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A first look at the genetic diversity of *Enteroctopus megalocyathus* (Cephalopoda: Enteroctopodidae) captured by the king crab fishery in the south of Chile

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Abstract

The octopus fishery in the southern tip of South America is based on *Enteroctopus megalocyathus*. It is fished on both the Atlantic and Pacific coasts, but no study has yet investigated the genetic variability of this octopus, which is frequently collected as bycatch. The genetic identity and diversity of *E. megalocyathus* from specimens caught by the king crab fishery along the Beagle Channel in southern Chile was investigated using sequences of three mitochondrial (16S rRNA, COI and COIII) and one nuclear (rhodopsin) markers. Homologous sequences from other Enteroctopodidae were included to determine the genetic variability of *E. megalocyathus*. In addition to *E. megalocyathus*, genetic data allowed us to identify *Muusoctopus eureka*, a species also collected by the king crab fishery. *Enteroctopus megalocyathus* was found to be genetically similar to *E. zealandicus*; the genetic distances between these two species were low, 0% (16S rRNA), 0.2% (COI) and 0.6% (COIII), which was also confirmed by the phylogenetic topologies, as both species are in the same clade. *Enteroctopus megalocyathus* has low levels of genetic diversity, as shown by haplotype and nucleotide diversity values for the mitochondrial markers (Hd = 0.06–0.32; π = 0.0001–0.003), and null diversity for the nuclear marker. All the haplotypic networks resolved with the mtDNA markers showed shared haplotypes among *E. megalocyathus*, *E. magnificus* and *E. zealandicus*. The low genetic diversity of *E. megalocyathus* can be attributed to both the geological history of South America and the life history of the species, rather than to the king crab fishery.

Introduction

Enteroctopus megalocyathus (Gould, 1852) is distributed along the Magellanic biogeographic province, from Chiloe Island in the Pacific Ocean to the San Matias Gulf in the Atlantic Ocean (Ré, 1998; Ibáñez *et al.*, 2009), and is an important fishery resource in the southern tip of South America, Chile and Argentina, where it is fished along the Atlantic and Pacific coasts with hooks inserted into rock crevices (Uriarte & Farías, 2014; Sauer *et al.*, 2019). Fishery management of *E. megalocyathus* does not exist in Chile but is currently under development (IFOP, 2019). In Argentina, this octopus supports a small-scale artisanal fishery (Ortiz & Ré, 2019). In addition, *E. megalocyathus* is a bycatch species of lobster (IFOP, 2019) and king crab fisheries in southern Chile (present study).

The king crab fishery in the southern tip of South America is an artisanal mixed fishery for centolla – *Lithodes santolla* (Molina, 1782) – and centollon – *Paralomis granulosa* (Hombron and Jacquinot, 1846). Both species are fished using traps and bait, a technique that also catches octopuses. Octopuses are a frequent component of the bycatch in pot and trap fisheries around the world (Brock & Ward, 2004; Groeneveld *et al.*, 2006; Conners & Levine, 2017). For instance, species of *Enteroctopus* Rochebrune & Mabille, 1889 are collected as bycatch in the Alaskan and South African fisheries (Groeneveld *et al.*, 2006; Barry *et al.*, 2013; Conners & Levine, 2017).

Studies on the genetic diversity of *Enteroctopus* are scarce (Strugnell *et al.*, 2011; Toussaint *et al.*, 2012; Barry *et al.*, 2013). Spatial genetic structure as well as low haplotype diversity have been detected in *Enteroctopus dofleini* (Wülker, 1910) in Alaska (Barry *et al.*, 2013). No study has yet investigated the genetic diversity in *E. megalocyathus*. The population genetic pattern of *E. dofleini* observed in Alaska may be mirrored in *E. megalocyathus* as both species share several characteristics; both species are merobenthic, have a similar paralarval period and similar paralarva size at hatching (Uriarte & Farías, 2014). Nevertheless, studies about the genetic structure of other molluscs, fish and crustaceans in the Magellanic province have shown low genetic diversity and/or no genetic structure (molluscs, de Aranzamendi *et al.*, 2011, 2014; fishes, Ceballos *et al.*, 2012; crabs, Barrera-García, 2016; González-Wevar *et al.*, 2016a, 2016*b*). A lack of genetic structure in these groups is attributed to two types of factors: (1)

historical, such as events that happened during the last glacial period (sea level regression, decrease in marine water temperature, ice sheet scouring and ice sheet calving); and (2) biological, such as larval dispersal, which is driven by currents. The aim of the present study is to determine the genetic diversity and identity of *Enteroctopus megalocyathus* in the southern tip of South America. Genetic data like these can be valuable for the management of fisheries because bycatch could lead to a reduction in the genetic diversity of this species.

Materials and methods

Sampling, DNA extraction, Polymerase Chain Reaction (PCR) and sequencing

We obtained samples from 34 octopuses caught by the king crab fishery in the Beagle Channel, in the southern tip of Chile (54°54′–55°07′S 65°50′–69°19′W; Figure 1, Supplementary material Table S1). All samples were stored in 96% ethyl alcohol and kept at 4°C for subsequent molecular analyses at Laboratorio de Genética y Genómica del CEQUA (Centro de Estudios del Cuaternario Fuego, Patagonia y Antártica).

Genomic DNA was extracted from arm muscle tissue and was subsequently purified using the QIAamp DNA Mini Kit (Qiagen) following the established manufacturer procedure. Polymerase chain reaction amplifications for the 16S rRNA, cytochrome *c* oxidase subunits I and III (COI and COIII, respectively) and the nuclear gene rhodopsin (Rho) were carried out. Each 25 µl reaction contained 2.5 µl of $MgCl_2$ (2.5 mM), 12 µM of each primer, 200 mM of each dNTP, 1× PCR buffer, and 1.25 U of GoTaq polymerase (Promega). Universal primers (16Sar and 16Sbr) were used for the amplification of 16S rRNA fragments (Palumbi, 1996). The COI (LCO1490 and HCO2198) and COIII (COIIIi3′ and COIIIi5′) primers used were those described by Folmer *et al.* (1994) and Barriga-Sosa *et al.* (1995), respectively; rhodopsin (Rhod1243octfwd and Rhod1793octbck) primers were those described by Strugnell (2004). Polymerase chain reactions were conducted in a Mycycler (Bio-Rad) thermocycler using annealing temperatures of 52°C for 16S rRNA, 49°C for COI, 38°C for COIII and 57°C for Rho, and the following conditions: an initial cycle of denaturing at 94°C for 5 min followed by 30 cycles at 94°C for 45 s, an annealing step for 45 s, an extension step at 72°C for 90 s, and finally, an extension cycle at 72°C for 15 min. Bidirectional sequencing reactions were performed by Macrogen (Seoul, South Korea) and utilizing the primers used for PCR amplifications. Sequences were visualized, concatenated, and edited with the program BioEdit 7.0 (Hall, 1999) and adjusted by eye. Sequence alignments were conducted in Clustal W (Thompson *et al.*, 1994) implemented in MEGA X (Kumar *et al.*, 2018) and revised with the respective translation of amino acids for COI, COIII and rhodopsin.

DNA-based octopuses bycatch identification

Preliminary identification of bycatch species was performed using a sequence similarity search in the Barcode of Life Data Systems (BOLD; https://www.boldsystems.org/) (for COI) and GenBank (for all sequences) using the Basic Local Alignment Search Tool (BLAST) for highly similar sequences (Mega-BLAST) and using only publicly available sequences. Species were assigned based on the percentage of maximum similarity (>99%). To corroborate this, MEGA X (Kumar *et al.*, 2018) was used for sequence divergence calculation between reference and sample sequences for each mitochondrial marker. The Tamura–Nei distance model for 16S rRNA, and the Tamura 3 parameter for COI and COIII were used for estimating genetic distances among the four species of *Enteroctopus*. The Tamura–Nei distance model was used for estimating genetic distances among species of *Muusoctopus*. All models were specified by jModeltest 2 (Darriba *et al.*, 2012).

Genetic analysis of *Enteroctopus megalocyathus*

The genetic diversity of *E. megalocyathus* in the southern tip of South America was investigated by estimating the number of segregating sites (S), haplotypes (K), nucleotide diversity (π) and haplotype diversity (Hd) in DnaSP 6 (Rozas *et al.*, 2017). The

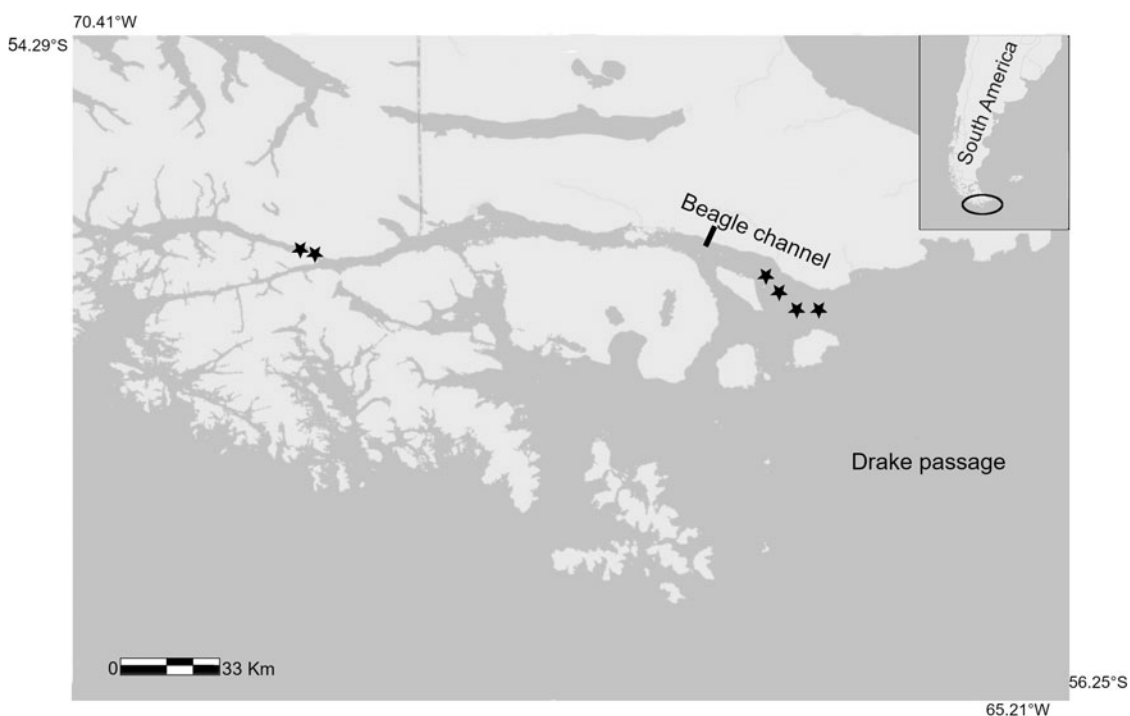

Fig. 1. King crab fishery localities in South of Chile where octopuses were caught as bycatch (see Supplementary material Table S1).

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values for π and Hd were compared with those reported by Goodall-Copestake *et al.* (2012) for a wide variety of animals, including molluscs, to determine the level of genetic diversity of *E. megalocyathus*. Tajima's test (D) and Fu's *Fs* were performed to quantify the significant departure from mutation-drift equilibrium in ARLEQUIN (Excoffier *et al.*, 2005). The same software was used to investigate the demographic expansion of the population of *E. megalocyathus* by comparing the distribution of pairwise differences among haplotypes with the expected distribution of a model of population expansion (mismatch distribution). For the latter, only the marker with more polymorphic sites was used because genes with high levels of polymorphism are better for inferring demographic histories (Grant, 2015). Haplotype networks were constructed using the median-joining algorithm network in Network 10.2 software (FluxusTechnology Ltd, www.fluxus-engineering.com) to investigate the genealogical relationships among haplotypes for the three species of *Enteroctopus* from the southern hemisphere. For these analyses, homologous sequences of *E. megalocyathus* from GenBank, from Chiloe Island, Puerto Williams, and Falkland Islands were used (see Table 1 for GenBank accession numbers). In addition, sequences of *E. magnificus* (Villanueva *et al.*, 1992) from South Africa and *E. zealandicus* (Benham, 1944) from New Zealand were included (see Table 1 for GenBank accession numbers).

Phylogenetic analysis

To determine the phylogenetic relationships between the four species of *Enteroctopus*, a phylogenetic analysis was conducted. Available public sequences for each gene were retrieved from GenBank for species of Enteroctopodidae and for the outgroup species of *Octopus vulgaris* Cuvier, 1797, and *Bathypolypus* Grimpe, 1921 (see Table 1 for GenBank accession numbers). These two species were used to root the phylogeny as several studies have shown that these outgroups are suitable for the Enteroctopodidae (Strugnell *et al.*, 2011; Ibáñez *et al.*, 2016, 2021; Sanchez *et al.*, 2018). *Octopus californicus* Berry, 1911, was included in the ingroup as it has been demonstrated to belong to the Enteroctopodidae (Strugnell *et al.*, 2011; Ibáñez *et al.*, 2021). jModeltest 2 (Darriba *et al.*, 2012) was used to select the best-fit model for each dataset (separate genes) based on the Bayesian information criterion (BIC). Given that sequence availability per gene differs in GenBank, phylogenetic reconstruction was inferred using each gene independently and the sampled

Table 1. GenBank sequences of species of family *Enteroctopodidae* and the outgroups, *Bathypolypus* and *Octopus vulgaris* used in the present study

	GenBank accession number			
Taxa	16S rRNA	COI	COIII	Rhod
Bathypolypus sp.	AY616972	HM572183	EF016320	GQ226024
Enteroctopus dofleini	AY545109	AB477017	X83103	AY545174
Enteroctopus megalocyathus	KT314274-KT314276, GQ226032, KM459453, KM459454, **MW509831**	KM459468, KM459469, KF774312, HM572167, MT216544, **MW549877**, **MW549878**	GQ226027, KM459483, KM459484, KC792304, KT314267- KT314269, **MW562308-MW562310**	**MW562315**
Enteroctopus zealandicus	MT216950-MT216955, **MW509832**	HM572175, MT216543, MT216545-MT216547, **MW549881**	MT225042-MT225046	**MW562317**
Enteroctopus magnificus	AJ252750	NA	NA	NA
Muusoctopus johnsonianus	HM572162	EF016333	HM572197	HM572225
Muusoctopus eicomar	KM459463, KM459464	KM459493	KM459485, KM459493, KM459495-97	NA
Muusoctopus eureka	EF016339, **MW509829, MW509830**	HM572168, EF016330, MW549879, MW549880	HM572190, EF016321, EF016322, **MW562311-MW562314**	HM572221, HM572222, **MW562316**
Muusoctopus normani	HM572153	EF016335	EF016325	HM572223
Muusoctopus oregonensis	FJ603543	HM572180	FJ603538	GQ226016
Muusoctopus yaquinae	FJ603539	HM572182	FJ603532	GQ226017
Muusoctopus longibrachus	HM572166	KM459471	KM459488	HM572219
Octopus californicus	AJ390322, HM572164	AF377968, MK649792-MK649795	HM572187, X83102, AJ250483	HM572214
Octopus vulgaris	EF016336	KT008578	KT008586	HM104297
Sasakiopus salebrosus	GQ226031	NA	GQ226028	GQ226025
Vulcanoctopus hydrothermalis	FJ603544	HM104264	FJ603533	HM572215

16S rRNA, large ribosomal subunit; COI, Cytochrome c oxidase subunit I; COIII, Cytochrome c oxidase subunit III; Rhod, Rhodopsin. Bold GenBank accession numbers are the sequences generated in the present study.

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Table 2. Genetic distances (%) for *Enteroctopus* and *Muusoctopus* species analysed in the present study

Taxa (GenBank sequences)	*Enteroctopus megalocyathus* (present study)		
	16S rRNA (TN)	COI (T3P)	COIII (T3P)
Enteroctopus dofleini	3.5	4.4–*4.5*–4.6	4.8–*5.6*–6.1
Enteroctopus magnificus	0.4	NA	NA
Enteroctopus megalocyathus	**0.2–0.4–0.6**	**0–*0.1*–0.2**	**0–*0.8*–2.3**
Enteroctopus zealandicus	**0**	**0–*0.2*–0.5**	**0–*0.6*–1.1**
Taxa (GenBank sequences)	***Muusoctopus eureka* (present study)**		
	16S rRNA (TN)	**COI (TN)**	**COIII (TN)**
Muusoctopus eicomar	4	7.2–*7.3*–7.4	11.3–*11.4*–11.5
Muusoctopus eureka	**0**	**0–*0.1*–0.2**	**0–*0.7*–1.5**
Muusoctopus johnsonianus	4.4	7.7–*7.8*–8	8.4–*8.6*–8.8
Muusoctopus longibrachus	4.1	6.6–*6.7*–6.8	8.0–*8.1*–8.3
Muusoctopus normani	4.4	8.2–*8.3*–8.4	13.6–*13.7*–13.8
Muusoctopus oregonensis	4.6	7.5–*7.6*–7.7	7.5–*7.6*–7.7
Muusoctopus yaquinae	4.6	8–*8.1*–8.3	9.5–*9.6*–9.7

Values given as Min-*Avg*-Max. Values ≤1 shown in bold. Abbreviations: TN, Tamura–Nei distance model; T3P, Tamura 3 parameter. All models were specified by jModeltest 2 (Darriba *et al.*, 2012).

sequences were collapsed into haplotypes using DnaSP 6 (Rozas *et al.*, 2017). The Bayesian analysis (BA) consisted of two independent Monte Carlo Markov Chain (MCMC) runs, each consisting of 10 million steps sampled every 1000 points. TRACER v1.6 (Rambaut *et al.*, 2018) was used to determine acceptable burn-in (25%) and to ensure the analysis had reached stationarity (we report values ≥0.6 for bpp). For the maximum-likelihood (ML) analysis, node supports were assessed using 1000 ultrafast bootstrap (bs) replicates (Hoang *et al.*, 2018), with values ≥60 reported in the present study. Inferences were performed in MrBayes 3.2 (Ronquist *et al.*, 2012) and IQ tree web server (Trifinopoulos *et al.*, 2016) for BA and ML analyses, respectively, and using only the mitochondrial markers. Because two best-fit models, TIM1 + G and TIM3 + G, cannot be implemented in MrBayes, the GTR + G model was used instead.

Results

DNA identification of octopus bycatch

A total of 129 sequences were obtained from 34 individuals with the following read lengths: 406 base pairs (bp) for Rho (MW562315–MW562317); 455 bp for COIII (MW562308–MW562314); 467 bp for 16S rRNA (MW509829–MW509832), and 600 bp for COI (MW549877–MW549881) sequences. Collapsed sequences yield three haplotypes for 16S rRNA, four for COI, seven for COIII, and two for rhodopsin. The haplotypes were identical or show >98% similarity to either *Muusoctopus eureka* (Robson, 1929) (99.8–100% for 16S rRNA, 99.8–100% for COI, 98.6–100% for COIII; six specimens) or *Enteroctopus megalocyathus* and *E. zealandicus* (99.8–100% for 16S rRNA, 99.8–100% for COI; 98.6–100% for COIII; 28 specimens) (Supplementary material, Tables S2–S4). Genetic divergence between sampled and reference sequences of *E. megalocyathus* ranged from 0–3.5% (16S rRNA), 0–4.6% (COI), and 0–6.1% (COIII). Genetic distances between *Muusoctopus eureka* and reference sequences of the species ranged from 0–4.6% (16S rRNA), 0–8.4% (COI) and 0–13.8% (COIII). The average genetic distances are shown in Table 2.

Genetic diversity analysis of Enteroctopus megalocyathus

Our results suggest that the population of *E. megalocyathus* from southern Chile has low genetic diversity (Table 3). For all the mitochondrial markers, *E. megalocyathus* shows low nucleotide (0.003–0.0001) and haplotype diversity (0.06–0.32). Results for rhodopsin show a lack of both nucleotide and haplotype diversity. The 467 bp fragment of 16s rRNA from 41 individuals of *E. megalocyathus* (28 specimens from the present study and 13 sequences from GenBank) yielded five haplotypes that differed at 10 sites

Table 3. Genetic parameters determined for *Enteroctopus megalocyathus* from South of Chile and best-fit models of substitution used in the phylogenetic analysis

Genetic diversity							Neutrality test		Substitution models			
Gen	*N*	bp	*S*	π	*K*	Hd	Tajima's *D*	Fu's *Fs*	Model	Parameters	BIC	lnL
16S rRNA	34	467	3	0.0007	4	0.17	−1.213^{ns}	−1.9839*	TIM3 + G	57	3733.2	1688.3
COI	33	600	1	0.0001	2	0.06	−1.1401^{ns}	−1.2903^{ns}	GTR + G	59	5692.1	2654.6
COIII	35	455	12	0.003	6	0.32	−1.68*	−0.5280^{ns}	TIM1 + G	67	5327.0	2453.4
Rhod	21	406	0	0	1	0	–	–	–	–	–	–

16S rRNA, large ribosomal subunit; COI, cytochrome c oxidase subunit I; COIII, cytochrome c oxidase subunit III; Rhod, Rhodopsin; N, number of individuals; bp, base pairs; S, number of segregating sites; π, nucleotide diversity; Hd, haplotype diversity; K, number of haplotypes. *$P < 0.05$.

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(Supplementary material, Table S2). The 600 bp fragment of COI from 39 individuals (28 specimens from the present study and 11 sequences from GenBank) yielded three haplotypes that differed at two sites (Supplementary material, Table S3). The 455 bp fragment of COIII from 40 individuals (28 specimens from the present study and 12 sequences from GenBank) yielded nine haplotypes that differed at 13 sites (Supplementary material, Table S4). These three mitochondrial markers resolved the correspondent haplotype H1 as the most frequent. The 406 bp fragment of rhodopsin from 21 individuals from the present study yielded a unique haplotype. Our results suggest that several of the resolved haplotypes from *E. megalocyathus* are shared with *E. zealandicus* (see Figure 2 and Supplementary material Tables S2–S4). Tajima's D test values were negative and not statistically significant for 16S rRNA and COI but were significant for COIII (see Table 3). Fu's *Fs* values were negative and not statistically significant for COI and COIII but were significant for 16S rRNA (see Table 3). The pairwise difference distribution among COIII haplotypes was L-shaped (Raggedness index = 0.368, $P = 0.52$; Figure 3).

The median-joining networks of mitochondrial genes (see Figure 2) included haplotypes of *Enteroctopus magnificus* (from South Africa) and *E. zealandicus* (from New Zealand) and show a single ubiquitous haplotype for each gene (see Figure 2B, C). These ubiquitous haplotypes occurred in 37 individuals for 16S rRNA and COI genes and in 29 individuals for COIII gen (see Supplementary material). The haplotypes from *E. megalocyathus* and *E. zealandicus* are different by one to three substitutions (see Figure 2A) in COIII, one or two substitutions in 16S rRNA, and one substitution in COI. The 16S rRNA and COI most frequent haplotypes are shared by *E. megalocyathus* and *E. zealandicus*. Unique haplotypes from *E. megalocyathus* are restricted to the Beagle Channel (COIIIH2–COIIIH5 and 16SH5) and Chiloe Island (16SH3 and 16SH4). The nuclear allele is shared by both *E. megalocyathus* and *E. zealandicus*. The only

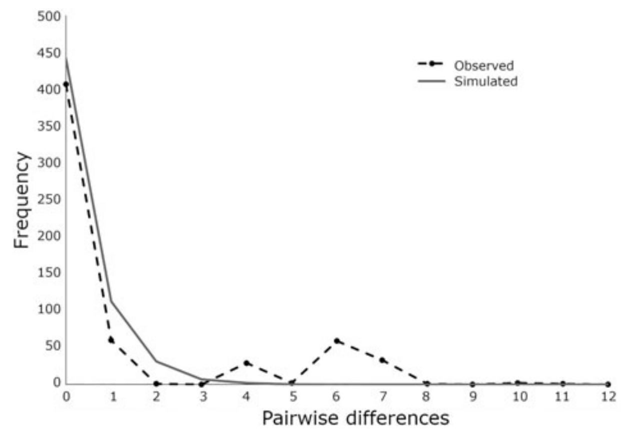

Fig. 3. Mismatch distribution of observed and expected pairwise differences among COIII haplotypes of *Enteroctopus megalocyathus.*

sequence for 16S rRNA available for *E. magnificus* yielded a unique haplotype that is separated from the most common 16SH1 haplotype by seven mutational sites. This haplotype is shared by *E. megalocyathus* and *E. zealandicus* (see Figure 2B).

Phylogenetic analysis

The resolved mitochondrial phylogenies (Figures 4 and 5) revealed two results about *Enteroctopus*: (1) the sequences from *E. megalocyathus* clustered with those from *E. zealandicus* in a well-supported monophyletic clade, except in the 16S rRNA ML phylogeny (Figure 5A); and (2) *E. dofleini* is the sister species of the *E. megalocyathus*/*E. zealandicus* clade. Interestingly, *E. magnificus* (AJ252750) was included in the *E. megalocyathus*/ *E. zealandicus* clade in the 16S rRNA tree (Bayesian posterior probability = 1, bs = 79). In addition, our analysis confirmed the

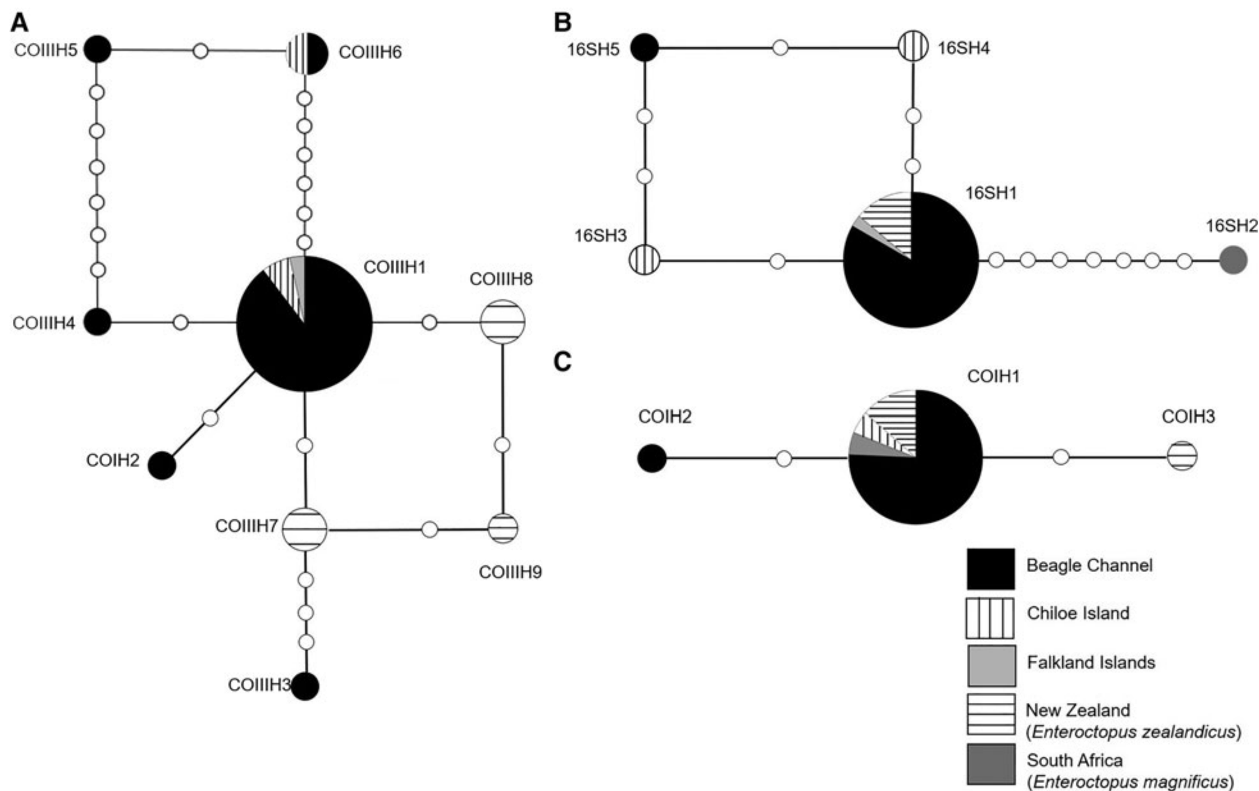

Fig. 2. Median-joining networks of haplotypes of *Enteroctopus megalocyathus*. (A) COIII, (B) 16S rRNA and (C) COI. Circles represent haplotype and their size is proportional to the number of individuals. White circles on branches represent one mutational substitution.

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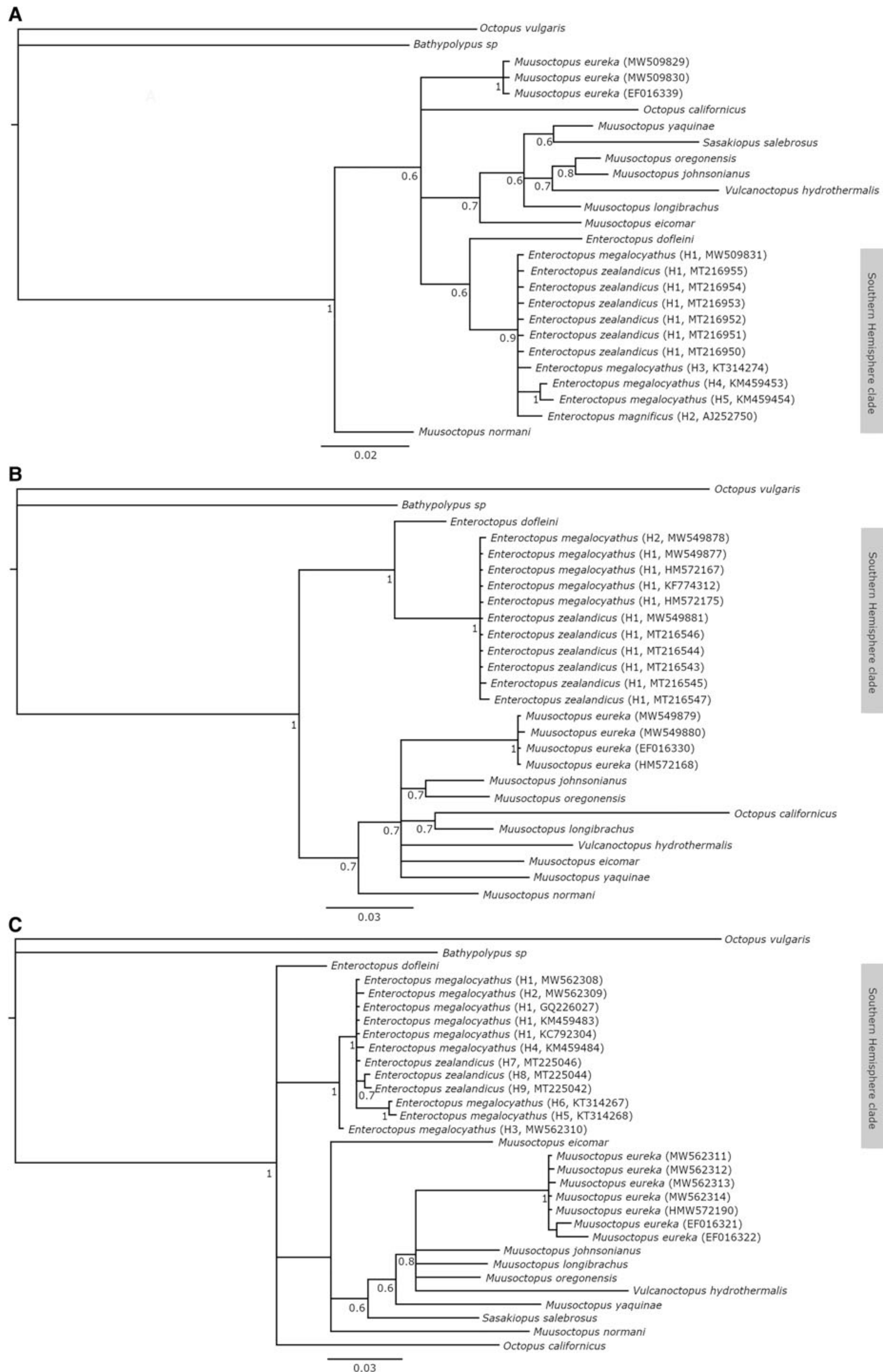

Fig. 4. Bayesian phylogeny of *Enteroctopus megalocyathus* based on homologous sequences of 16S rRNA (A), COI (B) and COIII (C). Bayesian posterior probability values (≥0.6) are shown beside nodes.

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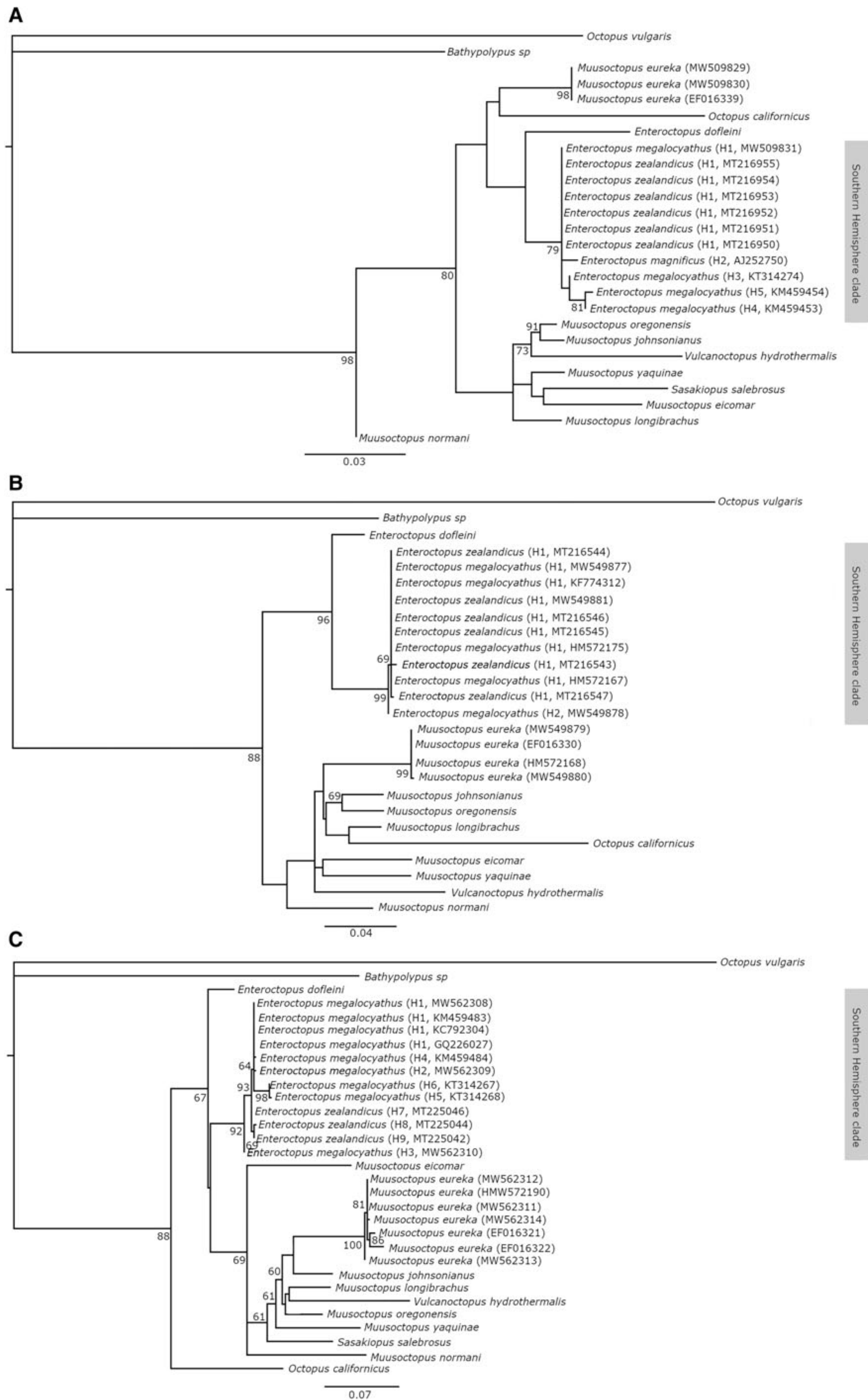

Fig. 5. Maximum likelihood phylogeny of *Enteroctopus megalocyathus* based on homologous sequences of 16S rRNA (A), COI (B) and COIII (C). Bootstrap values ($\geq$60) are shown beside nodes.

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phylogenetic position of *Muusoctopus eureka* as the haplotypes resolved in the present study cluster in a highly supported clade (both BA and ML) with the *M. eureka* sequences from GenBank.

Discussion

Genetic diversity of Enteroctopus megalocyathus

The estimates of π and Hd for samples of *Enteroctopus megalocyathus* fall below the median value of genetic diversity for several molluscs, crustaceans, and other animals (Goodall-Copestake *et al*., 2012); therefore, the genetic diversity of *Enteroctopus megalocyathus* could be considered as low. The low genetic diversity is not uncommon in octopuses and other molluscs from the same region; for instance, *Octopus mimus* (Gould, 1852), a merobenthic octopus from off Chile also shows low genetic diversity (Pardo-Gandarillas *et al*., 2018). Gastropods of the genus *Nacella* from the Magellanic province with similar ecological traits to *E. megalocyathus* (e.g. an adult benthic lifestyle and an early planktonic stage; see below) show low levels of genetic diversity (de Aranzamendi *et al*., 2011, 2014) (see Supplementary material, Table S5). These levels have been associated with a demographic expansion that occurred after the last glaciation, and to the major ocean currents that favour larval dispersal (de Aranzamendi *et al*., 2011, 2014; González-Wevar *et al*., 2016*a*, 2016*b*; Pardo-Gandarillas *et al*., 2018). These historical and ecological traits might also be responsible for the observed low genetic diversity of *E. megalocyathus*. Its paralarval stage can last up to three months (Uriarte & Farías, 2014), which is long enough for paralarvae to be dispersed by ocean currents. The low genetic diversity might also reflect a post-glacial recolonization of *E. megalocyathus* as has been suggested for several molluscs from South America (de Aranzamendi *et al*., 2011, 2014; González-Wevar *et al*., 2016*a*, 2016*b*; Pardo-Gandarillas *et al*., 2018). The hypothesis of a recent event of expansion in the population of *E. megalocyathus* is supported by the haplotype frequency distribution, by the negative *D* and *Fs* values, and by the unimodal mismatch distribution of pairwise differences among COIII haplotypes. The mitochondrial haplotype frequency pattern of *E. megalocyathus* is similar to the haplotype frequency of *Octopus mimus* from Chile (Pardo-Gandarillas *et al*., 2018) and to the haplotype frequency of *Nacella* spp. from the southern tip of South America (de Aranzamendi *et al*., 2011; González-Wevar *et al*., 2016*a*, 2016*b*), which show a ubiquitous haplotype and some singletons for each molecular marker. The presence of one dominant haplotype in *O. mimus*, *N. magellanica* (Gmelin, 1791) and *N. mytilina* (Helbling, 1779) along their distribution range suggests a recent geographic expansion (de Aranzamendi *et al*., 2011; González-Wevar *et al*., 2016*a*, 2016*b*; Pardo-Gandarillas *et al*., 2018).

Although the low genetic diversity of *Enteroctopus megalocyathus* is not associated with its fishery, the results are novel for the species and could be used for future studies on the genetic connectivity and structure of *E. megalocyathus* that could provide valuable information for the management of this fishery. Given that we use mitochondrial genes and one nuclear gene instead of a single locus, our results could be representative of the genetic diversity of *E. megalocyathus* in South America; however, further nuclear data such as microsatellite data or single nucleotide polymorphisms in addition to a larger sample from the Falkland Islands and the South of Chile could help corroborate the genetic pattern observed herein.

Species of Enteroctopus *from southern hemisphere*

The presence of common haplotypes is not rare in closely related sympatric species (e.g. gastropods, Kemppainen *et al*., 2009; de Aranzamendi *et al*., 2011) but is uncommon in allopatric species because introgressive hybridization is not possible. While *E. megalocyathus* has been described for South America, *E. zealandicus* has been for New Zealand; both species thus occur in non-overlapping geographic distributions. Two hypotheses could explain the observed genetic pattern presented. The first hypothesis is that *E. megalocyathus* and *E. zealandicus* share haplotypes due to incomplete lineage sorting evidenced by both species sharing mitochondrial haplotypes and a nuclear allele, and by both species exhibiting non-reciprocal monophyly. Nuclear markers have larger effective population size than mitochondrial markers, so lineage sorting takes longer to occur in nuclear genes (Sotelo *et al*., 2020). The second hypothesis is that *E. megalocyathus*, *E. magnificus* and *E. zealandicus* are conspecifics. This suggests that one *Enteroctopus* species occurs along the southern hemisphere, which is evidenced by the close relationship between the three taxa in the phylogenetic trees resolved in the present study, and previously stated in other studies (Hudelot, 2000 cited by Norman *et al*., 2014; Ibañez *et al*., 2020). However, both *E. megalocyathus* and *E. zealandicus* differ morphologically from each other in several characteristics (Ibáñez *et al*., 2020). Either hypothesis requires further investigation. In addition, we conclude that *Enteroctopus megalocyathus* has low genetic diversity because of its life history and historic events that occurred during the last glaciation rather than to the king crab fishery.

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Data. Data available within the article or its supplementary materials. The authors confirm that the data supporting the findings of this study are available within the article [and/or its supplementary materials].

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