

Morphological and molecular description of a new record of *Graneledone* (Cephalopoda, Octopodidae) in the southeastern Pacific Ocean

Descripción morfológica y molecular de un nuevo registro de *Graneledone* (Cephalopoda, Octopodidae) en el Océano Pacífico suroriental

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Resumen.- Los pulpos del género *Graneledone* habitan en aguas profundas y constituyen 8 especies reconocidas. Se realizaron análisis filogenéticos de 4 especies de *Graneledone* con 2 marcadores moleculares (16S y COI), y se informa sobre un nuevo registro de *Graneledone* para el Océano Pacífico frente a la zona centro-sur de Chile. Se obtuvieron 4 ejemplares de *Graneledone* sp. entre 436 y 1482 m de profundidad. Estos pulpos son de tamaño medio, no tienen saco de tinta y tienen una sola fila de ventosas en sus brazos, que son de igual longitud. Se caracterizan por tener de 43 a 45 ventosas en el brazo hectocotilizado (tercero derecho); 6 a 7 laminillas por demibranchia; órgano del sifón en forma VV y de 5 a 7 pliegues transversales en la lígula. Algunos rasgos morfológicos muestran una gran variación en comparación con las especies del Pacífico, Atlántico y la Antártida. Los análisis filogenéticos moleculares apoyan la hipótesis de monofilia de *Graneledone*.

Palabras clave: *Graneledone*, pulpos de aguas profundas, zona batial, Chile

Abstract.- Octopuses of the genus *Graneledone* inhabit in the deep-sea and are composed of 8 recognized species. Here we conduct phylogenetics analyses of 4 species of *Graneledone* using 2 molecular markers (16S and COI), and report a new record of *Graneledone* for the Pacific Ocean off south-central Chile. Four specimens of *Graneledone* sp. were collected from 436 to 1482 m depth. These octopuses are medium sized, have no ink sac and bear only one row of suckers in their arms, which are of similar length. They are characterized by having 43 to 45 suckers in the hectocotylized arm (third right); 6 to 7 lamellae per demibranch; VV-shaped funnel organ and 5 to 7 transverse creases in the ligulae. Some morphological traits show great variation compared with species from Pacific, Atlantic and Antarctic oceans. Molecular phylogenetic analysis supports the hypothesis of monophyly of *Graneledone*.

Key words: *Graneledone*, deep-sea octopuses, bathyal zone, Chile

INTRODUCTION

Octopuses of the genus *Graneledone* Joubin, 1898 generally live in the bathyal to abyssal zones ranging from 90 m to 2755 m depth and have been often reported occurring at particular ecosystems like hydrothermal vents and cold seeps (Voight 2000a, b, Guerrero-Kommritz 2000, 2006). *Graneledone* is composed of 8 recognized species (Norman & Hochberg 2005) found in all the oceans: *G. antarctica* Voss, 1976 and *G. macrotyla* Voss, 1976 from the Antarctica, *G. verrucosa* (Verrill, 1881) from the north Atlantic Ocean, *G. gonzalezi* Guerra, González & Chérel 2000 from the Kerguelen Island, *G. yamana* Guerrero-Kommritz, 2000 from the southwest Atlantic, *G.*

boreopacifica Nesis, 1982 from the north Pacific and *G. taniwha* O'Shea, 1999 and *G. challengerii* (Berry, 1916) from the south Pacific Ocean. The validity of certain species and sub-species is still controversial, as they have been classified based on highly variable morphological characters, making differentiating them very difficult. This genus is defined by having a body covered with warts, uniserial suckers, funnel organ VV shaped, no ink sac, crop reduced or absent, small gills, small posterior salivary glands, homodont radula, and in males terminal organ coiled in spiral and large ligulae in hectocotylus (O'Shea 1999, Guerrero-Kommritz 2000, Allcock *et al.* 2003). These

character states are derived among Octopodidae suggesting the hypothesis of monophyly of *Graneledone* (Voight 1997, 2000a). Molecular divergence time estimation suggests that *Graneledone* originated in the southern ocean and their radiation into deep-sea was facilitated by the thermohaline circulation (Strugnell *et al.* 2008a). However, this molecular analysis only included 3 species: *G. antarctica*, *G. verrucosa* and *G. boreopacifica*.

Until now, *Pareledone charcoti* Joubin, 1905, *Pareledone turqueti* Joubin, 1905, *Thaumeledone rotunda* (Hoyle, 1885) and *Graneledone antarctica* were the only octopuses with uniserial suckers reported from Chilean waters (18°S–56°S, Thore 1959, Rocha 1997, Vega *et al.* 2001, Vega 2009). However, recently specimens of *Graneledone* have been collected off south-central Chile, one of them in the vicinity of a bathyal methane seep site near Concepción (~37°S, Ibáñez *et al.* 2011). This site named Concepción Methane Seep Area (CMSA), has proven to be an abundance hotspot for both chemosymbiotic and heterotrophic fauna, and more than a hundred of megabenthic species have been recorded associated with it (Sellanes *et al.* 2004, 2008). Among this fauna, mollusks are one of the most important groups, with about 30 species present. In addition to common cephalopods (sepiolids and squids), unidentified incirrate octopod species belonging to *Muusoctopus* Gleadall 2004 (formerly *Benthoctopus*) and *Graneledone* genus occur in the CMSA and adjacent areas (Ibáñez *et al.* 2006, 2009, 2011).

In this study, we combine published data with new mitochondrial sequences to propose a phylogenetic hypothesis of the deep-sea octopuses of the genus *Graneledone*. Moreover, we describe a new record of *Graneledone*, comparing the type specimens both morphologically and genetically with their counterparts from the Pacific, Atlantic and Antarctic Oceans to further elucidate their taxonomic position.

MATERIALS AND METHODS

The present work was based on 4 *Graneledone* specimens captured off south-central Chile between 35°S and 38°S (Fig. 1). Octopuses of this genus are the only ones with uniserial suckers that have been reported so far for the Chilean margin (see Ibáñez *et al.* 2011). One of the specimens was collected by trawling during a cruise dedicated to the study of methane seep sites (29°S–45°S). The remaining 3 specimens were obtained as by-catch from commercial fishing activities, one from the long-line

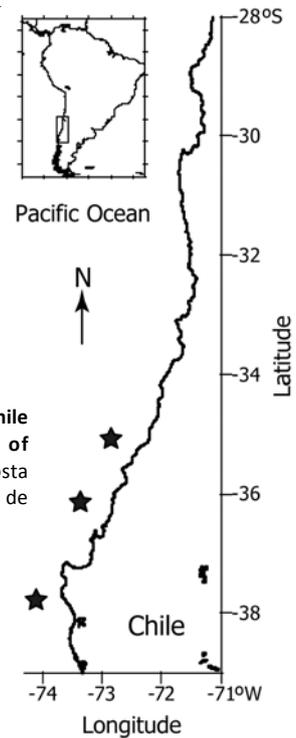


Figure 1. Map of the coast of Chile showing capture locations of *Graneledone* sp. / Mapa de la costa de Chile mostrando los lugares de captura de *Graneledone* sp.

fisheries targeting the Patagonian toothfish (*Dissostichus eleginoides* Smith, 1898), and the other 2 from shrimp trawling. Tissue samples were fixed in 96% ethanol for molecular analysis and the whole animal in 10% seawater formalin for later anatomic and morphological analysis. The radula of the specimen MNHNCL6640 was dissected, mounted on tape, critical-point dried, coated under vacuum with gold and examined with a JEOL T-300 scanning electron microscope (SEM).

Description, measurements and counts followed Roper & Voss (1983). Abbreviations used are the following: TL: total length; DML: dorsal mantle length; MW: mantle width; EO: eye diameter; HdL: head length; HdW: head width; AL: arm length 1 to 4R/L; WD: web depth A to E; ASC1-4: arm sucker count 1 to 4R/L; AS: arm sucker diameter; GiLC: gill lamella count; FuL: funnel length; FFL: free funnel length; CaL: calamus length; LL: ligula length; PAL: pallial aperture length; AW: arm base width; GiL: gill length; AF: arm formula; WF: web formula.

The specimens were deposited in the Museo Nacional de Historia Natural, Chile (MNHNCL), and Museo Zoológico de la Universidad de Concepción (MZUC-UCCC). The comparative material examined is deposited in Zoological Museum of Hamburg (ZMH), Germany.

Total DNA, to compare with other *Graneledone* species from GenBank, was extracted from 2 specimens following the saline extraction protocol (Aljanabi & Martinez 1997). PCR amplifications were carried out using for each sample 0.3 µl of *Taq* DNA polymerase and 2.5 µl commercially supplied buffer, with 2 µl dNTPs, and 0.5 µl of each primers LCO1490 and HCO2198 for Cytochrome Oxidase I (COI) and 16SF and 16SR for 16S rRNA (see primers in Allcock *et al.* 2008). After an initial denaturation (3 min at 94°C), the reaction mixtures were subjected to 35 cycles of 94°C (40 s), 50°C (40 s) for COI and 52°C (40 s) for 16S, and 72°C (60 s) followed by a final extension at 72°C (7 min) using a thermal cycler. PCR products were purified by the Wizard™ Prep system (Promega) following the manufacturer's protocols. Purified PCR products were sequenced by Macrogen Inc. Sequences were aligned using Clustal W implemented in MEGA 5.0 software (Tamura *et al.* 2011).

Phylogenetic reconstruction and distances between species were calculated from a matrix including the concatenated dataset (16S + COI). For this purpose, we determined the congruence on the phylogenetic signal of the genes with the ILD test (Farris *et al.* 1995), implemented in the partition homogeneity test of PAUP* version 4.0b (Swofford 2002). Parsimony (P) and maximum likelihood (ML) methods, and Bayesian inference (BI) were applied to the evaluation of phylogenetic relationships of *Graneledone* species. The evolutionary model that best fit the data was d GTR+Γ+I model (AIC = 4067.15, -lnL = 2016.57), determined by the Akaike Information Criterion (AIC) as implemented by JModelTest (Posada 2008). Bayesian analyses were conducted using MrBayes v3.2 (Ronquist *et al.* 2012) with four default heated chains, each with five million generations, sampled every 1000 generations. Runs were checked for convergence see the likelihood using the Tracer version 1.5 (Rambaut & Drummond 2009). The first 500 trees of each run were discarded as burn-in, and a consensus of the remaining trees was computed for the final outcome. Parsimony and ML analyses were carried out in PAUP* (Swofford 2002) (heuristic search, tree bisection-reconnection) and support for nodes was estimated by bootstrapping with 10,000 pseudo-replicates (Felsenstein 1985), providing an estimate of the confidence limits for the resulting topologies. Finally, we used FigTree 1.3.1 to edit the trees (Rambaut 2009). To construct these trees we used *Pareledone charcoti* (Joubin, 1905), *P. turqueti* (Joubin, 1905), *Megaleledone setebos* (Robson, 1932), *Thaumeledone peninsulae* Allcock *et al.* 2004, *T. rotunda*

Table 1. Octopod species included in the phylogenetic analyses / Especies de octópodos incluidas en los análisis filogenéticos

Species	COI	16S rRNA
<i>Graneledone</i> sp.1 MNHNCL 6641	JN800404	JN800402
<i>Graneledone</i> sp.2 MZUC-UCCC 32743	JN800403	JN800401
<i>Graneledone antarctica</i> Voss, 1976	AF377973	EU071436
<i>Graneledone verrucosa</i> 1 (Verrill, 1881)	EU071449	AY545111
<i>Graneledone verrucosa</i> 2 (Verrill, 1881)	AF000042	DQ093490
<i>Graneledone boreopacifica</i> 1 Nesis, 1982	EU071448	EU071435
<i>Graneledone boreopacifica</i> 2 Nesis, 1982	EU071449	---
<i>Thaumeledone rotunda</i> (Hoyle, 1885)	EU071445	EU071432
<i>Thaumeledone gunteri</i> Robson, 1930	AY557521	AF299266
<i>Thaumeledone peninsulae</i> Allcock <i>et al.</i> 2004	EU071446	EU071433
<i>Adelieledone polymorpha</i> (Robson, 1930)	EF102173	EF102194
<i>Adelieledone piatkowski</i> Allcock <i>et al.</i> 2003	EU071444	EU071431
<i>Pareledone charcoti</i> (Joubin, 1905)	EF102175	EF102196
<i>Pareledone turqueti</i> (Joubin, 1905)	EF102192	EF102213
<i>Megaleledone setebos</i> (Robson, 1932)	EF102174	EF102195

(Hoyle, 1885) and *T. gunteri* Robson, 1930 as outgroups and the phylogenetic tree is rooted using *Adelieledone polymorpha* (Robson, 1930) and *A. piatkowski* Allcock *et al.* 2003 since these species are basal to *Graneledone* (Strugnell *et al.* 2008 a,b). Sequences generated in this study are available from GenBank (Table 1).

SYSTEMATICS

Family Octopodidae d'Orbigny, 1840

Genus *Graneledone* Joubin, 1918

Graneledone sp. (Figs. 2-6)

DIAGNOSIS

Sucker row uniserial. Ink sac absent. Funnel organ VV-shaped. Hectocotylus clearly differentiated into calamus and ligula; the latter with or without creases. Terminal organ (penis) coiled in spiral. Gills small, with 5-9 lamellae per demibranch. Radula homodont or heterodont. Salivary glands small. Wart-like tubercles cover dorsal surfaces of mantle, head, arms and web.

Type species: *Graneledone verrucosa* (Verrill, 1881)

Synonymy: *Eledone verrucosa* Verrill, 1881, *Moschites verrucosa* Berry, 1917.

MATERIAL EXAMINED

Adult male 110 mm ML (North off Mocha island, 37°46.17'S, 74°07.29'W, southeastern Pacific, 1482 m depth) MNHNCL 6641. Collected by J. Sellanes, October 2007. Adult male 160 mm ML (off Constitución coast, caught by shrimp

trawling, 35°10'S, 72°55'W, 436 m) MNHNCL 6640. Collected by C.M. Ibáñez, October 2000. Adult female 165 mm ML (Northwest off Concepción, caught by a fishing line, 36°15.71'S, 73°43.48'W, 600 m) MZUC-UCCC 32743. Collected by M. Pedraza, March 2007. Adult male 80 mm ML (from southern Chile, caught by shrimp trawling) MNHNCL 300039 (without collecting data).

COMPARATIVE MATERIAL EXAMINED

Graneledone antarctica, adult male 55 mm ML (Antarctica, 74°31'54"S, 027°13'24"W, 2103 m) ZMH 12658, collected during RV Polarstern cruise Antarktis XV/3 at station 134 in 1998. *Graneledone antarctica*, adult female 60 mm ML (Antarctica, 69°25'00"S, 005°20'00"W, 1800 m) ZMH 72401, collected during RV Polarstern cruise Antarktis XXIII/2 at station 57 in 2005. *Graneledone verrucosa*, two females 46 and 17 mm ML, (NE Atlantic, 49°46'00"N, 012°31'00"W, 2000 m) ZMH 12657, collected during RV Walther Herwig cruise #47 at station 630 in 1981. *Graneledone verrucosa*, adult female 98 mm ML (N Atlantic, 1200 m), ZMH 12663, collected during RV Walther Herwig cruise #46 at station 591 in 1981. *Graneledone yamana*, adult male 50 mm ML (SE to Falkland Islands, 54°18'00"S, 56°10'00"W, 560 m) ZMH 12669, collected during RV Walther Herwig cruise #31 at station 584 in 1978. *Graneledone yamana*, adult male 58 mm ML (near to Falkland Islands, 50°18'00"S, 56°49'00"W, 515 m) ZMH 2789, collected during RV Walther Herwig cruise #45 at station 325 in 1966.

DIAGNOSIS

Medium sized octopus (TL = 520 to 810 mm) lacking ink sac. Suckers small and uniserial (6-8 mm maximum diameter). Eyes large (20% of DML) and projecting. Funnel organ VV shaped, free zone of the funnel corresponding to 53-62% of funnel length. First pair of arms always longest (75-78% of TL), with arm formula 1.2.3.4.; web very deep, sectors unequal; sector A and B always largest and sector E smallest. Third right arm of males hectocotylized with 43 and 45 suckers and shorter than opposite arm. The opposite arm with 98 to 107 suckers. Calamus of medium size (50% of the ligula) with deep median incision; ligula without copulatory lamellae, but with 5 to 7 creases. Six to 7 lamellae per demibranch. Radula bearing 7 teeth: 3 central, 2 lateral and 2 marginal plates in each transversal line. Body surface covered by complex papillose warts with many tiny spine-like structures covering body dorsally from mantle to arm tips. Thirty to 35 warts at the dorsal mantle midline from anterior to

posterior and 15 to 20 between the eyes. These warts composed of 1 to 5 individual processes. Two clusters of 18-22 warts above each eye.

DESCRIPTION

Adult specimens examined are of medium size (TL 520 to 810 mm). Mantle sacciform (up to 100 mm ML) (Fig. 2A). Body surface covered by complex papillose warts with many tiny spine-like structures covering body dorsally from mantle to arm tips (Figs. 2A, 5A-B). Thirty to 35 warts at the dorsal mantle midline and 15 to 20 between the eyes. These warts are clustered between 1 to 4 individual processes in the mantle and webs. Over each eye, two groups of 18 to 22 individual processes (Fig. 5B). These clusters are 1 to 5 mm in diameter. Pallial aperture moderately wide with respect to the mantle width (41-62% of ML). Head width similar to mantle (95 to 100% of MW). Eyes relatively large (diameter = 22 to 32 mm), located in a more lateral than frontal position, almost at the same level of the dorsal surface. Funnel medium sized (28 to 36% of ML); funnel free portion is very short represents 53 to 62% of the funnel total length. Funnel organ is 'VV' shaped, with internal components wider and longer than external ones (Figs. 2B, 5D).

Arms long and similar in length, generally not exceeding 78% of the TL, with the first pair always being the longest, and the fourth pair the shortest. Typical arm formula 1.2.3.4. Arms narrow and sharpened to the ends (14 to 18% of ML) (Table 2). Web short with E sector shortest. Web formula BCADE, ACDBE and ABCDE (Table 2).

Arms with uniserial suckers (Fig. 2A, 5C). Suckers small, tubular, sessile and without acetabulum aperture (4.2 to 5.4% of ML). Third right arm hectocotylized (53 to 48% of TL), being shorter in length than its opposite (57 to 64% of TL) (Table 2, Fig. 2A-C, 5E). Suckers on the hectocotylized arm number between 43 and 45; opposite arm carries between 98 and 107 (Table 2). Spermatophoric canal smooth and without pigmentation, extending along ventral face of hectocotylized arm ending in the calamus. Copulatory organ with a small ligula (3.2 to 4.1% of hectocotylized arm), with 5 to 7 transverse creases (Figs. 2C, 5E). Calamus (8 to 9 mm) without pigmentation and slightly long (50% of ligula length). Gills small with six to seven lamellae per demibranch.

Anterior salivary gland small (4 mm); posterior salivary glands small (6 mm); esophagus slender, pseudocrop without diverticulum, stomach muscular; caecum small and striated coiled in a single whorl; large digestive gland

Table 2. Measurements (mm) and counts of *Graneledone* sp. For abbreviations see Materials and Methods. *Incomplete or damaged organ / Medidas (mm) y conteos de *Graneledone* sp. Para abreviaciones ver Materiales y Métodos. *órgano dañado o incompleto

	MNHNCL 6640	MZUC-UCCC 32743	MNHNCL 6641	MNHNCL 30039
Sex	Male	Female	Male	Male
TL	810	760	520	330
AL1l	630	570	395	248
AL1r	565	564	380	245
AL2l	590	570	375	220*
AL2r	560	570	300*	240
AL3l	465	466	335	178
AL3r	390	495	278	170
AL4l	255	463	310	170
AL4r	439	442	300	160
AW	23	28	20	20
HW	88	101	90	60
HL	45	51	43	25
MW	93	130	90	75
DML	160	165	110	80
VML	95	100	93	50
PA	66	86	68	50
ED	32	32	22	12
SD	8	7	6	5
LL	16	---	9	6
CaL	8	---	8.5	4
FuL	47	47	40	35
FfuL	29	27	21	15
GiLC	7	6	7	7
WDA	90	105	78	60
WDBl	100	78	70	48
WDCl	90	100	65	38
WDDl	85	80	50	55
WDBr	114	78	55	56
WDCr	95	70	50	40
WDDr	85	57	55	59
WDE	85	48	30	65
WF	B>C>A>D=E	ACDBE	ABCDE	EABDC
AF	1234	1234	1234	1243
FO	VV	VV	VV	VV
ASC1R	100	87	102	73
ASC1L	61	99	95	75
ASC2R	104	97	45*	78
ASC2L	79	104	108	74
ASC3R	43	95	45	43
ASC3L	98	105	107	70
ASC4R	88	100	90	69
ASC4L	82	95	93	63

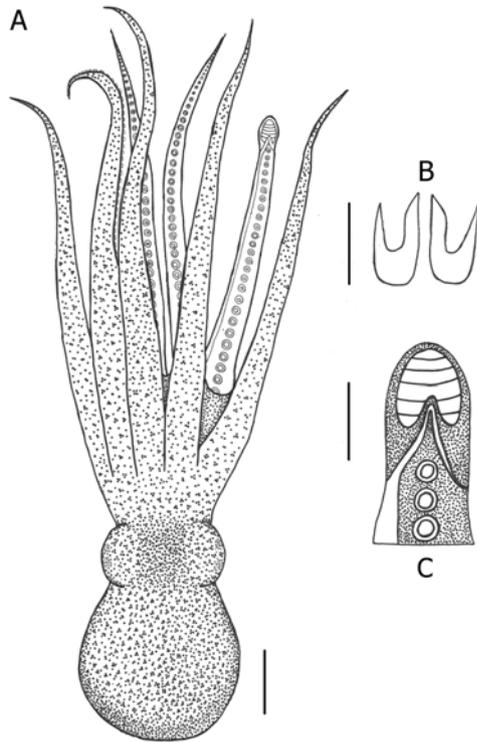


Figure 2. *Graneledone* sp. (MNHCL 6641). (A) adult male, (B) funnel organ, (C) tip of the hectocotylus. Scale bars: A, 30 mm; B, 30 mm; C, 8 mm / *Graneledone* sp. (MNHCL 6641). (A) macho adulto, (B) órgano del sifón, (C) hectocotilo. Escala de barras: A, 30 mm; B, 30 mm; C, 8 mm

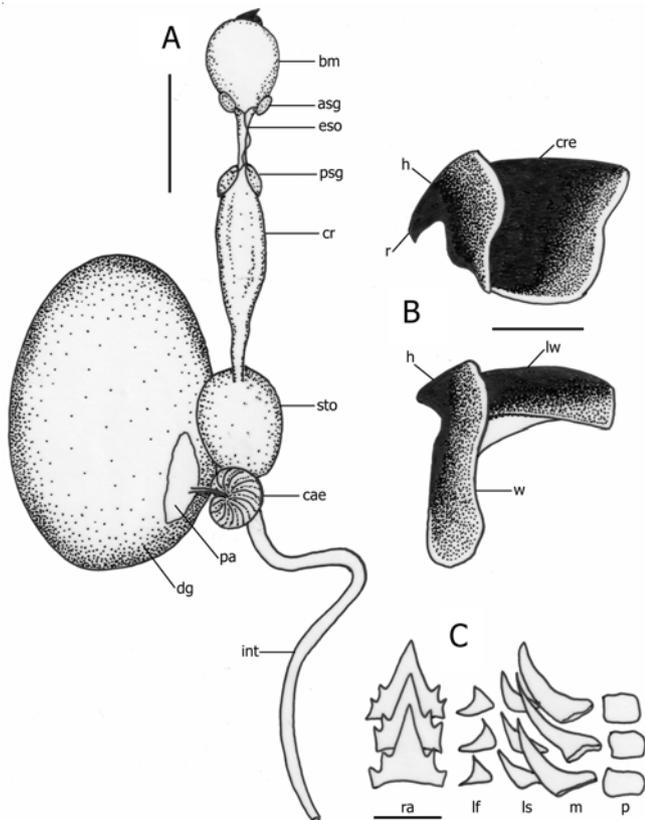


Figure 3. Digestive tract of *Graneledone* sp. (A) Digestive tract; asg, anterior salivary glands; eso, oesophagus; cr, crop; sto, stomach; cae, caecum; bm, buccal mass; psg, posterior salivary glands; dg, digestive gland; pa, pancreas; int, intestine, (B) beaks; r, rostrum; h, hood; cre, crest; lw, lateral wing; w, wing; (C) radula; p, plate; m, marginal tooth; ls, second lateral tooth; lf, first lateral tooth; ra, rachidian teeth. Scale bars: A, 10 mm; B, 20 mm; C, 1 mm / Tracto digestivo de *Graneledone* sp. (A) Tracto digestivo; asg, glándulas salivares anteriores; eso, esófago; cr, buche; sto, estómago; cae, ciego; bm, masa bucal; psg, glándulas salivares posteriores; dg, glándula digestiva; pa, páncreas; int, intestino, (B) picos; r, rostro; h, capucha; cre, cresta; lw, ala lateral; w, ala; (C) rádula; p, placa; m, diente marginal; ls, diente lateral secundario; lf, diente lateral primario; ra, diente raquídeo. Escala de barras: A, 10 mm; B, 20 mm; C, 1 mm

Figure 4. Reproductive system of *Graneledone* sp. (A) female reproductive system; do, distal oviduct; og, oviduct gland; po, proximal reproductive system; ov, ovary, (B) egg, (C) male reproductive system; ag, accessory gland; sgl, spermatid gland; tes, testis; ns, Needham's sac; to, terminal organ, (D) spermatophore. Scale bars: A, 10 mm; B, 2 mm; C, 20 mm; D, 15 mm / Sistema reproductivo de *Graneledone* sp. (A) sistema reproductivo de hembra; do, oviducto distal; og, glándula oviductal; po, oviducto proximal; ov, ovario, (B) huevo, (C) sistema reproductivo de macho; ag, glándula accesoria; sgl, glándula espermática; tes, testículo; ns, saco de Needham; to, órgano terminal, (D) espermatóforo. Escala de barras: A, 10 mm; B, 2 mm; C, 20 mm; D, 15 mm

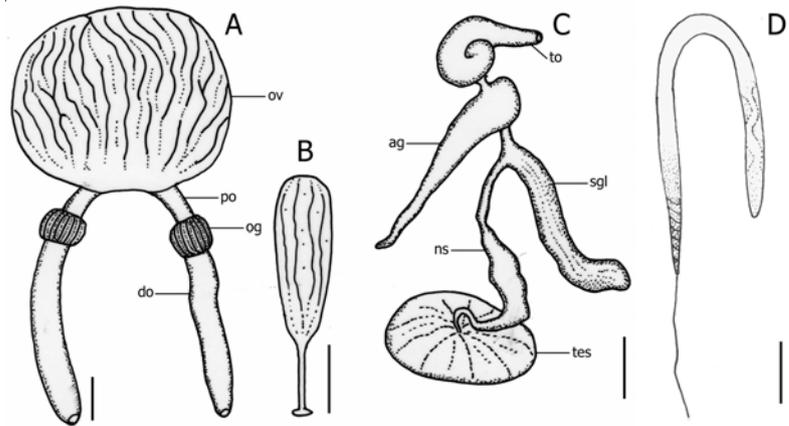


Figure 5. Photographs of *Graneledone* sp. (MNHCL 6641). (A) fresh specimen, (B) eye, (C) suckers, (D) funnel organ, (E) hectocotylus, (F) radulae. Scale bars: A, 30 mm; B, 20 mm; C, 5 mm; D, 20 mm; E, 10 mm; F, 1 mm / Fotografías de *Graneledone* sp. (MNHCL 6641). (A) espécimen fresco, (B) ojo, (C) ventosas, (D) órgano del sifón, (E) hectocotilo, (F) rádula. Escala de barras: A, 30 mm; B, 20 mm; C, 5 mm; D, 20 mm; E, 10 mm; F, 1 mm

(43 mm) roundish without ink sac; intestine long (107 mm) without anal flaps (Fig. 3A). Upper beak with strong deep jaw angle; lower beak with distinct groove along lower edge of insertion plate (Fig. 3B). Radula with a multicuspoid rachidian, and to each side two lateral teeth, one marginal tooth and the marginal plate (Fig. 3C, 5F, 6A, 6B).

Genitalia of female with large ovary (41 mm diameter); oviductal gland large (11 mm diameter); distal oviduct large (51 mm length) (Fig. 4A). 55 eggs (10-12 mm long) in ovary of maturing female (Fig. 4B).

Male reproductive system with short, thick proximal vas deferens; spermatophoral gland moderately long (60 mm) (Fig. 4C). Accessory gland large (40 mm), with terminal recurvature. Spermatophore sac long, containing four spermatophores (160-180 mm) (Fig. 4D). Poor preservation condition of the spermatophores did not allow further detailed description. Distal vas deferens short, penis diverticulum large with spiral, penis large (25 mm) (Fig. 4C).

Locations. Southeastern Pacific Ocean, north of Mocha island, Chile, 37°46.17'S, 74°07.29'W, at 1482 m (Fig. 1).

Distribution. Southeastern Pacific Ocean, from 35°S - 38°S, 436 to 1482 m depth (Fig. 1).

Habitat. Upper continental slope, associated with hard grounds (e.g., at methane seep sites).



Figure 6. Radula of *Graneledone* sp. (MNHNCL 6640). (A) general view, (B) detail of the rachidian tooth. Scale bars 0.1 mm / Rádula de *Graneledone* sp. (MNHNCL 6640). (A) vista general, (B) detalle del diente raquídeo. Escala de barras 0,1 mm

Table 3. Morphological comparison between *Graneledone* species / Comparación morfológica entre las especies de *Graneledone*

Species	Ocular cirri	Gills count	ASC	HASC	Warts mantle	Warts head	Reference
<i>Graneledone</i> sp.	Absent	6-7	61-108	43-45	30-35	15-20	This study
<i>G. antarctica</i>	Absent	5-6	49-63	39	25-36	23-27	Voss 1976, O'Shea 1999, this study
<i>G. gonzalezi</i>	Absent	6	29-72	32-40	20	10	Guerra <i>et al.</i> 2000
<i>G. yamana</i>	Present	5-7	35-80	26-38	10-15	4-6	Guerrero-Kommritz 2000, this study
<i>G. macrotyla</i>	Present	7	32-65	31-40	10-12	–	Voss 1976, Kubodera and Okutani 1994, Guerra <i>et al.</i> 2012
<i>G. boreopacifica</i>	Present	7-8	60-82	36-38	21-29	15-18	Voss and Percy 1990
<i>G. challengerii</i>	Absent	6-7	73-114	41-51	30-40	20-30	O'Shea 1999
<i>G. verrucosa</i>	Present	6-8	58-87	40-46	18-26	10-16	Allcock <i>et al.</i> 2003, this study
<i>G. taniwha taniwha</i>	Absent	7-8	58-97	39-45	24-29	12-15	O'Shea 1999
<i>G. taniwha kubodera</i>	Absent	7-8	71-99	44-48	23-25	11-14	O'Shea 1999

ASC = arm sucker count, HASC = hectocotylized arm sucker count

MORPHOLOGICAL COMPARISON

Some morphological characters distinguish *Graneledone* sp. from other congeneric species. The presence of supra-ocular cirri in *G. boreopacifica*, *G. verrucosa*, *G. macrotyla* and *G. yamana* contrasts with the absence of these structures in *G. sp.* Counts of cartilaginous clusters along the mantle and head are higher in individuals of *G. sp.* than in *G. gonzalezi*, *G. macrotyla*, *G. yamana*, *G. boreopacifica*, *G. verrucosa* and *G. taniwha*, but less than in *G. antarctica* and *G. challengerii* (Table 3). The numbers of wart processes within each cluster in *G. sp.* are higher (1-22 units) and larger (diameter 1-5 mm), than in *G. antarctica* (1-11 units) (diameter 0.3-0.9 mm), *G. gonzalezi* (1-12 units) (diameter 3.3 mm), *G. boreopacifica* (3-12 units) (diameter 1-3 mm), *G. verrucosa* (1-12 units) (diameter 1-3 mm) and *G. challengerii* (1-10 units) (diameter 1-2.7 mm) and less than *G. taniwha* (1-37 units) (diameter 0.5-8 mm). Moreover, *Graneledone* sp. have 5-7 transverse creases in the ligulae, while *G. gonzalezi*, *G. yamana*, *G. boreopacifica*, *G. verrucosa*, *G. challengerii* lacks them. *Graneledone taniwha taniwha* have 7-8 transverse creases in the ligulae, while *G. antarctica* have 9-13, higher count than in *G. sp.*, but *G. taniwha kubodera* have less (3-4) than *G. sp.* Hectocotylized sucker count is very close between *G. sp.* and *G. challengerii*, *G. verrucosa* and *G. taniwha*, but higher than other species (Table 3).

MOLECULAR PHYLOGENETIC ANALYSIS

Alignment lengths for COI and 16S rRNA fragments were 655 bp and 497 bp, respectively. Partition homogeneity test did not show significant differences between these molecular markers ($P = 0.21$), and sequences were combined and analyzed as a single data set. The combined data (16S + COI) of 1152 bp length contained 71 characters that were parsimony informative. The *Graneledone* sp. specimens differed by 9 substitutions (0.8%) from *G. boreopacifica*, and by 14 substitutions (1.2%) from *G. verrucosa* and 27 (2.3%) from *G. antarctica*. Comparing *G. boreopacifica* with *G. verrucosa* we found only 17 substitutions (1.5%). The only 16S rRNA sequence of *G. taniwha* from South West Pacific Ocean (GenBank accession number AJ311119) showed a substantial divergence (4.2%) with *G. sp.* Unfortunately, we are not sure if this sequence corresponds to some *G. taniwha* species because there is no collection information associated to the record.

All phylogenetic analyses (P, ML, BI) depict a similar tree topology with some slightly differences in node support by bootstrap and posterior probabilities (Fig. 7).

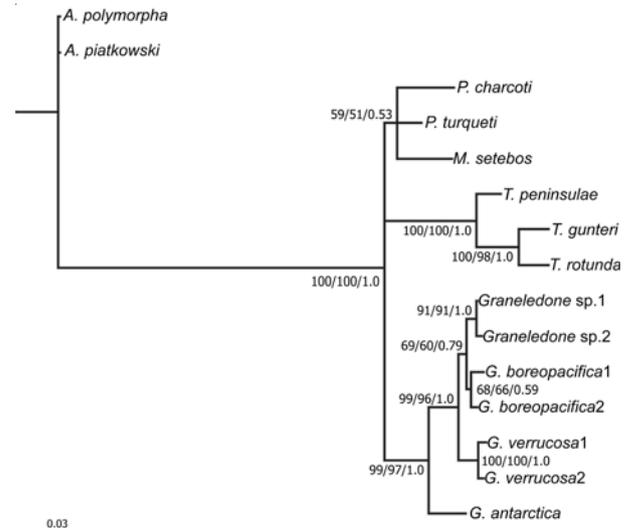


Figure 7. Bayesian phylogram of *Graneledone* species (16S+COI). Node values are bootstrap of 10,000 iterations of MP and ML, and posterior probabilities of BI / Filograma Bayesiano de las especies de *Graneledone* (16S+COI). Valores en los nodos son el resultado de bootstrap de 10.000 iteraciones de P y ML, y probabilidades a posteriori de BI

These high nodes values support the hypothesis of monophyly of *Graneledone* (Fig. 7). *Graneledone boreopacifica* was the sister species of *Graneledone* sp. (Fig. 7).

We found a ML tree of $-lnL = 1159.72$. Maximum parsimony analysis using the combined data set (16S+COI) returned one parsimonious tree, 212 steps in length (consistency index excluding uninformative sites = 0.761; retention index = 0.802).

DISCUSSION

Morphological, molecular and phylogenetic analysis of mtDNA sequences of the Chilean *Graneledone* specimens and those of North Pacific, North Atlantic and Antarctica supported the taxonomic distinctiveness of these specimens as a candidate species. However, further revision of the holotype and/or paratypes of the other species of *Graneledone* are needed to confirm the identity of *Graneledone* from Chile. Holotypes of *Graneledone* species are in France, Russia, England, Germany and New Zealand (Sweeney 2001) making them very difficult to access and study for Southern Hemisphere researchers.

The great genetic similarity between *Graneledone* species is due to their recent origin, estimated to be about 3 million years (Strugnell *et al.* 2008a). Similar low

differentiation between inter-species comparisons was detected in the related octopodid genus *Pareledone* (1-2%; Allcock *et al.* 2007) and *Thaumeledone* (2-3.3%; Strugnell *et al.* 2008b). The phylogenetic relationship is very consistent with the hypothesized monophyly of *Graneledone* (Voight 2000a) and the Antarctic origin of these deep-sea octopuses (Strugnell *et al.* 2008a), because *G. antarctica* is the most basal species of *Graneledone* and the Atlantic and Pacific species are the most derived and the divergence of Pacific species could be very recent. Sequences of all *Graneledone* species are needed to complete the phylogenetic reconstruction, establish their origin and the divergence of these deep-sea species.

The great variation in morphometric and meristic data in species of the genus *Graneledone* (Table 3) complicates the identification, given the overlap in counts of gills, suckers and warts among different species. Multivariate analysis of the morphological dataset suggests that these counts and indices, traditionally used for discriminating between cephalopods species, do not show great discrimination at species level, but provide excellent discrimination at the generic level (Allcock *et al.* 2008). The difficulty differentiating between *Graneledone* sp. and their relatives is consistent with the idea of cryptic species, and as mentioned earlier, could be a consequence of the recent origin of the group. Voight (2000a) commented on the possible existence of cryptic species of *Graneledone* within the north Pacific specimens collected at different depths. Cryptic speciation in octopuses has been reported in other genera like *Octopus* and *Pareledone* (Söller *et al.* 2000, Allcock *et al.* 2011), and could be more common than previously thought.

The records of octopus with uniserial suckers from Chile had many problems. Roper *et al.* (1984) mentioned *Pareledone* spp. with circumpolar distributions including the coast of southern Chile, Argentina and South Africa, leading Rocha (1997) to include *Pareledone charcoti* and *P. turqueti* in the check list of cephalopods from Chile. Guerrero-Kommritz (2006) reviewed the octopods with uniserial suckers from southwest Atlantic and finds a very diverse octopus fauna including *Eledone*, *Graneledone*, *Vosseledone*, *Thaumeledone*, and *Pareledone*. However, we studied up to 2500 km of Chilean coast and the only octopuses with uniserial suckers were *Graneledone* (see Ibáñez *et al.* 2011). Thore (1959) reported a specimen of *Bentheledone rotunda* (Hoyle 1885) (now *Thaumeledone rotunda*) off Valparaíso, Chile (33°S, 71°W), but he mentioned that the identification must be considered dubious, since H.M.S. Challenger only captured the arms

of the octopus. This specimen could have corresponded indeed to *Graneledone* sp., since at this latitude this species is so far the only reported deep-sea octopus with one row of suckers, hence, *T. rotunda* is not distributed in Chilean waters as has been reported (Rocha 1997, Vega *et al.* 2001, Vega 2009). Furthermore, the specimen identified as *G. antarctica* (MNHCL 300039) by Vega *et al.* (2001) from southern Chile corresponds to *Graneledone* sp. (Table 1).

Although some of the specimens have been found associated with hard grounds, like those present at methane seeps, no special adaptations to this type of habitat have been observed. We assume that the preference of this species for these sites is just a consequence of the locally enhanced abundance of potential prey and the availability of hard substrate generated by the carbonate reefs present at seep sites (Sellanes *et al.* 2008).

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LITERATURE CITED

- Aljanabi SM & I Martinez. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research* 25: 4692-4693.
- Allcock AL, MA Collins & M Vecchione. 2003. A redescription of *Graneledone verrucosa* (Verrill, 1881) (Octopoda: Octopodidae). *Journal of Molluscan Studies* 69: 135-143.
- Allcock AL, JM Strugnell, P Prodöhl, U Piatkowski & M Vecchione. 2007. A new species of *Pareledone* (Cephalopoda: Octopodidae) from the Antarctic Peninsula. *Polar Biology* 30: 883-893.

- Allcock AL, JM Strugnell & MP 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.
- Allcock AL, I Barratt, M Eléaume, K Linse, MD Norman, PJ Smith, D Steinke, DW Stevens & JM Strugnell. 2011.** Cryptic speciation and the circumpolarity debate: A case study on endemic Southern Ocean octopuses using the COI barcode of life. *Deep-Sea Research II* 58: 242-249.
- Farris JS, MKA Källersjöjärsjö, AG Kluge & C Bult. 1995.** Constructing a significance test for incongruence. *Systematic Biology* 44: 570-572.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- Guerra A, AF González & Y Cherel. 2000.** *Graneledone gonzalezi* sp. nov. (Mollusca: Cephalopoda): a new octopod from the Iles Kerguelen. *Antarctic Science* 12: 33-40.
- Guerra A, A Roura, MP Sieiro, JM Portela & JL Del Río. 2012.** New insights into the morphology, reproduction and distribution of the large-tuberculate octopus *Graneledone macrotyla* from the Patagonian slope. *Scientia Marina* 76: 319-328.
- Guerrero-Kommritz J. 2000.** A new species of *Graneledone* from the southwest Atlantic. *Journal of Molluscan Studies* 66: 543-549.
- Guerrero-Kommritz J. 2006.** Octopods (Cephalopoda: Octopoda) with uniserial suckers from the southwest Atlantic Ocean in the Zoological Museum Hamburg. *Mitteilungen Hamburgisches Zoologisches Museum und Institut* 103: 11-32.
- Ibáñez CM, RD Sepúlveda & J Chong. 2006.** A new species of *Benthooctopus* Grimpe 1921 (Cephalopoda: Octopodidae) from the southeastern Pacific Ocean. *Proceedings of the Biological Society of Washington* 119: 355-364.
- Ibáñez CM, PA Camus & FJ Rocha. 2009.** Diversity and distribution of cephalopod species of the coast off Chile. *Marine Biology Research* 5: 374-384.
- Ibáñez CM, MC Pardo-Gandarillas, D Párraga, M Ziruello & J Sellanes. 2011.** Cefalópodos recolectados en el talud continental de Chile central. *Amici Molluscarum* 19: 37-40.
- Kubodera T & T Okutani. 1994.** Eledonine octopods from Southern Ocean: systematics and distribution. *Antarctic Science* 6: 205-214.
- Norman MD & FG Hochberg. 2005.** The current state of *Octopus* taxonomy. *Phuket Marine Biology Center Research Bulletin* 66: 127-154.
- O'Shea S. 1999.** The marine fauna of New Zealand: Octopoda (Mollusca: Cephalopoda). NIWA. *Biodiversity Memoir* 112: 1-280.
- Posada D. 2008.** jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256.
- Rambaut A & AJ Drummond. 2009.** Tracer v1.5. [on line] <<http://tree.bio.ed.ac.uk/software/tracer>>
- 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, MA Suchard & JP Huelsenbeck. 2012.** MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* <doi: 10.1093/sysbio/sys029>
- Roper CFE & GL Voss. 1983.** Guidelines for taxonomic descriptions of cephalopod species. *Memoirs of the National Museum of Victoria* 44: 49-63.
- Roper CFE, MJ Sweeney & CE Nauen. 1984.** Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. *FAO Fisheries Synopsis* 125(3): 1-277.
- Sellanes J, E Quiroga & VA Gallardo. 2004.** First direct evidences of methane seepage and associated chemosynthetic communities in the bathyal zone off Chile. *Journal of the Marine Biological Association of the United Kingdom* 84: 1065-1066.
- Sellanes J, E Quiroga & C Neira. 2008.** Megafaunal community structure and trophic relationships of the recently discovered Concepción Methane Seep Area (Chile, ~36°S). *ICES Journal of Marine Science* 65: 1102-1111.
- Söller R, K Warnke, U Saint-Paul & D Blohm. 2000.** Sequence divergence of mitochondrial DNA indicates cryptic biodiversity in *Octopus vulgaris* and supports the taxonomic distinctiveness of *Octopus mimus* (Cephalopoda: Octopodidae). *Marine Biology* 136: 29-35.
- Strugnell JM, AL Allcock & MA Collins. 2008a.** Molecular evolutionary relationships of the octopodid genus *Thaumeledone* (Cephalopoda: Octopodidae) from the Southern Ocean. *Antarctic Science* 20: 245-251.
- Strugnell JM, AD Rogers, PA Prodöhl, MA Collins & AL Allcock. 2008b.** The thermohaline expressway: the Southern Ocean as a centre of origin for deep-sea octopuses. *Cladistics* 24: 853-860.
- Sweeney MJ. 2001.** Current Classification of Recent Cephalopoda. [on line] <<http://sirismm.si.edu/cephs/newclass.pdf>>
- Swofford DL. 2002.** PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4. Sinauer Associates, Sunderland. [CD ROM]
- Tamura K, D Peterson, N Peterson, G Stecher, M Nei & S Kumar. 2011.** MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 8: 2731-2739.
- Thore S. 1959.** Cephalopoda. Reports of the Lund University Chile Expeditions 1948-49 33: 1-20.

- Vega MA. 2009.** Sistemática, biogeografía y pesquería de cefalópodos de aguas chilenas, 288 pp. RIL Editores, Santiago.
- Vega MA, S Letelier & E Carreño. 2001.** Colección de cefalópodos del Museo Nacional de Historia Natural: catálogo especies de aguas chilenas. Boletín, Museo Nacional de Historia Natural Santiago Chile 57: 1-88.
- Voight JR. 1997.** Cladistic analysis of the order Octopoda based on anatomical characters. Journal of Molluscan Studies 63: 311-325.
- Voight JR. 2000a.** The distribution of octopuses of *Graneledone* (Cephalopoda: Octopodidae) in reference to deep-sea features. Malacologia 42: 63-74.
- Voight JR. 2000b.** A deep-sea octopus (*Graneledone* cf. *boreopacifica*) as a shell-crushing hydrothermal vent predator. Journal of Zoology of London 252: 335-341.
- Voss GL. 1976.** Two new species of octopods of the genus *Graneledone* (Mollusca: Cephalopoda) from the southern Ocean. Proceedings of the Biological Society of Washington 88: 447-458.
- Voss GL & WG Percy. 1990.** Deep-water octopods (Mollusca: Cephalopoda) of the Northeastern Pacific. Proceedings of the California Academy of Science 47: 47-94.

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