with a similar morphotype, different
726 from what we analysed in this study.

727

728 **Conclusions**

729 In this study we have found six additional MOTUs within *Eulalia*, which appear to be rarer and
730 mainly restricted to a particular region. Nevertheless, available data on *E. clavigera s.s.* continues to
731 indicate that this species is quite widespread in Europe. It is very abundant in temperate areas from the
732 western Mediterranean to the NE Atlantic, including the Savage islands, Azores and Canary islands. Despite
733 the close genetic proximity between the NE Atlantic and the Macaronesia populations, the lack of shared
734 haplotypes between these regions suggests that recent anthropogenic introduction through shipping may
735 not be the reason for this divergence, unlike the southern American population (Langeneck et al. 2019),
736 and instead, an older colonization of these islands could be possible. Its successful establishment in these
737 temperate and sub-tropical areas and recent observations of large populations in both regions, might change
738 trophic interactions within the native fauna. Given that *E. clavigera* is a predator feeding mostly on mussels
739 and barnacles (Rodrigo et al., 2015), with scavenger habits also observed (Morton, 2011), the demography
740 and effect of this species on local fauna deserve close monitoring.

741 Recently, a hidden biotechnological potential was uncovered in marine invertebrates, which might
742 offer a wide array of natural products, showing properties compatible with anaesthetics, fluorescent probes,
743 and even antibiotics and pesticides (Rodrigo and Costa, 2019). By analysing the phylogeny of toxin
744 mixtures, Rodrigo et al. (2021a) show that annelids are uniquely positioned in the evolution of animal
745 venoms. In particular, using the toxin-containing mucus present in the green *Eulalia*, which based on
746 collection site (mainland Portugal) corresponds to *E. clavigera s.s.* in our study, revealed possible
747 applications in anti-cancer therapeutics (Rodrigo et al., 2021b) and fluorescent probes for biotechnological
748 applications using a protein mixture from the mucus (Rodrigo, 2020). This once again highlights the
749 importance of formally describing cryptic complexes, since biochemical features might be unique to each
750 lineage and can have a range of distinct effects and applications.

751

752 **Conflict of interests**

753 The authors declare no conflicts of interest

754

755 **Declaration of funding**

756 This study was supported by the project River2Ocean – Socio-ecological and biotechnological
757 solutions for the conservation and valorisation of aquatic biodiversity in the Minho Region, with the

758 reference NORTE-01-0145-FEDER-000068, co-financed by the European Regional Development Fund
759 (ERDF), through Programa Operacional Regional do Norte (NORTE 2020). Thanks are due, for the
760 financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020), to Portuguese Foundation for
761 Science and Technology and Ministry of Education and Science (FCT/MEC) through national funds, and
762 the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. Marcos AL
763 Teixeira was supported by a PhD grant from FCT co-financed by ESF (SFRH/BD/131527/2017) and from
764 the DNAqua-Net STSM grant "Rich and hidden biodiversity not yet barcoded in the Canary archipelago
765 (Spain) as an opportunity to enrich the DNA barcode reference library for European polychaetes", under
766 the EU Cost action CA15219 - Developing new genetic tools for bioassessment of aquatic ecosystems in
767 Europe. Pedro Vieira work was supported by national funds through the Portuguese Foundation for Science
768 and Technology (FCT, I.P.) in the scope of the project (Early detection and monitoring of non-indigenous
769 species in coastal ecosystems based on high-throughput sequencing tools, PTDC/BIA-BMA/29754/2017).
770 Ascensão Ravara was funded by national funds, through FCT, I.P., in the scope of the framework contract
771 foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by
772 Law 57/2017, of July 19. Financial support to Arne Nygren from the Norwegian Taxonomy Initiative
773 [<http://www.biodiversity.no/Pages/135523>] (Cryptic polychaete species in Norwegian waters, knr 49-13,
774 pnr 70184228), the Swedish Taxonomy Initiative [[https://www.artdatabanken.se/en/the-swedish-
775 taxonomy-initiative/](https://www.artdatabanken.se/en/the-swedish-taxonomy-initiative/)] (Polychaete species complexes in Swedish waters, dnr 140/07 1.4 and 166/08 1.4),
776 and Kungliga Fysiografiska sällskapet Nilsson-Ehle donationerna [<https://www.fysiografen.se/sv/>].

777

778 **Acknowledgements**

779 The authors wish to thanks Nicolas Lavesque for the northern French *Eulalia* specimens, Jorge
780 Fraga and Beatriz Alfonso for all the assistance and knowledge provided during the Canary islands
781 sampling campaign, Julio Parapar for the northern Spanish specimens, and David Fenwicki for collecting
782 and providing live photos of the species *E. xanthomucosa* sp. nov..

783 Thanks are due to the Corsica programme. The CORSICABENTHOS expeditions (PI: Line Le
784 Gall), with a focus on the small benthic biota, are the marine component of the "Our Planet Reviewed"
785 programme. The Corsica programme is run by Muséum National d'Histoire Naturelle in partnership with
786 Université de Corse Pasquale Paoli and Office de l'Environnement de la Corse (OEC), with the support of
787 Office Français de la Biodiversité (OFB) and Collectivité Territoriale de Corse (CTC).
788 CORSICABENTHOS 2 took place in October 2020 in collaboration with Réserve Naturelle des Bouches
789 de Bonifacio. The organizers are grateful to Medeleine Cancemi, Jean-François Cubells, Jean-Michel
790 Culioli and Jean-Michel Palazzi for their support.

791

792 **Availability of data and materials**

793 New sequence data and specimen metadata were uploaded in the project "*Eulalia species complex*"
794 (DS-MTE) within BOLD (<http://v4.boldsystems.org/>) and in the following link: *upon paper acceptance*.
795 The alignments (FASTA and NEXUS formats) for each marker (COI, ITS and 28S) and the concatenated
796 one (COI+ITS+28S) are all publicly available online at Figshare (DOI: *upon paper acceptance*). GenBank
797 accession numbers: xxx-xxx (COI), xxx-xxx (ITS2), and xxx-xxx (28S) (*upon paper acceptance*). See

798 online supplemental Table S1 for more details. The new biological material is deposited at the Biological
799 Research Collection (Marine Invertebrates) of the Department of Biology of the University of Aveiro
800 (COBI at DBUA), Portugal. The specimen from Banyuls (France) belonging to *E. xanthomucosa* sp. nov
801 was donated to SCRIPPS Oceanography, while specimens from Corsica are deposited at the Muséum
802 national d'Histoire naturelle (MNHN). All specimens available upon request, including the ones from Arne
803 Nygren's personal collection.

804

805

806 **References**

807 Audouin, J. V. & Milne Edwards, H. (1833). [Part 3.] Classification des Annélides et description de celles
808 qui habitent les côtes de la France. *Annales des sciences naturelles*, Paris. (series 1), 29, 195-269

809 Barfuss, M. H. J. (2012). Molecular studies in Bromeliaceae: Implications of plastid and nuclear DNA
810 markers for phylogeny, biogeography, and character evolution with emphasis on a new
811 classification of Tillandsioideae. Vienna: University of Vienna, 244. Available from:
812 <http://othes.univie.ac.at/24037>

813 Bely, A. E., & Wray, G. A. (2004). Molecular phylogeny of nauid worms (Annelida: Clitellata) based on
814 cytochrome oxidase I. *Molecular Phylogenetic and Evolution* 30(1), 50–63.
815 [https://doi.org/10.1016/S1055-7903\(03\)00180-5](https://doi.org/10.1016/S1055-7903(03)00180-5)

816 Bianchi, C. N., & Morri, C. (2000). Marine Biodiversity of the Mediterranean Sea: Situation, Problems and
817 Prospects for Future Research. *Marine Pollution Bulletin*, 40(5), 367–376.
818 [https://doi.org/10.1016/S0025-326X\(00\)00027-8](https://doi.org/10.1016/S0025-326X(00)00027-8)

819 Bianchi, C. N., Morri, C., Chiantore, M., Montefalcone, M., Parravicini, V., & Rovere, A. (2012).
820 Mediterranean Sea biodiversity between the legacy from the past and a future of change. In
821 Stambler N. (Ed.), *Life in the Mediterranean Sea: A look at habitat changes* (pp. 1– 55). *Nova*
822 *Science Publishers Inc*

823 Bleidorn, C., Kruse, I., Albrecht, S., & Bartolomaeus, T. (2006). Mitochondrial sequence data expose the
824 putative cosmopolitan polychaete *Scoloplos armiger* (Annelida, Orbiniidae) as a species complex.
825 *BMC Evolutionary Biology*, 6(1), 47. <https://doi.org/10.1186/1471-2148-6-47>

826 Bonse, S., Schmidt, H., Eibye-Jacobsen, D., & Westheide, W. (1996). *Eulalia viridis* (Polychaeta:
827 Phyllodocidae) is a complex of two species in northern Europe: results from biochemical and
828 morphological analysis. *Cahiers de Biologie Marine*, 37, 33–48

829 Borges, L. M. S., Sivrikaya, H., Roux, A. le, Shipway, J. R., Cragg, S. M., & Costa, F. O. (2012).
830 Investigating the taxonomy and systematics of marine wood borers (Bivalvia : Teredinidae)
831 combining evidence from morphology, DNA barcodes and nuclear locus sequences. *Invertebrate*
832 *Systematics*, 26(6), 572–582. <https://doi.org/10.1071/IS12028>

- 833 Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A., &
834 Drummond, A. J. (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis.
835 *PLOS Computational Biology*, 10(4), e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>
- 836 Calvo, M., Templado, J., Oliverio, M., & Machardom, A. (2009). Hidden Mediterranean biodiversity:
837 molecular evidence for a cryptic species complex within the reef building vermetid gastropod
838 *Dendropoma petraeum* (Mollusca: Caenogastropoda). *Biological Journal of the Linnean Society*,
839 96(4), 898–912. <https://doi.org/10.1111/j.1095-8312.2008.01167.x>
- 840 Carr, C. M., Hardy, S. M., Brown, T. M., Macdonald, T. A., & Hebert, P. D. N. (2011). A Tri-Oceanic
841 Perspective: DNA Barcoding Reveals Geographic Structure and Cryptic Diversity in Canadian
842 Polychaetes. *PLOS ONE*, 6(7), e22232. <https://doi.org/10.1371/journal.pone.0022232>
- 843 Castresana, J. (2000). Selection of Conserved Blocks from Multiple Alignments for Their Use in
844 Phylogenetic Analysis. *Molecular Biology and Evolution*, 17(4), 540–552.
845 <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- 846 Çinar, M. E. & Gönlügür-Demirci, G. (2005). Polychaete assemblages on shallow-water benthic habitats
847 along the Sinop Peninsula (Black Sea, Turkey). *Cahiers de Biologie Marine*, 46, 253-263
- 848 Claparède, É. (1868). Les annélides chétopodes du Golfe de Naples. *Mémoires de la Société de Physique*
849 *et d'Histoire Naturelle de Genève*. 19(2), 313-584
- 850 Clement, M., Snell, Q., Walke, P., Posada, D., & Crandall, K. (2002). TCS: estimating gene genealogies.
851 In *Proceedings 16th International Parallel and Distributed Processing Symposium* (pp. 7).
852 Presented at the Proceedings 16th International Parallel and Distributed Processing Symposium.
853 IPDPS 2002, Ft. Lauderdale, FL: IEEE. <https://doi.org/10.1109/IPDPS.2002.1016585>
- 854 Cuesta, J. A., Almón, B., Pérez-Dieste, J., Trigo, J. E., & Bañón, R. (2016). Role of ships' hull fouling and
855 tropicalization process on European carcinofauna: new records in Galician waters (NW Spain).
856 *Biological Invasions*, 18(3), 619–630. <https://doi.org/10.1007/s10530-015-1034-9>
- 857 Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics
858 and parallel computing. *Nature Methods*, 9(8), 772–772. <https://doi.org/10.1038/nmeth.2109>
- 859 Delić, T., Trontelj, P., Rendoš, M., & Fišer, C. (2017). The importance of naming cryptic species and the
860 conservation of endemic subterranean amphipods. *Scientific Reports*, 7(1), 3391.
861 <https://doi.org/10.1038/s41598-017-02938-z>
- 862 Desiderato, A., Costa, F. O., Serejo, C. S., Abbiati, M., Queiroga, H., & Vieira, P. E. (2019). Macaronesian
863 islands as promoters of diversification in amphipods: The remarkable case of the family Hyalidae
864 (Crustacea, Amphipoda). *Zoologica Scripta*, 48(3), 359–375. <https://doi.org/10.1111/zsc.12339>
- 865 Ehlers, E. H. (1864). Die Borstenwürmer (Annelida Chaetopoda) nach systematischen und anatomischen
866 Untersuchungen dargestellt
- 867 Eibye Jacobsen, D. (1991). A revision of *Eumida* malmgren 1865 polychaeta phyllodocidae. *Steenstrupia*.
868 <https://eurekamag.com/research/006/964/006964164.php>.

869 Eiby-Jacobsen, D. (1993). On the phylogeny of the Phyllodocidae (Polychaeta Annelida): an alternative.
870 *Journal of Zoological Systematics and Evolutionary Research*, 31(3), 174–197.
871 <https://doi.org/10.1111/j.1439-0469.1993.tb00188.x>

872 Fernández-Palacios, J. M., Nascimento, L. de, Otto, R., Delgado, J. D., García-del-Rey, E., Arévalo, J. R.,
873 & Whittaker, R. J. (2011). A reconstruction of Palaeo-Macaronesia, with particular reference to
874 the long-term biogeography of the Atlantic island laurel forests. *Journal of Biogeography*, 38(2),
875 226–246. <https://doi.org/10.1111/j.1365-2699.2010.02427.x>

876 Fernandez-Triana, J.L. (2022). Turbo taxonomy approaches: lessons from the past and recommendations
877 for the future based on the experience with Braconidae (Hymenoptera) parasitoid wasps. *ZooKeys*
878 1087, 199–220. <https://doi.org/10.3897/zookeys.1087.76720>

879 Fišer, C., Robinson, C. T., & Malard, F. (2018). Cryptic species as a window into the paradigm shift of the
880 species concept. *Molecular Ecology*, 27(3), 613–635. <https://doi.org/10.1111/mec.14486>

881 Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of
882 mitochondrial cytochrome c oxidase subunit I from metazoan invertebrates. *Molecular Marine*
883 *Biology and Biotechnology* 3, 294–299

884 Fujisawa, T., & Barraclough, T. G. (2013). Delimiting Species Using Single-Locus Data and the
885 Generalized Mixed Yule Coalescent Approach: A Revised Method and Evaluation on Simulated
886 Data Sets. *Systematic Biology*, 62(5), 707–724. <https://doi.org/10.1093/sysbio/syt033>

887 Galil, B. S. (2009). Taking stock: inventory of alien species in the Mediterranean sea. *Biological Invasions*,
888 11(2), 359–372. <https://doi.org/10.1007/s10530-008-9253-y>

889 Grosse, M., Capa, M., & Bakken, T. (2021). Describing the hidden species diversity of *Chaetozone*
890 (Annelida, Cirratulidae) in the Norwegian Sea using morphological and molecular diagnostics.
891 *ZooKeys*, 1039, 139–176. <https://doi.org/10.3897/zookeys.1039.61098>

892 Gómez, A., & Lunt, D.H. (2007). Refugia within refugia: Patterns of phylogeographic concordance in the
893 Iberian Peninsula. In: Weiss S, Ferrand N, editors. *Phylogeography of Southern European Refugia*
894 (pp. 155–88). Dordrecht: Springer

895 Guindon, S., & Gascuel, O. (2003). A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies
896 by Maximum Likelihood. *Systematic Biology*, 52(5), 696–704.
897 <https://doi.org/10.1080/10635150390235520>

898 Hardy, S. M., Carr, C. M., Hardman, M., Steinke, D., Corstorphine, E., & Mah, C. (2011). Biodiversity and
899 phylogeography of Arctic marine fauna: insights from molecular tools. *Marine Biodiversity*, 41(1),
900 195–210. <https://doi.org/10.1007/s12526-010-0056-x>

901 Hassouna, N., Mithot, B., & Bachelier, J.-P. (1984). The complete nucleotide sequence of mouse 28S
902 rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher
903 eukaryotes. *Nucleic Acids Research* 12(8), 3563–3583. <https://doi.org/10.1093/nar/12.8.3563>

- 904 Hupało, K., Teixeira, M. A. L., Rewicz, T., Sezgin, M., Iannilli, V., Karaman, G. S., Grabowski, M., &
905 Costa, F. O. (2019). Persistence of phylogeographic footprints helps to understand cryptic
906 diversity detected in two marine amphipods widespread in the Mediterranean basin. *Molecular*
907 *Phylogenetics and Evolution*, 132, 53–66. <https://doi.org/10.1016/j.ympev.2018.11.013>
- 908 Hutchings, P., & Kupriyanova, E. (2018). Cosmopolitan polychaetes – fact or fiction? Personal and
909 historical perspectives. *Invertebrate Systematics*, 32(1), 1–9. <https://doi.org/10.1071/IS17035>
- 910 Jolly, M. T., Viard, F., Gentil, F., Thiébaud, E., & Jollivet, D. (2006). Comparative phylogeography of two
911 coastal polychaete tubeworms in the Northeast Atlantic supports shared history and vicariant
912 events. *Molecular Ecology*, 15(7), 1841–1855. <https://doi.org/10.1111/j.1365-294X.2006.02910.x>
- 913 Jörger, K. M., Norenburg, J. L., Wilson, N. G., & Schrödl, M. (2012). Barcoding against a paradox?
914 Combined molecular species delineations reveal multiple cryptic lineages in elusive meiofaunal
915 sea slugs. *BMC Evolutionary Biology*, 12(1), 245. <https://doi.org/10.1186/1471-2148-12-245>
- 916 Kato, T., Pleijel, F., & Mawatari, S. F. (2001). *Eulalia gemina* (Phyllodocidae: Polychaeta), a newspecies
917 from Shirahama, Japan. *Proceedings of the Biological Society of Washington*, 114, 381–388
- 918 Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7:
919 Improvements in Performance and Usability. *Molecular Biology and Evolution*, 30(4), 772–780.
920 <https://doi.org/10.1093/molbev/mst010>
- 921 Kumar, S., Stecher, G., Li, M., Nnyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary
922 Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35(6), 1547–
923 1549. <https://doi.org/10.1093/molbev/msy096>
- 924 Langeneck, J., Diez, M. E., Nygren, A., Salazar-Vallejo, S., Carrera-Parra, L. F., Vega Fernández, T.,
925 Badalamenti, F., Castelli, A., & Musco, L. (2019). Worming its way into Patagonia: an integrative
926 approach reveals the cryptic invasion by *Eulalia clavigera* (Annelida: Phyllodocidae). *Marine*
927 *Biodiversity*, 49(2), 851–861. <https://doi.org/10.1007/s12526-018-0864-y>
- 928 Langeneck, J., Scarpa, F., Maltagliati, F., Sanna, D., Barbieri, M., Cossu, P., Mikac, B., Galletti, M. C.,
929 Castelli, A., & Casu, M. (2020). A complex species complex: The controversial role of ecology
930 and biogeography in the evolutionary history of *Syllis gracilis* Grube, 1840 (Annelida, Syllidae).
931 *Journal of Zoological Systematics and Evolutionary Research*, 58(1), 66–78.
932 <https://doi.org/10.1111/jzs.12336>
- 933 Leigh, J. W., & Bryant, D. (2015). popart: full-feature software for haplotype network construction.
934 *Methods in Ecology and Evolution*, 6(9), 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- 935 Leite, B. R., Vieira, P. E., Teixeira, M. A. L., Lobo-Arteaga, J., Hollatz, C., Borges, L. M. S., Duarte, S.,
936 Troncoso, J. S., & Costa, F. O. (2020). Gap-analysis and annotated reference library for supporting
937 macroinvertebrate metabarcoding in Atlantic Iberia. *Regional Studies in Marine Science*, 36,
938 101307. <https://doi.org/10.1016/j.rsma.2020.101307>
- 939 Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism
940 data. *Bioinformatics*, 25(11), 1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>

- 941 Lobo, J., Teixeira, M. A. L., Borges, L. M. S., Ferreira, M. S. G., Hollatz, C., Gomes, P. T., Sousa, R.,
 942 Ravara, A., Costa, M. H., & Costa, F. O. (2016). Starting a DNA barcode reference library for
 943 shallow water polychaetes from the southern European Atlantic coast. *Molecular Ecology*
 944 *Resources*, 16(1), 298–313. <https://doi.org/10.1111/1755-0998.12441>
- 945 Lockett, M. M., & Gomon, M. F. (2001). Ship Mediated Fish Invasions in Australia: Two New
 946 Introductions and A Consideration of Two Previous Invasions. *Biological Invasions*, 3(2), 187–
 947 192. <https://doi.org/10.1023/A:1014584201815>
- 948 McIntosh, W. C. (1908). A monograph of British Annelids Part I. Polychaeta. Nephthydidae to Syllidae.
 949 *Ray Society of London*, II., 2, 1-232
- 950 Maggs, C. A., Castilho, R., Foltz, D., HENZLER, C., JOLLY, M. T., KELLY, J., OLSEN, J., PEREZ, K. E., STAM, W.,
 951 VÄINÖLÄ, R., VIARD, F., & WARES, J. (2008). Evaluating Signatures of Glacial Refugia for North
 952 Atlantic Benthic Marine Taxa. *Ecology*, 89(11), S108–S122. <https://doi.org/10.1890/08-0257.1>
- 953 Martin, D., Aguado, M. T., Fernández Álamo, M.-A., Britayev, T. A., Böggemann, M., Capa, M.,
 954 Faulwetter, S., Fukuda, M.V., Helm, C., Petti, M.A.V., Ravara, A., & Teixeira, M.A.L. (2021).
 955 On the Diversity of Phyllodocida (Annelida: Errantia), with a Focus on Glyceridae, Goniadidae,
 956 Nephtyidae, Polynoidae, Sphaerodoridae, Syllidae, and the Holoplanktonic Families. *Diversity*,
 957 13(3), 131. <https://doi.org/10.3390/d13030131>
- 958 Martin, D., Meca, M. A., Gil, J., Drake, P., & Nygren, A. (2017). Another brick in the wall: population
 959 dynamics of a symbiotic species of *Oxydromus* (Annelida, Hesionidae), described as new based
 960 on morphometry. *Contributions to Zoology*, 86(3), 181–211. [https://doi.org/10.1163/18759866-](https://doi.org/10.1163/18759866-08603001)
 961 08603001
- 962 Martin, D., Gil, J., Zanol, J., Meca, M.A., & Pérez Portela, R. (2020). Correction: Digging the diversity of
 963 Iberian bait worms *Marphysa* (Annelida, Eunicidae). *PLOS ONE*, 15, e0233825.
 964 <https://doi.org/10.1371/journal.pone.0233825>
- 965 Meyer, A. (1938). Der Rogen und die Entwicklung der trochophora von *Eulalia viridis*. *Biologia generalis*,
 966 14, 334-89
- 967 Miglietta, M. P., Faucci, A., & Santini, F. (2011). Speciation in the Sea: Overview of the Symposium and
 968 Discussion of Future Directions. *Integrative and Comparative Biology*, 51(3), 449–455.
 969 <https://doi.org/10.1093/icb/icr024>
- 970 Morgado, E. H., & Amaral, A. C. Z. (1983). Anelídeos poliquetos associados ao briozoário Schizoporella
 971 unicornis (Johnston): IV. Phyllodocidae e Hesionidae. *Revista Brasileira de Zoologia*, 2, 49–54.
 972 <https://doi.org/10.1590/S0101-81751983000200002>
- 973 Morton, B. (2011). Predator–prey–scavenging interactions between *Nucella lapillus*, *Carcinus maenas* and
 974 *Eulalia viridis* all exploiting *Mytilus galloprovincialis* on a rocky shore recovering from tributyl-
 975 tin (TBT) pollution. *Journal of Natural History*, 45(39–40), 2397–2417.
 976 <https://doi.org/10.1080/00222933.2011.596637>

- 977 Núñez, J., Brito, M. C., & Docoito, J. R. (2005). Annelid Polychaetes from Canary Islands: Catalogue of
978 species, distribution and habitats. *Vieraea*, 33, 297-32
- 979 Nygren, A., Eklöf, J., & Pleijel, F. (2009). Arctic-boreal sibling species of *Paranaitis* (Polychaeta,
980 Phyllodocidae). *Marine Biology Research* 5(4), 315–327.
981 <https://doi.org/10.1080/17451000802441301>
- 982 Nygren, A. (2014). Cryptic polychaete diversity: a review. *Zoologica Scripta*, 43(2), 172–183.
983 <https://doi.org/10.1111/zsc.12044>
- 984 Nygren, A., Eklöf, J., & Pleijel, F. (2010). Cryptic species of *Notophyllum* (Polychaeta: Phyllodocidae) in
985 Scandinavian waters. *Organisms Diversity & Evolution*, 10(3), 193–204.
986 <https://doi.org/10.1007/s13127-010-0014-2>
- 987 Nygren, A., Parapar, J., Pons, J., Meißner, K., Bakken, T., Kongsrud, J. A., Oug, E., Gaeva, D., Sikorski,
988 A., Johansen, R. A., Hutchings, P. A., Lavesque, N., & Capa, M. (2018). A mega-cryptic species
989 complex hidden among one of the most common annelids in the North East Atlantic. *PLOS ONE*,
990 13(6), e0198356. <https://doi.org/10.1371/journal.pone.0198356>
- 991 Nygren, A., & Pleijel, F. (2011). From one to ten in a single stroke – resolving the European *Eumida*
992 *sanguinea* (Phyllodocidae, Annelida) species complex. *Molecular Phylogenetics and Evolution*,
993 58(1), 132–141. <https://doi.org/10.1016/j.ympev.2010.10.010>
- 994 Olive, P. J. W. (1975). A vitellogenesis promoting influence of the prostomium in the polychaete *Eulalia*
995 *viridis* (müller) (phyllodocidae). *General and Comparative Endocrinology*, 26(2), 266–273.
996 [https://doi.org/10.1016/0016-6480\(75\)90145-8](https://doi.org/10.1016/0016-6480(75)90145-8)
- 997 Peijnenburg, K. T. C. A., Breeuwer, J. A. J., Pierrot-Bults, A. C., & Menken, S. B. J. (2004).
998 Phylogeography of the Planktonic Chaetognath *Sagitta setosa* Reveals Isolation in European Seas.
999 *Evolution*, 58(7), 1472–1487. <https://doi.org/10.1111/j.0014-3820.2004.tb01728.x>
- 1000 Pleijel, F. (1993). Polychaeta Phyllodocidae. *Marine Invertebrates of the Scandinavia*, 8, 1-159
- 1001 Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery
1002 for primary species delimitation. *Molecular Ecology*, 21(8), 1864–1877.
1003 <https://doi.org/10.1111/j.1365-294X.2011.05239.x>
- 1004 Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior Summarization in
1005 Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology*, 67(5), 901–904.
1006 <https://doi.org/10.1093/sysbio/syy032>
- 1007 Ratnasingham, S., & Hebert, P. D. N. (2013). A DNA-Based Registry for All Animal Species: The Barcode
1008 Index Number (BIN) System. *PLOS ONE*, 8(7), e66213.
1009 <https://doi.org/10.1371/journal.pone.0066213>
- 1010 Ravara, A., Ramos, D., Teixeira, M. A. L., Costa, F. O., & Cunha, M. R. (2017). Taxonomy, distribution
1011 and ecology of the order Phyllodocida (Annelida, Polychaeta) in deep-sea habitats around the

1012 Iberian margin. *Deep Sea Research Part II: Topical Studies in Oceanography*, 137, 207–231.
1013 <https://doi.org/10.1016/j.dsr2.2016.08.008>

1014 Read, G.; Fauchald, K. (Ed.) (2022). World Polychaeta Database. *Eulalia virens* Ehlers, 1864. Accessed
1015 through: World Register of Marine Species at:
1016 <https://www.marinespecies.org/aphia.php?p=taxdetails&id=339528> on 2022-03-30

1017 Rodrigo, A. P., Costa, M. H., Matos, A. P. A. de, Carrapiço, F., & Costa, P. M. (2015). A Study on the
1018 Digestive Physiology of a Marine Polychaete (*Eulalia viridis*) through Microanatomical Changes
1019 of Epithelia During the Digestive Cycle. *Microscopy and Microanalysis*, 21(1), 91–101.
1020 <https://doi.org/10.1017/S143192761401352X>

1021 Rodrigo, A. P., & Costa, P. M. (2019). The hidden biotechnological potential of marine invertebrates: The
1022 Polychaeta case study. *Environmental Research*, 173, 270–280.
1023 <https://doi.org/10.1016/j.envres.2019.03.048>

1024 Rodrigo A. P. (2020). The biotechnological value of a novel potent marine biotoxin from the polychaete
1025 worm *Eulalia viridis*: chemical and toxicological evaluation. Universidade Nova Lisboa. PhD
1026 thesis. Available at: <http://hdl.handle.net/10362/116178>.

1027 Rodrigo, A. P., Grosso, A. R., Baptista, P. V., Fernandes, A. R., & Costa, P. M. (2021a). A Transcriptomic
1028 Approach to the Recruitment of Venom Proteins in a Marine Annelid. *Toxins*, 13(2), 97.
1029 <https://doi.org/10.3390/toxins13020097>

1030 Rodrigo, A. P., Mendes, V. M., Manadas, B., Grosso, A. R., Alves de Matos, A. P., Baptista, P. V., Costa,
1031 P. M., & Fernandes, A. R. (2021b). Specific Antiproliferative Properties of Proteinaceous Toxin
1032 Secretions from the Marine Annelid *Eulalia* sp. onto Ovarian Cancer Cells. *Marine Drugs*, 19(1),
1033 31. <https://doi.org/10.3390/md19010031>

1034 Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed
1035 models. *Bioinformatics*, 19(12), 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>

1036 Sampieri, B. R., Vieira, P. E., Teixeira, M. A. L., Seixas, V. C., Pagliosa, P. R., Amaral, A. C. Z., & Costa,
1037 F. O. (2021). Molecular diversity within the genus *Laeonereis* (Annelida, Nereididae) along the
1038 west Atlantic coast: paving the way for integrative taxonomy. *PeerJ*, 9, e11364.
1039 <https://doi.org/10.7717/peerj.11364>

1040 Schimmenti, E., Musco, L., Brutto, S. L., Mikac, B., Nygren, A., & Badalamenti, F. (2016). A
1041 Mediterranean record of *Eulalia ornata* (Annelida: Phyllodoceidae) corroborating its fidelity link
1042 with the Sabellaria alveolata-reef habitat. *Mediterranean Marine Science*, 17(2), 359–370.
1043 <https://doi.org/10.12681/mms.1485>

1044 Schmitt, T., Fritz, U., Delfino, M., Ulrich, W., & Habel, J. C. (2021). Biogeography of Italy revisited:
1045 genetic lineages confirm major phylogeographic patterns and a pre-Pleistocene origin of its biota.
1046 *Frontiers in Zoology*, 18(1), 34. <https://doi.org/10.1186/s12983-021-00418-9>

1047 Schwindt, E., López Gappa, J., Raffo, M. P., Tatián, M., Bortolus, A., Orensanz, J. M., et al. (2014). Marine
1048 fouling invasions in ports of Patagonia (Argentina) with implications for legislation and

1049 monitoring programs. *Marine Environmental Research*, 99, 60–68.
1050 <https://doi.org/10.1016/j.marenvres.2014.06.006>

1051 Sosa, A., Núñez, J. & Bacallado, J.J. (1976). Contribución al estudio de los poliquetos en Canarias. I:
1052 Aphroditidae, Amphinomidae, Phyllocidae y Eunicidae. *Vieraea*, 6(2), 231-252

1053 Taboada, S., Leiva, C., Bas, M., Schult, N., & McHugh, D. (2017). Cryptic species and colonization
1054 processes in *Ophryotrocha* (Annelida, Dorvilleidae) inhabiting vertebrate remains in the shallow-
1055 water Mediterranean. *Zoologica Scripta*, 46(5), 611–624. <https://doi.org/10.1111/zsc.12239>

1056 Teixeira, M. A. L., Vieira, P. E., Pleijel, F., Sampieri, B. R., Ravara, A., Costa, F. O., & Nygren, A. (2020).
1057 Molecular and morphometric analyses identify new lineages within a large *Eumida* (Annelida)
1058 species complex. *Zoologica Scripta*, 49(2), 222–235. <https://doi.org/10.1111/zsc.12397>

1059 Teixeira, M. A. L., Vieira, P. E., Ravara, A., Costa, F. O., & Nygren, A. (2022). From 13 to 22 in a second
1060 stroke: revisiting the European *Eumida sanguinea* (Phyllocidae: Annelida) species complex.
1061 *Zoological Journal of the Linnean Society*, Efirst.

1062 Ushakov, P. L. (1972). Polychaetes of the sub-order Phyllociforma of the Polar Basin and the
1063 northwestern part of the Pacific. In Russian. Translated by the Israel Program for Scientific
1064 Translations: Jerusalem, 1974. *Fauna SSSR*, 102, 1- 271

1065 Vieira, P. E., Desiderato, A., Holdich, D. M., Soares, P., Creer, S., Carvalho, G. R., Costa, F. O., &
1066 Queiroga, H. (2019). Deep segregation in the open ocean: Macaronesia as an evolutionary hotspot
1067 for low dispersal marine invertebrates. *Molecular Ecology*, 28(7), 1784–1800.
1068 <https://doi.org/10.1111/mec.15052>

1069 Viéitez, J. M., Alós, C., Parapar, J., Besteiro, C., Moreira, J., Núñez, J., Laborda, A. J. & San Martín, G.
1070 (2004). Annelida Polychaeta I. In Ramos, M.A. et al. (Eds.). *Fauna Iberica*, Vol. 25 (530 pp).
1071 Museo Nacional de Ciencias Naturales CSIC, Madrid

1072 Weitschek, E., Fison, G., & Felici, G. (2014). Supervised DNA Barcodes species classification: analysis,
1073 comparisons and results. *BioData Mining*, 7(1), 4. <https://doi.org/10.1186/1756-0381-7-4>

1074 Wonham, M. J., Carlton, J. T., Ruiz, G. M., & Smith, L. D. (2000). Fish and ships: relating dispersal
1075 frequency to success in biological invasions. *Marine Biology*, 136(6), 1111–1121.
1076 <https://doi.org/10.1007/s002270000303>

1077 Zenetos, A., Meric, E., Verlaque, M., Galli, P., Boudouresque, C. F., Giangrande, A., Cinar, M., &
1078 Bilecenoglu, M. (2008). Additions to the annotated list of marine alien biota in the Mediterranean
1079 with special emphasis on Foraminifera and Parasites. *Mediterranean Marine Science*, 9(1), 119–
1080 166. <https://doi.org/10.12681/mms.146>

1081 Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with
1082 applications to phylogenetic placements. *Bioinformatics*, 29(22), 2869–2876.
1083 <https://doi.org/10.1093/bioinformatics/btt499>

1084

1085 **Table and figure captions**

1086 **Table 1** Number of specimens acquired for this study (n), the respective sampling area and code
1087 abbreviation for the different sampling locations

1088

1089 **Table 2** Primers and PCR conditions used in this study

1090

1091 **Table 3** Mean intra (in bold) and inter-MOTU genetic distances (K2P) for the three analysed markers (COI,
1092 ITS, 28S), for the 9 retrieved *Eulalia* MOTUs and two outgroups.

1093

1094 **Table 4** Indices of genetic diversity estimated for each *Eulalia* species and outgroups (OUTG), based on
1095 COI. Number of sequences (n); nucleotide diversity (π), number of haplotypes (h), haplotype diversity (Hd)
1096 and number of variables sites (S). Region abbreviations as stated in Table 1

1097

1098 **Table 5** Summary of the most relevant morphometric findings rating from 1 (smaller proportions) to 4
1099 (larger proportions), number of segments (NS), worm length (WL), worm width (WW), live and preserved
1100 coloration, depth and geographical range between the new described species and *E. clavigera* s.s..
1101 Abbreviations for the morphometric proportions as stated in the methods

1102

1103 **Fig. 1** Map with the sampling sites used for this study. Abbreviations as seen in Table 1.

1104

1105 **Fig. 2** Schematic of the *Eulalia clavigera* morphotype showing the measurements used in the morphometric
1106 analysis. **a** Anterior end. **b** Parapodia. Abbreviations: CLL, the length of the chaetigerous lobes; CLH, the
1107 height of the chaetigerous lobes; AL, the length of the antennae; PL, the length of the palps; MAL, the
1108 length of the middle antenna; DTL, dorsal tentacular cirri on segment 2; VTL, ventral tentacular cirri on
1109 segment 2; DCL, the length of the dorsal cirri; VCL, the length of the ventral cirri; HL, the length of the
1110 head; WWP, the width of the worm with parapodia; WW, the width of the worm without parapodia; HW,
1111 the width of the head; DCW, the width of the dorsal cirri; VCW, the width of the ventral cirri; DE, distance
1112 between the eyes.

1113

1114 **Fig. 3** Phylogenetic tree and respective COI haplotypes and MOTU locations. **a** Phylogenetic tree
1115 reconstructed using Bayesian inference based on concatenated COI, ITS regions and 28S sequences, with
1116 information regarding the different MOTU delineation methods. BINs were used only for COI. MOTU
1117 GB1 only have COI sequences and was not present in BOLD systems preventing BIN analysis. Only the
1118 bootstrap values over 0.85 BI and 85 ML support are shown. Each different consensus MOTU is
1119 represented by the respective number, with the different colours corresponding to the respective geographic
1120 distribution. Live photo belong to the specimen DBUA0002464.02, measuring around 45 mm in length and
1121 exhibiting greenish colour. **b** Haplotype network based on COI for all the analysed MOTUs and outgroups
1122 (OUTG). Each haplotype is represented by a circle and number of haplotypes are according to the displayed
1123 scale. Colours indicate the geographic location of the haplotype. Numbers correspond to the number of
1124 mutational steps between haplotypes. Lines without numbers means only one mutation between haplotypes.

1125

1126 **Fig. 4** Haplotypes networks based on ITS **(a)** and 28 **(b)** for all MOTUs and outgroups, except MOTU GB1.
1127 Each haplotype is represented by a circle and number of haplotypes are according to the displayed scale.
1128 Colours indicate the geographic location of the haplotype. Numbers correspond to the number of mutational
1129 steps between haplotypes. Lines without numbers means only one mutation between haplotypes.

1130

1131 **Fig. 5** Scatter plots with the most considerable proportions in distinguishing *E. clavigera* (populations from
1132 mainland Europe and Canary islands), *E. feliciae* sp. nov., *E. madeirensis* sp. nov. and *E. xanthomucosa*
1133 sp. nov. **a** Morphometric proportions between the length of the ventral cirri (VCL) and the length of the
1134 dorsal cirri (DCL). **b** between the length of the dorsal cirri (DCL) and the width of the dorsal cirri (DCW).
1135 **c** between the length of the chaetigerous lobe (CLL) and the length of the ventral cirri (VCL). **d** between
1136 the length of the head (HL) and the width of the head (HW). **e** between the length of the head (HL) and
1137 dorsal tentacular cirri on segment 2 (DTL). **f** between the length of the head (HL) and the length of the
1138 antennae (AL). **g** between the length of the head (HL) and length of the median antenna (MAL). **h** between
1139 the width of the worm of median segments (WW) and the number of segments (NS).

1140

1141 **Fig. 6** Live, relaxed *Eulalia* specimens exhibiting the different types of coloration corresponding to the new
1142 described species and information regarding the specimen size (WL: worm length). **a** *Eulalia feliciae* sp.
1143 nov., specimen DBUA0002468.07, dorsal view, exhibiting greenish colour. **b** *Eulalia xanthomucosa* sp.
1144 nov., specimen from the Natural History Museum, live photo by David Fenwicki (left) and specimen BI-
1145 2014/15-077 (right), dorsal view, exhibiting yellow colour. **c** *Eulalia madeirensis* sp. nov., specimen
1146 DBUA0002469.03, dorsal view, exhibiting a faint yellowish/light green colour.

1147

1148

1149 SUPPORTING INFORMATION

1150

1151 Additional Supporting Information can be found in the online version of this article at the publisher's web-
1152 site:

1153

1154 **Table S1** Voucher data, origin of the specimens and GenBank accession numbers for each of the analysed
1155 genetic markers original to this study and molecular metadata used for comparison purposes or as
1156 outgroups.

1157

1158 **Table S2** Measurements for all the specimens used in morphometry belonging to *Eulalia clavigera* with
1159 populations from the Macaronesia islands and mainland Europe, *Eulalia feliciae* sp. nov., *Eulalia*
1160 *madeirensis* sp. nov. and *Eulalia xanthomucosa* sp. nov.

1161