

1 **Reappraisal of the hyperdiverse European *Platynereis* (Annelida: Nereididae) species**
2 **complex, with the description of two new species**

3
4 Marcos A.L. Teixeira^{1,2*}, Joachim Langeneck³, Pedro E. Vieira^{1,2}, José Carlos Hernández⁴, Bruno
5 R. Sampieri⁵, Panagiotis Kasapidis⁶, Serena Mucciolo⁷, Torkild Bakken⁸, Ascensão Ravara⁹, Arne
6 Nygren¹⁰, Filipe O. Costa^{1,2}

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

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

12 ³ Dipartimento di Biologia, Università di Pisa, via Derna 1, I-56126 Pisa, Italy

13 ⁴ Biología Animal, Edafología y Geología, Universidad de La Laguna, Tenerife, Spain

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

16 ⁶ Hellenic Centre for Marine Research, Institute of Marine Biology, Biotechnology and
17 Aquaculture, Anávyssos, Greece

18 ⁷ Katedra Zoologii Bezkręgowców i Hydrobiologii, Uniwersytet Łódzki, ul. Banacha 12/16, 90-237,
19 Łódź, Poland

20 ⁸ Norwegian University of Science and Technology, NTNU University Museum. NO-7491
21 Trondheim, Norway

22 ⁹ Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of
23 Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

24 ¹⁰ Institutionen for marina vetenskaper, Göteborgs Universitet, Tjärnö, Strömstad, Sweden

25
26 **Corresponding author*

27 Mail: mark-us_teixeira@hotmail.com

28
29
30 **Running title:** Disentangling the European *Platynereis* complex

41 **Abstract**

42 Morphologically similar species are often overlooked, but molecular techniques have been
43 effective in signalling potential hidden diversity, thereby boosting the documentation of unique
44 evolutionary lineages and ecological diversity. *Platynereis dumerilii* and *Platynereis massiliensis*
45 are part of a recognized species complex, where only differences in the reproductive biology have
46 been identified so far. Recent studies integrating cytochrome c oxidase subunit I (COI) sequence
47 data with reproductive features and life-history observations, found evidence of additional
48 undescribed diversity for these species in the Mediterranean Sea.

49 Analyses of DNA sequence data (COI, 16S rDNA and D2 region of the 28S rDNA) of populations
50 of the *P. dumerilii* morphotype, obtained from a broader sampling area along European marine
51 waters and the Macaronesia islands (Madeira, Azores and Canaries), provided compelling
52 evidence for the existence of at least 10 divergent evolutionary lineages. Complementing the
53 genetic data, morphological observations of the better represented lineages revealed two major
54 groups with distinctive paragnath patterns. Other morphological characters, such as differences
55 in the size of the tentacular cirri, number of segments, shape of the parapodia, serration type in
56 the spiniger chaetae and pigmentation types, compared between topotypic material and from
57 other locations, were also useful in the erection of two new *Platynereis* species: *P. macaronensis*
58 sp. nov. widespread in the Macaronesia islands and *Platynereis jourdei* sp. nov., restricted to the
59 western Mediterranean. The previous unaccepted species *Nereis agilis* is emended as
60 *Platynereis agilis* comb. nov. for one of the lineages present both in the NE Atlantic and western
61 Mediterranean. *P. dumerilii* is also redescribed based on topotypic material. However, the
62 uncertainty in the identity of *P. massiliensis* due to the original incomplete description, and the
63 absence of type and topotypic material prevents its unequivocal assignment to the lineage
64 assumed in this and in other previous studies. The remaining five lineages are represented by
65 only a few small specimens with morphological features poorly preserved, thus were not
66 described in this study. Lastly, two small Nereidid species that share the same habitat and can
67 often be misidentified as *P. dumerilii* juveniles, one unique to the Macaronesia islands and the
68 other present both in the Mediterranean and Macaronesia, may be entirely new unreported
69 species or new pseudo cryptic lineages belonging to an existing group. Additional sampling effort
70 and further morphological examination are needed to clarify the status of these lineages.

71

72 **Additional keywords:** *Platynereis*, Nereididae, integrative taxonomy, cryptic species

73

74

75

76

77

78

79

80

81 Introduction

82

83 A growing number of studies have been challenging the broadly-distributed or
84 cosmopolitan-quality of multiple marine benthic invertebrates (e.g. Nygren *et al.* 2018; Hupało *et*
85 *al.* 2019; Sampieri *et al.* 2021), unveiling instead the occurrence of complexes of cryptic or
86 pseudo cryptic species with more restricted geographic distributions (Struck *et al.* 2018;
87 Hutchings and Kupriyanova 2018; Cerca *et al.* 2020). Morphologically similar species are often
88 overlooked, but molecular techniques have been extremely effective in signalling potential hidden
89 species. Their detection, complemented with further morphological examination, has the ability to
90 boost the documentation of unique evolutionary lineages and associated diversity of ecological
91 attributes (Nygren 2014; Langeneck *et al.* 2020; Martin *et al.* 2020).

92 Species with no clear and stable morphological differences, i.e. cryptic species, can
93 sometimes be distinguished by their life history traits. Evidence of this apparent morphological
94 stasis can be exemplified by the annelids *Platynereis dumerilii* (Audouin & Milne Edwards, 1833)
95 and *Platynereis massiliensis* (Moquin-Tandon, 1869). Based on previous descriptions, these
96 sibling species can only be distinguished by the different reproductive strategies (Hauenschild,
97 1951). *Platynereis dumerilii* is gonochoric and semelparous (with a single reproductive event in
98 life), with males and females being attracted to each other by pheromones (Zeeck *et al.* 1988;
99 Zeeck *et al.* 1998), transforming into a pelagic epitokous form called *heteronereis* (Zantke *et al.*
100 2014), dying after the process is over. The larval stage has a planktotrophic development (Zeeck
101 *et al.* 1988; Fischer and Dorresteyn 2004). *Platynereis massiliensis* shows no epitokous
102 transformation and is a protandrous hermaphrodite, characterized by egg brooding and
103 lecithotrophic larval stages with a semi-direct development (Schneider *et al.* 1992).

104 *Platynereis dumerilii* is a meso-herbivore species (Ricevuto *et al.* 2015) first described
105 from the French Atlantic coast (type locality: La Rochelle). It is also reported throughout the
106 Mediterranean inhabiting shallow hard bottoms covered by seaweeds (Giangrande 1988; Gambi
107 *et al.* 2000), where it is often mistaken for the apparent morphologically similar and sympatrically-
108 distributed *P. massiliensis* (type locality: Marseille, France). Outside the Mediterranean, *P.*
109 *dumerilii* has also been reported from other parts of the world such as the Gulf of Mexico, Cuba,
110 the English Channel, Norway, the Black Sea, Mozambique and South Africa (Kara *et al.* 2020). It
111 is considered a bioindicator of organic pollution (Bellan 1980), a model species for basic biology
112 and Evo-Devo studies (Fischer and Dorresteyn 2004; Helm *et al.* 2015; Özpölat *et al.* 2021) can
113 also be used as a model to address various aspects of acclimatization and adaptation to ocean
114 acidification (Wäge *et al.* 2017), as it is one of the dominant species present in volcanic CO₂ vents
115 (Ricevuto *et al.* 2015). Although reported in Naples (Hauenschild, 1951) and Banyuls (Schneider
116 *et al.* 1992), *P. massiliensis* is still not included in Mediterranean polychaete check-lists and
117 revisions (Arvanitidis 2000; Mikac 2015) due to the analysis of preserved specimens only and the
118 lack of ecological investigations complemented with molecular data. Based on reproductive
119 biology studies, Valvassori *et al.* (2015) found evidence of the occurrence of *P. massiliensis* in
120 the CO₂ vents system of the Italian island of Ischia.

121 Evidence of additional lineages belonging to the *P. dumerilii* complex were also found by
122 (Wäge *et al.* 2017) after integrating cytochrome c oxidase subunit I (COI) sequence data with
123 reproductive biology and life-history observations on some selected populations thriving in the
124 vent areas from the Italian islands of Ischia and Vulcano. This analysis highlighted the presence
125 of four distinct *Platynereis* lineages, two of them primarily present in CO₂ vents, and presumably
126 all brooders, and the other two clades dominating the non-acidified sites, appearing to be
127 epitokous free spawners. Based on this genetic data and the fact that there is no evidence of
128 accidental human translocation of *P. dumerilii* to other regions (Read 2007), it is highly probable
129 that at least some of the 19 previously synonymised species with *P. dumerilii* are actually valid
130 distinct species. These synonyms belong to 17 different type localities, ranging from the Atlantic
131 to the Pacific Ocean (Read and Fauchald 2021a) and might correspond to morphotype variants
132 within the *P. dumerilii* cryptic complex, that were inadequately synonymised. Recently, a South
133 African taxon formerly thought to be *P. dumerilii* was ascribed to a new species (*P. entshonae*
134 Kara, Santos, Macdonald & Simon, 2020) mainly based on molecular data, with principal
135 component analysis scores revealing no separation based on morphological characters (Kara *et*
136 *al.* 2020). However, a smaller postero-dorsal tentacular cirri (up to chaetigers 6–8) and an unique
137 bidentate chaetae type (notopodial homogomph falciger), distinguish this species from the original
138 *P. dumerilii* morphotype.

139 To investigate the possible existence of additional hidden *Platynereis* species within the
140 *P. dumerilii* morphotype, and attempting to resolve the current existing European complex in this
141 group, we used a multi-locus approach, as well as morphological data, to examine multiple
142 populations from Scandinavia, to the NE Atlantic, the Macaronesia islands (Azores, Madeira and
143 Canaries) and the Western and Eastern Mediterranean Sea.

144

145 **Methods**

146

147 *Taxon sampling*

148 Nereidid specimens were collected in several localities along the Atlantic and
149 Mediterranean coasts of Europe, including the Macaronesia islands, and at Mazagan (Morocco).
150 The Atlantic localities include: Norway (Stavanger, Bergen and Trondheim), Sweden (Tjärnö),
151 Great Britain (Plymouth), France (Morlaix Bay, La Rochelle, Arcachon Bay), Portugal (northern
152 beach of Canto Marinho, Azorean islands of Santa Maria, São Miguel and Terceira, Madeira and
153 Porto Santo islands), and Spain (Canary islands of Tenerife, Gran Canaria, El Hierro, La Palma,
154 Lanzarote and Fuerteventura). The Mediterranean localities include: France (Banyuls), Spain
155 (Calpe), Italy [Tuscany area (Calafuria, Antignano, Ardenza, Vada, Livorno and the islands of
156 Montecristo, Pianosa and Elba), Trieste (Adriatic Sea) and Taranto (Ionian Sea)], and Greece
157 (Mazoma and in Crete island (Paralia Skinaria)). The specimens were picked among algae in
158 rocky beaches, at low tide or by scuba diving down to 10 meters depths, and fixed in 96% ethanol.
159 Additionally, specimens from the Arrabida Natural Park (Lisbon, Portugal) were provided by the
160 National Museum of Science and Natural History (Portugal).

161

162 *Molecular procedures and data mining*

163 DNA sequences of the 5' end of the mitochondrial cytochrome oxidase subunit I (mtCOI-
164 5P, approximately 658 bp) were obtained for 193 *Platynereis* specimens and 33 Nereididae
165 species belonging to other genera. Sequences of 16S rDNA (approximately 368 bp) and D2
166 region of 28S rDNA (approximately 420 bp) were also obtained for a representative number of
167 specimens per location. Molecular data from *Pseudonereis palpata* (Treadwell, 1923) specimens,
168 collected at Crete island, were used as outgroup for all the analysed loci, as well as COI
169 sequences from *Perinereis marionii* (Audouin & Milne Edwards, 1833) specimens collected in NW
170 Portugal (Canto Marinho) and Great Britain (Plymouth). For comparison purposes, COI
171 sequences from the four *Platynereis* lineages obtained by Wäge *et al.* (2017) and *Platynereis*
172 sequences from Kara *et al.* (2019) and (Calosi *et al.* 2013) were mined from GenBank.
173 Additionally, COI sequences belonging to the outgroups *Neanthes fucata* (Savigny, 1822), *Nereis*
174 *zonata* (Malmgren, 1867), *Nereis pelagica* Linnaeus, 1758, *Nereis heterocirrata* Treadwell, 1931
175 and *Ceratonereis tantaculata* Kinberg, 1865 were mined from GenBank and completed the final
176 dataset used for the phylogenetic analysis. DNA was extracted, amplified, sequenced, and
177 assembled as described in (Lobo *et al.* 2016) and Nygren *et al.* (2018). PCR conditions and
178 primers used are detailed in Table S1. Sampling locations, GenBank accession numbers, and
179 voucher data are detailed in Table S2. As only a few parapodia or a small portion of the posterior
180 end were used for the DNA extraction, DNA voucher specimens are deposited at the Research
181 Collection of Marine Invertebrates of the Department of Biology of the University of Aveiro (COBI
182 at DBUA), Portugal, and available for further morphological or molecular study. Specimens which
183 were exhausted in the DNA analysis were assigned only with the Process ID from the BOLD
184 systems (<http://v4.boldsystems.org/>), corresponding to the ones from northern Greece
185 (MTPD194-20-MTPD201-20) and the specimens MTPD191-20 (France, Morlaix) and MTPD144-
186 20 (Spain, Gran Canaria). The specimens from Norway are deposited at NTNU University
187 Museum (Bakken *et al.* 2021). The full dataset (excluding the sequences from Calosi *et al.* (2013),
188 which cannot be found in BOLD) and its metadata can be accessed at BOLD Systems under the
189 project "*Platynereis* Species Complex (DS-MTPD)" and in the following link: (doi: *upon paper*
190 *acceptance*).

191

192 *Phylogenetic analysis*

193 The phylogenetic analyses of the different loci were performed through maximum
194 likelihood (ML) and Bayesian inference (BI). Sequences from the mtDNA COI-5P, rDNA 16S and
195 the D2 region of the rDNA 28S were aligned and concatenated in MEGA 10.0.5 software (Kumar
196 *et al.* 2018) with Clustal W (Thompson *et al.* 1994). MrBayes 3.1.2 (Ronquist and Huelsenbeck
197 2003) was used to conduct the Bayesian analysis. Best-fit models were selected using the Akaike
198 Information Criterion in the jModeltest software (Guindon and Gascuel 2003; Darriba *et al.* 2012).
199 For COI we applied the Hasegawa-Kishino-Yano gamma distributed rates across sites (HKY +G)
200 for the first two positions and the General Time Reversible model with gamma distributed rates

201 across sites (GTR +G) for the third position. The latter model was also applied to the remaining
202 loci (16S and 28S-D2). Number of generations was set to 10 000 000, and sample frequency to
203 500. Twenty-five percent of the samples were discarded as burn-in (burninfrac = 0.25). The
204 resulting tree files were checked for convergence in the effective sampling sizes (ESSs >200)
205 with Tracer 1.7 software (Rambaut *et al.* 2018) and then analysed in Figtree 1.4.3
206 (<http://tree.bio.ed.ac.uk/software/figtree/>). The final version of the tree was edited with the
207 software Inkscape 0.92.3 (<https://www.inkscape.org>). Maximum Likelihood phylogenies were
208 performed in MEGA 10.0.5 with 1000 bootstrap runs with the GTR model with gamma distributed
209 rates across sites (GTR +G) for the concatenated dataset. A maximum likelihood amino acid
210 radiation tree was also performed in MEGA 10.0.5, using the Jones-Taylor-Thornton model with
211 equal rates across sites (JTT) for all the COI *Platynereis* lineages to visualize amino acid
212 differences between lineages. The BI tree was displayed in the results with the addition of the ML
213 support values if a similar topology is found.

214 The alignments (fasta and nexus format) for each individual marker and the concatenated
215 one are all publicly available online at Figshare (doi: *upon paper acceptance*).

216

217 *MOTU clustering*

218 To depict Molecular Operational Taxonomic Units (MOTUs), three delineation methods
219 were applied to the concatenated dataset, except for COI where the Barcode Index Number (BIN)
220 implemented in BOLD (Ratnasingham and Hebert 2013) was also applied. The Automatic
221 Barcode Gap Discovery (ABGD, (Puillandre *et al.* 2012) was implemented on a web interface
222 (<http://www.wabi.snv.jussieu.fr/pic/abgd/abgdweb.html>) with default settings using the K2P distance
223 matrix. The Generalized Mixed Yule Coalescent (GYMC) single threshold model (Fujisawa and
224 Barraclough 2013), as well as Poisson Tree Processes (bPTP, (Zhang *et al.* 2013) were applied,
225 with both analyses performed on a web interface (<https://species.h-its.org/>). BEAST 2.4.6
226 (Bouckaert *et al.* 2014) was used to generate the Bayesian ultrametric tree for the GYMC with the
227 appropriate best model (based on AIC criteria; GTR equal rates), and four independent runs for
228 50 000 000 MCMC generations, sampled every 5,000 generations. Tracer 1.6 software was used
229 to estimate convergence ESSs > 200 for all parameters. The consensus tree was obtained using
230 TreeAnnotator 2.4.6 (Bouckaert *et al.* 2014) and loaded into the Figtree software. ML phylogenies
231 obtained above in the “phylogenetic analysis” section contributed for the bPTP results. A final
232 consensus MOTU was chosen using the majority rule (i.e. most common number of MOTUs
233 across different delimitation methods and in case of draw, MOTUs were separated if more than
234 3.5% COI genetic divergence was present).

235

236 *Genetic distances, diversity and structure*

237 The mean genetic distances (Kimura-2-parameters, K2P) within and between MOTUs
238 were calculated in MEGA 10.0.5. Haplotype networks were made for the original sequences
239 through the PopART software (Leigh and Bryant 2015) using the method of Templeton, Crandall
240 and Sing (TCS, (Clement *et al.* 2002) to evaluate the relationship between the haplotypes and

241 their geographical distribution. Indices of genetic diversity, namely number of haplotypes (h),
242 haplotype diversity (hd), polymorphic sites (S), nucleotide diversity (π), Fu & Li D and Tajima D
243 statistical tests, were estimated based on COI for each MOTU using DNASP 5.10 (Librado and
244 Rozas 2009).

245

246 *Morphological analysis*

247 Morphological observations were carried out with an Olympus stereo microscope
248 equipped with a camera lucida for line drawings. Stereo microscope images were taken with a
249 Canon EOS1100D camera. Compound microscope images of parapodia and chaetae were
250 obtained with a Zeiss Axioplan 2 imaging light microscope (Carl Zeiss, Oberkochen, Germany),
251 equipped with a DP70 Olympus camera (Olympus Corp., Tokyo, Japan), after mounting the
252 parapodia on a slide preparation using Aquamount (Gurr) liquid. The software Inkscape 0.92.3
253 (<https://www.inkscape.org>) was used to create the final images for the drawings of the parapodia,
254 pharynx and anterior part of the worm's body.

255 Parapodial and chaetal terminology in the taxonomic section follows (Bakken and Wilson
256 2005) with the modifications made by (Villalobos-Guerrero and Bakken 2018). Pharynx paragnath
257 terminology follows (Bakken *et al.* 2009). Chaetigers after segment 15 are considered part of the
258 worm's mid-body, with the first 15 segments considered the anterior region of the body.

259

260 **Results**

261

262 *Phylogenetic reconstruction*

263 The BI tree (Fig.1A) is split into two major clades. The first clade (Clade A, including
264 MOTUs 1-10) generally complies with the description of the *Platynereis dumerillii* pseudo cryptic
265 complex, while the second clade includes *P. entshonae*, a sibling species of *P. dumerillii*
266 distinguished mainly at the molecular level (Kara *et al.* 2020), a group of undetermined nereidids
267 that share the same habitat and some morphological similarities with juveniles of *Platynereis*
268 species (Clade B, including MOTUs 11-15), and all the outgroup species included in the analysis.
269 Clade A is further divided into three sub-clades (A1: MOTU 1; A2: MOTUs 2-6, A3: MOTUs 7-10)
270 based on close genetic distances, topology, information regarding the reproductive biology and
271 paragnath variations.

272 A total of 15 unique consensus MOTUs were obtained, four of which are singletons with
273 only one sequence available (MOTUs 8, 13, 14 and 15). The remaining MOTUs correspond to
274 monophyletic clades with low divergence (COI <3% K2P) and are collapsed in Figure 1A. Apart
275 from the outgroups, additional MOTUs from other studies are also represented in the tree (GB1-
276 4). From these, GB2 and GB3 (included in Clade A3), present low support values (<0.85) and
277 lack well-defined bifurcated clades, and might belong to MOTU 9. However, morphological
278 analysis would need to be done to confirm this. MOTU GB1 seems to be a new lineage from
279 South Africa and MOTU GB4 is the recently described species *P. entshonae*. In general, the
280 Macaronesia (particularly the Canary islands) and the whole Mediterranean Sea appear to be a

281 cryptic hotspot, with several localities with more than two sympatric MOTU's (see map on Fig.
282 1B).

283 Focusing only on Clade A (*P. dumerilii* complex), three MOTUs are unique to the
284 Macaronesia (MOTU 5, 7 and 8) of which one occurs exclusively in Porto Santo island (MOTU 8)
285 and two sympatric ones are present in the Gran Canaria and Lanzarote islands alone (MOTUs 5
286 and 7). Additionally, three lineages are present exclusively in the Mediterranean (MOTU 1 and
287 MOTU 6 in the western part and MOTU 3 in the Eastern part of the Sea) of which MOTU 1 was
288 only found at Banyuls. Three sympatric MOTUs were identified in the southeast of Spain (MOTUs
289 4, 6 and 10) and in the Northern Tyrrhenian Sea (MOTUs 4, 6 and 9). Four different MOTU's were
290 found in the NE Atlantic, three of them shared with the Mediterranean (MOTUs 4, 9 and 10) and
291 one exclusive to this part of the European coastline (MOTU 2). The specimens from the type
292 locality of *P. dumerilii* species (La Rochelle) grouped all within MOTU 4. This particular lineage is
293 the most widespread and easy to find among all the mainland samples, being present both in NE
294 Atlantic and the whole Mediterranean Sea, while MOTU 7 was the most widespread and abundant
295 one among the Macaronesia islands. A radiation amino acid tree based on COI sequences from
296 the 10 retrieved *Platynereis*' MOTUs was also able to separate the three main sub-clades (A1,
297 A2 and A3) found in the BI tree, with MOTUs 1, 2, 5 and 7 not sharing the same amino acids with
298 any of the remaining lineages.

299 A non-collapsed ML tree with 1000 bootstrap support can be seen in the supplementary
300 material (Fig. S1).

301

302 *Genetic distances*

303 The global mean genetic distances (K2P) for the clades A and B can be found in Table
304 1. Regarding only the *Platynereis* complex (clade A), the mean intra-MOTU distance was 0.2 (0
305 – 3.5)% for COI and 0.3 (0 – 1.4)% for 16S, while the average inter-MOTU distances were 19.4
306 (4.4 – 26.6)% and 6.2 (1.5 – 9.9)% respectively. For the 28S-D2 region, it ranged between 0.2 (0
307 – 1.4)% and 1.1 (0.1 – 3.9)% for intra- and inter-MOTU divergence, respectively. Detailed mean
308 genetic distances for the three genetic markers between each MOTU can be found in Table S3.
309 When comparing between major clade A and B, the maximum interspecific genetic distances are
310 significantly higher in all loci, especially for 16S and 28SD2. In this scenario, maximum
311 divergences of 32.6% COI, 35.7% 16S and 36.9% 28SD2 were recorded, as opposed to the
312 26.9%, 9.9% and 3.9% found only within clade A, based on the same respective loci.

313

314 *Haplotype networks and diversity*

315 All COI (Fig. 2A) and 16S (Fig. 2C) haplotypes were completely sorted among MOTUs,
316 i.e. no haplotypes were shared among more than one MOTU. However, some MOTUs (4, 5 and
317 6; 12 and 13; 14 and 15) shared the same haplotype in the 28S-D2 loci (Fig. 2B). The 28S-D2
318 network provided two major groups segregating clade B as seen in the BI, with more than 90
319 mutations separating it from clade A.

320 The COI network also revealed geographically structured populations within MOTU 9 and
321 10, corresponding to the 5 distinct BINs shown in the BI (Fig. 1A), except the populations from
322 North of France and south of Great Britain that did not split into separate BINs in MOTU 10. By
323 contrast not all populations from different Atlantic islands were completely sorted in MOTU 7, with
324 the presence of shared haplotypes between all islands, except Gran Canaria and La Palma.
325 Further geographic sorting in the COI network can also be identified within MOTU 4 regarding
326 populations from the western and eastern Mediterranean Sea.

327 For the most sampled MOTUs (4, 6, 7, 9, 10) COI haplotype diversity is relatively high
328 ($Hd > 0.89$ to 0.99 , Table 2), except for MOTU 6 ($Hd: 0.65$). The latter, together with MOTU 4, are
329 the only cases with a significant Tajima D and Fu and Li's D tests, where the negative values
330 indicate possible population expansion after a recent bottleneck or the occurrence of selective
331 sweeps, with the neutral model of nucleotide substitutions accepted for the remaining MOTUs.

332

333 *Platynereis dumerilii pseudo-cryptic complex (clade A): Morphological findings*

334 A compilation of European species currently considered as synonyms of *P. dumerilii* with
335 their main distinctive morphological traits based on the original descriptions is given in Table 3.
336 *Platynereis nadiae* Abbiati & Castelli, 1992 was included in this table, despite being currently
337 accepted by WoRMS, given the similarity of this species' description with juveniles from
338 *Platynereis dumerilii*. A similar summary was made for the ten different *Platynereis* MOTUs
339 analysed in this study (Table 4). Two new species are described in the taxonomic section, below,
340 corresponding to the MOTUs 6 (Figs. 3-4) and MOTU 7 (Figs. 5-6). Additionally, the previous
341 synonymized name *Nereis agilis* Keferstein, 1862 is reinstated as *Platynereis agilis* comb. nov.
342 for MOTU 10 and redescribed (Figs. 7-8). Amended descriptions of *P. dumerilii* (MOTU 4) and *P.*
343 *massiliensis* (MOTU 9) are also provided, using the specimens studied herein (Figs. 9-10 and
344 Figs. 11-12, respectively). The remaining MOTUs are represented by a smaller number of
345 specimens in suboptimal conditions and thus are not fully described here. However, they seem
346 to share the same morphological features from the respective phylogenetically nearest
347 neighbours (see Fig. 1A), except for a few different characteristics shown by MOTUs 2 and 5. In
348 MOTU 2 the morphology of parapodia and tentacular cirri is closer to MOTU 10 instead of the
349 remaining MOTUs from clade A2, while in MOTU 5 the tentacular cirri are similar to MOTU 9
350 (Table 4). Specimens from MOTU 3 were very small with the entire worm being used for DNA
351 extraction, thus only a very preliminary morphological analysis was done. MOTU 1 seems to be
352 morphologically similar to MOTU 4 (Table 4) and seems to share a similar pigmentation as the
353 Livorno population from MOTU 9.

354 All the analysed MOTUs from clade A seem to share the typical dorsal and ventral
355 parapodial cirri variation described in the topotypic material, with the dorsal cirrus being at least
356 twice the length of the corresponding ligule, whereas the ventral cirrus is short and may reach
357 half the size of the ventral ligule. Differences in the size of the tentacular cirri, paragnath patterns,
358 number of segments and serration type in the spiniger chaetae contributed for the main
359 differences between lineages. In our observations, pigmentation does not seem to be always a

360 useful character since it can sometimes be absent in very small specimens or completely lost
361 upon fixation in ethanol. However, generally speaking, it is possible to identify a designated MOTU
362 based on the pigmentation patterns as seen in the respective figures, except between MOTUs 4
363 and 6 where some specimens might share similar pigmentation density and pattern. Another
364 apparently relevant morphological character is the number of teeth in the jaws of adult specimens,
365 considering the stability of the reported numbers, either 8 or 11 (Table 3). Due to the difficulty of
366 dissecting small organisms such as *Platynereis* specimens, the pharynx and jaws of the studied
367 specimens could only be examined in a few worms. Nevertheless, generally, MOTUs from clade
368 A3 seem to have a higher number of teeth, between 7-8 against the 5-6 from clade A2 (Table 4).

369

370 *Undetermined nereidids (clade B): Morphological findings*

371 Five additional MOTUs, belonging to small sized nereidid specimens, were retrieved and
372 may be confused with small juvenile specimens of other *Platynereis* species. Apart from the
373 genetic evidence (Fig. 1A, Table 1) and considering morphological features alone (particularly,
374 the tentacular cirri and pharynx paragnaths), it is clear that MOTUs 11, 12 and 13 belong to a
375 different genus - either *Neanthes* Kinberg, 1865 or *Nereis* Linnaeus, 1758. Compared to
376 descriptions and figures in Fauvel (1923) and Fauna Iberica (Peral *et al.* 2004), MOTUs 12-13,
377 unique to the Macaronesia islands, are morphologically close to *Nereis zonata* Malmgren, 1867,
378 with similar proportions between the antennae in relation to the palps and very short tentacular
379 cirri. However, some differences in the parapodial structure were found and the pharynx has
380 different paragnath patterns. Based on photos deposited in BOLD (Zhou *et al.* 2010), MOTUs 12-
381 13 also showed high resemblance with specimens identified as *Nereis heterocirrata* Treadwell,
382 1931, grouping very closely in the phylogenetic tree as well (Fig. 1A). The most noticeable feature
383 of the latter species is represented by the two dorsal tentacular cirri extending about to the end
384 of the palp, while the posterior ventral one is considerably shorter (Treadwell 1931), which is also
385 observed in our specimens (Fig. 13). This species is only reported in Southeast Asia and no
386 reports in the Atlantic were found so far (Read & Fauchald 2021b,
387 <https://www.marinespecies.org/polychaeta/aphia.php?p=taxdetails&id=329658>).

388 MOTU 11 (Fig. 14) has similar paragnath patterns as described in some *Neanthes*
389 species, particularly *Neanthes fucata* (Savigny, 1822), as seen in the drawings from Peral *et al.*
390 (2004). However, parapodia from the posterior part do not have the characteristic leaf-like dorsal
391 ligules found in *N. fucata* or in most species belonging to *Neanthes*. Furthermore, the presence
392 of homogomph falcigers (Fig. 14G), which are lacking in *Neanthes*, resemble *Nereis* species
393 instead, in particular *Nereis zonata* (see Gravina *et al.* 2016). The latter species may also display
394 a high degree of variation in the paragnath arrangements, some of which may be similar to the
395 ones described for *Neanthes fucata* (Peral *et al.* 2004; Gravina *et al.* 2016).

396 The outgroups from GenBank identified as *N. fucata* grouped with our samples identified
397 as *Perinereis marionii* (Audouin & Milne Edwards, 1833). The latter species possess a
398 characteristic paragnath pattern in the oral ring with a dorso-ventral continuous band composed
399 of multiple small paragnaths and an irregular line of larger paragnaths in the anterior margin,

400 especially in area V with a large conical paragnath with triangular shape and areas VI with a small
401 transverse bar. Parapodia is also characterized with the presence of a very long dorsal ligule in
402 the posterior parapodia (Peral *et al.* 2004; see photo of our specimen from *P. marionii* in the
403 supplemental material Fig. S2). This result strongly suggest misidentifications in the genetic
404 databases for this group.

405 MOTUs 14 and 15 appear to share similar morphological features as MOTU 12,
406 especially regarding the tentacular cirri, being very small and all similar in size. However, since
407 the few available specimens lacked proper structural integrity, no further conclusions could be
408 taken.

409

410 *Taxonomic section*

411

412 ***Platynereis* Kinberg, 1865**

413

414 *Diagnosis (emended from Bakken and Wilson 2005)*

415 Prostomium cordiform with entire anterior margin, two pairs of eyes in trapezoid
416 arrangement, one pair of antennae, one pair of palps, four pairs of tentacular cirri with distinct
417 cirrophores. One apodous anterior segment, usually larger in length than chaetiger 1. Pharynx
418 maxillary and oral rings with rod-like paragnaths arranged in tight rows: Areas I and V – absent;
419 II – absent or present in small groups; III, IV and VI – present; VII and VIII – present, arranged in
420 isolated patches or in one or more irregular lines forming a continuous band. Jaws with dentate
421 cutting edge. Parapodia with dorsal ligule prechaetal notopodial lobe, median ligule, and ventral
422 ligule on anterior chaetigers. Neuropodial postchaetal lobe absent. Dorsal cirrus simple, lacking
423 basal cirrophore. Ventral cirri single. Notoaciculae absent from segments 1 and 2. Notochaetae:
424 homogomph spinigers, homogomph falcigers may be present. Neurochaeta, dorsal fascicle:
425 homogomph spinigers, heterogomph falcigers; ventral fascicle: heterogomph spinigers,
426 heterogomph falcigers.

427

428 *Remarks*

429 *Platynereis* was originally described and has been accepted as lacking paragnaths in
430 areas I, II and V of the pharynx (Kinberg 1865, Bakken and Wilson 2005). However, these
431 structures were found to be present in pharynx-area II of specimens belonging to *P. dumerilii* s.s.
432 and to a new species described herein. The diagnosis of the genus is therefore emended
433 accordingly.

434

435 ***Platynereis dumerilii* (Audouin & Milne-Edwards, 1833)**

436

(Figs. 9-10)

437

438 *Material examined*

439 *Neotype and hologenophore*: **France, La Rochelle**, 1 spm, DBUA0002438.01,
440 46°08'47.4"N - 1°12'36.0"W, low tide, among red algae, collected by Jérôme Jourde, 18/09/2020.
441 GenBank (COI): *upon paper acceptance*.

442

443 *Other material*: **Sweden, Tjärnö**, 10 spms, DBUA0002435.01-10, 58°52'27.6"N -
444 11°08'43.4"E, 3-5 meters, among algae, collected by Felicia Ultin and Marcos AL Teixeira,
445 20/12/2018; **Norway, Trondheim**, 1 spm, NTNU-VM-76216, 63°26'24.0"N - 10°30'14.4"E , 2
446 meters depth, among algae, collected by Torkild Bakken, 04/09/2018; **France, La Rochelle**, 16
447 spms, DBUA0002438.02-17, 46°08'47.4"N - 1°12'36.0"W, low tide, among red algae, collected
448 by Jérôme Jourde, 18/09/2020; **France, Arcachon Bay**, 1 spm, DBUA0002439.01, 44°39'44.2"N
449 - 1°09'10.0"W, low tide, among algae, collected by Nicolas Lavesque, 18/09/2020; **Portugal,**
450 **Canto Marinho**, 1 spm, DBUA0002436.01, 41°44'13.2"N - 8°52'33.6"W, low tide, among algae,
451 collected by Marcos AL Teixeira, 20/05/2019; **Spain, Calpe**, 2 spms, DBUA0002434.01-02,
452 38°38'23.8"N - 0°03'30.0"E, low tide, among algae, collected by Pedro E Vieira, 05/08/2019; **Italy,**
453 **Antignano**, 2 spms, DBUA0002437.01-02, 43°29'32.0"N - 10°19'01.2"E, 3 m, among algae,
454 collected by Joachim Langeneck, 10/09/2019; 3 spms, DBUA0002437.04-06, 43°29'32.0"N -
455 10°19'01.2"E, 6 m, among *Posidonia oceanica* rhizomes, collected by Joachim Langeneck,
456 20/09/2019; 1 spms DBUA0002437.03, 43°29'32.0"N - 10°19'01.2"E, 3 m, among algae, collected
457 by Joachim Langeneck, 27/06/2019; **Italy, Ardenza**, 5 spms, DBUA0002437.07-11, 43°30'43.3"N
458 - 10°18'52.3"E, 2 m, gravel with *Posidonia oceanica* debris, collected by Joachim Langeneck,
459 18/09/2019; **Italy, Vada**, 4 spms, DBUA0002437.12-15, 43°18'39.8"N - 10°25'54.6"E, 10 m,
460 among algae, collected by Joachim Langeneck, 26/10/2019; **Italy, Elba island**, 3 spms,
461 DBUA0002437.16-18, 42°48'41.1"N - 10°19'23.7"E, 3 m, among algae, collected by Joachim
462 Langeneck, 15/01/2020; **Italy, Montecristo island**, 7 spms, DBUA0002437.19-25, 42°20'05.9"N
463 - 10°17'22.3"E, low tide, among algae, collected by Joachim Langeneck, 05/09/2020; **Italy,**
464 **Taranto**, 1 spm, DBUA0002437.26, 40°27'59.0"N - 17°14'20.0"E, 12 m depth, on mud with shell
465 fragments, collected by Joachim Langeneck, 20/03/2019; **Italy, Trieste**, 1 spm,
466 DBUA0002437.27, 45°38'51.6"N - 13°45'32.9"E, low tide, among algae, collected by Joachim
467 Langeneck, 19/02/2020; **Greece, Mazoma**, 2 spm, MTPD200-20, MTPD201-20, 39°03'21.3"N -
468 20°50'00.5"E, low tide, among algae, collected by Katerina Vasileidou, 01/01/2017; **Greece,**
469 **Crete**, 1 spm, DBUA0002437.27, 35°09'57.6"N - 24°25'17.0"E, 5-10 meters, among algae,
470 collected by Giorgios Pace, 14/03/2020.

471

472 *Description*

473 Small-sized worms (1.5-26 mm long, 30-80 segments), tapering posteriorly. Neotype
474 complete, 26 mm for 76 chaetigers, high pigmentation density. Preserved specimens yellowish-
475 brown, with small pigmentation dots covering most of the anterior region and the prostomium area
476 adjacent to the eyes (Fig. 9A, Fig. 10A). The apodous anterior segment is similar in size to the
477 first chaetigers and lacks a well-defined ring-like dot pattern. Prostomium cordiform, with two pairs
478 of eyes in trapezoid arrangement. Antennae and palps similar in length (Fig. 9A). Palps consisting

479 of a palpophore and ovalshaped palpostyle. Four pairs of tentacular cirri usually longer than
480 body's width, with the longer postero-dorsal cirri reaching chaetiger 9-12 (Fig. 9A), rarely to
481 chaetiger 15. Pharynx maxillary and oral rings (Fig. 9A-B) with rod-like paragnaths arranged in
482 tight rows: Area I and V – absent, II - forming double parallel rows, III - forming a group of short
483 rows, IV - forming several long rows in pyramidal arrangement, VI - forming double parallel rows,
484 VII-VIII – arranged in double parallel short rows forming a continuous band. Jaws are finely
485 toothed until a short distance from the tip, usually with 5 or 6 teeth. Anterior parapodia (Fig. 9C)
486 with rounded to triangular ligules much shorter than in mid-body parapodia (Fig. 9D). Dorsal
487 notopodial ligule triangular from mid-body chaetigers, median notopodial ligule digitiform, equal
488 in length as dorsal ligule (Fig. 9D). Neuroacicular ligule short rounded in anterior chaetigers,
489 triangular and slightly shorter than ventral ligule from mid-body chaetigers. Dorsal cirri three times
490 longer than the parapodial dorsal ligule. Ventral cirri much shorter than ventral ligule (Fig. 9C-D).
491 Notochaetae: homogomph spinigers, serrations present in about $\frac{3}{4}$ length of blades; homogomph
492 falcigers, short blades incurved with a terminal tendon, serrated $\frac{1}{2}$ length of blade (Fig. 10E).
493 Neurochaeta, dorsal fascicle: homogomph spinigers, serrations present in about $\frac{3}{4}$ length of
494 blades (Fig. 10B), heterogomph falcigers short blades incurved with a terminal tendon, serrated
495 $\frac{2}{3}$ length of blade (Fig. 10C); ventral fascicle: heterogomph spinigers, serrations present in about
496 $\frac{3}{4}$ length of blades (Fig. 10D).

497

498 *Molecular data*

499 COI, 16S and 28SD2 sequences as in specimens DBUA0002434.01-02,
500 DBUA0002435.01-10, NTNU-VM-76216, DBUA0002436.01, DBUA0002438.01-17,
501 DBUA0002439.01, DBUA0002437.01-25, DBUA0002437.26, DBUA0002440.01,
502 DBUA0002437.27, MTPD200-20 and MTPD201-20 (Table S2). Phylogenetic relationship within
503 the *Platynereis dumerilii* pseudo cryptic complex as in Fig. 1A, belonging to MOTU 4, with high
504 support values and low intraspecific (<3%) genetic divergence for both the mitochondrial and
505 nuclear markers. Interspecific COI mean distances to the closest and distant neighbour are 8.2%
506 (K2P, MOTU 5) and 21.8% (K2P, MOTU 10) respectively. DOI for the species' neotype Barcode
507 Index Number (BIN): *upon paper acceptance*.

508

509 *Distribution and habitat:*

510 NE Atlantic, from Scandinavia to Mediterranean Sea, among green or red algae and
511 gravel with *Posidonia oceanica* rhizomes, in subtidal or intertidal areas.

512

513 *Reproduction:*

514 It is a gonochoric species, with a single reproductive event in life (semelparous)
515 transforming into a pelagic epitokous form (*heteronereis*) and a larval stage with planktotrophic
516 development (Wäge *et al.* 2017).

517

518 *Remarks:*

519 The holotype of *P. dumerillii* could not be found, thus preventing an effective
520 morphological or molecular comparison. The National Museum of Natural History (France), which
521 is home for major polychaete collections described by French authors, lack the type specimens
522 for this species. However, all the specimens collected in the type locality, presenting
523 morphological characteristics that fit the overall original description by Audouin & Milne-Edwards
524 (1833), grouped in a single MOTU (Fig. 1A, MOTU 4). Minor differences concern the pigmentation
525 pattern and pharynx jaws compared to the original description. The holotype was reported as
526 being yellowish with some brown spots at the basis of parapodia, although it is not clear whether
527 it refers to live or preserved organisms. Instead, the preserved specimens studied herein were
528 yellowish with brown pigmentation covering most of the anterior region. The pharynx and jaws
529 are incompletely described by Audouin & Milne-Edwards (1833), but from the original illustrations
530 (Pl. XIII, fig. 12), jaws seem to have 11 teeth far surpassing the 5-6 observed in the topotypes
531 examined herein. Furthermore, the original description presents some morphological
532 discrepancies compared to all other *Platynereis* species. The posterior parapodia are described
533 as having an overgrown neuracicular ligule and a clear separation between the notopodium and
534 neuropodium, suggesting that the specimens studied by those authors could be developing into
535 a *heteronereis* stage. The presence of paragnaths in Area II of the pharynx also distinguishes *P.*
536 *dumerillii* from the remaining *Platynereis* species described up to date. Given the apparent loss of
537 the holotype, and to provide taxonomic stability, a neotype was selected from among the
538 specimens collected in the type locality.

539 For a *Platynereis* review in the use of the species as a model system for genetics,
540 regeneration, reproduction biology, development, evolution, chronobiology, neurobiology,
541 ecology, ecotoxicology, and most recently also for connectomics and single-cell genomics, see
542 Özpolat *et al.* (2021).

543

544 ***Platynereis jourdei* Teixeira, Ravara, Langeneck and Bakken sp. nov.**

545 (Figs. 3-4)

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

547

548 *Material examined*

549 *Type material.* **Spain, Calpe**, 1 spm, DBUA0002431.02, 38°38'23.8"N - 0°03'30.0"E, low
550 tide, among algae, 05/08/2019, GenBank (COI): upon paper acceptance; 8 spms, paratypes and
551 paragenophores, DBUA0002431.01, DBUA0002431.03-09 38°38'23.8"N - 0°03'30.0"E, low tide,
552 among algae, collected by Pedro E Vieira, 05/08/2019.

553 *Other material.* **Italy, Pianosa island**: 5 spms, DBUA0002432.16-20, 42°34'59.8"N -
554 10°05'56.0"E, low tide, among algae, collected by Joachim Langeneck, 22/09/2020; **Italy,**
555 **Calafuria**, 1 spm, DBUA0002432.01, 38°29'52.8"N - 8°50'16.8"W, low tide, among algae,
556 collected by Joachim Langeneck, 11/01/2019; **Italy, Antignano**, 1 spm, DBUA0002432.02,
557 43°29'32.0"N - 10°19'01.2"E, 6 m in depth, among *Posidonia oceanica* rhizomes, collected by
558 Joachim Langeneck, 20/09/2019; 4 spms, DBUA0002432.03-06, 43°29'32.0"N - 10°19'01.2"E, 3

559 m depth, among algae, collected by Joachim Langeneck, 10/09/2019; 3 spms, DBUA0002432.07-
560 09, 43°29'32.0"N - 10°19'01.2"E, 3 m depth, among algae, collected by Joachim Langeneck,
561 27/06/2019; **Italy, Montecristo island**, 6 spms, DBUA0002432.10-15, 42°20'05.9"N -
562 10°17'22.3"E, low tide, among algae, collected by Joachim Langeneck, 05/09/2020; **France,**
563 **Banyuls**, 1 spm, DBUA0002433.01, 42°28'53.9"N - 3°08'00.3"E, low tide, among red algae,
564 collected by Felicia Ultin, 20/09/2020.

565

566 *Description.*

567 Small-sized worms (1.5-28 mm long, 30-71 segments), tapering posteriorly. Holotype
568 complete, 26 mm long for 71 chaetigers, very low pigmentation density. Preserved specimens
569 yellowish-brown, with fainted scattered pigmentation dots covering most of the anterior region
570 varying in density (when visible) and the prostomium area adjacent to the eyes (Fig. 3A, Fig. 4A).
571 The apodous anterior segment lacks a well-defined ring-like dot pattern, but this pattern may
572 appear after the first few segments, varying in terms of pigment density (Fig. 3E, Fig. 4B).
573 Prostomium cordiform, with two pairs of eyes in trapezoid arrangement. Antennae and palps
574 similar in length. Palps consisting of a palpophore and ovalshaped palpostyles. Four pairs of
575 tentacular cirri usually longer than the body width, with the longer postero-dorsal cirri reaching up
576 to chaetiger 9-12 (Fig. 3A). Pharynx maxillary and oral rings with rod-like paragnaths arranged in
577 tight rows (Figs. 3A-B): Area I and V – absent, II - forming double parallel rows, III - forming a
578 group of short rows, IV - forming several long rows in pyramidal arrangement, VI - forming double
579 parallel rows, VII-VIII – arranged in double parallel short rows forming a continuous band. Jaws
580 are finely toothed until a short distance from the tip, usually with five teeth. Anterior parapodia
581 with rounded to triangular ligules (Fig. 3C) slightly shorter than in mid-body parapodia, notopodial
582 ligules equal in length from mid-body chaetigers (Fig. 3D). Neuroacicular ligule short digitiform,
583 longer than a round ventral ligule in anterior chaetigers, triangular and equal in length as a
584 digitiform ventral ligule from mid-body chaetigers (Fig. 3C-D). Dorsal cirri three times the length
585 of parapodial dorsal ligule. Ventral cirri slightly shorter than ventral ligule (Fig. 3C-D).
586 Notochaetae: homogomph spinigers, serrated almost to the end of blade (Fig. 4C).
587 Neurochaetae, dorsal fascicle: homogomph serrated spinigers, heterogomph falcigers, incurved
588 with a distinct terminal tendon, serrated $\frac{1}{3}$ length of blade (Fig. 4E); ventral fascicles:
589 heterogomph serrated spinigers, heterogomph falcigers incurved with a terminal tendon, serrated
590 $\frac{2}{3}$ length of blade (Fig. 4D).

591

592 *Molecular data*

593 COI, 16S and 28SD2 sequences as in specimens DBUA0002431.01-09,
594 DBUA0002432.01-20 and DBUA0002433.01 (Table S2). Phylogenetic relationship within the
595 *Platynereis dumerilii* pseudo cryptic complex as in Fig. 1A, belonging to MOTU 6, with high
596 support values and low intraspecific (<3%) genetic divergence for both the mitochondrial and
597 nuclear markers. Interspecific COI mean distances to the closest and distant neighbour are 18.5%

598 (K2P, *P. dumerillii* s.s.) and 25.2% (K2P, MOTU 1) respectively. DOI for the species' holotype
599 Barcode Index Number (BIN): *upon paper acceptance*.

600

601 *Etymology:*

602 The species is named after Jérôme Jourde for his sampling efforts and kindness in
603 providing *Platynereis* specimens from the type locality on the behalf of the authors of this paper.

604

605 *Distribution and habitat:*

606 Western Mediterranean Sea, in subtidal or low tide among algae and *Posidonia oceanica*
607 rhizomes. Also present in CO₂ vents (Wäge et al. 2017).

608

609 *Reproduction:*

610 It is a gonochoric species, with a single reproductive event in life (semelparous)
611 transforming into a pelagic epitokous form (*heteronereis*) and a larval stage with planktotrophic
612 development (Wäge et al. 2017).

613

614 *Remarks:*

615 *Platynereis jourdei* sp. nov. is morphologically very similar and genetically close to *P.*
616 *dumerillii* s.s, with both species grouping close together within clade A2 of the phylogenetic tree
617 (Fig. 1A). The two species are barely distinguishable by body pigmentation of live specimens and
618 slightly different parapodia morphology. *Platynereis jourdei* sp. nov. usually presents lower
619 pigmentation density and some specimens may have ring-like pigment dot pattern in the anterior
620 region (excluding the first few segments) and parapodia from mid-body segments has shorter
621 triangular ligules. Despite the morphological and phylogenetic proximity of the two species, the
622 molecular interspecific difference between them (18.5%, COI K2P) justifies the erection of the
623 new species.

624 *Platynereis jourdei* sp. nov. and *P. dumerillii* s.s. are often sympatric in the western
625 Mediterranean Sea, thus requiring some caution in their identification.

626

627 ***Platynereis macaronensis* Teixeira, Ravara, Langeneck and Bakken sp. nov.**

628 (Figs. 5-6)

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

630

631 *Material examined*

632 *Type material. Spain - Canary islands, Tenerife:* 1 spm, holotype and hologenophore
633 DBUA0002429.03, 28°25'53.3"N - 16°32'57.2"W, low tide, among red algae, collected by Marcos
634 AL Teixeira, 10/04/2019, GenBank (COI): *upon paper acceptance*; 2 spms, paratypes and
635 paragenophores DBUA0002429.01-02, 28°25'53.3"N - 16°32'57.2"W, low tide, rocky beach
636 among red algae, 10/04/2019.

637 *Other material. Spain - Canary islands, Tenerife*, 3 spms, DBUA0002429.04-06,
638 28°34'17.1"N - 16°20'01.1"W, low tide, rocky beaches among algae, collected by Marcos AL
639 Teixeira, 05/04/2019; **Spain - Canary islands, Lanzarote**, 5 spms, DBUA0002429.07-11, low
640 tide, rocky beaches among algae, 29°13'05.3"N - 13°26'30.4"W, collected by Marcos AL Teixeira,
641 04/04/2019; **Spain - Canary islands, Gran Canaria**, 11 spms, DBUA0002429.12-22, low tide,
642 rocky beaches among algae, collected by Marcos AL Teixeira, 27°59'06.5"N - 15°22'33.0"W,
643 06/04/2019; **Spain - Canary islands, La Palma**, 5 spms, DBUA0002429.23-27, low tide, rocky
644 beaches among algae, 28°48'19.8"N - 17°45'41.6"W, collected by Marcos AL Teixeira,
645 09/04/2019; **Spain - Canary islands, Fuerteventura**, 5 spms, DBUA0002429.28-32, low tide,
646 rocky beaches among algae, 28°03'59.7"N - 14°30'24.9"W, collected by Marcos AL Teixeira,
647 02/04/2019; **Spain - Canary islands, El Hierro**, 1 spm, DBUA0002430.01, low tide, rocky
648 beaches among algae, 27°47'05.1"N - 18°00'41.7"W, collected by Pedro E Vieira, 2014;
649 **Morocco, Mazagan**, 2 spms, DBUA0002430.02-03, low tide, rocky beaches among algae,
650 33°15'50.5"N - 8°30'38.6"W, collected by Pedro E Vieira, 2014; **Portugal, Madeira**, 4 spms,
651 DBUA0002428.07-10, 32°38'46.0"N - 16°49'27.0"W, low tide, rocky beaches among algae,
652 collected by Pedro E Vieira, 2011; **Portugal - Azores, Terceira island**, 3 spms,
653 DBUA0002428.04-06, 38°40'60.0"N - 27°03'27.1"W, low tide, rocky beaches among algae,
654 collected by Pedro E Vieira, 2015; **Portugal - Azores, Santa Maria island**, 3 spms,
655 DBUA0002428.01-03, 36°56'59.7"N - 25°05'42.0"W, low tide, rocky beaches among algae,
656 collected by Pedro E Vieira, 2014.

657

658 *Description.*

659 Small-sized worms (5-18 mm long, 49 segments), tapering posteriorly. Holotype lacking
660 posterior end of the worm, 15 mm long for 44 chaetigers. Preserved specimens yellowish-red or
661 yellowish-brown, with a well-defined ring-like pigmentation pattern on the apodous segment and
662 semi ring-like pattern in other anterior segments (Fig. 5A, Fig. 6A). Pigmentation may not be
663 visible in some preserved specimens and may also be present in a prostomium area adjacent to
664 the eyes. Prostomium cordiform, with two pairs of eyes in trapezoid arrangement. Antennae and
665 palps similar in length. Palps consisting of a palpophore and ovalshaped palpostyles. Four pairs
666 of tentacular cirri usually as long as body width, with the longer postero-dorsal cirri reaching up
667 to chaetiger 6-8 (Fig. 5A). Pharynx maxillary and oral rings with rod-like paragnaths arranged in
668 tight rows (Fig. 5A-B): Areas I, II and V - absent. III - forming a group of short rows, IV - forming
669 several long rows in pyramidal arrangement. VI - forming a group of three transverse rows, VII-
670 VIII - arranged in single rows forming a continuous band. Jaws are finely toothed until a short
671 distance from the tip, usually with 7-8 teeth. Notopodial ligule digitiform in anterior parapodia,
672 median ligule rounded, similar in length (Fig. 5C). Mid-body parapodia similar in length, dorsal
673 ligule slightly triangular, median ligule digitiform (Fig. 5D). Neuroacicular ligule triangular, longer
674 than ventral digitiform ligule in anterior chaetiger, shorter than ventral ligule from mid-body
675 chaetigers. Dorsal cirrus more than twice the length of the dorsal ligule and ventral cirrus about
676 the same length or slightly shorter than ventral ligule (Fig. 5C-D). Notochaetae: homogomph

677 spinigers, serrated $\frac{2}{3}$ length of blade (Fig. 6B). Neurochaeta, dorsal fascicle: homogomph
678 spinigers, heterogomph falcigers short blades incurved with a small terminal tendon, serrated $\frac{2}{3}$
679 length of blade (Fig. 6C); ventral fascicle: heterogomph spinigers, heterogomph falcigers short
680 blades incurved with a distinct terminal tendon, serrated $\frac{1}{3}$ length of blade (Fig. 6D). Spiniger
681 chaetae lightly serrated (Fig. 6B).

682

683 *Molecular data*

684 COI, 16S and 28SD2 sequences as in specimens DBUA0002428.01-10,
685 DBUA0002429.01-32 and DBUA0002430.01-03 (Table S2). Phylogenetic relationship within the
686 *Platynereis dumerilii* pseudo cryptic complex as in Fig. 1A, belonging to MOTU 7, with high
687 support values and low intraspecific (<3%) genetic divergence for both the mitochondrial and
688 nuclear markers. Interspecific COI mean distances to the closest and distant neighbour are 13.5%
689 (K2P, MOTU 8) and 24% (K2P, *P. jourdei* sp. nov.) respectively. DOI for the species' holotype
690 Barcode Index Number (BIN): *upon paper acceptance*.

691

692 *Etymology:*

693 The species is named after the regional area (Macaronesia) it is restricted within.

694

695 *Distribution and habitat:*

696 Macaronesia islands (Madeira, Azores and Canary islands); it occurs in the western coast
697 of Morocco as well, in intertidal rocky beaches among green and red algae. It seems it is not
698 present in the island of Porto Santo (Madeira), being instead replaced by MOTU 8 (Fig. 1A-B),
699 although a greater sampling effort in Porto Santo is needed to confirm this.

700

701 *Remarks:*

702 *Platynereis macaronensis* sp. nov. can be easily distinguished from *P. dumerilii* s.s. by
703 the lower number of segments (almost half the number of segments for worms of similar size),
704 the shorter tentacular cirri (reaching chaetiger 8, instead of chaetiger 12), the higher number of
705 jaw teeth (with the presence of two or three more teeth) and the distinct paragnath arrangement
706 and pigmentation pattern (see Table 4). Regarding the latter two characters, *P. macaronensis* sp.
707 nov. is closer to *P. massiliensis* in having a ring-like pigmentation pattern and a similar paragnath
708 arrangement. However, these two species differ in the blades of the spinigerous chaetae, which
709 in *P. massiliensis* are coarsely serrated, while in *P. macaronensis* sp. nov. the blades are
710 narrower and the spinulation is lighter. Genetic distances (mean 15.5% COI K2P) and distinct
711 geographic distribution also distinguished these two species. Additionally, some pigmentation
712 details in the anterior segments are distinct from *P. massiliensis*, with the presence of semi ring-
713 like dot patterns.

714

715 Unlike most other species from the complex, that are widely distributed along the Atlantic
716 and Mediterranean coast of Europe, *P. macaronensis* sp. nov. is unique to the Macaronesia
717 islands and western coast of Morocco. No reproductive studies were done for this species, but

717 given the genetic proximity to the nearest neighbour (MOTU 9 – *P. cf. massiliensis*), it is probable
718 it shares the same hermaphrodite features, egg brooding and lecithotrophic larval stages.

719

720

Platynereis cf. massiliensis (Moquin-Tandon, 1869)

721

(Figs. 11-12)

722

723 *Material examined*

724

Portugal, Canto Marinho, 14 spms, DBUA0002424.01-03, DBUA0002425.01-11,

725

41°44'13.2"N - 8°52'33.6"W, low tide, among algae, collected by Marcos AL Teixeira, 20/05/2019.

726

Morocco, Mazagan, 1 spm, DBUA0002426.01, low tide, rocky beaches among algae,

727

33°15'50.5"N, 8°30'38.6"W, collected by Pedro E Vieira, 2014; **Italy, Livorno**, 3 spms,

728

DBUA0002427.01-03, 43°32'45.6"N - 10°18'07.2"E, marina, pontoon scrapings among algae,

729

23/10/2019.

730

731

Description.

732

Small-sized worms (3.5-26 mm long, 35-45 segments), tapering posteriorly. Preserved

733

specimens yellowish-brown, with a ring-like pigmentation pattern in most of the anterior segments

734

(Fig. 11A, Fig. 12A), or a high amount of dots scattered throughout the body, varying in dot size

735

and density except on the apodous segment (Fig. 11E, F). Pigmentation may also be present in

736

prostomium, adjacent to the eyes. Prostomium cordiform, with two pairs of eyes in trapezoid

737

arrangement. Antennae and palps similar in length. Palps consisting of a palpophore and

738

ovalshaped palpostyles. Four pairs of tentacular cirri usually as long as body width, with the longer

739

postero-dorsal cirri reaching up to chaetiger 6-8 (Fig. 11A). Pharynx maxillary and oral rings with

740

rod-like paragnaths arranged in tight rows (Fig. 11A-B): Areas I, II and V - absent. III - forming a

741

group of short rows, IV - forming several long rows in pyramidal arrangement. VI - forming a group

742

of three transverse rows, VII-VIII - arranged in single rows forming a continuous band. Jaws are

743

finely toothed until a short distance from the tip, usually with 7 teeth. Anterior dorsal parapodial

744

ligules (Fig. 11C) digitiform slightly longer than median triangular ligule, neuropodial acicular ligule

745

triangular, as long as a rounded ventral ligule. In mid-body chaetigers dorsal notopodial ligule

746

slightly longer than median digitiform ligule, neuropodial acicular ligule round shorter than

747

lanceolate ventral ligule (Fig. 11D). Dorsal cirrus more than twice the length of the dorsal ligule

748

and ventral cirrus about the same size or slightly shorter than ventral ligule (Fig. 11C-D).

749

Notochaetae: homogomph spinigers with coarsely serrated blades (Fig. 12B). Neurochaeta,

750

dorsal fascicle: homogomph spinigers (Fig. 12C), heterogomph falcigers with short blades

751

incurved with a distinct terminal tendon, serrated $\frac{1}{2}$ length of blade (Fig. 12D); ventral fascicle:

752

heterogomph spinigers (Fig. 12C), heterogomph falcigers short blades incurved with a distinct

753

terminal tendon, serrated $\frac{1}{2}$ length of blade (Fig. 12E).

754

755

Molecular data

756 COI, 16S and 28SD2 sequences as in specimens DBUA0002424.01-03;
757 DBUA0002425.01-11, DBUA0002426.01 and DBUA0002427.01-03 (Table S2). Phylogenetic
758 relationship within the *Platynereis dumerillii* pseudo cryptic complex as in Fig. 1A, belonging to
759 MOTU 9, with high support values and low intraspecific (<3%) genetic divergence for both the
760 mitochondrial and nuclear markers. Interspecific COI mean distances to the closest and distant
761 neighbour are 5% (K2P, MOTU 10) and 24% (K2P, MOTU 5) respectively. DOI for the species'
762 Barcode Index Number (BIN): *upon paper acceptance*.

763

764 *Distribution and habitat:*

765 NE Atlantic to the Western Mediterranean Sea, from Portugal and Morocco to western
766 Italy. Found in rocky beaches among algae in intertidal or subtidal habitats, including CO₂ vents
767 (Wäge *et al.* 2017).

768

769 *Reproduction:*

770 Reproduction without epitokous transformation; it is a protandrous hermaphrodite,
771 characterized by egg brooding and lecithotrophic larval stages with a semi-direct development
772 (Schneider *et al.* 1992, Wäge *et al.* 2017). In the original description by Moquin-Tandon (1869), it
773 was described as simultaneous hermaphrodite, instead.

774

775 *Remarks:*

776 The original description by Moquin-Tandon is very poor (type locality: Marseille, France),
777 and the identification of specimens as *P. massiliensis* is mostly tentative. Wäge *et al.* (2017)
778 genetically pinpointed two lineages sharing the same reproductive features as *P. massiliensis*
779 (egg brooders), mainly present in acidic waters. Despite the lack of the type material, the
780 congruence of their developmental observations with other studies (Hauenschild, 1951,
781 Schneider *et al.* 1992; Helm *et al.* 2015) suggests that their *Platynereis* population from Ischia
782 represents *P. massiliensis* (MOTU 9 in our study, Fig. 1). The Vulcano population (egg brooder),
783 grouped in our MOTU 1 (Fig. 1), which also have sequences from Banyuls. However, this MOTU
784 1 is closer to the original type locality reported for *P. massiliensis* (Marseille, France). Further
785 sampling and reproductive studies in the topotypic material is needed to confirm if our lineage
786 actually corresponds to specimens found in Marseille.

787 In our study, the MOTU attributed to *Platynereis massiliensis* differs from *P. dumerillii* s.s.
788 mainly in having much shorter dorsal tentacular cirri, different paragnath arrangement with
789 absence of paragnaths on area II, coarsely serrated chaetae, and different pigmentation in some
790 of its specimens. Additionally, high molecular distances (mean 21.6% COI K2P) and different
791 reproductive strategies and life history distinguishes this species from *P. dumerillii* s.s. (Wäge *et*
792 *al.* 2017).

793 This species possesses diverse pigmentation patterns, one of which is very distinct and
794 apparently unique to the population from Porto di Livorno (Italy). This pigmentation pattern has a
795 high amount of dots scattered throughout the body and is characterized by the larger dot size

796 (almost circular-like) when compared to the NE Atlantic populations. An independent COI clade
797 with 3.3% K2P mean distances distinguishes the Livorno variant against the NE Atlantic
798 populations, however without enough divergence to be separated by any of the applied MOTU
799 delineation methods (Fig. 1).

800

801 ***Platynereis agilis*** (Keferstein, 1862) **comb. nov.**

802 (Figs. 7-8)

803

804 *Material examined*

805 **Spain, Calpe**, 5 spms, DBUA0002421.01-05, 38°38'23.8"N, 0°03'30.0"E, low tide, among
806 algae, collected by Pedro E Vieira, 05/08/2019; **Portugal, Arrabida Natural Park (Lisbon)**, 15
807 spms, (*waiting voucher assignment for the specimens from the Portuguese National Museum of*
808 *Science and Natural History*), 38°26'13.1"N, 9°03'47.3"W, 9 m. in depth, among algae, kindly
809 provided by the National Museum of Science and Natural History (Portugal), 22/09/2014; **France,**
810 **Morlaix Bay**, 2 spms, DBUA0002422.01, MTPD191-20, 48°43'48.0"N, 3°59'09.6"W, low tide,
811 among algae, collected by Celine Houbin, 17/09/2020; **Great Britain, Plymouth**, 1 spm,
812 DBUA0002423.01, 50°21'35.4"N, 4°09'01.8"W, low tide, among algae, collected by Felicia Ultin,
813 27/03/2017.

814

815 *Description* (based on the original description of *Nereis agilis* Keferstein, 1862, emended).

816 Small-sized worms (5-20 mm long, 45-50 segments), tapering posteriorly. Preserved
817 specimens yellowish, with no pigmentation (Fig. 7A, Fig. 8A). Prostomium cordiform, with two
818 pairs of eyes in trapezoid arrangement. Antennae and palps similar in length. Palps consisting of
819 a palpophore and ovalshaped palpostyles. Four pairs of tentacular cirri at least as long as body
820 width, with the longer postero-dorsal cirri reaching up to chaetiger 10-15 (Fig.7A). Pharynx
821 maxillary and oral rings with rod-like paragnaths arranged in tight rows (Fig. 7A-B): Areas I, II and
822 V - absent. III - forming a group of short rows, IV - forming several long rows in pyramidal
823 arrangement. VI - forming a group of three transverse rows, VII-VIII - arranged in single rows
824 forming a continuous band. Jaws are finely toothed until a short distance from the tip, usually with
825 7-8 teeth. Dorsal notopodial ligule in anterior parapodia digitiform to triangular as long as median
826 triangular ligule (Fig. 7C), from mid-body parapodia dorsal ligule triangular similar in length as
827 median ligules (Fig. 7D). Neuroacicular ligule large triangular longer than ventral ligule in anterior
828 parapodia, triangular and shorter than ventral ligule from mid-body parapodia. Dorsal cirrus three
829 times longer than dorsal ligule (Fig. 7C-D). Ventral cirrus about the same size or slightly shorter
830 than ventral ligule (Fig. 7C-D).

831 Notochaetae: homogomph spinigers with coarsely serrated $\frac{3}{4}$ length of blade (Fig. 8B).
832 Neurochaeta, dorsal fascicle: homogomph spinigers serrated, heterogomph falcigers with distinct
833 tendon, serrated $\frac{1}{3}$ of the blade (Fig. 8D); ventral fascicle: heterogomph spinigers, heterogomph
834 falcigers with tendon, serrated $\frac{1}{2}$ length of the blade (Fig. 8C).

835

836 *Molecular data*

837 COI, 16S and 28SD2 sequences as in specimens (*waiting voucher assignment for the*
838 *specimens from the Portuguese National Museum of Science and Natural History*),
839 DBUA0002421.01-05, DBUA0002422.01, MTPD191-20 and DBUA0002423.01 (Table S2).
840 Phylogenetic relationship within the *Platynereis dumerilii* pseudo cryptic complex as in Fig. 1A,
841 belonging to MOTU 10, with high support values and low intraspecific (<3%) genetic divergence
842 for both the mitochondrial and nuclear markers. Interspecific COI mean distances to the closest
843 and distant neighbour are 5% (K2P, *P. cf. massiliensis*) and 24.2% (K2P, MOTU 5) respectively.
844 DOI for the species' Barcode Index Number (BIN): *upon paper acceptance*.

845

846 *Distribution and habitat:*

847 NE Atlantic to the Western Mediterranean Sea, from Great Britain to Mediterranean
848 Spain. Found in rocky beaches among algae in intertidal or subtidal habitats.

849

850 *Reproduction:*

851 The claim by Keferstein (1862) of hermaphroditism has not been confirmed by recent
852 studies, but given the genetic proximity for this species to the nearest neighbour (MOTU 9, *P. cf.*
853 *massiliensis*), it is possible it shares the same hermaphrodite features, egg brooding and
854 lecithotrophic larval stages (Wäge *et al.* 2017).

855

856 *Remarks:*

857 *Platynereis agilis*, originally described as *Nereis agilis* (Keferstein 1862) from St. Vaast
858 (North France) and until now considered as a junior synonym of *P. dumerilii*, is clearly part of the
859 *P. dumerilii* species complex, given its similar morphology and the genetic proximity to the other
860 species of the complex (Fig. 1, Table 4). However, visible differences can easily be found against
861 *P. dumerilii* s.s. with almost half the segments in worms of similar size, distinct paragnath
862 arrangement, no pigmentation (although this is not always a reliable character due to fixation in
863 ethanol), anterior parapodia with longer triangular ligules and spinigerous chaetae with coarsely
864 serrated blades. All these differences, along with the genetic distances (mean 21.8% COI K2P),
865 justify the removal from synonymy and re-establishment of the species. *Platynereis agilis* shares
866 a similar paragnath arrangement and the coarsely serrated chaetae with the species *P. cf.*
867 *massiliensis*, but greatly differs from the latter due to lack of pigmentation and regarding the longer
868 size of the postero-dorsal cirri, reaching up to chaetiger 15, instead of chaetiger 8. Despite the
869 low genetic COI distance (mean 5% K2P) compared to *P. cf. massiliensis*, the distinct
870 morphological differences justify the resurrection of this species.

871

872 **Discussion**

873

874 Some species within the family Nereididae have morphological features with very small
875 variations which can often lead to misidentifications (Bakken and Wilson 2005). This is especially

876 true when comparing small specimens belonging to different species where one is significantly
877 more abundant than the other. The variation found in the rarer and lesser known species, e.g. the
878 size of the tentacular cirri, might be attributed to damage from the sampling techniques or
879 juveniles and the pharynx might not be everted as well, which can lead to wrong taxonomic
880 conclusions. This was the case in our samples between the clades A and B, where molecular
881 data and a more careful morphological analysis found considerable differences between the two.
882 However, it is still possible to find *P. dumerilii* assigned to MOTU 11 (GenBank: KC591811.1) in
883 the genetic databases, and our earlier first-pass assessment of some specimens from clade B
884 led to incorrect identifications as well (Teixeira *et al.* 2021). Maximum genetic distances between
885 these two major clades were very high (see Table 1), especially in the 28SD2 locus where values
886 rose to 36.9%, as opposed to the 3.9% found between MOTUs in clade A. Other annelid studies
887 about cryptic complexes also reported similarly low 28S distances among neighbouring MOTUs
888 (Teixeira *et al.* 2020; Sampieri *et al.* 2021). This nuclear locus is known for its poor utility in
889 species-level discrimination in many groups of animals (Jörger *et al.* 2012), but it is very efficient
890 for reconstructing deeper phylogenies (Weitschek *et al.* 2014). The higher values between major
891 clades A and B might signal that species present in clade B do not belong to a complex of closely
892 related species, representing instead very distinct species with a distant ancestor from *P.*
893 *dumerilii*. This is also supported by the morphological data where it's clear that MOTUs 11, 12
894 and 13 belong to another genus, probably *Nereis*. Based on this preliminary data it is clear that
895 either entirely new unreported species, or new pseudo cryptic lineages belonging to an existing
896 group, were found, but a larger sampling effort and further morphological examination is needed
897 to confirm this.

898 Regarding the major clade A, the combined molecular data from three different loci
899 provided compelling evidence for the existence of at least 10 deeply divergent and completely
900 sorted lineages within the *P. dumerilii* complex in Europe. These deep genetic distances are a
901 strong indication of long term isolation, thereby the lineages involved can qualify for recognition
902 as separate species (Bickford *et al.* 2007; Churchill *et al.* 2014; Delić *et al.* 2017). Complementing
903 the molecular data, some morphological variations within the most abundant MOTUs (4, 6, 7, 9,
904 10) were found as well (Table 4). The genetic COI distances recorded in this clade (mean 19.8%,
905 K2P) fit within the range reported for congeneric distances in comprehensive studies of COI
906 variation targeting polychaetes. For example, mean COI distances (K2P) of 16.5%, 24.0% and
907 22% were found in the regional polychaete fauna of the Arctic (Carr *et al.* 2011), north-eastern
908 Atlantic (Lobo *et al.* 2016) or between cryptic populations of *Eurythoe complanata* (Pallas, 1766)
909 from eastern Pacific (Panama) and Atlantic samples (Barroso *et al.* 2010), respectively. The only
910 exception to this are MOTUs 5 and 10 where the COI distances to the nearest neighbours
911 (MOTUs 4 and 9) were much lower, namely 8.6% and 6.4% respectively, which is still a fair
912 genetic distance and much higher than the usual intraspecific variation found in Nereidids (Glasby
913 2005; Paiva *et al.* 2019).

914

915 *Untangling the Platynereis complex*

916 The original description for *P. massiliensis* is quite incomplete and does not include any
917 reliable morphological character or figures (Moquin-Tandon, 1869). Instead, only the main
918 reproductive features were highlighted to distinguish this species from *P. dumerilii*, suggesting
919 that the two species are morphologically identical when analysing the respective specimens.
920 Based on Wäge *et al.* (2017), it was possible to genetically pinpoint two lineages sharing the
921 same reproductive features as *P. massiliensis* (egg brooders), mainly present in acidic waters
922 and two other clades matching *P. dumerilii* (*heteronereis* stage), mostly living in non-acidic waters.
923 These two *P. dumerilii* clades grouped in our clade A2, more specifically in MOTUs 4 and 6, with
924 the first one occurring in the type locality. MOTU 4 (*P. dumerilii* s.s.) and MOTU 6 (*P. jourdei* sp.
925 nov.) have a distinct paragnath pattern from the ones found in clade A3 (MOTUs 7, 9 and 10),
926 where sequences of *P. massiliensis* from Wäge *et al.* (2017) grouped with. As stated by the
927 previous mentioned study, despite the lack of the type material, the congruence of their
928 developmental observations with other studies (Hauenschild, 1951, Schneider *et al.* 1992; Helm
929 *et al.* 2015) suggests that their *Platynereis* population from Ischia represents *P. massiliensis*, and
930 group together with our sequences specifically from MOTU 9. The Vulcano population, also a
931 brooder, grouped in MOTU 1 (clade A1) together with our two sequences from Banyuls, but it was
932 not possible to observe the pharynx and confirm if similar paragnath patterns to MOTU 9 could
933 be identified as well.

934 Given that MOTU 7 (*P. macaronensis* sp. nov.) and MOTU 8 are endemic to the
935 Macaronesia islands, MOTU GB1 has been reported from South Africa, and MOTUs GB2 and
936 GB3 probably belonging either to MOTU 9 or MOTU 10, among the analysed material only MOTU
937 10, present in the Western Mediterranean, could also qualify as possible source for the originally
938 described *P. massiliensis*. MOTU 10 is genetically close to MOTU 9 (max distances of 6.4% COI
939 K2P) and it's very likely that they share the same reproductive traits; however it shows some
940 visible morphological differences when compared to the latter. These differences seem to fit the
941 description of *Nereis agilis* Keferstein, 1862, described for the NE Atlantic (type locality: St. Vaast,
942 France) and hitherto considered as an unaccepted subjective synonym for *P. dumerilii*. In the
943 original description, the analysed specimens seem to be simultaneous hermaphrodites without
944 *heteronereis* stage, tentacular cirri and dorsal cirri are longer than the ones usually reported for
945 *P. dumerilii* and on parapodia four ligules are noticeable, although the third [starting from the
946 dorsal side] is very short, but no mention to the pharynx is done (Keferstein, 1862). Another
947 unaccepted subjective synonym described for the Gulf of Naples (Italy), *Nereis peritonealis*
948 Claparède, 1868, describes a similar paragnath pattern as the one presented here for the clade
949 A3. However, even though there is no detailed data on the reproductive mode, the reported small
950 size of mature eggs (Claparède, 1868) would suggest that this is not a species with direct
951 development, i.e. not a brooder, but it might have a planktonic larvae stage (Sato and Masuda
952 1997). This goes in line with MOTUs 4 and 6 instead, even though paragnath patterns do not
953 match. An interesting note regarding the description of *Nereis agilis* is that ovaries and testes are
954 separated in two different sectors of the body (Keferstein, 1862), while in *P. massiliensis* they
955 should occur in the same segments (Moquin-Tandon, 1869). From the biological point of view,

956 the latter arrangement is very surprising, as it would imply a high risk of self-fertilisation;
957 nonetheless, such discrepancies might depend on different interpretations of the same structures
958 by different scholars, and what is interpreted as a developing gonad might be a glandular structure
959 in other sources. This calls for a new observation on the reproductive features and a description
960 based on topotypic material to compare against our interpretation of *P. massiliensis*, and confirm
961 if the lineage identified in this and in other previous studies match the topotypic samples. Other
962 species with currently unaccepted names in European type localities are also available (Table 3)
963 but they are very incomplete and an unequivocal attribution to any of the MOTUs found in clade
964 A seems impossible. Three additional unaccepted species historically synonymized with *P.*
965 *dumerilii*, i.e., *Heteronereis fucicola* Örsted, 1843, *Nereilepas variabilis* Örsted, 1843, and
966 *Heteronereis malmgreni* Claparède, 1868, were not included in this table because all refer to
967 epitoke forms, that at the time were believed to be different species from the atoke forms. We
968 cannot possibly reconstruct their morphological correspondence to atoke specimens we studied,
969 but we can exclude that they are synonymous with *P. massiliensis*-like brooders. Taxa from
970 Denmark described by Örsted represent different stages of the epitoke modification or different
971 sexes, and based on distribution of MOTUs, might correspond either to *P. dumerilii* s.s. (MOTU
972 4) or to MOTU2. *Heteronereis malmgreni* was instead described for the Gulf of Naples and it is
973 probably a description of the epitoke form of *Nereis peritonealis*, which could also be the same
974 as *P. dumerilii* s.s..

975

976 *Reproduction strategies in Platynereis*

977 The suggested reproduction modes based on genetic proximity done in this study, being
978 fixed at the basis of the two major retrieved clades by Wäge *et al.* (2017), might not be correct.
979 Instances of reproductive plasticity were reported in other Nereididae species, e.g. the
980 suppression of epitoky as a probable answer to environmental pressures within the same lineage
981 (Prevedelli and Cassai 2001; Daas *et al.* 2011). However, as no genetic data complemented these
982 studies, this could also be a clue to unreported cryptic species as well. Several references,
983 pointed out in Daas *et al.* (2011), that stress the presence of atokous and epitokous "races" or
984 "forms" in *Perinereis cultrifera* (Grube, 1840) (e.g. Marcel, 1962; Zghal and Ben Amor, 1989;
985 Scaps *et al.* 1992; Rouhi *et al.* 2008), might actually be linked to the evidence of cryptic species
986 within this taxon, which was reported in other studies. For example, upon further examination,
987 *Perinereis* populations from North of France and Algeria have distinct alloenzymes, number of
988 paragnaths and number of teeth per half jaw (Scaps *et al.* 2000). Using a similar methodology to
989 the previous example, distinct populations from the Elba Island (Western Italy) were also found,
990 corresponding to two different habitat types between brackish waters and an adjacent marine site
991 (Maltagliati *et al.* 2001). Nevertheless, this would still question if sister lineages or other
992 phylogenetically close species might have, or not, completely different reproduction modes.
993 Without actual studies on reproductive biology complemented with genetic data, we should not
994 discard the possibility of different reproductive features within the *Platynereis* complex or even
995 possible reproductive plasticity within the same MOTU.

996 It is speculated that the low dispersal rate in many marine brooding species with a direct
997 or semi-direct development without planktonic larval stage can promote genetic divergence and
998 help to explain the genetic isolation of populations, while the free-swimming larvae easily migrate,
999 resulting in higher chances of gene flow among populations (Palumbi and Baker 1994; Teske *et*
1000 *al.* 2011). Evidence that stressful conditions (e.g. hydrothermal vents, port environments or
1001 brackish-water habitats) are better tolerated in the survival of *Platynereis* populations with a
1002 brooding strategy was noted in several studies (Lucey *et al.* 2015; Gambi *et al.* 2016; Wäge *et al.*
1003 2017). Being volcanic in origin, the Macaronesia islands harbour in its vicinities a large amount of
1004 CO₂ vents characterized by the low pH waters (Viveiros *et al.* 2020; González-Delgado *et al.*
1005 2021), which might favor the proliferation of brooder worms instead of free spawners. Sampling
1006 in the CO₂ vents could also provide additional *Platynereis* lineages yet to be explored, which could
1007 be unique to each island, given how important the Macaronesia archipelagos seem to be in the
1008 cryptic speciation of marine invertebrates (Desiderato *et al.* 2019; Vieira *et al.* 2019).

1009

1010 **Conclusions**

1011

1012 Among the 10 different *Platynereis* lineages from Europe uncovered with molecular data,
1013 seven of them had particular geographical distributions, either confined to the western (MOTUs 1
1014 and 6) or eastern (MOTU 3) Mediterranean Sea, NE Atlantic (MOTU 2), Macaronesia islands
1015 (MOTU 7) and sometimes exclusive to a single island (MOTU 8) or limited to a few islands within
1016 a single archipelago (MOTU 5), indicating also a high level of endemism. MOTUs 4, 9 and 10
1017 were sympatric with at least two other lineages from the group, with MOTUs 9 and 10 revealing
1018 geographically structured populations through their COI haplotypes. No considerable genetic
1019 structure was found in each sampled island within MOTU 7 despite the existence of two other
1020 lineages in this region of the Atlantic. These findings call for a better recognition of the role of both
1021 the Macaronesian archipelagos and the Mediterranean Sea as promoters of extensive
1022 diversification of marine invertebrates and emphasize the importance of the conservation of the
1023 biodiversity of the intertidal rocky shore of these regions. Despite the two new species erected in
1024 this study (*P. macaronensis* sp. nov. and *P. jourdei* sp. nov.) and further clarification regarding
1025 the status of the *P. agilis*, *P. dumerilii* and *P. massiliensis*, five other lineages still remain unnamed
1026 and in need of further sampling effort and morphological examination. In particular, MOTU 2, an
1027 apparently rarer lineage from the NE Atlantic, that seems to be easy to find in Norway based on
1028 sampling campaigns under the Norwegian projects (BIN: BOLD:AAC5474, BOLD Systems).
1029 Three other unaccepted synonyms are reported for this area as well (e.g. Denmark), but
1030 descriptions are very incomplete or referring only to epitoke forms. Topotypic specimens of *P.*
1031 *massiliensis* and further studies on its reproductive biology are also needed to pinpoint if this
1032 species actually corresponds to the lineage assumed in this, and in the previous studies (Wäge
1033 *et al.* 2017; Calosi *et al.* 2013; Kara *et al.* 2019).

1034 Failure to recognise this hidden biodiversity may compromise the accuracy and the
1035 interpretation of biomonitoring and ecological data for *Platynereis* and its use as a model species

1036 (Özpolat *et al.* 2021). Integrative taxonomy is thus essential to solve these uncertainties and to
1037 allow naming the involved undescribed species. Otherwise, most molecular data providing
1038 enough support for species hypothesis will continue to be unused, and large fractions of
1039 biodiversity will persist unnoticed.

1040

1041 **Conflict of interests**

1042 The authors declare no conflicts of interest

1043

1044 **Declaration of funding**

1045 This study was supported by the project Next generation monitoring of coastal
1046 ecosystems in a scenario of global change (NextSea, NORTE-01-0145-FEDER-000032), under
1047 the PORTUGAL 2020 Partnership Agreement, through the European Regional Development
1048 Fund (ERDF). Thanks are due, for the financial support of CESAM – Centro de Estudos do
1049 Ambiente e do Mar (UIDB/50017/2020+UIDP/50017/2020), to Portuguese Foundation for
1050 Science and Technology and Ministry of Education and Science (FCT/MEC) through national
1051 funds, and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete
1052 2020. The research leading to these results also received partial funding, from the European
1053 Union's Horizon 2020 research and innovation programme under grant agreement No 730984,
1054 ASSEMBLE Plus project (application n. 8229, 4th CALL, "Crypticism in the marine realm: DNA
1055 barcode-based outlook into selected invertebrate taxa of the Eastern Mediterranean"). Marcos AL
1056 Teixeira was supported by a PhD grant from FCT co-financed by ESF (SFRH/BD/131527/2017)
1057 and from the DNAqua-Net STSM grant "Rich and hidden biodiversity not yet barcoded in the
1058 Canary archipelago (Spain) as an opportunity to enrich the DNA barcode reference library for
1059 European polychaetes", under the EU Cost action CA15219 - Developing new genetic tools for
1060 bioassessment of aquatic ecosystems in Europe. Pedro Vieira work was supported by national
1061 funds through the Portuguese Foundation for Science and Technology (FCT, I.P.) in the scope of
1062 the project (Early detection and monitoring of non-indigenous species in coastal ecosystems
1063 based on high-throughput sequencing tools, PTDC/BIA-BMA/29754/2017). Ascensão Ravara
1064 was funded by national funds, through FCT, I.P., in the scope of the framework contract foreseen
1065 in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by
1066 Law 57/2017, of July 19. Financial support to Arne Nygren from the Norwegian Taxonomy
1067 Initiative [<http://www.biodiversity.no/Pages/135523>] (Cryptic polychaete species in Norwegian
1068 waters, knr 49-13, pnr 70184228), the Swedish Taxonomy Initiative
1069 [<https://www.artdatabanken.se/en/the-swedish-taxonomy-initiative/>] (Polychaete species
1070 complexes in Swedish waters, dnr 140/07 1.4 and 166/08 1.4), and Kungliga Fysiografiska
1071 sällskapet Nilsson-Ehle donationerna [<https://www.fysiografen.se/sv/>]. Financial support to
1072 Torkild Bakken from the Norwegian Taxonomy Initiative project Polychaetes in Norwegian Ports
1073 project no. 70184238.

1074

1075 **Acknowledgements**

1076 Special thanks to Giorgios Pace for scuba diving and proving nereidids from the island of
1077 Crete (Greece); Katerina Vasileidou for the northern Greek *Platynereis* specimens; Matilde
1078 Boschetti, Michela Del Pasqua, Chiara Ravaglioli, Jonathan Tempesti and Marco Zuffi for their
1079 assistance in obtaining macrobenthic samples from several Italian localities; Celine Houbin and
1080 Nicolas Lavesque for the northern French *Platynereis* specimens; Jérôme Jourde for the type
1081 locality samples of the *P. dumerilii* s.s. and Jorge Fraga for all the assistance and knowledge
1082 provided during the Canary islands sampling campaign. Lastly, to Andrea Desiderato for the
1083 assistance in the morphological analysis of the undetermined nereidids found in this study.
1084 Sample collection was performed under approved ethics guidelines and no special permits were
1085 needed for the sampling campaigns at the time.

1086

1087 **Availability of data and materials**

1088 New sequence data and specimen metadata were uploaded in the project “*Platynereis*
1089 *species complex*” (DS-MTPD) within BOLD (<http://v4.boldsystems.org/>) and in the following link:
1090 *upon paper acceptance*. The alignments (FASTA and NEXUS formats) for each marker (COI,
1091 16S and 28SD2) and the concatenated one (COI+16S+28SD2) are all publicly available online at
1092 Figshare (DOI: *upon paper acceptance*). The new biological material is deposited at the Biological
1093 Research Collection (Marine Invertebrates) of the Department of Biology of the University of
1094 Aveiro (COBI at DBUA), Portugal, while specimens from Norway are deposited at the Norwegian
1095 University of Science and Technology, NTNU University Museum. Specimens from the Arrabida
1096 Natural Park (Lisbon, Portugal) were provided by the National Museum of Science and Natural
1097 History (Portugal). All specimens are available upon request, with the exception of the ones
1098 exhausted in the DNA analysis mentioned in the Methods.

1099

1100 **References**

1101

1102 Abbiati M, Castelli A (1992). *Platynereis nadiae* sp. n. (Polychaeta: Nereididae) from Italian
1103 coasts. *Zoologica Scripta* **21**, 151-155

1104

1105 Arvanitidis C (2000). Polychaete fauna of the Aegean Sea: inventory and new information. *Bulletin*
1106 *of Marine Science* **66**, 73–96

1107 Audouin JV, Milne Edwards H (1833). [Part 3.] Classification des Annélides et description de
1108 celles qui habitent les côtes de la France. *Annales des sciences naturelles*, Paris (series
1109 1) **29**, 195-269, available online at <https://biodiversitylibrary.org/page/6044727>

1110 Bakken T, Glasby CJ, Wilson RS (2009). A review of paragnath morphology in Nereididae
1111 (Polychaeta). *Zoosymposia* **2**, 305-316

- 1112 Bakken T, Hårsaker K, Daverdin M (2021). Marine invertebrate collection NTNU University
 1113 Museum. Version 1.976. Norwegian University of Science and Technology. Occurrence
 1114 dataset <https://doi.org/10.15468/ddbs14> accessed via GBIF.org on 2021-09-27.
- 1115 Bakken T, Wilson RS (2005). Phylogeny of nereidids (Polychaeta, Nereididae) with paragnaths.
 1116 *Zoologica Scripta* **34**, 507–547
- 1117 Barroso R, Klautau M, Solé-Cava AM, Paiva PC (2010). *Eurythoe complanata* (Polychaeta:
 1118 Amphinomidae), the ‘cosmopolitan’ fireworm, consists of at least three cryptic species.
 1119 *Marine Biology* **157**, 69–80
- 1120 Bellan G (1980). Relationship of pollution to rocky substratum polychaetes on the French
 1121 Mediterranean coast. *Marine Pollution Bulletin* **11**, 318–321
- 1122 Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007). Cryptic
 1123 species as a window on diversity and conservation. *Trends in Ecology & Evolution* **22**,
 1124 148–155
- 1125 Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A,
 1126 Drummond AJ (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis.
 1127 *PLOS Computational Biology* **10**, e1003537
- 1128 Calosi P, Rastrick SPS, Lombardi C, de Guzman HJ, Davidson L, Jahnke M, Giangrande A,
 1129 Hardege JD, Schulze A, Spicer JI, Gambi M-C (2013). Adaptation and acclimatization to
 1130 ocean acidification in marine ectotherms: an in situ transplant experiment with
 1131 polychaetes at a shallow CO₂ vent system. *Philosophical Transactions of the Royal
 1132 Society B: Biological Sciences* **368**, 20120444
- 1133 Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN (2011). A Tri-Oceanic Perspective:
 1134 DNA Barcoding Reveals Geographic Structure and Cryptic Diversity in Canadian
 1135 Polychaetes. *PLOS ONE* **6**, e22232
- 1136 Castelli A, Bianchi CN, Cantone G, Çinar ME, Gambi MC, Giangrande A, Iraci-Saredi D, Lanera
 1137 P, Licciano M, Musco L, Sanfilippo R, Simonini R (2008). Annelida Polychaeta. In: Relini
 1138 G. (ed), Checklist della flora e della fauna dei mari italiani. Parte I. *Biologia marina
 1139 mediterranea* **15**, 323-373
- 1140 Cerca J, Meyer C, Purschke G, Struck TH (2020). Delimitation of cryptic species drastically
 1141 reduces the geographical ranges of marine interstitial ghost-worms (*Stygocapitella*;
 1142 Annelida, Sedentaria). *Molecular Phylogenetics and Evolution* **143**, 106663

- 1143 Churchill CKC, Valdés Á, Foighil DÓ (2014). Molecular and morphological systematics of
1144 neustonic nudibranchs (Mollusca : Gastropoda : Glaucidae : *Glaucus*), with descriptions
1145 of three new cryptic species. *Invertebrate Systematics* **28**, 174–195
- 1146 Çinar ME, Dağlı E, Kurt-Sahin G (2014). Checklist of Annelida from the coasts of Turkey. *Turkish*
1147 *Journal of Zoology* **38**, 734-764
- 1148 Claparède É (1868). Les annélides chétopodes du Golfe de Naples. *Mémoires de la Société de*
1149 *Physique et d'Histoire Naturelle de Genève* **19**, 313-584, plates I-XVI, available online at
1150 <https://www.biodiversitylibrary.org/page/14309905>
- 1151 Clement M, Snell Q, Walke P, Posada D, Crandall K (2002). TCS: estimating gene genealogies.
1152 In 'Proceedings 16th International Parallel and Distributed Processing Symposium'.
1153 Proceedings 16th International Parallel and Distributed Processing Symposium. IPDPS
1154 2002. pp. 7 pp. (IEEE: Ft. Lauderdale, FL)
- 1155 Daas T, Younsi M, Daas-Maamcha O, Gillet P, Scaps P (2011). Reproduction, population
1156 dynamics and production of *Nereis falsa* (Nereididae: Polychaeta) on the rocky coast of
1157 El Kala National Park, Algeria. *Helgoland Marine Research* **65**, 165–173
- 1158 Darriba D, Taboada GL, Doallo R, Posada D (2012). jModelTest 2: more models, new heuristics
1159 and parallel computing. *Nature Methods* **9**, 772–772
- 1160 Delić T, Trontelj P, Rendoš M, Fišer C (2017). The importance of naming cryptic species and the
1161 conservation of endemic subterranean amphipods. *Scientific Reports* **7**, 3391
- 1162 Desiderato A, Costa FO, Serejo CS, Abbiati M, Queiroga H, Vieira PE (2019). Macaronesian
1163 islands as promoters of diversification in amphipods: The remarkable case of the family
1164 Hyalidae (Crustacea, Amphipoda). *Zoologica Scripta* **48**, 359–375
- 1165 Fauvel P (1924). Faune de France 5: Polychètes errantes. *Nature* **113**, 528–529
- 1166 Fischer A, Dorresteijn A (2004). The polychaete *Platynereis dumerilii* (Annelida): a laboratory
1167 animal with spiralian cleavage, lifelong segment proliferation and a mixed benthic/pelagic
1168 life cycle. *BioEssays: News and Reviews in Molecular, Cellular and Developmental*
1169 *Biology* **26**, 314–325
- 1170 Fujisawa T, Barraclough TG (2013). Delimiting Species Using Single-Locus Data and the
1171 Generalized Mixed Yule Coalescent Approach: A Revised Method and Evaluation on
1172 Simulated Data Sets. *Systematic Biology* **62**, 707–724
- 1173 Gambi MC, Musco L, Giangrande A, Badalamenti F, Micheli F, Kroeker KJ (2016). Distribution
1174 and functional traits of polychaetes in a CO₂ vent system: winners and losers among
1175 closely related species. *Marine Ecology Progress Series* **550**, 121–134

- 1176 Gambi MC, Zupo V, Buia MC, Mazzella† L (2000). Feeding ecology of *Platynereis dumerilii*
 1177 (Audouin & Milne-Edwards) in the seagrass *Posidonia oceanica* system: The role of the
 1178 epiphytic flora (Polychaeta, nereididae). *Ophelia* **53**, 189–202
- 1179 Giangrande A (1988). Polychaete zonation and its relation to algal distribution down a vertical cliff
 1180 in the western Mediterranean (Italy): a structural analysis. *Journal of Experimental Marine*
 1181 *Biology and Ecology* **120**, 263–276
- 1182 Glasby CJ (2005). Polychaete distribution patterns revisited: an historical explanation. *Marine*
 1183 *Ecology* **26**, 235–245
- 1184 González-Delgado S, González-Santana D, Santana-Casiano M, González-Dávila M, Hernández
 1185 CA, Sangil C, Hernández JC (2021). Chemical characterization of the Punta de
 1186 Fuencaliente CO₂-enriched system (La Palma, NE Atlantic Ocean): a new natural
 1187 laboratory for ocean acidification studies. *Biogeosciences* **18**, 1673–1687
- 1188 Gravina MF, Lezzi M, Bonifazi A, Giangrande A (2015). The genus *Nereis* Linnaeus, 1758
 1189 (Polychaeta, Nereididae): State of the art for identification of Mediterranean species. *Atti*
 1190 *della Società Toscana di Scienze Naturali, Memorie Serie B* **122**, 147-164
- 1191 Grube AE (1850). Die Familien der Anneliden. *Archiv für Naturgeschichte, Berlin* **16**, 249-364.,
 1192 available online at <https://biodiversitylibrary.org/page/6958350>, page(s): 296
- 1193 Guindon S, Gascuel O (2003). A Simple, Fast, and Accurate Algorithm to Estimate Large
 1194 Phylogenies by Maximum Likelihood. *Systematic Biology* **52**, 696–704
- 1195 Hauenschild C (1951). Nachweis de sogenannten atoken Geschlechtsformdes Polychaeten
 1196 *Platynereis dumerilii* Aud et M Edw alseigene Art auf Grund von Zuchtversuchen. *Zoolo*
 1197 *Jahr Abteil all Zool Physiol der Tiere* **63**, 107–128
- 1198 Helm C, Adamo H, Hourdez S, Bleidorn C (2015). An immunocytochemical window into the
 1199 development of *Platynereis massiliensis* (Annelida, Nereididae). *International Journal of*
 1200 *Developmental Biology* **58**, 613–622
- 1201 Hupało K, Teixeira MAL, Rewicz T, Sezgin M, Iannilli V, Karaman GS, Grabowski M, Costa FO
 1202 (2019). Persistence of phylogeographic footprints helps to understand cryptic diversity
 1203 detected in two marine amphipods widespread in the Mediterranean basin. *Molecular*
 1204 *Phylogenetics and Evolution* **132**, 53–66
- 1205 Hutchings P, Kupriyanova E (2018). Cosmopolitan polychaetes – fact or fiction? Personal and
 1206 historical perspectives. *Invertebrate Systematics* **32**, 1–9

- 1207 Jörger KM, Norenburg JL, Wilson NG, Schrödl M (2012). Barcoding against a paradox?
 1208 Combined molecular species delineations reveal multiple cryptic lineages in elusive
 1209 meiofaunal sea slugs. *BMC Evolutionary Biology* **12**, 245
- 1210 Kara J, Santos CSG, Macdonald AHH, Simon CA, Kara J, Santos CSG, Macdonald AHH, Simon
 1211 CA (2020). Resolving the taxonomic identities and genetic structure of two cryptic
 1212 *Platynereis* Kinberg species from South Africa. *Invertebrate Systematics* **34**, 618–636
- 1213 Keferstein W (1862). Untersuchungen über niedere Seethiere. Zeitschrift für wissenschaftliche
 1214 Zoologie. **12**, 1-147 plates 1-11, available online at
 1215 <https://www.biodiversitylibrary.org/page/44977773>
- 1216 Kinberg JGH (1865). Annulata nova. [Continuatio.]. Öfversigt af Königlich.
 1217 Vetenskapsakademiens förhandlingar, Stockholm **22**, 167-179, available online at
 1218 <https://biodiversitylibrary.org/page/32339443>
- 1219 Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: Molecular Evolutionary Genetics
 1220 Analysis across Computing Platforms. *Molecular Biology and Evolution* **35**, 1547–1549
- 1221 Langeneck J, Scarpa F, Maltagliati F, Sanna D, Barbieri M, Cossu P, Mikac B, Galletti MC, Castelli
 1222 A, Casu M (2020). A complex species complex: The controversial role of ecology and
 1223 biogeography in the evolutionary history of *Syllis gracilis* Grube, 1840 (Annelida,
 1224 Syllidae). *Journal of Zoological Systematics and Evolutionary Research* **58**, 66–78
- 1225 Leigh JW, Bryant D (2015). popart: full-feature software for haplotype network construction.
 1226 *Methods in Ecology and Evolution* **6**, 1110–1116
- 1227 Librado P, Rozas J (2009). DnaSP v5: a software for comprehensive analysis of DNA
 1228 polymorphism data. *Bioinformatics* **25**, 1451–1452
- 1229 Lobo J, Teixeira MAL, Borges LMS, Ferreira MSG, Hollatz C, Gomes PT, Sousa R, Ravara A,
 1230 Costa MH, Costa FO (2016). Starting a DNA barcode reference library for shallow water
 1231 polychaetes from the southern European Atlantic coast. *Molecular Ecology Resources*
 1232 **16**, 298–313
- 1233 Lucey NM, Lombardi C, DeMarchi L, Schulze A, Gambi MC, Calosi P (2015). To brood or not to
 1234 brood: Are marine invertebrates that protect their offspring more resilient to ocean
 1235 acidification? *Scientific Reports* **5**, 12009
- 1236 Maltagliati F, Camilli L, Lardicci C, Castelli A (2001). Evidence for morphological and genetic
 1237 divergence in *Perinereis cultrifera* (Polychaeta: Nereididae) from two habitat types at Elba
 1238 Island. *Journal of the Marine Biological Association of the United Kingdom* **81**, 411–414

- 1239 Marcel R (1962). Cycle annuel de *Perinereis cultrifera* Grube (Annélide Polychète) à Alger.
1240 *Mémoires de la Société des Sciences Naturelles de Cherbourg* **19**, 39–54
- 1241 Martin D, Gil J, Zanol J, Meca MA, Portela RP (2020). Digging the diversity of Iberian bait worms
1242 *Marphysa* (Annelida, Eunicidae). *PLOS ONE* **15**, e0226749
- 1243 Mikac B (2015). A sea of worms: polychaete checklist of the Adriatic Sea. *Zootaxa* **3943**, 1–172
- 1244 Moquin-Tandon, G. (1869). Note sur une nouvelle annelide chetopode hermaphrodite (*Nereis*
1245 *massiliensis*). *Annales des sciences naturelles*, Paris (series 5) **11**, 134, available online
1246 at <https://www.biodiversitylibrary.org/page/33087914>
1247 Nygren A (2014). Cryptic polychaete diversity: a review. *Zoologica Scripta* **43**, 172–183
- 1248 Nygren A, Parapar J, Pons J, Meißner K, Bakken T, Kongsrud JA, Oug E, Gaeva D, Sikorski A,
1249 Johansen RA, Hutchings PA, Lavesque N, Capa M (2018). A mega-cryptic species
1250 complex hidden among one of the most common annelids in the North East Atlantic.
1251 *PLOS ONE* **13**, e0198356
- 1252 Örsted AS (1843). Annulorum danicorum conspectus. Auctore A.S. Örsted. Fasc. I. Maricolæ.
1253 (Quæstio ab universitate Hafniensi ad solvendum proposita et proemio ornata). ,
1254 available online at <http://www.biodiversitylibrary.org/bibliography/11849>
- 1255 Özpolat BD, Randel N, Williams EA, Bezares-Calderón LA, Andreatta G, Balavoine G, Bertucci
1256 PY, Ferrier DEK, Gambi MC, Gazave E, Handberg-Thorsager M, Hardege J, Hird C,
1257 Hsieh Y-W, Hui J, Mutemi KN, Schneider SQ, Simakov O, Vergara HM, Vervoort M,
1258 Jékely G, Tessmar-Raible K, Raible F, Arendt D (2021). The Nereid on the rise:
1259 *Platynereis* as a model system. *EvoDevo* **12**, 10
- 1260 Paiva PC, Mutaquilha BF, Coutinho MCL, Santos CSG (2019). Comparative phylogeography of
1261 two coastal species of *Perinereis* Kinberg, 1865 (Annelida, Polychaeta) in the South
1262 Atlantic. *Marine Biodiversity* **49**, 1537–1551
- 1263 Palumbi SR, Baker CS (1994). Contrasting population structure from nuclear intron sequences
1264 and mtDNA of humpback whales. *Molecular Biology and Evolution* **11**, 426–435
- 1265 Peral GSM, Martín JMV, Calvo CA (2004). Annelida: Polychaeta I. In: Ramos M.A. et al. (eds),
1266 Fauna Iberica. Vol. 25. Museo Nacional de Ciencias Naturales. CSIC, Madrid: 530 pp.
- 1267 Prevedelli D, Cassai C (2001). Reproduction and larval development of *Perinereis rullieri* Pilato
1268 in the Mediterranean Sea (Polychaeta: Nereididae). *Ophelia* **54**, 133–142
- 1269 Puillandre N, Lambert A, Brouillet S, Achaz G (2012). ABGD, Automatic Barcode Gap Discovery
1270 for primary species delimitation. *Molecular Ecology* **21**, 1864–1877

- 1271 Quatrefages A (1866). Histoire naturelle des Annelés marins et d'eau douce. Annélides et
 1272 Géphyriens. *Librarie Encyclopédique de Roret. Paris* **1**, 1-588, available online at
 1273 <http://books.google.com/books?id=FV9IAAAAYAAJ>
- 1274 Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018). Posterior Summarization in
 1275 Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology* **67**, 901–904
- 1276 Ratnasingham S, Hebert PDN (2013). A DNA-Based Registry for All Animal Species: The
 1277 Barcode Index Number (BIN) System. *PLOS ONE* **8**, e66213
- 1278 Read G and Fauchald K (Ed.) (2021a). World Polychaeta Database. *Nereis heterocirrata*
 1279 Treadwell, 1931. Accessed at:
 1280 <https://www.marinespecies.org/polychaeta/aphia.php?p=taxdetails&id=329658> on 2021-
 1281 05-12-2021
- 1282 Read G and Fauchald K (Ed.) (2021b). World Polychaeta Database. *Platynereis dumerilii*
 1283 (Audouin & Milne Edwards, 1833). Accessed through: World Register of Marine Species
 1284 at: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=130417> on 05-12-2021
- 1285 Read GB (2007). Taxonomy of sympatric New Zealand species of *Platynereis*, with description of
 1286 three new species additional to *P. australis* (Schmarda) (Annelida: Polychaeta:
 1287 Nereididae). *Zootaxa* **1558**, 1–28
- 1288 Ricevuto E, Benedetti M, Regoli F, Spicer JI, Gambi MC (2015). Antioxidant capacity of
 1289 polychaetes occurring at a natural CO₂ vent system: Results of an in situ reciprocal
 1290 transplant experiment. *Marine Environmental Research* **112**, 44–51
- 1291 Ronquist F, Huelsenbeck JP (2003). MrBayes 3: Bayesian phylogenetic inference under mixed
 1292 models. *Bioinformatics* **19**, 1572–1574
- 1293 Rouhi A, Gillet P, Deutsch B (2008). Reproduction and population dynamics of *Perinereis*
 1294 *cultrifera* (Polychaeta: Nereididae) of the Atlantic coast, El Jadida, Morocco. *Cahiers De*
 1295 *Biologie Marine* **49**, 151–160
- 1296 Sampieri BR, Vieira PE, Teixeira MAL, Seixas VC, Pagliosa PR, Amaral ACZ, Costa FO (2021).
 1297 Molecular diversity within the genus *Laeonereis* (Annelida, Nereididae) along the west
 1298 Atlantic coast: paving the way for integrative taxonomy. *PeerJ* **9**, e11364
- 1299 Sato M, Tsuchiya M (1991). Two patterns of early development in nereidid polychaetes keying
 1300 out to *Neanthes japonica* (Izuka). *Ophelia* **5**, 371–382
- 1301 Sato M, Masuda Y (1997). Genetic differentiation in two sibling species of the brackish-water
 1302 polychaete *Hediste japonica* complex (Nereididae). *Marine Biology* **130**, 163–170

- 1303 Scaps P, Retière C, Desrosiers G, Miron G (1992). Dynamique d'une population de *Perinereis*
1304 *cultrifera* (Grube) de la côte nord de Bretagne. *Cahiers De Biologie Marine* **33**, 477–494
- 1305 Scaps P, Rouabah A, Leprêtre A (2000). Morphological and biochemical evidence that *Perinereis*
1306 *cultrifera* (Polychaeta: Nereididae) is a complex of species. *Journal of the Marine*
1307 *Biological Association of the United Kingdom* **80**, 735–736
- 1308 Schneider S, Fischer A, Dorresteyn AWC (1992). A morphometric comparison of dissimilar early
1309 development in sibling species of *Platynereis* (Annelida, Polychaeta). *Roux's archives of*
1310 *developmental biology* **201**, 243–256
- 1311 Struck TH, Feder JL, Bendiksbj M, Birkeland S, Cerca J, Gusarov VI, Kistenich S, Larsson K-H,
1312 Liow LH, Nowak MD, Stedje B, Bachmann L, Dimitrov D (2018). Finding Evolutionary
1313 Processes Hidden in Cryptic Species. *Trends in Ecology & Evolution* **33**, 153–163
- 1314 Teixeira MAL, Vieira PE, Pleijel F, Sampieri BR, Ravara A, Costa FO, Nygren A (2020). Molecular
1315 and morphometric analyses identify new lineages within a large *Eumida* (Annelida)
1316 species complex. *Zoologica Scripta* **49**, 222–235
- 1317 Teixeira MAL, Nygren A, Ravara A, Vieira PE, Hernández JC, Costa FO (2021). The small
1318 polychaete *Platynereis dumerillii* revealed as a large species complex with fourteen
1319 MOTUs in European marine habitats. *ARPHA Conference Abstracts* **4**, e64937.
- 1320 Teske PR, von der Heyden S, McQuaid CD, Barker NP (2011). A review of marine
1321 phylogeography in southern Africa. *South African Journal of Science* **107**, 43–53
- 1322 Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of
1323 progressive multiple sequence alignment through sequence weighting, position-specific
1324 gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680
- 1325 Treadwell AL (1931). Three new species of polychaetous annelids in the collections of the United
1326 State National Museum. Available at: <http://repository.si.edu/xmlui/handle/10088/16029>
- 1327 Valvassori G, Massa-Gallucci A, Gambi MC (2015). Reappraisal of *Platynereis massiliensis*
1328 (Moquin-Tandon) (Annelida Nereididae) a neglected sibling species of *Platynereis*
1329 *dumerillii* (Audouin & Milne Edwards). *Biologia marina mediterranea* **22**, 113–116
- 1330 Vieira PE, Desiderato A, Holdich DM, Soares P, Creer S, Carvalho GR, Costa FO, Queiroga H
1331 (2019). Deep segregation in the open ocean: Macaronesia as an evolutionary hotspot for
1332 low dispersal marine invertebrates. *Molecular Ecology* **28**, 1784–1800
- 1333 Viéitez JM, Alós C, Parapar J, Besteiro C, Moreira J, Núñez J, Laborda AJ, San Martín G (2004).
1334 Annelida Polychaeta I. In: Ramos M.A. *et al.* (eds), Fauna Iberica. Vol. 25. Museo
1335 Nacional de Ciencias Naturales. CSIC, Madrid: 530 pp

- 1336 Villalobos-Guerrero TF, Bakken T (2018). Revision of the *Alitta virens* species complex (Annelida:
1337 Nereididae) from the North Pacific Ocean. *Zootaxa* **4483**, 201-257-201–257
- 1338 Viveiros F, Chiodini G, Cardellini C, Caliro S, Zanon V, Silva C, Rizzo AL, Hipólito A, Moreno L
1339 (2020). Deep CO₂ emitted at Furnas do Enxofre geothermal area (Terceira Island,
1340 Azores archipelago). An approach for determining CO₂ sources and total emissions
1341 using carbon isotopic data. *Journal of Volcanology and Geothermal Research* **401**,
1342 106968
- 1343 Wäge J, Valvassori G, Hardege JD, Schulze A, Gambi MC (2017). The sibling polychaetes
1344 *Platynereis dumerilii* and *Platynereis massiliensis* in the Mediterranean Sea: are
1345 phylogeographic patterns related to exposure to ocean acidification? *Marine Biology* **164**,
1346 199
- 1347 Weitschek E, Fiscon G, Felici G (2014). Supervised DNA Barcodes species classification:
1348 analysis, comparisons and results. *BioData Mining* **7**, 4
- 1349 Zantke J, Bannister S, Rajan VBV, Raible F, Tessmar-Raible K (2014). Genetic and Genomic
1350 Tools for the Marine Annelid *Platynereis dumerilii*. *Genetics* **197**, 19–31
- 1351 Zeeck E, Hardege J, Bartels-Hardege H, Wesselmann G (1988). Sex pheromone in a marine
1352 polychaete: Determination of the chemical structure. *Journal of Experimental Zoology*
1353 **246**, 285–292
- 1354 Zeeck E, Harder T, Beckmann M (1998). Uric Acid: The Sperm-Release Pheromone of the Marine
1355 Polychaete *Platynereis dumerilii*. *Journal of Chemical Ecology* **24**, 13–22
- 1356 Zghal F, Ben Amor Z (1989). Sur la présence en Méditerranée de la race épitoque de *Perinereis*
1357 *cultrifera* (Polychète). *Archives de l'Institut Pasteur de Tunis* **66**, 293–301
- 1358 Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013). A general species delimitation method with
1359 applications to phylogenetic placements. *Bioinformatics* **29**, 2869–2876
- 1360 Zhou H, Zhang Z, Chen H, Sun R, Wang H, Guo L, Pan H (2010). Integrating a DNA barcoding
1361 project with an ecological survey: a case study on temperate intertidal polychaete
1362 communities in Qingdao, China. *Chinese Journal of Oceanology and Limnology* **28**, 899–
1363 910

1364

1365 **Table and figure captions**

1366

1367 **Table 1.** Mean intra and inter-MOTU genetic distances (K2P) for the three analysed markers
1368 (COI, 16S, 28S-D2), either only for the 10 MOTUs corresponding to the *Platynereis dumerilii*

1369 pseudo cryptic complex (Clade A, Fig. 1A), or using the additional 5 MOTUs from the
1370 undetermined Nereidids (Clade B, Fig. 1A).

1371

1372 **Table 2.** Indices of genetic diversity estimated for each MOTU, based on COI. Number of
1373 sequences (n); nucleotide diversity (π), number of haplotypes (h), haplotype diversity (Hd) and
1374 number of variable sites (S). Region abbreviations: LP, La Palma; EH, El Hierro; TE, Tenerife;
1375 GC, Gran Canaria; FV, Fuerteventura; LA, Lanzarote; TER, Terceira; SMA, Santa Maria; SMI,
1376 São Miguel; MA, Madeira; PS, Porto Santo; PTC, Portugal – Canto Marinho; PTA, Portugal –
1377 Arrabida Natural Park; FRA, France – Arcachon Bay; FRR, France – La Rochelle; FRM, France,
1378 Morlaix Bay; GBP, Great Britain – Plymouth; NOT, Norway - Trondheim; NOB, Norway - Bergen;
1379 SWT, Sweden – Tjärnö; SPC, Spain – Calpe; FRB, France – Banyuls; ITT, Italy – Tuscany Area;
1380 ITR, Italy - Trieste; ITTA, Italy – Taranto; GRA, Greece, Amvrakikos lagoon; GRC, Greece – Crete
1381 island.

1382

1383 **Table 3.** List of currently unaccepted European synonyms of *P. dumerilii* based on WoRMS
1384 database, with their main distinctive morphological traits based on the original descriptions.

1385

1386 **Table 4.** Summary of the main morphological observations for the 10 different *Platynereis* MOTUs
1387 analysed in this study. Species in bold correspond to the ones described in the taxonomic section.

1388

1389

1390 **Fig. 1.** A, Phylogenetic tree reconstructed using Bayesian inference based on concatenated COI,
1391 16S and 28S-D2 sequences, with information regarding the different MOTU delineation methods.
1392 BINs were used only for COI. Outgroups (“OUTG” and “GB”), with the exception of *Pseudonereis*
1393 *palpata*, only have COI sequences. Collapsed clades have less than 3.5% genetic divergence.
1394 Only the bootstrap values over 0.85 BI and 85 ML support are shown. Each different consensus
1395 MOTU is represented by the respective number, with the different colors corresponding to the
1396 respective geographic distribution. B, Geographic distribution in Europe for the 15 retrieved
1397 MOTUs. C, Maximum likelihood amino acid (a.a.) radiation tree based on COI sequences
1398 belonging to MOTUs 1-10 (clade A). Region abbreviations: LP, La Palma; EH, El Hierro; TE,
1399 Tenerife; GC, Gran Canaria; FV, Fuerteventura; LA, Lanzarote; TER, Terceira; SMA, Santa
1400 Maria; SMI, São Miguel; MA, Madeira; PS, Porto Santo; PTC, Portugal – Canto Marinho; PTA,
1401 Portugal – Arrabida Natural Park; FRA, France – Arcachon Bay; FRR, France – La Rochelle;
1402 FRM, France, Morlaix Bay; GBP, Great Britain – Plymouth; NOT, Norway - Trondheim; NOB,
1403 Norway - Bergen; SWT, Sweden – Tjärnö; SPC, Spain – Calpe; FRB, France – Banyuls; ITT, Italy
1404 – Tuscany Area; ITR, Italy - Trieste; ITTA, Italy – Taranto; GRA, Greece, Amvrakikos lagoon;
1405 GRC, Greece – Crete island.

1406

1407 **Fig. 2.** Haplotypes networks based on COI (A), 28S-D2 (B) and ITS (C) for all the 15 MOTUs
1408 based on the original *Platynereis* and *Nereis* data and *Pseudonereis palpata* as outgroup. Each

1409 haplotype is represented by a circle and number of haplotypes are according to the displayed
1410 scale. Colours indicate the geographic location of the haplotype. Numbers correspond to the
1411 number of mutational steps between haplotypes. Lines without numbers means only one mutation
1412 between haplotypes. Country abbreviations: PT, Portugal; SP, Spain; FR, France; GB, Great
1413 Britain; NO, Norway; SW, Sweden; IT, Italy; GR, Greece; MOR, Morocco

1414

1415 **Fig. 3.** Drawing of the main morphological features in the anterior region, pigmentation and
1416 parapodia in *P. jourdei* sp. nov. (MOTU 6). A, dorsal view of the anterior region with dot-like
1417 pigmentation; prostomium and pharynx; B, ventral view of the pharynx; C, 10th parapod, posterior
1418 view; D, 30th parapod, posterior view; E, Pigmentation absent in the apodous anterior segment
1419 and well-defined ring-like dot pattern present after the first few segments.

1420

1421 **Fig. 4.** Dorsal view of the anterior region and chaetae types in *P. jourdei* sp. nov. (MOTU 6). A,
1422 Pigmentation as seen in a preserved specimen, with high dot density scattered around the
1423 anterior region. B, Pigmentation as seen in a preserved specimen, with a ring-like dot pattern in
1424 the anterior segments. C, Notochaetae, chaetiger 30: homogomph spinigers lightly serrated. D,
1425 Notochaetae, ventral fascicles: heterogomph falcigers, chaetiger 30. E, Neurochaetae, dorsal
1426 fascicle: heterogomph falcigers (1), homogomph spinigers (2), chaetiger 30.

1427

1428 **Fig. 5.** Drawing for the main morphological features in the anterior region, pigmentation and
1429 parapodia in *P. macaronensis* sp. nov. (MOTU 7). A, dorsal view of the anterior region with a well-
1430 defined ring-like dot pigmentation in the apodous anterior segment; prostomium and pharynx. B,
1431 ventral view of the pharynx. C, 10th parapod, posterior view. D, 30th parapod, posterior view.

1432

1433 **Fig. 6.** Dorsal view of the anterior region and chaetae types in *P. macaronensis* sp. nov. (MOTU
1434 7). A, Pigmentation as seen in a preserved specimen, with presence of well-defined ring-like dot
1435 pattern. B, Notochaetae: homogomph spinigers lightly serrated, chaetiger 10. C, Neurochaeta,
1436 ventral fascicle: heterogomph falcigers (1), chaetiger 10. D, Neurochaeta, ventral fascicle:
1437 heterogomph falcigers, chaetiger 30.

1438

1439 **Fig. 7.** Drawing of the main morphological features in the anterior region, pigmentation and
1440 parapodia in *P. agilis* comb. nov. (MOTU 10). A, dorsal view of the anterior region with absence
1441 of pigmentation; prostomium and pharynx. B, ventral view of the pharynx. C, 10th parapod,
1442 posterior view. D, 28th parapod, posterior view.

1443

1444 **Fig. 8.** Dorsal view of the anterior region and chaetae types in *P. agilis* comb. nov. (MOTU 10).
1445 A, Absence of pigmentation as seen in a preserved specimen. B, Notochaetae: homogomph
1446 spinigers with coarsely serrated blades, chaetiger 10. C, Neurochaeta, ventral fascicle:
1447 heterogomph falcigers, chaetiger 10. D, Neurochaeta, ventral fascicle: heterogomph falcigers,
1448 chaetiger 28.

1449

1450 **Fig. 9.** Drawing of the main morphological features in the anterior region, pigmentation and
1451 parapodia in *P. dumerilii* s.s. (MOTU 4). All terminology used is based on the references
1452 mentioned in the Methods. A, dorsal view of the anterior region with dot-like pigmentation;
1453 prostomium and pharynx. B, ventral view of the pharynx. C, 10th parapod, posterior view. D, 30th
1454 parapod, posterior view.

1455

1456 **Fig. 10.** Dorsal view of the anterior region and chaetae types in *P. dumerilii* s.s. (MOTU 4). A,
1457 pigmentation as seen in a preserved specimen, with high dot density scattered around the anterior
1458 region. B, Neurochaeta, dorsal fascicle: homogomph spinigers with lightly serrated blades (1),
1459 chaetiger 30. C, Neurochaeta, ventral fascicle: heterogomph falcigers, chaetiger 30. D,
1460 Neurochaeta, ventral fascicle: heterogomph spinigers (1), chaetiger 30. E, Notochaetae:
1461 homogomph falciger, chaetiger 57.

1462

1463 **Fig. 11.** Drawing of the main morphological features in the anterior region, pigmentation and
1464 parapodia in *P. c.f. massiliensis* (MOTU 9). A, dorsal view of the anterior region with ring-like dot
1465 pigmentation pattern; prostomium and pharynx. B, ventral view of the pharynx. C, 10th parapod,
1466 posterior view. D, 30th parapod, posterior view. E, Pigmentation absent in the apodous anterior
1467 segment and large circular-like dot patterns scattered after the first few segments, typically found
1468 in populations from Porto di Livorno (Italy). F, Pigmentation absent in the apodous anterior
1469 segment and scattered dot patterns after the first few segments.

1470

1471 **Fig. 12.** Dorsal view of the anterior region and chaetae types in *P. cf. massiliensis*. A,
1472 pigmentation as seen in a preserved specimen, with ring-like dot pigmentation pattern present in
1473 the apodous anterior segment and in the remaining anterior segments. B, Notochaetae:
1474 homogomph spinigers with coarsely serrated blades, chaetiger 30. C, Neurochaeta ventral
1475 fascicle: heterogomph spinigers (1); dorsal fascicle: homogomph spinigers (2), chaetiger 10. D,
1476 Neurochaeta, dorsal fascicle: heterogomph falcigers, chaetiger 30. E, Neurochaeta, ventral
1477 fascicle: heterogomph falcigers, chaetiger 30.

1478

1479 **Fig. 13.** Drawing for the main morphological features in the anterior region, pigmentation and
1480 parapodia in *Nereis aff. heterocirrata* (MOTUs 12 and 13). A, dorsal view of the anterior region
1481 with absence of pigmentation; prostomium and pharynx. B, ventral view of the pharynx. C, 11th
1482 parapod, posterior view. D, 24th parapod, posterior view. E, Neurochaeta, chaetiger 1:
1483 heterogomph falciger. F, Neurochaeta, chaetiger 24: heterogomph spiniger (3), heterogomph
1484 falciger (2), homogomph spiniger (4); Notochaetae homogomph falciger (1). G, Notochaetae
1485 chaetiger 11, homogomph spiniger (1); Neurochaeta parapod 11, heterogomph falciger (2).

1486

1487 **Fig. 14.** Drawing for the main morphological features in the anterior region, pigmentation and
1488 parapodia in *Nereis aff. zonata* (MOTU 11). A, dorsal view of the anterior region with absence of

1489 pigmentation; prostomium and pharynx. B, ventral view of the pharynx. C, 10th parapod, posterior
1490 view. D, 31th parapod, posterior view. E, Notochaetae, chaetiger 10: heterogomph spiniger (1),
1491 homogomph spiniger (2). F, Neurochaeta, chaetiger 30: heterogomph falciger. G, Notochaetae,
1492 chaetiger 30: homogomph falciger.

1493

1494

1495 SUPPORTING INFORMATION

1496

1497 Additional Supporting Information can be found in the online version of this article at the
1498 publisher's web-site:

1499

1500 **Table S1.** Primers and PCR conditions used in this study

1501

1502 **Table S2.** Voucher data, origin of the specimens and GenBank accession numbers for each of
1503 the analysed genetic markers original to this study and molecular metadata from other studies
1504 used for comparison purposes or as outgroups.

1505

1506 **Table S3.** Inter and intra (in bold) specific distances (K2P) with 1000 bootstraps for COI, 16S and
1507 the D2 region of 28S, respectively, for the ten retrieved *Platynereis* MOTUs.

1508

1509

1510 **Fig. S1.** Non-collapsed maximum likelihood phylogenies for the concatenated (COI, 16S and
1511 28SD2) dataset.

1512

1513 **Fig. S2.** Specimen (DBUA0002454.01) belonging to *Perinereis marionii* with focus on the
1514 pharynx, dorsal view.

1515