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Phylogeny and biogeography of *Muusoctopus* (Cephalopoda: Enteroctopodidae)

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Deep-sea octopuses of the genus *Muusoctopus* are thought to have originated in the Pacific Northern Hemisphere and then diversified throughout the Pacific and into the rest of the World Ocean. However, this hypothesis was inferred only from molecular divergence times. Here, the ancestral distribution and dispersal routes are estimated by Bayesian analysis based on a new phylogeny including 38 specimens from the south-eastern Pacific Ocean. Morphological data and molecular sequences of three mitochondrial genes (16S rRNA, COI and COIII) are presented. The morphological data confirm that specimens newly acquired from off the coast of Chile comprise two species: *Muusoctopus longibrachus* and the poorly described species, *Muusoctopus eicomar*. The latter is here redescribed and is clearly distinguished from *M. longibrachus* and other closely related species in the region. A gene tree was built using Bayesian analysis to infer the phylogenetic position of these species within the species group, revealing that a large genetic distance separates the two sympatric Chilean species. *M. longibrachus* is confirmed as the sister species of *Muusooctopus eureka* from the Falkland Islands; while *M. eicomar* is a sister species of *Muusoctopus yaquinae* from the North Pacific, most closely related to the amphi-Atlantic species *Muusoctopus januarii*. Molecular divergence times and ancestral distribution analyses suggest that genus *Muusoctopus* may have originated in the North Atlantic: one lineage dispersed directly southward to the Magellan region and another dispersed southward along the Eastern Pacific to the Southern Ocean and Antarctica. The *Muusoctopus* species in the Southern Hemisphere have different phylogenetic origins and represent independent invasions of this region.

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Introduction

The field of phylogenetic biogeography focuses on how processes such as geological and climatic change influence speciation, extinction and the geographical distribution of species in a phylogenetic framework (Wiley & Lieberman 2011). This enables ancestral geographical distributions to be reconstructed and the centre of origin of a group of species can be estimated. It is then possible to better understand how vicariance and/or dispersal have influenced present distribution patterns (see, for example, Ridgway *et al.* 1998; Anderson 2000; Collado *et al.* 2011).

In marine ecosystems, four centres of origin of marine fauna have been recognized: the Antarctic, the North Pacific, the East Indies and the Southern Caribbean (Briggs 2003, 2006). Each centre has produced dominant, successful species that have spread over large geographical areas functioning as 'evolutionary engines' (Briggs 2003, 2006). Strugnell *et al.* (2008) proposed the Southern Ocean as the centre of origin of deep-sea octopods of several genera (e.g. *Graneledone*, *Thaumeledone*) and suggested that the global thermohaline circulation has acted as an evolutionary driver. Studies with other deep-sea octopods of the genus *Muusoctopus* indicate that this species group originated in relatively shallow waters of the Northern Hemisphere and their dispersion to Southern Hemisphere appears to represent independent invasions of this region based on divergence times and non-overlapping distributions of three independent clades (Strugnell *et al.* 2011; Gleadall 2013). However, the source of this distribution is not known: it could be the result of several dispersal events; or of vicariance processes spread over the last 25 My.

Octopuses of the species groups *Graneledone* and *Muusoctopus* inhabit continental slopes and bathyal zones, including extreme environments such as hydrothermal vents, cold seeps, regions of tectonic activity and also hydrocarbon deposit sites in the North Pacific Ocean at depths that may exceed 2200 m (Voight 2000a, 2008). *Graneledone*, *Muusoctopus* and *Vulcanoctopus* have been found in local areas near these extreme environments brooding eggs and foraging (Voight 2000a,b, 2005, 2008; Voight & Grehan 2000; Voight & Drazen 2004; Sellanes *et al.* 2008; Ibáñez *et al.* 2011).

The genus *Muusoctopus* contains around 28 known species that inhabit deep waters of all oceans of the world from the equator to polar seas at depths reaching 3850 m (Strugnell *et al.* 2011). The genus *Muusoctopus* Gleadall 2004 was proposed to replace genus name *Benthoctopus* (not *Benthoctopus sensu* Grimpe) because *Octopus piscatorum* Verrill, 1879 (the type species of this genus) was identified by Muus (2002) as a junior synonym of *Bathypolypus bairdii* (Verrill, 1873), and therefore genus *Benthoctopus* as a junior synonym of *Bathypolypus* Grimpe (see Gleadall *et al.* 2010). Muus (2002) suggested *Benthoctopus januarii* (Hoyle, 1885), as an alternative type species for the genus *Benthoctopus* (requiring an application to the ICZN to designate the new type species), but Gleadall (2004) instead proposed a new genus name designating the amphi-Atlantic species *Muusoctopus januarii* (Hoyle, 1885) as its type species. In a study of *Muusoctopus* species found in waters off the Falkland Islands, Gleadall *et al.* (2010) redescribed *Muusoctopus eureka* (Robson 1930), synonymizing two species (previously identified as *Benthoctopus*: *B. eureka* and *B. megallanicus* Robson 1930) and described a new species, *M. bizikovi*, and a new subspecies of the Chilean species, *M. longibrachus* (Ibáñez *et al.* 2006). The new subspecies was named *M. longibrachus akambei*, based on eight males and four females of large size (mantle length up to 165 mm; Gleadall *et al.* 2010).

In the south-eastern Pacific Ocean off Central Chile (36–37°S), *M. longibrachus longibrachus* is so far the only formally described species, based on 12 adult males and two females of medium size (mantle length between 75 and 140 mm; Ibáñez *et al.* 2006). However, Villarroel *et al.* (2001) and Ibáñez *et al.* (2011) recognized two different *Muusoctopus* morphotypes from the Chilean coast between 22°S and 45°S at depths between 241 and 922 m. One of them is *M. longibrachus longibrachus* and the other was recently described as *Benthoctopus eicomar* Vega 2009. Unfortunately, the type material for the latter was not found in the stated depository (confirmed lost following correspondence directly with M.A. Vega and curators at MNHNCL), and the species was poorly described: the diagnosis and description are brief and the type locality is imprecise (a range of 17 degrees of latitude during 3–4 years of collecting); and no original museum registration numbers were recorded (see Vega 2009).

Voss (1988a), based on the work of Robson (1930) and Voss's unpublished data, speculated that the geographical distribution of *M. eureka* (as *Benthoctopus magellanicus*) ranges throughout the Magellanic biogeographical region (i.e. from the Valdes peninsula, in Argentina, to Chiloe Island, in southern Chile at 42°S). However, it seems likely that the specimens from southern Chile considered in that study probably corresponded to the then undescribed species (*M. eicomar*): no *Muusoctopus* specimens have been recorded from the Cape Horn Province of the Magellanic region; and in recent surveys, *M. eureka* has been recorded neither in the Cape Horn Province nor in the Peruvian Province (Villarroel *et al.* 2001; Ibáñez *et al.* 2006, 2011; Gleadall *et al.* 2010; Gleadall 2013).

Gleadall (2013) proposed that the ancestors of *M. longibrachus longibrachus* could have arrived in Chile either by

direct migration southwards from the North Pacific; or possibly via Cape Horn after first moving through the Atrato Seaway into the Atlantic, then diverging from *M. januarii* and migrating south along the eastern coast of South America to arrive at the present pattern of distribution. Which of these routes was used by *M. longibrachus longibrachus* to arrive off Chile and Patagonia remains ambiguous.

Here, the phylogeny and divergence times of *Muusoctopus* species are inferred to estimate the origin and diversification of these deep-sea octopuses. Morphological and molecular approaches are combined to evaluate the systematic status of the two sympatric Chilean species of *Muusoctopus* and data available in GenBank are combined with new sequences to elucidate phylogenetic relationships within the genus.

Materials and methods

Sampling

A total of 38 octopuses were collected and examined for this study (Fig. 1). Twenty were taken during three major cruises (VG04, VG06 and VG07) on board *AGOR Vidal Gormáz*, all of which aimed to study the methane seep sites off south-central Chile. Sampling was by Agassiz trawl (mouth opening 1 × 0.4 m, mesh size 10 × 10 mm at the cod-end) operated in 20-min hauls. The main target area during the cruises was the Concepcion Methane Seep Area (CMSA) (36°21′W, 73° 43′W). Additional samples were collected at two other nearby potential seep sites (36°02′S, 73°38′W and 37°56′S, 74°01′W) at depths ranging from 608 to 922 m. The remaining five specimens were collected in the framework of the FIP 2005-61 project (National Fund for Fisheries Research), aimed at characterizing the benthic habitat of the Chilean margin between 29° and 38°S at 100–450 m depth. Five specimens were obtained as by-catch from commercial shrimp trawling near Valparaiso (33°23′S, 71°53′W). Eight additional specimens were collected during the INSPIRE cruise, on board *R/V Melville* in 2010, at sites ranging from off Peninsula Taitao, southern Chile (~46°55′S, 75°35′W, 460–697 m depth) to off El Quisco (~33°22′S, 71°52′W; ~340 m depth).

Tissue samples were fixed in 96% ethanol for molecular analysis. While smaller whole animals were also preserved in 96% ethanol, tissue subsamples were taken from larger animals before the remaining specimens were fixed in a buffered 10% formaldehyde solution for anatomical and morphological analysis.

Taxonomic descriptions are in the supplementary material.

DNA extraction, PCR amplification and sequencing

Total DNA was extracted from 15 specimens following the saline extraction protocol (Aljanabi & Martinez 1997). PCR

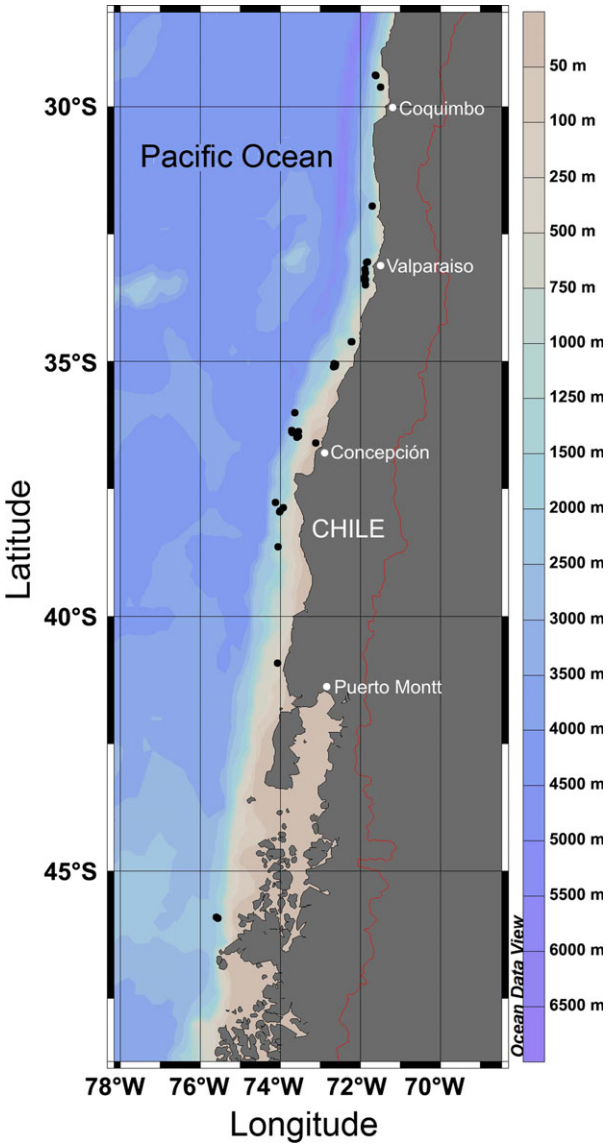

Fig. 1 Sites along the Chilean coast where *Muusoctopus* specimens were collected.

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 each of primers of Cytochrome Oxidase I (COI), Cytochrome Oxidase III (COIII) and 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), [51 °C (40 s) for COI; 55 °C (40 s) for 16S; 40 °C (40 s) for COIII], 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 (Wisconsin, USA) following the manufacturer's protocols. Purified PCR products were sequenced by

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Macrogen (Seoul, South Korea) Sequences were aligned by Clustal W (Thompson *et al.* 1994) implemented in MEGA ver. 5.0 software (Tamura *et al.* 2011). Sequences generated in this study are available from GenBank (Table S1). Genetic distances (p-distance and K2P) between species were calculated for each gene (COI, COIII, 16S rRNA) in MEGA ver. 5.0 software to describe and compare the variation between and within species.

Phylogenetic analysis

Previous to phylogenetic analysis, two preliminary steps were performed. Firstly, a saturation test of each gene was performed in DAMBE ver. 6.0 (Xia 2013). This analysis found little saturation of codifying genes (COI: Iss = 0.107 < Iss.c = 0.734, $P < 0.001$ and COIII: Iss = 0.125 < Iss.c = 0.712, $P < 0.001$). Secondly, the best substitution model for each gene was estimated with jModelTest (Posada 2008) using Bayesian information criteria (BIC, Table S2).

Phylogenetic reconstruction was inferred from a partition matrix with a different substitution model for each gene and another matrix including the concatenated data set (16S + COI + CIII) using the most complex model (GTR + G + I) to reduce the chance that the method would concentrate too much probability in too few trees (Huelsenbeck and Rannala, 2004). Both analyses were compared by means of Bayes Factors (BF, Kass and Raftery, 1995) in Tracer ver. 1.5 (Rambaut & Drummond 2009). This analysis showed strong support for the combined matrix above the partition matrix (BF > 8). Bayesian Inference (BI) was applied to the evaluation of phylogenetic relationships of *Muusoctopus* species using a combined matrix (16S + COI + CIII) with the most complex model of substitution (GTR + G + I). Bayesian analyses were conducted using MrBayes ver. 3.2 (Ronquist *et al.* 2012) with four chains, each with five million generations, sampled every 1000 generations. Bayesian analyses were performed three times to compare the likelihood values of each run using Tracer version 1.5 (Rambaut & Drummond 2009). In these analyses, a birth–death prior of branch lengths was used to avoid polytomies and improve the posterior probability of the nodes (Kuhn *et al.* 2011). 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. FigTree ver. 1.4 was used to edit the trees (Rambaut 2009). The phylogenetic trees were rooted using *Octopus vulgaris* (Lamarck 1798), *Bathypolypus* and *Enteroctopus* as outgroups (see Strugnell *et al.* 2011).

A Bayesian MCMC analysis using BEAST ver. 1.8.2 (Drummond *et al.* 2012) was performed for molecular clock estimation using a concatenated matrix (16S + COI + COIII). We used the same substitution models for phylogenetic reconstructions (Table S2). The relaxed molecular clock with uncorrelated exponential distribution was the model that best fitted the data (log10 Bayes Factor > 6.0). The Birth–Death model served as the tree prior, and parameters were logged every 1000 iterations, sampling a total of 20 000 000 generations. With burn-in set to 10%, the convergence of all parameters was finally checked using Tracer. The relaxed molecular clock was calibrated using the estimated divergence time of *Enteroctopus* – *Muusoctopus* with a normal distribution prior (22 ± 2.2 my, Gleadall 2013).

Biogeographical analyses

Biogeographical data are based on present-day distributions of *Muusoctopus* species (see legend of Fig. 3). The Bayesian Binary Method (BBM, Yu *et al.* 2014) was used to estimate ancestral states implemented in the RASP package (Reconstruct Ancestral States in Phylogenies ver. 3.02, Yu *et al.* 2015). The BBM calculates the probabilities of ancestral ranges using the probabilities of each unit area generated by the average probability of the presence (1) and absence (0) over all sampled generations of the ancestral species in the area (Yu *et al.* 2014). Ancestral area analyses were carried out on Maximum Credibility Tree (MCCT) from BEAST analysis, and information on nodes was summarized and plotted as pie charts. Bayesian analyses were conducted using four heated chains, each with five million generations, sampled every 1000 generations.

Results

Genetic distances

The concatenated data (COI + COIII + 16S) of 1748 bp contained 422 characters that were parsimony informative. The two *Muusoctopus* species from Chile differed by 3.4% (K2P) within 16S rRNA; 7.2% (K2P) within COI; and 9.7% (K2P) within COIII. Comparing *Muusoctopus eicomar* with all species, the differences ranged over 3.6–6.5% within 16S rRNA, 6.0–9.4% within COI and 7.3–13.7% within COIII. *Muusoctopus longibrachus longibrachus* from Chile and *M. longibrachus akambei* from the Falkland Islands showed the lowest genetic differentiation (0.5% K2P) within 16S rRNA; 1.1% (K2P) within COI; and 0.8% (K2P) within COIII. Comparing *M. longibrachus akambei* with all other *Muusoctopus* species, the differences ranged over 2.4–5.3% with 16S rRNA, 4.7–8.4 with COI and 5.3–10.4% with COIII. The intraspecific genetic distances (p-distance) were lower than intersubspecific and interspecific distances for all genes (Table 1).

Phylogenetic analysis

Phylogenetic analysis of the concatenated sequences (16S + COI + COIII) revealed that the genus *Muusoctopus* is paraphyletic by the presence of *Vulcanoctopus* inside the

Table 1 Genetic distance (p-distance) between species and subspecies of *Muusoctopus*

Model	16S rRNA	COI	COIII
Intraspecies	0.0005 (0.000–0.006)	0.003 (0.000–0.027)	0.003 (0.000–0.017)
Intersubspecies	0.0035 (0.003–0.004)	0.009 (0.008–0.011)	0.007 (0.006–0.008)
Interspecies	0.0280 (0.006–0.054)	0.063 (0.029–0.088)	0.078 (0.038–0.127)

principal clade (Fig. 2). The consensus tree indicates that *M. longibrachus longibrachus* off Chile is the sister subspecies of *M. longibrachus akambei* from the Falkland Shelf Province (PP = 1.0) and this lineage is recovered as a monophyletic group (Fig. 2). Both *M. longibrachus* subspecies (*longibrachus* and *akambei*) are sister to *M. eureka* from the Falkland Islands (PP = 0.78). *Muusoctopus eicomar* is the sister species of *M. yaquinae* from the North Pacific (PP = 0.93; Fig. 2).

Divergence times are consistent with previous studies (Strugnell *et al.* 2011; Gleadall 2013). The origin of *Muusoctopus* + *Vulcanoctopus* was during the Miocene (~17 My, clade 1), and the radiation in the Southern Ocean occurred during the Pliocene (~4.7 My, clade 8) (Fig. 3). The estimation of divergence times had an error between 5 and 10 My (Table 2).

Biogeographical analysis

The BBM found 21 events of dispersal and 10 events of vicariance along the *Muusoctopus* phylogeny (Fig. 3). Estimate of the ancestral locality for the basal node of

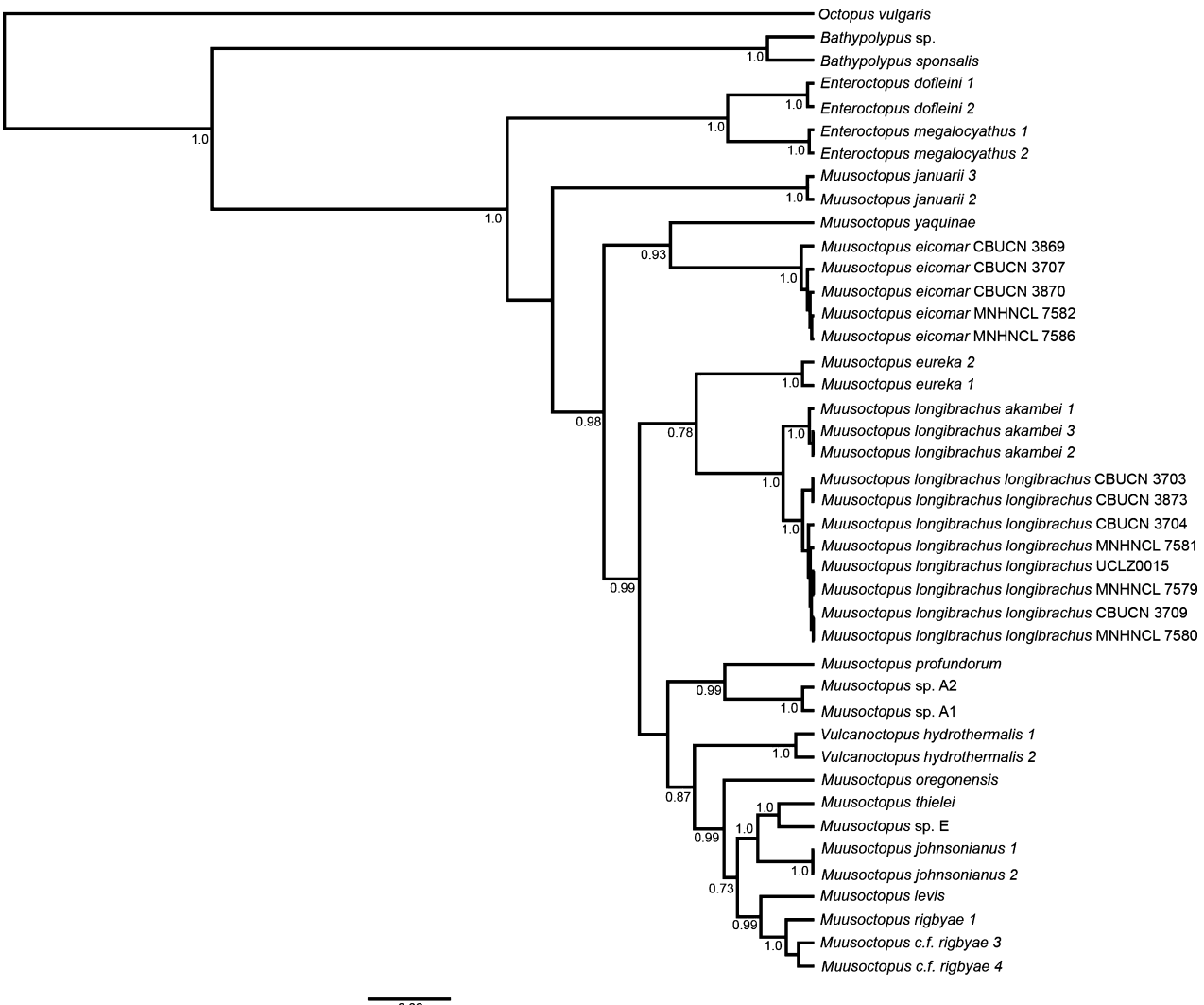

Fig. 2 Bayesian phylogram of *Muusoctopus* species from the concatenated data set (16S + COI + COIII). Node values only show posterior probabilities above 0.70.

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Muusoctopus showed that the most recent common ancestor of the genus (clade 1) probably occupied a broad area including the North Atlantic (PP = 0.82) during the Miocene (~17 My, Fig. 3). Clade 2 showed a mixture of possible ancestral distributions, with the North Pacific (PP = 0.43) and Magellan regions (PP = 0.29) having the highest probabilities (Fig. 3). Clade 3, composed only of *M. yaquinae* and *M. eicomar*, was the most probable part of the phylogenetic tree from which the *Muusoctopus* lineage could have originated in the North Pacific (PP = 0.59) during the upper Miocene (~8 My, Fig. 2). Clade 4 has a high probability that the Magellan province (PP = 0.85) is its ancestral distribution, while clade 5 has a combined probability of origins in either the Magellan region (PP = 0.49) or North Pacific (PP = 0.42; Fig. 3). In clade 6, both subspecies of *M. longibrachus* (*longibrachus* and *akambei*) have a common ancestor inhabiting the Magellan province (PP = 0.77) during the Pleistocene (~1.3 My, Fig. 3). The entire clade 7 originated in the North Pacific (PP = 0.99) during the Upper Miocene (~8.5 My, Fig. 3) and dispersed to the Southern Hemisphere. Clade 8 shows combined probabilities among localities between the Southern Ocean (PP = 0.36) and the North Pacific (PP = 0.54; Fig. 3). It seems that this clade originated in the North Pacific and since the Pliocene (about ~4.7 My) has colonized the Southern Ocean, including localities in the seas around New Zealand, South Australia, the Kerguelen Plateau and Antarctica.

Discussion

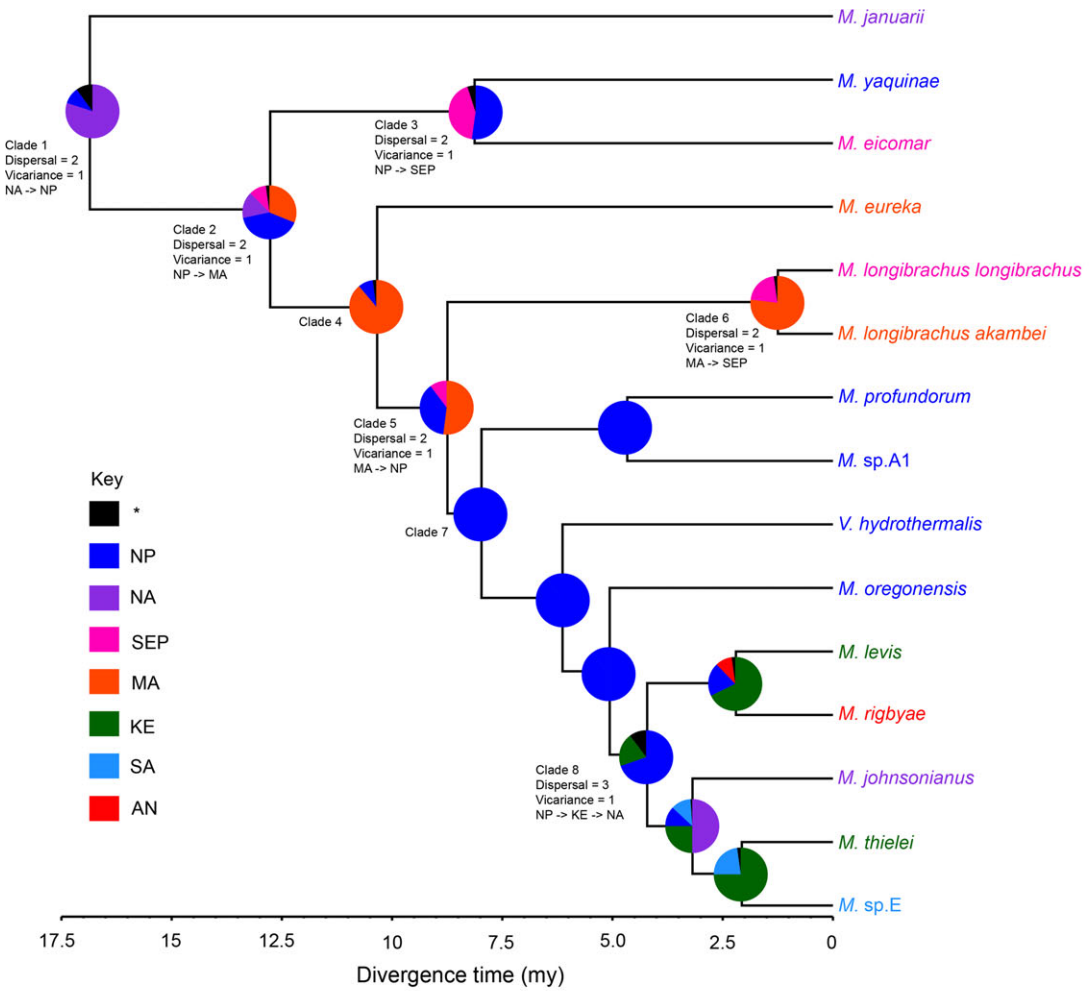
Molecular divergence times and ancestral distribution analyses confirm that *Muusoctopus* species could have originated in the Northern Atlantic, with one lineage dispersing southward to the Magellan region and another southward via the Eastern Pacific to the Southern Ocean and around Antarctica. Previous studies support a Northern Hemisphere origin for this group but with a different dispersal route (Atlantic by Strugnell *et al.* 2011; Pacific by Gleadall 2013). The analysis in the present study supports the hypothesis that *Muusoctopus* species in the Southern Hemisphere represent an invasion of this region independent of the presence of other groups of octopus (Strugnell *et al.* 2011). The previous studies (Strugnell *et al.* 2011; Gleadall 2013) only proposed a dispersion route based on molecular phylogeny and divergence times estimated, but they did not estimate the ancestral distribution or related biogeographical processes. The results presented here are stronger because they include an evaluation combining phylogeny and distribution data to infer the ancestral distribution and dispersion/vicariance events using the Bayesian binary method. *Muusoctopus eicomar* and *M. yaquinae* (along with *M. januarii* in the Atlantic) seem likely to represent the more direct remnants of the ancestral population subjected to vicariance by closure of the Atrato Seaway (Fig. 3B). Based on the results, two principal dispersion routes are proposed: the first from Northern Atlantic to Southern Ocean, and the second crossing the Atrato Seaway to the Pacific Ocean where some species dispersed northward and others southward (Fig. 3B). The inclusion of *M. eicomar* in the analysis changes the phylogenetic relationships of some species, the previously suggested centre of origin and routes of dispersal (cf. for example Strugnell *et al.* 2011; Gleadall 2013). For this reason, it is important to include as many species of *Muusoctopus* as possible to optimize estimations of the origin and diversification of this genus.

Morphological, molecular and phylogenetic analysis of mtDNA sequences of the Chilean *Muusoctopus* specimens supports the existence of two species, *Muusoctopus longibrachus longibrachus* and *M. eicomar*, the last one redescribed here. Comparison of these two species with those from the North Pacific, Atlantic and Antarctic waters suggests that these two species have different phylogenetic and biogeographical origins. *Muusoctopus longibrachus longibrachus* has a southern Atlantic vicariant subspecies, while *M. eicomar* has closer affinities with the northern Pacific, as its sister species is *M. yaquinae* from the Oregon region.

The most prominent morphological character to differentiate the two south-eastern Pacific (Chilean) *Muusoctopus* species is the length of the first arm pair. The molecular distances between the two species are very high (3.4–9.7%), and the phylogenetic analysis confirms that both species have different origins. The phylogenetic results reported here confirm the common ancestry of *M. longibrachus s.s.* and *M. longibrachus akambei* and support the subspecies level designation proposed by Gleadall *et al.* (2010). The modest genetic distance (0.5–1.1%) and the lack of shared haplotypes suggest a recent divergence around 29 000 years ago (Gleadall 2013).

Two undescribed species of the genus *Muusoctopus* were previously reported from the continental shelf of Chile (21°S–35°S) from depths between 180 and 500 m (Villarroel *et al.* 2001). These species probably corresponded to *M. longibrachus* and *M. eicomar*: Gleadall (2013) speculated that one of these (here identified as *M. eicomar*) was probably closely related to *M. januarii*, and the data in the present paper confirm this. Also, Nesis (1973, 1987) reported *Benthoctopus* sp. aff. *januarii* Nesis 1973; from Cocos Island off northern Peru, at depths between 570 and 1850 m, although it might have been *M. longibrachus* given that Cardoso & Hochberg (2014) recently reported *M. longibrachus* off northern Peru (~5–9°S): the exact distributions of *M. longibrachus* and *M. eicomar* require confirmation.

Reviewing the different species previously identified in the literature as *Benthoctopus* (*eureka*, *bizikovi*, *hokkaidensis*,


M. januarii
M. yaquinae
M. eicomar
M. eureka
M. longibrachus longibrachus
M. longibrachus akambei
M. profundorum
M. sp.A1
V. hydrothermalis
M. oregonensis
M. levis
M. rigbyae
M. johnsonianus
M. thielei
M. sp.E
Clade 1
Dispersal = 2
Vicariance = 1
NA -> NP
Clade 2
Dispersal = 2
Vicariance = 1
NP -> MA
Clade 3
Dispersal = 2
Vicariance = 1
NP -> SEP
Clade 4
Clade 5
Dispersal = 2
Vicariance = 1
MA -> NP
Clade 6
Dispersal = 2
Vicariance = 1
MA -> SEP
Clade 7
Clade 8
Dispersal = 3
Vicariance = 1
NP -> KE -> NA
Key
*
NP
NA
SEP
MA
KE
SA
AN
17.5
15
12.5
10
7.5
5.0
2.5
0
Divergence time (my)

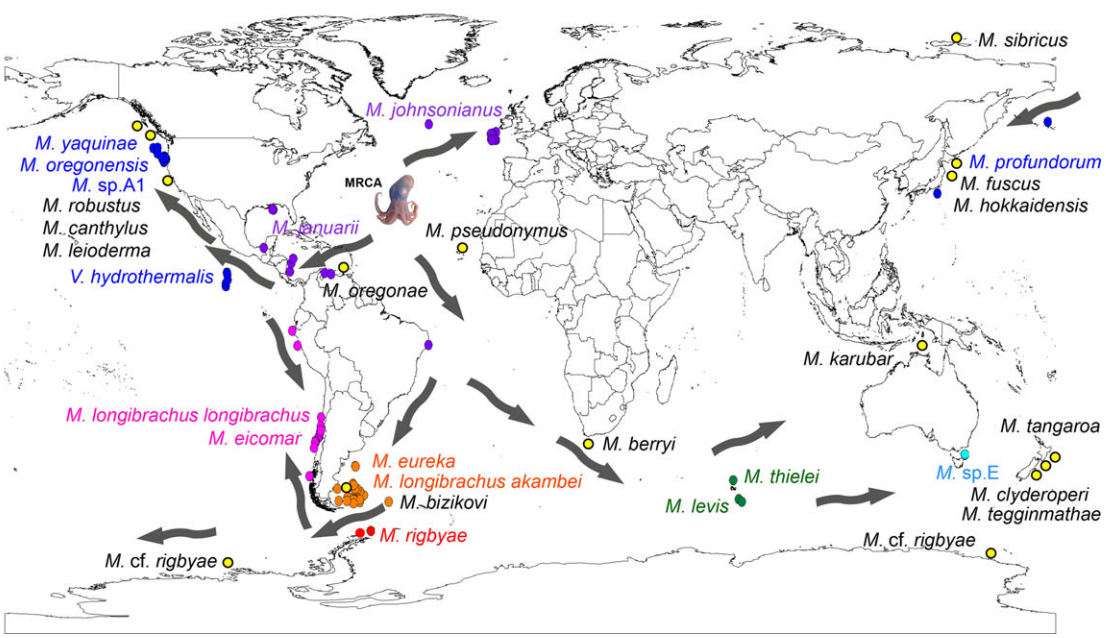

M. sibricus
M. johnsonianus
M. yaquinae
M. oregonensis
M. sp.A1
M. robustus
M. canthylus
M. leioderma
V. hydrothermalis
MRCA
M. januarii
M. pseudonymus
M. oregonae
M. profundorum
M. fuscus
M. hokkaidensis
M. karubar
M. longibrachus longibrachus
M. eicomar
M. eureka
M. longibrachus akambei
M. bizikovi
M. rigbyae
M. berryi
M. thielei
M. levis
M. tangaroa
M. sp.E
M. clyderoperi
M. tegginmathae
M. cf. rigbyae
M. cf. rigbyae

Fig. 3 Historical biogeography of *Muusoctopus*. —A. Divergence times and reconstruction of ancestral areas along the phylogeny of the *Muusoctopus* group species. Pie charts at each node show posterior probabilities of alternative ancestral distributions. The colours represent possible ancestral distribution at different nodes: AN, peri-Antarctica; KE, Kerguelen Plateau; MA, Magellanic region; NA, North Atlantic; NP, North Pacific; SA, off Southern Australia; SEP, South-Eastern Pacific (SEP); black (asterisk) represents wide ancestral distribution. The name of each species is written using the same colour key, which corresponds to the species present distribution. —B. World map showing the present/actual/known distribution of *Muusoctopus* species. Colour dots are the same as in the phylogeny. Yellow-black dots represent species not included in this study. The grey arrows represent the dispersion routes of *Muusoctopus* species. MRCA: Most Recent Common Ancestor.

Table 2 Divergence times (My) estimated for *Muusoctopus* species

Clade number	Median age	HPD 95%
Clade 1	16.7	9.6–23.5
Clade 2	13.1	6.6–19.5
Clade 3	8.2	2.3–15.3
Clade 4	10.8	5.5–16.9
Clade 5	9.4	3.9–14.8
Clade 6	1.3	0.1–4.8
Clade 7	8.5	3.9–14.1
Clade 8	4.7	1.7–8.5

yaquinae, *leioderma*, *longibrachus*, *eicomar*, *tegginmathae*, *clyderoperi* and *tangaroa*) from Chile, Canada, USA, the Falkland Islands and New Zealand, all are here identified as species of *Muusoctopus* (Table S5). This identification is based both on the conservative internal morphology of the pseudophallus of these species (data not shown but see also Gleadall 2004, 2013; and Gleadall *et al.* 2010) and the mtDNA data for some species. In the pseudophallus, the spermatophoric duct opens into the anteromedial chamber through a triangular orifice anterior to this arch, as in *M. januarii*, *M. eureka* and *M. bizikovi* (Gleadall 2004; Gleadall *et al.* 2010). The phylogenetic analysis reveals that all former *Benthoctopus* species (*januarii*, *yaquinae*, *johnsonianus*, *rigbyae*, *oregonensis*, *thielei*, *levis* and *profundorum*) and *Vulcanoctopus hydrothermalis* are part of the same clade. The pseudophallus and hectocotylus morphology of *V. hydrothermalis* is typical of the group (Gleadall, unpublished data; Janet Voight, personal communication), but there are many apomorphies for which the species has been placed in its own genus (González *et al.* 1998, 2002; Gleadall *et al.* 2010). Originally, this species was described from two specimens with diagnostic characters suggested to be adaptations of these animals to hydrothermal vents (e.g. white coloration, eyes without iris). Strugnell *et al.* (2009) dismissed morphological characters that would warrant the distinction of *Vulcanoctopus* from *Muusoctopus*, and Voight (2012) identified *V. hydrothermalis* as a species of *Muusoctopus*. Gene sequence analyses, including that of the present study, place *V. hydrothermalis* within the *Muusoctopus* clade.

The two Chilean *Muusoctopus* species reported in the present study have been found associated with methane seeps, presumably as a consequence of the locally enhanced abundance of potential prey and the availability of shelter generated by carbonate reefs (Sellanes *et al.* 2008), although their morphological characters do not correspond with those of *V. hydrothermalis* (characters purportedly associated with this habitat). More generally, the bathymetric distribution of species in the genus *Muusoctopus* has been associated with cold waters with relatively high oxygen concentration (Villarroel *et al.* 2001). These are typical of the Antarctic Intermediate Waters (AIW), which at the Chilean margin flow below 300–400 m depth. This oceanographic characteristic may explain the modest size of the gills and the low number of gill lamellae, comparing with shallow-water species, as metabolic activity is lower in colder waters, therefore enabling tolerance of a relatively smaller branchial surface for respiration (Ibáñez *et al.* 2006). The resulting reduction in respiratory structures would then probably represent a modification to life at depth *sensu* Robson (1932) and Voss (1988b).

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Ethical standards

This research was approved by the Universidad de Chile ethical committee and the Chilean government through FONDECYT. The manuscript has not been submitted to more than one journal for simultaneous consideration nor has it been published previously.

Conflict of interest

The authors declare that they have no conflict of interest with any other projects, researchers or organizations, commercial or otherwise.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. *Muusoctopus longibrachus longibrachus*.

Fig. S2. *Muusoctopus eicomar*.

Fig. S3. Line drawings of *Muusoctopus eicomar* based on the neotype.

Fig. S4. Line drawings of the reproductive system of *Muusoctopus eicomar*.

Fig. S5. Juveniles of *Muusoctopus* sp. at 702 m depth in the continental slope of Chile off Concepción (~36°21′S, 73°42′W; 700–854 m depth).

Table S1. Octopod species included in the phylogenetic analyses and their genebank code for each mitochondrial gene.

Table S2. Results of substitution model selection for each mitochondrial gene.

Table S3. Measurements (mm) and counts of *Muusoctopus longibrachus longibrachus*. For abbreviations see Materials and Methods. *Incomplete or damaged organ.

Table S4. Measurements (mm) and counts of *Muusoctopus eicomar*. For abbreviations see Materials and Methods. *Incomplete or damaged organ.

Table S5. Morphological comparison between *Muusoctopus* species.

Data S1. Taxonomic.