Genetic diversification and life-cycle of the polychaete Capitella spp. from the English Channel: evidence for sympatric cryptic species and alternative reproductive strategies

Céline Boidin-Wichlacz, Didier Jollivet, Claire Papot, Lolita Roisin, François Massol, Aurélie Tasiemski

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

Céline Boidin-Wichlacz[1, 2]¥, Didier Jollivet[3]¥, Claire Papot[1], Lolita Roisin[1], François Massol[1, 2], Aurélie Tasiemski[1, 2]

[1]Univ. Lille, CNRS, UMR 8198 - Evo-Eco-Paleo, F-59000 Lille, France.

[2]Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille, F-59000 Lille, France.

[3]Sorbonne Université, CNRS UMR 7144 'Adaptation et Diversité en Milieux Marins' (AD2M), Team 'Dynamique de la Diversité Marine' (DyDiv), Station biologique de Roscoff, Place G. Teissier, 29680 Roscoff, France.

¥ These authors equally contributed to this work

Boidin-Wichlacz: 0000-0002-0242-868X

Jollivet: 0000-0001-8042-1857

Massol: 0000-0002-4098-955X

Tasiemski: 0000-0003-3559-5115

Correspondence should be addressed to celine.wichlacz@univ-lille.fr and jollivet@sb-roscoff.fr

Abstract

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

Declarations

Data Availability Statement

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

Code availability

Not applicable

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Introduction

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

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

reared individuals from various geographic localities. Because the concept of species heavily relies on reproductive isolation, the very high variation in reproductive modes, mating seasons or ecological preferences in the complex of species associated with *Capitella capitata* is likely to be a potent factor affecting speciation and explaining such a high cryptic diversity at a small spatial and/or regional scale. So far, barcode records of *C.* aff. *capitata* published in the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007) mostly include specimens collected in Canada, United States and India. Additional sequences also evidenced the existence of new phylogenetic lineages in the Mediterranean Sea and along the Brazilian coast (Livi et al. 2017; Silva et al. 2017). *Capitella* species have been also well documented along the European Atlantic coastlines in muddy habitats of harbors and estuaries. Despite being often used as a bio-indicator species of pollution in the North Sea, this species has yet to be genetically described and its life cycle has to be properly examined in relation with its genotype and morphology.

Morphological traits traditionally used to identify species of the Capitellidae family include the overall size, shape and the relative size of the prostomium and peristomium (as a complete or an incomplete ring), the number and distribution of capillary chaetae and hooded hooks along the thorax, the morphology, the number and the size of the genital spines and the shape of the pygidium (Blake et al. 2009). Using these morphological characters and sequences of the mitochondrial *Cox-1* gene, the present study provides new insights about the genetic status of *Capitella* spp. inhabiting the muddy sediments of the intertidal zone of the North European coastlines. To this extent, we first examined the spatial distribution of barcoded specimens along the French coasts of the Atlantic and English Channel and, then defined more precisely morphological and developmental diagnostic traits (i.e. morphologies, reproductive modes, larval developments and juvenile growth rates) for one of the three genetically-differentiated *Capitella* lineages present in Roscoff (Brittany, France), i.e. the most dominant one, the C-Channel1, (*Capitella* English Channel). Specimens present ~~at~~ in Roscoff not sorted completely during sampling, species isolation was achieved by rearing them in small aquaria over several generations. The conditions to rear and breed this specific genetic lineage over several generation were achieved in the laboratory.

Material and methods

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

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

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

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

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

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

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

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

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

Genetic identification of lineages

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

PCR amplification: A 569 bp fragment of the mitochondrial cytochrome oxidase subunit 1 (*Cox1*) gene was amplified using newly -designed primers CO1Forward (5′- GTACAGAACTTGCGCGTTCCT-3′) and CO1Reverse (5′- CCACCACCAGTAGGATCAAA -3′). Amplifications were carried out with a GoTaq® G2 DNA Polymerase (Promega). The reaction mixture (25 µL) for PCR amplification contained 10 µM of each primer, 10 µM of each desoxynucleotide triphosphate (dNTP), 1X Go Taq® Flexi buffer (Promega), and 5U of GoTaq G2 Flexi DNA polymerase (Promega). ~~The final volume was adjusted to 25 µL with DEPC water.~~ DNA amplification was performed ~~on a thermocycler (Eppendorf)~~ under the following conditions: (1) ~~an initial denaturation step at~~ 95 °C for 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 °C for 5 min ~~for final elongation~~. PCR products were ~~then visualized on a 1.5% agarose gel with ethidium bromide following electrophoresis at 100 volts for 30 min, and~~ subsequently purified with nucleofast 96 PCR cleanup kit. Purified products were then Sanger-sequenced on an ABI 3100 capillary sequencer using BigDye (PerkinElmer) terminator chemistry following the manufacturer's protocol (Applied Biosystems, Foster City, CA).

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

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

Other statistical tests

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

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

Results

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

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

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

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

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

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

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

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

The effect of gender on the width of the 5th setiger changed when analyses were performed on all sampled worms or within MOE groups. With all individuals, a linear model including 'sex' as an explanatory variable was significantly better at modelling the width of the 5th setiger than one without it ($F_{2.152} = 6.85$, p = 0.00142). However, when the same analysis was performed on the group 1 or the group 2 as inferred by the best MOE model, the addition of 'sex' did not significantly improve the goodness-of-fit in either case (for the group 1 with 11 females, 31 males and 50 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 = 0.0605). In other words, once morphospecies membership, as inferred by MOE models, is taken into account, the size of males and females do not differ.

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

As opposed to *C. capitata* from Greenland, which produces a high number of pelagic larvae, species living in the English Channel have a "lecithotrophic ~~indirect~~" development with the hatching of the larvae occurring inside the parental tube at the trochophore stage, which further develops into metatrochophores that freely swim in the water ~~column~~ tank. The genus *Capitella* contains at least 16 lecithotrophic species, three of them being hermaphrodites. *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.~~apitella~~ 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) l 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

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

Conclusion

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

Acknowledgements

This work was funded by the FRB Region Hauts-de-France (VERMER project, 2014-2016), the Region Hauts-de-France (AniMo project, 2013), the University of Lille (BQR emergence) and the Total Foundation (PIONEER project, 2016-2018). Authors are grateful to Nicolas Gayet (IFREMER, LEP Plouzané) for taking the SEM photos. Christophe Calarnou and Sylvie Flourez for their technical help in the Lab and on the field. Stéphane Hourdez and Virginie Cuvillier-Hot are deeply acknowledged for sampling and discussion.

References

Adkins M, Schulze A (2011) Development of *Capitella sp.* G from Galveston Bay, Texas. Mar Biol Res 7:202–207. doi: 10.1080/17451000.2010.489612

Albano MJ, da Cunha Lana P, Bremec C, Elías R, Martins CC, Venturini N, Muniz P, Rivero S, Vallarino EA, Obenat S (2013) Macrobenthos and multi-molecular markers as indicators of environmental contamination in a South American port (Mar del Plata, Southwest Atlantic). Mar Pollut Bull 73:102–114. doi: 10.1016/j.marpolbul.2013.05.032

Biernacki C, Celeux G, Govaert G (2006) Assessing a Mixture Model for Clustering with the Integrated Classification Likelihood. INRIA 3521:27.

Bierne N, Borsa P, Daguin C, Jollivet D, Viard F, Bonhomme F, David P (2003) Introgression patterns in the mosaic hybrid zone between Mytilus edulis and M. galloprovincialis. Mol Ecol 12:447–61.

Blake JA (2009) Redescription of *Capitella capitata* (Fabricius) from West Greenland and designation of a neotype (Polychaeta, Capitellidae). Zoosymposia 2:55–80. doi: 10.1016/j.ejop.2011.07.004

Blake JA, Grassle JP, Eckelbarger KJ (2009) *Capitella teleta*, a new species designation for *Capitella* sp . I, with a review of the literature for confirmed records. Zoosymposia 2:25–53.

Boidin-Wichlacz C, Vergote D, Slomianny C, Jouy N, Salzet M, Tasiemski A (2012) Morphological and functional characterization of leech circulating blood cells: role in immunity and neural repair. Cell Mol Life Sci 69:1717–1731.

Chia F-S, Gibson G, Qian P-Y (1996) Poecilogony as a reproductive strategy of marine invertebrates. Oceanol Acta 19:203–208. doi: 10.1016/j.bios.2013.08.031

Eckelbarger KJ, Grassle JP (1983) Ultrastructural Differences in the Eggs and Ovarian Follicle Cells of *Capitella* (Polychaeta) Sibling Species. Biol Bull 165:379–393.

Eckelbarger KJ, Grassle JP (1987) Interspecific Variation in genital spine, sperm, and larval morphology in six sibling species of *Capitella*. BIOL soc WASH pp. 62-76:62–76. doi: 10.1080/00364827.1980.10431474.

Egea E, David B, Choné T, Laurin B, Féral JP, Chenuil A (2016) Morphological and genetic analyses reveal a cryptic species complex in the echinoid *Echinocardium cordatum* and rule out a stabilizing selection explanation. Mol Phylogenet Evol 94:207–220. doi: 10.1016/j.ympev.2015.07.023

Ferrier David E.K. (2012) Evolutionary crossroads in developmental biology: annelids. Development 139:2643–2653. doi: 10.1242/dev.074724

Gamenick I, Abbiati M, Giere O (1998) Field distribution and sulphide tolerance of *Capitella capitata* (Annelida: Polychaeta) around shallow water hydrothermal vents off Milos (Aegean Sea). A new sibling species? Mar Biol 130:447–453. doi: 10.1007/s002270050265

Gouy M, Guindon S, Gascuel O (2010) Sea view version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27:221–224. doi: 10.1093/molbev/msp259

Grassle JP, Grassle FJ (1976) Sibling Species in the Marine Pollution Indicator *Capitella* (Polychaeta). Science (80-.). 192:567–569.

673 Grassle JP, Gelfman C, Mills S (1987) Karyotypes of *Capitella* sibling species, and a several species in the related
674 genera Capitellides and Capitomastus (Polychaeta). Bull Biol Soc Washingt 7:77–88.

675 Hartman O (1959) Capitellidae and Nereidae (Marine Annelids) from the Gulf Side of Florida, with a Review of
676 Freshwater Nereidae. Bull Mar Sci 9:153-168(16).

677 Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos Trans R Soc B Biol Sci
678 359:183–195. doi: 10.1098/rstb.2003.1388

679 Holte B, Oug E (1996) Soft-bottom macrofauna and response to organic enrichment in the subarctic waters of
680 Tromso, Northern Norway. J Sea Res 36:227–237.

681 Hourdez S, Boidin-Wichlacz C, Jollivet D, Massol F, Rayol MC, Bruno R, Zeppilli D, Thomas F, Lesven L, Billon
682 G, Duperron S, Tasiemski A (2021, in press) Investigation of *Capitella* spp. symbionts in the context of
683 varying anthropic pressures: First occurrence of a transient advantageous epibiosis with the giant bacteria
684 Thiomargarita sp. to survive seasonal increases of sulfides in sediments.

685 Jolly M, Viard F, Gentil F, Thiébaut E, Jollivet D (2006) Comparative phylogeography of two coastal polychaete
686 tubeworms in the Northeast Atlantic supports shared history and vicariant events. Mol Ecol 15:1841–55.

687 Kesäniemi JE, Mustonen M, Boström C, Hansen BW, Knott KE (2014) Temporal genetic structure in a
688 poecilogonous polychaete: The interplay of developmental mode and environmental stochasticity. BMC Evol
689 Biol 14:1–16. doi: 10.1186/1471-2148-14-12

690 Knowlton N, Weigt LA (1998) New dates and new rates for divergence across the Isthmus of Panama. Proc R Soc B
691 Biol Sci 265:2257–2263. doi: 10.1098/rspb.1998.0568

692 Kruse I, Reusch TBH, Schneider M V. (2003) Sibling species or poecilogony in the polychaete *Scoloplos armiger*?
693 Mar Biol 142:937–947. doi: 10.1007/s00227-002-1007-2

694 Kruse I, Strasser M, Thiermann F (2004) The role of ecological divergence in speciation between intertidal and
695 subtidal *Scoloplos armiger* (Polychaeta, Orbiniidae). J Sea Res 51:53–62. doi: 10.1016/j.seares.2003.05.004

696 Lardicci C, Ceccherelli G (1994) Dinamica di popolazione di una specie del complesso *Capitella capitata* in un
697 piccolo bacino salmastro dell'isola d'Elba. Biol Mar Mediterr 1:355–356.

698 Leigh JW, Bryant D (2015) Full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–
699 1116. doi: 10.1111/2041-210X.12410

700 Lewis C, Watson GJ (2012) Expanding the ecotoxicological toolbox: The inclusion of polychaete reproductive
701 endpoints. Mar Environ Res 75:10–22. doi: 10.1016/j.marenvres.2011.08.002

702 Linke-Gamenick I, Forbes VE, Mendez N (2000) Effects of chronic fluoranthene exposure on sibling species of
703 *Capitella* with different development modes. Mar Ecol Prog Ser 203:191–203. doi: 10.3354/meps203191

704 Livi S, Tomassetti P, Vani D, Marino G (2017) Genetic evidences of multiple phyletic lineages of *Capitella capitata*
705 (Fabricius 1780) complex in the Mediterranean Region. J Mediterr Ecol 15:5–11.

706 Luttikhuizen PC, Drent J, Baker AJ (2003) Disjunct distribution of highly diverged mitochondrial lineage clade and
707 population subdivision in a marine bivalve with pelagic larval dispersal. Mol Ecol 12:2215–2229. doi:
708 10.1046/j.1365-294X.2003.01872.x

709 Maggs CA, Kelly J, Castilho R, Foltz D, Henzler C, Perez KE, Jolly MT, Olsen J, Stam W, Väinölä R, Viard F,
710 Wares J (2008) Evaluating signatures of glacial refugia for north atlantic benthic marine taxa. Ecology 89:108–
711 122. doi: 10.1890/08-0257.1

712 Martin D, Grémare A (1997) Secondary production of *Capitella* sp. (Polychatea: Capitellide) inhabiting different
713 organically enriched environments. Sci Mar 61:99–109.

714 Martín DS (1991) Macroinfauna de una bahía mediterránea. Estudio de los niveles de organización de las
715 poblaciones de anélidos poliquetos. PhD Thesis. Faculty of Biology, University of Barcelona. 456.

716 Martin JP, Bastida R (2006) Life history and production of *Capitella capitata* (Capitellidae: Polychaeta) in Rio de la
717 Plata estuary (Argentina). Thalassas 22:25–38.

718 Méndez N (2002) Experimental evidence of polymorphysm of sexual development in *Capitella* sp. B (Polychaeta:

719 Capitellidae) from Barcelona, Spain. Sci Mar 66:103–110. doi: 10.3989/scimar.2002.66n2103

720 Méndez N (2006) Life cycle of *Capitella* sp. Y (Polychaeta: Capitellidae) from Estero del Yugo, Mazatlán, Mexico.
721 J Mar Biol Assoc UK 86:263. doi: 10.1017/S0025315406013117

722 Méndez N (2016) Laboratory development of *Capitella* sp. A (Annelida: Capitellidae) from a NW Mediterranean
723 fish farm reared under different organic enrichment conditions. Sci Mar 80:535. doi:
724 10.3989/scimar.04450.08B

725 Méndez N, Romero J, Flos J (1997) Population dynamics and production of the polychaete *Capitella capitata* in the
726 littoral zone of Barcelona (Spain, NW Mediterranean) * ,. 218:263–284.

727 Méndez N, Linke-Gamenick I, Forbes VE (2000) Variability in reproductive mode and larval development within
728 the *Capitella capitata* species complex. Invertebr Reprod Dev 38:131–142. doi:
729 10.1080/07924259.2000.9652448

730 Méndez N, Hilliard J, Schulze A (2019) Early development of two *Capitella species* (Annelida: Capitellidae) from
731 the Gulf of Mexico. J Mar Biol Assoc United Kingdom 99:1557–1568. doi: 10.1017/S0025315419000687

732 Meyer NP, Carrillo-Baltodano A, Moore RE, Seaver EC (2015) Nervous system development in lecithotrophic larval
733 and juvenile stages of the annelid *Capitella teleta*. Front Zool 12:1–27. doi: 10.1186/s12983-015-0108-y

734 Murphy K, Murphy TB (2019) Gaussian parsimonious clustering models with covariates and a noise component.
735 Adv Data Anal Classif 14:293–325. doi: 10.1007/s11634-019-00373-8

736 Muths D, Davoult D, Gentil F, Jollivet D (2006) Incomplete cryptic speciation between intertidal and subtidal
737 morphs of Acrocnida brachiata (Echinodermata: Ophiuroidea) in the Northeast Atlantic. Mol Ecol 15:3303–18.

738 Pardo EV, Teixeira LLS, Amaral ACZ (2010) Morphometric analysis of *Capitella capitata* (Polychaeta,
739 Capitellidae). Iheringia Série Zool 100:13–18. doi: 10.1590/S0073-47212010000100002

740 Pearson M, Pearson TH (1991) Variations in populations of *Capitella capitata* (Fabricius, 1780) (Polychaeta) from
741 the west coast of Scotla. Ophelia Suppl 5:363–370.

742 Petraitis PS (1985) Females Inhibit Males'Propensity To Develop Into Simultaneous Hermaphrodites in *Capitella*
743 *Species* I (Polychaeta). Biol Bull 168:395–402. doi: 10.2307/1541520

744 Petraitis PS (1991) The effects of sex ratio and density on the expression of gender in the polychaete *Capitella*
745 *capitata*. Ecol Evol 12:393–404.

746 Prevedelli D, Simonini R (2003) Life cycles in brackish habitats: Adaptive strategies of some polychaetes from the
747 Venice lagoon. Oceanol Acta 26:77–84. doi: 10.1016/S0399-1784(02)01232-X

748 Prevedelli D, N'Siala GM, Simonini R (2006) Gonochorism vs. hermaphroditism: Relationship between life history
749 and fitness in three species of *Ophryotrocha* (Polychaeta: Dorvilleidae) with different forms of sexuality. J
750 Anim Ecol 75:203–212. doi: 10.1111/j.1365-2656.2006.01040.x

751 Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary
752 species delimitation. Mol Ecol 21:1864–77. doi: 10.1111/j.1365-294X.2011.05239.x

753 Qian PY, Chia FS (1991) Fecundity and egg size are mediated by food quality in the polychaete worm *Capitella* sp. J
754 Exp Mar Bio Ecol 148:11–25. doi: 10.1016/0022-0981(91)90143-K

755 Qian PY, Chia FS (1992) Effects of diet type on the demographics of *Capitella* sp. (Annelida: Polychaeta):
756 lecithotrophic development vs. planktotrophic development. J Exp Mar Bio Ecol 157:159–179. doi:
757 10.1016/0022-0981(92)90160-C

758 Ratnasingham S, Hebert PDN (2007) Barcoding, Bold : The Barcode of Life Data System (www.barcodinglife.org).
759 NPR Mol Ecol Notes 7:355–364. doi: 10.1111/j.1471-8286.2006.01678.x

760 Rawson PD, Secor CL, Hilbish TJ (1996) The effects of natural hybridization on the regulation of DUI mtDNA in
761 Mytilus spp. Genetics 144:241–248.

762 Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sanchez-Gracia A
763 (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34:3299–3302. doi:
764 10.1093/molbev/msx248

765 Schikorski D, Cuvillier-Hot V, Leippe M, Boidin-Wichlacz C, Slomianny C, Macagno E, Salzet M, Tasiemski A
766 (2008) Microbial Challenge Promotes the Regenerative Process of the Injured Central Nervous System of the
767 Medicinal Leech by Inducing the Synthesis of Antimicrobial Peptides in Neurons and Microglia. J Immunol
768 181:1083–1095. doi: 10.4049/jimmunol.181.2.1083

769 Schrevel J, Philippe M (1993) Parasitic Protozoa, Academic P. California

770 Schweigkofler M, Bartolomaeus T, Von Salvini-Plawen L (1998) Ultrastructure and formation of hooded hooks in
771 Capitella capitata (Annelida, Capitellida). Zoomorphology 118:117–128. doi: 10.1007/s004350050062

772 Seaver EC (2016) Annelid models I: *Capitella teleta*. Curr Opin Genet Dev 39:35–41. doi:
773 10.1016/j.gde.2016.05.025

774 Silva CF, Shimabukuro M, Alfaro-lucas JM, Fujiwara Y, Sumida PYG, Amaral ACZ (2016) A new *Capitella*
775 polychaete worm (Annelida : Capitellidae) living inside whale bones in the abyssal South Atlantic. Deep Res
776 Part I 108:23–31. doi: 10.1016/j.dsr.2015.12.004

777 Silva CF, Seixas VC, Barroso R, Di Domenico M, Amaral ACZ, Paiva PC (2017) Demystifying the *Capitella*
778 *capitata* complex (Annelida, Capitellidae) diversity by morphological and molecular data along the Brazilian
779 coast.

780 Sukwoo C, Steimle FW, Reid RN, Fromm SA, Zdanowicz VS, Pikanowski RA (1992) Association of benthic
781 macrofauna with habitat types and quality in the New York Bight. Mar Ecol Prog Ser 89:237–251. doi:
782 10.3354/meps089237

783 Tsutsumi H (1987) Population dynamics of *Capitella capitata* (Polychaeta; Capitellidae) in an organically polluted
784 cove. Mar Ecol Prog Ser 36:139–149. doi: 10.3354/meps036139

785 Tsutsumi H, Fukunaga S, Fujita N, Sumida M (1990) Relationship between growth of *Capitella* sp. and organic
786 enrichment of the sediment. Mar Ecol Prog Ser 63:157–162. doi: 10.3354/meps063157

787 Warren LM (1976) A population study of the polychaete *Capitella capitata* of Plymouth. Mar Biol 216:209–216.

788 Willcox MS, Nickell TD (1998) Field evidence of poecilogony in *Capitella capitata*. Ophelia 49:141–145. doi:
789 10.1080/00785326.1998.10409378

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 αLRT option or 100 bootstraps using 5 randomly-choosen
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.

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

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

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

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

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

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

Tables

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

	C-Channel1	C-Atlantic	C-Channel2	*C.capitata* Canada/ India	*C.capitata* Mediterra -nean	*C.capitata* Canada/ Japan	*C.teleta* France	*C.teleta* Japan/ USA
C-Channel1	0.001							
C-Atlantic	0.021	0.002						
C-Channel2	0.142	0.141	0.000					
C.capitata Canada/India	0.257	0.262	0.227	0.011				
C.capitata Mediterranean	0.286	0.291	0.311	0.349	0.124			
C.capitata Canada/Japan	0.245	0.238	0.229	0.299	0.338	0.067		
C. teleta France	0.308	0,326	0.259	0.403	0.395	0.292	0.011	
C.teleta Japan/USA	0.238	0.236	0.259	0.288	0.337	0.225	**0.265**	0.018

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

Clade	**N**	**S**	**Hd**	**π**	**θw**	**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.

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