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Short Communication

New records of early life-stages of cephalopods in the Chiloé Interior Sea

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ABSTRACT. Early life stages of cephalopods were sampled with zooplankton nets in southern Chile (41°-43°S) during the CIMAR 11 Fiordos cruise, November 2005. A total of 52 individuals were collected, comprising three families (Octopodidae, Sepiolidae, Onychoteuthidae) and four species (*Robsonella fontaniana, Enteroctopus megalocyathus, Semirossia patagonica,* an undetermined Onychoteuthidae species). Paralarvae of Octopodidae have been previously recorded in northern and southern Chile; however, for Sepiolidae and Onychoteuthidae, this is the first record of early life stages in Chilean waters.

Keywords: cephalopods, paralarvae, Octopodidae, Sepiolidae, Onychoteuthidae, fjords, southern Chile.

Nuevos registros de estadios de vida tempranos de cefalópodos en el mar interior de Chiloé

RESUMEN. Estadios tempranos de cefalópodos fueron obtenidos con redes de zooplancton en el sur de Chile (41°-43°S) durante el crucero CIMAR 11 Fiordos en noviembre de 2005. Un total de cincuenta y dos individuos fueron recolectados, comprendiendo tres familias (Octopodidae, Sepiolidae and Onychoteuthidae) y cuatro especies (*Robsonella fontaniana, Enteroctopus megalocyathus, Semirossia patagonica,* y una especie indeterminada de Onychoteuthidae). Si bien existen descripciones previas de paralarvas de Octopodidae para el norte y sur de Chile, este trabajo provee el primer registro de estadios tempranos de vida de Sepiolidae y Onychoteuthidae para aguas chilenas.

Palabras clave: cefalópodos, paralarvas, Octopodidae, Sepiolidae, Onychoteuthidae, fiordos, sur de Chile.

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The life cycle of most nektonic cephalopod species starts with planktonic stages called "paralarvae" (Young & Harman, 1988). At this first stage after hatching, young cephalopods resemble miniature adults; however, their mode of life differs from that of juveniles and older conspecifics (Rocha *et al*., 1999; Barón, 2003). The identification of these young stages is confusing and problematic, mainly due to the inefficiency of nets for sampling juvenile forms, difficulties for recognizing consistent systematic characters, and technological obstacles for rearing early stages in laboratory (Sweeney *et al*., 1992). However, obtaining information on the distribution of paralarval forms in oceanographic surveys is an important component of life-history studies, and can be essential for determining when and where adults spawn, and to establish recruitment patterns for each species (Sweeney *et al*., 1992; Bower *et al*., 1999; Rocha *et al*., 1999).

Several studies have shown that early life stages of many cephalopods species are closely associated with oceanic current systems, where eggs masses, paralarvae and even juveniles, can actively be transported from spawning areas to their feeding grounds (reviewed by Rocha *et al*., 1999). Although such studies are of major relevance, the distribution and role of early life-history stages of cephalopods in marine trophodynamics (*i.e.* as predators or prey) is not yet fully understood (Boyle & Rodhouse, 2005; Jackson *et al*., 2008).

For most of the ~80 cephalopod species recorded in Chilean waters (Rocha, 1997; Ibáñez *et al*., 2009; Vega 2009), young forms are still not known, and field studies on taxonomy, abundance and distribution of paralarval or juvenile stages remain limited (Vega *et al*., 1999, 2000) (Table 1). The current study provides additional information about taxonomic identification, size structure, abundance and geographic distribution of early life stages of cephalopods collected in a small section of the Chilean fjord region (41°-43°S).

Young stages of cephalopods were collected aboard the vessel AGOR "Vidal Gormaz" during CIMAR 11 Fiordos cruise (November 2005). Sampling was conducted at 19 oceanographic stations between Seno de Reloncaví (41º31'S) and Boca del Guafo (43º39'S), southern Chile (Fig. 1a). Oblique hauls were performed from 200 m depth to the surface using a Bongo net (350 µm mesh; 60 cm diameter) or a Tucker trawl (300 µm mesh; 1 m^2 diameter) equipped with a flowmeter to estimate the volume of sea-water filtered, ranging from 140 m^3 (range: 101 to 179 m^3) in stations where paralarvae were collected, to 98 m^3 (range: 57 to 188 m^3) in stations where paralarvae were not found. Samples were preserved in 5% formalin in sea water solution until further examination. Each individual was identified to the lowest taxonomic level by comparison with descriptions available in the literature (Sweeney *et al*., 1992; Ortiz *et al*., 2006; Bolstad 2008; González *et al*., 2008; Ortiz & Ré, 2010; Uriarte *et al*., 2010) using standardized morphological characters, including dorsal mantle length (DML, mm), number and distribution of suckers (S), tentacular club (TC) and chromatophore patterns. Abundance of paralarvae was expressed as ind 1000 m^{-3} and their spatial distribution were plotted in a map of the area provided by the Chilean Army. When possible, samples were compared with adult specimens stored at Museo Nacional de Historia Natural, Chile and reported as MNHNCL with their corresponding storage number.

A total of fifty two specimens were collected, from the families Octopodidae D'Orbigny, 1840; Sepiolidae Leach, 1817 and Onychoteuthidae Gray, 1849 (Table 2). The highest abundances and spatial distributions were observed in oceanic stations (*e.g.* 38, 47, 49, 50) around Boca del Guafo and Corcovado Gulf, with more than 18 ind 1000 m^{-3} (Figs. 1b-1d). Octo-pods identified as *Robsonella fontaniana* (D'Orbigny, 1834) were the most abundant, with 46 paralarvae ranging from 2.3 to 4.6 mm DML (Table 2, Fig. 1b). These individuals displayed a muscular body, covered by an unpigmented layer (especially smaller sizes) surrounding the funnel, mantle, eyes and arms. The eyes were large, and the mantle was short and rounded (Figs. 2a-2b). Arrangements of brown chromatophores covered the dorsal and ventral surfaces of the head, mantle, arms (~4) and dorsal surface of the perivisceral epithelium (> 18). Arms were shorter than the mantle. All paralarvae presented an uniserial line of four suckers of similar size (Figs. 2c-2d) and larger individuals also presented, in addition to the uniserial line of four, a variable number of small biserial suckers in development (ranging from 2 to 10) depending on the size of the paralarvae.

Four larger octopods were collected (5.0-12.1 mm DML, Table 2, Fig. 1b), and identified as *Enteroctopus megalocyathus* (Gould, 1852). An unpigmented layer also surrounded their bodies, only interrupted by the funnel orifice, mantle aperture, and sucker openings. Eyes were large and prominent. The mantle was muscular, broad and rounded (Figs. 2e-2f, 2f). Bodies were dorsally and ventrally spotted with brown chromatophores; however, less numbers were observed in the ventral surface. Arms had a slightly smaller size compared with the mantle, showing 18-36 biserial suckers (depending of DML) arranged in a zig-zag row that gently decreased in size from the mouth towards the tip (Figs. 2g-2h).

Only one paralarva of Sepiolidae was collected (5.5 mm DML), and identified as *Semirossia patagonica* (Smith, 1881). The mantle was short and broad. Fins were large and separated. Dorsal and ventral surfaces of the body were covered by round and brown chromatophores, except for the edge of the fins (Figs. 2i-2j). Arms were shorter than the mantle, with small biserial suckers markedly enlarged at the medial and distal portion of the series on arms. The TC showed small and numerous suckers that gently decreased in size towards the tips (Figs. 2k-2l).

One 19 mm-DML squid was identified as Onychoteuthidae, but its taxonomic status could not be identified to the species level. The specimen had a muscular body, with a relatively broad mantle pointing out posteriorly. Eyes were large and prominent (Fig. 2m). Dark chromatophores covered the head and dorsal surface of the mantle. The gladius was visible beneath the skin in the dorsal midline (Fig. 2n). Rhombic fins in the posterior end were bisected by the gladius (Fig. 2o). Arms were shorter than the mantle, with stalked suckers (~50-60) decreasing in size from the mouth towards the tip (see schemes:

Table 1. Previous records of early life stages of cephalopods collected along the Chilean coast.
Tabla 1. Previos registros de estadios tempranos de cefalópodos recolectados a lo largo de la costa de Chile.

Date	Area	Family	Species	Number of paralarvae	MDL range (mm)	Reference
1998	Mejillones, northern Chile	Gonatidae	*Gonatus* sp.	6	3.9 - 17	Vega *et al*. (1999)
	(23°S)	Bolitaenidae	*Japetela* sp.	4	2.9 - 9.8	
		Ommastrepidae	Ommastrepidae ind.	1	3.9	
		Chiroteuthidae	*Ciroteuthis* sp.	1	-	
		Cranchiidae	*Teuthowenia* sp.	1	-	
		Octopodidae	Octopodidae ind.	1	-	
1998-99	Canal Moraleda, southern Chile	Octopodidae	*Octopus* sp.	13	2.1 - 8.3	Vega *et al*. (2000)
	(43°S-46°S)	Gonatidae	*Gonatus antarcticus*	1	5	

(-) Unknown, specimens were not in a good enough condition to estimate DML.

Table 2. Early life stages of cephalopods collected in the present study, southern Chile (CIMAR 11 Fiordos cruise, 41°S-43°S).
Tabla 2. Estadios tempranos de cefalópodos recolectados en el presente estudio, Sur de Chile (Crucero CIMAR 11 Fiordos, 41°S-43°S).

Station	Depth (m)	Gear	Date	Family	Species	Number of paralarvae	DML range (mm)
16	0 - 20	Bongo net	8/11/2005	Octopodidae	*Enteroctopus megalocyathus*	1	6.5
				Onychoteuthidae	Onychoteuthidae indet.	1	19
32	0 - 20	Bongo net	8/11/2005	Octopodidae	*Enteroctopus megalocyathus*	1	8.6
				Sepiolidae	*Semirossia patagonica*	1	5.5
38	0 - 100	Bongo net	19/11/2005	Octopodidae	*Enteroctopus megalocyathus*	2	5.3 - 12.1
				Octopodidae	*Robsonella fontaniana*	6	2.3 - 3.1
47	0 - 150	Bongo net	14/11/2005	Octopodidae	*Robsonella fontaniana*	1	3.6
				Octopodidae	*Enteroctopus megalocyathus*	1	5.0
49	0 - 200	Bongo net	14/11/2005	Octopodidae	*Robsonella fontaniana*	3	3.2 - 3.9
50	0 - 70	Tucker trawl	14/11/2005	Octopodidae	*Robsonella fontaniana*	13	2.6 - 4.0
50	0 - 100	Tucker trawl	14/11/2005	Octopodidae	*Robsonella fontaniana*	22	2.5 - 4.6

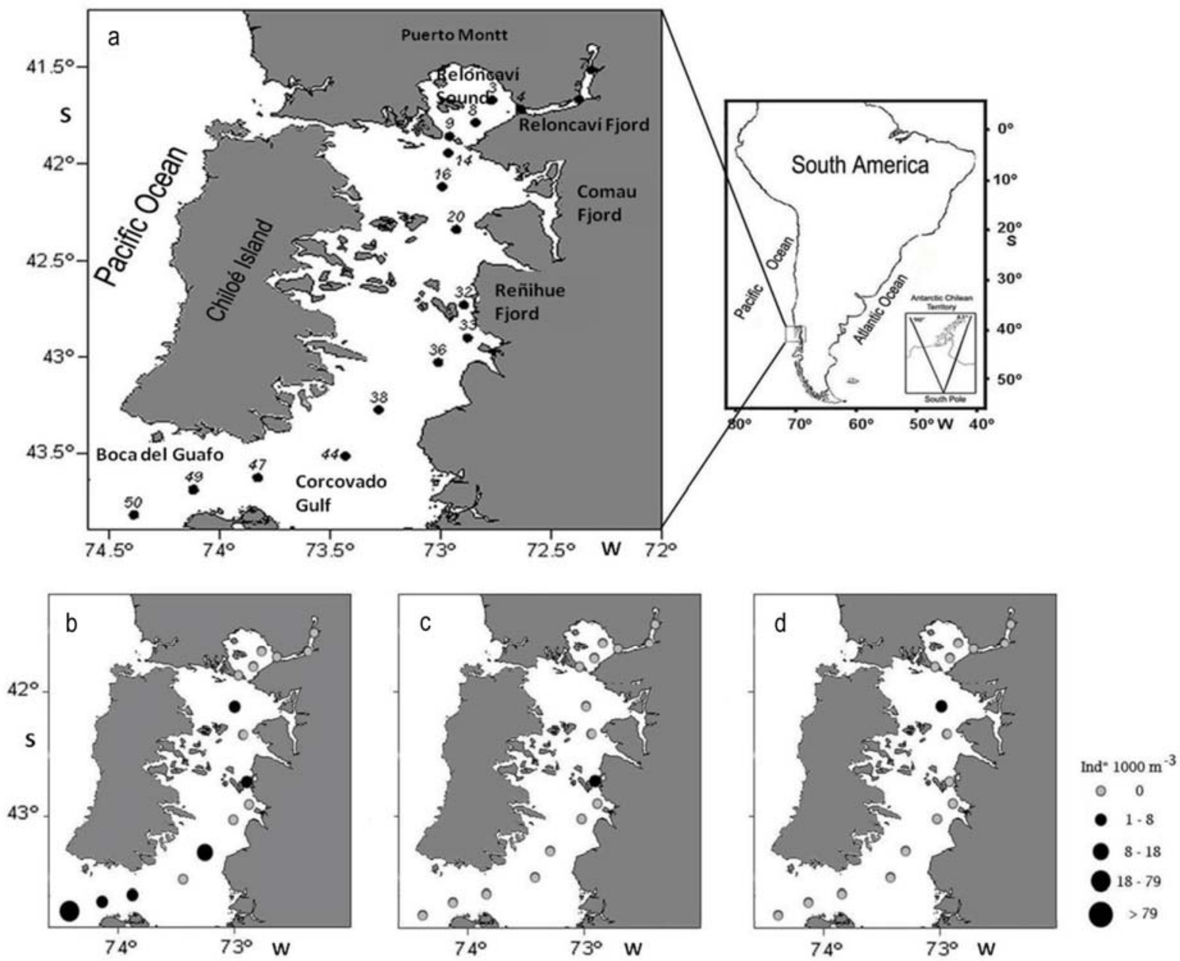

Figure 1. Sampling stations and spatial distribution of early life stages of cephalopods collected during CIMAR 11 Fjords cruise. a) Geographic position, b) Octopodidae abundance, c) Sepiolidae abundance, d) Onychoteuthidae abundance.

Figura 1. Estaciones de muestreo y distribución especial de estadios tempranos de cefalópodos recolectados durante el crucero CIMAR 11 Fiordos. a) Posición geográfica, b) abundancia de Octopodidae, c) abundancia de Sepiolidae, d) abundancia de Onychoteuthidae.

Figs. 2p-2q). Tentacles were slightly longer than arms. TC showed a single row of 9 hooks in the manus, surrounded by ~40 stalked circular suckers with a central aperture. The carpal pad displayed 10 suckers (Fig. 2r).

The early life stages of cephalopods collected during this survey belong to species that are common in Chilean waters; however, with the exception of some octopuses (*e.g*. *O. mimus, R. fontaniana, E. megalocyathus*) and squids (*e.g*. *D. gigas*, *L. gahi*) (Warnke, 1999; Guerra *et al*., 2001; Castro *et al*., 2002; Ortiz *et al*., 2006; González *et al*., 2008; Staaf *et al*., 2008; Ortiz & Ré 2010; Uriarte *et al*., 2010), detailed information on morphological patterns of hatchlings and juveniles has not previously been described. Although the abundance of young cephalopods in our samples seems to be low, similar values have been previously recorded in this geographic area by Vega *et al*. (2000) and Balbontín *et al*. (2004). Causes of these low captures could be their patchy distribution, low densities, or their potential ability to avoid the plankton nets (Sweeney *et al*., 1992). The comparatively large captures of octopods recorded in this survey at oceanic stations could reflect either the active movements of these visual predators in searching for food, or alternatively, an accumulation of paralarvae due to currents between inshore and offshore waters. In fact, currents play a major role in the dynamic of this complex hydrographic system, attaining values of 66.5 cm s^{-1} in areas close to the sampling site (*i.e*. Canal Moraleda, Balbontín *et al*., 2009). The presence of cephalopod paralarvae in previous surveys conducted in this region suggest that Chilean fjords and channels could

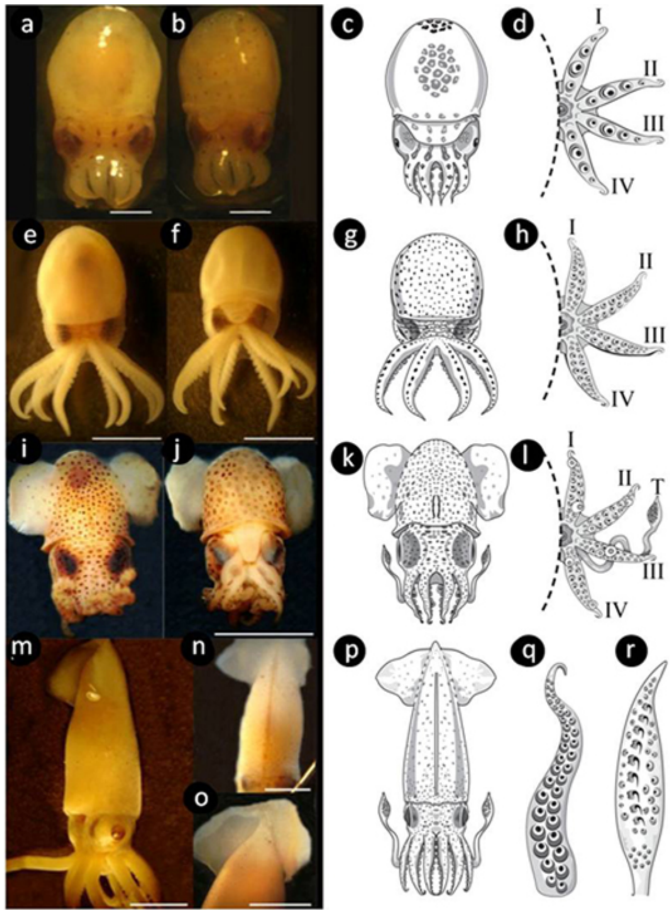

Figure. 2. Early life stages of cephalopods collected during CIMAR 11 Fjords cruise. From top to bottom: Octopodidae, Sepiolidae and Onychoteuthidae. a, b, c, d) *Robsonella fontaniana*, where a and b) dorsal and ventral views, scale bar: 0.75 mm; c and d) external morphology and oral view. e, f, g, h) *Enteroctopus megalocyathus*, where e-f) dorsal and ventral views, scale bar: 10 mm; g-h) external morphology and oral view. i, j, k, l) *Semirossia patagonica*, where i-j) dorsal and ventral views, scale bar: 5 mm; k, l) external morphology and oral view. m, n, o, p, q, r) Onychoteuthidae ind., where m, n, o) complete specimen, gladius visible in dorsal midline and protruding rostrum of gladius, scale bar: 5 mm. p, q, r) external morphology, arm and tentacle, respectively. All schemes maintain the same scale than original samples.

Figura. 2. Estadios tempranos de cefalópodos recolectados durante el crucero CIMAR 11 Fiordos. De arriba a abajo: Octopodidae, Sepiolidae y Onychoteuthidae. a, b, c, d) *Robsonella fontaniana*, donde a-b) vistas dorsal y ventral, escala: 0,75 mm; c-d) morfología externa y vista oral. e, f, g, h) *Enteroctopus megalocyathus*, donde e-f) vistas dorsal y ventral, escala: 10 mm; g-h) morfología externa y vista oral. i, j, k, l) *Semirossia patagonica*, donde i-j) vistas dorsal y ventral, escala: 5 mm; k, l) morfología externa y vista oral. m, n, o, p, q, r) Onychoteuthidae ind., donde m, n, o) espécimen completo, gladius visible en línea media del dorso y rostro sobresaliente del gladius, escala: 5 mm. p, q, r) morfología externa, brazo y tentáculo, respectivamente. Todos los esquemas mantienen las mismas escalas que las muestras originales.

be important for hatching and breeding not only for benthic octopuses, but also for other species of cephalopods inhabiting this particular area.

The morphological characters suggested by Sweeney *et al*. (1992) for species recognition were useful for identifying the young stages collected. Comparisons among laboratory reared paralarvae of the three most common coastal octopuses from Chilean waters: *O. mimus*, *R. fontaniana* and *E. megalocyathus* [DML (1.69 *vs* 3.18 *vs* 8.38), number of suckers (3 *vs* 4-5 *vs* 16-27), number of visceral chromatophores (6-8 *vs* 19-33 *vs* 100-210)] (Warnke, 1999; Castro *et al*., 2002; Ortiz *et al*., 2006; González *et al*., 2008; Ortiz & Ré, 2010; Uriarte *et al*., 2010), in addition with the geographic distribution of adult specimens, allowed us to determine the taxonomic identification of the paralarvae collected. From the three above named species, *O. mimus* is the only one that does not occur in southern Chile (Vega *et al*., 2001; Ibáñez *et al*., 2009; Vega, 2009). From the other two, *E. megalocyathus* is the only large octopod (~1 m total length [TL]) recorded in this area (Osorio *et al*., 2006; Häussermann & Försterra, 2009), with paralarvae around 8 mm DML and a planktonic-benthic period of dispersal (Ortiz *et al*., 2006), which could explain the large size of the paralarvae collected. By contrast, for *R. fontaniana* smaller adult sizes (28 cm TL), smaller hatchling sizes (~4.0 mm TL), extended planktonic periods (~72 days) and large benthic stage size (~11 mm TL) have been recorded (González *et al*., 2008; Ortiz & Ré, 2010; Uriarte *et al*., 2010), supporting the identity of the small paralarvae collected. A skin film covering the body of the paralarvae has been reported in several species of octopuses, including *E. megalocyathus* (see Villanueva & Norman, 2008); however, it was not detected by Ortiz & Ré (2010) in *R. fontaniana* hatchlings. The presence of this loose skin in the samples of *R. fontaniana* collected could be an artefact of the fixative process, and should be re-evaluated in live field-collected individuals. Although the function of this skin film is unknown, it is possible that such layer is used to attain neutral buoyancy, potentially aiding passive dispersion (Villanueva & Norman, 2008).

In relation to Sepiolidae, two species have been recorded in southern Chile, *Rossia glaucopis* and *Semirossia patagonica* (Ibáñez *et al*., 2009; Vega, 2009); however, only adults of the latter are know from the study area (Valdovinos *et al*., 2005; Osorio *et al*., 2006; Häusermann & Försterra, 2009). For *S. patagonica* there is no previously published information about embryonic development or paralarval stages in Chilean waters. Sweeney *et al*. (1992) described for Sepiolidae, subfamily Rossiinae, a size of 5-7 mm in eggs, similar to the size of the young stage collected in this survey. *S. patagonica* might be confused with *R. galucopis*; however, only the former possess enlarged suckers in the arms, a clearly visible trait in both the paralarva collected and in one adult specimen stored at Museo Nacional de Historia Natural, Chile (MNHNCL, N°1979).

For Onychoteuthidae, records from the southeastern Pacific coast (*i.e*. 42°S-56°S) describe the occurrence of five species in Chilean waters (*i.e*. *Onychoteuthis banksii*, *Kondakovia longimana*, *Moroteuthis ingens*, *M. knipovitchi* and *M. robsoni*) (Rocha, 1997; Vega *et al*., 2001; Ibáñez *et al*., 2009; Vega, 2009). A recent taxonomic revision of the family also suggested the occurrence of *Onykia* (*Moroteuthopsis*) *ingens* between 30°S and 60°S, which, however, would be a new combination for *Moroteuthis ingens* (Bolstad, 2008). Despite difficulties in Onycoteuthid's systematics, the juvenile collected in this survey showed the typical morphological characters used to identify members of this family, such as the gladius visible as a dark line running length of the dorsal mantle with a pointed rostrum bisecting the fins. The TC showed a single row of hooks in the manus (double row in adults); however, because hooks begin to develop around 15 mm (Bolstad, 2008), it is possible that they were just partly developed in this juvenile squid, which probably corresponds to *Onykia* sp. (K. Bolstad, *pers. comm.*). Nevertheless, because there is no information available on young onychoteuthis from Chilean waters, detailed studies and sampling are still needed to clarify the specific identity of this individual.

The knowledge of the marine flora and fauna of this fjord region is still in a discovery phase, and has been hampered by difficulties in sampling this relatively isolated area. Results from previous oceanographic surveys (*e.g*. CIMAR 1, 3, 4, 7, 9, 11, 12) and from recent biodiversity expeditions (Häussermann & Försterra, 2009) have substantially increased the information available for this region. The efforts made in the present survey have also provided new and valuable information to partially fulfill the current knowledge gap about early life stages of cephalopods from Chilean and southeastern Pacific waters.

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