Redescription of the flapjack octopod, *Opisthoteuthis bruuni* (Cephalopoda: Opisthoteuthidae) from the southeastern Pacific Ocean and evolutionary relationships of cirrate octopods

M. Cecilia Pardo-Gandarillas^{1*}, Mariana Díaz-Santana-Iturrios², Mark Fenwick³, Roger Villanueva⁴, Christian M. Ibáñez²

1.- Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile.

2.- Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andres Bello, Avenida República 440, Santiago, Chile.

3.- National Institute of Water and Atmospheric Research, Ltd., Wellington, New Zealand.

4.- Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta 37-49, 08003-Barcelona, Spain.

Morphology and phylogeny of *Opisthoteuthis bruuni*

***Corresponding author:** pardogandarillas@gmail.com

CONFLICT OF INTEREST STATEMENT: The authors declare that there is no conflict of interest.

ABSTRACT

Cirrate octopods are considered to resemble the ancestor of all octopuses. Cirrates inhabit the deep ocean and are characterized by the presence of fins, a cartilaginous inner shell and a single row of suckers alternating with pairs of cirri thus comprising uniserial suckers and biserial cirri. The objective of this contribution is to improve the taxonomy of *Opisthoteuthis bruuni* from the southeastern Pacific Ocean and suggest a new hypothesis of cirrate phylogeny. Given that the most complete molecular data set for cirrates available in public databases is comprised almost exclusively of 16S

rRNA gene sequences, we compared morphological and mitochondrial gene 16S rRNA data (generated in this study) from the genus *Opisthoteuthis* from the southeastern Pacific Ocean. Additionally, we sequenced *Opisthoteuthis chathamensis*, *Opisthoteuthis mero* and *Luteuthis dentatus* from the southwestern Pacific, as along with *Cirroctopus mawsoni* from Antarctic waters. The morphological data and the phylogenetic analysis confirm the status of *Opisthoteuthis bruuni* as a member of genus *Opisthoteuthis* and the family Opisthoteuthidae. This re-description includes the first morphological characterization of the male and female adult stage of *O. bruuni*, in addition to reporting geographic and bathymetric range extensions. *Opisthoteuthis bruuni* presents conspicuous morphological features (small terminal fins, short cirri and U-shaped shell). Our phylogenetic tree supports three families: Cirroctopodidae, Cirroteuthidae, and Opisthoteuthidae; which is different from the phylogenetic arrangements previously reported.

KEYWORDS: 16S, cirrata, finned octopods, cephalopods, morphology.

INTRODUCTION

Cirrate octopods inhabit depths ranging from 300 to 7000 m in all oceans of the world (Vecchione *et al.*, 2002; Collins and Villanueva, 2006; Jameson and Vecchione, 2020). The group is characterized by the presence of fins, a cartilaginous inner shell, and a single row of suckers alternating with pairs of cirri thus comprising uniserial suckers and biserial cirri (Voss, 1988a; Collins and Villanueva, 2006). Within the Octopodiformes Berthold and Engeser, 1987, cirrates are considered to resemble the ancestor of all octopuses (Young *et al.*, 1998), thus, knowledge regarding their phylogenetic relationships is crucial to understanding cephalopod evolution.

The classifications and phylogenetic arrangements of cirrate octopods have historically embraced significant modifications in the number, names, and relationships of families. Robson (1932) recognized three cirrate families (Opisthoteuthidae Verrill, 1896, Cirroteuthidae Keferstein, 1866 and Stauroteuthidae Grimpe, 1916) according to morphological observations, which Voss (1998a) later confirmed based on web and shell types. O'Shea (1999) in his monograph carried out

measurements and counts and calculated indices for cirrates, and classified them into the families Opisthoteuthidae and Cirroteuthidae, and introduced the new families Luteuthidae O'Shea, 1999 and Grimpoteuthidae O'Shea, 1999. According to a phylogeny of cirrate octopuses obtained from 16S rRNA gene sequences, Piertney *et al.* (2003) suggested a division of cirrates into four families: Cirroteuthidae, Grimpoteuthidae and Opisthoteuthidae, and a new undescribed family. Based on morphological information, Collins and Villanueva (2006) described the fourth family, the Cirroctopodidae Collins and Villanueva, 2006, and specified which genera for each family. However, Vecchione *et al.* (2016), summarized the work by Piertney *et al.* (2003) and Collins and Villanueva (2006) and suggested modifications to the classifications by proposing the following four families: Opisthoteuthidae, Stauroteuthidae, Cirroteuthidae, and Cirroctopodidae. Despite these contrasting systematic treatments, cirrate classification and recognition of taxa continue to be problematic, especially at the species level. There are gaps in knowledge concerning their biology, ecology and behavior, that make understanding their phylogenetic relationships difficult.

In addition, the most often cited diagnostic morphological traits for cirrate species and families include shell shape, gill shape, and shape of the anterior end of the mantle, but these features were described based on only a few specimens, or the juvenile stages exclusively (*i.e.*, *Opisthoteuthis bruuni* (Voss, 1982), and in some cases, a single specimen in poor condition (*e.g.*, *Luteuthis dentatus* O'Shea, 1999). Hence, these morphological characteristics must be confirmed with analytical methods such as molecular phylogenetic reconstructions in order to evaluate the reliability of their classification.

To achieve this, more molecular sequences of cirrate octopods have been made available in the GenBank repository since the phylogeny proposed by Piertney *et al.* (2003), although most of these sequences are from the mitochondrial 16S rRNA gene. Therefore, a new phylogenetic reconstruction has been carried out by us using this gene and applying accurate phylogenetic models as a first step towards addressing systematic problems with cirrate octopods.

Voss (1982) examined 16 juvenile cirrate octopods collected off Antofagasta in Chile (23° 41'S, 70° 34'W), and concluded that the specimens belonged to the genus *Grimpoteuthis* Robson, 1932. Using this material, he described a new species, *Grimpoteuthis bruuni* Voss, 1982, noting that it was morphologically similar to *Grimpoteuthis grimaldi* (Joubin, 1903), collected in the Atlantic Ocean (Joubin, 1903). However, in a subsequent revision, Villanueva *et al.* (2002) suggested that the generic status of *G. bruuni* needed clarification after reviewing the morphological traits in the original description. Consequently, Collins (2003) assigned the species to the genus *Opisthoteuthis* Verrill, 1883 based on morphological attributes (suckers, web and shell), and hence, the former *G. bruuni* is currently recognized as *Opisthoteuthis bruuni*.

Based on literature reports, museum material and fisheries bycatch, Rocha (1997), Vega *et al.* (2001), Villarroel *et al.* (2001), Ibáñez *et al.* (2009), Vega (2009) and Ibáñez *et al.* (2011) published lists of cephalopods found in Chilean waters and detected the following cirrate octopods: *Cirroteuthis? hoylei* Robson, 1932, *Cirrothauma murrayi* Chun, 1911, *Grimpoteuthis bruuni* and *Opisthoteuthis* sp. However, generally information is scarce, and the taxonomic keys available lack quality, which make identifications difficult and diagnoses doubtful, especially at the species level for *Opisthoteuthis* spp. Therefore, it seems sensible to pool efforts and examine as many cirrate specimens from this area as possible, to determine their identity, and provide information about their phylogenetic relationships. The objective here is to improve the morphological characterization of *Opisthoteuthis bruuni* by analyzing adult specimens deposited in the National Museum of Natural History, Santiago, Chile (MNHNCL), including the specimens collected by Ibáñez *et al.* (2011). In addition, we provide a new phylogenetic reconstruction of the relationships among cirrates and suggest a new systematic and taxonomic treatment.

MATERIALS AND METHODS

For this study we examined three *Opisthoteuthis* females collected by Ibáñez *et al.* (2011) taken as by-catch from commercial shrimp trawling during 2007-2009 on the continental slope off Valparaíso, central Chile, between 33° 03'S, 71° 50'W and 33°

21°S, 71° 54'W, at 337 - 512 m depths (Fig. 1). The specimens were fixed in 96% Ethanol and deposited at the National Museum of Natural History (MNHNCL), Santiago, Chile (specimens MNHNCL 300138 to 300140). The species identifications were determined using the original description by Voss (1982) and the specialized key by Hochberg *et al.* (2016). In addition, other specimens from MNHNCL were examined and identified at the species level by the presence of small terminal fins, short cirri and U-shaped shell, but their poor condition (*i.e.*, incomplete mantle and missing arms and suckers) limited us to recording morphometric data and only two specimens were measured completely.

Material examined

Opisthoteuthis bruuni: A female 39 mm DML (MNHNCL 300138), off Algarrobo coast, Chile, 33°21'03"S, 71°54'07"W, at 504 m depth, March 2007, coll. Diana Párraga. Two females 36 mm (MNHNCL 300139) and 50 mm (MNHNCL 300140) DML, off Algarrobo coast, Chile, 33°21'05"S, 71°54'06"W, at 512 m depth, 15 November 2009, coll. Diana Párraga. A female 35 mm DML (MNHNCL 300014-A) and a male 32 mm DML (MNHNCL 300014-B), off Los Vilos coast, Chile, 31°56'99" S, 71°54'99" W, at 400 m depth, 23 January 1979, coll. María Codoceo. A female 30 mm DML (MNHNCL 300162) off Los Vilos coast, Chile, 31°56'99" S, 71°54'99" W, at 420 m depth, 23 January 1979, st. 1, B/C Goden Wind, coll. María Codoceo. Three unsexed specimens (MNHNCL 5698, 5701, 5702) off Los Vilos coast, Chile, 31°36'S, 71°31'W, at 300-400 m depth, 24 October 1976, st. 1, B/C Goden Wind, coll. María Codoceo. Two unsexed specimens (MNHNCL 300156) off Papudo coast, Chile, 32°31'00"S, 71°22'00"W, at 250-300 m depth, 29 March 1979, st. 3, B/C Goden Wind, coll. María Codoceo. Two unsexed specimens (MNHNCL 300157, 300161) off Papudo coast, Chile, 32°31'00"S, 71°22'00"W, at 250-280 m depth, 29 November 1976, st. 3, B/C Goden Wind, coll. María Codoceo. One unsexed specimen (MNHNCL 5711) off Papudo coast, Chile, 32°31'00"S, 71°22'00"W, at 250-280 m depth, 29 March 1979, st. 3, B/C Goden Wind, coll. María Codoceo. One unsexed specimen (MNHNCL 5697) off Montemar coast, Chile, 33°01'S, 71°38'W, at 300-350 m depth, 10 November 1976, B/C Goden Wind, coll. María Codoceo. One unsexed specimen

(MNHNCL 300220) off Antofagasta coast, Chile, 23°41'00" S, 70°34'00" W, at 400 m depth, 4 August 1999, coll. Juan Villarroel.

Morphometric data analysis

External measurements were recorded following Roper and Voss (1983) as follows: total length (TL), dorsal mantle length (DML), ventral mantle length (VML), mantle width (MW), head length (HdL), head width (HdW), arm length (from 1 to 4 R / L, AL), web depth (sectors A to E, WD), sucker diameter (SD), eye diameter (ED), funnel length (FuL), arm base width (AW), fin length (FL), fin width (FW) and arm sucker count (ASC). Specimens in poor condition were not measured.

Molecular and phylogenetic analyses

A piece of mantle tissue was obtained from two specimens of *Opisthoteuthis bruuni* (MNHNCL 300138 and 300139), one specimen of *Opisthoteuthis chathamensis* O'Shea, 1999 (one unsexed specimen NZ22, Chatham Rise, New Zealand (NZ) at 43°11'00" S, 175°52'00" E, 2012, RV Tangaroa), *Opisthoteuthis mero* O'Shea, 1999 (unsexed specimen NIWA 1060931, Chatham Rise, New Zealand at 43°11'1.8" S, 175°52'27.6" E at 438 m depth, 15 August 2016, st. TAN1511/78, RV Tangaroa), *Luteuthis dentatus* O'Shea, 1999 (unsexed specimen NIWA 95257, Challenger Plateau, Tasman Sea, at -37°17'60" S, 168°18'4" E, 17 February 2016, st. TRIP4618/29, F.V. Voyager) and *Cirroctopus mawsoni* (Berry, 1917) (two unsexed specimens NZ16 and NZ18, from Ross Sea, Antarctica at 75°01'00" S, 175°55'00" E, 2012, RV Tangaroa) deposited in the National Institute of Water and Atmospheric Research (NIWA), New Zealand. Genomic DNA was extracted from tissue samples following the saline extraction protocol (Aljanabi and Martinez 1997). The mitochondrial 16S rRNA gene was sequenced using the primers (16SF and 16SR) used by Allcock *et al.* (2008). Amplifications by PCR were conducted in 25 µl reactions as follows: 0.3 µl of Taq DNA polymerase (1.5 units), 2.5 µl of commercial buffer 10X (50mM KCl, 10mM Tris-HCl, pH 8.0), 1.0 µl 50mM MgCl₂, 2 µl of dNTPs (10 µM), and 0.5 µl (10 pg/µl) of each primer. The thermal cycler conditions were as follows: initial denaturation for 3 min at 94°C, followed by 35 cycles at 94°C (40 s),

52°C (40 s) and 72°C (60 s), and a final extension at 72°C (7 min). PCR products were purified and sequenced by MacroGen Inc. using the same primers employed for PCR. To infer phylogenetic relationships sequences were compared to other 16S rRNA gene sequences from cirrate octopods obtained from GenBank (Table 1). Sequences were aligned using the MUSCLE algorithm implemented in MEGA X software (Kumar *et al.* 2018). Genetic uncorrected distances (p-distance) between species, genera and families were calculated for 16S rRNA gene in MEGA X to determine and compare the genetic variation.

Bayesian inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP) methods were employed to evaluate the phylogenetic relationships among the species. Phylogenetic reconstructions were carried out using GTR+ Γ +I evolutionary model (selected by Akaike Information Criterion (AIC) = 4067.15, -lnL = 2016.57) implemented in MEGA X software. Bayesian analyses were performed using MrBayes v3.2 (Ronquist *et al.* 2012) with four default heated chains, running ten million generations of Markov Chain Monte Carlo (MCMC), and sampling every 1000 generations to obtain parameters and phylogenetic trees. Convergence was checked for runs according to likelihood values and Effective Sample Size (ESS) using the Tracer v1.5 software (Rambaut and Drummond 2009). The first 1000 trees were discarded as burn-in and a consensus of the remaining trees was computed as outcome. Maximum Likelihood and Maximum Parsimony analyses were carried out in PAUP* (Swofford, 2002) using heuristic search and tree bisection-reconnection methods, the node support was estimated using 5000 bootstrap pseudo-replicates (Felsenstein, 1985), from which the final confidence limits were obtained.

Vampyroteuthis infernalis Chun, 1903 (Vampyromorpha) was used as outgroup to root the trees. This species is considered to be the sister group of the Octopodiformes (Lindgren *et al.*, 2012). The sequences generated in this study are available in GenBank (Table 1).

RESULTS

Using the morphological characteristics stated in the original description by Voss (1982) and the specialized key by Hochberg *et al.* (2014), the specimens analyzed in

our study were identified as *Opisthoteuthis bruuni* (Voss, 1982), therefore, we provide the following detailed characterizations:

Systematics

Genus: *Opisthoteuthis* Verrill, 1883

Species: *Opisthoteuthis bruuni* (Voss, 1982)

Synonym: *Grimpoteuthis bruuni* Voss, 1982, pages: 426-432, figures: 1-2.

Diagnosis

Small species of *Opisthoteuthis* (see Verrill, 1883) with moderate antero-posterior body compression (bell-shaped); mantle, head, and aboral surface of arms without spots. Optic lobe spherical, optic nerve passes through the white body (violet in color) (Voss, 1982). Fins flap-like, relatively short (FLI 22.9-41); mantle and nuchal constriction poorly developed; arms long (ALI 42.6-74.7), web nodules absent, web deep (WDI 61.5-180); male with sucker enlargement in proximal field of all arms and distal fields of arms III and IV, enormous near the web edge; enlarged suckers present in males. Suckers few, ASC 21-44.

Description

The specimens measured are small TL 98 to 206 mm. Mantle sacciform (DML 24 to 50 mm) with small fins (FL 6 to 13 mm, Fig. 2A, B, E, F). Body, brown with gelatinous smooth texture (Fig. 2A, B, C, E, F, G). Internal shell U-shaped (Fig. 2D). Head wide (82 to 162% DML). Eyes large, laterally oriented (diameter = 13 to 19 mm) (Fig. 2A, B, E, F). Arms approximately equal in length, almost three times DML (120 to 295% DML) (Table 2), with 21 to 44 suckers of small diameter (1 to 5 mm) and short cirri along arms. Male sucker disks bigger and wider than female sucker disks (Fig. 2C, G). Cirri begin at the first suckers. Webs large (62 to 180% of DML) extending to suckers 18 to 20 (Table 2; Fig. 2B). Measurements (ALI and HWI) and counts (ASC and Gills) are concordant with the original description (Voss, 1982) of *Opisthoteuthis bruuni* (Table 3). Digestive gland large compared to stomach (20 vs 7 mm); esophagus with distinct crop; intestine narrow compared to esophagus (Fig. 3Aa-d).

Gills large (20 mm), half-orange shaped with four primary lamellae per side and six secondary lamellae per demibranch (Fig. 3B). Rostral tip of the upper beak sharp; jaw edge curved and almost perpendicular to jaw angle; shoulder and wing small; free crest barely angled extending to the posterior tip of the crest; crest barely curved, 16 mm in length; hood and rostrum curved (Fig. 3C, D). Rostral tip of the lower beak blunt and short; jaw angles wide, extending to the wing; wing fold not conspicuous; crest 9 mm in length; hood without conspicuous groove (Fig. 3E, F). Male genitalia composed of large terminal organ; convoluted seminal vesicle complex; large and oval accessory gland; and large and oval testicle (Fig. 4Aa-d). Female genitalia composed of one large distal oviduct that leads from an oviducal gland; large ovary containing numerous oocytes of different sizes as follows: minute/small= 0.5-1.5 mm; medium= 2-4 mm; large= 5-7 mm (Fig. 4Ba-c, 4C).

Molecular and phylogenetic analyses

The original 16S rRNA fragment lengths ranged from 450 to 465 bp. After alignment the length of the sequences was 478 bp (including indels), of which 113 bp (23.7%) were parsimony informative. The three phylogenetic analyses (MP, ML and BI methods) produced similar tree topologies with high support in most clades, although it is worth noting that nodes of some *Opisthoteuthis* species lacked phylogenetic resolution according to MP and BI; ML in two nodes within Cirroctopodidae did not show phylogenetic resolution (Fig. 5). The MP analysis resulted in one most parsimonious tree, 252 steps in length (consistency index = 0.60; retention index = 0.88). Most nodes of the parsimony consensus tree had high values (> 90% bootstrap), except for few nodes within the Opisthoteuthidae and Cirroctopodidae clades (Fig. 5). The ML tree topology (-lnL = 2114.19) and its bootstrap support are closer to the Bayesian consensus tree than to the parsimony tree. The Bayesian consensus tree shows high posterior probabilities (> 0.95) in most nodes including those that are not supported by MP and ML (Fig. 5).

The phylogenetic analyses indicate that the three cirrate families (Cirroteuthidae, Cirroctopodidae, and Opisthoteuthidae) are monophyletic (Bootstrap ML and MP > 60%, PP > 0.73, Fig. 5). Overall, the phylogenetic tree is well-supported at the family

and genus level, except for some nodes within the Opisthoteuthidae (Fig. 5). Clade 1 is comprised by *Cirroteuthis* Eschricht, 1836, *Cirrothauma* Chun, 1911 and *Stauroteuthis* Verrill, 1879. Clade 2 includes *Cirroctopus hochbergi* O'Shea, 1999, *Cirroctopus glacialis* Robson, 1930 and *Cirroctopus mawsoni*. Clade 3 includes *Opisthoteuthis*, *Luteuthis* O'Shea, 1999 and *Grimpoteuthis* Robson, 1932 (Fig. 5). *Opisthoteuthis bruuni* is included within the Opisthoteuthidae and *Opisthoteuthis*, which supports its current taxonomic status at the family and genus levels. *Opisthoteuthis bruuni* was found within a subclade comprising species from New Zealand, Chile and Eastern Atlantic; *O. mero* from New Zealand was its sister species. In addition, according to MP, ML, and BI values, the genus *Opisthoteuthis* is a monophyletic group.

The maximum genetic distance between *Opisthoteuthis* species was 5.6 % (Table 4). Within the Opisthoteuthidae, the genetic distances between genera ranged from 6.5 to 7.9 %, in Cirroctopodidae from 4.6 to 4.9 % between *Cirroctopus* species, while in Cirroteuthidae, from 4.6 to 6.7 % between genera (Table 4). By comparing families, the highest genetic distances were found between Cirroctopodidae and Cirroteuthidae (15 %), and the lowest between Cirroteuthidae and Opisthoteuthidae (9.5 %, Table 4).

DISCUSSION

The morphometric and meristic measurements presented here support the identification of three specimens collected by us near Valparaíso (Chile) and eleven from the MNHNCL, also collected off central Chile, as *Opisthoteuthis bruuni*. Previous studies did not describe the adult stage of *O. bruuni*, therefore, here, we describe not only the external morphological attributes of adult *O. bruuni*, but also characterize the internal organs and oocytes. In regard to these features, it may be worth noting that the distal oviduct of the female genitalia is distinctly larger (in width and length) than in other *Opisthoteuthis* (i.e., *Opisthoteuthis calypso* Villanueva, Collins, Sánchez and Voss, 2002, *Opisthoteuthis agassizii* Verrill, 1883, *Opisthoteuthis dongshaensis* Lu, 2010, and *Opisthoteuthis massyae* (Grimpe, 1920), Villanueva *et al.*, 2002; Lu, 2010)

despite the ovarian oocytes being relatively small, similar to those of *O. agassizii*, *O. mero*, *O. chathamensis* and *O. calypso*, and smaller than *O. massyae*, *Opisthoteuthis californiana* Berry, 1949, and *Opisthoteuthis albatrossi* (Sasaki, 1920) (Villanueva *et al.*, 2002; Collins and Villanueva, 2006).

Previous studies analyzed several octopod specimens from northern Chile and Peru and identified them only as *Opisthoteuthis* sp. (Nesis 1987; Villarroel *et al.* 2001; Ibáñez *et al.* 2011; Cardoso and Hochberg 2012). In this study, we examine the only specimen deposited in the MNHNCL by Villarroel *et al.* (2001) and identify it as *O. bruuni* (MNHNCL 300220). Therefore, we infer that all the specimens evaluated by these authors correspond to *O. bruuni*, since they all presented similar morphology according to Villarroel *et al.* (2001).

Opisthoteuthis bruuni was previously only known from the type locality, off Antofagasta, Chile, at a depth range of 250-360 m (Voss, 1982). We now have records from central Chile extending the latitudinal distribution from 5°S – 33°S and the bathymetric distribution as 250 – 512 m. Moreover, the gill morphology of *O. bruuni* is similar to that of other cirrates (e.g. *Opisthoteuthis agassizii*) that inhabit the deep-sea in low oxygen conditions and present large gills and a reduced number of gill lamellae, features adapted to provide an enhanced oxygen intake efficiency (Young and Vecchione, 2002; Collins and Villanueva, 2006). Indeed, these morphological traits are suited to the oceanographic conditions of the water masses inhabited by *O. bruuni*, that have low temperature, medium salinity and low dissolved oxygen (Ahumada *et al.*, 2000).

There are certain aspects concerning the putative species of cirrate octopods reported by Rocha (1997), Vega *et al.* (2001), Villarroel *et al.* (2001), Ibáñez *et al.* (2009), Vega (2009) and Ibáñez *et al.* (2011) for Chilean waters that should be taken into consideration. The first aspect is that Guerra *et al.* (1998) did not recognize *Cirrotheuthis? hoylei* as a valid cirrate species in Chilean waters, instead suggesting that the specimen examined could only be recognized at the genus level. However, given the characteristics available to describe the specimen, which Robson (1932) used with great reserve, and considering the taxonomic updates for cirrate octopods compiled by Jereb *et al.* (2014), we suggest that this specimen can only be identified

as a cirrate in general, since it presented cirri. The second consideration is regarding *Cirrothauma murrayi*, which Rocha (1997) included in his report of cephalopods in Chilean waters citing Voss (1988a). However, Voss referred the occurrence of this species in this area to Aldred *et al.* (1983), who in turn had cited Roper and Brundage (1972). These authors report three sampling locations off Chile for specimens of the genus *Cirrothauma* (without determining the species) but according to Voss (1967) no specific information indicates any species of this genus or any other cirrate is present in Chilean waters.

In view of the imprecise historical classifications of cirrate diversity from Chilean waters, and considering the findings of this study, we consider this research to establish a baseline taxonomy of Chilean cirrates. The veracity of our identification is supported by morphometric, meristic and molecular evidence and these methods should be employed in further studies of this group of cephalopods. In addition, we have presented evidence that the only cirrate that is currently recognized for Chilean waters is *Opisthoteuthis bruuni*.

Opisthoteuthis bruuni is a data deficient species in the IUCN Red List of threatened species (Lyons and Allcock 2014). This species is taken as by-catch in trawl fisheries due to its benthic distribution in the continental shelf (200-500 m depth) (Villarroel *et al.* 2001). The highest frequency as by-catch for *O. bruuni* is CPUE 1.7 - 27 kg/h in the crustacean fishery between Antofagasta and Caldera (22°S – 27°S, Villarroel *et al.* 2001). We do not have access to CPUE data for other cirrates, although sampling data of *O. agassizi* and *Opisthoteuthis vossi* Sanchez and Guerra, 1989 indicates abundances of 6-16 ind/Km² daytime and 18-23 ind/Km² nighttime for the former and 202-337 ind/Km² daytime and 256-499 ind/Km² nighttime for the latter (Villanueva and Guerra, 1991). While other *Opisthoteuthis* species are catalogued as vulnerable (i.e., *O. calypso*, *O. massyae*), endangered (i.e., *O. mero*) and critically endangered (i.e., *O. chathamensis*) in the IUCN (Lyons and Allcock 2014), and are also under fisheries pressure in trawling areas (Collins and Villanueva, 2006). We suggest that urgent research is needed to determine the taxonomic status of cirrates and the possible effects of fisheries on their populations. In this sense, the information

that we present in this study could be informative for the categorization of *O. bruuni* in the IUCN.

The monophyly of the Order Octopodiformes has been confirmed by molecular and morphological methods (Voight, 1997; Young *et al.*, 1998; Vecchione *et al.*, 2000, Strugnell *et al.*, 2005; Lindgren *et al.*, 2012). However, the internal relationships of cirrates remain controversial due to significant changes in their family and genus classifications. From this perspective, our phylogenetic analysis is the most complete in terms of representation of taxa and phylogenetic methods (MP, ML and BI), but remains limited to a single gene. The phylogeny in our study supports the existence of three families of cirrate octopods (Cirroteuthidae, Cirroctopodidae and Opisthoteuthidae), which is different from the arrangements of four families previously suggested by O'Shea (1999), Piortney *et al.* (2003), and Vecchione *et al.* (2016). At the family level, recognizing Stauroteuthidae (as in Vecchione *et al.* (2016) would implicate to consider the Cirroteuthidae as paraphyletic, which would lack systematic rationale.

Much more biological knowledge is needed about cirrate octopods. Considering that most of the sequences available in public data bases are from 16S rRNA gene, we suggest further studies should include different molecular markers to gain a better understanding of the evolutionary relationships of this important group of cephalopods.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

This work was funded with resources provided by INACH RG 50-18 project. Authors thank Oscar Gálvez and David López for their support to review the specimens from the Museum of Natural History of Santiago, Chile (MNHNCL). Authors are also grateful to Diana Párraga (IFOP), Darren Stevens (NIWA) and Stephanny Curaz (UNAB) for donating samples and providing laboratory support. Mariana Díaz-

Santana-Iturrios wishes to thank CONACYT for the grant provided during the postdoctoral stay within the framework of the call Estancias Posdoctorales en el Extranjero_2019.

REFERENCES

- ALDRED, R.G., M. NIXON & J.Z. YOUNG, 1983, *Cirrothauma murrayi* Chun, a finned octopod. *Philosophical Transactions of the Royal Society B*, 301: 1-54.
- ALJANABI, S.M. & I. MARTINEZ, 1997, Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25: 4692-4693.
- ALLCOCK, A.L., J.M. STRUGNELL & M.P. JOHNSON, 2008, How useful are the recommended counts and indices in the systematics of the Octopodidae (Mollusca: Cephalopoda). *Biological Journal of the Linnean Society*, 95: 205-218.
- CARDOSO, F. & F.G. HOCHBERG, 2012, 'Some deep-sea octopods found off Peru.' (Cephalopod International Advisory Council Symposium: Florianopolis, Brazil.)
- COLLINS, M.A., 2003, The genus *Grimpoteuthis* (Octopoda: Grimpoteuthidae) in the north-east Atlantic, with descriptions of three new species. *Zoological Journal of the Linnean Society*, 139: 93–127.
- COLLINS, M.A. & R. VILLANUEVA, 2006, Taxonomy, ecology and behaviour of the cirrate octopods. *Oceanography and Marine Biology*, 44: 277-322.
- COLLINS, M.A., V. LAPTIKHOVSKY & J.M. STRUGNELL, 2010, Expanded description of *Opisthoteuthis hardyi* based on new specimens from the Patagonian slope. *Journal of the Marine Biological Association of the United Kingdom*, 90(3): 605-611.
- CUCCU, D., M. MEREU, R. CANNAS, M.C. FOLLESA, A. CAU & P. JEREB, 2009, Morphology, biology and molecular characterizations of *Opisthoteuthis calypso* (Cephalopoda: Octopoda) from the Sardinian Channel (central western Mediterranean). *Journal of the Marine Biological Association of the United Kingdom*, 89(8): 1709-1715.

- GIRIBET, G., A. OKUSU, A.R. LINDGREN, S.W. HUFF, M. SCHRODL & M.K. NISHIGUCHI, 2006, Evidence for a clade composed of molluscs with serially repeated structures: monoplacophorans are related to chitons. *PNAS*, 103(20): 7723-7728.
- GUERRA, A., R. VILLANUEVA, K.N. NESIS & J. BEDOYA, 1998, Redescription of the deep-sea cirrate octopod *Cirroteuthis magna* Hoyle, 1885, and considerations on the genus *Cirroteuthis* (Mollusca: Cephalopoda). *Bulletin of Marine Science*, 63(1): 51-81.
- FELSENSTEIN, J., 1985, Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39: 783–791.
- FUCHS, D., 2009, Octobranchia – a dyphyletic taxon? *Berliner Paläobiologische Abhandlungen*, 10: 182-192.
- HOCHBERG, F.G., M.D. NORMAN & J.K. FINN, 2014, Family Opisthoteuthidae. In 'Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 3. Octopods and Vampire Squids'. (Eds P. Jereb, C.F.E. Roper, M.D. Norman, and F.K. Finn) pp. 248-261. (FAO Species Catalogue for Fishery Purposes: Rome.)
- IBÁÑEZ, C.M., P.A. CAMUS & F. ROCHA, 2009, Diversity and distribution of cephalopod species of the coast off Chile. *Marine Biology Research*, 5: 374-384.
- IBÁÑEZ, C.M., M.C. PARDO-GANDARILLAS, D. PÁRRAGA, M. ZILLERUELO & J. SELLANES, 2011, Cefalópodos recolectados en el talud continental de Chile central. *Amici Molluscarum*, 19: 37-40.
- JAMESON, A. & VECCHIONE, M. 2020. First in situ observation of Cephalopoda at hadal depths (Octopoda: Opisthoteuthidae: *Grimpoteuthis* sp.) *Marine Biology*, 167(82): 1-5.
- JEREB, P., C.F.E. ROPER, M.D. NORMAN & J.K. FINN, 2014, *Cephalopods of the world. An annotated and illustrated catalogue of species known to date. Octopods and Vampire squids*. FAO Species Catalog for Fishery Purposes, Rome, Vol. 3, 352 pp.
- LINDGREN, A.R., M.S. PANKEY, F.G. HOCHBERG & T.H. OAKLEY, 2012, A multi-gene phylogeny of Cephalopoda supports convergent morphological evolution

- in association with multiple habitat shifts in the marine environment. *BMC Evolutionary Biology*, 12(1): 129.
- LU, C-C., 2010, A new species of *Opisthoteuthis*, *O. dongshaensis* sp. nov., from the South China Sea (Octopoda: Cirrata: Opisthoteuthidae). *Zoological Studies*, 49(3): 405–420.
- LYONS, G. & A.L. ALLCOCK, 2014, *Opisthoteuthis bruuni*. (The IUCN Red List of Threatened Species: U.K.)
- NESIS, K.N., 1987, *Cephalopods of the world*. (T.F.H. Publications Inc.: New Jersey, U.S.A.)
- O'SHEA, S., 1999, 'The marine fauna of New Zealand: Octopoda (Mollusca: Cephalopoda).' (NIWA Biodiversity Memoir: New Zealand.)
- PIERTNEY, S., C. HUDELOT, F.G. HOCHBERG & M.A. COLLINS, 2003, Phylogenetic relationships among cirrate octopods (Mollusca: Cephalopoda) resolved using mitochondrial 16S ribosomal DNA sequences. *Molecular Phylogenetics and Evolution*, 27: 348–353.
- RAMBAUT, A. & A.J. DRUMMOND, 2009, 'Tracer v1.5.' Available at <http://tree.bio.ed.ac.uk/software/tracer>.
- ROBSON, G.C., 1932, A monograph of the recent cephalopoda based on the collections in the British museum (natural history). Part II. The Octopoda. (Richard Clay & Sons: London.)
- ROCHA, F., 1997, Cephalopods in Chilean waters, a review. *Malacological Review*, 30: 101-113.
- RONQUIST, F., M. TESLENKO, P. VAN DER MARK, D. AYRES, A. DARLING, S. HÖHNA, B. LARGET, L. LIU, M.A. SUCHARD & J.P. HUELSENBECK, 2012, MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 22: 539-542.
- ROPER, C.F.E. & W.L. BRUNDAGE, 1972, Cirrate octopods with associated Deep-Sea organisms: new biological data based on deep benthic photographs (Cephalopoda). (Smithsonian Contributions to Zoology: U.S.A.)
- ROPER, C.F.E. & G.L. VOSS, 1983, Guidelines for taxonomic descriptions of cephalopod species. *Memoirs of the National Museum of Victoria*, 44: 49-63.

- STRUGNELL, J., M. NORMAN, A.J. DRUMMOND & A. COOPER, 2004, Neotenous origin of pelagic octopuses. *Current Biology*, 14(8): R300-1.
- STRUGNELL, J., M. NORMAN, J. JACKSON, A.J. DRUMMOND & A. COOPER, 2005, Molecular phylogeny of coleoid cephalopods (Mollusca: Cephalopoda) using a multigene approach; the effect of data partitioning on resolving phylogenies in a Bayesian framework. *Molecular Phylogenetics and Evolution*, 37(2): 426-441.
- SWOFFORD, D.L., 2002, PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4. (Sinauer Associates: Sunderland.)
- TAKUMIYA, M., M. KOBAYASHI, K. TSUNEKI & H. FURUYA, 2005, Phylogenetic relationships among major species of Japanese coleoid cephalopods (Mollusca: Cephalopoda) using three mitochondrial DNA sequences. *Comparative study*, 22(2): 147-155.
- TANABE, K., A. MISAKI, Y. HIKIDA & T. NISHIMURA, 2017, New records of coleoid cephalopod jaws from the Upper Cretaceous of Hokkaido, Japan, and their paleobiogeographic and evolutionary implications. *Cretaceous Research*, 70: 128-141.
- VECCHIONE, M., R.E. YOUNG & D.B. CARLINI, 2000, Reconstruction of ancestral character states in neocoloid cephalopods based on parsimony. *American Malacological Bulletin*, 15(2): 179-193.
- VECCHIONE, M., M.A. COLLINS & M.J. SWEENEY, 2002, Systematics, ecology and biology of cirrate octopods: workshop report. *Bulletin of Marine Science*, 71: 79-94.
- VECCHIONE, M., Mangold, K.M., Young, R.E. 2016. Cirrata Grimpe, 1916. Finned Octopods. Version 27 February 2016. Accessed at: <http://tolweb.org/Cirrata/20086/2016.02.27>
- VEGA, M.A., S. LETELIER & E. CARREÑO, 2001, Colección de cefalópodos del Museo Nacional de Historia Natural: catálogo especies de aguas chilenas. *Museo Nacional de Historia Natural Santiago Chile*, 57: 1-88.
- VEGA, M.A., 2009, Sistemática, Biogeografía y Pesquería de Cefalópodos de Aguas Chilenas. (RIL Editores: Santiago, Chile.)

- VILLANUEVA, R. & A. GUERRA, 1991, Food and prey detection in two deep-sea cephalopods: *Opisthoteuthis agassizi* and *O. vossi* (Octopoda: Cirrata). *Bulletin of Marine Science*, 19(1-2): 288-299.
- VILLANUEVA, R., M.A. COLLINS, P. SÁNCHEZ & N. VOSS, 2002, Systematics, distribution and biology of the cirrate octopods of the genus *Opisthoteuthis* (Mollusca, Cephalopoda) in the Atlantic Ocean, with description of two new species. *Bulletin of Marine Science*, 71(2): 933–985.
- VILLARROEL, J.C., M.A. VEGA & E. ACUÑA, 2001, Cefalópodos recolectados en la pesquería de crustáceos de la zona norte y centro-sur de Chile. *Revista de Biología Marina y Oceanografía*, 36: 83-97.
- VOIGHT, J.R., 1997, Cladistic analysis of the octopods based on anatomical characters. *Journal of Molluscan Studies*, 63: 311-325.
- VOSS, G.L., 1967, The biology and bathymetric distribution of Deep-Sea cephalopods. (Proceedings of the International Conference on Tropical Oceanography: U.S.A.)
- VOSS, G.L., 1982, *Grimpoteuthis bruuni*, a new species of finned octopod (Octopoda: Cirrata) from the southern Pacific. *Bulletin of Marine Science*, 32: 426-433.
- VOSS, G.L., 1988a, The biogeography of the Deep-Sea octopoda. *Malacologia*, 29(1): 295-307.
- VOSS, G.L., 1988b, Evolution and phylogenetics relationship of deep-sea octopods (Cirrata and Incirrata). In 'The Mollusca. Vol. 12. Paleontology and Neontology of Cephalopods'. (Eds M. R. Clarke and E. R. Trueman.) pp. 253-276. (Academic Press: London, U.K.)
- YOUNG, R.E. & M. VECCHIONE, 2002, Evolution of the gills in the octopodiformes. *Bulletin of Marine Science*, 71(2): 1003-1017.
- YOUNG, R.E., M. VECCHIONE & D.T. DONOVAN, 1998, The evolution of coleoid cephalopods and their present biodiversity and ecology. *South African Journal of Marine Science*, 20: 393-420.

Tables

Table 1. Octopodiform species included in the phylogenetic analyses. For collection codes see material and methods section. * Specimen sequenced for this study

Species	Distribution	GenBank Accession number	Reference
<i>Vampyroteuthis infernalis</i>	Worldwide	AY686586	Cuccu <i>et al.</i> (2009)
<i>Opisthoteuthis bruuni</i> MNHNCL 300138*	South Eastern Pacific	MT725733	This study
<i>Opisthoteuthis bruuni</i> MNHNCL 300139*	South Eastern Pacific	MT725734	This study
<i>Opisthoteuthis chathamensis</i> NZ22*	Chatham Rise, New Zealand	MT216982	This study
<i>Opisthoteuthis mero</i> NIWA 106093*	Chatham Rise, New Zealand	MT216997	This study Ibáñez <i>et al.</i> 2020
<i>Opisthoteuthis mero</i> NIWA 95194	Chatham Rise, New Zealand	MT216998	
<i>Opisthoteuthis calypso</i> 1	Eastern Atlantic and Mediterranean	FJ403542	Cuccu <i>et al.</i> (2009)
<i>Opisthoteuthis calypso</i> 2	Eastern Atlantic and Mediterranean	FJ403541	Cuccu <i>et al.</i> (2009)
<i>Opisthoteuthis calypso</i> NIWA	Eastern Atlantic and Mediterranean	AJ315374	Piertney <i>et al.</i> (2003)
<i>Opisthoteuthis massyae</i> NIWA	SE Atlantic (NWA)	AJ315371	Piertney <i>et al.</i> (2003)
<i>Opisthoteuthis massyae</i> SBMNH	North Eastern Atlantic	AJ315372	Piertney <i>et al.</i> (2003)
<i>Opisthoteuthis massyae</i> NMSZ 1999158.076	North Eastern Atlantic	AF487298	Piertney <i>et al.</i> (2003)
<i>Opisthoteuthis massyae</i> NMSZ 1999158.077	North Eastern Atlantic	AF487299	Piertney <i>et al.</i> (2003)
<i>Opisthoteuthis hardyi</i> BMNH 20080844	South Atlantic, Shag Rocks	FJ785404	Collins <i>et al.</i> (2010)
<i>Opisthoteuthis hardyi</i> BMNH 20080845	South Atlantic, Shag Rocks	FJ785403	Collins <i>et al.</i> (2010)
<i>Opisthoteuthis hardyi</i> NMSZ1999158.088	South Atlantic, Shag Rocks	AF487302	Collins <i>et al.</i> (2010)
<i>Opisthoteuthis depressa</i>	North Western Pacific, Japan	AB191117	Takumiya <i>et al.</i> (2005)
<i>Opisthoteuthis californiana</i>	North Pacific	AJ315373	Piertney <i>et al.</i> (2003)
<i>Grimpoteuthis</i> sp. NMSZ 1999158.160	North Eastern Atlantic	AF487310	Piertney <i>et al.</i> (2003)
<i>Grimpoteuthis</i> sp. NMSZ 1999158.162	North Eastern Atlantic	AF487309	Piertney <i>et al.</i> (2003)

<i>Grimpoteuthis</i> sp. AU 13925a	North Eastern Atlantic	AF487305	Piertney <i>et al.</i> (2003)
<i>Grimpoteuthis</i> sp. AU 13925b	North Eastern Atlantic	AF487306	Piertney <i>et al.</i> (2003)
<i>Cirrothauma murrayi</i> AU 13924	North Eastern Atlantic	AF487283	Piertney <i>et al.</i> (2003)
<i>Cirrothauma murrayi</i> AU 13945	North Eastern Atlantic	AF487282	Piertney <i>et al.</i> (2003)
<i>Cirroteuthis muelleri</i> AU 13945	North Eastern Atlantic	AF487284	Piertney <i>et al.</i> (2003)
<i>Stauroteuthis syrtensis</i>	North Eastern Atlantic	DQ280042	Giribet <i>et al.</i> (2006)
<i>Stauroteuthis syrtensis</i> AU Scotia 404	North Eastern Atlantic	AF487290	Piertney <i>et al.</i> (2003)
<i>Stauroteuthis syrtensis</i> AU 13919a	North Eastern Atlantic	AF487285	Piertney <i>et al.</i> (2003)
<i>Stauroteuthis gilchristi</i> NMSZ 1999158.013	South West Atlantic	AF487292	Piertney <i>et al.</i> (2003)
<i>Stauroteuthis gilchristi</i> NMSZ 1999158.006	South West Atlantic	AF487291	Piertney <i>et al.</i> (2003)
<i>Luteuthis dentatus</i> NMNS 002157-00088	North Eastern Pacific	AJ315377	Piertney <i>et al.</i> (2003)
<i>Luteuthis dentatus</i> NIWA 95257	Ross Sea	MT725732	This study
<i>Cirroctopus glacialis</i> NMSZ	South West Atlantic	AF487304	Piertney <i>et al.</i> (2003)
<i>Cirroctopus hochbergi</i> NIWA	South West Pacific	AJ315376	Piertney <i>et al.</i> (2003)
<i>Cirroctopus mawsoni</i> NZ16*	Antarctica, Ross Sea	MT725730	This study
<i>Cirroctopus mawsoni</i> NZ18*	Antarctica, Ross Sea	MT725731	This study

Table 2. Measurements (mm) and counts of *Opisthoteuthis bruuni* examined during the present study. For abbreviations see Materials and Methods.

	MNHNCL 300014-A	MNHNCL 300014-B	MNHNCL 300138	MNHNCL 300139	MNHNCL 300140
Sex	female	male	female	female	female
TL	98	135	154	128	206
AL1l	46	71	80	82	113
AL1r	47	77	72	88	104
AL2l	44	66	79	86	117
AL2r	42	66	79	82	111
AL3l	42	72	99	81	125
AL3r	48	73	67	73	131
AL4l	48	72	102	78	113
AL4r	52	69	115	71	123
AW	5	5	7	8	10
HW	34	52	35	42	41
HL	42	46	-	-	-
MW	34	39	30	33	39
DML	35	32	39	36	50
VML	39	34	32	33	44
PA	8	16	10	15	20
ED	17	17	13	15	15
SD	1	4	1	2	3
FL	7/6	13/11	9/7	8/7	13/11
FW	16/15	33/31	20/19	15/17	26/25
FuL	9	10	11	17	35
WDA	32	56	41	49	68
WDbl	31	53	24	53	71
WDcl	26	52	42	43	83
WDdl	29	55	39	36	90
WDbr	35	42	43	37	63
WDcr	33	57	41	39	80
WDdr	31	52	34	36	78

WDe	23	46	63	28	81
ASC1R	36	32	21	27	39
ASC1L	35	27	24	31	44
ASC2R	33	27	26	28	38
ASC2L	34	31	23	31	41
ASC3R	34	36	21	31	43
ASC3L	33	32	26	28	38
ASC4R	38	35	25	30	41
ASC4L	40	33	27	29	42

Table 3. Comparison of indices and counts of *Opisthoteuthis bruuni* from this study and the original description (Voss, 1982), n = number of specimens.

Indices	Females Voss (1982) n = 3	Females this study n = 4	Males Voss (1982) n = 13	Males this study n = 1
DML	13 – 19	36 – 50	13 - 29	32
HWI	126.3 – 176.9	82 – 116.6	133.3 – 158.6	162.5
ALI	-	42.9 – 74.7	42.6 – 63	48.9 - 57
FLI	22.9 – 34.8	39 – 41	26 – 36.9	40
FWI	90 – 90.9	65 – 88	58.8 – 105.7	103
WDI	78.9 – 92.9	61.5 – 180	70 – 86.9	65.7 - 100
SDI	3.1 – 3.9	2.6 – 6.0	2.7 – 4.8	2.8
ASC1	27 – 32	21 – 44	28 – 35	27 - 36

Table 4. Uncorrected genetic distances (pairwise) between cirrates. Raw minimum and maximum values.

GENERA	Min.	Max.
<i>Opisthoteuthis</i> – <i>Opisthoteuthis</i>	0.012	0.056
<i>Cirroctopus</i> – <i>Cirroctopus</i>	0.002	0.049
<i>Stauroteuthis</i> – <i>Stauroteuthis</i>	0.009	0.009
<i>Opisthoteuthis</i> – <i>Luteuthis</i>	0.046	0.065
<i>Opisthoteuthis</i> – <i>Grimpoteuthis</i>	0.060	0.079
<i>Opisthoteuthis</i> – <i>Cirroctopus</i>	0.088	0.118
<i>Opisthoteuthis</i> – <i>Cirrothauma</i>	0.104	0.134
<i>Opisthoteuthis</i> – <i>Stauroteuthis</i>	0.118	0.144
<i>Opisthoteuthis</i> – <i>Cirroteuthis</i>	0.097	0.130
<i>Grimpoteuthis</i> – <i>Luteuthis</i>	0.019	0.021
<i>Grimpoteuthis</i> – <i>Cirroctopus</i>	0.104	0.116
<i>Grimpoteuthis</i> – <i>Stauroteuthis</i>	0.118	0.123
<i>Grimpoteuthis</i> – <i>Cirrothauma</i>	0.109	0.116
<i>Grimpoteuthis</i> – <i>Cirroteuthis</i>	0.097	0.100
<i>Luteuthis</i> – <i>Cirroctopus</i>	0.102	0.113
<i>Luteuthis</i> – <i>Stauroteuthis</i>	0.118	0.120
<i>Luteuthis</i> – <i>Cirrothauma</i>	0.109	0.111
<i>Luteuthis</i> – <i>Cirroteuthis</i>	0.095	0.095
<i>Cirroctopus</i> – <i>Stauroteuthis</i>	0.130	0.150
<i>Cirroctopus</i> – <i>Cirrothauma</i>	0.111	0.137
<i>Stauroteuthis</i> – <i>Cirrothauma</i>	0.042	0.046
<i>Stauroteuthis</i> – <i>Cirroteuthis</i>	0.065	0.067
<i>Cirrothauma</i> – <i>Cirroteuthis</i>	0.060	0.060

Figure captions

Figure 1. Sampling localities, museum and literature records of *Opisthoteuthis bruuni* along southeastern Pacific.

Figure 2. General features of *Opisthoteuthis bruuni*. A) Fresh female, MNHNCL 300139; B) and C) Fresh female, MNHNCL 300138; D) U-shaped shell, specimen MNHNCL 300260; E) and F) Male, specimen MNHNCL 300014 preserved in ethanol; G) circumoral arrangement of arms, specimen MNHNCL 300162 preserved in ethanol. Scale bars (A, B, C, E, F, G) = 20 mm; (D) = 6 mm.

Figure 3. Digestive tract and gills of *Opisthoteuthis bruuni*. A) Digestive system, Specimen MNHNCL 300014, a: oesophagus, b: digestive gland, c: stomach, d: intestine; B) Demibranchs, specimen MNHNCL 300260; C) Dorsal view of upper beak, specimen MNHNCL 300161; D) Lateral view of upper beak, specimen MNHNCL 300161; E) Lateral view of lower beak, specimen MNHNCL 300161; F) Dorsal view of lower beak, specimen MNHNCL 300161. Scale bars (A) = 10 mm; (B) = 2 mm; (C, D, E, F) = 6 mm.

Figure 4. Reproductive system of *Opisthoteuthis bruuni*. A) Male genitalia, specimen MNHNCL 300014, a: terminal organ, b: seminal vesicle complex, c: accessory gland d: testicle. B) Female genitalia, specimen MNHNCL 300260, a: terminal oviduct, b: oviducal gland, c: dissected ovary with oocytes; C) oocytes, specimen MNHNCL 300260. Scale bars (A) = 10 mm; (B) = 10 mm; (C) = 5 mm.

Figure 5. Bayesian consensus phylogram of cirrate octopods. Node values represent bootstrap support for parsimony and likelihood analyses, and posterior probabilities for Bayesian analysis.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5