Highlights

1. Cephalopod paralarval richness was 2x higher in Moroccan than Iberian waters
2. Three planktonic dispersal patterns were identified in the Iberian–Canary current upwelling system
3. The interaction between vertical behaviour and oceanography led to these 3 dispersal patterns
4. Each planktonic pattern had different haplotype and nucleotide genetic signatures
5. *Octopus vulgaris* paralarvae shift within upwelling filaments from coast to ocean


Coastal-Oceanic
Upwelling filaments
Coastal
Oceanic
Continental shelf
200 m
500 m
Cephalopod paralarvae planktonic dispersal patterns at the Canary Current Eastern Boundary upwelling Ecosystem
1 Octopus vulgaris; 2 Illex coindetii;
3 Todaropsis eblanae; 4 Loligo vulgaris;
5 Alloteuthis subulata; 6 Alloteuthis media;
7 Rondeletiola minor; 8 Sepiola tridens;
9 Sepiola atlantica; 10 Sepiola ligulata;
11 Heteroteuthis dispar; 12 Ommastrephidae;
13 Todarodes sagitattus; 14 Gonatus steenstrupi;
15 Ancistrocheirus alessandrinii; 16 Pyroteuthis margaritifera; 17 Ancistroteuthis lichtensteinii;
18 Brachioteuthis riisei; 19 Abraliopsis morisii;
20 Liocranchia reinhardtii; 21 Mastigopsis hjorti.

Oceanographic processes shape genetic signatures of planktonic cephalopod paralarvae in two upwelling regions

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Abstract

The planktonic paralarval stage of cephalopods (octopus, squids and cuttlefishes) is an important dispersal phase, particularly of benthic species, that lasts from days to months. Cephalopod paralarvae modify their vertical position in the water in upwelling ecosystems and such behaviour influences their spatial distribution and genetic structure, but to what extent? In this work specific water masses were sampled with Lagrangian buoys in two contrasting upwelling systems (Iberian Peninsula and Morocco) of the Iberian–Canary current eastern boundary upwelling (ICC) in order to: i) identify the cephalopod assemblage in the different upwelling systems ii) define their planktonic dispersal patterns and iii) analyse the effect of different dispersal patterns on genetic structure and connectivity. Cephalopod paralarvae were identified using the cytochrome *c* oxidase subunit I gene (COI), revealing 21 different species and F_{st} values showed no population structure between both upwelling systems. Cephalopod species richness was two times higher in the Moroccan upwelling than in the Iberian Peninsula, with an undescribed Ancistrocheiridae species identified in Moroccan waters. Three common planktonic dispersal patterns were identified in the ICC: coastal, coastal-oceanic and oceanic. Coastal and oceanic dispersal patterns favoured spatio-temporal paralarval retention or "schooling" of different cohorts over the continental shelf and continental slope in 9 and 11 species, respectively. Such spatio-temporal retention was reflected in the complex haplotype networks and high nucleotide / haplotype diversity recorded for these two groups. The only cephalopod species displaying a coastal-

oceanic dispersal pattern was *Octopus vulgaris,* where low nucleotide and haplotype diversity was observed. The observed decline in genetic structure resulted from the dispersal of similar cohorts within upwelling currents and upwelling filaments to the oceanic realm. Seascape analysis revealed that cephalopod paralarvae from two coastal upwelling ecosystems of the ICC display three planktonic dispersal patterns with contrasting distributions and signatures at the genetic level.

Keywords: upwelling filaments, cephalopod paralarvae, seascape genetics, eastern boundary upwelling system, planktonic dispersal patterns, *Octopus vulgaris,* Northeastern Atlantic.

Author Contributions

Conceived and designed the experiment: AR, AFG, EDB; collected samples: AR, AFG; processed the samples: AR; analysed the data: AR, MA, JMS; contributed reagents / materials / analysis tools: AFG, JMS and AG; AR wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

All authors declare no conflict of interest and have approved the final version of the manuscript.

1. Introduction

Understanding larval exchange between populations of marine organisms is a fundamental aspect of population ecology and essential for the management of fishery stocks. Among the different types of larvae present in the water, planktotrophic larvae spend more time in the plankton than their lecithotrophic counterparts (Vance, 1973). It is expected that longer pelagic duration would result in increased dispersal, increased gene flow and, consequently, decreased levels of population genetic structure. Although there are many studies that suggest this hypothesis (Bohonak, 1999; Siegel et al., 2003), Weersing and Toonen (2009) concluded from a meta-analysis of 87 studies of pelagic larval duration (PLD) and population genetic estimates - measured as global F_{st} - that mean PLD is a poor predictor of connectivity. Most models assume that planktotrophic larvae behave as passive particles (e.g. Teske et al. 2015), however when coupling vertical migration behaviour with mesoscale or sub-mesoscale circulations, long distance dispersal can be minimized and larvae are retained in close proximity to the area that they hatched (Shanks and Eckert, 2005; Queiroga et al. 2007; Morgan and Fisher, 2010).

In cephalopods, there are two major life-history dispersal patterns based on egg morphology. The “holobenthic” dispersal pattern, utilized by cuttlefishes and most sepiolids (Boletzky, 2003), is the production of relatively few, large eggs (>10-12% of adult mantle length) resulting in young cephalopods that adopt the benthic mode of life after hatching. Conversely, the “merobenthic” dispersal pattern of octopods, loliginids, ommastrephids or oceanic squids (Boletzky 2003; Villanueva and Norman 2008) is the production of numerous small eggs that hatch into free-swimming, planktonic hatchlings called paralarvae (Young and Harman, 1988). These cephalopod paralarvae actively migrate up and down through the water column (Passarella and Hopkins, 1991; Shea and Vecchione, 2010), affecting their horizontal distribution along the continental shelf (Roura et al. 2016). The horizontal distances travelled post-hatching range from less than one kilometre in small bottom-dwelling species with no distinct planktonic phase (e.g. sepiolid squids of the subfamily Sepiolinae, Boyle and Boletzky, 1996) to probably hundreds of kilometres in pelagic species, especially teuthid squids (Roberts, 2005) and octopods (Villanueva and Norman, 2008; Roura et al., 2017). The different dispersal patterns of merobenthic paralarvae seem to result from a complex interaction between diel vertical behaviour and mesoscale processes, and horizontal dispersal is inversely related with the size of the planktonic hatchlings for squids and octopods (Villanueva et al. 2016). However, the effect of such dispersal at the genetic level

remains to be studied in cephalopods, especially in highly dynamic ecosystems like the upwelling regions.

The Iberian–Canary current eastern boundary upwelling system (ICC) constitutes one of the four main eastern boundary upwelling systems of the world's oceans (Barton, 1998). The ICC covers the latitudinal range between 12–43º N and it is divided into five sub-regions (Arístegui et al., 2009). This work is centred in the Galician, Portuguese and the Moroccan sub-regions. The western Iberian sub-region (Galician and Portuguese coasts) is the northernmost part of the ICC. During spring and summer (from March–April to September–October) north-easterly winds predominate in the Iberian basin and mesoscale upwelling filaments develop intermittently in association with irregularities in the coastline like capes (Barton et al., 2001; Joint et al., 2001; Peliz et al., 2005; Cordeiro et al. 2015). The Moroccan sub-region, on the other hand, experiences year-round upwelling varying seasonally, occurrence of extended upwelling filaments, absence of freshwater inputs and massive dust inputs from the adjacent Sahara Desert (Navarro-Pérez and Barton, 1998; Arístegui et al., 2009).

Throughout the ICC, coastal upwelling is enhanced in the vicinity of topographic features such as capes, producing filaments of upwelled water that export coastal biomass into the open ocean (Van Camp et al., 1991). In this sense, the cool filaments of Cape Silleiro (NW Iberian Peninsula, 42-43º N) and Cape Ghir (30-31º N; W Morocco) export 4 and 31 x 10^8 kg of organic carbon per year to the adjacent shelf, which corresponds to 20 and 60 % of the phytoplankton primary production (Álvarez-Salgado et al., 2007), respectively. These filaments break the strongly stratified oceanic waters - thermocline and nutricline located at 80-100 m depth - creating clear community gradients that contrast with the open ocean communities (Hernández-León et al. 2002). Studies of larval distribution in the ICC suggest that vertical behaviour is one of the contributing mechanisms for retention over the shelf (Queiroga and Blanton, 2004; Peliz et al., 2007; Queiroga et al., 2007), as well as dispersion (Rodríguez et al. 2001, Bécognée et al., 2006).

The study of cephalopod paralarvae in the ICC has been mostly carried out in continental shelf waters off the NW Iberian Peninsula (Rocha et al., 1999; González et al. 2005; Otero et al. 2008; Olmos-Pérez et al. 2017a, b; Roura et al., 2012, 2016), the Portuguese coast (Moreno et al., 2009) and Mauritanian waters (Faure et al. 2000). Collectively, these studies showed that cephalopod paralarvae are scarce in the zooplankton and positively linked with the upwelling index. Marked changes in their horizontal and vertical distributions suggest that different dispersal patterns may be co-occurring within the

cephalopod assemblage of the ICC (Roura et al., 2016). However, difficulties in the taxonomic identification of most cephalopod paralarvae, especially within the loliginid, sepiolid and ommastrephid groups (Fernández-Álvarez et al. 2016; Olmos-Pérez et al. 2017a, b), hamper the accurate interpretation of the different adaptations of the paralarvae to the oceanography of the ICC. Accordingly, an integrative approach combining oceanographic, behavioural, morphological and genetic data, known as seascape genetics (Selkoe et al. 2008), is required to help elucidate the spatial ecology of cephalopod paralarvae in the ICC upwelling ecosystems.

Here, we explore the interaction between cephalopod paralarvae dispersal patterns and their genetic diversity / population structure in two contrasting upwelling systems: the seasonal upwelling of Cape Silleiro (NW Iberian Peninsula) and the permanent upwelling of Cape Ghir (W Morocco). We identified planktonic cephalopod species via DNA barcoding and morphological taxonomy, then categorized them into different planktonic dispersal patterns. Genetic diversity and population structure were then evaluated for each of these patterns under the oceanographic scenario of the ICC. This integrative approach combining fine-scale oceanography, morphology, behaviour and genetic data shed light on the effects of different planktonic dispersal patterns as drivers of genetic diversity and population structure of cephalopod paralarvae from one of the most important upwelling ecosystems of the world.

2. Material and methods

Data acquisition for this study was carried out under the framework of the multidisciplinary project "**Ca**naries–**Ib**erian Marine Ecosystem **Ex**changes (CAIBEX)" in 2009 on board the RV *Sarmiento de Gamboa* in the seasonal upwelling system off Cape Silleiro (41-43ºN, CAIBEX-I) from July 7 to 24, and the permanent upwelling system off Cape Ghir (30-32ºN, CAIBEX-III) from August 16 to September 5 (Fig. 1). High-resolution mapping of the study area determined the oceanographic conditions (temperature, salinity and Chl-fluorescence) *in situ* using a towed vehicle (SeaSoar) that undulates between the surface and 400 m depth. The information collected with the SeaSoar, together with real time satellite images of sea surface temperature (SST) and chlorophyll provided by the Plymouth Marine Laboratory (NEODAAS), helped to determine the location of upwelled water masses. Once detected, we aimed to follow the course of the upwelled water mass carrying out three Lagrangian experiments with an instrumented drifting buoy (IDB), with a length of 100m, which was deployed in the core of the upwelled water. The IDB was equipped with Global

Positioning System and Iridium communication, an Acoustic Doppler Current Profiler (ADCP) at 2 m to determine current direction and velocities down to 100 m depth, and temperature sensors at 10, 20, 40, 60, 65, 80 and 100 m depth.

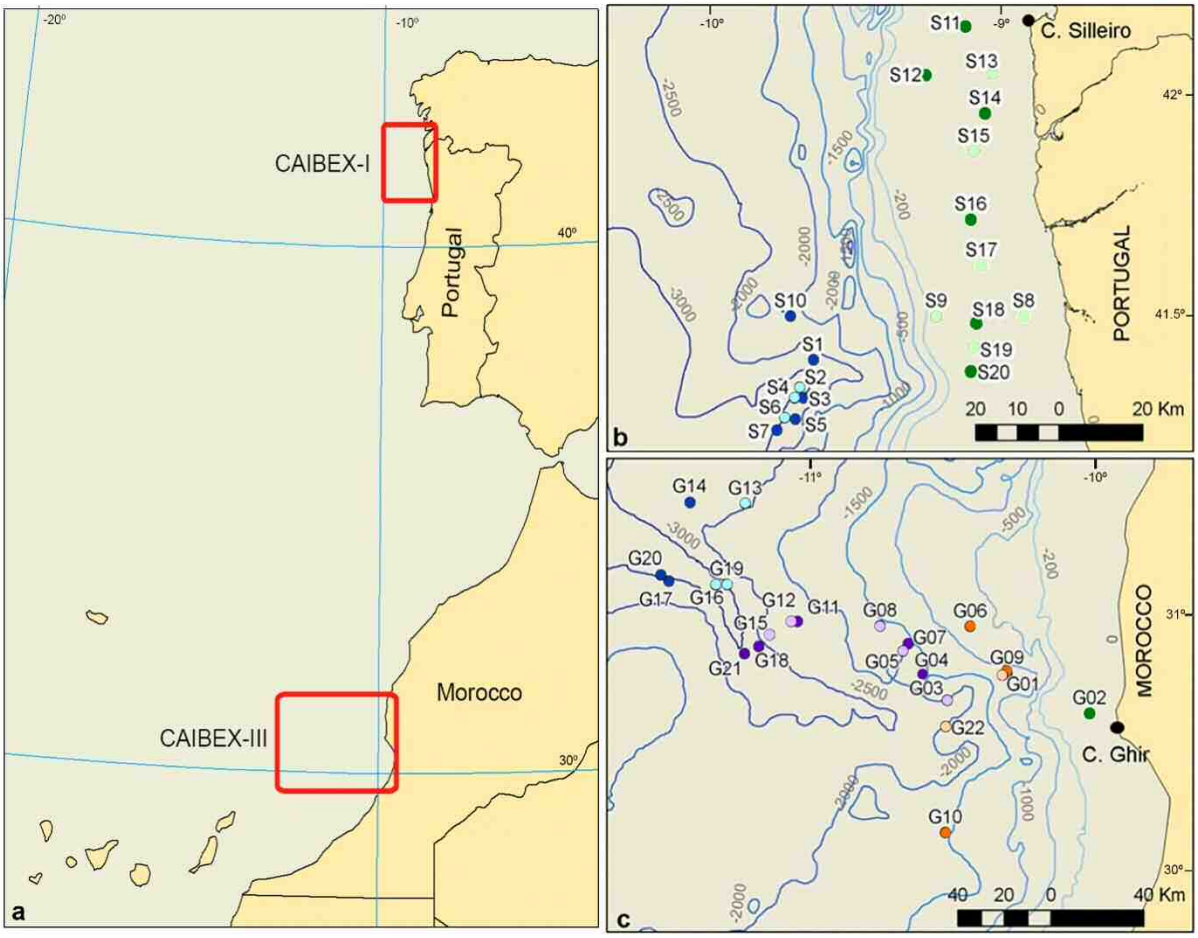

Fig. 1. a) Schematic map of the Iberian-Canary Upwelling system showing the areas sampled (red boxes). b) Zooplankton samples collected during CAIBEX-I off the NW coast of the Iberian Peninsula, around Cape Silleiro (42ºN). Samples S1 to S7 correspond to the first Lagrangian experiment (L1) carried out in the open ocean (blue dots) and S13 to S20 to the second Lagrangian experiment (L2) carried out over the continental shelf (green dots). c) Zooplankton samples collected during CAIBEX-III off the NW Africa coast, around Cape Ghir (30ºN, G1-G22). Samples were collected over the continental shelf at night (green dots, <200 m depth), in an area affected by the upwelling in the open ocean (orange dots, >200 m depth), following the upwelling filament during the third Lagrangian experiment (violet dots, L3) and in the open ocean (blue dots). Light / dark colours represent day / night samplings. See Table A.1 for details.

2.1 Physical and biological sampling

Meteorological conditions resulted in only weak development of upwelling during CAIBEX-I (Cordeiro et al., 2018). We employed a Lagrangian sampling approach, whereby

contrasting water masses were tracked and sampled: 1) oceanic waters over the continental slope around 41º 25'N in the relaxation period after a brief upwelling event (L1: July 10-13 inclusive, samples S1-S7 in blue Fig. 1b); and 2) an incipient coastal upwelling with alongshore transport over the shelf was sampled from 42ºN to 41º23N (L2: July 17-20 inclusive, samples S13-S20, Fig. 1b). Between these two Lagrangian experiments samples were collected following a coastal-ocean gradient off the Portuguese coast (S8-S10), as well as two samples in the continental shelf of Galicia (S11 and S12). Details of the sampling can be found in Table A.1.

In contrast, constant NE winds during CAIBEX-III allowed the development of a strong upwelling filament (Troupin et al., 2012, Sangrá et al., 2015). Once the core of the filament was identified the IDB was deployed in the core of the filament, allowing sampling of the upwelled water as it was advected from the coast into the ocean during the third Lagrangian experiment (L3: August 23-31 inclusive, samples in violet, Fig. 1c). Samples were also collected over the shelf (in green, Fig. 1c), in an area affected by the upwelling over the continental slope (in orange, Fig. 1c) and in the open ocean (in blue, Fig. 1c), to investigate the zooplankton communities surrounding the upwelling filament. Details of the sampling can be found in Table A.2.

A CTD was deployed to 500 m depth in the open ocean and to 10 m above the sea-bottom over the continental shelf (<200 m depth) before each zooplankton sampling. Mesozooplankton samples were collected close to the IDB both at midnight and midday, to identify vertical migrations, with two 750 mm diameter bongo nets equipped with 375 μm mesh and a mechanical flow-meter. At a ship speed of 2.5 knots three double-oblique tows were carried out at each station over the continental slope (>200m depth): at the deep scattering layer (DSL: 500 m), at 100 m and at the surface (0-5 meters). Over the continental shelf, samples were collected at 100 m (or 10 m above bottom when shallower) and at the surface (0-5 m). The bongo net was first lowered to the desired depth, towed for 30 minutes and subsequently hauled up at 0.5 m s^{-1}. Plankton samples were fixed with 96% ethanol and stored at -20ºC to allow DNA preservation.

2.2 Cephalopod identification and barcoding

Cephalopod paralarvae were sorted from the samples and were classified to the lowest taxonomic level according to Sweeney et al. (1992) and Vecchione et al. (2001). The dorsal mantle length (DML), width (W) and total length (TL) of each individual was recorded to the

nearest 0.1 mm using the software NIS-Elements 3.0 connected to a digital camera (Nikon DXM1200F) under a binocular microscope (Nikon SMZ800). Furthermore, length of the right tentacle (TeL) was measured in all decapod paralarvae and the number of suckers per arm was counted in every *Octopus vulgaris* paralarva (Sweeney et al. 1992).

The soft body of most cephalopod paralarvae is typically damaged during capture, especially in oceanic and neritic squid families, hampering the identification process. Three animals for which the mantle was not present were not measured. Cephalopod paralarvae of certain groups like oegopsids, loliginids or sepiolids lack morphological characters present in juveniles and adults - such as photophores, hooks or developed tentacles - thus preventing their identification to species level (Vecchione et al., 2001). Therefore, genetic identifications were done with the barcoding gene cytochrome *c* oxidase I (COI) (Hebert et al., 2003), to allow comparison with the vast database of cephalopod COI sequences available on GenBank. A project called "Cephalopod paralarvae of the Eastern Atlantic" (CEPAR) was created in collaboration with the Barcode of Life Data System (BOLD). A 654-bp region of the COI gene (Ratnasingham and Hebert, 2007) was sequenced from a small piece of mantle of each paralarvae. DNA extraction and sequencing were carried out at the University Guelph, Canada. Prior to obtaining the COI sequences, a visual database from each specimen was created using dorsal, ventral and lateral photographs. Sequence data are available on the Barcode of Life Data System (project folder Cephalopod paralarvae of the Eastern Atlantic "CEPAR").

2.3 Molecular analyses

Sequence data were compared against those held in publicly available databases (BOLD and GenBank) using the BLASTn algorithm. Species level identifications were based on homologies above 97% and the taxonomic position of those below 97% was assessed according to their location in a phylogenetic tree (Hebert et al., 2003). Cephalopod sequences were collapsed into unique haplotypes using DnaSP (Librado and Rozas 2009). jMODELTESTv.3.8 (Posada, 2008) was used to determine the most appropriate model of sequence evolution for phylogenetic analyses. The AIC (Akaike information criterion, Akaike 1974) favored the GTR + G + I model. A maximum likelihood (ML) method of phylogenetic inference was used to construct a phylogenetic tree including all the different haplotypes present using *Phy*ML v3.1 (Guindon et al. 2010). The strength of support for internal nodes of the ML phylogeny was measured using 1000 bootstrap replicates. Bayesian

marginal posterior probabilities were calculated using MrBayes v3.2 (Ronquist and Huelsenbeck, 2003). Model parameter values were treated as unknown and were estimated. Random starting trees were used and the analysis was run for 15 million generations, sampling the Markov chain every 1000 generations. The program Tracer v1.3 (Rambaut and Drummond, 2003) was used to ensure Markov chains had reached stationarity and to determine the correct 'burn-in' for the analysis.

2.4 Planktonic dispersal patterns: vertical and horizontal distribution

Once the paralarvae were identified, cephalopod assemblages were examined with multivariate techniques using the software packages PRIMER6 & PERMANOVA+ (Anderson MJ et al., 2008) to identify patterns of distribution. Abundance numbers were transformed using the function log (x + 1) to reduce the contribution of highly abundant species (Clarke and Green, 1988). The Bray-Curtis similarity matrix, which reflects differences in relative abundance as well as in species composition, was used to calculate the resemblance matrix among samples. A non-parametric permutational ANOVA (PERMANOVA) analysis running 999 permutations was used to test for vertical migrations using a two-factor nested design (factor day/night with two levels and strata, three levels: surface, 0-100, 0-500 m), and also to test the different horizontal distribution patterns observed (factor dispersal pattern, three levels: coast, coast-ocean, ocean). Non-parametric analyses using Mann-Whitney U tests were also conducted to test whether the cephalopod paralarvae abundances and DML varied significantly between the different habitats sampled. These analyses were used in order to explore the relationship between the different planktonic dispersal patterns displayed by the paralarvae in the genetic diversity metrics / neutrality statistics / haplotype networks.

2.5 Genetic diversity and population structure

Ambiguous nucleotides were visually edited using the chromatograms available in BOLD and using the aligned sequences as a reference. Only sequences >600bp, with no stop codons, were used in analyses (n = 318). Of these, only the species for which there were more than five sequences (Goodall-Copestake et al., 2012) were retained for further genetic analysis. Individual sequences smaller than 620 bp were deleted and the last 20 bp of the 3' end of all sequences were removed. As a result, a region of 624 bp was retained and used to calculate genetic diversity and neutrality tests (11 species, n = 285 specimens). DnaSP

(Librado and Rozas 2009) was used to calculate genetic diversity metrics for each species: haplotype number (H), number of polymorphic sites (S), haplotype diversity (h) and nucleotide diversity per site (π). The validity of the diversity metrics obtained was assessed using the 95th percentile boundary of the function $\pi = 0.0081h^2$ (Goodall-Copestake et al., 2012).

Historical fluctuations in population size were investigated using Fu's Fs and Tajima's D to test if the sequences were evolving neutrally or under selection using ARLEQUIN v.3.5.1.2 (Excoffier et al. 2005). A randomization procedure using 1,000 samples was used to test the significance of Fu's Fs and Tajima's D. Haplotype networks were generated from COI sequence data using Network v4.6 (Bandelt et al. 1999) to visualize the relationships between the existing haplotypes of a given species under the following criteria: with at least three haplotypes and a minimum of five individuals.

Three species (*Octopus vulgaris*, *Alloteuthis subulata* and *A. media*) were present in both surveys (NW Iberian Peninsula and W Morocco, Tables 1 and 2). Pairwise F_{ST} (Excoffier et al. 2005) was calculated between these populations using ARLEQUIN in order to know the extent of genetic differentiation between populations of both upwelling ecosystems.

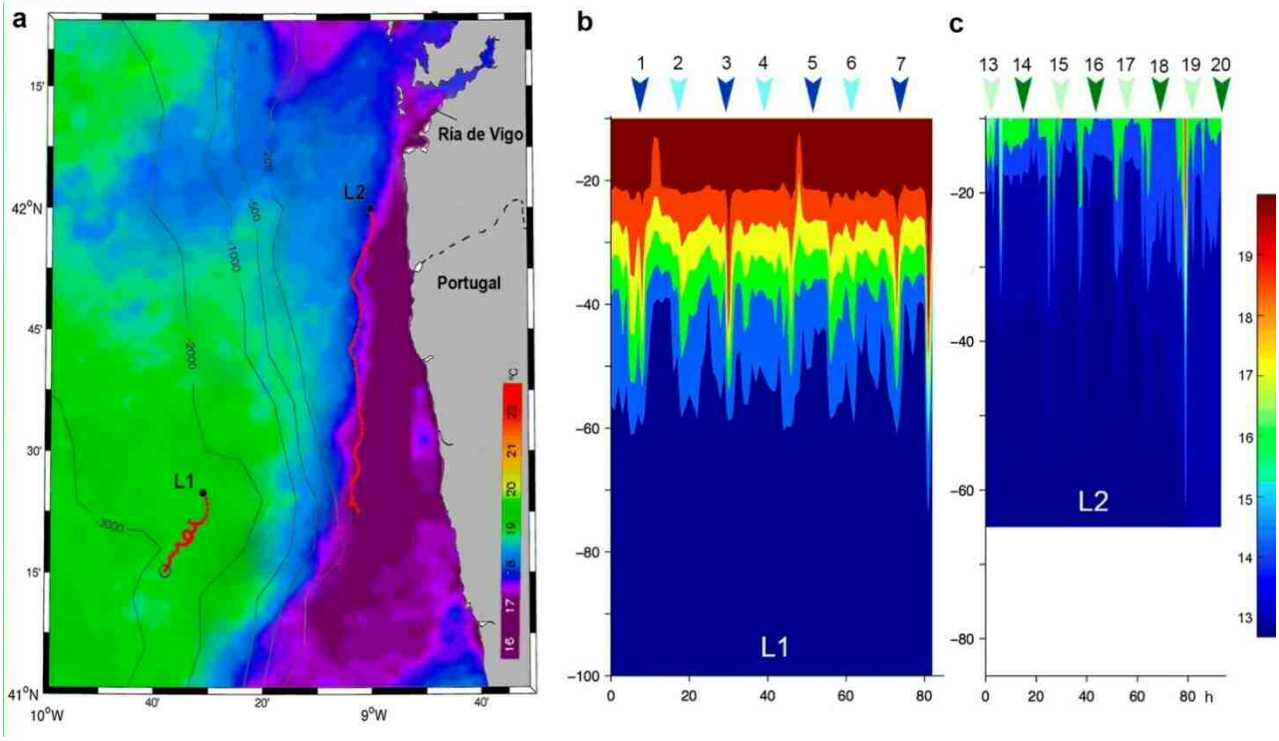

Fig. 2. a) Trajectory of the buoys during the first (L1, July 10-13) and second Lagrangian experiment (L2, July 17-20) in Iberian waters overlaid on sea surface temperature (SST) at

the end of L2 (July 20th). Temperature profiles recorded by the physical buoy during b) the first (L1) and c) second Lagrangian experiment (L2) of CAIBEX-I. Zooplankton samplings are shown with dark colour indicating night sampling.

3. Results

3.1 Oceanographic context

CAIBEX-I: The first Lagrangian experiment (L1, Fig. 2) was conducted over the continental slope of Portugal with depths ranging between 2,667 and 3,105 m, under northerly winds during the first half of the experiment and southerly winds onward. Wind velocities were low and the IDB was displaced slowly south-westward (Fig. 2a). Vertical temperature profiles recorded by the thermistor chain of the IDB showed that the water column was strongly stratified with a small increase in temperature when the winds shifted (Fig. 2b).

Experiment L2 was carried over the Iberian shelf of Galicia and Portugal between 90 and 150 m depth). Strong northerly winds during the first three days transported the IDB equatorward and slightly offshore, but weakening and then reversal of the wind to southerly in the last two days slowed and almost stopped the drift of the IDB (Fig. 2a). The temperature recorded by the IDB (Fig. 2c) shows the presence of upwelled water over the shelf.

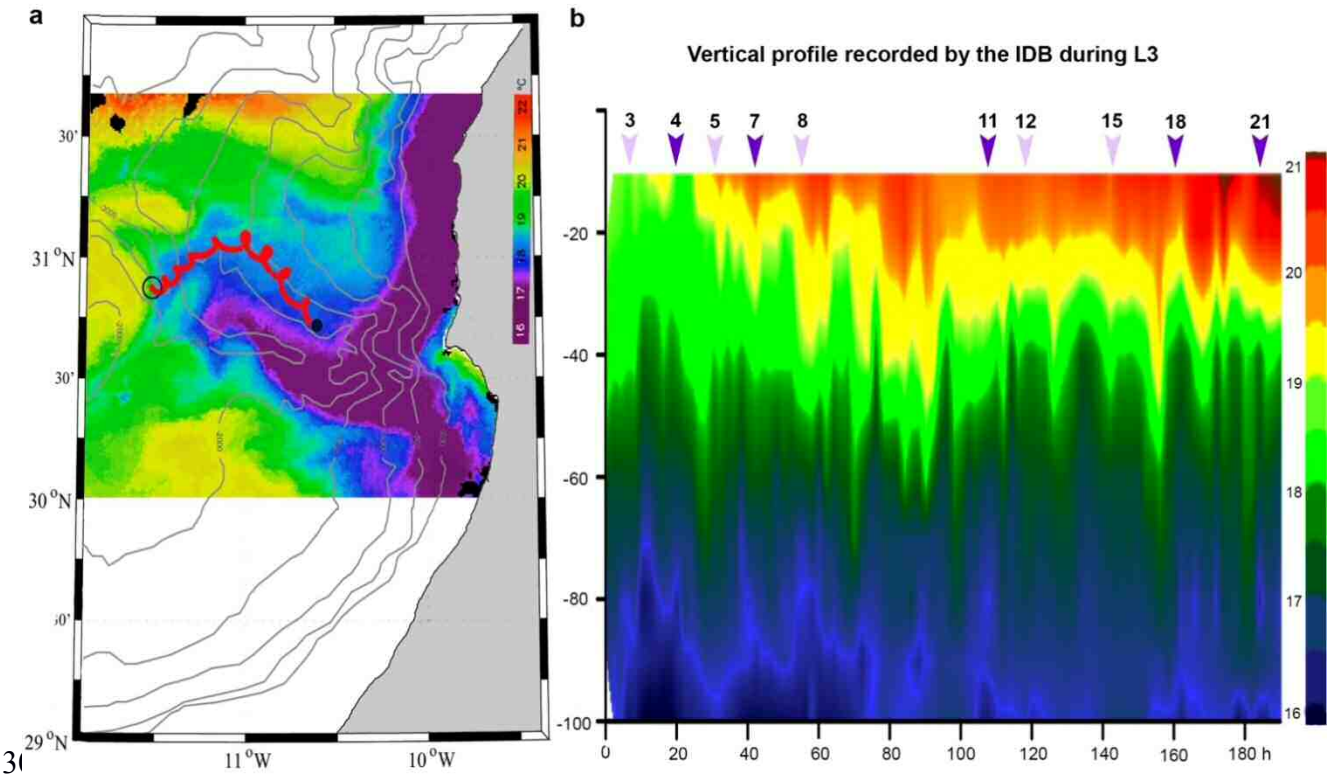

Fig. 3. a) Trajectory of the buoy during the third Lagrangian experiment in Moroccan waters (L3, August 23-31) overlaid on sea surface temperature (SST) at the beginning of the experiment (August 24th). b) Temperature profile recorded by the physical buoy during CAIBEX-III. Zooplankton samplings are shown, with dark colour indicating night sampling.

CAIBEX-III: Experiment L3 was carried out in waters 1540 – 3020 m deep. The IDB drifted north-westward offshore by 172 km within the core of an upwelling filament - 60 m deep and 25 km wide - and then shifted to the southwest (Fig. 3a). As recorded by the IDB thermistor chain, the drifters began in cold upwelled waters that were progressively heated by the warm and stratified surrounding oceanic waters (Fig. 3b). The temperature showed regular oscillation of the isotherms, at the local inertial period.

3.2 Cephalopod paralarvae identification

One adult (9.77 mm DML) and 134 cephalopod paralarvae and juveniles (ranging from 1.49 to 8.17 mm DML) were found in 48 samples from CAIBEX I (Table A.3). Four families were present. The most abundant were the octopods, with 99 paralarvae, representing 73.3% of the paralarvae collected, followed by the loliginids (16 paralarvae, 11.9%), ommastrephids (15 rhynchoteuthion, 11.1%) and sepiolids (four juveniles and one adult, 3.7%). COI sequences were obtained for 124 paralarvae from a total of 135 individuals (91.9%, Fig. 4) with similarities higher than 99% against sequences present on GenBank (Table 1). All the octopods sequenced were *Octopus vulgaris* (88 paralarvae). Eleven octopod paralarvae did not amplify correctly, but were also identified as *O. vulgaris* based on similarities of morphology and chromatophore pattern. *Octopus vulgaris* was the only species that was found in both coastal and oceanic samples. All loliginid paralarvae were identified and were assigned to the following species: 10 *Alloteuthis subulata*, four *A. media* and two *Loligo vulgaris*. Sequence data were obtained for all ommastrephid paralarvae: 12 were identified as *Todaropsis eblanae,* two were *Todarodes sagitattus* and one was *Illex coindeti*. Finally, all five sepiolids were identified as *Sepiola tridens*.

Planktonic dispersal pattern	Species	Shelf (L2)				Ocean (L1)					
		day		night		day			night		
		100	5	100	5	500	100	5	500	100	5
Coastal	*Alloteuthis media*	2		1	1						
	Alloteuthis subulata			7	3						
	Loligo vulgaris			2							
	Sepiola tridens			5							
	Illex coindetii				1						
	Todaropsis eblanae	8		3	1						
Coast-ocean	*Octopus vulgaris*	7	4	12	28	5	4		14	10	15
Oceanic	*Todarodes sagitattus*						1			1	

Table 1. Cephalopod paralarvae present in the different Lagrangian studies (L1 and L2) carried out off Cape Silleiro (41ºN, NW Iberian Peninsula) during CAIBEX-I. Values 5, 100 and 500 indicate the tow depth.

A total of 246 cephalopods were obtained from 65 samples in CAIBEX-III: 245 early stages (paralarvae or juveniles) ranging from 0.93 to 13.82 mm dorsal mantle length (DML, Table A.3) and one adult of *Mastigopsis hjorti* 9.77 cm DML. Overall, 53.7% of the cephalopods collected were obtained from the two coastal samples (< 100 m depth), while the remaining 46.3% were obtained from the oceanic samples (800–3050 m depth), of which 64.2% belonged to mesopelagic families. COI sequences were obtained for 227 cephalopods out of 246 (92.3%, Fig. 4). Within the neritic families, the most abundant group were the loliginids (n = 115, 46.8%), with 107 individuals being *Alloteuthis media* and eight *A. subulata* (homology 100%) found in the only two samples collected near the coast (Table 2). The next most abundant group were the octopods (n = 35, 14.2%), exclusively represented by *Octopus vulgaris* (homology 100%). This species was found in all the locations sampled: coastal (n = 9), upwelling (n = 4), filament (n = 10) and ocean (n = 12). Sepiolids (n = 10, 4%) were found in coastal (n = 7, represented by five *Rondeletiola minor,* one *Sepiola atlantica* and one *S. ligulata*), upwelling (n = 1, unidentified) and filament (n = 2, *Heteroteuthis dispar*). Ommastrephid rhynchoteuthions (n = 4, 1.6%) were only found in the filament samples and a single sequence was obtained showing low homology against the genetic database (*Todarodes sagitattus* 91.6%).

Dispersal pattern	Species	Shelf		Upwelling						Filament						Ocean					
		Night		Day			Night			Day			Night			Day			Night		
		100	5	500	100	5	500	100	5	500	100	5	500	100	5	500	100	5	500	100	5
Coastal	*Alloteuthis media**	91	16																		
	Alloteuthis subulata	8																			
	Rondeletiola minor	4	1																		
	Sepiola atlantica	1																			
	Sepiola ligulata	1																			
Coast-ocean	*Octopus vulgaris**	1	8		2		1	1		2			1	2	5	1	1		1	2	7
Oceanic	*Abraliopsis morisii*													1	1	3	1		1		1
	Ancistrocheirus alessandrinii												1		4	3	2	1			1
	Ancistroteuthis lichtensteinii									1	1		2	2	4		1		2	5	
	Brachioteuthis riisei												1	2		4	1			4	6
	Enoploteuthidae*									1					1		1				
	Gonatus steenstrupi																1				
	Heteroteuthis dispar													2							
	Liocranchia reinhardti															1*	1			1	
	*Mastigopsis hjorti**																		1		
	Oegopsida*																				1

	Ommastrephidae*										2		1		1							
	Onychoteuthidae*																				3	
	Pyrotheuthis margaritifera									1	1		1				4		2	2	
	Pyrotheutidae																	1		1*	1
	Sepiolidae*				1																

Table 2. Cephalopod paralarvae present in the different locations sampled off Cape Ghir (31ºN, W Morocco) during CAIBEX-III. Asterisk indicates species/taxa that lack genetic identification. Values 5, 100 and 500 indicate tow depth.

The oceanic families were present in the filament and oceanic samples. These included by order of abundance: onychoteuthids (n = 21, 8.5%) of which 18 were *Ancistroteuthis lichtensteinii* (100% homology); brachioteuthids (n = 18, 7.3%) represented by *Brachioteuthis riisei* (100% homology); pyroteuthids (n = 14, 5.7%) with eleven sequences corresponding to *Pyroteuthis margaritifera* (99% homology) and two undefined Pyroteuthidae with the closest match being *P. addolux*/*Pterygioteuthis* (90%); ancistrocheirids (n = 12, 4.9%), with ten sequences showing homologies below 90% with *Ancistrocheuirus lesueurii*; enoploteuthids (n = 11, 4.5%) eight identified as *Abraliopsis pfefferi* (99%), which is a junior synonym of *A. morisii* (Ropert and Jereb, 2010); cranchids (n = 3, 1.2%) two of which had 100% homologies with *Liocranchia reinhardti*; a gonatid with the closest match being with *Gonatopsis japonicus* (88.3%), however based on morphological characters and its distribution we identified it as *Gonatus steenstrupi*; an unidentified oegopsid (0.4%); and an adult mastigoteuthid morphologically identified as *Mastigopsis hjorti.*

The taxonomic status of the unknown ancistrocheirid paralarvae (n = 10) found on CAIBEX-III was further explored according to their position in a Kimura 2 parameter distance model tree constructed in BOLD (Fig. A.1). Our samples were grouped in a clade including two other ancistrocheirids, being 88.6 - 89.3% similar to seven sequences from an undefined Ancistrocheiridae collected in South Africa (private sequences) and 86.3 - 87.2% similar to available sequences of *Ancistrocheirus lesueurii* specimens from the Indo-Pacific. Accordingly, we suggest that the paralarvae collected off Morocco belong to *Ancistrocheirus* but represent a new species within the monotypic family Ancistrocheiridae. Our genetic results suggest that our species is different from that known from the Indo-Pacific - named *A. lesueurii* (d'Orbigny [in Férussac & d'Orbigny], 1842) -, therefore we would have to assign the ancistrocheirid paralarvae found off the coast of Morocco to the former species described

by Vérany in the Mediterranean, which is *Ancistrocheirus alessandrinii* (Vérany, 1847), as indicated in the discussion section.

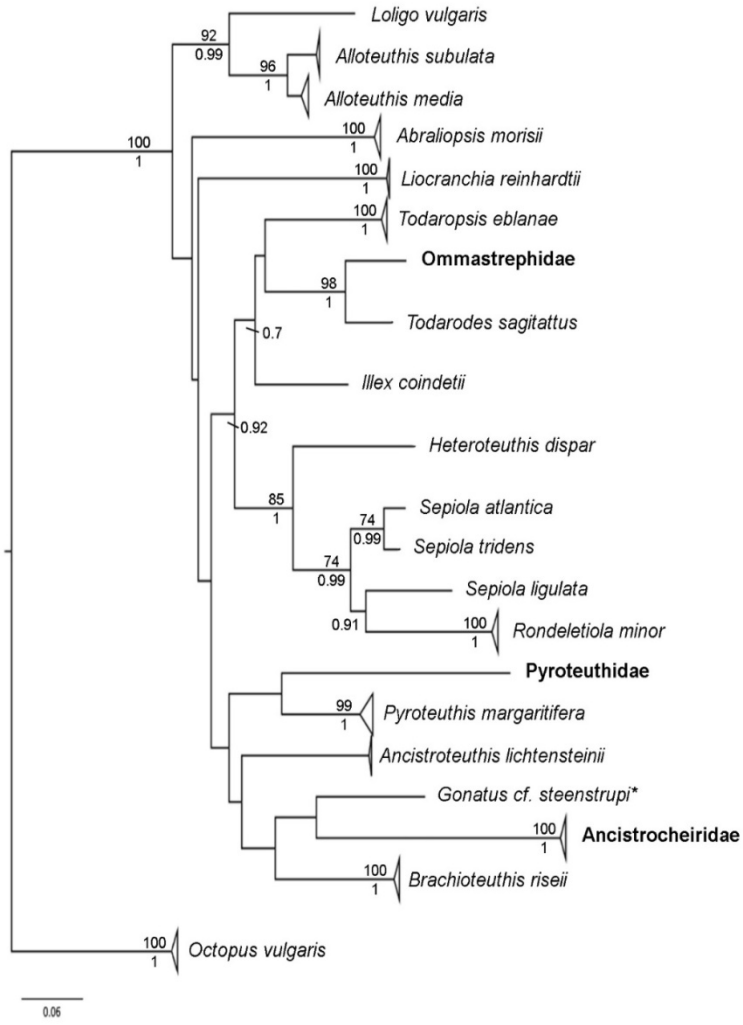

Fig. 4. Maximum likelihood (ML) phylogenetic tree showing the different cephalopod paralarvae species identified in the surveys CAIBEX-I and III. Species marked with an asterisk are not present in the genetic database but were assigned to a species based on their morphology. Species in bold represent those cephalopod paralarvae that are not present in the genetic database and were not possible to identify morphologically. Bootstrap values >60 after 1,000 replications and posterior probabilities >0.6 are shown above and below the nodes, respectively.

3.3 Planktonic dispersal patterns and vertical behaviour

The spatial distribution of cephalopod paralarvae in both upwelling systems showed a similar trend (Fig. 5) and three different planktonic dispersal patterns were identified:

- A “coastal” dispersal pattern followed by the loliginids *Alloteuthis media*, *A. subulata* and *L. vulgaris*; the sepiolids *Sepiola tridens*, *S. atlantica*, *S. ligulata* and *Rondeletiola minor*; and the ommastrephids *Todaropsis eblanae* and *Illex coindeti.* The wide size range measured - especially in loliginids and sepiolids (Table A.3) - provides evidence for the retention of these paralarvae between the coast and the limit of the continental shelf (200 m depth).

- A “coastal-oceanic” dispersal pattern followed exclusively by *O. vulgaris* in both upwelling ecosystems, with early stages (3 suckers per arm) found over the continental shelf; while advanced stages (between 3 to 15 suckers per arm) were found beyond the continental shelf in the upwelling filament and the ocean (Tables 1-3, Fig. 5). A positive relationship was recorded between distance from shore and *O. vulgaris* body size, showing that the paralarvae are growing as they are transported by upwelling filaments into the ocean (Fig. 6).

- An “oceanic” dispersal pattern followed by ommastrephids like *Todarodes sagitattus* in CAIBEX-I (Table1), oceanic sepiolids like *Heteroteuthis dispar* as well as true mesopelagic squids only found in samples with bottom depths greater than 2,000 m off the coast of Morocco (Table 2, Fig. 5). This mesopelagic squid assemblage is composed by *Brachioteuthis riseii*, *Ancistroteuthis lichtensteinii*, *Abraliopsis morisii*, *Pyroteuthis margaritifera*, *Ancistrocheirus alessandrinii*, *Liocranchia reinhardtii*, *Gonatus steenstrupii*, *Mastigopsis hjorti* and other undefined pyroteuthids, ommastrephids and oegopsids. Most of these species include a wide range of sizes (Table A.3) and their abundance increases progressively with the distance from the coast, especially in the filament and oceanic areas (Fig. 5).

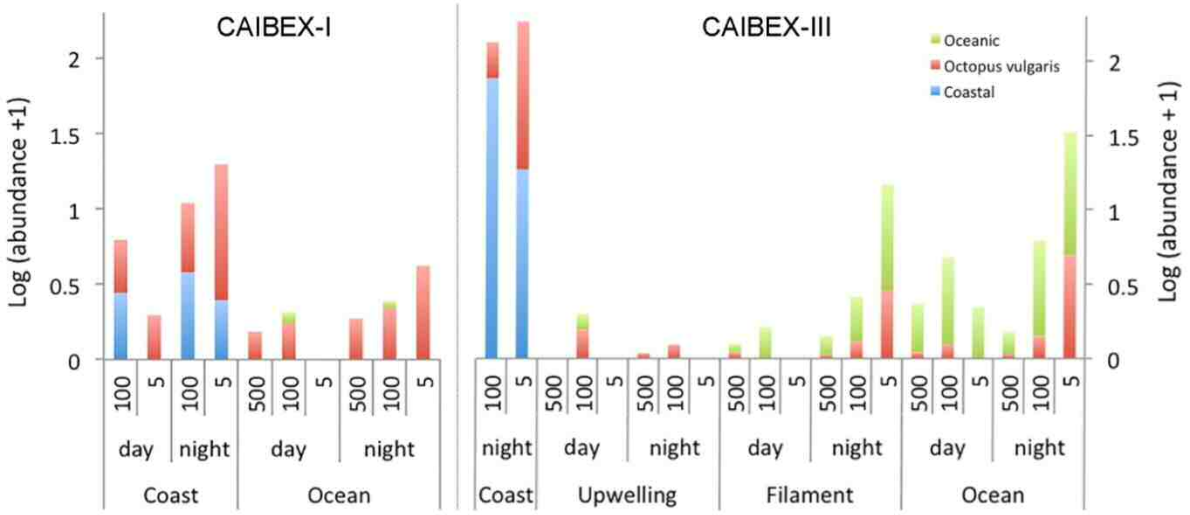

Fig. 5. Spatio-temporal distribution of the cephalopod paralarvae found in CAIBEX-I and III grouped according to the planktonic dispersal pattern and the location sampled. Paralarval abundances are calculated as individuals per 1,000 m^3 and represented as log (abundance + 1).

PERMANOVA analyses showed differences in the vertical behaviour between day and night for all planktonic dispersal patterns ($p<0.001$). Overall, cephalopod paralarvae were more abundant during the night, with increased abundance at the surface ($p<0.01$) decreasing gradually with depth (Fig. 5). In contrast, cephalopod paralarvae are absent from the surface in both coastal and oceanic environments during the day (with the exception of four very small octopus paralarvae collected in CAIBEX-I, Table 1, and two oceanic paralarvae collected in CAIBEX-III, Table 2) with the highest abundances recorded at 100 m.

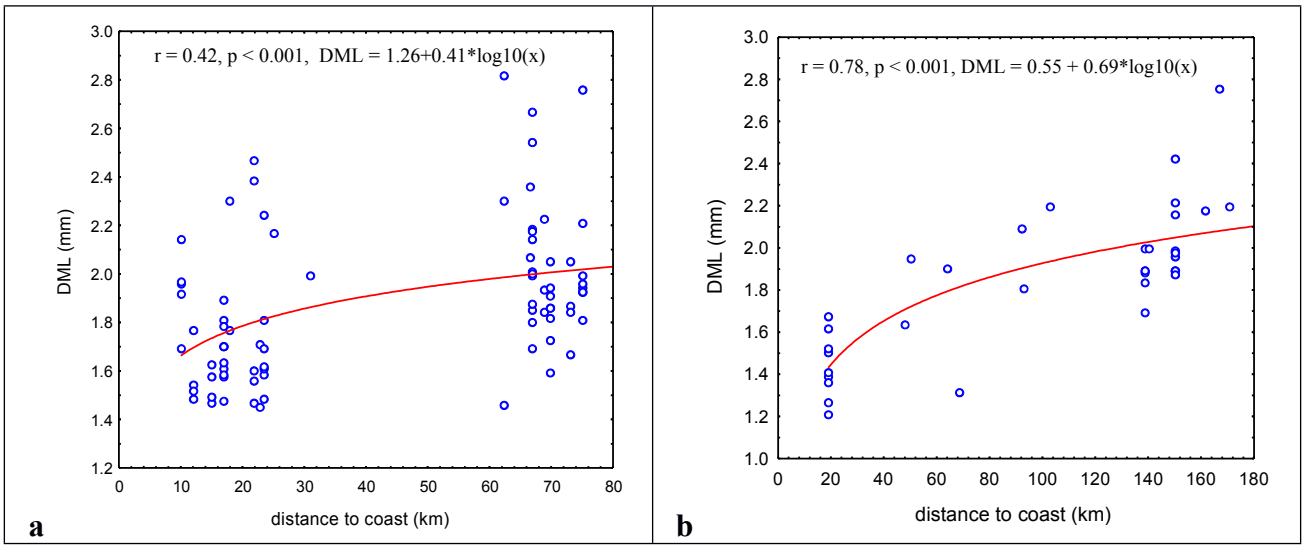

Fig. 6. Scatter plot showing the relationship between the distance to the coast (km) and the DML (mm) in *Octopus vulgaris* paralarvae collected during CAIBEX-I (a) and III (b).

Survey	Location	n	DML (mm)	Sucker nº	Depth (m)	Distance to coast (km)
CAIBEX I	Coast	51	1.75 ± 0.27	3 - 4	62 - 147	10 - 31
	Ocean	48	2.02 ± 0.28	3 - 5	1,940 - 3,105	62 - 75
CAIBEX III	Coast	9	1.44 ± 0.15	3	88 - 90	19
	Upwelling	4	1.66 ± 0.26	3 - 4	787 - 2,328	48 - 93
	Filament	10	1.96 ± 0.15	3 - 6	1,526 - 2,720	50 - 162
	Ocean	12	2.13 ± 0.33	4 - 15	2,418 - 3,110	140 - 171

Table 3. Average dorsal mantle length range (DML ± standard deviation) and sucker counts of *Octopus vulgaris* paralarvae found at the different locations sampled in CAIBEX I and III surveys. The range of depths and distances to coast is shown for the locations sampled.

3.4 Cephalopod paralarvae genetic diversity and population structure

Only 11 out of 21 different putative species identified with COI gene met the criteria to study their genetic diversity (at least five individuals per species and ≥ three haplotypes). The number of haplotypes per species ranged between 1 and 22, with a positive relationship with the number of sequences analysed ($R^2 = 0.63$). No diversity metrics could be calculated for *Sepiola tridens* (n = 5) and *Pyroteuthis margaritifera* (n = 6) as all sequences represented the same haplotype. Nucleotide diversity for the 624 bp data set ranged from 0.00046 to 0.00609 and haplotype diversity ranged from 0.243 to 0.8 (Table 4). Values for these COI diversity metrics were evenly distributed within the 95th percentile boundaries around the fitted model that relates these two variables (Fig. 7), thus indicating that there were no outliers despite the low number of sequences analysed for some species. According to the reference values calculated by Goodall-Copestake et al. (2012) as a cut-off for qualitative descriptions, three cephalopod species had high haplotype diversity ($h > 0.7013$): the coastal species *Alloteuthis media* and *Rondeletiola minor*, and the oceanic one *Brachioteuthis riseii* (Table 4). Conversely, only two species had high nucleotide diversity ($\pi > 0.00356$): *R. minor* and *B. riseii*. Significant differences ($p<0.001$) were found among planktonic dispersal patterns, with high nucleotide and haplotype diversity for both coastal and oceanic patterns and low diversity for the coastal-oceanic distribution displayed by *Octopus vulgaris* (Fig. 8).

Significant deviance from neutrality was revealed in both statistics analysed (Tajimas's D and Fu's Fs) for *Alloteuthis media*, *A. subulata* and *O. vulgaris* (Table 4). These species showed negative values of both statistics, suggesting that the sequences analysed were evolving under a non-random process, likely a recent population expansion (e.g. after a bottleneck or a selective sweep). A negative value for Fu's Fs provides evidence for an excess number of alleles relative to simulations, as would be expected from a recent population expansion or from genetic hitchhiking. Negative Tajima's D implies an excess of low frequency polymorphisms relative to expectation, also pointing in the direction of population size expansion and /or purifying selection. Remarkably, these three species were the only ones that were captured in both upwelling regions (Tables 1 and 2) and the observed significant deviances from neutrality tests could indicate that the sampled specimens belong

to different populations. However, pairwise F_{st} tests revealed that such population structure was not supported for *O. vulgaris* and *A. subulata* ($p > 0.05$), despite being separated by more than 2,000 km. Although *A. media* was also captured in both upwelling ecosystems the four sequences obtained from NW Iberian Peninsula were smaller than 624 bp and were not included in the analysis.

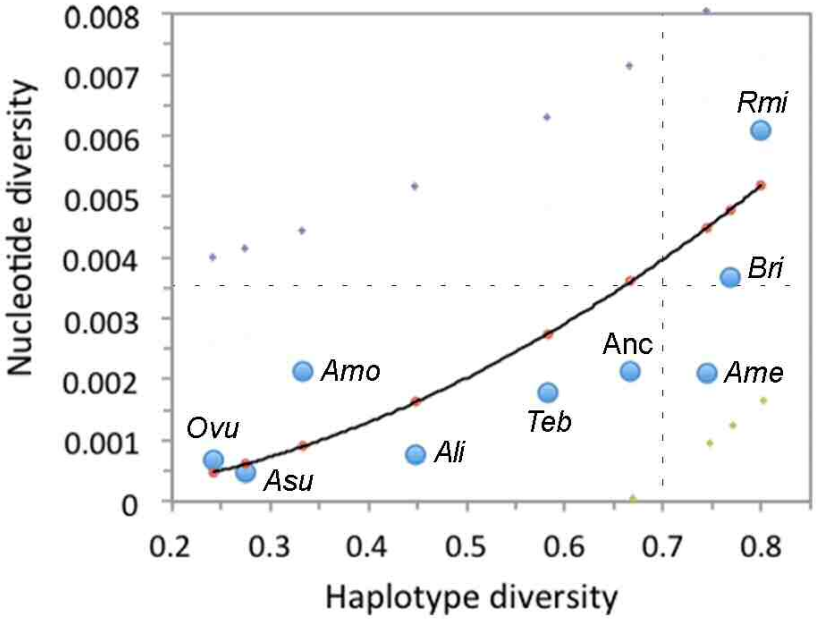

Fig. **7**. Nucleotide-haplotype diversity relationship obtained from a homologous 624-base region of COI for the following cephalopod paralarvae: Anc, *Ancistrocheirus alessandrinii*; *Ali, Ancistroteuthis lichtensteinii; Ame*, *Alloteuthis media*; *Amo, Abraliopsis morisii; Asu, Alloteuthis subulata; Bri, Brachioteuthis riseii; Ovu, Octopus vulgaris; Rmi, Rondeletiola minor; Teb, Todaropsis eblanae*. Red dots represent the expected nucleotide diversity values (π, with the 95th percentiles above and below) according to the model $\pi=0.0081h^2$ (Goodall-Copestake et al. 2012), based on our measured haplotype diversity (h). Dashed lines represent the median values of nucleotide and haplotype diversity obtained by Goodall-Copestake et al. (2012) that are used to represent low and high diversity.

The haplotype networks calculated from the common 624-data set are shown in Fig. 9 grouped according to their dispersal pattern. The star-shaped haplotype network found in the school of *A. media* (Fig. 9a) is in agreement with the recent population expansion event detected with neutrality tests, where many unique haplotypes (n = 16) radiate from few common haplotypes. The same star-shaped pattern is observed in *O. vulgaris* (Fig. 9b), but with fewer number of different haplotypes despite greater number of individuals included (n = 115). The case of *A. subulata,* for which there were fewer specimens analysed (n = 14),

Dispersal pattern	Species	n	H	S	Hd	π	Tajima's D	Fu's Fs
Coastal	*Alloteuthis media*	94	22	24	0.745 ± 0.037	0.00209 ± 0.00024	**- 2.16 (p=0.002)**	**- 19.94 (p<0.001)**
	A. subulata	14	3	2	0.275 ± 0.148	0.00046 ± 0.00026	**- 1.48 (p=0.048)**	**- 1.47 (p=0.007)**
	Todaropsis eblanae	9	4	3	0.583 ± 0.183	0.00178 ± 0.00063	0.03 (p=0.577)	- 0.82 (p=0.158)
	Sepiola tridens	5	1	0	0	0	-	-
	Rondeletiola minor	5	3	7	0.800 ± 0.164	0.00609 ± 0.00133	0.91 (p=0.803)	1.78 (p=0.799)
Coastal - Oceanic	*Octopus vulgaris*	115	9	11	0.243 ± 0.053	0.00068 ± 0.00021	**- 2.03 (p=0.001)**	**- 7.36 (p=0.001)**
Oceanic	*Brachioteuthis riisei*	14	6	8	0.769 ± 0.089	0.00368 ± 0.00048	- 0.33 (p=0.423)	- 0.60 (p=0.353)
	Ancistroteuthis lichtensteinii	15	3	2	0.448 ± 0.134	0.00076 ± 0.00025	- 0.59 (p=0.299)	- 0.52 (p=0.261)
	Pyroteuthis margaritifera *	6	1	0	0	0	-	-
	Abraliopsis morisii	6	2	4	0.333 ± 0.215	0.00214 ± 0.00138	- 1.29 (p=0.064)	2.14 (p=0.82)
	Ancistrocheirus alessandrinii	7	3	3	0.667 ± 0.16	0.00214 ± 0.00051	0.40 (p=0.688)	0.54 (p=0.543)

also showed evidence of non-neutral processes, which were not evident from the calculated haplotype network (Fig. 9a). Contrarily, in other haplotype network topologies – such as *Rondeletiola minor, Brachioteuthis riseii* and the new species of Ancistrocheiridae (Figs. 9a, c) - the observed haplotypes seem to be derived from an ancestral haplotype that may have not been sampled or may have been lost because of a recent population bottleneck.

Table 4. Genetic diversity calculated from a fragment of the COI gene of 624 bp from those cephalopod paralarvae (n) with at least 5 sequences per species: number of haplotypes (H), number of polymorphic sites (S), haplotype diversity (h ± SD), nucleotide diversity per site (π ± SD). Tajima's D and Fu's Fs tests of neutrality were calculated and its significance was obtained after 1,000 simulated samples (p-value).

4. Discussion

The multidisciplinary seascape approach sheds light on the processes that shape genetic diversity/population structure of planktonic cephalopod paralarvae according to their different life dispersal patterns. We used COI to identify cephalopod paralarvae and to explore the genetic diversity within species and among planktonic dispersal patterns. This barcoding approach revealed 21 different species and was particularly useful to identify early stages of cephalopod paralarvae <4 mm, where conspicuous taxonomic features were absent or not yet formed and would have limited the taxonomic identification above family level, especially within the families Loliginidae, Sepiolidae, Enoploteuthidae and Ommastrephidae.

4.1 Cephalopod paralarval diversity

Eight cephalopod species were identified in the upwelling ecosystems of NW Iberian Peninsula (CAIBEX-I) and 16 in W Morocco (CAIBEX-III), showing a marked latitudinal change in the cephalopod assemblage. The main reasons for the absence of tropical and sub-

tropical oceanic cephalopod paralarvae during CAIBEX-I (between 41º15'N and 42º6'N) may be the persistent fronts between the shelf and deep waters close to Cape S. Vicente (37ºN), which represent a temperature boundary to poleward dispersion (Peliz et al., 2005; Moreno et al., 2009). In fact, Moreno et al. (2009) found only 22 oceanic paralarvae belonging to four different species north of 40ºN in 57 surveys carried from 1986 to 2004. Of these, three were tropical species (belonging to two families; Onychoteuthidae and Mastigoteuthidae) found from January to June and *Teuthowenia megalops*, a sub-Arctic and northern temperate Atlantic species found only in winter months (Collins et al., 2001; Moreno et al., 2009). The only record of a tropical species found north of 40ºN is of *Brachioteuthis riseii* paralarvae, a cosmopolitan oceanic species found west of the British Isles between March and July (Collins et al., 2001). Accordingly, the absence of oceanic squid paralarvae during CAIBEX-I was a normal result for the area sampled, which is north of their spawning grounds off southern Portugal, and for the month sampled (July), which is too warm for northern temperate Atlantic species.

An unexpected finding of CAIBEX-I paralarval barcoding was the presence of *Sepiola tridens* in samples collected off the Portuguese shelf at night in bottom depths ranging from 100 to 148 m. This species, described from specimens collected in the North Sea, Ireland and NW Spain (de Heij and Goud, 2010), has been recently identified in the Ría de Vigo (Olmos-Pérez et al., 2017). Its presence off the Portuguese coast increases the southern limit of its range to 41º23'N. Morphologically, juveniles of this species cannot be separated with certainty from *S. atlantica;* however, *S. tridens* inhabits deeper waters (average depth 81.8m) than *S. atlantica* (average depth 37.4 m, de Heij and Goud, 2010).

Analyses based on morphological characters and identification guides (González et al. 2010) indicated that the loliginid paralarvae present in the Ría de Vigo was *Loligo vulgaris*. However, this deduction is likely incorrect, because in our genetic sampling off the coast of the NW Iberian Peninsula only 2 out of 16 loliginids were *L. vulgaris* (Table 1). Moreover, in a recent study carried out by Olmos-Pérez (2018) in the Ría de Vigo between 2012 and 2014, the most abundant loliginid was *A. media* (57.5%), followed by *A. subulata* (21%) and *L. vulgaris* (14.5%). Although adult specimens of *Alloteuthis* can be differentiated by the size of the central club sucker (Anderson FG et al. 2008), this characteristic is not yet present in paralarvae. They can be identified only on the basis of the chromatophore pattern of fresh individuals (Sweeney et al. 1992), which is a delicate character lost in fixed specimens. Our morphological study showed *A. subulata* paralarvae to be significantly bigger for all the body lengths measured than *A. media* (Table A.3). Recent morphological studies

found that tentacle length may be a good character to discriminate between both *Alloteuthis* species (Olmos-Pérez, 2018).

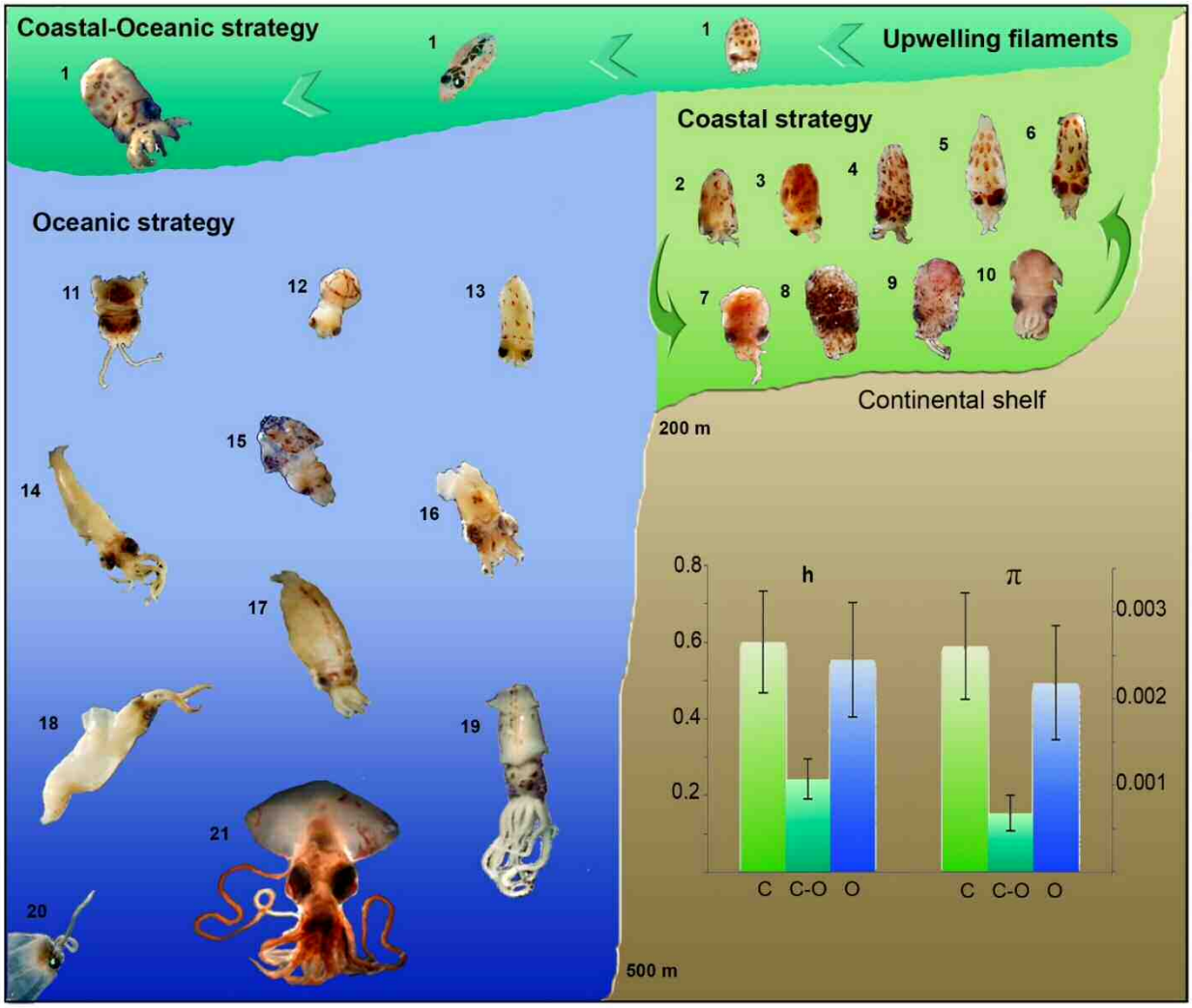

Fig. 8. Cephalopod paralarvae and their different planktonic dispersal patterns in the Iberian-Canary Upwelling System: coastal (C, light green); coastal-oceanic (C-O, dark green) and oceanic (O, blue). Average haplotype (h) and nucleotide (π) diversity estimated for the different planktonic dispersal patterns. Bars indicate standard deviations. 1 *Octopus vulgaris*; 2 *Illex coindetii*; 3 *Todaropsis eblanae*; 4 *Loligo vulgaris*; 5 *Alloteuthis subulata*; 6 *A. media*; 7 *Rondeletiola minor*; 8 *Sepiola tridens*; 9 *S. atlantica*; 10 *S. ligulata*; 11 *Heteroteuthis dispar*; 12 Ommastrephidae; 13 *Todarodes sagitattus*; 14 *Gonatus steenstrupi*; 15 *Ancistrocheirus alessandrinii*; 16 *Pyroteuthis margaritifera*; 17 *Ancistroteuthis lichtensteinii*; 18 *Brachioteuthis riisei*; 19 *Abraliopsis morisii*; 20 *Liocranchia reinhardtii*; 21 *Mastigopsis hjorti*.

The adults of most of the cephalopod paralarvae found during CAIBEX-III are common inhabitants of the tropical and subtropical waters of the Atlantic Ocean (Roper and

Young, 1975; Collins et al., 2001; Diekmann and Piatkowski, 2004; Clarke, 2006; Jereb and Roper, 2010). Nonetheless, there were records new for the eastern Atlantic, like the juvenile of *Sepiola ligulata*, collected on the Moroccan shelf (31º0.02'N, 10º0.78'W) at 80 m depth. *Sepiola ligulata* is a species that, to the best of our knowledge, was only found in the Mediterranean Sea (Jereb and Roper, 2010). Moreover, the specimen of *S. atlantica* found in the coastal sample off Cape Ghir extends the southern limit of this species to 31ºN. To our knowledge, the early life stages of *Ancistroteuthis lichtensteinii* (n = 18, 1.7 - 10.1 mm DML) collected during CAIBEX-III are the smallest ever found in the Atlantic (Vecchione et al., 2010) and their vertical distribution demonstrate that this species displays vertical migration at least during their early life stages (Table 2).

Ancistrocheirus lesueurii is presently deemed to be panoceanic and the only representative of the family Ancistrocheiridae (e.g. Roper and Jereb, 2010), whereas in the past two different species were ascribed to that family, namely *A. lesueurii* and *Thelidioteuthis alessandrinii* (e.g. Clarke, 1966). In the 1980s *A. lesueurii* was believed to be a cosmopolitan animal and *T. alessandrinii* was suspected to be the juvenile form of the same species (Roper et al., 1984). Since some ambiguities existed about the correct name to indicate the supposed unique ancistrocheirid species, Bello (1992) showed that it was *Ancistrocheirus lesueurii* (d'Orbigny [in Férussac & d'Orbigny], 1842), with type locality the Indo-Pacific Ocean. Based on the present genetic data, we suggest that the paralarvae collected in Morocco may represent a new species congeneric to, but different from, *A. lesueurii,* within the hitherto monotypic Ancistrocheiridae family. This suggestion is in agreement with differences in paralarval morphology between Atlantic and Pacific specimens that suggest that more than one species likely exist (Young et al., 1998), and also with the phylogenetic tree shown in Fig. A1 that shows a third species collected in South Africa within the Ancistrocheiridae.

According to the geographical origin of the ancistrocheirid paralarvae collected in this study, i.e. close to the entrance to the Mediterranean, we can safely suppose that these paralarvae belong to the same ancistrocheirid species that occurs in the Mediterranean (all Mediterranean oegopsid squids are also distributed in the eastern Atlantic; cf. Jereb & Roper, 2010). *A. lesueurii* was described by Vérany (1847), who named it *Loligo alessandrinii*, based on a paralarval specimen collected in the harbour of Messina (type locality: Strait of Messina, western Mediterranean). More than a century later, its presence was confirmed by the finding of the first adult female in the type locality, which was identified as *A. lesueurii* (Bello et al., 1994). Our genetic results suggest that the eastern Atlantic-Mediterranean

species is different from that known from the Indo-Pacific (namely *A. lesueurii,* Fig. A.1), therefore we would have to assign the ancistrocheirid paralarvae found off the coast of Morocco to the former species that is *Ancistrocheirus alessandrinii* (Vérany, 1847). However, a morphological and genetic study with adults needs to be undertaken to uncover the true diversity within the Ancistrocheiridae family.

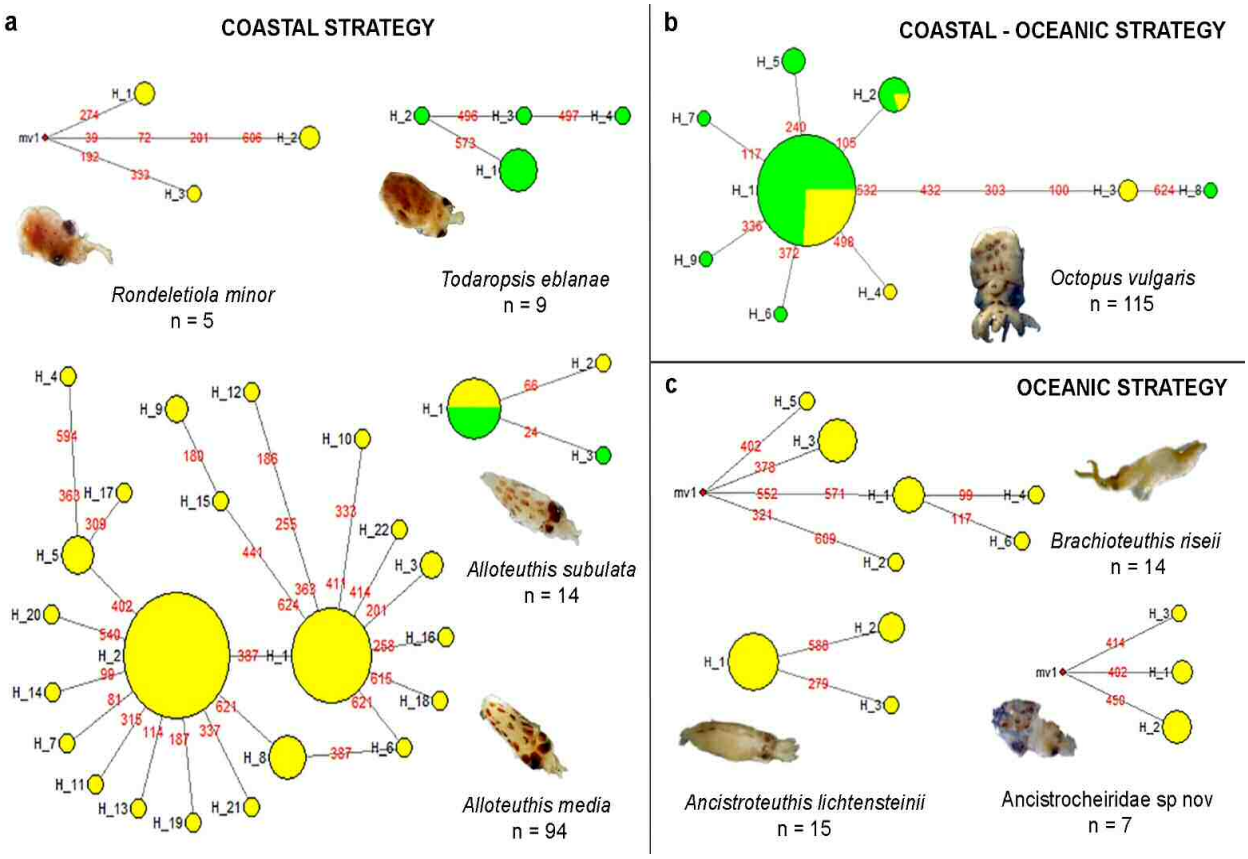

Fig. 9. Haplotype networks obtained for those cephalopod species with more than 5 individuals and more than 3 different haplotypes, grouped according to their dispersal pattern. Colours represent the upwelling regions sampled: green, cape Silleiro (NW Iberian Peninsula); yellow, Cape Ghir (W Morocco).

According to the geographical origin of our paralarvae, i.e. close to the entrance to the Mediterranean, we can safely suppose that these paralarvae belong to the same ancistrocheirid species that occurs in the Mediterranean (all Mediterranean oegopsid squids are also distributed in the eastern Atlantic; cf. Jereb & Roper, 2010). *A. lesueurii* was described by Vérany (1847), who named it *Loligo alessandrinii*, based on a paralarval specimen collected in the harbour of Messina (type locality: Straits of Messina, western Mediterranean). More than a century later, its presence was confirmed by the finding of the

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4.2 Planktonic dispersal patterns in coastal upwelling systems: interplay between oceanography and vertical behaviour

Changes in the vertical behaviour of the cephalopod paralarvae under the same oceanographic conditions in the upwelling regions of NE Atlantic lead to different dispersal/retention capabilities that likely affect the patterns of genetic diversity detected. Three major life dispersal patterns were identified for the planktonic stage of cephalopods: coastal, coastal-oceanic and oceanic (Fig. 8). The coastal dispersal pattern is followed by members of the families Loliginidae, Sepiolidae and Ommastrephidae, distributed from the coast to the edge of the continental shelf (200m). This distribution was only detected in *Octopus vulgaris*, with hatchings found in the coastal-shelf area and older paralarvae in the open ocean. The oceanic dispersal pattern was observed for certain species of coastal families like Sepiolidae (*Heteroteuthis dispar*) and Ommastrephidae (*Todarodes sagittatus*) together with oceanic midwater families including Onychoteuthidae, Brachioteuthidae, Pyroteuthidae, Ancistrocheiridae, Enoploteuthidae, Gonatidae, Mastigoteuthidae and Cranchiidae.

Cephalopod paralarvae with a coastal dispersal pattern are characterised by retention of different developmental stages, which is likely achieved by adjusting their vertical behaviour in accordance with the oceanographic conditions (Roura et al., 2016). Such retention was supported by the schooling behaviour of the loliginid paralarvae (*Alloteuthis media* and *A. subulata*, n = 99 that included specimens with TL ranging from 1.85 - 13.2 mm, Table A.3) found in the water-column sample off the coast of Morocco (Table 2). Other loliginid paralarvae (*A. media*, n = 16) were also found in the same coastal sample at the surface and, therefore, would be expected to occur within the filament as it flowed seaward. However, the lack of any loliginid paralarvae beyond the continental shelf in both upwelling ecosystems (observed in this study and previous ones, e.g. Rocha et al., 1999; Moreno et al., 2009) can be explained through an active behaviour controlling their vertical position - in this case moving downward to evade the offshore surface flow - effectively limiting offshore dispersal while favouring alongshore retention, as suggested by other studies (Otero et al.

2009; Roura et al. 2016). Such behaviour has also been observed in crustacean larvae in the ICC (Queiroga and Blanton, 2004; Queiroga et al., 2007) as well as in other upwelling ecosystems (Roberts, 2005; Shanks and Shearman, 2009; Morgan and Fisher, 2010). Consequently, the spatio-temporal aggregation of different life stages from different origins likely contributes to high nucleotide (π) and haplotype (h) diversities for cephalopod paralarvae displaying coastal dispersal patterns (Fig. 8). A similar effect has been observed in the cardinal reef fish (*Ostorhinchus doederleini*) which has strong homing behaviour – high aggregation and low dispersal – and displayed the highest genetic structure recorded to date for relatively small scales (<20 km) as pointed out by Gerlach et al. (2007). The exception to this general trend is represented by *A. subulata,* which inhabits deeper waters than its congener *A. media*. The low π and h diversity might reflect the small number of sequences obtained for this species, but a similar haplotype network with only three haplotypes was obtained from 37 specimens collected in the Ría de Vigo between 2012 and 2014 (Olmos-Pérez, 2018).

Contrary to the coastal dispersal pattern, the coastal-oceanic migration of *Octopus vulgaris* requires a tight coupling of their vertical behaviour with the upwelled surface waters to be transported offshore far from the coastal area, as suggested by Roura et al. (2016). One of the mechanisms proposed for such migration would be offshore transport in the oceanward upwelling filaments, as was observed for neritic ichthyoplankton larvae south of the Canary Islands (Rodríguez et al., 1999). Despite the limitations of interpreting vertical data collected with bongo nets, our results support the proposed migration mechanism, because octopus paralarvae were mainly present at the surface at night and absent from the surface layer during the day (Tables 1-2). Detailed vertical studies carried out in the Ría de Vigo (NW Iberian Peninsula) with a Multinet between 2012 and 2014 revealed that *O. vulgaris* paralarvae are mostly found during the day between 5 - 20 m and between 0 to 5 m at night (Olmos-Pérez, 2018). Such positioning would allow octopus paralarvae to be transported seaward within the upwelling filaments, which in our study was 60 m deep and 25 km wide (Sangrá et al. 2015). In fact, *O. vulgaris* was the only cephalopod paralarvae that was found in all the locations sampled in this study – *i. e*. the coastal area, the upwelling zone, the filament and open ocean (Tables 1-2) – as a result of this offshore transport and dispersal. Supporting this migratory behaviour is the positive relationship found between the distance to coast and the size of the paralarvae (Fig. 6), showing that the paralarvae are feeding correctly during this migration and are growing towards the open ocean. Species with large hatchlings

such as *Enteroctopus dofleini* have also been found distributed in both shallow and oceanic waters in the north Pacific Ocean (Villanueva and Norman, 2008).

Moreover, age estimations based on beak ring increments showed that octopus paralarvae collected from the open ocean were older (up to 28 days, Perales-Raya et al., 2017) than those collected close to the coast (less than 8 days, Garrido et al., 2016; Perales-Raya et al., 2017). Additional evidence of the seaward migration is that the 3,739 *O. vulgaris* paralarvae found in the ICC to date have been collected close to the coast over the continental shelf with only three suckers per arm (Rocha et al., 1999; González et al., 2005; Otero et al., 2008; Moreno et al., 2009; Garrido et al., 2016; Roura et al., 2013; Lourenço et al., 2017). However, up to 74 *O. vulgaris* individuals were collected beyond the edge of the continental shelf during CAIBEX-I and -III (bottom depths ranging from 787 to 3110 m) and 58 of them had more than three suckers per arm. These results support the hypothesis that *O. vulgaris* hatchlings are advected oceanward by coupling their vertical distribution with the prevailing oceanographic conditions to leave the coastal area, because paralarvae with more than 3 suckers have only been found far from the continental shelf.

The genetic data provided further evidence of the mechanism underpinning such dispersal in *O. vulgaris* paralarvae. Despite being found in all the locations sampled through a coastal-oceanic distance of more than 171 km and an alongshore extent covering more than 2,000 km (from 31º to 42ºN), the spatial genetic diversity obtained for *O. vulgaris*, compared with the rest of cephalopod paralarvae (Fig. 8), can only be explained by means of within-cohort sampling. This pattern of relatedness / low genetic diversity may result from paralarvae with similar origins remaining together throughout their dispersal stage, in this case in the upwelling waters and filament. Planes et al. (2002) revealed that as recruit cohorts of the blue spine unicorn fish (*Naso unicornis*) aged, their internal mean relatedness decreased. However, the large spatial scale that separates the two upwelling ecosystems (~2,000 km) and the different haplotypes detected clearly indicates that different hatching events were sampled. Low levels of population differentiation were also observed in the velvet swimming crab around the Iberian Peninsula (Sotelo et al. 2009). This species also has a pelagic stage of around two months suggesting that gene flow could be also operating in this area to maintain the genetic homogeneity observed.

In order to investigate whether the genetic patterns detected in CAIBEX-I and III resulted from schooling behaviour of larval *O. vulgaris*, the genetic diversity of the paralarvae collected in this study was compared to that of the species across its distribution range. COI samples were selected from GenBank based on the distribution of *O. vulgaris*

sensu stricto (Amor et al. 2014; 2017): three from Galicia (NW Spain: DQ683221-683223), two from Portugal (KF844042-844043), three from Senegal (DQ683224-683226), two from west Mediterranean (France: DQ683227, KF774311) and two from east Mediterranean (Turkey: KC311412, KC789315). The obtained sequences of *O. vulgaris sensu stricto* had a common region of 482 bp and the same fragment was trimmed in our sequences for direct comparison. Genetic diversities for *O. vulgaris sensu stricto* were $h = 0.530 \pm 0.076$ and $\pi = 0.0033 \pm 0.00048$, while for our paralarvae were $h = 0.166 \pm 0.047$ and $\pi = 0.00057 \pm 0.00019$. Such marked difference, suggest that the genetic pattern observed in the paralarvae was the result of sampling closely related individuals that were transported within the same water mass. This is a clear example of how a multidisciplinary approach that combines oceanography, behaviour, morphometry and genetics can shed light to explain low diversity values that would be otherwise inexplicable (Selkoe et al. 2008).

The stability and age of the oceanic pelagic ecosystem has enabled the evolution of many species, with subtle niche differences (Hopkins and Sutton, 1998). The oceanic dispersal pattern was followed by oceanic squids and oceanic species of coastal families like the Sepiolidae. These oceanic paralarvae were present within samples with bottom depths above 2000 m and were absent from the samples collected in the coastal and upwelling areas. All the oceanic species found in this study complete their development in the oceanic realm and have been observed elsewhere in the Atlantic (Diekmann and Piatkowski, 2004, Vecchione et al. 2010). Genetic diversity in these paralarvae was also high, as a result of sampling different specimens from different developmental stages and different origins. Mesopelagic cephalopods, together with fishes and decapods, are key zooplankton predators in the ocean that display diel vertical migration to feed close to the surface at night (Passarella and Hopkins, 1991). Interestingly, onychoteuthid and ancistrocheirid paralarvae were concentrated in the surface waters of the samples collected within the filament at night (Table 2), likely attracted by the increased zooplankton biomass (Hernández-León et al., 2002), as observed in other mesopelagic predators like shrimps or fishes (Hopkins and Sutton, 1998).

New comprehensive studies are needed to investigate the coupling of larval abundance in the plankton with settlement and post-settlement mortality on the shore for diverse taxa and locations across upwelling coasts to determine the underlying mechanisms responsible for the observed spatial and temporal patterns of recruitment. Such investigations

are essential for further advancing our understanding of processes that regulate marine populations and communities.

5. Conclusions

- The multidisciplinary approach undertaken in this study sheds light on the processes that shape genetic diversity/population structure of planktonic cephalopod paralarvae in two upwelling ecosystems of the Canary Current eastern boundary upwelling ecosystem.
- Eight cephalopod species were genetically identified in the upwelling ecosystem of NW Iberian Peninsula and 16 in W Morocco, showing a marked latitudinal change in the cephalopod assemblage.
- An undescribed species within the monotypic Ancistrocheiridae family was genetically identified in Moroccan waters and named *Ancistrocheirus alessandrinii* (Vérany, 1847).
- Genetic diversity in the COI gene revealed no genetic structure between the two upwelling regions for *Alloteuthis subulata* and *Octopus vulgaris*.
- Changes in the vertical behaviour of the cephalopod paralarvae under the same oceanographic conditions lead to different dispersal/retention capabilities and three different planktonic dispersal patterns were identified: coastal, coastal-oceanic and oceanic.
- Coastal and oceanic dispersal patterns displayed high levels of nucleotide and haplotype diversity as a result of spatio-temporal retention of different life stages, while the low levels registered for the coastal-oceanic dispersal pattern resulted from the advection of closely related specimens within upwelled waters / filaments into the ocean.
- *Octopus vulgaris* is the only cephalopod that display a coastal-oceanic dispersal pattern in the ICC as shown by the presence of paralarvae with more than three suckers per arm far from the continental shelf, the significant positive correlation between size and distance to coast and the low genetic diversity measured.

6. Acknowledgements

We are indebted to the captain, crew and technicians of R/V “Sarmiento de Gamboa”, for their assistance in collecting the zooplankton samples and hydrographical data. We acknowledge the help of Félix Álvarez sorting the paralarvae, as well as Mariana Rivas for preparing the cephalopod samples for barcoding. Special thanks to Dr. Dirk Steinke, who offered the facilities of the Barcoding of Life Data (BOLD) to sequence the cephalopods. Furthermore, we thank Marcos Regueira and Rocío Graña for their help preparing figures 1,

and 2 and 3, respectively. This study was supported by the project CAIBEX (Spanish Ministry of Innovation and Science CTM2007-66408-C02) and the first author by a "Fundación Barrié de la Maza" postdoctoral fellowship (3003197/2013) and a Securing Food, Water and the Environment Research Focus Area grant (La Trobe University) during writing.

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Appendices

Table A.1. Detailed information about the mesozooplankton samplings carried out during CAIBEX-I. Abbreviation: first (L1) and second (L2) Lagrangian experiments.

Sample	Date	CTD	Latitude	Longitude	Depth (m)	Volume (m^3)	Station	Day/night
S1	10-7-09	CS017	41º24.52'N	9º31.04'W	2667	6159	L1	night
S2	11-7-09	CS021	41º21.16'N	9º33.44'W	2885	5881	L1	day
S3	11-7-09	CS026	41º19.87'N	9º32.78'W	2784	7144	L1	night
S4	12-7-09	CS029	41º19.86'N	9º34.26'W	2294	6481	L1	day
S5	12-7-09	CS035	41º17.18'N	9º34.14'W	2924	6050	L1	night
S6	13-7-09	CS038	41º17.38'N	9º35.96'W	3100	5014	L1	day
S7	13-7-09	CS045	41º15.8'N	9º37.29'W	3105	4840	L1	night
S8	14-7-09	CS047	41º30.01'N	8º54.89'W	62	1019	Shelf	day
S9	14-7-09	CS050	41º30'N	9º10'W	134	2192	Shelf	day
S10	14-7-09	CS055	41º29.94'N	9º35.09'W	1940	5039	Ocean	night
S11	15-7-09	CS067	42º5.99'N	9º5.04'W	141	975	Shelf	night
S12	16-7-09	CS083	41º59.92'N	9º11.94'W	147	1796	Shelf	night
S13	17-7-09	CS089	42º0.15'N	9º0.35'W	108	1997	L2	day
S14	17-7-09	CS094	41º55.17'N	9º1.58'W	108	1335	L2	night
S15	18-7-09	CS096	41º50.47'N	9º3.57'W	114	1268	L2	day
S16	18-7-09	CS106	41º42.05'N	9º4.06'W	106	2616	L2	night
S17	19-7-09	CS108	41º36.35'N	9º2.16'W	96	1390	L2	day
S18	19-7-09	CS119	41º29.17'N	9º3.1'W	90	1496	L2	night
S19	20-7-09	CS121	41º26.2'N	9º3.54'W	90	1898	L2	day
S20	20-7-09	CS131	41º23.18'N	9º4.05'W	102	2261	L2	night

Table A.2. Detailed information about the mesozooplankton samplings carried out during CAIBEX III. Most of the samples were obtained from the core of the filament during the third Lagrangian experiment (L3).

Sample	Date	CTD	Latitude	Longitude	Depth (m)	Volume (m^3)	Station	Day/night
G1	20-8-09	FIL7	10º19.99'N	30º50.06'W	1437	7501	upwelling	day
G2	21-8-09	FIL10	10º0.78'N	31º0.02'W	90	2247	coast	night
G3	23-8-09	FIL13	10º36.01'N	30º43.32'W	1837	5509	L3	day
G4	24-8-09	FIL17	10º43'N	30º49.20'W	1532	4423	L3	night
G5	24-8-09	FIL18	10º48.12'N	30º54.20'W	1671	7007	L3	day
G6	24-8-09	FIL20	10º30.10'N	30º59.93'W	808	6436	upwelling	night
G7	25-8-09	FIL21	10º46.77'N	30º55.82'W	1823	5057	L3	night
G8	25-8-09	FIL23	10º54.47'N	30º59.75'W	1640	6325	L3	day
G9	26-8-09	FIL26	10º19.92'N	30º49.53'W	1104	6592	upwelling	night
G10	27-8-09	FIL40	10º36.02'N	30º13.75'W	1974	5648	upwelling	night
G11	28-8-09	FIL41	11º16.75'N	31º0.37'W	2095	5290	L3	night
G12	28-8-09	FIL42	11º18.48'N	31º0.38'W	2200	5107	L3	day
G13	28-8-09	FIL44	11º31.33'N	31º26.42W	2472	4965	ocean	day
G14	29-8-09	FIL44	11º22.50'N	30º57.27'W	2561	7735	ocean	night
G15	29-8-09	FIL46	11º25'N	30º57.25'W	2410	5534	L3	day
G16	29-8-09	FIL47	11º35.90'N	31º8.15'W	3042	5243	ocean	day
G17	29-8-09	FIL48	11º51.77'N	31º8.15'W	2688	6384	ocean	night
G18	30-8-09	FIL49	11º27.10'N	30º54.57'W	2505	6518	L3	night
G19	30-8-09	FIL51	11º36.18'N	31º8.27'W	2888	5473	ocean	day
G20	30-8-09	FIL52	11º54.11'N	31º9.89'W	2983	7214	ocean	night
G21	31-8-09	FIL53	11º30.88'N	30º52.70'W	2891	6188	L3	night
G22	3-9-09	FIL55	10º36.35'N	30º37.43'W	2006	7774	upwelling	day

Table A.3. Morphological variables measured on the cephalopod paralarvae collected during CAIBEX-I and III that were identified genetically: total length (TL), width (W), dorsal mantle length (DML) and tentacle length (TeL).

Survey	Species	n	TL	W	DML	TeL
CAIBEX I	*Alloteuthis media*	4	4.52 (2.88 – 5.52)	1.52 (1.13 – 1.9)	3.13 (2.11 – 3.66)	1.13 (0.61 – 1.61)
	Alloteuthis subulata	10	8.55 (3.59 – 12.22)	2.70 (1.52 – 3.77)	5.93 (2.67 – 8.17)	2.15 (0.67 – 3.43)
	Illex coindetii	1	3.66	1.63	2.72	0.90
	Loligo vulgaris	2	5.99 (5.18 – 6.79)	2.04 (1.84 – 2.25)	4.10 (3.69 – 4.51)	1.58 (1.34 – 1.81)
	Octopus vulgaris	88	2.58 (1.99 – 3.58)	1.42 (1.14 – 1.85)	1.89 (1.45 – 2.76)	
	Sepiola tridens	5	8.49 (4.97 – 18.18)	4.64 (3.22 – 9.32)	4.93 (3.22 – 9.77)	2.76 (1.17 – 7.09)
	Todarodes sagitattus	2	4.28 (2.48 – 6.08)	1.89 (1.53 – 2.26)	3.53 (2 – 5.07)	0.77 (0.58 – 0.97)
	Todaropsis eblanae	12	2.98 (2.37 – 5.36)	1.52 (0.98 – 2.88)	2.34 (1.65 – 3.88)	0.59 (0.35 – 1.2)
CAIBEX III	*Abraliopsis morisii*	8	7.03 (3.01 – 15.65)	1.34 (0.38 – 2.03)	3.25 (1.04 – 6.08)	4.00 (1.32 – 8.77)
	Alloteuthis media	100	5.45 (1.85 – 13.2)	1.59 (0.55 – 3.59)	3.69 (1.27 – 9.05)	1.30 (0.33 – 2.83)
	Alloteuthis subulata	8	7.42 (3.9 – 12.29)	2.38 (1.33 – 3.73)	5.25 (2.97 – 8.71)	1.54 (0.68 – 2.72)
	Ancistrocheiridae	10	4.97 (2.77 – 9.51)	1.58 (0.84 – 3.13)	3.07 (1.69 – 6.45)	1.71 (0.48 – 3.75)
	Ancistroteuthis lichtensteinii	18	4.62 (2.47 – 10.62)	1.33 (0.62 – 2.74)	3.43 (1.78 – 8.1)	1.00 (0.44 – 1.96)
	Brachioteuthis riisei	18	7.39 (4.05 – 16.09)	1.8 (1.14 – 3.01)	5.19 (2.43 – 10.05)	1.89 (0.75 – 5.53)
	Gonatopsis japonicus	1	14.36	2.54	8.07	5.37
	Heteroteuthis dispar	2	7.31 (6.7 – 7.91)	3.13 (3.01 – 3.24)	2.65 (2.34 – 2.95)	3.88 (3.8 – 3.96)
	Liocranchia reinhardtii	2	14.33 (5.91 – 22.76)	2.41 (0.98 – 3.83)	8.74 (3.67 – 13.82)	5.48 (2.19 – 8.77)
	Octopus vulgaris	33	2.59 (1.66 - 5)	1.39 (0.99 – 2.74)	1.85 (1.21 – 3.04)	
	Ommastrephidae	1	1.79	0.51	0.93	0.69
	Pyroteuthidae	2	6.57 (3.88 – 9.27)	3.03 (1.51 – 4.66)	3.95 (2.26 – 5.63)	2.31 (1.31 – 3.31)
	Pyroteuthis margaritifera	11	5.87 (1.4 – 13.37)	2.45 (0.51 – 9.24)	3.28 (0.97 – 6.55)	1.66 (0.37 – 4.01)
	Rondeletiola minor	5	4.11 (1.93 – 5.97)	1.34 (0.58 – 2.12)	1.90 (0.98 – 2.69)	1.83 (0.78 – 2.9)
	Sepiola atlantica	1	5.26	2.01	3.01	1.73
	Sepiola ligulata	1	5.25	2.40	2.90	1.72

Fig. A.1. Kimura 2 parameter distance model tree obtained from BOLD database to assess the phylogenetic position of the ten Ancistrocheiridae paralarvae (in red) collected on CAIBEX-III. Sequences in black are private and cannot be accessed while sequences in blue are publicly available.