1 **From thirteen to twenty-two in a second stroke: revisiting the European *Eumida sanguinea***
2 **(Phyllodocidae: Annelida) species complex**

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4 Marcos A.L. Teixeira[1,2*], Pedro E. Vieira[1,2], Ascensão Ravara[4], Filipe O. Costa[1,2], Arne Nygren[3]

5 [1] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of
6 Minho, Campus de Gualtar, 4710-057, Braga, Portugal
7 [2] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus de
8 Gualtar, 4710-057, Braga, Portugal
9 [3] Institutionen for marina vetenskaper, Göteborgs Universitet, Tjärnö, Strömstad, Sweden
10 [4] Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of
11 Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal.

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13 *Corresponding author*
14 Mail: mark-us_teixeira@hotmail.com

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16 **Running Title:** Revisiting the *Eumida sanguinea* species complex

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42 ABSTRACT

43 *Eumida sanguinea* is a recognized polychaete species complex which, in previous studies, has

44 been reported to have additional undescribed diversity. We detected nine additional lineages by

45 analysing DNA sequence (mitochondrial: COI, 16S rRNA and nuclear loci: ITS region and 28S

46 rRNA) of *E. sanguinea* morphotype populations from a broader sampling effort in European

47 marine waters. Customary morphological features failed to provide consistent differences or

48 unique characters that could be used to distinguish these *Eumida* species. However, by

49 complementing DNA data with morphometrics, geographic range, colour, and pigmentation

50 patterns, we revealed five new species. Two of these undescribed species derived from the

51 previously signalled *Eumida* lineages S21 and GB22, which are now named as *E. schanderi* sp.

52 nov. and *E. fenwicki* sp. nov., respectively. Three other species based on newly discovered

53 lineages, namely *E. gretathunbergae* sp. nov., *E. pleijeli* sp. nov., and *E. langenecki* sp. nov. From

54 the six new lineages remaining, three are represented by less than two exceptionally well-

55 preserved specimens, which prevented further comprehensive analysis. The last three lineages

56 were only distinct with mitochondrial markers. Integrative taxonomy is essential to elucidate

57 evolutionary phenomena and eventually allow informed use of species complexes, exhibiting

58 stasis in biomonitoring or other ecological studies.

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60 ADDITIONAL KEYWORDS: *Eumida*, Phyllodocidae, pigmentation, morphological stasis

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INTRODUCTION

The species *Eumida sanguinea* (Örsted, 1843) was originally described from the Danish coast (WoRMS Editorial Board, 2021) and has been commonly reported in the Atlantic northern hemisphere (Eibye-Jacobsen, 1991), including the northern Iberian Peninsula (Leite *et al.,* 2019), as well as in Madagascar, Mozambique (Day *et al.,* 1967), and New Zealand (Glasby *et al.,* 2009). It is usually found in sandy-muddy substrates or gravel and among algae in shallow subtidal habitats, ranging from a few to hundreds of meters in depth (Eibye-Jacobsen, 1991), including estuaries and coastal lagoons (Walker & Rees, 1980). As a phyllodocid, it is believed to be a carnivore (Jumars, Dorgan, & Lindsay, 2015), but no published study has yet described its specific feeding habits. Although its planktotrophic larvae enable a large-scale dispersal (Pleijel, 1993; Rouse, 2006) its cosmopolitan status has recently been challenged. In European seas, 13 different lineages have already been reported to belong to the *Eumida sanguinea* species complex (*Essc*). By combining multi-locus molecular data with the white pigmentation pattern observed in live animals, Nygren & Pleijel (2011) defined nine of those lineages as nominal species: *Eumida sanguinea s.s.*; *Eumida notata* (Langerhans, 1880); *Eumida alkyone* Nygren & Pleijel, 2011; *E. asterope* Nygren & Pleijel, 2011; *E. elektra* Nygren & Pleijel, 2011; *E. kelaino* Nygren & Pleijel, 2011; *E. maia* Nygren & Pleijel, 2011; *E. merope* Nygren & Pleijel, 2011; and *E. taygete* Nygren & Pleijel, 2011. More recently, Teixeira *et al.* (2020) delineated another species from the complex, *Eumida mackiei* Teixeira, Pleijel & Nygren, by adding quantitative morphometric analyses to the methodology used in the previous study. The remaining putative species [*Eumida* F22 and *Eumida* S21 from Nygren & Pleijel (2011)], and *Eumida* GB22 from Teixeira *et al.* (2020), could not be described because only one specimen of each was available, which is not ideal, especially when the description is heavily based on molecular data (Churchill *et al.*, 2014; Delić *et al.*, 2017) and morphometric analyses (Ravara, *et al*., 2010; Martin *et al.*, 2017). All these putative species appeared to be sympatric with at least one other species of the complex, except for *E. notata* that is exclusive to Madeira Island (Portugal) and possesses a unique white pigmentation pattern among the *Essc*. However, there was no consensus between mitochondrial and nuclear loci sequences (Teixeira *et al.*, 2020) in the segregation of *E. notata* from its sister Mediterranean species, *Eumida merope,* as separate molecular operational taxonomic units (MOTUs).

The new *Essc* illustrated in Nygren & Pleijel (2011) were described based on systematic molecular analyses, an approach applicable when there are no evident morphological differences (cryptic species). Apart from the white pigmentation pattern in live worms, the morphology of antennae, anterior cirri, and parapodia provided no consistent differences to be used to distinguish species. Moreover, all setae are composite within the entire genus (Pleijel, 1993). As seen in Teixeira *et al.* (2020), slight differences in shape and length of dorsal and ventral cirri, in the anterior appendages, or distance between eyes and head width can be further explored by morphometric analyses. However, intraspecific and size-dependent variations are large and can

120 fail to produce independent clusters. The presence and arrangement of proboscis papillae may
121 be distinctive traits; however, the proboscis is often not everted, and papillae are sometimes
122 difficult to detect in preserved specimens. Nevertheless, Teixeira *et al.* (2020) reported the
123 presence of proboscis papillae in *E. maia* as opposed to the smooth appearance described for *E.*
124 *sanguinea s.s.* (Pleijel, 1993). Reproductive features and gametogenesis may be a useful
125 alternative in discriminating closely related species, as seen in Sampieri *et al.* (2020), in which
126 two cryptic *Laeonereis* (family Nereididae) lineages were distinguished using both COI and
127 histological data. However, specimens have to be directly preserved in a special preservation
128 solution (e.g., 10% glutaraldehyde) instead of ethanol, which, in turn, may affect DNA
129 amplification success.

130 Morphological stasis has been pointed to as a possible justification for morphological
131 similarities within cryptic complexes, wherein some members retain a high degree of
132 morphological similarity over extended periods (Costa & Carvalho, 2010; Cerca *et al.*, 2020b).
133 Although it has been investigated by combining comprehensive data on genomic and phenotypic
134 traits to statistically test for significant differences in rates of phenotypic disparity between cryptic
135 and non-cryptic species (Struck *et al.*, 2018), stasis remains a controversial issue in evolutionary
136 biology (Crossman *et al.*, 2016; Fraïsse *et al.*, 2016; Fišer *et al.* 2018). Morphological characters
137 and their variation are important to identify and discriminate specimens and species; therefore,
138 their absence is often interpreted as a potential failure to capture and study biodiversity (Futuyma,
139 2010). Finding new cryptic lineages and combining molecular tools with occasional small
140 morphological trait changes in lineages displaying stasis is essential to help comprehend this
141 evolutionary phenomenon.

142 In this study, nine new *Essc* lineages were uncovered in the European NE Atlantic and
143 Mediterranean Sea, with five of them being erected to accommodate the previously undescribed
144 *Eumida* S21 (Nygren & Pleijel, 2011) and *Eumida* GB22 (Teixeira *et al.*, 2020), and three of them
145 unravelled for the first time. The lineages were defined based on four different loci and
146 supplemented by data on morphometrics, geographic range, colour, and pigmentation patterns.
147 Furthermore, new sequences were provided for the previously described species, both from
148 populations already located such as *E. maia* from Great Britain (Plymouth), *E. taygete* from
149 France (Banyuls), and *E. alkyone* from Norway (Bergen and Drøbak), as well as unreported
150 locations like *E. kelaino* from Great Britain (Plymouth), France (Roscoff), and Norway (Sandefjord
151 and Bergen); *E. merope* from Great Britain (Plymouth) and France (Roscoff); *E. elektra* from
152 France (Roscoff); *E. sanguinea s.s.* from Great Britain (Plymouth); and lastly *E. taygete* from
153 Great Britain (Plymouth) and western Italy (Ischia). The close molecular similarity between some
154 of the new lineages was discussed from an evolutionary perspective, and the *Essc* case was used
155 to investigate links between morphological stasis and cryptic diversity.

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157 MATERIAL AND METHODS
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159 TAXON SAMPLING, IMAGE CAPTURE, AND MOLECULAR DATA RETRIEVAL

160   One hundred and forty-eight *Eumida* specimens were collected from Norway (Agdenes –
161   NOA; Bergen – NOB; Drøbak – NOD; and Sandefjord - NOS), Sweden (Bohuslän - SWB), France
162   (Roscoff - FRR and Banyuls - FRB), Great Britain (Plymouth – GBP and Cornwall - GBC), and
163   Italy (Ischia – ITI; Taranto – ITT; Antignano – ITA; Naples – ITN; and Orbetello - ITO) and fixed
164   in 96% ethyl alcohol for molecular analysis. Photographs of live and preserved specimens were
165   taken with a Canon EOS1100D camera. The specimens from Norway are deposited at the
166   University Museum of Bergen (ZMBN), and the remaining ones at the Biological Research
167   Collection (Marine Invertebrates) of the Department of Biology of the University of Aveiro (COBI
168   at DBUA), Portugal. Additional specimens of *E. merope* and *E. notata* were kindly loaned by the
169   Swedish Museum of Natural History (SMNH), and COBI-DBUA. The two specimens of *E. taygete*,
170   MTANE128-19 and MTANE129-19, had all their tissue used for DNA extraction purposes, and no
171   voucher is available.

172   We obtained sequences of mitochondrial cytochrome oxidase subunit I (mtCOI-5P) from
173   all the new available 148 specimens, and mitochondrial 16S rRNA, nuclear ITS-regions (i.e., ITS1,
174   5.8S rRNA, and ITS2), and 28S rRNA for a representative number of specimens per location. For
175   comparison purposes, a compilation of 149 published sequences from the mtCOI, as well as 100
176   sequences from the ITS-regions and 28S rRNA corresponding to the *Essc*, and the respective
177   outgroups were mined from the GenBank, originally from the studies of Nygren & Pleijel (2011)
178   and Teixeira *et al.* (2020). Moreover, 35 novel 16S sequences were retrieved during this work
179   from specimens used in the previous studies. Molecular data from *Eumida bahusiensis*
180   Bergström, 1914; *Eumida ockelmanni* Eibye-Jacobsen, 1987; and *Sige fusigera* Malmgren, 1865
181   were used as outgroups for all alignments to comprise the final dataset. The full dataset and
182   associated metadata can be accessed at the Barcode of Life Data Systems (BOLD), under the
183   project "Five new species - *Eumida sanguinea* complex (DS-MTANE2)" and in the following link:
184   DOI: dx.doi.org/10.5883/DS-MTANE2*.* Table S1 details the sampling locations, GenBank
185   accession numbers, and voucher data. DNA was extracted, amplified, sequenced, and
186   assembled as described in Nygren *et al.* (2018). Table 1 displays the PCR conditions and primers
187   used.

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189   PHYLOGENETIC ANALYSIS AND GENETIC DISTANCES
190   A methodology similar to that of Teixeira *et al.* (2020) was applied for the phylogenetic
191   analysis of the different loci by maximum likelihood (ML) and Bayesian inference (BI). In brief,
192   mitochondrial markers (COI and 16S) were concatenated and aligned in MEGA 10.0.5 software
193   (Kumar *et al.*, 2018) with Clustal W (Thompson *et al.*, 1994). Nuclear markers (ITS regions and
194   28S) were also concatenated and aligned with MAFFT online (https://
195   mafft.cbrc.jp/alignment/server/; Katoh & Standley, 2013). Table 1 included all marker sequence
196   lengths. Highly variable regions, extensive gaps, and poorly aligned positions, which were
197   extensively present only in the concatenated nuclear alignment, were eliminated using Gblocks
198   0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html; Castresana, 2000). The
199   options for a less stringent selection and to not allow many contiguous non-conserved positions

200 were selected, making it more suitable for phylogenetic analysis. We used MrBayes 3.1.2
201 (Ronquist & Huelsenbeck, 2003) to conduct the Bayesian analysis. Best-fit models were selected
202 using the Akaike Information Criterion in the JModeltest software (Guindon & Gascuel, 2003;
203 Darriba *et al.*, 2012). For COI, we applied the Hasegawa-Kishino-Yano with gamma-distributed
204 rates across sites (HKY +G) for the third position and the General Time-Reversible (GTR) model
205 with equal rates across sites (GTR) for the first two positions. The latter was also applied to the
206 16S analysis. Regarding the concatenated ITS region with 28S, we applied the GTR model with
207 gamma-distributed rates across sites (GTR +G). The number of generations was set to 10 000
208 000, and the sampling frequency to 500. Twenty-five per cent of the samples were discarded as
209 burn-in (burninfrac = 0.25). The resulting tree files were successfully checked for convergence in
210 Tracer 1.6 software (Rambaut *et al.*, 2018) and then analysed in Figtree 1.4.3
211 (http://tree.bio.ed.ac.uk/software/figtree/). The final version of the trees for each alignment was
212 edited with the software Inkscape 0.92.3 (https://www.inkscape.org). Maximum likelihood
213 phylogenies were performed in MEGA 10.0.5 with 1 000 bootstrap runs, using the GTR model
214 with equal rates across sites for both concatenated datasets. Only the BI tree was displayed in
215 the results and, in the case of a similar topology, with the addition of the ML support values. The
216 alignments (FASTA and NEXUS formats) for each marker and the concatenated ones are all
217 publicly available online at Figshare (DOI: 10.6084/m9.figshare.12114528).
218 The mean genetic distances (Kimura-2-parameters, K2P) within and between MOTUs
219 were calculated in MEGA 10.0.5, using the same GBlock alignment from above for nuclear loci.
220

221 MOTU CLUSTERING

222 To depict MOTUs, we applied three delineation methods to both the concatenated
223 mitochondrial and nuclear alignments except for COI, to which we also applied the Barcode Index
224 Number (BIN), implemented in BOLD (Ratnasingham & Hebert, 2013), which is exclusive to this
225 locus. The Automatic Barcode Gap Discovery (ABGD, Puillandre *et al.*, 2012) approach was
226 implemented on a web interface (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with
227 default settings and K2P distance matrix. Both Generalized Mixed Yule Coalescent (GYMC)
228 single threshold model (Fujisawa & Barraclough, 2013) and Poisson Tree Processes (bPTP,
229 Zhang *et al.*, 2013) were applied on a web interface (https://species.h-its.org/). BEAST 2.4.6
230 (Bouckaert *et al.*, 2014) was used to generate a Bayesian ultrametric tree for the GYMC, with the
231 appropriate best model (based on AIC criteria; GTR equal rates) and four independent runs for
232 50 000 000 MCMC generations, sampled every 5 000 generations. Tracer 1.6 software was used
233 to estimate convergence in effective sampling sizes (ESSs > 200) for all parameters. A consensus
234 tree was obtained using TreeAnnotator 2.4.6 (Bouckaert *et al.*, 2014) and loaded into the Figtree
235 software. The ML phylogenies obtained in the "phylogenetic analysis" section contributed to the
236 bPTP results. Consensus MOTUs were defined based on the majority rule and, in case of a draw,
237 an intermediate MOTU was chosen.
238

239 GENETIC DIVERSITY AND STRUCTURE

240   To evaluate the relationship between haplotypes and their geographical distribution,

241   haplotype networks were built through the PopART software (Leigh & Bryant, 2015) using the

242   TCS method (Clement *et al.*, 2002). No GBlocks were applied in this analysis to avoid

243   underestimating the number of nuclear haplotypes. Indices of genetic diversity, namely number

244   of haplotypes (h), haplotype diversity (HD), polymorphic sites (S), nucleotide diversity (π), and Fu

245   & Li D and Tajima D statistical tests, were estimated based on COI for each MOTU, using the

246   DNASP 5.10 software (Librado & Rozas, 2009).

247

248   MORPHOMETRY

249   Two objectives were proposed for morphometric analysis, namely: (1) to explore if

250   genetically similar species belonging to *E. notata*, *E. merope*, and the new British lineage *E. aff.*

251   *merope* can be separated using the methodology and (2) to complement molecular results with

252   morphometric data to help describe the new species E*. schanderi* sp. nov., *E. fenwicki* sp. nov.,

253   and *E. gretathunbergae* sp. nov. Since in the previous study (Teixeira *et al.*, 2020), *E. sanguinea*

254   *s.s.* showed high morphometric intraspecific variability and failed to produce an independent

255   morphometric cluster, we used new samples of *E. elektra* for comparison purposes. This species

256   was chosen for being usually located in the middle of the phylogenetic tree and within the average

257   *Essc* genetic distances.

258   The remaining lineages were represented by very small and/or less than three specimens

259   and thus were not used for this analysis. At least nine preserved specimens under ideal conditions

260   (i.e., with the morphological characters proposed herein and, if possible, of similar sizes) were

261   chosen per population. All the different morphological characters were measured directly from the

262   specimen, without dissecting specific structures.

263   The following characters were selected and measured (Fig. 1A-B): number of segments

264   (NS); the lengths (in mm) of worm (WL), chaetigerous lobes (CLL), terminal antennae (AL), palps

265   (PL), median antenna (MAL), cirri on segment 1 and dorsal cirri on segment 2 (CS1L, DCS2L),

266   dorsal and ventral cirri on median segments (DCL, VCL), and head (HL); the widths (in mm) of

267   worm with parapodia (WWP) and without parapodia (WW), head (HW), and dorsal and ventral

268   cirri of median segments (DCW, VCW); and distance between eyes (DE), as well as height (mm)

269   of chaetigerous lobes (CLH). Although the first two segments are fused, we refer to them as

270   segments 1 and 2, with the latter having a pair of cirri (dorsal and ventral). WW and WWP were

271   measured from the worm's widest part, usually from either segment 27 or 40, depending on the

272   worm's size. The distance between eyes was measured from the centre of the eyespots to avoid

273   possible different individual responses to fixation as is the case of hesionids (Martin *et al.*, 2017).

274   To minimize bias based on size variability, measurements taken for inter-population analysis were

275   converted to ratios and used to create scatter plots for the following pairs of morphological

276   characters: AL/STL, AL/LTL, AL/HL, AL/HW, AL/PL, AL/MAL, PL/MAL, HL/MAL, HW/MAL,

277   STL/LTL, LTL/HL, STL/HL, PL/STL, PL/LTL, DE/HL, DE/WW, HL/HW, WW/WWP, WW/NS,

278   WL/NS, WW/WL, HL/MAL, DCL/VCL, DCL/DCW, VCL/VCW, DCL/CLL, VCL/CLL, and CLL/CLH.

279   The scatter plots were produced using Microsoft Excel (Office 365 ProPlus).

280   Although not used in the above analysis due to lack of available specimens in optimal
281   conditions to allow the creation of morphometric clusters, additional measurements were also
282   collected for two Italian lineages (*E. pleijeli* sp. nov. and *E. langenecki* sp. nov.). The ratio of
283   common morphological structures used to separate *Eumida* species might provide additional
284   information to be used as differential diagnoses against the remaining analysed lineages.
285   Emphasis was given to: antennae, palps, cirri on segment 1, dorsal cirri on segment 2, dorsal cirri
286   of median segments and ventral cirri of median segments.

287   All measurements were done with a LEICA MC170 HD stereo microscope, with an
288   incorporated measurement software. Supplementary Table S2 shows detailed morphometric
289   values for each specimen.

290

291   RESULTS

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293   PHYLOGENY RECONSTRUCTION
294   The BI phylogenetic trees (Fig. 2A, B) were created from a dataset of 297 COI, 94 16S,
295   192 ITS, and 28S sequences belonging to specimens of the *Essc* and four outgroup species (*E.*
296   *bahusiensis*, *E. ockelmanni*, *S. fusigera*, *and E. aff. ockelmanni*). Support values over 0.85 are
297   shown in the BI trees. Since BI and ML trees display a different topology, ML bootstrap values
298   are not shown in the BI tree. Detailed ML trees with 1 000 bootstrap support values, for both
299   mitochondrial and nuclear concatenated datasets, are available in the supplementary material
300   (Fig. S1 and Fig. S2, respectively).

301   Both mitochondrial and nuclear loci showed evidence of at least five new *Eumida* MOTUs
302   compared to the previous studies, with mitochondrial markers also revealing a distinct British
303   MOTU close to *E. merope,* hereafter referred to as *E. aff. merope* (MOTU 11, Fig. 2A); a new
304   Mediterranean MOTU close to *E. kelaino*, hereafter referred to as *E. aff. kelaino* (MOTU 17, Fig.
305   2A)*;* another British MOTU close to *E. gretathunbergae* sp. nov.*,* hereafter referred to as *E. aff.*
306   *gretathunbergae* (MOTU 13, Fig. 2A); and lastly an additional unnamed Italian lineage *Eumida*
307   ORB997 (MOTUs 2 and 23, Fig. 2A, B) close to the new species *E. pleijeli* sp. nov.

308   Apart from outgroups, the number of consensus MOTUs ranged between 18 (Fig. 2B)
309   and 22 (Fig. 2A). Most of them were present either in Great Britain, Scandinavia, or southern
310   France. The newly described species, *Eumida gretathunbergae* sp. nov., was present in the
311   British Isles and northern France (MOTU 12 and 25, Fig. 2A, B); *E. pleijeli* sp. nov. (MOTUs 3 and
312   23, Fig. 2A, B) *and E. langenecki* sp. nov. (MOTUs 5, Fig. 2A, B) were both in Western Italy; *E.*
313   *schanderi* sp. nov. [previously referred to as *Eumida* unnamed species S21 from Nygren & Pleijel
314   (2011)] exclusively in Norway and Sweden (MOTUs 22 and 26, Fig. 2A, B); and lastly *E. fenwicki*
315   sp. nov. [previously named *Eumida* unnamed species GB22 from Teixeira *et al.* (2020)] in both
316   Scandinavia and Great Britain (MOTU 6, Fig. 2A, B)*.*

317   The closely related species *E. notata* (MOTU 9, Fig. 2A) and *E. merope* (MOTU 10, Fig.
318   2A), including the new British lineage *E. aff. merope* (MOTU 11, Fig. 2A), could be completely
319   sorted using mitochondrial loci, forming highly supported clades in the BI tree. However, only one

320   of the clustering algorithms could split these lineages into three distinct MOTUs by using nuclear

321   markers, with the remaining ones being clustered together in a low supported monophyletic clade

322   instead (MOTU 24, Fig. 2B). A similar pattern was also observed between *Eumida pleijeli* sp. nov.

323   and *Eumida* ORB997 (MOTU 23, Fig. 2B), between *E. aff. gretathunbergae* and *E. gretathunbergi*

324   sp. nov. (MOTU 25, Fig. 2B), and between *E. aff. kelaino* and *E. kelaino* (MOTU 27, Fig. 2B).

325   Distinct marker-dependent MOTU sorting cases were also observed for *E. schanderi* sp.

326   nov., in which MOTU 26 was delimited only with nuclear markers. This sorting is recorded

327   independently for both ITS and 28S, as evidenced in the haplotype networks detailed further

328   below.

329

330   GENETIC DISTANCES AND *EUMIDA AFF. OCKELMANNI*

331   Assuming *E. aff. merope, E. aff. kelaino*, and *E. aff. gretathunbergae* as valid species,

332   the global mean genetic distances for the whole *complex* can be found in Table 2, including the

333   distances of the most similar and divergent MOTUs for the nearest and farthest neighbours,

334   respectively. The mean intraspecific distances were 0.59 (0 – 3.8) % for COI and 0.18 (0 – 0.8)

335   % for 16S, while average congeneric distances are 16.7 (5.5 – 23.4) % and 7.6 (0.3 – 15.1) %

336   respectively. The distances for ITS-region ranged between 0.44 (0 – 5.8) % and 9.2 (0.5 – 18.1)

337   % for intra- and interspecific divergence, respectively, whereas for 28S, the corresponding

338   distances were 0.06 (0 – 0.8) % and 1.7 (0 – 4.5) %, respectively. The two MOTUs found in *E.*

339   *schanderi* were responsible for the high intraspecific maximum distances reported for the nuclear

340   loci.

341   At first, *E. aff. ockelmanni* was assigned to the *Essc* based on morphological similarity;

342   however, genetic distances and BI phylogenetic tree topology signalled otherwise. The two

343   available specimens were very small (less than 2 mm), which can sometimes lead to

344   misidentifications in *Eumida*. Upon a more careful morphological analysis, we concluded that this

345   MOTU is closer to the outgroup belonging to *E. ockelmanni.* This seems to corroborate the

346   molecular data, in which we observed unusually high molecular distances compared to the

347   remaining *Essc.* This is true especially regarding nuclear markers (maximum distances up to 38.2

348   and 9.7% for ITS region and 28S, respectively), and yet much closer to *E. ockelmanni* (maximum

349   distances up to 13.8 and 1.8% for ITS region and 28S, respectively), which might indicate a

350   species complex still undescribed for this group as well.

351

352   HAPLOTYPE NETWORKS

353   All haplotype networks (COI, Fig. 3; ITS, 28S, 16S, Fig. 4A-C) show that the five new

354   species, as well as the new unnamed *Eumida* lineages, are completely sorted from each other

355   and the remaining *Essc*. This is even observed in 28S haplotypes (Fig. 4B), which is a slowly

356   evolving gene and may fail to exhibit complete classification when others do, especially when

357   dealing with closely related species. The only exception to this pattern is observed in the

358   Mediterranean and British lineages from *E. merope* and *E. aff. merope,* which shared haplotypes

359   both in 28S and ITS loci. The low number of mutational steps between nuclear haplotypes, such

as evidenced in the ITS and 28S networks (Fig. 4A, B), may be responsible for their lower phylogenetic resolution when it comes to delineating MOTUs 23, 24, 25, and 27 (Fig. 2B). All of the MOTUs were sympatric with at least one other MOTU within the *Essc*, except the MOTUs from Italy and *E. notata*.

Additionally, two distinct ITS and 28S haplotypes for *E. schanderi* sp. nov. were found, which correspond to different MOTUs in the BI tree (22 and 26, Fig. 2B). Also, two distinct groups of haplotypes for *E. alkyone* could be distinguished based on ITS alone, in which no sharing is observed between Norwegian and Swedish specimens. Three completely sorted COI haplotype groups were also found within *E. taygete,* splitting the Mediterranean and British populations and adding a unique shared haplotype in samples from both regions, with seven mutations apart from the remaining ones.

No MOTU has a central position from which every other derived in any of the networks, and a large amount of circular COI mutation paths are found mainly in *E. notata*, *E sanguinea s.s.*, *E. alkyone*, *E merope*, and *E. aff. merope.*

Haplotype diversity within the *Essc* is relatively high for COI (Table 3), with *E. fenwicki* sp. nov., *E. mackiei*, and *E. maia* being the only ones with significant negative Tajima D or Fu and Li's D tests. Therefore, the population might be in expansion after a recent bottleneck or linkage to a swept gene, with the neutral model of nucleotide substitutions being accepted for the remaining MOTUs. *Eumida mackiei* and *E. gretathunbergi* sp. nov. have the highest haplotype diversity (HD [COI]: 0.99) and segregating sites (S= 37 and 39 respectively).

LIVE PHOTOGRAPHS AND PIGMENTATION DATA

A summary of the different white pigmentation combinations [types A to H, following Nygren & Pleijel (2011)] observed for all the species in the complex is given in Table 4. Live photographs of specimens exhibiting white pigmentation patterns and colour, belonging to the newly described species and unnamed *Eumida* lineages (RO174-180, ANT002, and ORB997), including *E. aff. merope*, *E. aff. kelaino* and *E. aff. gretathunbergae* can be found in Fig. 5A-F, Fig. 6A-E and Fig. 7A-C. Three of the five new species (*E. schanderi* sp. nov., *E. fenwicki* sp. nov., and *E. gretathunbergae* sp. nov.) share type B pigmentation, which corresponds to the absence of white pigmentation. However, *E. schanderi* sp. nov. (Fig. 5A) and *E gretathunbergae* sp. nov. (Fig. 5D) are polymorphic, with some specimens also exhibiting type D (dorsally on segment 2 only, Fig. 5B) and F (Fig. 5C) pigmentation, respectively (see Table 4). Type F pigmentation was defined by Nygren & Pleijel (2011) as a single longitudinal line of white pigmentation and erroneously assigned to one specimen designated as *Eumida* unnamed species S21, here named as *E. schanderi* sp. nov. This specimen presents type B pigmentation, i.e., no white pigmentation. Type F pigmentation is here redefined as white transverse dorsal lines present on most segments. *Eumida fenwicki* sp. nov. also possesses type B pigmentation (Fig. 5E), while *E. pleijeli* sp. nov. (Fig. 6D) has a green colour with type C, characterized by the presence of a longitudinal white line together with white pigmentation dorsally on segment 2.

399   Two other pigmentation types are newly defined in this study, namely: type G, with white
400   pigmentation from the prostomium to the middle of the eyes of worms, similar to type E, but with
401   the addition of small dorsal transverse white dots, which seems to be unique to *E. langenecki* sp.
402   nov. (Fig. 6C); and type H, spotted in the currently unnamed *Eumida* ORB997 (Fig. 6E), defined
403   by the presence of white pigmentation dorsally on segment 2 but with a non-white eye-like pattern
404   dorsally between segments along the whole body of the worm. *Eumida* ORB997 has a very
405   distinct pigmentation among all members of the *Essc,* including *E. pleijeli* sp. nov., even though
406   these two species share the same nuclear MOTU.
407   *Eumida aff. merope* is also polymorphic and shares the same type D (Fig. 6B) and A (Fig.
408   6A) white pattern as the Mediterranean counterpart. *Eumida aff. grethathunbergae* possesses
409   both type B and F (Fig. 7C) pigmentation types, following the same pattern as its sister lineage
410   *E. gretathunbergae* sp. nov.. *Eumida. aff. kelaino* has type C (Fig. 7B) similar to *E. kelaino*, while
411   the unnamed *Eumida* RO174-180 has type B (Fig. 5F) and lastly the unnamed *Eumida* ANT002
412   (Fig. 7D) has type D. The new British *E. taygete* population has an additional pigmentation (type
413   B) compared to the Mediterranean populations (type D). *E. elektra* population from northern
414   France also has a distinct pigmentation (type D) when compared to the Scandinavian populations
415   (type B). Apart from *E. schanderi* sp. nov., *E. elektra*, *E. merope, E. aff. merope*, *E. taygete*, *E.*
416   *gretathunbergae* sp. nov., and *E. aff. gretathunbergae*, the remaining lineages of the *Essc* only
417   have a single pigmentation type as far as we know.
418   In total, the *Essc* is composed of eight variable pigmentation types distributed among 22
419   distinct COI clades. Based on geographic distribution and pigmentation types, *Essc* belonging
420   species can be significantly narrowed down without using molecular data, distinguishing some
421   only based on these criteria (see the *Essc* key in the taxonomic section).
422

423   MORPHOMETRIC MEASUREMENTS
424   The different morphometric proportions seen in the scatter plots in Fig. 8A-H are the only
425   ones displaying significant visible differences, with the formation of independent clusters among
426   the analysed MOTUs. A variation of either nine or ten specimens per lineage were analysed. The
427   use of morphometric proportions of antenna length (AL) against head length or width (HL; HW),
428   palp length (PL), cirri on segment 1 or dorsal cirri on segment 2 (CS1L; DCS2L), and median
429   antenna length (MAL) seems to be effective in distinguishing *E. gretathunbergae sp. nov., E.*
430   *schanderi sp. nov., E. fenwicki sp. nov.*, *and E. elektra* from each other (main morphometric
431   findings summarized in Table 5). The larger number of segments and worm length is also very
432   distinct in *E. gretathunbergae sp. nov.* (Fig. 8G, H). The short antennal length recorded for one
433   of the *E. elektra* specimens (around 0.158 mm), which might be due to damages during sampling,
434   could be the reason for the overlap with the remaining analysed species. Even though there are
435   not enough available specimens to form morphometric clusters for *Eumida pleijeli* sp. nov. and *E.*
436   *langenecki* sp. nov., these species can still be described with unique features that distinguish
437   them from the remaining *Essc*. To do so, a combination of pigmentation type, live colouration,

438   and geographic range (Table 5) is needed and complemented with the molecular data seen above
439   (Fig. 2).
440   As for finding possible morphometric variations between the sister lineages *E. merope*
441   and *E. aff. merope*, our results (Fig. 9A-F) reveal high intraspecific variation within *E. aff. merope*,
442   whose morphometric measurements are scattered around the other analysed species for most of
443   the proportions, except when comparing antennae (AL) and palp (PL) lengths (Fig. 9B). Some
444   partial overlaps between *E. notata* and *E. merope* are also observed. However, *E. merope*, *E.*
445   *schanderi* sp. nov., and *E. gretathunbergae sp. nov*. seem to have palps longer than antennae.
446   This is contrary to the remaining species analysed in ours or other previous studies, which either
447   have antennae larger than palps or of the same proportion. Besides AL/PL ratio, *E. notata* can be
448   differentiated from *E. elektra, E. merope*, and *E. aff. merope* by comparing worm width (WW) with
449   worm width with parapodia (WWP) (Fig. 9A). Some morphometric clusters may also overlap with
450   *E. elektra,* probably due to how genetically close this species is against the remaining analysed
451   ones. Moreover, the mean COI distances between this species and the closest neighbours are
452   shared with *E. notata, E aff. merope*, *and E. alkyone*, with K2P values of 13.8, 13.5, and 12.6 %,
453   respectively.
454   A detailed description of the five new species can be found in the taxonomic section
455   below, with their respective Zoobank lsid registration codes.
456
457   TAXONOMY
458
459   *Eumida sanguinea* species complex (*Essc*)
460
461   *Diagnosis* (amended from Nygren & Pleijel, 2011)
462   *Eumida* with cordate dorsal cirri, near-symmetrical along the longitudinal axis, 1.25–1.9
463   times longer than wide. Colour varies between light yellow, yellowish-brown and green, distributed
464   among eight different pigmentation types (Table 4). Small to medium-sized worm, usually
465   between 3 to 30 mm in length and 30 to 110 segments. High intraspecific morphometric variation.
466
467   *Remarks*
468   *Eumida sanguinea* species complex is an informal name for a clade that includes fifteen
469   described species in north-east Atlantic waters (Nygren & Pleijel 2011; Teixeira *et al.* 2020)
470   including the new ones described herein, with an addition of four undescribed lineages (*Eumida*
471   F22, RO174-180, ANT002, and ORB997) and three distinct mitochondrial sister lineages (*E. aff.*
472   *merope*, *E. aff. kelaino*, and *E. aff*. *gretathunbergae*). This designation should be applied for
473   identifications based on the morphology of preserved specimens, in which white pigmentation
474   has disappeared and no molecular data is available.
475   Recorded egg sizes are 85–95 µm for specimens from Danish waters (Eibye-Jacobsen,
476   1991) and 90 µm for specimens from the English Channel and Sweden (Pleijel, 1993). Egg sizes
477   up to 110 µm were also observed by Nygren & Pleijel (2011) for some members of the complex.

478   Cazaux (1970) described the development from trochophore to newly settled stages from
479   Bordeaux in France.
480   *Eumida bahusiensis* Bergstrom, 1914 is phylogenetically very close to *Essc* species and
481   can therefore be part of it. The species can be distinguished morphologically by its broader dorsal
482   and ventral cirri distally pointed and by the green colour with white type A pigmentation in live
483   animals (Nygren *et al*. 2017). However, it can often be confused with *Eumida mackiei* (Teixeira,
484   Nygren and Pleijel, 2020), which has the same background colour and pigmentation, as well as
485   median ventral cirri, approaching the broader form of *E. bahusiensis*. The two species are
486   genetically very distinct with 21% COI average divergence and are not sister species.
487
488   A simple *Essc* key based on pigmentation types and geographic distribution can be seen
489   below. This key should only be used for identifications where pigmentation and colour of live
490   specimens were recorded. Table 4 displays the pigmentation types.
491

492   **1) Scandinavia**
493   **a)** Type A pigmentation...*E. alkyone*
494   **b)** Type B pigmentation
495   **b1)** Palps longer than antennae…………….…….…………………………..*E. schanderi*
496   **b2)** Palps shorter than antennae…………….……………………………*E. elektra*, *E. fenwicki*
497   (The distinction between these two species is only possible with molecular data)
498   **c)** Type C pigmentation ……………………………………………………………..*E. kelaino*
499   **d)** Type D pigmentation …………………………………………. *E. sanguinea* s.s., *E. schanderi*
500   (The distinction between these two species is only possible with molecular data)
501

502   **2) Great Britain + Brittany, France**
503   **a)** Type A pigmentation
504   **a1)** Greenish colour
505   **a1.1)** Palps as long as antennae …………………...*E. mackiei*
506   **a1.2)** Palps shorter than antennae …………...*E. maia*
507   **a2)** Yellowish-brown colour
508   **a2.1)** Palps longer than antennae …………..... *E. gretathunbergae*; *E. aff. gretathunbergae*;
509   (The distinction between these two species is only possible with molecular data)
510   **a2.2)** Palps shorter than antennae ……………………….………...……*E. aff. merope*
511   **b)** Type B pigmentation …………………….............*E. taygete*, *Eumida* RO174-180, *E. fenwicki*
512   (The distinction between these three species is only possible with molecular data)
513   **c)** Type C pigmentation ………………………………………………..…………*E. kelaino*
514   **d)** Type D pigmentation ……………………………*E. sanguinea* s.s., *E. elektra*; *E. aff. merope*
515   (The distinction between these three species is only possible with molecular data)
516   **e)** Type F pigmentation ……………………………..... *E. gretathunbergae*; *E. aff. gretathunbergae*
517   (The distinction between these two species is only possible with molecular data)

518

3) Madeira Island (Portugal)

  a) Type E pigmentation ……………………………………………….………*E. notata*

521

4) Western Mediterranean

  a) Type A pigmentation ……………………………………………….………… *E. maia*

  b) Type B pigmentation ……………………………...………………...… *E. asterope*

  c) Type C pigmentation

   c1) Green colour……………………………………………….........*E. pleijeli*

   c2) Yellowish colour…………………………………………...…*E. aff. kelaino*

  d) Type D pigmentation …………………. *E. merope*, *Eumida* F22, *E. taygete*; *Eumida* ANT002

      (The distinction between these four species is only possible with molecular data)

  e) Type G pigmentation ……………………………………………… *E. langenecki*

  f) Type H pigmentation ……………………………………………. *Eumida* ORB997

532

5) Eastern Mediterranean

  a) Type A pigmentation ………………………………………………… *E. merope*

  b) Type D pigmentation ………………………………………………… *E. taygete*

536

537

***Eumida fenwicki* sp. nov.**

urn:lsid:zoobank.org:act: DA689D94-E20B-4126-8DA4-575576EB5C86

540

Material examined

    Type material. Great Britain, Cornwall: 1 spm, holotype and hologenophore, DBUA0002396.01, 50º21.5'N, 04º08.9'W, 10m, coarse shell gravel, dredge, 16-03-2011; 1 spm, paratype and paragenophore, DBUA0002396.02, 50º21.5'N, 04º08.9'W, 10m, coarse shell gravel, dredge, 16-03-2011. The type specimens were collected by David Fenwick.

    Other material. Great Britain, Cornwall: 5 spms, DBUA0002397.01-05, 50º06'12.0"N, 5º32'49.4"W, pontoon scrapings, 14/04/2015, 02/04/2015, 04/05/2015, 07/05/2015 and 28/05/2015 respectively; 1spm, DBUA0002397.06, 50º06'12.0"N, 5º32'49.4"W*,* pontoon scrapings, 13/07/2016; Norway, Agdenes: 9 spms, ZMBN_134523 - 134530; DBUA0002398.01, 63º35.721'N, 9º33.100'E, 10m, sand, shell-fragments, dredge, 05/09/2016; 3 spms, ZMBN_134531 - 134533, 63º35.721'N, 9º33.100'E, 2m, coarse gravel and rocks, dredge, 05/09/2016; France, Roscoff: 2 spms, DBUA0002399.01-02, 48º44'55.2"N, 3º54'23.3"W, 45m, gravel, 01/02/2018. British specimens were collected by David Fenwick, while the Norwegian ones were collected by the students from the ForBio programme. All specimens are preserved in ethanol 96%. The specimens from Norway are deposited at the University Museum of Bergen (ZMBN), and the remaining ones, including the holotype and paratype, at the Biological Research

Collection (Marine Invertebrates) of the Department of Biology of the University of Aveiro (COBI at DBUA), Portugal.

Diagnosis

Member of *Essc* with type B pigmentation (Table 4), i.e., without white pigmentation (Fig. 5E). Live specimens present light-yellowish colouration. Antennae slightly longer than palps. Proportions between antenna length and cirri length on segment 1, dorsal cirri length on segment 2, or head length and width smaller than those found in *E. gretathunbergae* sp. nov. and *E. elektra*, but greater than those of *E. schanderi* sp. nov. Palp length, cirri on segment 1 and dorsal cirri on segment 2, and head length and width are considerable smaller when compared to the same length of *E. gretathunbergae* sp. nov. antennae. Dorsal cirri on segment 2 usually twice as long as cirri on segment 1. Head wider rather than longer. Dorsal cirri of median segments 1.5 times longer rather than wider. Ventral cirri of median segments twice as long rather than wider. Proboscis not observed. Worms small, usually between 3 to 10 mm long, with 45 to 75 segments.

Molecular data

COI, 16S, ITS, and 28S sequences as in specimens DBUA0002396.01-06; ZMBN_134523 to 134533; DBUA0002398.01; DBUA0002399.01-02 (Table S1). Phylogenetic relationship as in Fig. 2A-B, with high support values and low intraspecific (<3%) genetic divergence for both mitochondrial and nuclear markers. Mean interspecific COI distances to the nearest and farthest neighbours are 14.8% (K2P, *E. aff. merope*) and 21.1% (K2P, *E. schanderi* sp. nov.), respectively. DOI for the species' Barcode Index Number (BIN): dx.doi.org/10.5883/BOLD:ADG3938.

Etymology

The new species is named after David Fenwick to recognize his kindness in collecting and photographing a large number of *Eumida* specimens on the behalf of the last author of this paper.

Distribution and habitat:

Atlantic Ocean – from Norway to the British Isles, 2 to 10 m depth, on coarse gravel and rocks.

Remarks

Morphologically similar to *E. sanguinea sensu stricto* (Örsted, 1843), except for pigmentation pattern. Pigmentation type B shared with *E. asterope* Nygren & Pleijel, 2015; *E. elektra* Nygren & Pleijel, 2015; some specimens of *E. schanderi* sp. nov.; the British population of *E. taygete* Nygren & Pleijel, 2011; *Eumida* RO174-18; and some specimens from *E. gretathunbergae* sp. nov. and *E. aff. gretathunbergae*. However, those species differ from *E. fenwicki* sp. nov. at the molecular level, with mean interspecific COI distances (K2P, %) of 19.4,

597 15.1, 21.1, 17.3, 17.7, 16.6, and 16.5 respectively. Morphometric proportions of the antennal
598 length against either head length or width, palp length, cirri on segment 1, and dorsal cirri on
599 segment 2 seem to be effective in distinguishing this species from *E. gretathunbergae, E.*
600 *schanderi*, and *E. elektra.*

601

602 ***Eumida schanderi* sp. nov.**
603 urn:lsid:zoobank.org:act: 4780C5B7-EC23-44A5-B2CC-88E9FE8C5891

604

605 *Material examined*

606 *Type material.* Norway, Bergen: 1 spm, holotype and hologenophore, ZMBN_134556,
607 60°14'11.9"N, 5°12'02.1"E, 27m, algae, gravel, triangular dredge, 24/07/2014; 11 spms,
608 paratypes and paragenophores, ZMBN_134550 - 134555; ZMBN_134557 - 134561,
609 60°14'11.9"N, 5°12'02.1"E, 27m, algae, gravel, triangular dredge, 24/07/2014. The type
610 specimens were collected by the crew aboard R/V Hans Brattström owned by the University of
611 Bergen and operated by the Institute of Marine Research.

612 *Other material*. Sweden, Bohuslän: 1spm, SMNH 110614, 58°52'00.0"N, 11°06'00.0"E,
613 40m, gravel*,* dredge, 12/05/2005. Collected by Fredrik Pleijel. All specimens, including the
614 holotype and paratypes, are preserved in ethanol 96% and deposited at the University Museum
615 of Bergen (ZMBN), with the exception of the Swedish one, which is deposited in the Swedish
616 Museum of Natural History (SMNH).

617

618 *Diagnosis*

619 Member of *Essc* with type D pigmentation (Table 4), i.e., with white pigmentation present
620 dorsally on segment 2 and anterior cirri (Fig. 5B). Type B pigmentation, i.e., without white
621 pigmentation (Fig. 5A) also observed in some paratypes and other analysed material. Live
622 specimens present greenish colouration. Antennae slightly shorter than palps. Proportions
623 between the antenna length and head length or width, median antenna length, cirri on segment
624 1, and dorsal cirri on segment 2 smaller than those in *E. gretathunbergae* sp. nov*., E. fenwicki* sp.
625 nov., and *E. elektra*. Palp length, cirri on segment 1, and dorsal cirri on segment 2, or head width
626 similar to those of *E. fenwicki*. Head almost twice as wide as long. Dorsal cirri on segment 2
627 usually twice as long as cirri on segment 1. Dorsal cirri of median segments very large, almost
628 twice as long rather than wider. Ventral cirri of median segments longer rather than wider, usually
629 twice as long. Proboscis with numerous minute papillae evenly distributed (Fig. 5B). Worms small,
630 usually between 3 to 7 mm long, with 40 to 60 segments.

631

632 *Molecular data*

633 COI, 16S, ITS, and 28S sequences as in specimens ZMBN_134550 to 134561 and
634 SMNH 110614 (Table S1). Phylogenetic relationship as in Fig. 2A, B, with high support values
635 and low intraspecific (<3%) genetic divergence for mitochondrial loci. However, introgression of
636 mtDNA from a non-sampled lineage may be present in nuclear markers with two strongly-

637 supported sister MOTUs with 5.8 and 0.6% mean genetic divergence for ITS and 28S,
638 respectively. Mean interspecific COI distances to the nearest and farthest neighbours are 15.6%
639 (K2P, *E. aff. merope*) and 21.8% (K2P, *E. langenecki* sp. nov.), respectively. DOI for the species'
640 Barcode Index Number (BIN): dx.doi.org/10.5883/BOLD:ACQ6378.

642 *Etymology*
643   The new species is named to honour the memory of Christoffer Schander (1960-2012),
644 a much-appreciated former colleague to the last author of this paper.

646 *Distribution and habitat:*
647   Atlantic Ocean – Norway and Sweden, from 27 to 40 m depth, on gravel with algae.

649 *Remarks*
650   Morphologically similar to *E. sanguinea sensu stricto* (Örsted, 1843), including
651 pigmentation pattern. Pigmentation type D shared with *E. sanguinea* s.s.; *E. merope* Nygren &
652 Pleijel, 2015; *E. aff. merope*; *Eumida* F22 Nygren & Pleijel, 2011; *Eumida* ANT002; and the
653 Mediterranean population of *E. taygete* Nygren & Pleijel, 2011. Pigmentation type B shared with
654 *E. fenwicki* sp. nov.; *E. asterope* Nygren & Pleijel, 2011; *E. elektra* Nygren & Pleijel, 2011; *E.
655 gretathunbergae* sp. nov.; and the British population of *E. taygete* Nygren & Pleijel, 2011.
656 However, those species differ from *E. schanderi* at the molecular level, with mean interspecific
657 COI distances (K2P, %) of 15.1, 17.3, 15.6, 18.6, 16.5, 21.1, 16.5, 18.8, and 18.4, respectively.
658 Proboscis has papillae (Fig. 5B), unlike the one reported in *E. sanguinea* s.s., which is almost
659 smooth with sparsely distributed minute papillae, arranged in six more-or-less distinct rows
660 (Pleijel, 1993). Morphometric proportions of the antenna-palp ratio and antenna length against
661 head length or width, palp length, cirri on segment 1, and dorsal cirri on segment 2, and median
662 antenna seem to be effective in distinguishing this species from *E. gretathunbergae* sp. nov.*, E.
663 schanderi* sp. nov., *and E. elektra.*

665   **Eumida gretathunbergae sp. nov.**
666   urn:lsid:zoobank.org:act:B974C7EA-E00D-4D8A-B791-5A82BAEAAADE

668 *Material examined*
669   *Type material.* Great Britain, Plymouth: 1 spm, *holotype and hologenophore*,
670 DBUA0002400.01, 50°21'30.0"N, 4°08'54.0"W, 15m, coarse shell gravel, dredge, 16/03/2011; 8
671 spms, paratypes and paragenophores, DBUA0002400.02-09, 50°21'30.0"N, 4°08'54.0"W, 15m,
672 coarse shell gravel, dredge, 16/03/2011. Collected by the crew aboard R/V SEPIA (Marine
673 Biological Association) and Fredrik Pleijel.
674   *Other material*. Great Britain, Plymouth: 17 spms, DBUA0002400.10-26, 50°21.59″N,
675 4°09.03″W, 8 - 13m, coarse shell gravel, dredge, 27/03/2017. Collected by the crew aboard R/V
676 SEPIA (Marine Biological Association) and Fredrik Pleijel. All specimens, including the holotype

677 and paratypes, are preserved in ethanol 96% and deposited at the Biological Research Collection
678 (Marine Invertebrates) of the Department of Biology of the University of Aveiro (COBI at DBUA),
679 Portugal.
680
681 *Diagnosis*
682   Member of *Essc* with type F pigmentation (Table 4), i.e., with transverse dorsal lines
683 across segments (Fig. 5C). Type B pigmentation, i.e., without white pigmentation (Fig. 5D) also
684 observed in some paratypes and other analysed material. Live specimens present yellowish-
685 brown colouration. Antennae are shorter than palps. Proportions of the antenna length against
686 head length or width, median antenna length, cirri on segment 1, and dorsal cirri on segment 2
687 larger than those of *E. schanderi* sp. nov. and *E. fenwicki* sp. nov., but smaller than those of *E.*
688 *elektra*. Despite a significantly larger worm size, antenna length with similar morphometric
689 measurements as *E. fenwicki* sp. nov. Head wider than longer. Dorsal cirri on segment 2 usually
690 twice as long as cirri on segment 1. Dorsal cirri of median segments large, 1.5 times longer rather
691 than wider. Ventral cirri of median segments twice longer than wider. Proboscis with numerous
692 minute papillae evenly distributed (Fig. 5C). Worms small- to medium-sized, usually between 10
693 to 20 mm long, with 80 to 105 segments.
694
695 *Molecular data*
696   COI, 16S, ITS, and 28S sequences as in specimens DBUA0002400.01-26 (Table S1).
697 Phylogenetic relationship as in Fig. 2A, B, with high support values and low intraspecific (<3%)
698 genetic divergence for both mitochondrial and nuclear markers. Mean interspecific COI distances
699 to the nearest and farthest neighbours are 13% (K2P, *E. aff. gretathunbergae*) and 21.8% (K2P,
700 *Eumida* ORB997), respectively. DOI for the species' Barcode Index Number (BIN):
701 dx.doi.org/10.5883/BOLD:AEA3142.
702
703 *Etymology*
704   The new species is named after Greta Thunberg to honour her achievement in gathering
705 attention from the general public towards climate change.
706
707 *Distribution and habitat:*
708   Atlantic Ocean – British Isles, 8-15 m depth, on coarse shell gravel.
709
710 *Remarks*
711   Morphologically similar to *E. sanguinea sensu stricto* (Örsted, 1843), except for
712 pigmentation pattern. Type F pigmentation is unique for this species (including *E. aff.*
713 *gretathunbergae*). Type B pigmentation is shared with *E. fenwicki* sp. nov.; *E. asterope* Nygren &
714 Pleijel, 2011; *E. elektra* Nygren & Pleijel, 2011; *E. aff. gretathunbergae*; *Eumida* RO174-180;
715 some specimens of *E. schanderi sp. nov*.; and the British population of *E. taygete* Nygren &
716 Pleijel, 2011. However, those species differ from *E. gretathunbergae* sp. nov. at the molecular

717 level, with mean interspecific COI distances (K2P, %) of 16.6, 15.5, 16.6, 13.0, 14.5, 18.4, and
718 16.0, respectively. Proboscis has papillae (Fig. 5C), unlike the one reported in *E. sanguinea* s.s.,
719 which is almost smooth with sparsely distributed minute papillae, arranged in six more-or-less
720 distinct rows (Pleijel, 1993). The number of segments, worm length, antennae/palps ratio, as well
721 as morphometric proportions of the antenna length against head length or width, palp length, cirri
722 on segment 1, dorsal cirri on segment 2, and median antenna, seem to be very effective in
723 distinguishing this species from *E. fenwicki* sp. nov., *E. schanderi* sp. nov., and *E. elektra*.
724
725 ***Eumida pleijeli* sp. nov.**
726 urn:lsid:zoobank.org:act: F2B43974-0771-4B9A-9CC9-42FF33CEB454
727
728 *Material examined*
729   *Type material.* Italy, Naples: 1 spm, holotype and hologenophore, DBUA0002407.02,
730 40°49'48.0"N, 14°14'13.2"E, 6m, coarse shell gravel, dredge, 05/05/2010; 1 spm, paratype and
731 paragenophore, DBUA0002407.01, 40°49'48.0"N, 14°14'13.2"E, 6m, coarse shell gravel, dredge,
732 05/05/2010. Collected by Joachim Langeneck.
733   All type specimens are preserved in ethanol 96% and deposited at the Biological
734 Research Collection (Marine Invertebrates) of the Department of Biology of the University of
735 Aveiro (COBI at DBUA), Portugal.
736
737 *Diagnosis*
738   Member of *Essc* with green colouration mixed with type C pigmentation (Table 4), i.e.,
739 white pigmentation dorsally on segment 2 and anterior cirri, and with a longitudinal mid-dorsal line
740 (Fig. 6D). Antennae longer than palps. Dorsal cirri on segment 2 almost three times as long as
741 cirri on segment 1, unlike the smaller ratio (twice as long) in *E, fenwicki* sp. nov., *E. schanderi* sp.
742 nov, *E gretathunbergae* sp. nov. *E. langenecki* sp. nov.and *E. elektra*. Dorsal cirri of median
743 segments large, 1.5 times longer rather than wider, with similar ratio as *E, fenwicki* sp. nov., *E*
744 *gretathunbergae* sp. nov. *E. langenecki* sp. nov. and *E. elektra,* but smaller than *E. schanderi* sp.
745 nov. (usually twice as long). Ventral cirri of median segments 1.5 times longer rather than wider,
746 but with smaller ratio (usually twice as long) as *E, fenwicki* sp. nov., *E. schanderi* sp. nov, *E*
747 *gretathunbergae* sp. nov. *E. langenecki* sp. nov. and *E. elektra.* Proboscis not observed. Worms
748 small- to medium-sized, usually between 10 to 15 mm long, with 55 to 65 segments.
749
750 *Molecular data*
751   COI, 16S, ITS, and 28S sequences as in specimens DBUA0002407.01-02 (Table S1).
752 Phylogenetic relationship as in Fig. 2A, B, with high support values and low intraspecific (<3%)
753 genetic divergence for both mitochondrial and nuclear markers. However, nuclear markers (ITS
754 and 28S) group this species into the same MOTU as the unnamed *Eumida* ORB99, even though
755 high interspecific COI divergence is found (18%, K2P). Mean interspecific COI distances to the
756 nearest and farthest neighbours are 16.8% (K2P, *E. mackiei*) and 21.6 (K2P, *E. langenecki* sp.

757   nov.), respectively. DOI for the species' Barcode Index Number (BIN):
758   *dx.doi.org/10.5883/BOLD:AEH2033*

759

760   *Etymology*

761   The new species is named after Fredrik Pleijel to honour his passion and dedication to
762   the study of the Phyllodocidae.

763

764   *Distribution and habitat:*

765   Western Mediterranean Sea – Italy, 6 m depth, on coarse shell gravel.

766

767   *Remarks*

768   Morphologically similar to *E. sanguinea sensu stricto* (Örsted, 1843), except for
769   pigmentation pattern. Type C pigmentation is shared only with *E. kelaino* and its Mediterranean
770   counterpart *E. aff. kelaino*. However, *E. kelaino* and *E. aff. kelaino* differ greatly from *E. pleijelii*
771   sp. nov. at the molecular level, with mean interspecific COI distances (K2P, %) of 19.0 and 19.1,
772   respectively, and share a different colouration. This species can share the same nuclear MOTU
773   (ITS+28S) as the unnamed *Eumida* ORB997, but the latter has a very distinct pigmentation (Type
774   H) among all members of the *Essc. E. pleijelii* sp. nov. can be identified based only on colour,
775   pigmentation, and geographic distribution jointly.

776

777   **_Eumida langenecki_ sp. nov.**
778   urn:lsid:zoobank.org:act: 3E870E7A-C1D6-4918-BCE0-737E5B81418A

779

780   *Material examined*

781   *Type material.* Italy, Antignano: 1 spm, holotype and hologenophore*,* DBUA0002409.02,
782   43°29'31.2"N, 10°19'01.2"E, 6m, coarse shell gravel, dredge, 22-05-2020; 2 spms, paratypes and
783   paragenophores, DBUA0002409.04-05, 43°29'31.2"N, 10°19'01.2"E, 6m, coarse shell gravel,
784   dredge, 22-05-2020. Collected by Joachim Langeneck.

785   *Other material.* Italy, Ischia: 1 spm, DBUA0002408.01, 40°44'42.0"N, 13°56'20.4"E, 6m,
786   coarse shell gravel, dredge, 10/05/2010; Italy, Antignano: 1 spm, DBUA0002409.01,
787   43°27'57.6"N, 10°20'24.0"E, 4m, coarse shell gravel, dredge, 05/05/2010; 1 spm,
788   DBUA0002409.03, 43°29'31.2"N, 10°19'01.2"E, 6m, coarse shell gravel, dredge, 22-05-2020..
789   Collected by Joachim Langeneck. All specimens, including the holotype and paratypes, are
790   preserved in ethanol 96% and deposited at the Biological Research Collection (Marine
791   Invertebrates) of the Department of Biology of the University of Aveiro (COBI at DBUA), Portugal.

792

793   *Diagnosis*

794   Member of *Essc* with type G pigmentation (Table 4), i.e., with white pigmentation dorsally
795   on prostomium and white transverse dorsal dots across segments (Fig. 6C). Live specimens
796   present yellow colouration. Antennae slightly longer than palps. Dorsal cirri on segment 2 twice

797   as long as cirri on segment 1, with similar ratio as *E, fenwicki* sp. nov., *E. schanderi* sp. nov, *E*

798   *gretathunbergae* sp. nov and *E. elektra,* but smaller than *E. pleijeli* sp. nov. (almost three times

799   as long). Dorsal cirri of median segments large, 1.45 times longer rather than wider, sharing a

800   similar ratio as *E, fenwicki* sp. nov., *E. pleijeli sp. nov.*, *E gretathunbergae* sp. nov and *E. elektra,*

801   but smaller than *E. schanderi* sp. nov (twice as long). Ventral cirri of median segments almost

802   twice as long rather than wider, sharing a similar ratio as *E, fenwicki* sp. nov., *E. schanderi* sp.

803   nov., *E gretathunbergae* sp. nov and *E. elektra,* but greater than *E. pleijeli sp. nov* (1.5 times as

804   long). Proboscis not observed. Worms small, usually between 7 to 11 mm long, with 70 segments.

805

806   *Molecular data*

807   COI, 16S, ITS, and 28S sequences as in specimens DBUA0002408.01;

808   DBUA0002409.01-05 (Table S1). Phylogenetic relationship as in Fig. 2A, B, with high support

809   values and low intraspecific (<3%) genetic divergence for both mitochondrial and nuclear

810   markers. Mean interspecific COI distances to the nearest and farthest neighbours are 15.4%

811   (K2P, *E. maia*) and 22.0 (K2P, *E. mackiei*), respectively. DOI for the species' Barcode Index

812   Number (BIN): *dx.doi.org/10.5883/BOLD:AEH2035*

813

814   *Etymology*

815   The new species is named after Joachim Langeneck for his sampling efforts and kindness

816   in providing unique Mediterranean *Eumida* specimens on the behalf of the authors of this paper.

817

818   *Distribution and habitat*

819   *Western* Mediterranean Sea – Italy, 3 – 6 m depth, on coarse shell gravel.

820

821   *Remarks*

822   Morphologically similar to *E. sanguinea sensu stricto* (Örsted, 1843), except for

823   pigmentation pattern. Type G pigmentation is unique among the *Essc* and can solely be used to

824   identify this species. Type G and E pigmentation, are the only pigmentation types with white

825   pigmentation dorsally on the prostomium up to the middle of the eyes. The latter being exclusive

826   to *E. notata* found only in Madeira Island (Portugal).

827

828   DISCUSSION

829

830   NEW SPECIES AND UNNAMED LINEAGES

831   All members of the *Essc*, including the five new species, displayed COI genetic distances

832   comparable to those found among established species of polychaetes (e.g., Carr *et al.*, 2011;

833   Lobo *et al.*, 2016; Sampieri *et al.*, 2021). However, the results of nuclear markers in *E. schanderi*

834   sp. nov. are unexpected due to their customary low divergence with COI. Vieira *et al.* (2019) also

835   found a similar occurrence when comparing COI and 18S rRNA for one of the MOTUs of

836   *Dynamene magnitorata* Holdich, 1968 (Isopoda), reporting evidence of cryptic lineages between

the Iberian Peninsula and Macaronesia islands. This could be a case of heterozygosity at nuclear loci (Sota & Vogler, 2003); however, overlapping spikes were not found when analysing the trace files for *E. schanderi* sp. nov. Besides, the distance between these haplotypes was perhaps too large to be attributed to heterozygotic variation. Another more plausible scenario is that hybridization and introgression of mtDNA from a non-sampled lineage could explain the presence of the same COI haplotype in two different sister lineages (Bachtrog *et al.*, 2006).

Although our molecular data support species hypothesis for most new lineages, there are a few exceptions in the nuclear MOTUs 23, 24, 25, and 27. Each of them is composed of at least two corresponding distinct mitochondrial lineages. At first, these patterns can either be explained by hybridization or differential substitution rates among loci. It is because some loci would display more consolidated lineage sorting stages than others. However, except for *E. merope* and *E. aff. merope* (MOTU 24), no nuclear haplotypes are shared. Therefore, although broader sampling and balanced representation of sequences from different loci are needed, hybridization could be discarded. MOTU 24 also did not share haplotypes between *E. notata* and either *E merope* or *E. aff. merope*. However, regardless of the species status, it is evident these lineages have diverged recently. When considering only the COI genetic distances (for which there is extensive data), they appear to be on the lower boundary of customary congeneric distances reported either within consolidated *Eumida* species (Teixeira *et al.*, 2020) or even compared to polychaetes in general (Nygren *et al.*, 2009; Ravara *et al.*, 2017). Indeed, *E. aff. merope* displays a mean COI genetic distance of 10 and 8% to *E. merope* and *E. notata*, respectively, with limited morphometric differences (Fig. 9) as well. Similar values can be found between *E. kelaino* (MOTU 18) and *E. aff. kelaino* (MOTU 17) or between *E. grethathunbergae* sp. nov. (MOTU 12) and *E. aff. gretathunbergae* (MOTU 13), with 12 and 13% mean COI divergence, respectively, and each pair sharing the same nuclear MOTU. A comparative analysis by Teixeira *et al.* (2020) indicated that *Essc* species with COI divergence below 10% may have little or no differentiation in ITS or 28S. In our study, the same was observed for 16S since the mean distances range between those two nuclear markers (Table 2). Some exceptions to this pattern can be found in *E. pleijeli* sp. nov. (MOTU 3) and *Eumida* ORB997 (MOTU 2). In these, a mean COI divergence of 18% was observed associated with different pigmentation types, but only 1.5% in the ITS region. Alternative divergence patterns between nuclear and mitochondrial markers have been reported for other cryptic species as well (e.g., *Notophylum* Örsted, 1843). In this regard, although low mean COI K2P distances were found between shallow and deep water populations (8.5%), mean distances for ITS1 (4.9%) were still higher compared to some *Eumida* species analysed here (Nygren *et al.*, 2010).

Morphometric data also failed to separate MOTUs with comparatively low genetic distances between them (<14% COI, Fig. 9). Rice *et al.* (2008) compared genetic distances and reproductive compatibility in *Polydora cornuta* Bosc, 1802 populations. They reported that signs of partial larval development can be found between populations with 8% mean COI distances, but not between those with COI divergence above 15%. Some exceptional cases have also been reported in marine invertebrates, namely in copepods (Handschumacher *et al.*, 2010). This study

showed that, despite the genetic COI distances of above 23% between the Pacific population of *Tigriopus californicus* (Baker, 1912) and Icelandic *Tigriopus brevicornis* (Müller O.F., 1776), their crossing can produce mature F1 and F2 hybrids. This, in turn, challenges the restrictive biological species concept.

PHYLOGEOGRAPHIC INSIGHTS

Most *Essc* species have extreme COI haplotype diversity (Table 2). This is comparable to *Terebellides* Sars, 1835 in Nygren *et al.* (2018), wherein almost all specimens sampled and sequenced had a unique haplotype in some species. Therefore, additional larvae may have been recruited from other populations (Meibner *et al.*, 2014). Other polychaete species, such as *Hediste* Malmgren, 1867, have also shown more than 80 haplotypes in 100 sequences recorded in species "A" and "B" for both *H. diversicolor* (O.F. Müller, 1776) and *H. atoka* Sato & Nakashima, 2003 (Tosuji *et al.*, 2019). Such high haplotype diversity within species could also be related to the Pleistocene glaciation (initiated circa 2.8 MY, Maggs *et al.* 2008). Isolated northern ice-free areas may have allowed pockets of diversity to persist (Stewart & Lister, 2001; Rowe *et al.*, 2004; Provan & Bennett, 2008). These glacial refuges are areas where some plants or animals survived during this unfavourable period, with organisms of the same kind extinguished nearby or retracted southwards to more favourable locations (Andersen & Borns, 1994). It has been proposed that the Western English Channel is one of the possible locations of coastal glacial refuges (Maggs *et al.*, 2008), thereby close to the Plymouth and Cornwall area, which is home to eight different *Essc* species, including two of the new species (MOTUs 6 and 12, Fig. 2A, B). Isolation into refugia reduces geographical ranges and population sizes, resulting in high genetic diversity and high dissimilarity between refugee populations (Comes & Kadereit, 1998; Willis *et al.*, 2004).

The high genetic distance between MOTUs within the *Essc* of the northeast Atlantic suggests that their diversification likely pre-dates the Pleistocene glaciations. This potential survival of divergent lineages in common refugia may explain the high level of sympatry currently observed in this region (Highsmith, 1985; Desiderato *et al.*, 2019).

PIGMENTATION DATA, THE *ESSC* AND MORPHOLOGICAL STASIS

Recent studies have suggested that cryptic complexes may remain morphologically identical due to long periods of morphological stasis (Struck *et al.*, 2018). For example, rates of morphological evolution in the *Stygocapitella* complex are significantly slower than in closely related non-cryptic taxa from Nerillidae Levinsen, 1883 and Orbiniidae Hartman, 1942 (Cerca *et al.*, 2020b). Besides the geographic distribution, colouration, and pigmentation, all the new *Eumida* species examined in this study fail to display any stable and diagnostic morphological differences, even though slight morphometric variations on the size and shape of the cirri and prostomial appendages can be found at least between 4 lineages (Table 5). Notably, the outgroup *E. bahusiensis*, which normally occurs paraphyletically within the *Essc* (Fig. 2), possesses the same white pigmentation pattern (type B) and the distinct greenish band as does *E. mackiei* (Teixeira *et al.*, 2020). However, the dorsal cirrus has a visible and larger width that can be

identified through traditional morphological approaches (Nygren *et al.,* 2017). Such micromorphological variations within cryptic and pseudo-cryptic species are seldom detected. For instance, most *Stygocapitella* lineages lack diagnostic characters and morphological differences that could allow an unambiguous identification to the species level, including morphometric data (Cerca *et al.*, 2020a). In a previous study (Teixeira *et al.*, 2020), *E. sanguinea s. s.* also failed to produce a separated morphometric PCA cluster against three other species from the complex. Even though in this particular case it could be attributed to bias towards juveniles among the examined specimens, the likelihood of finding overlapping morphometric variation is still high when dealing with more than fifteen different *Eumida* species. This PCA result and, in particular, our new morphometric data for *E. aff. merope* (Fig. 9) could also be indicative of phenotypic plasticity (e.g., in the proportions of several morphological structures). This phenomenon is widespread across invertebrates since different phenotypes occur associated with particular environmental conditions (Fusco & Minelli, 2010; Forsman, 2015), but still scarcely studied in polychaetes (Nygren & Pleijel, 2011; Syomin *et al.*, 2017). Environmental features could be an explanation for this variation. In the *Syllis gracilis* Grube, 1840 complex (Langeneck *et al.*, 2020), a univariate analysis of morphological characters showed that marine specimens sampled on intertidal algal communities are differentiated from brackish-water and *Sabellaria*-associated individuals.

Pronounced phenotype changes without molecular divergence are also patent in the *Essc*. Aside from the lack of apparent correlation between geographic occurrence and species with colour polymorphism (such as *E. merope*, *E aff. merope*, *E. schanderi* sp. nov., *E. gretathunbergae* sp. nov., and *E. aff. gretathunbergae*), *E. elektra* and *E. taygete* populations had all their sequences grouped in the same respective MOTU, but geographically different populations had distinct pigmentations. The latter had an exceptionally wide geographical range within the *Essc*, from Great Britain to the western and eastern Mediterranean. Yet, the individuals from the British population possess a distinct pigmentation type (Table 4) and several unique haplotypes (Fig. 3). Interestingly, reports have often attributed the deep divergence in invertebrates between eastern and western Mediterranean to the Messinian salinity crisis around 6 MY (e.g., Hupało *et al.,* 2019; Rögl, 1999). The same is not observed in *E. taygete*, suggesting recent colonization of the Mediterranean. In this case, neither the morphotype nor geographic location alone could be indicative of a new species within the *Eumid*a complex unless complemented with molecular data. Such a contrast, for example between *Eumida pleijeli* sp. nov. and *Eumida langenecki* sp. nov., a combination among collection location, live colouration, and white pigmentation type is sufficient to successfully identify these species within the *Essc*.

CONCLUSIONS

The combination of morphometric and genetic data successfully validated the existence of three new undescribed species within the *Essc*, namely *E. schanderi* sp. nov., *E. fenwicki* sp. nov., and *E. gretathunbergae* sp. nov. Since morphometric scatter plots seem to be informative only for at least five specimens with optimal conditions, such methodology cannot be used for the

957 remaining eight newly detected MOTUs. However, combining colour, white pigmentation types,
958 and geographic distribution was enough to successfully identify two additional new species, *E.*
959 *pleijeli* sp. nov. and *E. langenecki* sp. nov., rising to fifteen the total number of species described
960 within this complex. Our results also suggest that morphometric data alone may not provide
961 enough resolution for the most genetically close *Eumida* species (i.e., with about less than 14%
962 COI divergence) and/or cases where nuclear data fail to split into the same number of MOTUs as
963 mtDNA. Moreover, the probability of finding overlapping morphometric variation for any of the
964 analysed proportions is high when dealing with more than fifteen different *Eumida* species.
965 Although genetically similar, the sister species *E. notata* and *E. merope* had at least two different
966 morphometric markers, did not share haplotypes, and also differed in pigmentation type and
967 geographic distribution, strengthening their status as independent species. Ideally, studies
968 examining reproductive compatibility between populations could help clarify the species status of
969 the lineages referred to as *E. aff. merope, E aff. kelaino*, and *E. aff. gretathunbergae* compared
970 to their respective sister species. The remaining three new undescribed *Eumida* lineages in this
971 work (RO174-180, ANT002 and ORB997) will join *Eumida* F22 from Nygren & Pleijel (2011) as
972 putative species within the *Essc,* with further sampling efforts still needed to clarify their status.
973 The underlying mechanisms behind morphological stasis are still unknown and remain
974 controversial in evolutionary biology (Fišer *et al.*, 2018). In this sense, combining molecular
975 phylogenetic tools and examination of small morphological changes can help understand stasis
976 in species complexes. This can eventually allow for more formal and widespread recognition of
977 cryptic and pseudo-cryptic biodiversity in biomonitoring and ecological studies.
978
979 CONFLICT OF INTERESTS
980 The authors declare no conflicts of interest
981
982 REFERENCES
983

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Marker	Primer	Fragment	Direction (5'- 3')	PCR thermal cycling conditions	Reference
COI	PolyLCO	658bp	(F) GAYTATWTTCAACAAATCATAAAGATATTGG	1) 94 °C (1 min); 2) 5 cycles: 94 °C (40 s), 45 °C (40 s), 72 °C (1 min); 3) 35 cycles: 94 °C (40 s), 51 °C (40 s), 72 °C (1 min); 4) 72 °C (5 min).	Carr *et al.* (2011)
	PolyHCO		(R) TAMACTTCWGGGTGACCAAARAATCA		
COI	LCO1490	658bp	(F) GGTCAACAAATCATAAAGATATTGG	1) 94 °C (1 min); 2) 5 cycles: 94 °C (30 s), 45 °C (1 min 30 s), 72 °C (1 min); 3) 35 cycles: 94 °C (30 s), 51 °C (1 min 30 s), 72 °C (1 min); 4) 72 °C (5 min).	Folmer *et al.* (1994)
	HCO2198		(R) TAAACTTCAGGGTGACCAAAAAATCA		
	COI-E		(R) TATACTTCTGGGTGTCCGAAGAATCA		Bely *et al.* (2004)
16S	16SAR-L	c.368bp	(F) CGCCTGTTTATCAAAAACAT	1) 94 °C (3 min); 2) 40 cycles: 94 °C (30 s), 47 °C (30 s), 72 °C (1 min); 3) 72 °C (7 min).	Kessing *et al.* 1989
	16SANN-F		(F) GCGGTATCCTGACCGTRCWAAGGTA		
	16SBR-H		(R) CCGGTCTGAACTCAGATCACGT		
ITS1	ITS18Sfa	c.675bp	(F) GAGGAAGTAAAAGTCGTAACA		Barfuss (2012)
	ITS5.8Sra		(R) GTTCAATGTGTCCTGCAATTC		
ITS2	ITS5.8SF	c.375bp	(F) ATGCTTAAATTCAGCGGGT	1) 96 °C (4 min); 2) 45 cycles: 94 °C (30 s), 48 °C (30 s), 72 °C (1 min); 3) 72 °C (8 min).	Nygren *et al.* (2009)
	28SR		(R) GAATTGCAGGACACATTGAAC		
28S	28sC1	c.791bp	(F) ACCCGCTGAATTTAAGCAT		Hassouna *et al.* (1984)
	28s-D2		(R) TCCGTGTTTCAAGACGG		

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Table 1. Primers and PCR conditions used in this study.

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	Marker	MOTUs	Minimum Distance (%)	Mean Distance (%)	Maximum distance (%)
Within MOTUs	COI	All	0	0.59	3.8
	16S		0	0.18	0.8
	ITS		0	0.44	5.8*
	28S		0	0.06	0.8*
Between MOTUs	COI	All	5.5	16.7	23.4
	16S		0.3	7.6	15.1
	ITS		0.5	9.2	18.1
	28S		0	1.7	4.5
Most similar MOTUS	COI	9 vs 11	7.6	8.2	8.7
		9 vs 10	5.5	6.7	7.4
		10 vs 11	9.8	10.2	10.6
	16S	10 vs 11	0.3	0.3	0.5
		9 vs 11	0.4	0.4	0.5
		9 vs 10	0.5	0.7	0.8
	ITS	17 vs 18	0.5	0.5	0.9
		2 vs 3	1.5	1.5	1.5
		12 vs 13	1.4	1.9	3.4
	28S	10 vs 11	0	0	0
		20 vs 16	0.1	0.1	0.1
		17 vs 18	0.3	0.3	0.4
Most distant MOTUs	COI	1 vs 13	20.5	21.7	23.4
		1 vs 5	21.1	22	22.6
		2 vs 12	21.1	21.8	22.9
	16S	2 vs 15	15.1	15.1	15.1
		2 vs 22	14.1	14.4	14.4
		1 vs 15	13.7	13.8	14.1
	ITS	1 vs 14	17.4	17.6	18.1
		3 vs 14	15	15.1	15.2
		2 vs 14	15.4	15.5	15.6
	28S	2 vs 12	3.7	3.8	4.5
		2 vs 13	3.5	3.6	3.7
		5 vs 13	3.4	3.5	3.6

Table 2. Mean intra and interspecific genetic distances (K2P) among all the *Essc* for the four analysed markers (COI, 16S, ITS and 28S), with focus on the distances between MOTUs in relation to the three closest and distant neighbours

	Region	N	h	Hd	S	Pi	Fu and Li's D	Tajima's D
E. schanderi sp. nov.	BSW, BNO	13	6	0.72	4	0.0015	-1.60955 P > 0.10	-1.43759 P > 0.10
E. fenwicki sp. nov.	ANO, CGB, PGB, RFR	23	10	0,73	11	0,0022	-1,33065 P > 0.10	**-1,87001 P < 0.05**
E. gretathunbergae sp. nov.	PGB	26	24	0.99	39	0.0089	-2.10801 0.10 > P > 0.05	-1.77253 0.10 > P > 0.05
Eumida RO174-180	RFR	2	2	1.0	1	0,0016	-	-
E. aff. gretathunbergae	RFR, PGB	2	2	1.0	3	0,0048	-	-
E. aff. kelaino	BFR	2	1	0.0	-	-	-	-
Eumida ANT002	AIT	1	1	-	-	-	-	-
E. pleijeli sp. nov.	NIT	2	1	0.0	-	-	-	-
E. langenecki sp. nov.	AIT	5	5	1.0	11	0,0073	-0,92693 P > 0.10	-0,92693 P > 0.10
Eumida ORB997	OIT	1	1	-	-	-	-	-
E. aff. merope	RFR, PGB	13	8	0,86	11	0,0033	-1,94450 0.10 > P > 0.05	-1,70303 0.10 > P > 0.05
E. merope	BFR, ICR	10	8	0.93	32	0,0060	0,19975 P > 0.10	0,84167 P > 0.10
E. notata	FPT	11	10	0,98	24	0,0092	-1,27851 P > 0.10	-1,47163 P > 0.10
E. mackiei	PGB	28	26	0,99	37	0,0081	**-2,69971 P < 0.05**	-1,71525 0.10 > P > 0.05
E. sanguinea	BSW, FNO, BNO, PGB, SGB, HDEN	31	14	0,82	26	0,0073	-0,06960 P > 0.10	-1,06336 P > 0.10
E. maia	PGB, CGB, BFR	39	24	0,91	42	0,0084	**-2,61094 P < 0.05**	-1,71386 0.10 > P > 0.05
E. alkyone	BNO, DNO, BSW	8	7	0,96	16	0,0082	-1,09777 P > 0.10	-0,85599 P > 0.10
E. elektra	BNO, RFR	15	4	0,70	5	0,0030	0,48090 P > 0.10	0,76339 P > 0.10
E. taygete	BFR, CGB, PGB, IIT	22	18	0,98	35	0,0111	-0,49631 P > 0.10	-1,07113 P > 0.10
E. kelaino	BSW, PGB, SNO, BNO, RFR	21	10	0,81	10	0,0038	-1,00506 P > 0.10	-0,50678 P > 0.10
E. asterope	BFR	2	2	1.0	1	0,0016	-	-
Eumida F22	BFR	1	1	-	-	-	-	-

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Table 3. Indices of genetic diversity estimated, based on COI for each MOTU. Number of
1221 sequences (n); nucleotide diversity (π), number of haplotypes (h), haplotype diversity (Hd) and
1222 number of variables sites (S). Region abbreviations as stated in the methods, with the addition of:
1223 DENH, Denmark, Helsingør; CRI, Croatia, Istra; GBS, Great Britain, Scilly Islands and PTM,
1224 Portugal, Madeira island.

Combination Type	On Prostomium	Dorsally on Segment 2[a]	Dorsal Transverse Lines[b]	Dorsal Longitudinal Line	Dorsal Transverse Dots	Dorsal Eye-like Pattern	Species
A	-	X	X	-	-	-	*E. alkyone; E. maia;* **E. merope**; **E. aff. merope**, *E. mackiei;*
B	-	-	-	-	-	-	*E. asterope;* **E. elektra**; **E. taygete**; **E. gretathunbergae sp. nov.** *;* **E. schanderi sp. nov**.*; E. fenwicki* sp. nov.; *Eumida* RO174-180; **E. aff. gretathunbergae**
C	-	X	-	X	-	-	*E. kelaino; E.aff. kelaino; Eumida pleijeli* sp. nov.
D	-	X	-	-	-	-	*E. sanguinea;* **E. merope**; *E aff. merope,* **E. taygete**, *Eumida* F22; **E. schanderi sp. nov**.*; Eumida* ANT002; **E. elektra**
E	X	-	-	-	-	-	*E. notata*
F	-	-	X	-	-	-	**E. gretathunbergae sp. nov**.; **E. aff. gretathunbergae**
G	X	-	-	-	X	-	*E. langenecki* sp. nov.
H	-	X	-	-	-	X	*Eumida* ORB997

[a] Specimens with pigmentation dorsally on segment 2 may also have various amounts of pigmentation on the anterior cirri and in the prostomium.
[b] Transverse lines in some specimens from *Eumida merope, Eumida cf merope and E. aff. gretathunbergae* are very short, approaching spots.

Table 4. Patterns of white pigmentation in the *Eumida sanguinea* species complex with eight unique combinations (types A–H). Species in bold have polymorphic pigmentation types.

	E. fenwicki sp. nov.	*E. schanderi* sp. nov.	*E. gretathunbergae* sp. nov.	*E. elektra*	*E. pleijeli* sp. nov.	*E. langenecki* sp. nov.
AL/PL	*2* (**AL > PL**)	*1* (**AL < PL**)	*3* **(AL < PL)**	*4* (AL > PL)	AL > PL	AL > PL
AL/HL	**2**	**1**	3 (larger head)	4	-	-
AL/HW	**2**	**1**	3 (larger head)	4	-	-
AL/MAL	2	**1**	2	3	-	-
AL/STL	**2**	**1**	3 (larger tentacles)	4	-	-
AL/LTL	**2**	**1**	3 (larger tentacles)	4	-	-
NS (mean)	56	45	**94**	61	70	59
DCL>DCW	1,5x	**1.9x**	1.5x	1.5x	1.5x	1.45x
VCL>VCW	2x	2x	2x	2x	1.5x	2x
DCS2L>CS1L	2x	2x	2x	2x	**2.7x**	2x
WL (mean, mm)	5	4	**14**	7	9	12
Pigmentation	B	B and D	B and **F**	B	**C**	**G**
Live Coloration	Light Yellow	**Greenish**	Yellowish-brown	Yellowish	**Green**	Yellow
Distribution	NE Atlantic	**Scandinavia**	**Great Britain**	NE Atlantic	**Western Mediterranean Sea**	**Western Mediterranean Sea**

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1231 **Table 5.** Summary of the most relevant morphometric findings rating from 1 (smaller proportions) to 4 (larger proportions), number of segments (NS), ratio

1232 between the length and width of the dorsal cirri of median segments (DCL>DCW), ventral cirri of media segments (VCL>VCW) and the length between the

1233 dorsal cirri on segment 2 against the cirri on segment 1 (DCS2>CS1L), worm length (WL), pigmentation type, live coloration and geographical range regarding

1234 the new described species and *E. elektra*. Data in bold has the most distinct differences when combined

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Table and figure captions

Table 1. Primers and PCR conditions used in this study.

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Fig.1. *S*chematic of the *E. sanguinea* morphotype (modified from Teixeira *et al.* (2020)) showing the measurements used in the morphometric analysis (A, B). A, Anterior end. B, Parapodia. Abbreviations: CLL, the length of the chaetigerous lobes; CLH, the height of the chaetigerous lobes; AL, the length of the antennae; PL, the length of the palps; MAL, the length of the middle antenna; CS1L, cirri on segment 1; DCS2L, dorsal cirri on segment 2; DCL, the length of the dorsal cirri; VCL, the length of the ventral cirri; HL, the length of the head; WWP, the width of the worm with parapodia; WW, the width of the worm without parapodia; HW, the width of the head; DCW, the width of the dorsal cirri; VCW, the width of the ventral cirri; DE, distance between the eyes.

Fig.2. Phylogenetic tree reconstructed using Bayesian inference for the *Essc* (A, B), comparing 296 COI and 94 16S concatenated mitochondrial sequences (A) against 192 combined nuclear markers from the ITS-region and 28S sequences (B), with information regarding the different MOTU delineation methods. BINs were used only for COI. Collapsed clades have less than 3.5% genetic divergence. Numbers in parenthesis indicate the number of sequences used for each MOTU and in the case of the mitochondrial markers the first correspond to COI and the second

to 16S. *Eumida ockelmanni*, *Eumida aff. ockelmanni*, *Sige fusigera* and *Eumida bahusiensis* as outgroups. Only the bootstrap values over 0.85 BI support are shown. Each different consensus MOTU is represented by the respective number, with the colored ones corresponding to the described species and new lineages found in this study. Abbreviations: Cons MOTU, Consensus MOTU; OUTG, Outgroup.

Fig.3. Haplotypes networks based on COI for all the *Essc* and respective outgroups. Each haplotype is represented by a circle and number of haplotypes are according to the displayed scale. Colours indicate the geographic location of the haplotype. Numbers correspond to the number of mutational steps between haplotypes. Lines without numbers means only one mutation between haplotypes.

Fig.4. Haplotypes networks based on ITS (A), 28S (B) and 16S (C) for all the *Essc* and respective outgroups. Each haplotype is represented by a circle and number of haplotypes are according to the displayed scale. Colours indicate the geographic location of the haplotype. Numbers correspond to the number of mutational steps between haplotypes. Lines without numbers means only one mutation between haplotypes.

Fig.5. Live, relaxed *Eumida* specimens exhibiting different types of white pigmentation patterns and coloration (A-F). A, *Eumida schanderi* sp. nov., specimen ZMBN_134559 (size: 3.3 mm), with green coloration and type B pigmentation and (B) specimen ZMBN_134556 (size: 3.7 mm, holotype) with type D pigmentation and focus on the prostomium. C, *Eumida gretathunbergae* sp. nov., specimen DBUA0002400.01 (size: 15 mm, holotype) exhibiting type F pigmentation, and (D) specimen DBUA0002400.03 (size: 13 mm) exhibiting type B pigmentation. E, *Eumida fenwicki* sp. nov., specimen DBUA0002396.01 (size 5 mm, holotype) exhibiting type B pigmentation. F, *Eumida* RO174-180, specimen DBUA0002403.01 (size: 10 mm) exhibiting type B pigmentation. Darker yellow color results from the stomach content.

Fig.6. Live, relaxed *Eumida* specimens exhibiting different types of white pigmentation patterns and coloration (A-E). A, *Eumida aff. merope*, specimen DBUA0002393.01 exhibiting type A pigmentation and (B) specimen DBUA0002395.02 displaying type D pigmentation. C, *Eumida langenecki* sp. nov., specimen DBUA0002408.01, with type G pigmentation. D, *Eumida pleijeli* sp. nov., specimen DBUA0002407.02, displaying its characteristic green coloration mixed with type C pigmentation. E, *Eumida* ORB997, specimen DBUA0002410.01, with type H pigmentation. All the specimens are similar in size measuring around 12 mm, except for DBUA0002410.01 measuring around 6.3 mm.

Fig.7. Live, relaxed *Eumida* specimens exhibiting the different types of white pigmentation patterns and coloration (A-C). A, *Eumida aff. kelaino*., specimen DBUA0002404.01 exhibiting type C pigmentation. B, *Eumida aff gretathunbergae*, specimen DBUA0002401.01 displaying type F

1321 pigmentation with very small transverse lines. C, *Eumida* ANT002, specimen DBUA0002405.01
1322 exhibiting type D pigmentation. All the specimens are similar in size measuring around 14 mm,
1323 except for DBUA0002405.01 measuring around 3 mm.
1324
1325 **Fig.8.** Scatter plots with the most significative proportions in distinguishing *E. fenwicki* sp. nov.,
1326 *E. schanderi* sp. nov., *E. gretathungergae* sp. nov and *E. elektra* from each other (A-H).
1327 Morphometric proportions between the length of the antennae - AL and (A) short tentacular length
1328 - STL; (B) large tentacular length - LTL; (C) head width - HW; (D) head length - HL; (E) palp length
1329 - PL and (F) the length of the middle antenna - MAL. Measurements between the number of
1330 segments - NS against (G) the worm width – WW and (H) worm length - WL.
1331
1332 **Fig.9.** Scatter plots with the most significative proportions in distinguishing *E. notata, E. merope,*
1333 *E. aff. merope* and *E. elektra* from each other (A-F). Morphometric measurements between (A)
1334 worm width - WW and worm width with parapodia - WWP; (B) antennae length - AL and palp
1335 length - PL; (C) head length - HL with antennae length - AL; (D) distance between the eyes – DE
1336 and head width - HW; (E) ventral cirri length - VCL with dorsal cirri length - DCL and (F)
1337 chaetigorous lobe length – CLL against the ventral cirri length - VCL.
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1340 SUPPORTING INFORMATION
1341
1342 Additional Supporting Information can be found in the online version of this article at the
1343 publisher's web-site:
1344
1345 **Table S1.** Voucher data from Nygren *et al*. (2016), Teixeira *et al*. (2020) and this study. Origin of
1346 the specimens used in the molecular work, pigmentation types, vouchers and GenBank accession
1347 numbers for each of the analysed genetic markers. Separate accession numbers were assigned
1348 for ITS1 and ITS2 regarding data from Teixeira *et al.* (2020), while the records from Nygren and
1349 Pleijel (2011) and this study have a single accession number for the entire ITS region.
1350
1351 **Table S2.** Morphometric measurements for all the specimens belonging to the new species *E.*
1352 *schanderi* sp. nov., *E fenwicki* sp. nov., *E. gretathunbergae* sp. nov., *E. pleijeli* sp. nov., E.
1353 *langenecki* sp. nov., as well for *E. notata*, *E. merope*, *E aff. merope* and *E. elektra*.
1354
1355 **Fig. S1.** Maximum likelihood phylogenies for the concatenated mitochondrial (COI and 16S)
1356 dataset
1357
1358 **Fig. S2.** Maximum likelihood phylogenies for the concatenated nuclear (ITS and 28S) dataset
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