



Paralarvae of cephalopods in channels and fjords of the southern tip of Chile (46–53°S)



M. Cecilia Pardo-Gandarillas^{a,*}, Christian M. Ibáñez^b, J. Francisco Ruiz^c,
Claudia A. Bustos^d, Fabiola A. Peña^e, Mauricio F. Landaeta^d

^a Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

^b Departamento de Ecología y Biodiversidad, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello, Santiago, Chile

^c Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile

^d Laboratorio de Ictioplancton (LABITI), Facultad de Ciencias del Mar y de Recursos Naturales, Universidad de Valparaíso, Chile

^e Faculty of Life Sciences, Utrecht University, Utrecht, The Netherlands

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ABSTRACT

Cephalopods are common in Chilean waters; however, there is little information on the distribution and abundance of their early life stages. In the austral spring seasons of 2008 and 2009 zooplankton samples were collected during two oceanographic cruises (CIMAR 14, CIMAR 15) in Patagonian fjords and channels of southern Chile between 46°S and 53°S. Zooplankton sampling was conducted at 82 stations with Bongo (60 cm diameter, 300 μm mesh size) and Tucker (1 m² diameter, 300 μm mesh size) trawl nets. The paralarvae were preliminarily identified by morphology and pigment patterns. Subsequently, their identity was confirmed through mitochondrial DNA (16S rRNA and COIII) analysis comparing them with the common adult octopus species from Chile which also have planktonic paralarvae (*Octopus mimus*, *R. fontaniana* and *Enteroctopus megalocyathus*) by phylogenetic analysis. We obtained 12 octopus and 1 squid paralarvae in 2008 and 16 octopus and 1 squid paralarvae in 2009. The paralarvae were caught exclusively in areas with higher oceanic influence and were absent in areas with greater freshwater discharge and/or ice melting. Southern Chile is characterized by geomorphological discontinuity due to fragmentation of the south coast starting at 41°30'S, where there is a large freshwater influence in estuaries promoting fjord-like characteristics. Thus, it is not surprising that cephalopod paralarvae were not found in such places, because they are stenohaline. Both morphometric and molecular data confirmed that 26 paralarvae belonged to *R. fontaniana*; one paralarva was identified as *Enteroctopus megalocyathus*; *Doryteuthis gahi* and Onychoteuthidae were also collected. Our results expand the known range of cephalopod paralarvae to areas where they have never been reported in the southern tip of Chile. The presence of paralarvae in these fjords supports the hypothesis of their use as spawning grounds for many species of squid and octopus of different families.

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1. Introduction

The extreme south of South America constitutes the marine unit of the Magellan Biogeographical Province (Boschi and Gavio, 2005; Spalding et al., 2007; Balech and Ehrlich, 2008). This province is characterized by a cold biota of Tertiary origin (Camus, 2001) and a Subantarctic fauna; the Pacific and Atlantic coasts are very similar (Boschi and Gavio, 2005; Balech and Ehrlich, 2008). The Pacific coast, from north of Chiloé Island (~42°S) to Cape Horn (~56°S), is described as a large insular system (the Chilean Archipelago) char-

acterized by numerous gulfs, fjords, channels, small river basins and many conspicuous glaciers, which are the product of the tectonic processes and ice ages; this makes it a complex landscape with difficult access both by land and by sea (Pickard, 1971; Camus, 2001). Little information is available about the biodiversity, biogeography and physical and ecological processes of this Pacific area, in contrast with what is known for the Atlantic side (Pickard, 1971; Silva and Neshiba, 1979; Strub et al., 1998; Acha et al., 2004). The geomorphological discontinuity of this zone (from 41° 30'S to the south) produces an elevated input of fresh water into the coastal water masses; conditions are estuarine, with high precipitation, large tidal flux, low temperature and low salinity (Ahumada et al., 2000; Camus, 2001; Ibáñez et al., 2009). These hydrological conditions generate a semi-isolated system in the fjords, which according

* Corresponding author.

E-mail address: pardogandarillas@gmail.com (M.C. Pardo-Gandarillas).

to Antezana (1999) could affect the continuity of the distribution range of species. However, Lancellotti and Vásquez (2000) suggest that the low salinity condition in the interior waters of fjords which is the product of precipitation and continental rivers would be progressively re-established at depths of 20 m, and in the gradient from east (interior waters) to west (exposed western coast).

During the last 20 years the CIMAR-Fiordos cruises have produced a substantial increase in knowledge of the biological diversity of plankton (Avaria et al., 1997, 1999; Palma and Rosales, 1997; Mujica and Medina, 1997; Balbontín and Bernal, 1997; Palma et al., 1999; Bernal and Balbontín, 1999), fish (Pequeño and Lamilla, 1997; Pequeño, 1999, 2000), decapod crustaceans (Retamal and Gorny, 2001), paralarvae of cephalopods (Vega et al., 2000; Carrasco et al., 2012) and other mollusks of the interior channels (Osorio and Reid, 2004; Osorio et al., 2005) and the exterior or oceanic zones (Osorio et al., 2006). The studies performed in the exterior channel (Tuamapu, King, Memory, Ninualac, Darwin and Pulluche, with direct connection to the Pacific Ocean) have suggested that these are natural migration routes of marine species between the open ocean and interior waters; and where supposedly the processes of reproduction, spawning, larval development and growth occur, which are vital for the maintenance of these ecosystems (Osorio et al., 2006). The extraordinary reproductive activity (greater concentrations of recently hatched eggs, larvae and paralarvae) of marine fish and cephalopods observed (in early pelagic stages) in strongly stratified areas near frontal zones (Balbontín and Bernal, 1997; Vega et al., 2000; Bustos et al., 2008, 2015; Landaeta and Castro, 2006) has led to the proposal that these are areas where a mixing of species of different origin occur (oceanic, pelagic, demersal and intertidal) (Landaeta and Castro, 2006). The presence of early stages of cephalopods (paralarvae), in particular, is an indication that the channels and fjords could be important areas for incubation and growth, not only for benthonic octopuses, but also for other species of cephalopods which inhabit this region (Carrasco et al., 2012).

Cephalopods are common in the southeast Pacific coast; they have a wide oceanic and coastal distribution (Rocha, 1997; Ibáñez et al., 2009). Their maturity and sexual development are linked to environmental factors including temperature, salinity and photoperiod (Hanlon and Messenger, 1996; Boyle and Rodhouse, 2005). A female may deposit from a few to over a thousand eggs, depending on the species; these hatch as miniature cephalopods commonly called paralarvae, which do not undergo metamorphosis (Sweeney et al., 1992; Jereb and Roper, 2005). The majority of young cephalopods in the early stages of growth, are difficult to capture, due to the lack of adequate sampling methods; their identification to the level of species continues to be uncertain or inadequate (Sweeney et al., 1992; Rodhouse et al., 1992; Vecchione, 1987; Vega et al., 2000; Boyle and Rodhouse, 2005).

The taxonomic keys to identify paralarvae usually use adult characters and patterns of distribution, size and number of chromatophores in different body regions as taxonomic characters which allow the identification of species (Kubodera and Okutani, 1981; Young et al., 1989; Hochberg et al., 1992; Messenger, 2001; Ortiz and Ré, 2010). Also, other morphological characters such as the relative length of different arms and the number, arrangement and relative size of suckers could be useful to separate species (Young et al., 1989; Hochberg et al., 1992). The spawning sites of adult females have also provided information about biogeographic patterns and reproductive characteristics (Boletzky, 1998; Barón, 2001; Ortiz et al., 2006), and with this avoiding further potential erroneous identification of species and excessive simplification of the biological diversity in a region (Villanueva and Norman, 2008). A final very useful tool for the identification of paralarvae is the use of DNA sequences (Gilly et al., 2006; Wakabayashi et al., 2006,

2007), since they can be compared with adult individuals and/or sequences obtained from GenBank.

The objective of this study is to provide the first morphological and genetic characterization for the specific identification of the cephalopod paralarvae from Patagonian fjords and channels in southern Chile.

2. Materials and methods

2.1. Sample collection and oceanographic data

From the first to 19 November 2008 and from 11 October to 19 November 2009 sampling was performed in the fjords and channels of the Chilean Archipelago (XI Región), during two oceanographic cruises in the AGOR Vidal Gormaz, as part of the program CIMAR-Fiordo 14 and 15. The first cruise, CIMAR 14 (2008) was between 47° and 50°S latitude and the second cruise CIMAR 15 (2009) was between 50° and 53°S. On these cruises 44 stations were sampled in 2008 and 38 in 2009 (Fig. 1). At each station, environmental data from the water column (temperature, salinity and density) were collected from the surface down to 800 m depth or 10 m above the seafloor using a Seabird SBE-19CTD profiler. Plankton samples were taken with oblique tows using a Bongo net with 60 cm mouth diameter (mesh size) and a calibrated TSK flow meter, from the surface to 200 m depth or 10 m above the bottom. Some plankton samples were fixed in 95% alcohol (19), others in 4% formaldehyde buffered with sodium borate (11). The mantle tissue was extracted from each paralarva for DNA analysis.

The two transects shown in Fig. 2 were made in order to estimate the oceanographic conditions in the water column of the studied area, one from Penas Gulf (open ocean) to Baker channel (river input influence, ~47–48 °S, Fig. 2A,B) and the other from Trinidad Gulf (open ocean) to a large glacier, the Southern Ice Field (ice melting influence, ~50–51 °S, Fig. 2C,D).

The relationship between presence and absence of paralarvae and the environmental variables (temperature and salinity) was explored with Generalized Linear Models (GLM) in the R package (R Development core Team, 2015). Therefore, to analyze paralarvae presence/absence, we used a binomial distribution with a logit link function. Akaike's information criterion (AIC) was estimated to explore which environmental variables best fit with the presence/absence of paralarvae.

2.2. Morphology of the paralarvae

The preliminary identification of the paralarvae was performed visually using bibliographic information, mainly Sweeney et al. (1992), Ortiz et al. (2006) and Ortiz and Ré (2010). The following body characters were measured (mm): total length (TL), dorsal mantle length (DML), ventral mantle length (VML), mantle width (MW), head width (HW) and length of the left (ALL 1–4) and right (ALR 1–4) arms. The number of suckers on the arms (ASC 1–4) and the number of chromatophores on the funnel, mantle (dorsal and ventral), head (dorsal and ventral) and arms were also recorded.

2.3. Molecular analysis

DNA was extracted from mantle tissue using the salting-out technique (Alhjanabi and Martinez, 1997). The primers used were those proposed by Allcock et al. (2008) to amplify the mitochondrial genes cytochrome oxidase III (COIII) and 16S rRNA. PCR amplifications for each paralarvae used the following protocol: 0.3 µl *Taq* DNA polymerase (1.5 U), 2.5 µl 10X (50 mM KCl, 10 mM Tris-HCl, pH 8.0) commercial buffer, 2 µl dNTPs (10 µM), 1.0 µl 50 mM MgCl₂ and 0.5 µl (10pg/µl) of each primer (CO3FP and CO3RP, 16SFP and 16SRP, Allcock et al., 2008). The optimum amplification conditions

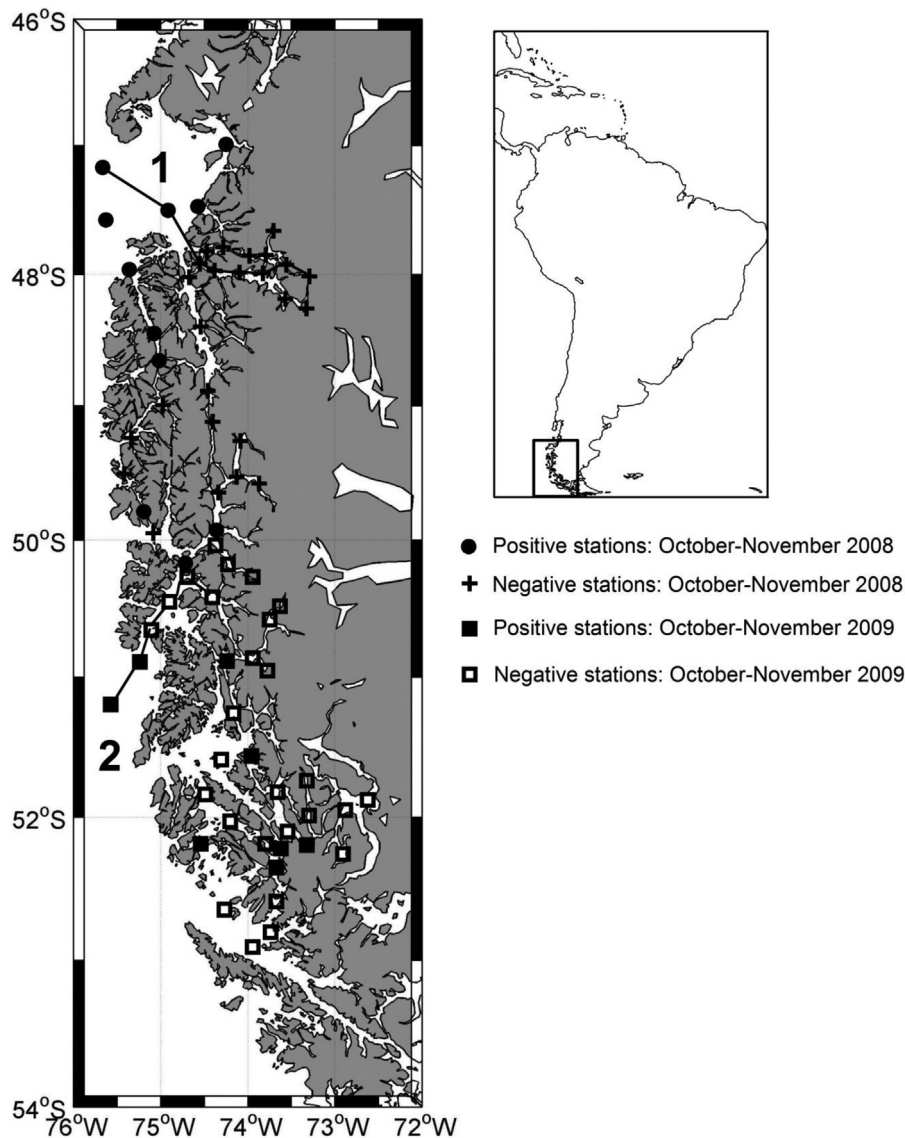


Fig. 1. Spatial distribution of the cephalopod paralarvae collected in the CIMAR 14 and 15 cruises. Lines 1 and 2 correspond to transects that are show in Fig. 2.

were used in a thermocycler: initial denaturation at 94 °C for 3 min, followed by 35 amplification cycles of denaturation at 94 °C for 40 sec, annealing at 40 °C (40 s) for COIII and 55 °C (40 s) for 16S rRNA and 72 °C for 60 s, with a final extension at 72 °C for 7 min (Allcock et al., 2008). The amplicons were purified and sequenced by Macrogen, Inc. (South Korea). Finally, the sequences were edited and aligned by eye using the program ProSeq version 2.9 (Filatov, 2002). The same program was used to compare sequences of paralarvae obtained with adult *R. fontaniana* (Coquimbo, Valparaíso and Lenga), *Octopus mimus* (Antofagasta and Caleta Punta Arenas) and *Enteroctopus megalocyathus* (Ancud and Puerto Williams), since they also have planktonic paralarvae. Only 16 of the 26 paralarvae of *R. fontaniana* were preserved in alcohol, DNA analysis was performed with these. We obtained genetic information for two genes (16S rRNA + COIII) from five paralarvae, which were used for an analysis of combined genes to perform phylogenetic analysis.

To evaluate evolutionary relationships we used a phylogenetic hypothesis based on a Bayesian framework using Mr. Bayes v3.2 (Ronquist et al., 2012) to obtain a sample of trees. The phylogenetic reconstruction was performed using the concatenated (16S rRNA + COIII) data matrix. The model of evolution which best adjusted to the data with the Akaike (CIA) criterion was

the GTR + Γ + I model (AIC = 4067.15, $-\ln L$ = 2016.57), which was determined in JModelTest (Posada, 2008). A Bayesian analysis was performed using four chains with temperatures by default, each with 5 million generations, with sampling every 1000 generations. The runs were checked with likelihood convergence in the program Tracer version 1.5 (Rambaut and Drummond, 2009). The first 500 trees of each run were burned, and a consensus tree was calculated from the remaining trees for the final result. Finally, the FigTree 1.3.1 program (Rambaut, 2009) was used to edit the trees. *Vampyroteuthis infernalis* was used as an outgroup in the construction of these trees. The sequences generated in this study will be available in GenBank (Table 1).

3. Results

3.1. Physical settings

Water column structure was variable depending on the coastal geography and topographic features of the area. The open ocean (left side of each panel in Fig. 2) showed a vertically mixed water column, both in temperature and salinity (around 8–10 °C and 32 PSU at 47–48 °S, Fig. 2A,B; 7.5 °C and 32–33 PSU at 50–51 °S,

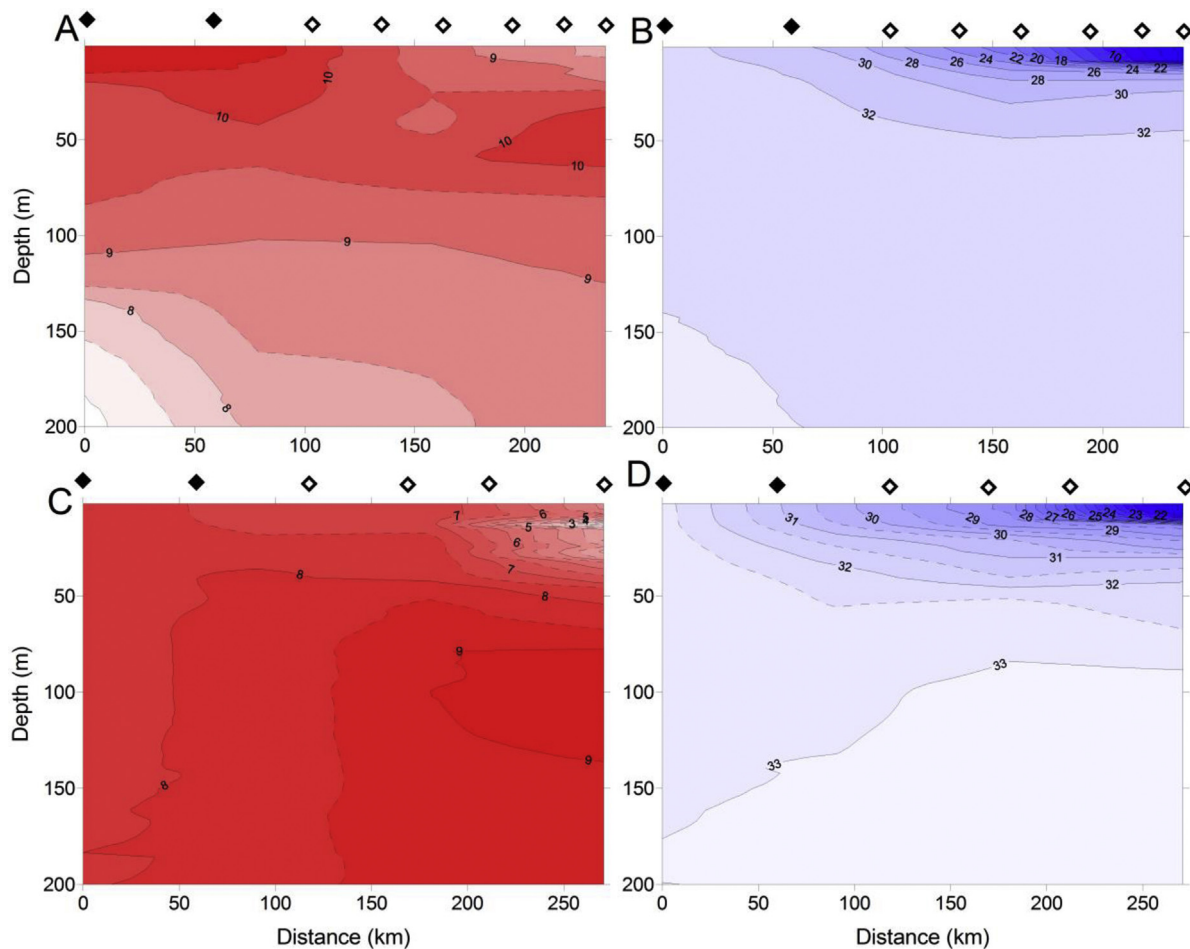


Fig. 2. Vertical sections transects of hydrographic conditions of the water column in the studied area. A, C, temperature ($^{\circ}\text{C}$) and B, D, salinity in southern Chile. October–November 2008 (A, B, transect 1) and October–November 2009 (C, D, transect 2). Diamonds in the upper panel indicates location of bio-oceanographic stations, and color (black, white) indicate time of the sampling (night, day, respectively). See Fig. 1 for general location of the transects.

Table 1

Species collected in particular geographical locations which were used in the phylogenetic analysis. GenBank accession numbers for each specimen are presented.

Species	Latitude	Longitude	16S rRNA	COIII
<i>Octopus mimus</i> Antofagasta 1	23°27'19.26"S	70°37'7.11"W	KT314270	KT314265
<i>Octopus mimus</i> Antofagasta 2	23°27'19.26"S	70°37'7.11"W	KT314271	KT314266
<i>Octopusmimus</i> Caleta Punta Arenas 1	21°38'1.79"S	70°8'40.41"W	KT314272	KT314263
<i>Octopusmimus</i> Caleta Punta Arenas 2	21°38'1.79"S	70°8'40.41"W	KT314273	KT314264
<i>Enteroctopus megalocyathus</i> Ancud	41°47'58.59"S	73°50'44.73"W	KT314274	KT314267
<i>Enteroctopus megalocyathus</i> Puerto Williams 1	54°54'21.69"S	68°24'36.93"W	KT314275	KT314268
<i>Enteroctopus megalocyathus</i> Puerto Williams 2	54°54'21.69"S	68°24'36.93"W	KT314276	KT314269
<i>Robsonellafontaniana</i> Coquimbo 1	29°26'56.40"S	71°18'19.63"W	KT314286	KT314256
<i>Robsonellafontaniana</i> Coquimbo 2	29°26'56.40"S	71°18'19.63"W	KT314287	KT314257
<i>Robsonellafontaniana</i> Valparaíso	33°01'04.94"S	71°38'20.64"W	KT314282	KT314252
<i>Robsonellafontaniana</i> Lenga 1	36°45'38.72"S	73°10'25.85"W	KT314283	KT314253
<i>Robsonellafontaniana</i> Lenga 2	36°45'38.72"S	73°10'25.85"W	KT314284	KT314254
<i>Robsonellafontaniana</i> Lenga 3	36°45'38.72"S	73°10'25.85"W	KT314285	KT314255
Paralarvaestation 80-3	49°55'48.00"S	74°58'12.00"W	KT314279	KT314260
Paralarvaestation 80-4	49°55'48.00"S	74°58'12.00"W	KT314280	KT314261
Paralarvaestation 80-5	49°55'48.00"S	74°58'12.00"W	KT314281	KT314262
Paralarvae estación 89	43°36'36.00"S	74°58'48.00"W	KT314278	KT314259
Paralarvaestation 44	50°28'48.00"S	75°07'12.00"W	KT314277	KT314258

Fig. 2C,D). In longitudinal channels each water column was vertically stratified by salinity in the north and south studied areas, showing strong vertical gradients (i.e., halocline) at 20–25 m depth (Fig. 2B,D). Temperature inside channels was rather stable, from 8.5 to 10 $^{\circ}\text{C}$ and 7 to 8.5 $^{\circ}\text{C}$ in the north and south area, respectively. Areas influenced by river discharge and ice melting showed strong horizontal and vertical gradients. Salinity showed the lowest val-

ues in areas near the Baker River, and the freshwater input caused large salinity gradients (Fig. 2B). Near the glacier, low temperatures (1.4 $^{\circ}\text{C}$) and salinities (14.62 PSU) were detected (Fig. 2C,D), producing water with low density (11.47 units of sigma-t). Also, a thermal inversion was observed in the vicinity of the glacier between 5 and 20 m, with low water temperatures (1–3 $^{\circ}\text{C}$) caused by ice melting (Fig. 2C).

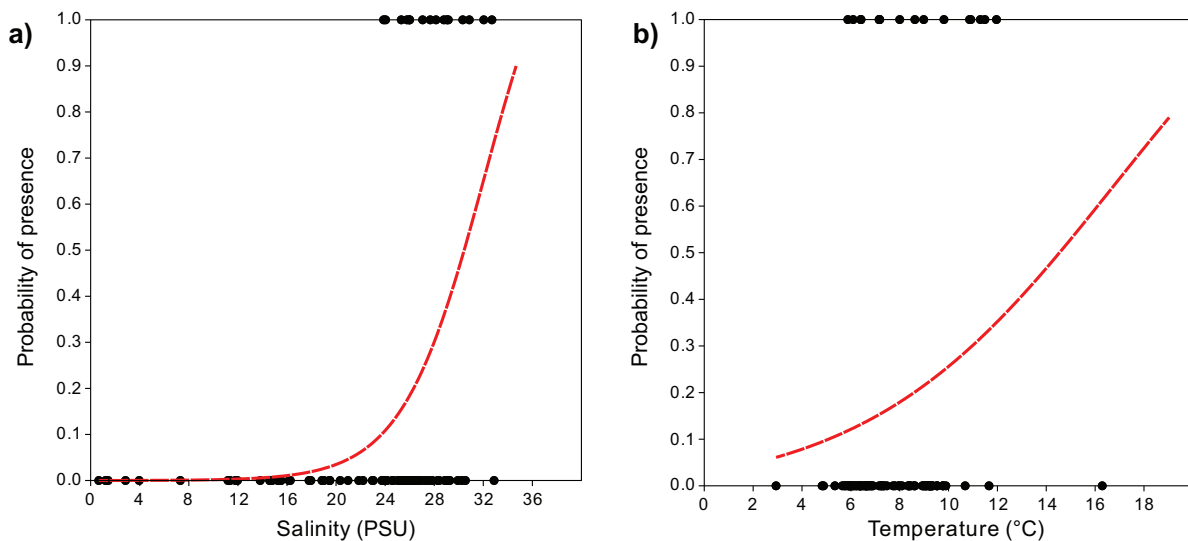


Fig. 3. Relationship between probability of presence/absence of paralarvae and salinity and temperature.

Table 2

Results of Generalized Linear Models (GLM) applied to presence/absence of paralarvae as function of salinity and temperature.

	Estimate	S.E.	Z-value	P	AIC
Salinity	0.315	0.107	2.959	0.003	69.15
Temperature	0.237	0.136	1.745	0.080	84.32

The Generalized Linear Models (GLM) showed that the salinity emerged as the strongest predictor for the presence/absence of paralarvae (Table 2); and that the probability of capture increased as salinity increased from 24 to 32 PSU (Fig. 3). However, temperature did not show a significant relationship with presence/absence of paralarvae (Table 2, Fig. 3).

3.2. Paralarvae morphology

The majority of the paralarvae obtained in the cruises CIMAR-Fiordo 14 and 15 belong to the Octopodidae family; only one member of the Loliginidae and Onychoteuthidae family were found. The squid paralarvae of Onychoteuthidae could not be identified morphologically to the level of genus or species, nor could genetic analysis be performed, since they were preserved in formalin. Of all the octopus paralarvae, 26 were identified morphologically as *R. fontaniana*, and one large paralarvae as *Enteroctopus megalocyathus*. This last paralarvae was fixed in formalin and could not be sequenced. However, its morphological and morphometric measurements were compared to the paralarvae described by Ortiz et al. (2006) from Argentina.

In the CIMAR 14 cruise a total of 13 cephalopod paralarvae were collected at 10 stations (23.8%). Twelve of the 13 paralarvae were *R. fontaniana*, and one belonged to the Loliginidae family. In the CIMAR 15 cruise a total of 17 paralarvae were sampled from 7 stations (18.4%). Of these 17 paralarvae, fifteen were *R. fontaniana*, one was *Enteroctopus megalocyathus* and the last belonged to the Onychoteuthidae family.

The paralarvae of *Doryteuthis gahi* was large in size (4.75 mm TL); its mantle was longer than wide (ML = 2.75 mm, MW = 1.65 mm), with small lateral fins (0.3 mm) located on the terminal region of the mantle; the arms were short (AL = 0.4–0.75 mm) and the tentacles were twice as long as the arms (1.24 mm) (Fig. 4A). The mantle had 8 large dorsal chromatophores and 29 small ventral chromatophores. The head had 6 dorsal chromatophores and 8 large

ventral chromatophores arranged in three lines; the arms had 2–5 large chromatophores.

The paralarvae of the Onychoteuthidae was large in size (30 mm TL); its mantle was longer than wide (ML = 20 mm, MW = 8 mm), with large lateral fins (7 mm) located on the terminal region of the mantle; the arms were of similar length (AL = 6–9 mm) and the tentacles were twice as long as the arms (12 mm). The mantle had 20 small dorsal chromatophores and 29 small ventral chromatophores. The head had 4 dorsal chromatophores; the arms had 4–6 large chromatophores.

The paralarvae of *Enteroctopus megalocyathus* was large in size (26 mm TL); its mantle was ovoid and similar in length and width (ML = 12.8 mm, MW = 11 mm); the arms were as long as the mantle (AL = 10–12 mm) (Fig. 4B). The mantle had 128 small dorsal chromatophores and 75 small ventral chromatophores. The head had 41 dorsal chromatophores and 18 large ventral chromatophores; the arms had 17–25 small chromatophores and there were 18 small chromatophores on the funnel.

The morphometric and meristic measurements (Table 3) showed that the paralarvae of *R. fontaniana* collected in the CIMAR 14 cruise, had a larger body size (TL = 3.0–8.5 mm, DML = 1.8–5.8 mm), with longer arms and more suckers than those collected in CIMAR 15 (TL = 3.2–6.0 mm, DML = 2.3–4.0 mm). The fixed paralarvae of *R. fontaniana* had chromatophores with the appearance of a group of round to ovalate dots. On the dorsal mantle, small tegumental chromatophores were present in the anterior and posterior margins, but absent in the intermediate regions (Fig. 4C). Over the perivisceral epithelium, 17–20 large tegumental chromatophores covered the dorsal surface of the visceral mass. In the ventral region of the mantle there were 8–10 bands with 41–73 chromatophores (Fig. 4D). In the dorsal region of the head there were 8–14 chromatophores arranged in three lines, while in the ventral region of the head there were two large chromatophores, one on each side, adjacent to the funnel. The funnel had 2 or 3 chromatophores and the arms had 4–9, which were smaller towards the tips.

3.3. Molecular analysis

The results of the phylogenetic analysis confirmed that the majority of the octopus paralarvae belong to the species *R. fontaniana*; the clade formed with the paralarvae and adult octopuses of this species had a high posterior probability (PP = 1.0) (Fig. 5).

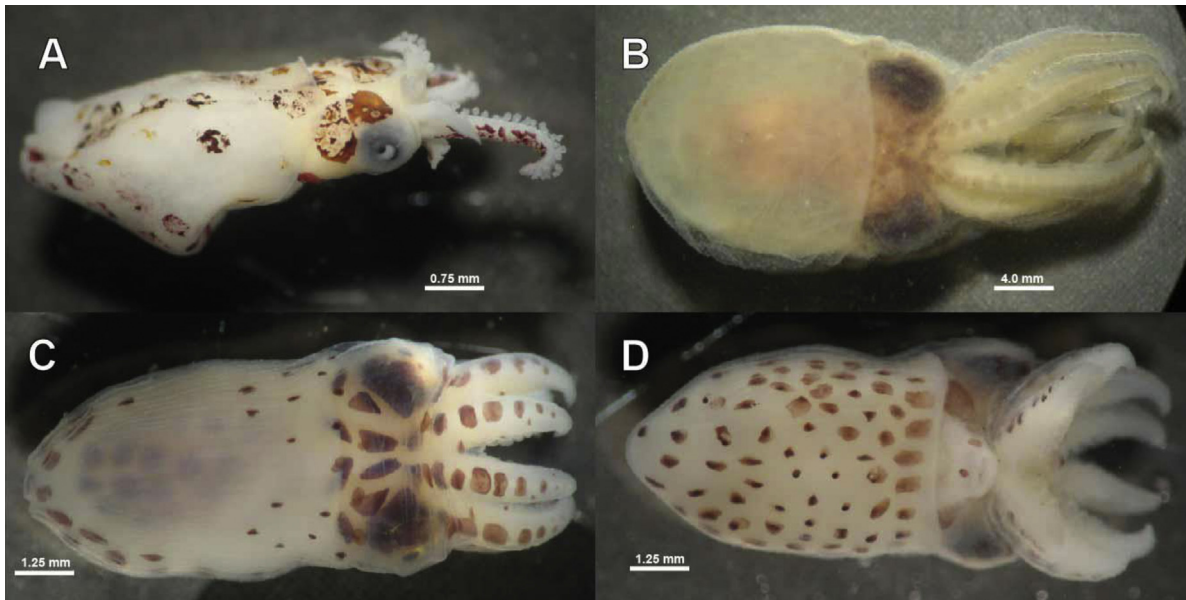


Fig. 4. Imagen of cephalopods paralarvae corresponding to A: dorsal view of Lolliginidae, B: dorsal view of *Enteroctopus megalocyathus*, C-D: dorsal and ventral views of *Robsonella fontaniana* respectively.

Table 3
Morphometry of the paralarvae species collected in CIMAR 14 and CIMAR 15 from Patagonian fjords and channels of southern Chile. The data values are average and standard deviation on brakets. N: individuals number.

	CIMAR 14		CIMAR 15			
Species	<i>R. fontaniana</i>	<i>D. gahi</i>	<i>R. fontaniana</i>	Ocyroteuthidae	<i>E. megalocyathus</i>	
Morphometry (mm)	N= 12	N= 1	N= 16	N= 1	N=1	
Total length	5.73 (2.0)	4.8	5.98 (0.8)	30.0	26.0	
Dorsal mantle length	3.62 (1.2)	2.8	3.7 (0.5)	20.0	12.8	
Mantle length	3.04 (1.0)	2.3	3.05 (0.6)	24.0	10.0	
Mantle width	2.71 (0.7)	1.7	3.23 (0.3)	8.0	11.0	
Head width	2.57 (0.7)	1.4	2.78 (0.3)	6.0	9.0	
Arms length:						
1 right	1.48 (0.6)	0.5	1.97 (0.2)	6.0	12.0	
2 right	1.39 (0.5)	0.8	1.84 (0.2)	8.0	10.0	
3 right	1.5 (0.6)	1.3	1.92 (0.3)	9.0	10.4	
4 right	1.41 (0.5)	0.4	1.85 (0.2)	7.0	10.0	
1 left	1.53 (0.6)	0.5	1.89 (0.2)	6.0	11.0	
2 left	1.47 (0.5)	0.5	1.95 (0.2)	8.0	11.0	
3 left	1.5 (0.5)	1.3	1.93 (0.3)	7.0	10.2	
4 left	1.43 (0.5)	0.4	1.85 (0.2)	6.6	10.0	

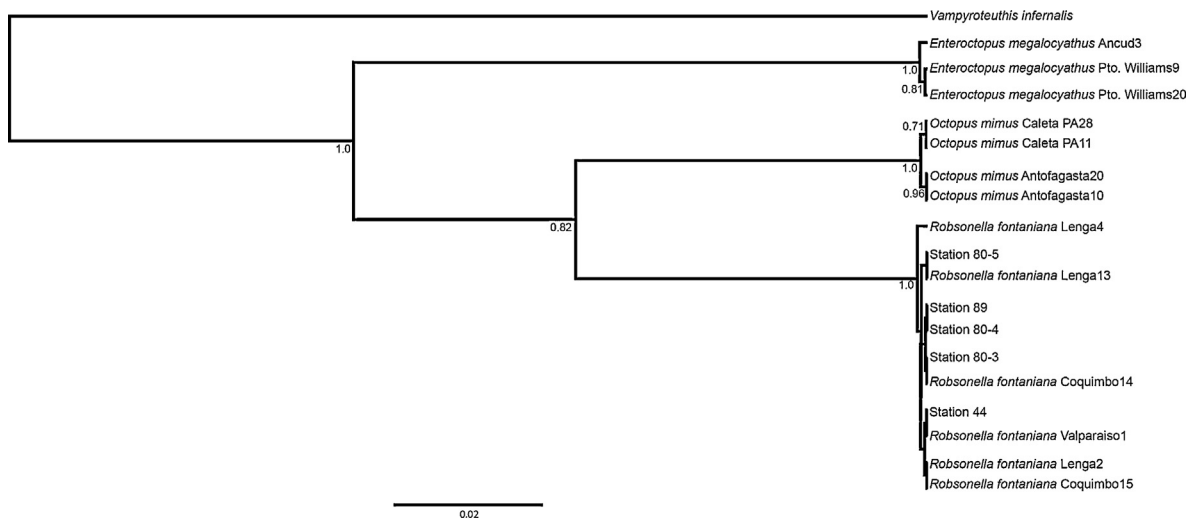


Fig. 5. Consensus phylogenetic tree of the paralarvae of *Robsonella fontaniana* and three species of adult octopus from the southern coast of Chile.

The genetic differentiation between the paralarvae and adults of *R. fontaniana* was very low (0.0–0.1%), while between the paralarvae of *R. fontaniana* and adults of *O. mimus* it was 17.3%, and with adults of *E. megalocyathus* it was 14.2%.

4. Discussion

Fjords are productive ecosystems which connect the open sea with freshwater derived from land drainage and ice melting, and consequently they are characterized by strong fluctuations and spatial variability in salinity and density. In this study we collected four species of cephalopods, mainly in the open ocean and channels of Chilean Patagonia, where high salinity values were also found. At the head of the fjords, where salinity and density of the seawater showed the lowest values and a high vertical stratification, paralarvae were absent. We suggest that the absence of paralarvae in low salinity areas would be due to the fact that most cephalopod species are stenohaline and eurythermic, and thus salinity would be a main determinant of their geographical distribution (Boyle and Rodhouse 2005; Jebb and Roper 2005). Furthermore, some studies suggest that the plankton and micronekton segregate along salinity/density gradients, and differences in salinity alone can potentially affect recruitment success by causing changes in food availability, growth and condition of larval stages of marine organisms (León et al., 2008; Landaeta et al., 2011, 2012).

Only one paralarvae from the Onychoteuthidae family could not be morphologically or genetically identified to the genus or species level. The octopus paralarvae were either identified morphologically as *Enteroctopus megalocyathus* or *R. fontaniana*. The morphometry, distribution, size and numbers of chromatophores in *E. megalocyathus* paralarvae coincided with those reported by Ortiz et al. (2006) from Argentina. The meristic and morphometric data were also congruent with the ranges described by Ortiz and Ré (2010) for *R. fontaniana* from Argentina. The body size of *R. fontaniana* paralarvae found in this study was similar to those found in previous studies performed in more northern latitudes (41–46°S, Vega et al., 2000; Carrasco et al., 2012). According to the records of daily growth in experimental conditions (Uriarte et al., 2010), the paralarvae showed ML between 2.3 and 4.0 mm, thus we could assume that they have a planktonic phase of 40–50 days, which then would change to the benthonic phase between 72 and 78 days later (Uriarte et al., 2010).

This study found the same paralarvae species which have been recorded in other studies made between 41°–45°S (Vega et al., 2000; Carrasco et al., 2012). However, our paralarvae were caught in the most southern part of the Patagonian fjords, thus our records extend the known distribution of paralarvae of these species. Both octopus species *Enteroctopus megalocyathus* and *R. fontaniana* identified in this study are commonly found as adults along the Magellan Province (Ibáñez et al., 2009; Vega, 2009; Ortiz et al., 2006; Ortiz and Re, 2010), and also the Peruvian Province for *R. fontaniana* and the Patagonian squid *Doryteuthis gahi* (Ibáñez et al., 2009; Vega, 2009). The presence of cephalopod paralarvae in fjords and channels reinforces the suggestion that both squids and octopuses use these areas as spawning sites and nursery grounds (Vega et al., 2000; Carrasco et al., 2012). Furthermore, this area also has been suggested as an important spawning and nursery area for larvae of several fish species (Bustos et al., 2008, 2011; Landaeta et al., 2011, 2012).

The genetic distance and phylogenetic analyses confirm that the paralarvae identified morphologically as *R. fontaniana* had a 99% of genetic similarity with *R. fontaniana* adult individuals. Furthermore, our molecular data from paralarvae *R. fontaniana* share the same haplotype with other adult individuals caught in Lenga, Valparaíso and Coquimbo, suggesting that this species has high dis-

persal potential. According to the phylogeny, we can say that *R. fontaniana* is closely related to *O. mimus* rather than to *E. megalocyathus*, the latter belongs to a new family recently described (Strugnell et al., 2014).

5. Conclusion

In this study we found paralarvae of the same species that have been recorded previously in southern Chile. However this study collected paralarvae in the channels of the southern tip of Chile, between 46°S to 53°S. Thus our data expand the known distribution of cephalopod paralarvae to areas where they have never been reported. Also, the presence of cephalopod paralarvae in the channels support the idea that these areas are spawning sites for various species of cephalopods, both squids and octopuses of different families. The absence of paralarvae near the areas influenced by freshwater input and/or ice melting could be the result of vertical advection and/or high mortality rates due to physiological stress.

Conflict of interest

We confirm that there are no conflicts of interest of the authors or for any one type.

Author contributions

Zooplankton samples collected by MFL, JFR and CAB. Morphological identification of samples by CMI and MCP-G. Photographs by JFR. Genetic work lab by FAP. Genetic Analyzed data by CMI and MCP-G. Contributed reagents/materials/analysis tools by CMI, MCP-G and MFL. Wrote the paper MCP-G, JFR, MFL and CMI.

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