Polar Biol
DOI 10.1007/s00300-014-1623-6

ORIGINAL PAPER

Low level of genetic divergence between *Harpagifer* fish species (Perciformes: Notothenioidei) suggests a Quaternary colonization of Patagonia from the Antarctic Peninsula

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Received: 16 January 2014 / Revised: 14 November 2014 / Accepted: 17 November 2014

Abstract The evolution of the marine benthic fauna of Antarctica has been shaped by geological and climatic atmospheric factors such as the geographic isolation of the continent and the subsequent installation of the Antarctic Circumpolar Current (ACC). Despite this isolation process, strong biogeographic links still exist between marine fauna from the Antarctic Peninsula and southern South America. Recent studies in different taxa have shown, for example, that shallow benthic organisms with long larval stages maintained contact after the physical separation of the continents and divergence may be associated with the intensification of the ACC in the late Miocene—early Pliocene. In this context, here we performed phylogenetic reconstructions and estimated the level of molecular divergence between congeneric species of *Harpagifer*, a marine notothenioid from the Antarctic Peninsula (*Harpagifer antarcticus*) and Patagonia (*H. bispinis*) using the mitochondrial control region. Phylogenies were reconstructed using Maximum Parsimony and Bayesian Inference, while the divergence time of *H. antarcticus* and *H. bispinis* was estimated following a relaxed Bayesian approach and assuming a strict molecular clock hypothesis. According to our estimation, the divergence between *H. bispinis* and *H. antarcticus* is more recent than expected if it was associated with the intensification of the ACC during the mid to late Miocene. We propose that climatic and oceanographic changes during the coldest periods of the Quaternary (i.e., Great Patagonian Glaciation, 1–0.9 Ma) and the northward migration of the Antarctic Polar Front may have assisted the colonization of southern South America by *Harpagifer*, from the Antarctic Peninsula via the Scotia Arc Islands.

Keywords mtDNA control region · Southern Ocean · Antarctic Polar Front · Great Patagonian Glaciation · Long-distance dispersal

Electronic supplementary material The online version of this article (doi:10.1007/s00300-014-1623-6) contains supplementary material, which is available to authorized users.

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Published online: 27 November 2014

Introduction

The Southern Ocean (SO) and particularly the coastal Antarctic waters constitutes a unique marine environment for biogeographic studies, considering its isolation and the high levels of endemism of the fish fauna (Bargelloni et al. 2000; Clark et al. 2004; Peck et al. 2005; Aronson et al. 2007). Geological processes in the SO since the Mesozoic influenced oceanographic and climatic changes that are responsible for the current biogeography of the region (Crame 1999; Zachos et al. 2001; Griffiths et al. 2009). For instance, the opening of the Tasman Gateway and the Drake Passage were responsible for the separation of Antarctica, Australia and South America (Lawver et al. 2003; Barker et al. 2007). The fragmentation and dispersion of the continental blocks that formed Gondwana allowed the formation of the Antarctic Circumpolar Current (ACC). According to Crame (1999), this oceanographic feature represents a barrier since the Eocene–Oligocene boundary. Concurrently, the distance between continents allowed a gradual bathymetric isolation through the formation of deep zones around Antarctica (Clarke et al. 2005).

The oxygen ($\delta^{18}O$) isotope in the deep sea shows an overall cooling trend of about 14 °C since the early Eocene (Zachos et al. 2008) resulting in the expansion of the Antarctic cryosphere in the late Eocene (*Oi-1* glaciation; Zachos et al. 1996, 2001; DeConto and Pollard 2003; Mackensen 2004; Pagani et al. 2005). During the mid-Miocene, Sea Surface Temperatures (SST), salinity and ice volume trends suggest an intensification of the ACC that presumably affected the meridional heat/vapor transport, triggering global cooling and the initiation of subzero polar conditions in the SO (Nong et al. 2000; Zachos et al. 2001; Shevenell et al. 2004; Lewis et al. 2008). The Pliocene (5.33–2.58 Ma) is marked by the onset of climate variability that increased particularly during the Quaternary glacial cycles (1.8 Ma–10 ka) (Zachos et al. 2001). These glacial–interglacial processes have caused fluctuations in temperature and sea level, as well as ice sheet advances and retreats over the continental shelves at higher latitudes (Hewitt 2000, 2004; Thatje et al. 2005). In this context, the SO marine benthic fauna has been shaped by the interaction of geological, oceanographic and climatic events, manifested for example in the absence of durophagous predators such as decapod crustaceans and most teleost fish (Aronson et al. 2007). However, some invertebrate groups including bryozoans, polychaetes, amphipods, pycnogonids and echinoderms are abundant and diverse, suggesting that environmental changes have not impeded their evolutionary success (Clarke and Johnston 1996). One of the most remarkable processes of diversification in the Antarctic realm is the evolution of the cold-adapted notothenioid fishes (Clarke and Johnston 1996). While most of the teleost groups were completely eradicated from Antarctica, this suborder dominates in diversity, abundance and biomass (Eastman 2005). The evolutionary success of the Notothenioidei in subzero ecosystems has been explained by the presence of antifreeze glycoproteins, a key innovation in this Antarctic group (Cheng and DeVries 1991; Eastman 1993; Cheng 1998). Nevertheless, recent molecular study indicates that the most species-rich lineages within the notothenioids diversified and evolved during the late Miocene (11.6–5.3 Ma), 10 Ma after the acquisition of antifreeze glycoproteins (Near et al. 2012).

Despite the historical isolation of the Antarctic, a strong biogeographic link exists between the marine benthic fauna of Antarctica and the southern tip of South America (Arntz et al. 2005). The accepted explanation for this affinity relies on the fact that these continents were contiguous until the opening of the Drake Passage and drifted apart progressively (Crame 1999). Several authors have suggested that this connection was possible through the seamounts of the Scotia Arc (Clarke and Crame 1997; Bargelloni et al. 2000; Clarke et al. 2005; Strugnell et al. 2008; Díaz et al. 2011). Different taxa including marine invertebrates such as *Euphausia* sp. (Patarnello et al. 1996) and fish (Clarke and Johnston 1996) exhibit high levels of genetic divergence between provinces of the SO, supporting vicariance speciation associated with continental drift. However, recent molecular studies in marine organisms with high dispersal potential from Antarctica and South America suggest more recent divergence, providing evidence of connectivity between these provinces after their physical separation (Helmuth et al. 1994; Page and Linse 2002; Thornhill et al. 2008; Wilson et al. 2009; Díaz et al. 2011; González-Wevar et al. 2012; Poulin et al. 2014). Recent studies in the Central Scotia Ridge indicate that a remnant, now submerged volcanic arc may have formed a barrier to deep eastward circulation until ~11.6 Ma (Dalziel et al. 2013). The formation of a full deep ACC may have played a key role in the subsequent expansion of the Antarctic cryosphere (Lear et al. 2000; Zachos et al. 2001), as well as in an intensification of the ACC associated with an increase in the strength of westerly winds (Flower and Kennett 1994; Shevenell et al. 2004). Hence, the full development of a deep ACC was not achieved during the Eocene/Oligocene boundary (~33.9 Ma), as long surmised, but only in the late Miocene (Dalziel et al. 2013). It may be envisioned that such changes created the characteristic polar conditions of the region that are responsible for the thermal and physical barriers that isolated the marine fauna of the Southern Ocean continental shelves. As previously stated, molecular-based analyses comparing Antarctic and South American congeneric taxa (González-Wevar et al. 2012; Poulin et al. 2014) suggest more recent separation

processes associated with major oceanographic changes related to the final establishment of a full deep ACC.

In this study, we performed phylogenetic reconstruction and estimated the levels of molecular divergence and genetic diversity between two congeneric species from the Antarctic Peninsula and southern South America of the spiny plunderfish (Harpagiferinae, as recently proposed by Duhamel et al. 2014) a marine notothenioid that has one genus, *Harpagifer,* with 10 species currently described. The distribution of the other eight species is south of the sub-Antarctic Front and is restricted to the sub-Antarctic islands and islands of the southern Scotia Arc (Duhamel et al. 2014). Harpagiferids are small (7–10 cm standard length) benthic fishes generally characterized by narrow and shallow bathymetric ranges (Eastman 1993) and by the presence of a long free-living larval stage (Kock and Kellermann 1991; White and Burren 1992). Species of the genus have a wide head armed with opercular and subopercular spines. The Antarctic species *Harpagifer antarcticus* (Nybelin 1947) and its Magellanic relative *H. bispinis* (Hureau 1990) are very similar in terms of their ecology and morphology (Eastman 2005); however, *H. antarcticus* presents a supraorbital ridge with two low knobs, while the top of the head of *H. bispinis* is smooth except for a small swelling above the rear edge of the eye in most individuals (Hureau 1990). The information contained in their DNA sequences will permit us to test the prediction of congruence between molecular divergence and the intensification of the ACC during the mid- to late Miocene, as recently proposed by Poulin et al. (2014). Similarly, DNA comparisons between Antarctic and South American congeneric species will help us to better understand the tempo and modes of differentiation in benthic marine organisms of the SO.

Materials and methods

Sample collection, DNA extraction, PCR amplification, sequence alignment and genetic diversity analysis

Individuals of *H. bispinis* ($n = 63$) were collected in the intertidal zone in four localities along the Magellanic Province from 2008 to 2012. Individuals of *H. antarcticus* ($n = 54$) were collected in inter- and sub-tidal zone in three localities at King George Island, South Shetland Islands, western Antarctic Peninsula, from 2011 to 2012 (Fig. 1; Online Resource 1). All specimens were fixed in 95 % ethanol, and DNA was extracted from muscle tissue using the salting-out method described by Aljanabi and Martínez (1997). A partial fragment of the mitochondrial control region (D-loop) was amplified using specific primers: CR-HarpaF (5′-TTA AAC CCC TCC CTA CCG CT-3′; CR-HarpaR (5′-AGA GTG AAG GGG TGT CAG AAG C-3′) based on *H. antarcticus* control region sequences (Zhuang and Cheng 2010). Amplifications were performed in a 25-µl reaction containing 5 µl 5× Buffer (50 mM KCl, 10 mM Tris–HCl, pH 8.0), 2.0 µl 25 mM $MgCl_2$, 100 mM dNTPs, 0.5 µl each primer (10 pg/µl), 5 U Taq (Promega), 12.2 µl double-distilled water and 25 ng DNA. Thermal cycling parameters included an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 90 s, 53 °C for 90 s, 72 °C for 90 s, and a final extension of 5 min at 72 °C. PCR amplicons were visualized in agarose gels (1.5 %) stained with Ethidium Bromide. Double-strand amplicons were purified using QIAquick Gel Extraction Kit (QIAGEN) and sequenced in both directions using an automatic sequencer ABI3730 × 1 at Macrogen Inc. (Seoul, South Korea). Sequences were manually edited using Proseq v.2.91 (Filatov 2002) and aligned with ClustalW (Larkin et al. 2007). All sequences were deposited in GenBank under Accession Numbers KM669173–KM669214, and a list of haplotypes can be found in Online Resource 2. We determined the levels of genetic polymorphism in *H. bispinis* and *H. antarcticus* using standard diversity indices: haplotype number (k), segregating sites (S), haplotype diversity (H), average pairwise sequence differences (Π) and nucleotide diversity (π) for each species, using DnaSP v.5.00.07 (Librado and Rozas 2009).

Phylogenetic analysis

Phylogenetic relationships in Harpagiferinae were estimated using Maximum Parsimony (MP) and Bayesian Inference (BI). MP reconstructions were performed using PAUP*, with the following assumptions: Characters were treated as equally weighed using a heuristic search and tree bisection reconnection (TBR), with the branch-swapping option. The steepest descent option was set to off and MULTREES to on with random-taxon addition sequences to search for optimal trees. Node support values were computed using nonparametric bootstrapping (BS) with a full heuristic search option and 1,000 pseudo-replicates (Felsenstein 1981). Nucleotide substitution models for BI analyses were selected using the Bayesian Information Criterion (BIC; Schwarz 1978) determined by JModelTest v.2.1 (Posada 2008). Bayesian analyses were used to obtain posterior probabilities for the nodes in the phylogenetic trees using the HKY + G + I substitution model. Posterior probability values of sampled trees were obtained using the Metropolis coupled Markov Chain Monte Carlo algorithm (MCMC) implemented in MrBayes v.3.2 (Ronquist et al. 2011). Four chains were run twice in parallel for 50 million generations, and trees were sampled every thousand generations. We considered the chains stationary when the

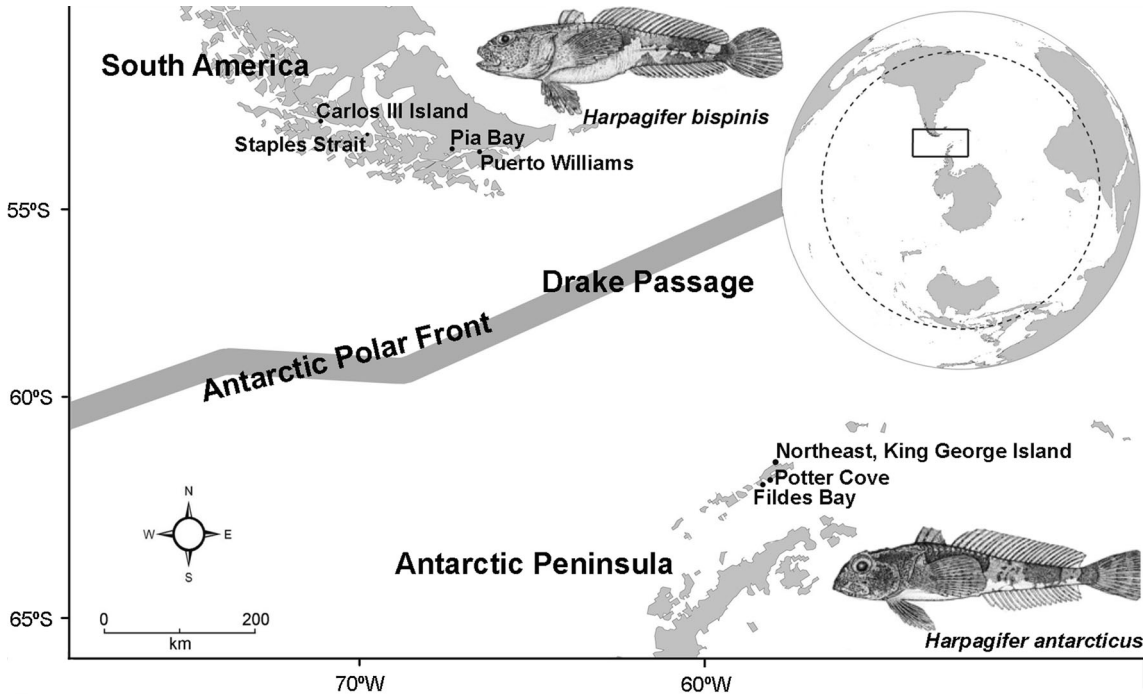

Fig. 1 Sampling localities of *Harpagifer bispinis* in Patagonia, South America and *H. antarcticus* in King George Island, Antarctica

average standard deviation of split frequencies was less than 0.01 (Ronquist and Huelsenbeck 2003). Bayesian Posterior Probabilities (BPP) were estimated as the optimal posterior distributions of trees (after burn-in) that showed that specific node. Nodes in the phylogenetic tree were considered highly supported with BPP values $\geq$0.95 and bootstrap support (BS) $\geq$ 75 %. The posterior probability density of the combined tree and log files was summarized as a maximum clade credibility tree using TreeAnnotator v.1.7.1 (http://beast.bio.ed.ac.uk/TreeAnnotator) prior to its visualization in FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree).

Molecular divergence

To evaluate molecular divergence, we included in the analyses four randomly chosen D-loop haplotypes for each species (*H. bispinis* and *H. antarcticus*). For phylogenetic purposes, we included seven D-loop sequences belonging to notothenioid species from four Antarctic subfamilies, (*Notothenia coriiceps* GU214214.1, *Champsocephalus gunnari* GU217680.1, *Chaenocephalus aceratus* GU214227.1, *Chionodraco myersi* GU214228.1, *Racovitzia glacialis* GU214226.1, *Pogonophryne scotti* GU214223.1) and one sub-Antarctic subfamily (*Eleginops maclovinus*, GU214211.1). We performed a DNA saturation analysis following Xia and Xie (2001) in DAMBE to evaluate how saturation of transitions is accumulated in relation to nucleotide divergence in the entire data set. Following Matschiner et al. (2011) and Near et al. (2012), we used as outgroup the South American notothenioid *E. maclovinus*. We estimated a specific mutation rate for Harpagiferinae using time-calibrated phylogenies with BEAST v.1.7.1 (Drummond and Rambaut 2007). Age priors with a normal distribution were applied following Matschiner et al. (2011) and Near et al. (2012), including the most recent common ancestor (MRCA) of the Antarctic Clade (mean, 22.4 Ma; 95 % highest probability density (HPD): 19.7–25.1 Ma). We used as internal calibration date the MRCA of Channichthyinae (mean 6.3 Ma; 95 % HPD: 4.8–7.8 Ma). Divergence estimations used an uncorrelated lognormal relaxed molecular clock model and a strict molecular clock model (Drummond et al. 2006). We used a birth–death speciation prior for branching rates along the phylogeny (Gernhard 2008). BEAST runs were conducted five times with each run consisting of 3×10^7 generations that were sampled every 1,000 iterations. Replicates were combined using LogCombiner v.1.7.1 (Drummond and Rambaut 2007) after removing the first 10 % of the trees as burn-in. Convergence of model parameter values and estimated node heights were confirmed by effective sample size (ESS) > 1,200 using Tracer v.1.5 (Drummond and Rambaut 2007). Maximum clade credibility trees were generated using TreeAnnotator v.1.7.1 (Drummond and Rambaut 2007). Tree settings were compared with Bayes Factor (BF), using the marginal likelihood of each model as implemented in Tracer. We also estimated the divergence between the species using the number of pairwise differences and assuming a strict

molecular clock hypothesis. For this purpose, we used the previously estimated substitution rate for Harpagiferinae. Haplotype genealogical relationships were built following the method described in Salzburger et al. (2011), using phylogenetic tree reconstruction with the maximum likelihood (ML) method implemented in Phylip v.3.69 (Felsenstein 1989) and the model of sequence evolution selected by the BIC (Posada 2008).

Results

Genetic diversity and phylogenetic analyses

Mitochondrial D-loop sequences in *Harpagifer* were 581 bp long. Only 45 positions were variable (7.7 %), and 30 of these were parsimoniously informative; sequences were A–T rich (60.2 %). The number of polymorphic sites (S) varied between 21 (*H. antarcticus*) and 26 (*H. bispinis*). Both species exhibited 21 different and non-shared haplotypes (Table 1). Levels of genetic diversity, estimated as the average number of nucleotide differences and nucleotide diversity, were greater in the Antarctic *H. antarcticus* ($\Pi = 5.99$; $\pi = 0.01$) than in its Patagonian relative ($\Pi = 2.04$; $\pi = 0.003$; Table 1).

The maximum likelihood haplotype genealogy of *H. bispinis* (Fig. 2) showed a typical star-like topology in which the central haplotype was the most frequent (47.6 %). Two haplotypes located no more than two mutational steps away from the central haplotype showed intermediate frequencies (6.35 %). The remaining haplotypes occurred in low frequencies, and we identified 14 unique haplotypes. In contrast, *H. antarcticus* showed a more complex and expanded topology, with 3 main haplotypes separated by 6–10 mutation steps and mainly eccentric in the network. One haplotype was the most frequent (25.92 %) and two haplotypes showed intermediate frequency (16.7 %). The rest were found in low frequencies; there were 14 unique haplotypes (Fig. 2). No significant topological incongruences were detected using the two reconstruction methods (MP and BI). The monophyly of Harpagiferinae and the reciprocal monophyly of *H. bispinis* and *H. antarcticus* were highly supported (BS = 100 %) and BI (BPP = 1.0; Fig. 2). Within the Harpagiferinae, reconstructions recognized two main clades; *H. antarcticus* from the Antarctic Peninsula and *H. bispinis* from South America.

Table 1 Genetic diversity indices in *H. bispinis* and *H. antarcticus*

Species	*n*	*K*	*H*	*S*	Π	π
H. bispinis	63	21	0.77	26	2.04	0.003
H. antarcticus	54	21	0.88	21	5.99	0.010

n = number of sampled specimens; K = number of haplotypes; H = haplotype diversity; S = polymorphic sites; Π = average number of nucleotide differences; π = nucleotide diversity

Molecular divergence

Our control region sequences were not saturated in the species, validating their use in phylogenetic reconstruction. According to the divergence estimations, a first cladogenic event separating the South American notothenioid *E. maclovinus* from the MRCA of the Antarctic Clade occurred during the Eocene around 36.7 Ma. Then, a second split within the Antarctic Clade occurred during the early Miocene ~19.8 Ma (95 % HPD 17.2–22.7 Ma; Fig. 3), that separated the Nototheninae from the most derived groups (Artedidraconinae, Bathydraconinae, Channichthyinae and Harpagiferinae). Subsequently, a third separation between Harpagiferinae/Artedidraconinae and Bathydraconinae/Channichthyinae occurred in the mid-Miocene around 16 Ma (95 % HPD 14.4–18.6 Ma). Divergence estimations indicate a separation during the middle Miocene (15 Ma, 95 % HPD 12.6–17.5 Ma) between Harpagiferinae and Artedidraconinae. Finally, the separation between Antarctic *H. antarcticus* and South American *H. bispinis* occurred during the early Pleistocene around 1.7 Ma (95 % HPD 1.1–2.5 Ma; Fig. 3). The haplotype genealogy constructed in Harpagiferinae corroborated phylogenetic reconstructions showing two main haplogroups (Fig. 2); the first including *H. antarcticus* (Antarctic Peninsula) individuals and the second those of *H. bispinis* (South America). We observed an average of 14.6 pairwise differences and a total of 8 fixed mutations between the species. Using molecular clock calibration in the group (nucleotide changes/sites/Myr), the substitution rate for D-loop in *Harpagifer* was 0.016 (95 % HPD 0.027–0.008 Ma). Assuming this rate, the onset of the divergence between *H. antarcticus* and *H. bispinis* occurred during the Pleistocene, approximately 0.78 Ma (1.6–0.5 Ma).

Discussion

Southern Ocean biogeographers recognize three main periods that deeply influenced the distribution of the biota in this region (Mackensen 2004). (1) The Eocene/Oligocene boundary (~34 Ma), which coincides with the onset of the Antarctic isolation and the initiation of the ACC (Zachos et al. 2001; Barker and Thomas 2004; Livermore et al. 2005; Pfuhl and McCave 2005). (2) The middle Miocene (~14 Ma) associated with an intensification of the ACC and the re-establishment of an ice sheet in the

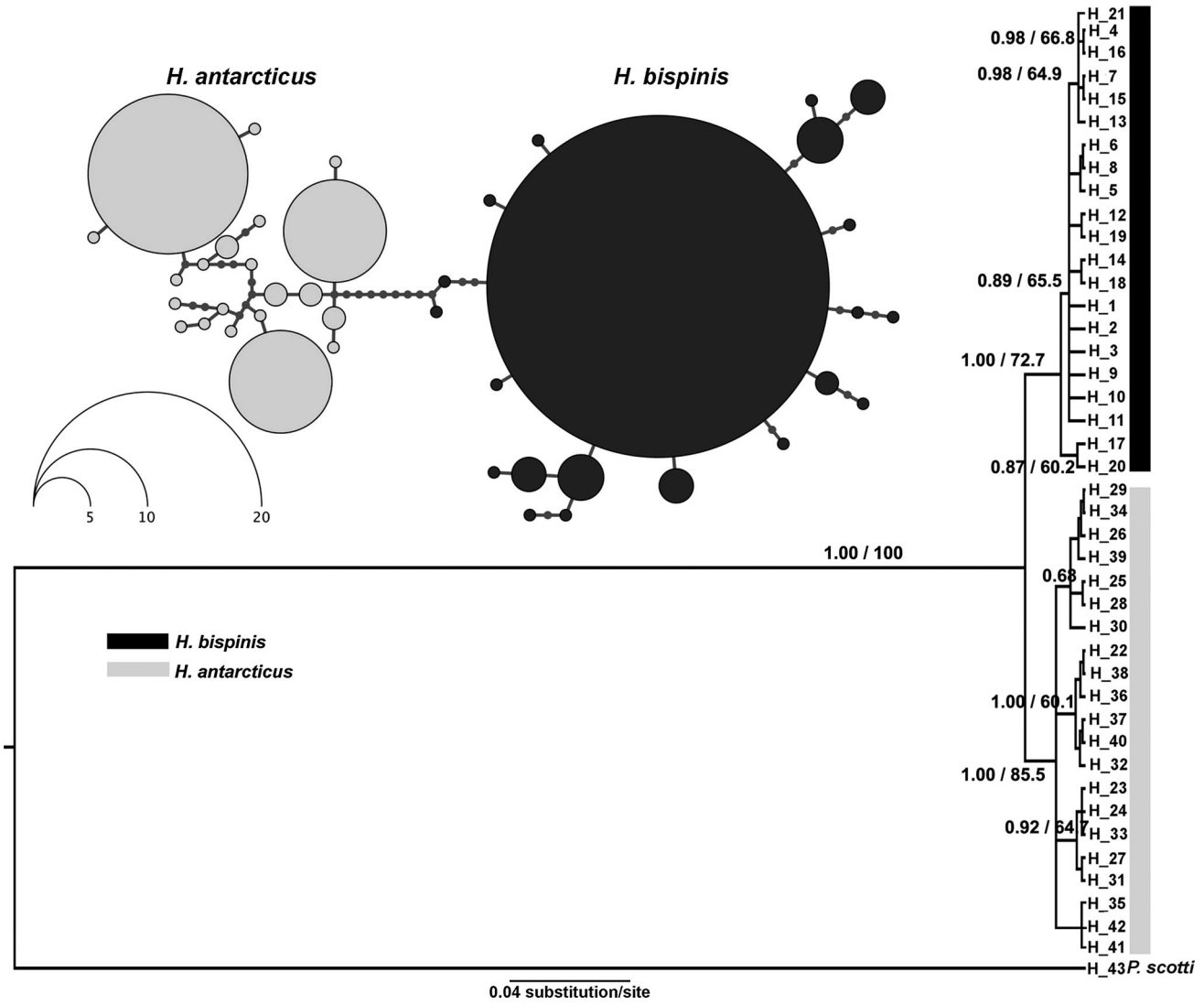

Fig. 2 Haplotype genealogy including 63 *H. bispinis* (*black*) and 54 *H. antarcticus* (*gray*) mtDNA control region sequences. Circle sizes are proportional to haplotype frequencies. Phylogenetic relationships based on mitochondrial DNA control region haplotypes reconstructed using Bayesian Inference (BI) and Maximum Parsimony (MP) for *Harpagifer bispinis* (*black*) and *H. antarcticus* (*gray*). *Pogonophryne scotti* was used as outgroup. Node support values are presented as Bayesian posterior probabilities and parsimony bootstrap support

East Antarctic and along the Pacific margin of West Antarctica (Lawver et al. 2003; Verducci et al. 2009). (3) The Quaternary characterized by the alternation between glacial and interglacial periods that greatly affected the seasonality and intensity of sea ice formation (Barker and Thomas 2004; Aronson et al. 2007). In recent decades, studies based on molecular markers have tried to associate patterns of diversification of marine benthic fauna with major change events in the Southern Ocean. For example, some groups such as *Euphausia* (Patarnello et al. 1996) and fish (Clarke and Johnston 1996) exhibit large genetic differences that are associated with the separation of the continents, while other marine organisms show much more recent divergences (González-Wevar et al. 2010, 2012; Poulin et al. 2014).

Phylogenetic relationships within the suborder Notothenioidei are well resolved and reasonably understood (Near and Cheng 2008; Janko et al. 2011; Rutshmann et al. 2011). At the same time, several studies have estimated the divergence among notothenioid lineages (Bargelloni et al. 1994; Chen et al. 1998; Bargelloni et al. 2000; Near 2004; Matschiner et al. 2011; Rutshmann et al. 2011; Near et al. 2012). Most of the current diversity in nothothenioid species-rich clades (*Trematomus*, Channichthynae and Artedidraconinae) occurred after the middle Miocene (11.6–5.3 Ma), more than 10 Ma after the origin of this Antarctic suborder (Near et al. 2012). However, most of these studies are biased toward the relationships within the Antarctic Clade rather than in the association among lineages inside and outside the Polar Front, and particularly

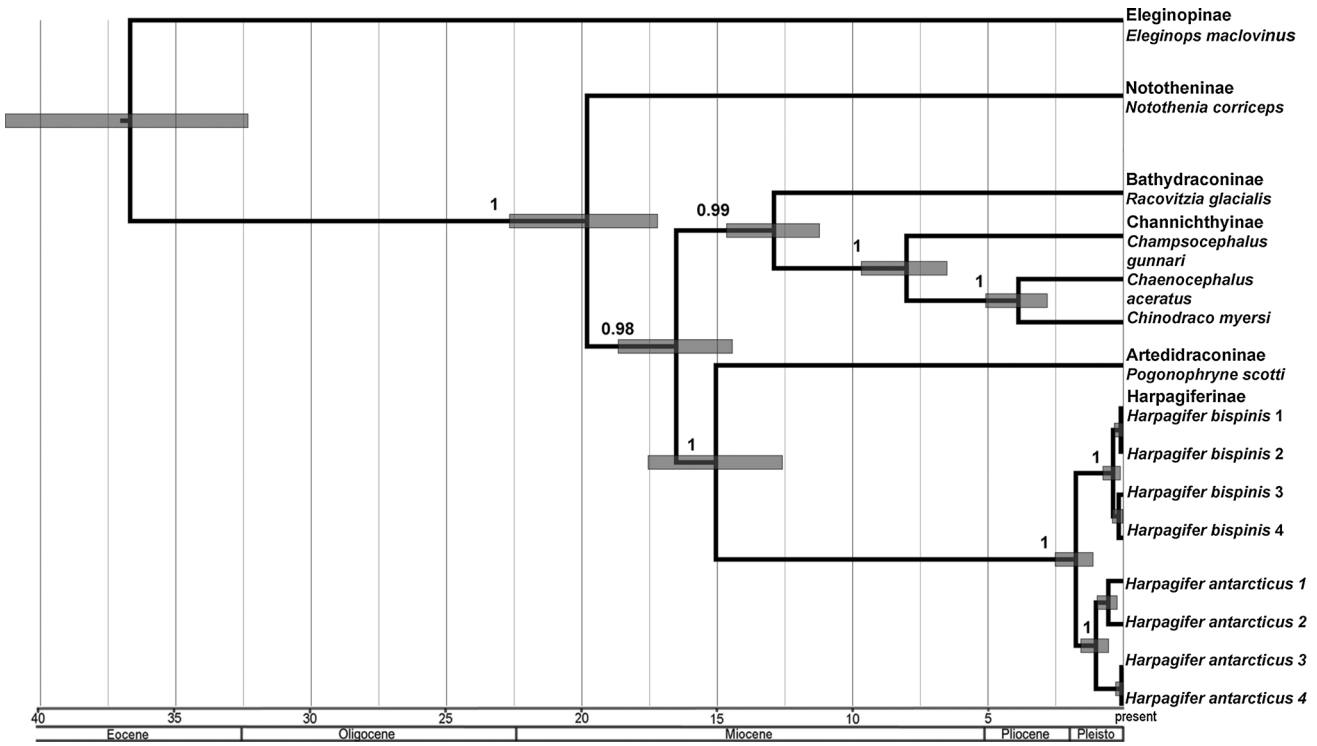

Fig. 3 BI time-calibrated phylogeny of nine notothenioid species. *Bars at nodes* indicate 95 % highest posterior density intervals of age estimates. Support values represent Bayesian posterior probabilities (above the branches)

with those species from southern South America. Therefore, the evolutionary history of non-Antarctic notothenioids remains not well established, particularly in the subfamily Harpagiferinae. This study constitutes the first attempt to establish the levels of genetic divergence and genetic diversity in congeneric species of *Harpagifer* from the Antarctic Peninsula and southern South America.

Phylogenetic reconstructions support the monophyly of the Antarctic Clade (BS = 81 %; BPP = 1.0; Fig. 3), as well as the monophyly of *Harpagifer* (BS = 100 %; BPP = 1.0), corroborating previous studies (Derome et al. 2002; Near and Cheng 2008). Similarly, all the reconstructions recovered the reciprocal monophyly and the relationship of *H. antarcticus* (Antarctic Peninsula) and *H. bispinis* (South America) with high levels of support (BS = 100 %; BPP = 1.0) and corroborate previous morphological (Hureau 1990) and ecophysiological studies (Johnston et al. 2002; Brodeur et al. 2003; Pérez et al. 2003). Divergence times inferred from molecular analysis include some uncertainty since the calibration points are related to ancient fossil records used by Matschiner et al. (2011). However, divergence estimations in notothenioids are in agreement with recent studies in this suborder (Matschiner et al. 2011; Rutshmann et al. 2011; Near et al. 2012).

According to our results, the MRCA of Nototheninae occurred about 19.8 Ma (95 % HPD 17.2–22.7), a result that is very similar and comparable to those obtained by Matschiner et al. (2011), 21.4 Ma (95 % HPD 15.3–28.2), Rutshmann et al. (2011) 18.6 Ma (95 % HPD 13.4–24.0) and Near et al. (2012) 22.4 Ma (95 % HPD 19.7–25.1). Thus, the diversification within the Antarctic Clade, including Nototheninae, Channicthyinae, Bathydraconinae and Artedidraconinae, occurred during the mid-Miocene between 16 and 12 Ma (Fig. 3). The diversification within the Antarctic Clade occurred more than 10 Ma after the origin of this cold-adapted suborder (Near et al. 2012). The diversification of the notothenioids in the SO occurred long after the physical separation of Antarctica and South America, estimated between 41 (Livermore et al. 2005) and 23.9 Ma (Eagles and Livermore 2002; Pfuhl and McCave 2005; Scher and Martin 2006; Lyle et al. 2007). A Pleistocene (1.7–0.8 Ma) separation between *H. antarcticus* (Antarctic Peninsula) and *H. bispinis* (South America) is much more recent than previous estimations of divergence between Antarctic and sub-Antarctic notothenioid genera, which range from 9 Ma (Bargelloni et al. 2000) to 6.1 Ma (Stankovic et al. 2002), following the onset of intensified cooling conditions in the SO (Near et al. 2012). Similarly, the divergence estimated in congeneric species of

Harpagifer from the Antarctic Peninsula and South America is more recent than those estimated for congeneric species of marine benthic invertebrates with dispersive potential including bivalves, patellogastropods and echinoids (González-Wevar et al. 2012). Considering the divergence estimated in *Harpagifer* and life history traits, we propose that the separation between *H. antarcticus and H. bispinis* may be related to a recent dispersion process from Antarctica to South America. This hypothesis is supported by (1) the terminal position of Harpagiferinae within the notothenioid phylogeny and (2) by the presence of antifreeze activity in *H. bispinis* from South America and *H. antarcticus* from the Antarctic Peninsula, a key innovation of Antarctic species (Arthur L. DeVries, pers. comm; Cheng et al. 2003).

Following this, we propose that climatic and oceanographic changes during the Pleistocene, particularly during the coldest periods (i.e., Great Patagonian Glaciation, 1–0.9 Ma, Rabassa 2008) produced the ideal conditions for the colonization of South America from Antarctica, especially for organisms with high dispersal potential such as *Harpagifer* (Kock and Kellermann 1991). During the coldest periods, the position of the ACC and the APF underwent major northward latitudinal shifts (Gersonde et al. 2005; Clark et al. 2006; Rabassa 2008; Kemp et al. 2010). In particular, during the middle Pleistocene (0.9 Ma), the northward shift of the APF was at least 7° latitude over a period of about 150 Ka (Kemp et al. 2010). This northward migration of the APF may have facilitated the dispersion of high latitude *H. antarcticus* toward South America through the Scotia Arc. As stated by Near et al. (2012), ecological opportunities resulting from repeated creation of open niches through the extinction of potential competitors, over substantial expanses of geologic time could result in the evolution of the unusual pattern of substantial morphological and ecological disparities within Antarctic notothenioid subclades. Antifreeze protection in *Harpagifer* could be responsible for the successful colonization of Patagonia during glacial and interglacial periods of the Quaternary following by the recent differentiation of *H. bispinis*. The presence of the AFGP could have conferred to *H. bispinis* the ability to resist harsh habitat conditions and reach unoccupied shores during glacial maxima. The presence of this key innovation would have bestowed a selective and adaptive advantage for *Harpagifer* against other intertidal Patagonian species that do not present AFGP activity (Cheng and Detrich 2007; Fraser et al. 2009; Waters et al. 2013). During this period, the 12 months of larval life span in *H. antarcticus* (Kock and Kellermann 1991; White and Burren 1992) may have allowed the connectivity between Antarctica and South America. The following interglacial period would have put an end to the northward displacement of the APF that resulted in the definitive separation of Antarctic and South American populations of *Harpagifer*. Genealogical reconstructions in *Harpagifer* characterized by reciprocal monophyly between *H. bispini* and *H. antarcticus* support this scenario, indicating that long-distance dispersal, or at least gene flow between Antarctica to South America was definitively interrupted, even during the following glacial periods. In allopatry, Antarctic and South American populations may have taken different evolutionary trajectories under contrasting environments, as suggested by differences in their genetic diversity patterns. Considering the genetic diversity indices and the haplotype genealogies of *H. antarcticus* and *H. bispinis* from King George Island and Patagonia, respectively, our results suggest contrasting demographic histories of these species. In this regard, we found high levels of nucleotide diversity and a more complex haplotype network in *H. antarcticus* than in *H. bispinis*. However, this preliminary result should be confirmed with sampling effort in other localities of Patagonia, Antarctic Peninsula and Scotia Arc Islands.

In conclusion, the mitochondrial control region provides new evidence on the differentiation process between Antarctic and South American marine benthic near-shore organisms with long dispersive larval potential. Based on our results, we propose that the northward migration of the APF during the coolest periods of the Pleistocene may have facilitated the colonization of southern South America by *Harpagifer*, from the Antarctic Peninsula across the Scotia Arc Islands. Such dispersal processes may have been favored by the long larval phase described in these species. Following the coolest periods of the Pleistocene, an abrupt southward return of the APF occurred, to a location similar to its modern position, where it constitutes an effective barrier to gene flow between Antarctic and sub-Antarctic provinces. Future studies in *Harpagifer* will include population level comparisons between the Antarctic Peninsula region and southern South America to further understand the genetic legacy of the Quaternary glaciations in contrasting areas of the SO. Similarly, a robust phylogenetic reconstruction using different genetic markers and including more species of the genus is required to confirm the evolutionary origin and the biogeography of this important fish group.

Acknowledgments We would like to thank Christina Cheng for constructive comments on the original manuscript. The following Projects supported this study: MSc thesis project INACH M_10–11 and Institute of Ecology and Biodiversity (IEB) PFB-23-2008.

References

Aljanabi S, Martínez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucl Acids Res 25:4692–4693

Arntz W, Thatje S, Gerdes D, Gili J-M, Gutt J, Jacob U, Montiel A, Orejas C, Teixidó N (2005) The Antarctic-Magellan connection:

macrobenthos ecology on the shelf and upper slope, a progress report. Sci Mar 69(Suppl 2):237–269
Aronson R, Thatje S, Clarke A, Peck L, Blake D, Wilga C, Seibel B (2007) Climate change and invisibility of the Antarctic benthos. Annu Rev Ecol Evol S 38:129–154
Bargelloni L, Ritchie P, Patarnello T, Battaglia B, Lambert D, Meyer A (1994) Molecular evolution at subzero temperature: mitochondrial and nuclear phylogenies of fishes from Antarctica (Suborden Notothenioidei), and the evolution of antifreeze glycopeptides. Mol Biol Evol 11(6):854–863
Bargelloni L, Marcato S, Zane L, Patarnello T (2000) Mitochondrial phylogeny of notothenioids: a molecular approach to antarctic fish evolution and biogeography. Syst Biol 49(1):114–129
Barker P, Thomas E (2004) Origin, signature and palaeoclimatic influence of the Antarctic Circumpolar Current. Earth Sci Rev 66:143–162
Barker P, Filipelli G, Florindo F, Martin E, Scher H (2007) Onset and role of the Antarctic Circumpolar Current. Deep-Sea Res Pt II 54:2388–2398
Brodeur J, Calvo J, Clarke A (2003) Myogenic cell cycle duration in *Harpagifer* species with sub-Antarctic and Antarctic distributions: evidence for cold compensation. J Exp Biol 206:1011–1016
Chen W, Bonillo C, Lecointre G (1998) Phylogeny of the Channichthyidae (Notothenioidei, Teleostei) based on two mitochondrial genes. In: di Prisco G, Pisano E, Clarke A (eds) Fishes of Antarctica: a biological overview. Springer, Milano, pp 287–298
Cheng C-HC (1998) Origin and mechanism of evolution of antifreeze glycoproteins in polar fishes. In: di Prisco G, Pisano E, Clarke A (eds) Fishes of Antarctica: a biological overview. Springer, Milano, pp 311–328
Cheng C-HC, Detrich W (2007) Molecular ecophysiology of Antarctic notothenioid fishes. Philos T R Soc B 362:2215–2232
Cheng C-HC, DeVries A (1991) The role of antifreeze glycopeptides and peptides in the freezing avoidance of cold water fishes. In: di Prisco G (ed) Life under extreme conditions. Springer, Berlin, pp 1–14
Cheng C-HC, Chen L, Near T, Jin Y (2003) Functional antifreeze glycoprotein genes in temperate-water new Zealand nototheniid fish infer an Antarctic evolutionary origin. Mol Biol Evol 20(11):1897–1908
Clark M, Clarke A, Cockell C, Convey P, Detrich HW, Fraser K, Johnston I, Methe B, Murray A, L Peck, Römish K, Rogers A (2004) Antarctic genomics. Comp Funct Genom 5:230–238
Clark P, Archer D, Pollard D, Blum J, Rial J, Brovkin V, Mix A, Pisias N, Roy M (2006) The middle Pleistocene transition: characteristics, mechanisms, and implications for long-term changes in atmospheric pCO_2. Quat Sci Rev 25:3150–3184
Clarke A, Crame JA (1997) Diversity, latitude and time: patterns in the shallow sea. In: Ormond RFG, Gage JD, Angel MV (eds) Marine biodiversity: causes and consequences. Cambridge University Press, Cambridge, pp 122–147
Clarke A, Johnston I (1996) Evolution and adaptive radiation of Antarctic fishes. Trends Ecol Evol 11(5):212–218
Clarke A, Barnes D, Hodgson D (2005) How isolated is Antarctica? Trends Ecol Evol 1:1–3
Crame JA (1999) An evolutionary perspectiva on marine faunal connections between southernmost south America and Antarctica. Sci Mar 63(Suppl 1):1–14
Dalziel IW, Lawver L, Pearce J, Barker P, Hastie A, Barfod D, Schenke H-W, Davis M (2013) A potential barrier to deep Antarctic circumpolar flow until the late Miocene? Geology 41:947–950
DeConto RM, Pollard D (2003) Rapid Cenozoic glaciation of Antarctica induced by declining atmospheric CO_2. Nature 421:245–249
Derome N, Chen W, Dettai A, Bonillo C, Lecointre G (2002) Phylogeny of Antarctic dragonfishes (Bathydraconidae, Notothenioidei, Teleostei) and related families based on their anatomy and two mitochondrial genes. Mol Phylogenet Evol 24:139–152
Díaz A, Féral J-P, David B, Saucède T, Poulin E (2011) Evolutionary pathways among shallow and deep sea echinoids of the genus *Sterechinus* in the Southern Ocean. Deep-Sea Res Pt II 58(1–2):205–211
Drummond AJ, Rambaut A (2007) *BEAST*: bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214
Drummond AJ, Ho S, Phillips M, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. PLoS Biol 4(5):699–710
Duhamel G, Hulley P-A, Causse R, Koubbi P, Vacchi M, Pruvost P, Vigetta S, Irisson J-O, Mormède S, Belchier M, Dettaï A, Detrich W, Gutt J, Jones CD, Kock K-H, Lopez LJ, Van de Putte A (2014) Chapter 7. Biogeographic patterns of fish. In: De Broyer C, Koubbi P, Griffiths HJ, Raymond B, Udekem d'Acoz Cd' et al (eds) Biogeographic Atlas of the Southern Ocean. Scientific Committee on Antarctic Research, Cambridge, pp 327–361
Eagles G, Livermore R (2002) Opening history of Powell Basin, Antarctic Peninsula. Mar Geol 185:195–205
Eastman J (1993) Antarctic fish biology: evolution in a unique environment. Academic Press, San Diego
Eastman J (2005) The nature of the diversity of Antarctic fishes. Polar Biol 28:93–107
Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376
Felsenstein J (1989) PHYLIP-phylogeny inference package (version 3.2). Cladistics 5:164–166
Filatov DA (2002) ProSeq: a software for preparation and evolutionary analysis of DNA sequence data sets. Mol Ecol Notes 2:621–624
Flower B, Kennett J (1994) The middle Miocene climatic transition: east Antarctic ice sheet development, deep ocean circulation and global carbon cycling. Palaeogeogr Palaeocl 108:537–555
Fraser C, Nikula R, Spencer H, Waters J (2009) Kelp genes reveal effects of subantarctic sea ice during the last glacial maximum. P Natl Acad Sci USA 106(9):3249–3253
Gernhard T (2008) The conditioned reconstructed process. J Theor Biol 253:769–778
Gersonde R, Crosta X, Abelmann A, Armand L (2005) Sea-surface temperature and sea ice distribution of the Southern Ocean at the EPILOG last glacial maximum—a circum-Antarctic view based on siliceous microfossil records. Quat Sci Rev 24:869–896
González-Wevar C, Nakano T, Cañete J, Poulin E (2010) Molecular phylogeny and historical biogeography of *Nacella* (Patellogastropoda: Nacellidae) in the Southern Ocean. Mol Phylogenet Evol 56:115–124
González-Wevar C, Díaz A, Gérard K, Cañete J, Poulin E (2012) Divergence time estimations and contrasting patterns of genetic diversity between Antarctic and southern South America benthic invertebrates. Rev Chil Hist Nat 85:445–456
Griffiths H, Barnes D, Linse K (2009) Towards a generalized biogeography of the Southern Ocean benthos. J Biogeogr 36:162–177
Helmuth B, Veit R, Holberton R (1994) Long-distance dispersal of a subantarctic brooding bivalve (*Gaimardia trapesina*) by kelp-rafting. Mar Biol 120:421–426
Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405:907–913
Hewitt G (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos T R Soc B 359:183–195
Hureau J (1990) Harpagiferidae. In: Gon O, Heemstra PC (eds) Fishes of the Southern Ocean. JLB Smith Institute of Ichthyology, Grahamstown, pp 357–363

Janko K, Marshall C, Musilová Z, Van Houdt J, Couloux A, Cruaud C, Lecointre G (2011) Multilocus analyses of an Antarctic fish species flock (Teleostei, Notothenioidei, Trematominae): phylogenetic approach and test of the early-radiation event. Mol Phylogenet Evol 60:305–316

Johnston I, Vieira V, Fernandez DA, Abercromby M, Brodeur J, Peck L, Calvo J (2002) Muscle growth in polar fish: a study of *Harpagifer* species with sub-Antarctic and Antarctic distributions. Fish Sci 68:1023–1028

Kemp A, Grigorov I, Pearce R, Garabato N (2010) Migration of the Antarctic Polar Front through the mid-Pleistocene transition: evidence and climatic implications. Quat Sci Rev 29:1993–2009

Kock K, Kellermann A (1991) Reproduction in Antarctic notothenioid fish. Rev Antarct Sci 3(2):125–150

Larkin M, Blackshields G, Brown N, Chenna R, McGettigan P, McWilliam H, Valentin F, Wallace I, Wilm A, Lopez R, Thompson J, Gibson T, Higgins D (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947–2948

Lawver L, Gahagan L, Campbell D (2003) Evolution of Cenozoic seaways in the circum-Antarctic region. Palaeogeogr Palaeocl 198:11–37

Lear C, Elderfield H, Wilson P (2000) Cenozoic deep-sea temperatures and global ice volumes from Mg/Ca in benthic foraminiferal calcite. Science 287:269–272

Lewis A, Marchant D, Ashworth A, Hedena L, Hemming S, Johnson J, Leng M-J, Machlus M, Newton A, Raine J, Willenbring J, Williams M, Wolfe A (2008) Mid-Miocene cooling and the extinction of tundra in continental Antarctica. P Natl Acad Sci USA 105:10676–10680

Librado P, Rozas J (2009) DnaSp v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Livermore R, Nankivell A, Eagles G, Morries P (2005) Paleogene opening of Drake Passage. Earth Planet Sci Lett 236:459–470

Lyle M, Gibbs S, Moore T, Rea D (2007) Late Oligocene initiation of the Antarctic Circumpolar Current: evidence from the South Pacific. Geology 35:691–694

Mackensen A (2004) Changing Southern Ocean paleocirculation and effect on global climate. Antarct Sci 16:369–384

Matschiner M, Hanel R, Salzburger W (2011) On the origin and trigger of the Notothenioid adaptive radiation. PLoS One 6(4):1–9

Near T (2004) Estimating divergence times of notothenioid fishes using a fossil-calibrated molecular clock. Antarct Sci 16:37–44

Near T, Cheng C-HC (2008) Phylogenetics of notothenioid fishes (Teleostei: Acanthomorpha): Inferences from mitochondrial and nuclear gene sequences. Mol Phylogenet Evol 47:832–840

Near T, Dornburg A, Kuhn K, Eastman J, Pennington J, Patarnello T, Zame L, Fernández DA, Jones C (2012) Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. P Natl Acad Sci USA 109:3434–3439

Nong GT, Najjar RG, Seidov D, Peterson WH (2000) Simulation of ocean temperature change due to the opening of Drake Passage. Geophys Res Lett 27:2689–2692

Nybelin O (1947) Antarctic fishes. Scientific results of the Norwegian Antarctic Expeditions 1927–1928 et spp. Nor Vidensk-Akad Oslo Arbok 2(26):1–76

Pagani M, Zachos JC, Freeman KH, Tipple B, Bohaty S (2005) Marked decline in atmospheric carbon dioxide concentrations during the Paleogene. Science 309:600–603

Page T, Linse K (2002) More evidence of speciation and dispersal across the Antarctic Polar Front through molecular systematics of Southern Ocean *Limatula* (Bivalvia: Limidae). Polar Biol 25:818–826

Patarnello T, Bargelloni L, Varotto V, Battaglia B (1996) Krill evolution and the Antarctic Ocean currents: evidence of vicariant speciation as inferred by molecular data. Mar Biol 126:603–608

Peck L, Clark M, Clarke A, Cockell C, Convey P, Detrich W III, Fraser K, Johnston I, Methe B, Murray A, Römish K, Rogers A (2005) Genomics: applications to Antarctic ecosystems. Polar Biol 28:351–365

Pérez AF, Calvo J, Tresguerres M, Luquet C (2003) Aglomerularism in *Harpagifer bispinis*: a subantarctic notothenioid fish living at reduced salinity. Polar Biol 26:800–805

Pfuhl H, McCave N (2005) Evidence for late Oligocene establishment of the Antarctic Circumpolar Current. Earth Planet Sci Lett 235:715–728

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25(7):1253–1256

Poulin E, González-Wevar C, Díaz A, Gérard K, Hüne M (2014) Divergence between Antarctic and South American marine invertebrates: what molecules tell us about Scotia Arc geodynamics and the intensification of the Antarctic Circumpolar Current. Glob Planet Change. doi:10.1016/j.gloplacha.2014.07.017

Rabassa J (2008) Late Cenozoic glaciations in Patagonia and Tierra del Fuego. In: Rabassa J (ed) Late Cenozoic of Patagonia and Tierra del Fuego. Developments in Quaternary science. Elsevier, Amsterdam, pp 151–204

Ronquist F, Huelsenbeck J (2003) MRBAYES 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Ronquist F, Huelsenbeck J, Teslenko M (2011) MrBayes (Bayesian analysis of phylogeny), Version 3.2. http://mrbayes.net

Rutshmann S, Matschiner M, Damerau M, Muschick M, Lehmann M, Hanel R, Salzburger W (2011) Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Mol Ecol 22:4707–4721

Salzburger W, Ewing G, Haeseler A (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. Mol Ecol 20:1952–1963

Scher H, Martin E (2006) Timing and climatic consequences of the opening of Drake Passage. Science 312:428–430

Schwarz G (1978) Estimating the dimension of a model. Ann Stat 6:461–464

Shevenell A, Kennett J, Lea D (2004) Middle Miocene Southern Ocean cooling and Antarctic cryosphere expansion. Science 305:1766–1770

Stankovic A, Spalik K, Kamler E, Borsuk P, Weglenski P (2002) Recent origin of sub-Antarctic notothenioids. Polar Biol 25:203–205

Strugnell J, Rogers A, Prodohl P, Collins M, Allcock A (2008) The thermohaline expressway: the Southern Ocean as a centre of origin for deep-sea octopuses. Cladistics 24:1–8

Thatje S, Hillenbrand C-D, Larter R (2005) On the origin of Antarctic marine benthic community structure. Trends Ecol Evol 20:534–540

Thornhill D, Mahon A, Norenburg J, Halanych K (2008) Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae). Mol Ecol 17:5104–5117

Verducci M, Foresi L, Scott G, Sprovieri M, Lirer F, Pelosi N (2009) The Middle Miocene climatic transition in the Southern Ocean: evidence of paleoclimatic and hydrographic changes at Kerguelen plateau from planktonic foraminifers and stable isotopes. Palaeogeogr Palaeocl 280:371–386

Waters J, Fraser C, Hewitt G (2013) Founders takes all: density-dependent processes structure biodiversity. Trends Ecol Evol 28(2):78–85

White M, Burren P (1992) Reproduction and larval growth of *Harpagifer antarcticus* Nybelin (Pises, Notothenioidei). Antarct Sci 4:421–430

Wilson N, Schrödl M, Halanych K (2009) Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial

lineages in the Antarctic sea slug Doris kerguelenensis (Mollusca, Nudibranchia). Mol Ecol 18:965–984

Xia X, Xie Z (2001) DAMBE: data analysis in molecular biology and evolution. J Hered 92:371–373

Zachos J, Quinn T, Salamy K (1996) High resolution (104 years) deep-sea foraminiferal stable isotope records of the Eocene–Oligocene climate transition. Paleoceanography 11:251–266

Zachos J, Pagani L, Sloan E, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693

Zachos J, Dickens G, Zeebe R (2008) An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. Nature 451:279–283

Zhuang X, Cheng C-HC (2010) ND6 gene "Lost" and found: evolution of mitochondrial gene rearrangement in Antarctic Notothenioids. Mol Biol Evol 27(6):1391–1403