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1 **Genetic diversification and life-cycle of the polychaete *Capitella* spp.**
2 **from the English Channel: Evidence for sympatric cryptic species**
3 **and alternative reproductive strategies**

4
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18 **Abstract**

19 Polychaetes belonging to the *Capitella* genus are known to represent a complex of opportunistic cryptic species that
20 dominates the macrobenthos of organically enriched sediments such as muddy areas particularly rich in sulfide. With
21 the exception of the species, *Capitella capitata* (Fabricius) from West Greenland and *Capitella* spp. from the
22 European Atlantic coast, have never been accurately characterized and were often reported as *capitata* in the
23 literature. The life cycle of this European worm has not been described properly either, despite its widespread use as
24 bio-indicators in ecological studies. The present study provides ~~here~~ the first morphological description and genetic
25 diversification of *Capitella* collected along the Brittany coast and the English Channel up to the entrance of the
26 North Sea. Both morphological and molecular data are congruent and supported the co-occurrence of cryptic species
27 of *Capitella* at the tip of Brittany. The most frequent French mt lineages, C-Channel1, C-Atlantic and C-Channel2
28 although well distinct from *Capitella teleta*, are also divergent but closer to *C. capitata* initially described from
29 Greenland. The most abundant species (C-Channel1) was isolated and reared in the laboratory to describe its life
30 cycle in order to predict its dispersal ability and ecological success in the sulfidic muddy habitats of the French
31 harbors.

32

33 **Declarations**

34 **Data Availability Statement**

35 The dataset analyzed during the current study is available from the corresponding author on reasonable request.

36 **Code availability**

37 Not applicable

38 **Conflict of interest**

39 The authors declare that they have no conflict of interest.

40 **Ethics approval**

41 Not applicable

42 **Consent to participate**

43 Not applicable

44 **Consent for publication**

45 Not applicable

46

47 **Introduction**

48

49 Species that belong to the genus *Capitella* represent opportunistic inhabitants of marine muddy sediments and are
50 considered as important ecological indicators of eutrophication due to their its high densities in polluted ecosystems
51 (Tsutsumi et al. 1990; Sukwoo et al. 1992; Albano et al. 2013). Because of its fast growth rate, benthic development
52 and short generation time, ~~it~~ this annelid ~~has also becomes~~ also constitutes a model organism for many
53 ecotoxicological (Linke-Gamenick et al. 2000; Lewis and Watson 2012) and developmental studies (Seaver 2016).
54 For a long period of time, the lack of reliable morphological diagnostic traits has led to the view that this polychaeta
55 taxon was made of a few cosmopolitan species, colonizing a great variety of environments (e.g., muddy soft-bottom,
56 egg capsules, whale-bones) across a wide spectrum of depths (e.g., from the intertidal zone to abyssal depths) with a
57 worldwide distribution (Adkins and Schulze 2011; Silva et al. 2017). Population genetics however clearly showed
58 that it presumably does not represent few cosmopolitan species but rather a highly diversified geographic species.
59 Since the 1970's, the ecological indicator *Capitella capitata* species I originated from Massachusetts (USA) is
60 considered as a complex of six cryptic species (Grassle and Grassle 1976). Together with these molecular
61 differences, the six genetic lineages also displayed different numbers of chromosomes (Grassle et al. 1987). Such a
62 high level of genetic divergence was also observed by Silva in 2017 among a series of *Capitella* species and
63 corroborates the idea that many *Capitella* lineages almost morphologically similar are reproductively isolated (Silva
64 et al. 2017).

65 Cryptic species attributed to *Capitella capitata* (Fabricius, 1780) exhibit striking differences in life-history traits
66 including (i) the period of breeding, (ii) the egg sizes, (iii) the reproductive modes (occurrence of hermaphroditism,
67 self-fertilization), (iv) the post-embryonic development (direct or indirect), (v) the production of lecithotrophic or
68 planktotrophic larvae and (vi) the dispersal duration of the larvae (Grassle and Grassle 1976; Chia et al. 1996). They
69 also display differences in gametes and in larval ultrastructures (Eckelbarger and Grassle 1983). The duration of the
70 life cycle of *Capitella* aff. *capitata* ranges from one-two months in lab-reared populations to nine months in some
71 wild populations of the Mediterranean Sea (Tsutsumi et al. 1990; Méndez 2016). The potential longevity also varies
72 between cryptic species from five months in a Mediterranean population sampled in the estuary of Ebro (Spain) to
73 one year in a population sampled from Warren Point on the estuary of the River Yealm, Devon (England) (Warren
74 1976; Martín 1991). All together these criteria were used to define at least 19 cryptic species identified in laboratory-

75 reared individuals from various geographic localities. Because the concept of species heavily relies on reproductive
76 isolation, the very high variation in reproductive modes, mating seasons or ecological preferences in the complex of
77 species associated with *Capitella capitata* is likely to be a potent factor affecting speciation and explaining such a
78 high cryptic diversity at a small spatial and/or regional scale. So far, barcode records of *C. aff. capitata* published in
79 the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007) mostly include specimens collected in
80 Canada, United States and India. Additional sequences also evidenced the existence of new phylogenetic lineages in
81 the Mediterranean Sea and along the Brazilian coast (Livi et al. 2017; Silva et al. 2017). *Capitella* species have been
82 also well documented along the European Atlantic coastlines in muddy habitats of harbors and estuaries. Despite
83 being often used as a bio-indicator species of pollution in the North Sea, this species has yet to be genetically
84 described and its life cycle has to be properly examined in relation with its genotype and morphology.
85 Morphological traits traditionally used to identify species of the Capitellidae family include the overall size, shape
86 and the relative size of the prostomium and peristomium (as a complete or an incomplete ring), the number and
87 distribution of capillary chaetae and hooded hooks along the thorax, the morphology, the number and the size of the
88 genital spines and the shape of the pygidium (Blake et al. 2009). Using these morphological characters and
89 sequences of the mitochondrial *Cox-1* gene, the present study provides new insights about the genetic status of
90 *Capitella* spp. inhabiting the muddy sediments of the intertidal zone of the North European coastlines. To this extent,
91 we first examined the spatial distribution of barcoded specimens along the French coasts of the Atlantic and English
92 Channel and, then defined more precisely morphological and developmental diagnostic traits (i.e. morphologies,
93 reproductive modes, larval developments and juvenile growth rates) for one of the three genetically-differentiated
94 *Capitella* lineages present in Roscoff (Brittany, France), i.e. the most dominant one, the C-Channel1, (*Capitella*
95 English Channel). Specimens present at in Roscoff not sorted completely during sampling, species isolation was
96 achieved by rearing them in small aquaria over several generations. The conditions to rear and breed this specific
97 genetic lineage over several generation were achieved in the laboratory.

98

99 **Material and methods**

100

101 **Field sampling and rearing conditions of the *Capitella* collection in the laboratory**

102

103 **Field sampling:** To assess the genetic diversity and the spatial distribution of *Capitella* spp. along the Atlantic and
104 English Channel coastlines of France ~~and assess their spatial distribution, we collected~~ *Capitella* specimens were
105 collected at five distinct localities along Brittany and Eastern Channel coastlines (Port-La-Forêt, Concarneau,
106 Roscoff, Boulogne and Dunkerque as show in Fig. 1a and Table S1). We then ~~assessed~~ determined both the
107 proportion of the putative cryptic species along the French coastline and their overall local density (all species
108 together) at a much smaller spatial scale within the harbor of Roscoff according to the habitat by sampling animals at
109 three (in triplicate) distinct sites (Fig. 1b). An additional site with a coarser sediment (namely site 4) was also
110 sampled once at the boundary of the harbor of Roscoff for ecological comparison. Replicates within each site were
111 equally treated. Specimens were collected by shoveling the first three cm of the superficial layer of the sediment. The
112 sediment was sieved through a 0.5 mm mesh and the retained worms were then sorted out and sexed under a
113 binocular in the laboratory. Following Pardo et al. recommendations (Pardo et al. 2010), ~~we measured~~ the width at

114 the 5th setigerous segment was measured as a size-estimator for each *Capitella* specimen because the thoracic region
115 is almost always present and this parameter is easily scorable.

116 **Rearing conditions:** A subset of *Capitella* worms coming from the Roscoff harbor were maintained in tanks
117 (30*15*20 cm) containing a 2-3 cm deep layer of soft sediments for the breeding experiment. Because of logistical
118 issues, the animals from other localities (Port-La-Forêt, Concarneau, Boulogne and Dunkerque) were only used for
119 the genetic study and not used for rearing. Sieved sediments were obtained from the Roscoff sampling sites and used
120 after being kept frozen at - 80 °C for at least nine months. Animals were kept in sterilized oxygenated seawater
121 (Instant Ocean at a salinity of 33‰) at 18 °C under natural light (Méndez 2002). Animals of each tank were fed
122 weekly with 0.5 g of a mixture consisting of an equal ratio of frozen sediments and a ground commercial dried baby
123 crop (HiPP Biologique, France). Animals were kept under these conditions for four years (about 18 generations of
124 worms) for breeding purposes. After this period, the genetic survey of the aquaria worms has revealed an abundant
125 the dominance of one of the three genetic lineages. Series of developmental stages from the newly formed embryos
126 still in eggs, the hatched larvae brooding in the females' tubes to the fully developed and mature adults' stages able
127 to reproduce were sampled from this last generation of reared worm's for subsequent descriptions.

128

129 **Morphological, reproductive and developmental traits in *Capitella* spp.**

130

131 **Identification of diagnostic morphological traits:** All specimens were examined using an optical microscope
132 (Zeiss Axio Imager M2 or Axio lab A1) and a stereomicroscope (Zeiss Stemi 305). To facilitate the observation,
133 animals were immersed in cold sea water containing 10% ethanol (Schikorski et al. 2008; Boidin-Wichlacz et al.
134 2012). The morphological traits chosen are traits that can be easily visualized when sorting the animals. The shape
135 of the prostomium (conical or rounded) and the presence/absence of a thoracic–abdominal transition was examined
136 on *Capitella* spp. sampled from the four Roscoff sites (1, 2, 3 and 4). When the transition from the thorax to the
137 abdomen was present with a narrower 10th setiger, individuals exhibit thoracic setigers becoming much wider from
138 the 8th setiger to the head. Drawings were made from a collection of these images. To evaluate the size of each
139 individual, the width of the 5th setigerous segment was photographed at least three times under a microscope (Axio
140 lab A1, Zeiss) with a camera and the Zen 2011 module of Zeiss for image analysis and the measures were averaged.
141 The distribution of the width of the 5th setiger among individuals was used to jointly infer the existence of different
142 clusters of individuals with common morphological traits and to assess the potential effects of the habitat, sex and
143 size to discriminate putative species-diagnostic morphological features.

144 **Identification of gender:** Individuals that presented testicular masses with genital spines between the 8th and 9th
145 setigerous segments were considered to be males. Gravid individuals were considered to be females. Alternatively,
146 individuals were only scored as females if oocytes were observed by transparency, other specimens that did not
147 meet these criteria were called juveniles. The presence of oocytes/sperm masses visible through the tegument was
148 observed under binocular.

149 **Morphological diagnosis of *Capitella* spp. reared in the laboratory:** At the end of the rearing experiment forty
150 specimens (20 males and 20 females) isolated of the tank were examined using an optical microscope (Zeiss Axio
151 Imager M2 or Axio lab A1) and a stereomicroscope (Zeiss Stemi 305) to determine the fecundity as well as to
152 diagnose morphological traits following the procedure described before. These individuals were also used to assess
153 egg size variations within and between females. To this extent, 10 brooding tubes from the 20 females were opened

154 carefully with needles under a dissecting microscope and were examined with a camera and the Zen 2011 module
155 (Zeiss) for image analyses. To visualize their internal structures, the 20 males were fixed in 2.5% glutaraldehyde and
156 then dehydrated in a series of ethanol solutions. Specimens were then embedded in a paraplast Plus® containing
157 medium and cut in sections down to 7 µm thickness. Slices were colored using toluidine blue (0.1%) for 4 min after
158 being dewaxed and rehydrated. Nuclear DNA was then labelled using a 4, 6-diamidino-2-phenylindole (DAPI,
159 Sigma) solution (at 0.5 µg/mL of PBS, ROTH) for 20 min in the dark at room temperature.

160

161 **Genetic identification of lineages**

162

163 **DNA extraction:** Genomic DNA was extracted from 161 individuals collected on the field along the Brittany and
164 the English Channel coasts (five localities see Table 1) and from a subset of 192 experimented *Capitella* coming
165 from Roscoff but reared in tanks for reproductive purposes. In addition, 96 juveniles coming from the progeny of the
166 20 pairs selected to study the mode of reproduction (20% of the total progeny) were also sequenced for further
167 identifying the different OTUs. Extractions were performed from the whole worm using a NucleoSpin Tissue XS kit
168 (Macherey-Nagel) according to the manufacturer's protocol.

169 **PCR amplification:** A 569 bp fragment of the mitochondrial cytochrome oxidase subunit 1 (*Cox1*) gene was
170 amplified using newly -designed primers CO1Forward (5'- GTACAGA AACTTGCGCGTTCCT-3') and CO1Reverse
171 (5'- CCACCACCAGTAGGATCAAA -3'). Amplifications were carried out with a GoTaq® G2 DNA Polymerase
172 (Promega). The reaction mixture (25 µL) for PCR amplification contained 10 µM of each primer, 10 µM of each
173 desoxynucleotide triphosphate (dNTP), 1X Go Taq® Flexi buffer (Promega), and 5U of GoTaq G2 Flexi DNA
174 polymerase (Promega). ~~The final volume was adjusted to 25 µL with DEPC water.~~ DNA amplification was
175 performed ~~on a thermocycler (Eppendorf)~~ under the following conditions: (1) ~~an initial denaturation step at 95 °C for~~
176 ~~15 min without enzyme,~~ (2) ~~a series of 39 cycles of~~ at 95 °C for 30 s, at 56 °C for 30 s, at 72°C for 1 min and (3) 72
177 °C for 5 min ~~for final elongation.~~ PCR products were ~~then visualized on a 1.5% agarose gel with ethidium bromide~~
178 ~~following electrophoresis at 100 volts for 30 min,~~ and subsequently purified with nucleofast 96 PCR cleanup kit.
179 Purified products were then Sanger-sequenced on an ABI 3100 capillary sequencer using BigDye (PerkinElmer)
180 terminator chemistry following the manufacturer's protocol (Applied Biosystems, Foster City, CA).

181 **Sequence analysis:** Chromatograms were checked manually using SeqScape V2.5. The sequence data were aligned
182 manually with BioEdit v.7.2.5 and/or SeaView v.5 (Gouy et al. 2010). Sequences of *C. capitata*, *C. teleta* and other
183 *Capitella* found worldwide were recovered from Genbank and the DDBJ (DNA Data Bank of Japan) to position our
184 sequences within the genus *Capitella* for phylogenetic purposes. Data collection of specimens, museum codes and
185 GenBank accession numbers (<https://www.ncbi.nlm.nih.gov/genbank>) are detailed in Table S2. Maximum likelihood
186 tree reconstructions were performed over the whole set of sequences using the software SeaView 5.0 (Gouy et al.
187 2010). The phylogenetic tree was then redrawn with FigTree 1.4.4 and Inkscape beta2 2019 to add annotations.
188 Mitochondrial sequences related to the French *Capitella* clades were then used to perform a haplotype network
189 following the Neighbor Joining (NJ) algorithm of the software PopART (Leigh and Bryant 2015). The net
190 divergence between the French mitochondrial clades was estimated with the package ComputeNet of the Mega6
191 software using the K2P substitution model, and tested with the automatic detection of barcode gaps implemented in
192 the online ABGD software developed by Puillandre (Puillandre et al. 2012). This application examines the

193 distribution of JC or K2P distances within and between putative OTUs in order to detect species breaks. Gene
194 diversity departures from neutral expectations were also tested using both Tajima'D and Fu&Li' F statistics with the
195 software DNAsp v.6 (Rozas et al. 2017), and subsequently checked against 1000 simulations of neutral coalescents.

196

197 **Other statistical tests**

198

199 Tests of two-way relationships between categorical variables (e.g. association between prostomium shape and
200 sampling site) were performed using chi-squared tests, with p-values computed from the results of 10,000 Monte-
201 Carlo simulations (as performed by function 'chisq.test' in R). The effect of an explanatory variable (categorical or
202 continuous) on a continuous response variable was assessed using an analysis of deviance, tested through the value
203 of its F statistic (using function 'anova' in R).

204 The width of the 5th setiger was modeled using Mixture Of Experts (MOE) models, as implemented in the R package
205 'MoEClust' (Murphy and Murphy 2019). In brief, this method allows to regress a variable (here, the width of the 5th
206 setiger) on multiple explanatory variables (the 'expert' variables) while at the same time inferring groups of
207 individuals which share a common intercept of the regression and common variance (here, cryptic species or, at
208 least, groups of species with similar morphological traits). Groups are inferred from the intrinsic structure of data and
209 external variables (the 'gating' variables). The width of the 5th setiger was assumed to depend on gender (three
210 modalities), presence/absence of a marked thoracic–abdominal transition (two modalities) and the shape of the head
211 (two modalities) and the sampling station (four modalities) typifying the habitat. All variables were allowed to adjust
212 the model as expert and/or gating variable, except 'station' which was only allowed as gating variable – the sampling
213 locality was assumed to affect the probability of sampling individuals from different groups, but not directly affect
214 their size. Different MOE models were trained and compared using the Integrated Completed Likelihood (ICL)
215 (Biernacki et al. 2006) and the best model was retained to assess correlations.

216

217 **Results**

218

219 **Four distinct clades of cryptic *Capitella* spp. along the English Channel coast**

220

221 Phylogenetic analyses using *Cox-1* sequences of *Capitella* specimens collected worldwide clearly indicated that
222 previously well recognized morphologically based species such as *C. capitata* are sub-divided into several genetic
223 clades. Among them, worms collected along the coasts of Brittany and the Eastern Channel are split in four distinct
224 clades (Clades C1, C2, C3 and C4) with robust bootstrap values (88-100%, Fig. 2). Because most of the genetic
225 variation is linked to the third codon position of the mitochondrial gene, further analyses on either amino-acids or the
226 first and second positions of codons did not significantly improve the robustness of the deepest nodes of the tree. The
227 net divergence between these clades estimated using the Maximum Composite Likelihood model (Mega 6) is high
228 with values ranging from 0.021 (between the two closest lineages) to 0.338 (between the most divergent ones, Table
229 1). The most divergent clade (C4) is closely related to the cluster of sequences associated with *C. teleta* from USA
230 (Massachusetts) and Japan. Although found at the two ends of the English Channel, this clade was rather rare and

231 only sampled inside the harbors of Concarneau, and Dunkerque on coarser sediments. The three other
232 Brittany/English Channel clades (C-Atlantic, C-Channel1 and C-Channel2) are genetically closer to the well
233 diversified group of *C. capitata* where the holotype described by Fabricius (1780) in Greenland is positioned. These
234 three clades however clearly differ from the reference sequences of *C. capitata* on the phylogenetic tree. Because of
235 the high rate of molecular evolution of the *Cox-1* gene and sequence saturation, the resolution of deep nodes is
236 however weak with bootstrap values often lower than 50%, suggesting that our clades can only be viewed as separate
237 lineages to *C. capitata*. As a consequence, they should be named as *Capitella* spp. for the first three French clades
238 and *C. aff. teleta* for the last one. The haplotype network obtained with PopART allowed us to discriminate *Capitella*
239 specimens according to their geography and refine more precisely their distribution along the French coastline (Fig.
240 1c). Twenty-three haplotypes were recovered from 161 individuals of *Capitella* spp. These haplotypes are split into
241 three main clades separated by 8 and 62 fixed substitutions (Fig. 1c). A barcode-gap automatic detection (ABGD)
242 analysis of the sequence dataset clearly indicated that they represent three distinct OTUs (Fig. S1). The two most
243 closely related clades are geographically distinct with one clade located in the English Channel and referred as C-
244 Channel1 and the other in the Atlantic and referred as C-Atlantic. However, these two clades overlap at in Roscoff
245 with a series of derived Atlantic haplotypes uniquely found at this specific location (Fig. 1c). The most divergent
246 clade is composed of only one haplotype with a restricted number of sampled specimens only collected in the
247 English Channel and thus referred as C-Channel2. The most widespread Clade C-Channel1 is well diversified with a
248 star-like shape typifying population expansion. In this clade, the hypothesis of demographic expansion is well
249 supported by both Tajima's D and Fu&Li's F tests with highly significant negative values (Table 2). Conversely, the
250 C-Atlantic clade displays a more structured genetic diversity but almost no departure to the neutral evolution of the
251 gene polymorphism. For this latter clade, Fu&Li's tests remained significant at the 5% threshold but significance
252 was further not supported by the simulations of neutral coalescents. Haplotype frequencies of the two most dominant
253 clades were greatly structured between Concarneau/Port-La-Forêt and Roscoff, indicating the presence of a physical
254 barrier to dispersal at the entrance of the English Channel (Fig. 1c).

255

256 **Statistical analysis of morphological variables of C-Atlantic, C-Channel1 and C-Channel2**

257 The different species of *Capitella* spp. (C-Atlantic, C-Channel1 and C-Channel2) live syntopically at different
258 densities in the four sampled sites of Roscoff (see Table S3). The density of *Capitella* is greater in sites 1 and 2 than
259 in sites 3 and 4 where the sediments are coarser and less eutrophicated. Sites 1 and 2 were indeed located in the most
260 sheltered area of the harbour near fishing boats that dump waste and offer more organic food to the worms. From the
261 154 *Capitella* spp. individuals collected at in Roscoff (March 2014), nearly half of them (48.1%) were sexually
262 undetermined or immature, and may represent juveniles (Table 3). Since the ratio of females to males is not
263 significantly different from 1:1 (binomial test, $p = 0.927$), the sex ratio is somehow balanced in the Roscoff
264 population.

265 Within the four sites of the harbor of Roscoff, two different shapes of prostomium (round Fig. 3a or conical Fig. 3b)
266 were observed from our *C. aff. capitata* specimens, regardless of their position in the harbor. At In Roscoff, the
267 proportions of round and conical shapes varied locally (Fig 3c). Individuals with a rounded prostomium are less
268 abundant at sites 1 and 4 while individuals with a round prostomium dominate at site 2. However, a Chi-squared test
269 performed on the prostomium \times station contingency table did not show any link between the locality and prostomium
270 shape ($\chi^2 = 6.45$, $df = 3$, $n = 154$, $p = 0.09529$). We observed as many females with conical prostomium as females

271 with rounded prostomium (Fig. 3d, Table S4). Males seemed, however, to have a rounded prostomium more often,
272 while the prostomium of the juveniles generally appears to be conical. This was reinforced by a Chi-squared test
273 which supports a link between the sex of the worm and the shape of prostomium ($\chi^2 = 12.97$, $df = 2$, $n = 154$, $p =$
274 0.0013).

275 Of the 154 *Capitella* collected, 64 individuals had no thoracic–abdominal transition in the thoracic metamere,
276 compared with 90 individuals who have a thoracic–abdominal transition (Fig. 4ap). The majority of *Capitella* from
277 sites 1 and 4 did not have a thoracic–abdominal transition in the thoracic metamere, while most of those from sites 2
278 and 3 did (Fig. 4l). The presence of a marked thoracic–abdominal transition might be linked to the locality ($\chi^2 =$
279 13.73 , $df = 3$, $n = 154$, $p = 0.0018$) and the gender ($\chi^2 = 12.04$, $df = 2$, $n = 154$, $p = 0.0017$), but not with the shape of
280 the prostomium ($\chi^2 = 0.30$, $df = 1$, $n = 154$, $p = 0.626$). The width of the 5th setiger displayed a general bimodal
281 distribution (Fig. 4f), which was confirmed by the outcome of the MOE model: we found two groups of sampled
282 individuals (Fig. 4a), partly defined by their thoracic–abdominal transition (Fig. 4d and m) and their sampling
283 locality (Fig. 4c and i), but did not evince the effect of the other variables (sex, head shape) on the width of the 5th
284 setiger. In other words, both the sampling station and the presence/absence of a thoracic–abdominal transition
285 determined the group each individual belongs to, and each of the two groups had its own mean and variance for the
286 width of the 5th setiger (Fig. 4b and e). Pairwise relationships between the inferred groups, the 5th setiger
287 measurements, and the two gating variables (thoracic–abdominal transition and locality) are given in Figure 5. The
288 first group (in blue) contained 92 individuals (Fig. 4a), with an average width of 201.6 μm and a standard deviation
289 of 43.7 (Fig. 4e); the second group (in red) comprised of 62 individuals (Fig. 4a), with an average width of 373.6 μm
290 and a standard deviation of 109.7 (Fig. 4e). The four stations had different 5th setiger size distributions, as most
291 individuals from the sites 1, 3 and 4 belong to the group 1 while the site 2 is mainly composed of individuals from
292 the group 2 (Fig. 4i), *i.e.* worms sampled in the site 2 had larger 5th setiger (Fig. 4g, j). The presence of a thoracic–
293 abdominal transition was indicative of the second group (Fig. 4m), with individuals with a thoracic–abdominal
294 transition having a larger 5th setiger (Fig. 4h and n). The second-best MOE model (with an ICL 0.94 points worse
295 than the best one) only differed by including prostomium shape as an additional gating variable. The inferred groups
296 of the second-best MOE model were strongly consistent with the ones found by the first model – randomly assigning
297 groups to individuals according to the memberships inferred by the two models gave a 6.7% mismatch rate between
298 the two classifications, *i.e.* only 10 *Capitella* individuals (out of 154) are expected to be classified differently by the
299 two best models.

300 The effect of gender on the width of the 5th setiger changed when analyses were performed on all sampled worms or
301 within MOE groups. With all individuals, a linear model including ‘sex’ as an explanatory variable was significantly
302 better at modelling the width of the 5th setiger than one without it ($F_{2,152} = 6.85$, $p = 0.00142$). However, when the
303 same analysis was performed on the group 1 or the group 2 as inferred by the best MOE model, the addition of ‘sex’
304 did not significantly improve the goodness-of-fit in either case (for the group 1 with 11 females, 31 males and 50
305 juveniles: $F_{2,90} = 0.33$, $p = 0.7195$; for the group 2 with 23 females, 15 males and 24 juveniles: $F_{2,60} = 2.94$, $p =$
306 0.0605). In other words, once morphospecies membership, as inferred by MOE models, is taken into account, the
307 size of males and females do not differ.

308

309 **Molecular identification of *Capitella* spp. reared in the laboratory**

310

311 A final alignment of a 522 nucleotides *Cox-1* fragment was performed on 192 *Capitella* spp. from the last generation
312 of worms obtained after four years of rearing (18th generation). Phylogenetic analyses clearly indicate that these
313 specimens belong to the three major clades C-Atlantic, C-Channel1 and C-Channel2, ~~but also indicate~~ and that the C-
314 Channel1 clade dominated the rearing collection with 88% of the genotyped individuals. In contrast, C-Channel2, C-
315 Channel1 and Atlantic only represented 3 and 9% of the whole set of genotyped samples, respectively. This clade is
316 also the most frequently encountered ~~at~~ in Roscoff.

317

318 **Morphology and life cycle of *Capitella* spp. reared in the laboratory**

319

320 A rounded prostomium and a semi-translucent head with two eyes was observed for all 40 *Capitella* (20 males and
321 20 females) examined in the aquarium (Fig. S2a). These worms were morphologically identical with a slender,
322 cylindrical body, of roughly 60–80 segments, capable of considerable expansion and contraction. Their bodies were
323 clearly sub-divided into a thoracic region made of nine setigerous segments and a finer abdomen with markedly
324 longer segments (*i.e.* presence of a clear thoracic-abdomen transition). Based on the barcoding of 192 individuals,
325 these individuals were likely to represent the C-Channel1 clade. To confirm this hypothesis a subset of 96 randomly
326 picked juveniles were genotyped from the progeny of these 40 *Capitella* spp. All these juveniles belong to the clade
327 C-Channel1. These results strongly support that the life cycle and the morphology of the *Capitella* further described
328 below, can be attributable to the C-Channel1 clade, although we cannot firmly exclude the possibility that a very few
329 of them might belong to another clade.

330 At the time of our observations, the selected individuals of *Capitella* spp. have all segments 1-6 with capillary
331 chaetae only, the seventh segment exhibits both capillary chaetae and hooks and the following segments have hooks
332 only. Several studies have demonstrated that hooks can be replaced by capillary chaetae with age (Schweigkofler et
333 al. 1998). A single genital pore opens mid-dorsally between setigers 8 and 9. In females, the pore opens as an oval
334 swelling whereas in males its extends as a middle stout chaeta (genital spines or genital hooks). The thorax is white or
335 cream with underlying pinkish color proportional to the concentration of hemoglobin-containing cells (Fig. S2b).
336 The abdominal setigers are translucent, light to bright red, depending on the abundancy of red cells circulating into
337 the coelomic cavity. The gut contains black or brown sediment, sometimes with the presence of monocytyd gregarine
338 parasites, *Ancora sagittata* (Fig. S2b,c), visible through the body wall (Schrevel and Philippe 1993). The coloration
339 may change from intense red to white with brown to black pigmentation presumably indicating that specimens are
340 becoming old without any taxonomical incidences (Fig. S2d). The pygidium displays a simple lobe as described for
341 the *C. capitata* holotype from Greenland (Blake 2009). All of these morphological traits were observed in
342 individuals reared in lab.

343 Mature males display genital spines between the eighth and ninth thoracic setigers (Fig. 5a). The genital spines of 20
344 males of CChannel1 were examined by histology to determine the intra- and inter-specific variation of
345 gametogenesis (Fig. 5). In all males, the number of spines is lower on the setiger 9 than on the setiger 8 with
346 approximately four genital spines on the former segment. The average number of spines on the setiger 8 is of six for
347 the examined individuals. These spines have a light-yellow glossy appearance in alive materials, and are slightly
348 falcate distally. Males possess a sac of paired genital ducts suspended in the lateral coelom between the seventh and
349 the eighth thoracic setigers, and a prominent copulatory organ located between the paired genital spines of the setiger
350 9 (Fig. 5b). Serial sections of the prominent copulatory organ provided evidences of structure, similar to the

351 zymogen-like secretory granules already described in *Capitella capitata* by Blake (Fig. 5c). Serial sections of the sac
352 genital paired ducts (Fig. 5d) showed numerous mature sperm bundles (Fig. 5e). The mature spermatozoon displayed
353 a conical acrosome, an elongated, tapering nucleus and a middle piece composed of a cytoplasmic sleeve or collar,
354 which extends posteriorly along the proximal portion of the flagellum (Fig. 5f).

355 Ovaries are well visible by transparency in mature females ~~through the body wall~~. They are paired yellowish sac
356 organs, usually with four or more oocytes at different stages of development. The ovaries are suspended by
357 mesenteries in the ventral coelomic cavity throughout the mid-body segments (Fig. 6a). Mature oocytes enter the
358 coelom, and float freely before being spawned by the female (Fig. 6b). Females of clade C-Channel1 build brooding
359 tubes made of mucus, sediment and organic material (Fig. 6c). After copulation, the fertilized eggs are deposited on
360 the inner wall of the tube where incubation of the embryos occurs (Fig. 6d). C-Channel1 produced a limited number
361 of eggs (between 67 and 169). Eggs (from 120 to 580 μm in diameter) are spherical and creamy-white to pale
362 yellow. To examine egg size variation within and between females, 10 brooding tubes coming from the set of the 20
363 females previously used for reproduction were carefully opened (see methods). The brooding tubes used come from
364 females of the same size/age and were sampled during the same breeding period. Egg size variation between females
365 is reported in Figure 6e. Although the general morphology of the females was similar, striking differences of the size
366 of fertilized eggs can be noticed between tubes. Six females had small eggs (140 to 220 μm), two females had
367 intermediate eggs (280 μm to 360 μm), and two females had larger eggs (460 μm to 560 μm). The identity of the
368 brooding tube explained 4.69e6 units of deviance, thus significantly improving the goodness-of-fit of a linear model
369 of egg size ($F = 646.42$, $p < 2.2\text{e-}16$). There was however no relationship between egg counts and the average egg
370 size among brooding tubes ($F = 1.044$, $p = 0.3367$). Differences of egg size may be partly explained by the fact that
371 some females did not deposit all their eggs in the brooding tube (Fig. S3a). Females indeed still have mature ~~eggs~~
372 oocytes in their coelomic cavity ~~during the time~~ while embryos ~~are~~ were developing into the eggs deposited in the
373 brooding tube. This suggests that the C-Channel1 lineage is able to spawn several times during the breeding season
374 leading to different cohorts of eggs that may differ in size depending of the female's reproductive investment.

375 In a limited number of observations (2%), some individuals with genital spines on both setigers 8 and 9 also
376 exhibited oogenesis in the abdominal setigers (Fig. S3b). Some males ~~thus~~ turn into females suggesting a
377 protandrous hermaphroditism.

378 Embryonic development of specimens from clade C-Channel 1, starts at the two-cell stage with divisions occurring
379 approximately every hour in a roughly synchronous pattern between quadrats. The four-cell stage was observed 2
380 hours later (Fig. 7a). All observed samples develop through an indirect developmental cycle with a trochophore and a
381 metatrochophore larva before their metamorphosis into juvenile. C-Channel 1 has an indirect-developing,
382 lecithotrophic, non-feeding larval stage as defined by David E. K. Ferrier (Ferrier 2012).~~These trochophore and~~
383 ~~metatrochophore larva were observed inside or outside the brood tube.~~ In our study, trochophore larvae are oval-
384 shaped (Fig. 7b) and bear two small eyes and two ciliary rings (Fig. 7c). These rings allow larval movements inside
385 the tube and/or in the water column ~~after hatching~~. At day 4, the gut appears (Fig. 7c). The metatrochophore stage
386 can be seen inside the tube or freely swimming in the tank water. It has 13–14 segments with a visible ventral
387 stomodeal concavity, which is not connected to the gut confirming a lecithotrophic development. Metatrochophores
388 metamorphose into juveniles within a period of seven days. By contrast with larvae, juveniles were only observed in
389 the water tank, outside the brood tubes.

390 The juvenile stage of 600 μm length on average, is characterized by a vermiform shape with a complete
391 segmentation, a clear separation of the thorax from the abdomen, the presence of two small eyes (Fig. 7d). From
392 anterior to posterior, the alimentary tract consists of a mouth, buccal cavity, pharynx, esophagus, midgut, hindgut,
393 and a posterior terminal anus. The mouth of juveniles opens on the ventral surface posterior to the prostomium,
394 adjacent to the junction of the peristomium (Fig. 7e). Juveniles undergo a substantial growth before being sexually
395 mature (adults) after a period of about two months.

396

397 **Discussion**

398

399 **Taxonomy and recent evolutionary history of *Capitella* spp. in France**

400

401 The use of DNA sequences for species identification (barcode) has been widely developed over the last decade with
402 universal ‘applicable’ markers such as the cytochrome c oxidase 1 (*Cox1*) or genes encoding the ribosomal units
403 (Leigh and Bryant 2015). As the mitochondrial genome evolves generally faster than nuclear genes, observed
404 divergence between individuals with the same morphology often reflects past population isolation for a long period
405 of time, while fixed morphological differences between groups of individuals improves the robustness of species
406 isolation. Further discrepancies in allele coalescence of nuclear genes, life history traits and especially reproduction
407 dynamics and larval development are however needed prior to conclude about reproductive isolation of genetically-
408 differentiated *mt* lineages and assess whether pre- or post-zygotic isolation processes are likely involved to initiate
409 gene divergence. In the present study, both morphological and molecular data are congruent and supported the co-
410 occurrence of cryptic species of *Capitella* at the tip of Brittany. The most frequent French *mt* lineages, C-Channel1,
411 C-Atlantic and C-Channel2, although well distinct from *Capitella teleta*, are also divergent but closer to *C. capitata*
412 (Fabricius, 1780) originally described from Greenland and previously sampled in Canada, India and the
413 Mediterranean Sea, with divergences well above the expected threshold separating distinct morphological species.
414 These first three French *mt* lineages may be even more closely related to *C. capitata* and differ from the reference
415 *capitata* species by 23% suggesting that they represent new taxa typifying the northern part of Europe. The fourth
416 French *mt* lineage (C4) is closer to *C. teleta* but also differs from it by 26%, and thus also represents a new divergent
417 species in the genus *Capitella*. This finding therefore indicates that the worldwide *Capitella* diversification is very
418 old (several to tens of millions of years ago) with parallel biogeographic histories between *C. teleta* and *C. capitata*,
419 at least in the Northern Atlantic. Surprisingly, the four genetic lineages examined in the present study are all found in
420 sympatry in the harbor of Roscoff at different proportions according to habitat. This species superimposition is also
421 reinforced by our MOE model of the width 5th setiger (unpublished data), which advocated the existence of at least
422 two different morphospecies, heterogeneously distributed among sampling locations and partly predicted by the
423 existence of a thoracic-abdominal transition. Further morpho-genetic analyses of reared individuals and their
424 offspring clearly indicated that the morphospecies with the thoracic-abdominal transition corresponded to individuals
425 of the C-Channel1 clade.

426 The finding of four discrete *Capitella* OTUs at the entrance of the English Channel reinforces our view that the tip of
427 Brittany represents a crossroad between different biogeographical provinces and thus a species mixing point where
428 populations overlap and possibly interact. More specifically, the geographic distribution of haplotypes clearly

429 indicates the presence of a genetic break between the populations located in the southern part of Brittany
430 (Concarneau/Port-La-Forêt: C-Atlantic) and those sampled in the Eastern Channel (C-Channel1), with a contact zone
431 located at the tip of Brittany (Roscoff) where populations overlapped locally. Because both Tajima's and Fu&Li
432 tests indicated that C-Channel1 is presently expanding, rapid recolonization of muddy sediments of the English
433 Channel after its opening may have favored secondary contacts with other refugial populations such as the C-
434 Atlantic one. The occurrence of a biogeographic barrier at the entrance of the English Channel for both invertebrate
435 fauna and algae has been widely documented in the past for a series of marine invertebrates and algae (Jolly et al.
436 2006; Maggs et al. 2008). With a divergence of 2%, genetic isolation of these two sibling units should have
437 coincided with the beginning of the Last Glacial Maximum (LGM) during which most of marine populations
438 contracted in multiple refuges, some of which being located either along the Iberic Peninsula, North of Scotland or
439 the Irish Sea (Maggs et al. 2008). Similar patterns of geographic divergence have been indeed observed for the
440 complexes of hybridizing blue mussels *Mytilus edulis/galloprovincialis* (Rawson et al. 1996; Bierne et al. 2003) and
441 the clam *Limecola balthica* (Luttikhuizen et al. 2003) in the North Sea/Baltic and English Channel, which may share
442 histories partly similar to *Capitella*. More unexpectedly, our results also report the co-occurrence of two well-
443 ~~divergent~~ diverged lineages (C-Channel1 and C-Channel2) at nearly all localities of the Eastern Channel with 14%
444 of divergence. The co-occurrence of cryptic species at the tip of Brittany with divergences ranged between 16 to 20%
445 was also reported in other polychaetes living in muddy-sand habitats of estuaries and bays such as *Pectinaria koreni*
446 and *Owenia fusiformis* (Jolly et al. 2006) but also in echinoderm species such as *Acrocnida brachiata/spatulissima*
447 (Muths et al. 2006), or the sea urchin *Echicardium cordatum* (Egea et al. 2016) living in soft sediments. According to
448 calibration dates used for *Cox-1* (Knowlton and Weigt 1998), these divergence may predates the opening of the
449 Bering strait (about 3.5 Mya during the Mio-pliocene transition) and the subsequent colonization of the Northern
450 Atlantic by Pacific lineages with different routes of colonization and several episodes of glaciations (Hewitt 2004).
451 Although partly explained by the demographic history of taxa since the Last Glacial Maximum, the superimposition
452 of four distinct *Capitella* OTUs at the tip of Brittany may be also due to differences in ecological niches and life-
453 history traits. Because some of them are involved in a mutualistic interaction with sulfo-oxidizing bacteria at small
454 spatial scales, depending on local conditions of eutrophication (~~unpublished data~~) (Hourdez et al. 2021), the second
455 aim of the present work was to search for differences in the morphology and life-history traits of these worms that
456 could explain their non-exclusive distributions. DNA sequence datasets provided thus a basis for further
457 phylogeographic studies and population genetics within this species complex and a way of discriminating species to
458 describe their life-history traits and reproductive dynamics.

459

460 **Life-history traits and growth rate of *Capitella*-Channel1**

461

462 Life-history traits of the species complex *Capitella capitata* has been extensively studied for a series of individuals
463 coming from very distinct geographic regions (Adkins and Schulze 2011; Méndez 2016; Silva et al. 2016). Despite a
464 few fixed life history features (brood tubes, trochophore and metatrochophore larvae), there is a remarkable diversity
465 and plasticity in *Capitella* life cycles according to environmental conditions as observed in eutrophicated areas
466 (Petraitis 1991; Gamenick et al. 1998; Silva et al. 2017). These worms indeed display a heterogeneous distribution of
467 densities linked to organic carbon and/or sulfidic enrichments (Gamenick et al. 1998; Blake 2009). The harbor of
468 Roscoff is a patchwork of enriched-areas with organic matter, due to the presence of decaying crab or fish carcasses

469 (Hourdez et al. 2021, in press). Variations in the density of *Capitella* spp. presently depicted in this study can be
470 therefore attributed to the opportunistic behavior of the worm that allows the rapid and nearby colonization of
471 organically-enriched and/or disturbed soft habitats of the harbor of Roscoff. In order to understand more precisely
472 how these worms can react rapidly to environmental fluctuations and expanded since the last glacial episode, our
473 effort focused on characterizing more specifically the life-history traits of *Capitella*-Channel1, the most abundant
474 representative of the three French *mt* lineages at in Roscoff. The general morphology in shape, size and colour of C-
475 Channel1 is similar to that described for *Capitella capitata* (Fabricius, 1780) but the lineage also shared additional
476 morphological similarities for the mature sperm and genital spines as described by Eckelbarger & Grassle (1987)
477 (Eckelbarger and Grassle 1987) C-Channel1 has however a semi-translucent head with two eyes on all specimens, as
478 opposed to *Capitella capitata floridana* which is generally devoid of eyes (Hartman 1959).

479 Individuals from natural populations of *Capitella* aff. *capitata* have a long life cycle (up to one year) at Plymouth
480 (England), Elba Island (Italy) or from Estero del Yugo (Mexico) (Warren 1976; Lardicci and Ceccherelli 1994;
481 Méndez 2006), respectively when compared to the duration of the life cycle of *Capitella* sp. from the west coast of
482 Kyushu (Japan), which varies from 1 to 2 months in cultured populations (Tsutsumi et al. 1990). Here the clade C-
483 Channel1 exhibits a life cycle of two months in aquaria under a constant temperature of 18 °C. Detailed information
484 about the duration of the life cycle of species belonging to the genus *Capitella* are scarce and differ widely according
485 to the reference species and their geographical origin (Méndez et al. 2000). The rather short life cycle of C-Channel1
486 is consistent with Adkins & Schulze's work which (Adkins and Schulze 2011) concluded that plasticity in
487 developmental timing is an adaptation for living in frequently disturbed environments: a situation often encountered
488 in the harbor of Roscoff. As pointed out by Grassle and Grassle (1976) *C. capitata* is able to modify its life cycle
489 depending on environmental conditions. These authors ~~indeed~~ has demonstrated that the population density of this
490 species mainly depends on its life history which may vary in organically polluted areas (Tsutsumi 1987; Méndez et
491 al. 1997; Martin and Bastida 2006). Because of its short life cycle, potential of this new species for explosive
492 demographic expansion is therefore considerably higher than that of many other long-lived benthic animals that are
493 found in more stable habitats. Together with a very short life span, the ability of C-Channel1 to lay its eggs within
494 tubes where metatrochophore larvae may develop freely inside or outside of the tube within a week before their
495 metamorphosis in juveniles also explains the rapid and local fluctuations of demography associated with this worm.
496 Our observations clearly indicated that a non-negligible proportion of metatrochophores were able to escape tubes to
497 freely swim in the experimental tanks before metamorphosis. Brooding represents a great advantage for the rapid
498 replenishment of the population by favoring the local retention of larvae and juveniles in the vicinity of parents
499 where food is abundant. Favoring local retention fits well the aggregative and patchy distribution of *Capitella* spp.
500 along the shore when local conditions are optimal. This however imposes a great limitation to larval dispersal and the
501 colonization of distant territories and could explain the high genetic diversification found in *Capitella*. Short-term
502 metatrochophore dispersal represents however an alternative to escape local conditions when they are not appropriate
503 enough due to the flexibility of the *Capitella* larvae to develop inside or outside the brood tube.

504 Concerning the worm's fecundity, the analysis of the size and number of eggs within a brooding tube indicated that
505 the dominant *Capitella* lineage from Roscoff (*i.e.* C-Channel1) releases a rather restricted number of small to large
506 eggs. Such a strategy highly contrasts with *Capitella* Ct (from Cranford, Ireland) and *Capitella* K (from Kimelford,
507 Scotland) which produce a high number of small eggs. This small production of eggs also greatly differs to the
508 reproductive effort of *Capitella* populations from temperate climates, such as in Germany, England, and Scotland

509 (Méndez et al. 2000). Here, despite the small number of eggs produced by C-Channel1 females, embryo
510 development is indirect ~~but~~ and larvae are brooded inside the maternal tube. However, both the egg size and the
511 number of eggs per release seem to greatly vary between females. In *Capitella capitata*, Pearson & Pearson (1991)
512 split the species into two distinct types. Type 1 comprised large eggs of a size equal to or greater than 250µm and
513 type 2 comprised small eggs of a size equal to or smaller than 182µm (Pearson and Pearson 1991). A majority of
514 individuals spawned more than a hundred of small eggs (around 160 µm) in a way similar to *Capitella* sp S and
515 *Capitella* sp L from Germany (Méndez et al. 2000), which have respectively an average of 50 to 250 eggs per female
516 release. Some females from Roscoff lineage however atypically produce a lower number of larger eggs ranged
517 between 280 and 560 µm. This may be due to differences in the age or the reproductive investment of females of the
518 same species. ~~Since, only 20% of the whole studied progeny was genotyped, we cannot fully exclude the low~~
519 ~~possibility that a small fraction of brooding females might come from another genetic lineage.~~ Some females still
520 have mature ~~eggs~~ oocytes in their coelomic cavity ~~during the time~~ while embryos are developing in the brooding
521 tube. This suggests that the C-Channel1 lineage is able to spawn several times during the breeding season leading to
522 different cohorts of eggs that may differ in size depending of the female's reproductive investment. By contrast,
523 females of *Capitella* aff. *capitata* from Tomioka bay (Japan) grown, reproduced only once in laboratory and died
524 soon after they had finished to brood (Tsutsumi 1987), but Pei-Yuan Qian also showed that reproductive variability
525 can occur among siblings in *Capitella* spp. from a single spawn (Qian and Chia 1992). Within our set of
526 experimental conditions, the extensive variation of egg size among females and the number of offspring can be also
527 explained by the density of worms in aquaria and the amount of food but not on temperature, which remained
528 constant throughout the experimentation. This partly fits previous findings of Qian and Chia (1991) who reported
529 that egg size of siblings can vary depending on environmental conditions (Qian and Chia 1991) and we cannot rule
530 out the possibility that the variation in the size of eggs can be environmentally-driven in *Capitella* C-Channel1. This
531 questions whether the C-Channel1 clade ~~is likely to~~ may display alternative ~~modes of larval development or whether~~
532 ~~the different genetic lineages have alternative~~ reproductive modes. Alternative modes of reproduction within a given
533 species have been explained by the co-occurrence of cryptic species in *Spionidae* (Kruse et al. 2003, 2004;
534 Kesäniemi et al. 2014). In the specific case of the Capitellidae, Holte and Oug (Holte and Oug 1996) however noted
535 that *C. aff. capitata* from Norway displays two modes of gamete development depending on the female size; (i) large
536 eggs (250 µm) release from small individuals and (ii) high numbers of smaller eggs from larger individuals. Similar
537 egg variation has been reported in British waters (Pearson and Pearson 1991). In the present study, all *Capitella*
538 females studied were morphologically similar (of the same size) and met the same rearing conditions. Together with
539 the fact that individuals have about 90% chance of coming from the C-Channel1 lineage, such variation in egg size is
540 likely to reflect an intrinsic reproductive property of the species. Our observations on the development however did
541 not support the hypothesis that ~~the species~~ one given female can produce two kinds of offspring as all the progeny
542 investigated had the same lecithotrophic larval developmental mode and thus refutes the hypothesis of a poecilogonic
543 species.

544 As opposed to *C. capitata* from Greenland, which produces a high number of pelagic larvae, species living in the
545 English Channel have a "lecithotrophic ~~indirect~~" development with the hatching of the larvae occurring inside the
546 parental tube at the trochophore stage, which further develops into metatrochophores that freely swim in the water
547 ~~column~~ tank. The genus *Capitella* contains at least 16 lecithotrophic species, three of them being hermaphrodites.
548 *C. aff. capitata* does not generally feed in the plankton (lecithotrophy), and is likely to exhibit a short-range dispersal.

549 Metatrochophore larvae have been also observed inside parental tubes in other *C. capitata* populations from the
550 littoral of Barcelona (Spain) (Méndez et al. 1997; Méndez 2002) as well as in ~~*C. capitata-teleta*~~ from Setauket
551 Harbor (New York, US) (Blake et al. 2009), shallow hydrothermal vent areas of Milos (Greece) (Gamenick et al.
552 1998) and the Gulf of Mexico (Méndez et al. 2019). Like *C. teleta* (Ferrier 2012; Meyer et al. 2015), *Capitella* spp.
553 described in this study has an indirectly developing, lecithotrophic larva, the *Capitella* larvae hatch as a trochophore
554 in the brooding tube and then are free to remain or leave the tube as a metatrochophore. Juveniles are always
555 observed in the water tank and never inside the brooding tubes ~~Here, the offspring emerged as juveniles from the~~
556 ~~parental tubes after 11 days on average, and followed a complete larval development from trochophore to~~
557 ~~metatrochophore stages inside the brooding tube. However, a~~ A non-negligible proportion of metatrochophore larvae
558 were observed in the water column suggesting that larvae may shift from a benthic to a pelagic development
559 depending on environmental conditions. Trochophore larvae are characterized by an oval shape without obvious
560 segmentation, two ciliary rings and two eyes, and thus able to swim and orientate in the water column. In the coast of
561 Barcelona, Méndez (2002) showed that *Capitella* sp. B has a lecithotrophic pelagic development together with the
562 simultaneous presence of trochophore and metatrochophore larvae in the maternal tube and non-simultaneous release
563 of both types of larvae (Méndez 2006). In general, the metatrochophore stage only lasted four days before
564 metamorphosis. Similar observations were made in the laboratory for *Capitella* sp. G of Galveston Bay (USA)
565 (Adkins and Schulze 2011) ~~but~~ and also in Italy where metatrochophores can develop even more rapidly in a few
566 hours to one day (Gamenick et al. 1998). For most of these species benthic larvae are favoured when local resources
567 are abundant (Willcox and Nickell 1998) and retention of larvae inside the brooding tubes can allow the rapid build-
568 up of a population where food supply is not limited and when dispersal to colonize new habitats is not essential
569 (what is the case for animals reared in under lab conditions). In this study, some kind of reproductive bet hedging
570 was spatially observed among females that do not lay all of their embryos in the same tube. In a few cases,
571 trochophore larvae have been seen to develop outside the tube and we cannot rule out the fact that more larvae could
572 freely develop in the water column under different environmental conditions. Méndez (2019) demonstrated that
573 larvae can survive until metamorphosis when forced to move outside the tube as trochophores or metatrochophores,
574 but not before reaching the trochophore stage (Méndez et al. 2019). Offspring that were forced to move out of the
575 tube at the metatrochophore stage, has however the same larval duration as to the undisturbed broods.

576 Planktotrophic development patterns were also observed in *Capitella* K and Ct and a first description of the life
577 cycle of *Capitella* sp. G from Galveston Bay, suggests that larvae may be facultatively feed in the plankton (Adkins
578 and Schulze 2011). Plasticity of larvae to stay in maternal tubes or feed in the plankton may be a way to face
579 changing environments. Our *Capitella* spp. species are not able to feed in the plankton but are often present in
580 estuaries, where the sediment is frequently disturbed by the tides and in harbors where anthropogenic activities are
581 likely to alter the sediment habitat. This may allow the short-term dispersal (a few day) of offspring when the
582 brooding tubes are destroyed or when the environmental conditions are not good enough to sustain the population.
583 The uncertainty in environmental conditions act as a selective force in the evolution of life-history traits of *Capitella*
584 spp. (Chia et al. 1996).

585 In our study, the low-investment of C-Channel1 in reproduction can allow adults to survive after the first
586 reproductive event and to restart gametogenesis for a new breeding period. We ~~indeed~~ did not observe the systematic
587 death of females after spawning. Seasonal fluctuations and organic enrichment of sediments are the main factors
588 affecting reproduction (Tsutsumi et al. 1990). Experimental studies have demonstrated that low temperatures can

589 diminish sensibly the reproductive activity of *C. capitata* Type I and the extremely low temperatures registered in
590 Punta Rasa could inhibit the reproduction of this species during the coldest season (Martin and Bastida 2006).
591 *Capitella* sp. I reproduction is complex with female worms remaining as functional females throughout their life.
592 *Capitella* aff. *capitata* from Tomioka Bay (Japan) reproduces throughout the whole year and the presence of
593 reproductive characteristics indicated that the ovaries of females matured a second time during the brooding period
594 (Tsutsumi et al. 1990). *Capitella* C-Channel1 also seems to adjust the proportion of males and females according to
595 the environmental conditions to the expected proportions of 1:1 and is likely to become a protandrous hermaphrodite
596 when needed. Sex ratio plasticity can represent a powerful tool for the maintenance of an optimal effective
597 population size over seasons. Summer period can be considered as extremely favorable for the population growth
598 because bottom water and sediment of sheltered areas such as harbours become rapidly warm and anaerobic with the
599 accelerated decay of organic matter and the stagnation of the bottom water. *Capitella* individuals collected at
600 Roscoff also have an equal percentage of females and males in natural populations with a possible environment-
601 driven shift towards females when some males turn into females. Similar reproductive dynamics have also been
602 observed in *C. capitata* from U.K and from Spain in studies dealing with the population dynamics of the worm
603 (Warren 1976; Martin and Grémare 1997). According to some authors (Petraitis 1985, 1991; Méndez 2006),
604 hermaphroditism occurs under certain conditions, when the population density is low, especially in protandrous
605 species. It is suggested that hermaphroditism, as well as the ability of self-fertilization observed in *Capitella* sp.Y for
606 example, is likely to enhance the success of the population in natural environments (Méndez 2006). Previous
607 laboratory experiments indicated that populations with low densities can trigger the development of female gonads in
608 *Capitella* males resulting in simultaneous hermaphroditic animals (Silva et al. 2016). Many studies already noticed
609 that sex ratio is quite variable and flexible in polychaetes (Prevedelli and Simonini 2003). The expression of sexual
610 phenotypes is very plastic in annelids and many polychaete species produce not only males and females but also
611 hermaphrodites. Gonochorism is considered the ancestral sexual system in polychaetes, and hermaphroditism is a
612 secondary acquisition in this perspective (Prevedelli et al. 2006). In the light of this series of articles, we could infer
613 that sex ratio may evolve together with the mode of development of the worm to adapt to local conditions. When
614 local conditions are good enough or when the number of individuals exploiting the habitat is small, the worm may
615 promote benthic development of larvae in tubes while increasing the number of females and hermaphrodites. On the
616 contrary, larvae may become pelagic by moving outside the tube at either the trochophore or metatrochophore stages
617 with a more balanced sex ratio of 1:1 to escape its local conditions and colonize new territories.

618

619 **Conclusion**

620 *Capitella* spp. collected in the English Channel represent a complex of three well-divergent mitochondrial lineages,
621 which can be viewed as potential cryptic species. Amongst them, the most frequent one at the entrance of the English
622 Channel (C-Channel1) seems to exhibit some variation in egg size and a dispersal strategy due to the fact that
623 metatrochophore larvae may either develop inside the maternal tube or outside in the water column. This may favor
624 the colonization of new habitats while promoting larval retention and limited dispersal ability depending on the
625 dynamic of environmental conditions at local spatial scales. Levels of mud eutrophication may indeed change rapidly
626 in space during the year. Such highly varying environmental conditions may act as a driving force in the evolution of
627 life-history traits in *Capitella* spp. at some locations and should promote speciation processes in the face of physical
628 barriers to gene flow.

629

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636

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790

791 **FIGURE LEGENDS**

792 **Fig. 1** Study areas and cryptic species distribution of *Capitella*. **a** Position of sampling locations along the coasts of
 793 the English Channel and the North Atlantic. **b** Satellite image of the four Roscoff localities sampled during this
 794 study. **c** Haplotype network of mitochondrial haplotypes of *Capitella* spp. using the Minimum Spanning Network
 795 method implemented in PoPART. The size of the circle is proportional to the number of shared haplotypes. The links
 796 and black bars represent a number of mutations between two distinct haplotypes. Black circles represent missing
 797 haplotypes.

798 **Fig. 2** Maximum likelihood (ML) tree for 108 individuals representing a selection of *Capitella* taxa for which a
 799 mitochondrial *Cox-1* gene sequence is available in Genbank together with our set of specimens. The ML tree of
 800 *Capitella* spp. was obtained using PhyML with either the α LRT option or 100 bootstraps using 5 randomly-chosen
 801 starting BioNJ trees, the Tamura-Nei 1993 +G+I substitution model and the NNI & SPR swapping option with
 802 Seaview 5.0 software. Sequences come from individuals of the Eastern Channel (Dunkerque-Boulogne), Western
 803 Channel (Roscoff) and Atlantic (Port-la Forêt/Concarneau) and specimens related to several *Capitella* species,
 804 including *C. aff. capitata* and *C. teleta* sampled worldwide (Japan, Canada, United States, India, Mediterranean). C1
 805 to C4 indicates the 4 clades of the study and the arrows the reference species including *teleta*, *biota*, *aracaensis*,
 806 *nonatoi*, *aciculata*, *neoaciculata* and *capitata* from Greenland. Slashed values above branches correspond to the
 807 statistical supports of nodes with posterior probability and bootstrap information.

808 **Fig. 3** Morphological analyses of *C. aff. capitata*. collected at in Roscoff. **a** Images and drawings of anterior end, left
809 lateral view of the shape of the head prostomium (conical or rounded). **b** Images and drawings of the dorsal view
810 showing the presence or not of the thoracic–abdominal transition .**c** Histogram of the percentages of individuals
811 according to the shape of their prostomium and their geographic repartition. **d** Histogram of the percentages of
812 individuals by sex depending on the shape of the prostomium. Cap S, capillary setae; Eye; Per, peristomium; Pro,
813 prostomium; Set, setiger.

814 **Fig. 4** Pairwise relationships among variables related to the mixture of expert’s model of the width of the 5th setiger.
815 **a** Proportions of individuals in the two groups (1 in blue with 92 individuals, 2 in red with 62 individuals). **b** & **e**
816 Distribution of the 5th setiger width among the two groups (**b**: jittered points, **e**: classic boxplots). **c** Distribution of
817 the stations of sampling for individuals from the group 1 (above) and the group 2 (below). **d** Distribution of the
818 presence/absence of the thoracic–abdominal transition marked for individuals from the group 1 (above) and the
819 group 2 (below). **f** Overall distribution of the 5th setiger. **g** & **j** distribution of the 5th setiger width between the four
820 sites (**g**: jittered points colored by group, **j**: classic boxplots). **h** & **n** Distribution of the 5th setiger width depending on
821 the presence/absence of a thoracic–abdominal transition marked (**h**: jittered points colored by group, **n**: classic
822 boxplots). **i** Distribution of the two groups in the four stations, with the station 1 on top and the station 4 at the
823 bottom. **k** Distribution of individuals by sampling station. The number of individuals per station is 61, 76, 11 and 6. **l**
824 Presence/absence of a thoracic–abdominal transition by station (absence on the left, presence on the right, and
825 stations ordered from top to bottom). **m** Distribution of the two groups according to the presence/absence of a
826 thoracic–abdominal transition on the 9th setigers, with absence above and presence below. **o** Distribution of sample
827 site by the presence/absence of a thoracic–abdominal transition on the 9th setiger (absence above, presence below,
828 and the four sites ordered from left to right). **p** Distribution of individuals by the presence with 64 individuals (P)
829 /absence with 90 individuals (A) of a thoracic–abdominal transition (absence on the left, presence on the right).

830 **Fig. 5** Male reproductive organ. **a** Light micrograph of dorsal view of eighth and ninth setigers of live *Capitella*
831 Channel1 showing genital spines. **b** cross section of a male *Capitella* Channel1 at the 8th and 9th setigers, stained with
832 toluidine blue. **c** Cross-section of a male showing the genital spines and copulatory organ stained with toluidine blue
833 (nucleic acids are labelled in blue (DAPI)). The white field is superimposed to see the shadows of the structures. **d**
834 Section of the sac-like genital ducts stained with toluidine blue. The second image shows the nuclear labelling
835 (DAPI), evidencing a large quantity of sperm. **e** Microscopic observation of one sperm with nucleic acids labelled in
836 blue (DAPI). Abbreviations: Cap S, capillary setae; Cg, sac genital ducts; Co, copulatory organ; Dg, digestive tract;
837 Gs, genital spines; Set, setiger.

838 **Fig. 6** Female reproductive organ. **a** High magnification of ovaries in dorsal view. Dotted lines mark the boundaries
839 between adjacent segments. **b** Dorsal view of mature oocytes freely floating in the coelom. **c** A brood tube
840 containing eggs and the female in dorsal view. **d** Enlargement of a portion of the brooding tube with eggs. **e**
841 Distributions of egg size (y-axis) by female (x-axis). Distributions are presented as violin plots, with white dots
842 representing median values, the black solid lines indicating the second and third quartiles, thin lines extending to the
843 extremes of the distribution, and the width of the violins proportional to the distribution of egg size values.

844 **Fig. 7** Light micrographs of developmental stages of *Capitella* Cc-Channel1. **a** Embryo at a spiral stage of four cells.
845 **b** Dorsal view of trochophore larva, anterior end is up. **c** Dorsal view of metatrochophore larva, anterior end is up. **d**
846 Juvenile specimen of Cc-Channel1 in lateral view with anterior to the left and ventral down. **e** Observation of
847 different sizes of juveniles from the same brood. **c** Schematic of embryonic and larval stages for *Capitella* sp.

848 Abbreviations: An, anus; Br, brain; Ec, ectoderm; En, endoderm; Es, esophagus; Gl, gut lumen; Hg, hindgut; Mg,
849 midgut; Ph, pharynx; Pro, prostomium; Pt, prototroch; Pyg, pygidium; Te, telotroch; Rc, rectum.

850

851 **Tables**

852 Table 1 Genetic distances between the different sequence groups (clades/lines) for the mitochondrial gene Cox-1
853 calculated using the MEGA6 software.

	C-Channel1	C-Atlantic	C-Channel2	<i>C.capitata</i> Canada/India	<i>C.capitata</i> Mediterranean	<i>C.capitata</i> Canada/Japan	<i>C.teleta</i> France	<i>C.teleta</i> Japan/USA
C-Channel1	0.001							
C-Atlantic	0.021	0.002						
C-Channel2	0.142	0.141	0.000					
<i>C.capitata</i> Canada/India	0.257	0.262	0.227	0.011				
<i>C.capitata</i> Mediterranean	0.286	0.291	0.311	0.349	0.124			
<i>C.capitata</i> Canada/Japan	0.245	0.238	0.229	0.299	0.338	0.067		
<i>C. teleta</i> France	0.308	0.326	0.259	0.403	0.395	0.292	0.011	
<i>C.teleta</i> Japan/USA	0.238	0.236	0.259	0.288	0.337	0.225	0.265	0.018

854

855 Table 2 Genetic diversity descriptors for the different clades and statistical test values to detect a difference in
856 neutrality for the mitochondrial gene Cox-1 in the 3 clades. N: number of sequences, S: number of sites varying,
857 ***p<0.01, **p<0.02, *p<0.05, NS: not significant, NA: not applicable.

Clade	N	S	Hd	π	θ_w	Fu&Li'D	Fu&Li'F	Tajima'D
C-Channel1	108	17	0.417±0.059	0.00123±0.00024	0.00620±0.00207	-5.13**	-4.89**	-2.44**
C-Atlantic	26	6	0.6±0.00983	0.00158±0.00034	0.00301±0.0015	-2.24*	-2.526*	-1.25(NS)
C-Channel2	11	0	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

858

859 Table 3 Percentages of *Capitella* spp. individuals by gender and site at the Roscoff harbor.

Number (%)		
Site 1	Juveniles	65.57
	Females	8.20

	Males	26.23
Site 2	Juveniles	28.95
	Females	35.53
	Males	35.53
Site 3	Juveniles	72.73
	Females	18.18
	Males	9.09
Site 4	Juveniles	66.67
	Females	0
	Males	33.33

860