ORIGINAL ARTICLE

Following the Antarctic Circumpolar Current: patterns and processes in the biogeography of the limpet *Nacella* (Mollusca: Patellogastropoda) across the Southern Ocean

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ABSTRACT

Aim We use an integrative biogeographical approach to further understand the evolution of an important Southern Ocean marine benthic element, the limpet genus *Nacella* (Mollusca: Patellogastropoda).

Location Southern Ocean.

Methods We used multi-locus time-calibrated phylogeny of *Nacella* at the scale of the whole Southern Ocean to elucidate the underlying processes involved in the origin and diversification of the genus.

Results Divergence-time estimates suggest that soon after its origin during the mid-Miocene (*c.* 12.5 Ma), *Nacella* separated into two main lineages currently distributed in (1) South America and (2) Antarctica and the sub-Antarctic islands. We identified two pulses of diversification, during the late Miocene (8 to 5.5 Ma) and the Pleistocene (< 1 Ma).

Main conclusions Major periods of climatic and oceanographical change strongly affected the biogeography of *Nacella* and demonstrate both the long- and short-term influence of the Antarctic Circumpolar Current across the Southern Ocean. Our analyses support the validity of all currently recognized *Nacella* species and reveal a new South-American lineage. This work constitutes the most detailed molecular-based study of an ecologically important, near-shore invertebrate Southern Ocean group and in so doing contributes to the improved understanding of the underlying patterns and processes in the origin and diversification of marine benthic fauna across this globally important region.

Keywords
Antarctic Circumpolar Current, biogeography, lineage-through-time plots, long-distance dispersal, *Nacella*, Southern Ocean

INTRODUCTION

The origin, evolution and biogeography of the Southern Ocean biota have long been controversial. Substantial differences of opinion on the relative roles of vicariance versus dispersal and the relationships among different areas have characterized the literature since the early 1900s (Clarke *et al.*, 1992; Aronson *et al.*, 2007; Waters, 2008). New research, as a consequence of extensive sampling campaigns and modern molecular laboratory techniques, has significantly enhanced our knowledge of the biogeography of the region (Page & Linse, 2002; Strugnell *et al.*, 2008; González-Wevar *et al.*, 2010; Díaz *et al.*, 2011; Terauds *et al.*, 2012; Saucède *et al.*, 2014). Consensus is now growing about the combined significance of vicariance and dispersal in explaining observed distributional patterns (Patarnello *et al.*, 1996; Crame, 1999; Linse *et al.*, 2006; Strugnell *et al.*, 2008; Fraser *et al.*, 2009; González-Wevar *et al.*, 2010, 2012; Nikula *et al.*, 2010; Mortimer *et al.*, 2011; Cumming *et al.*, 2014; Poulin *et al.*, 2014).

Early works relying on oceanographical evidence suggested that the Antarctic fauna was highly endemic and clearly divided into sub-Antarctic, low Antarctica and Antarctic/high Antarctic areas (Ekman, 1953; Hedgpeth, 1969). Recent analyses based on faunal similarities, however, have argued that large-scale biogeographical patterns differ depending on the taxonomic group (Griffiths *et al.*, 2009; Downey *et al.*, 2012; Pierrat *et al.*, 2013; Griffiths & Waller, 2016). Moreover, approaches that integrate data from fossil, climatic, oceanographical and genetic sources are revealing just how these differences play out among different taxonomic groups, while also providing insights into substantial cryptic diversity and spatial structuring within many of the groups (Leese *et al.*, 2008; Strugnell *et al.*, 2008; González-Wevar *et al.*, 2010, 2016; Arango *et al.*, 2011; Baird *et al.*, 2011; Díaz *et al.*, 2011; Allcock & Strugnell, 2012).

Comprehensive molecular studies of marine the fauna across the Southern Ocean nonetheless remain rare (e.g. Thornhill *et al.*, 2008; Fraser *et al.*, 2009; González-Wevar *et al.*, 2010; Díaz *et al.*, 2011). Indeed, most studies have been restricted to limited areas of the Southern Ocean (Mahon *et al.*, 2008; Wilson *et al.*, 2009; Hunter & Halanych, 2010; Leese *et al.*, 2010; Janosik *et al.*, 2011). Hence, much scope exists for multidisciplinary approaches using broadly distributed taxa to improve understanding of the biogeography and evolutionary history of this important region (Chown *et al.*, 2015).

The patellogastropod limpet genus, *Nacella* Schumacher, 1817, occurs on many of the intertidal and subtidal shores around the Southern Ocean and includes several species endemic to one or two geographically isolated sub-Antarctic islands (Powell, 1973). South America, and in particular the Magellanic Province, shows the highest taxonomic richness, with at least eight nominal species (Powell, 1973; Valdovinos & Rüth, 2005). As a consequence, Powell (1973) argued that this region was the centre of origin for the genus, from which seaweed-associated species spread eastward, driven by the West Wind Drift. Nevertheless, the role of long-distance dispersal in the evolution of the Southern Ocean's biota has been controversial (Waters, 2008). Even Powell (1955) was critical of this hypothesis and wrote: 'too much stress ... laid on the apparent circum-sub-Antarctic range of molluscs that are presumed to owe their distribution to the West Wind Drift'. As for many Southern Ocean molluscan (and other) groups that show conservative morphologies, including *Yoldia* (González-Wevar *et al.*, 2012; Poulin *et al.*, 2014), *Trophon/Trophonella* (Barco *et al.*, 2012), *Doris* (Wilson *et al.*, 2009) and *Margarella* (Williams *et al.*, 2010; Williams, 2012), molecular studies have improved the understanding of the evolution of *Nacella* (González-Wevar *et al.*, 2010, 2011; De Aranzamendi *et al.*, 2011). Geographically distant species of *Nacella* generally exhibit greater genetic differentiation, Patagonian species being clearly separated from species of the Antarctic Peninsula, Central Chile and sub-Antarctic Heard Island (González-Wevar *et al.*, 2010). In spite of these advances, our understanding of the relationships between sub-Antarctic *Nacella* species and those from Antarctica and South America remains poor. There is still no certainty about the number of species that are widespread and those that are more narrowly distributed. For instance, the kelp-dwelling *N. mytilina* is recorded from the southern tip of South America, the Falkland/Malvinas Islands and Kerguelen Island, more than 8000 km away (Powell, 1973). Based on the previous molecular studies showing that geographically distant species fall in different genetic lineages (González-Wevar *et al.*, 2010), it might be expected that more than one species is involved here. Nevertheless, considering the wide geographical distribution of macroalgae (Fraser *et al.*, 2009; Macaya & Zuccarello, 2010), and many of their associated invertebrates (Helmuth *et al.*, 1994; Waters, 2008; Nikula *et al.*, 2010, 2012; Cumming *et al.*, 2014), it is possible that *N. mytilina* has a broad range across the sub-Antarctic. Similar issues apply to *N. macquariensis* and *N. kerguelenensis* recorded on the Kerguelen Plateau and at Macquarie Island, some 5000 km apart.

In this study, we applied multi-locus molecular phylogenetic reconstructions, divergence-time estimations including all the species currently found around the Southern Ocean. We derive a clear picture of the evolutionary relationships in *Nacella*, the taxonomic status of different populations within species and biogeography of the entire genus. We also compared the distribution of the evolutionary lineages in *Nacella* with the large-scale faunal provinces proposed for Southern Ocean gastropods (Griffiths *et al.*, 2009; Pierrat *et al.*, 2013; Koubbi *et al.*, 2014). Addressing these specific issues in the context of this dominant benthic group provides fresh insights of the biogeography and evolution of the Southern Ocean marine biota.

MATERIALS AND METHODS

Sample collection

Nacella species were collected from across the Southern Ocean (see Appendix S1 in Supporting Information) and identified following Powell (1973), Valdovinos & Rüth (2005) and González-Wevar *et al.* (2010, 2011; Fig. 1). Individuals from Heard Island identified as *N. macquariensis* (González-Wevar *et al.*, 2010) are here referred as *N.* cf. *macquariensis* (Appendix S1). Two specimens from Kerguelen Island were sampled from living kelps, and based on shell morphology (Powell, 1973), they are labelled as *N.* aff. *mytilina* Appendix S1 in Supporting Information. Finally, specimens (*n* = 3) of a putative new species recently recorded at the Beagle Channel, southern South America, were also included in the analysis.

DNA preparation and sequence edition

Whole specimens were fixed in ethanol (95%), and DNA was prepared from the mantle using a standard salting-out DNA method (Aljanabi & Martinez, 1997). A fragment of
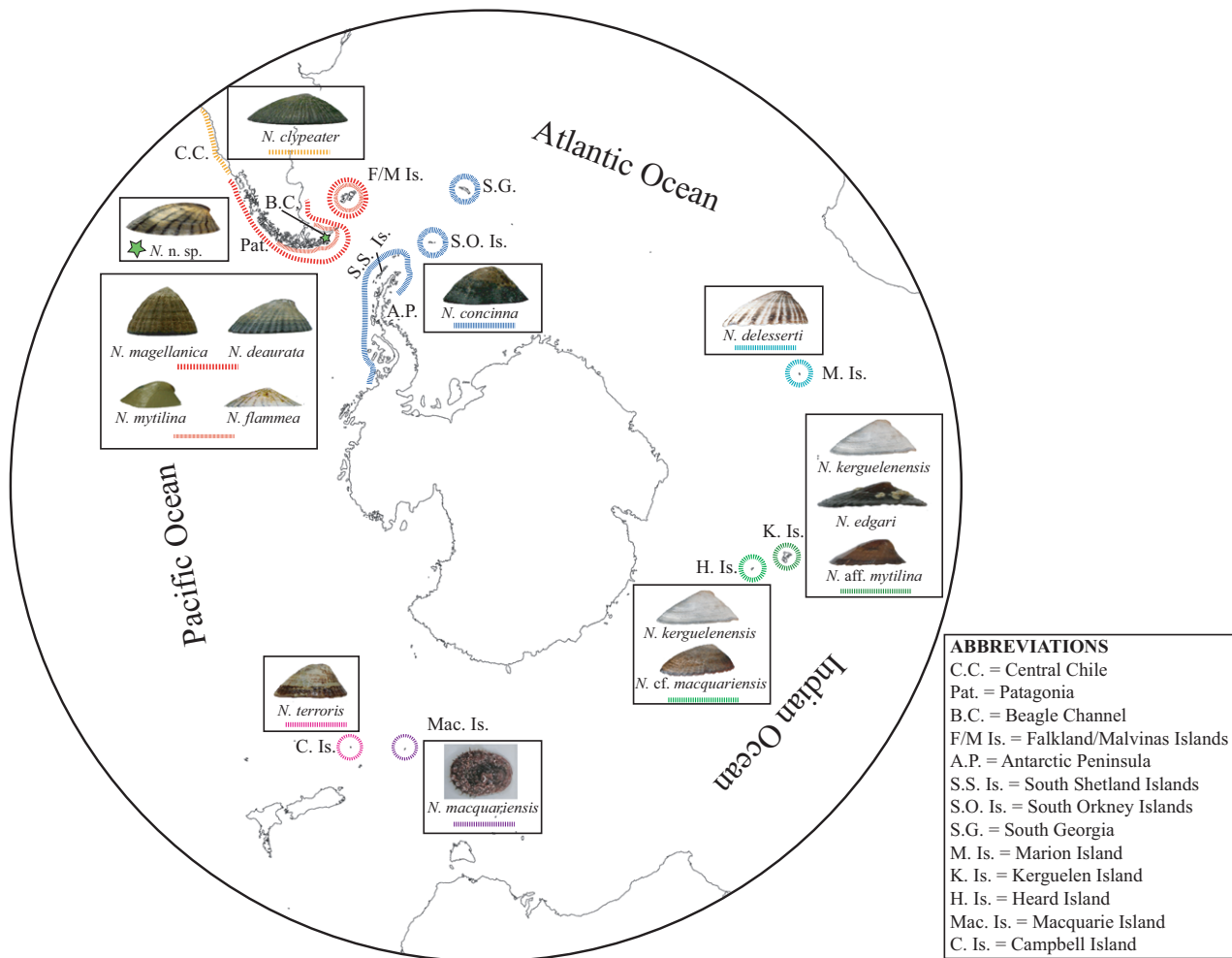
Figure 1 Nominal species of *Nacella* and their respective distributions in the Southern Ocean including South America, Antarctica and sub-Antarctic islands.

the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified using universal (Folmer *et al.*, 1994) and specific primers (González-Wevar *et al.*, 2010). Similarly, a fragment of the mitochondrial gene cytochrome b was amplified using universal primers (Merrit *et al.*, 1998). Finally, a fragment of the 28S rRNA gene was amplified using universal primers LSU5′ and LSU 1600R (Littlewood *et al.*, 2000). Sequences were assembled independently and edited using Geneious 5.1.7 (Kearse *et al.*, 2012). Multiple alignments were obtained using MUSCLE (Edgar, 2004), base composition of nucleotide sequences was analysed with MEGA 6 (Tamura *et al.*, 2013), and codon usage was determined using the effective number of codons value (ENC; Wright, 1990) from DnaSP 5.0 (Librado & Rozas, 2009). This statistic has values between 20 (extremely biased codon usage) and 61 (totally random codon usage).

Phylogenetic reconstructions

Mitochondrial DNA reconstructions included all the recognized species of *Nacella*, 10 species of the sister genus *Cellana* and other patellogastropods (*Cymbula*, *Helcion*, *Scutellastra* and *Patella*; Appendix S1). Nuclear reconstructions included 28S rRNA sequences of different patellogastropods (Appendix S1). Phylogenetic relationships were estimated using maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses (BA). MP analyses were performed using PAUP* (Swofford, 2002), following González-Wevar *et al.* (2010). Nucleotide substitution models for ML and BA analyses were selected using the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 2.0 (Darriba *et al.*, 2012), respectively. The GTR+I+G (mtDNA) and GTR+G (28S rDNA) substitution models were used for ML and BA analyses using the programs PHYML (Guindon & Gascuel, 2003) and MrBayes 3.1.2. (Huelsenbeck & Ronquist, 2001). Nonparametric bootstrap (BS) values were used to infer nodal support for both the MP and ML (1000 pseudo-replicates; Felsenstein, 1981). Bayesian inference posterior probabilities were estimated using the Metropolis coupled Markov chain Monte Carlo algorithm (MCMC) running four chains for 100×10^6 generations with trees

sampled every 1000 generations. Stationarity was inferred when the average standard deviation of split frequencies was less than 0.01 (Huelsenbeck & Ronquist, 2001). The initial 10% of the trees was discarded (burn-in) and posterior probabilities were estimated as the fraction of trees showing a particular node. Posterior probability density was summarized as a maximum clade credibility tree using TREEANNOTATOR 1.6.1 (http://beast.bio.ed.ac.uk/TreeAnnotator) and then visualized with FIGTREE 1.4 (http://tree.bio.ed.ac.uk/software/figtree).

Divergence-time estimations

Relaxed molecular clock analyses were used for mtDNA genes with an uncorrelated lognormal (ucln) model of molecular evolutionary rate heterogeneity and the GTR+I+G substitution model implemented in BEAST 1.6.2 (Drummond & Rambaut, 2007; Drummond *et al.*, 2012). An age prior with a normal distribution was applied to the time to the most recent common ancestor (tmrca) of Nacellidae (mean, 38; SD, 3.8), based on an Upper Eocene fossil of *Cellana ampla* (Lindberg & Hickman, 1986). We included two priors within *Nacella*, (1) the tmrca of *N. concinna* (mean, 5; SD, 0.5; Jonkers, 1998) and (2) the tmrca of *N. clypeater*-like fossil (mean, 4, SD, 0.4) from southern Peru (DeVries, 2009). A birth–death speciation prior was used for branching rates in the phylogeny, four chains were run twice for 50×10^6 generations, and trees were sampled every 1000 generations. Convergence of model parameter was estimated by plotting the marginal posterior probabilities versus the generation state in TRACER 1.5 (http://beast.bio.ed.ac.uk/Tracer). Effective sample size values were estimated for each parameter to ensure adequate mixing of the MCMC (ESSs > 1000).

Diversification rates and lineage-through-time plots

Net diversification rate within *Nacella* was estimated using 'geiger' (Harmon *et al.*, 2008) in the R environment (R Core Team, 2014). To evaluate temporal variations in diversification rates, we compared the AIC scores of constant-rate (e.g. pure birth, birth–death) and rate-variable models with 'laser' (Rabosky, 2006). To evaluate the best-fit model, we performed a statistical test for constancy of diversification rates using the fitdAICrc function based on ΔAIC. This function considers differences in the scores between AIC_{RC} (best-fit constant-rate model) and AIC_{RV} (best-fit rate-variable model; Rabosky, 2006). A test of significance of ΔAIC was conducted through 10,000 simulations. A lineage-through-time (LTT) plot was generated in 'ape' (Paradis *et al.*, 2004) using a birth–death model and several extinction rates to determine the stability of the model. The birth-rate model was determined from the branching times, and the expected number of lineages was estimated under a constant model (i.e. pure birth) using 1000 simulations in 'phytools' (Revell, 2012). Even when our lineage-through-time models were inferred from phylogenetic reconstructions, extinction rates

were considered in the analyses (Nee *et al.*, 1994). Finally, we tested for simultaneous divergence across different populations pairs (Antarctica, South America and the Kerguelen Plateau) spanning the same barrier to gene flow (the ACC) using MTML-MSBAYES (Hickerson *et al.*, 2007; Huang *et al.*, 2011). We estimated the Y divergence times across Y ancestral populations and descendant population pairs. Then, we estimated the number of possible divergence events (Ψ, $1 < Ψ < Y$), the mean divergence times [E(τ)] and the ratio of the variance to the mean in these Y divergence time(s) ($\Omega = \mathrm{Var}(\tau)/\mathrm{E}(\tau)$). Calculations were carried out using 10×10^6 simulated draws from the prior and a posterior sample of 1000 with a post-acceptance adjustment using local linear regression.

RESULTS

No indels or stop codons were identified in *Nacella*'s COI (659 bp) and Cytb (410 bp) sequences, respectively. No amino-acid substitution was detected within *Nacella* for the COI gene, whereas Cytb exhibited four substitutions (positions 16, 48, 101 and 102). The combined mtDNA alignment (1069 bp) included 207 variable positions (19.4%) of which 193 (93.2%) were parsimony informative. Nuclear 28S rRNA (839 bp) was more conserved with 55 variable positions (6.5%) of which 50 (90.9%) were parsimony informative. Mitochondrial sequences were A-T rich (61.8%); nuclear ones were G-C rich (64.2%). Mitochondrial and nuclear sequences were not saturated at any position. No evidence for codon bias was detected in either COI (ENC = 36.37) or Cytb (ENC = 45.17).

All the reconstructions discriminated major taxonomic groupings of patellogastropods with high bootstrap and posterior probabilities (Appendices S2 and S3). Similarly, the monophyly of Nacellidae and the respective monophyly of *Nacella* and *Cellana* were highly supported (Appendices S2 and S3). Mitochondrial and nuclear reconstructions resolved the relationships within *Nacella* lineages without topological inconsistencies (Fig. 2a). Reconstructions recognized an early separation in the genus into two main clades: (*a*) comprising lineages from Antarctica and sub-Antarctic Islands (Marion, Kerguelen, Heard, Macquarie and Campbell) and (*b*) consisting of lineages from South America (Fig. 2a).

Within lineage *c* all the reconstructions grouped Heard Island individuals (*N.* cf. *macquariensis*) with *N. edgari* from Kerguelen Island (purple, Fig. 2). Similarly, all the methods and markers recognized *N. macquariensis* from Macquarie Island as reciprocally monophyletic (mauve, Fig. 2) with respect to all other populations and sister to *N. terroris* from Campbell Island (pink, Fig. 2). We conclude, therefore, that individuals from Heard Island previously ascribed to *N. macquariensis* (e.g. Powell, 1973; González-Wevar *et al.*, 2010) are in fact *N. edgari* and that true *N. macquariensis* is restricted to Macquarie Island.

Lineage *d* (green, Fig. 2) comprises *N. kerguelenensis* from Kerguelen and Heard Islands and kelp-associated individuals
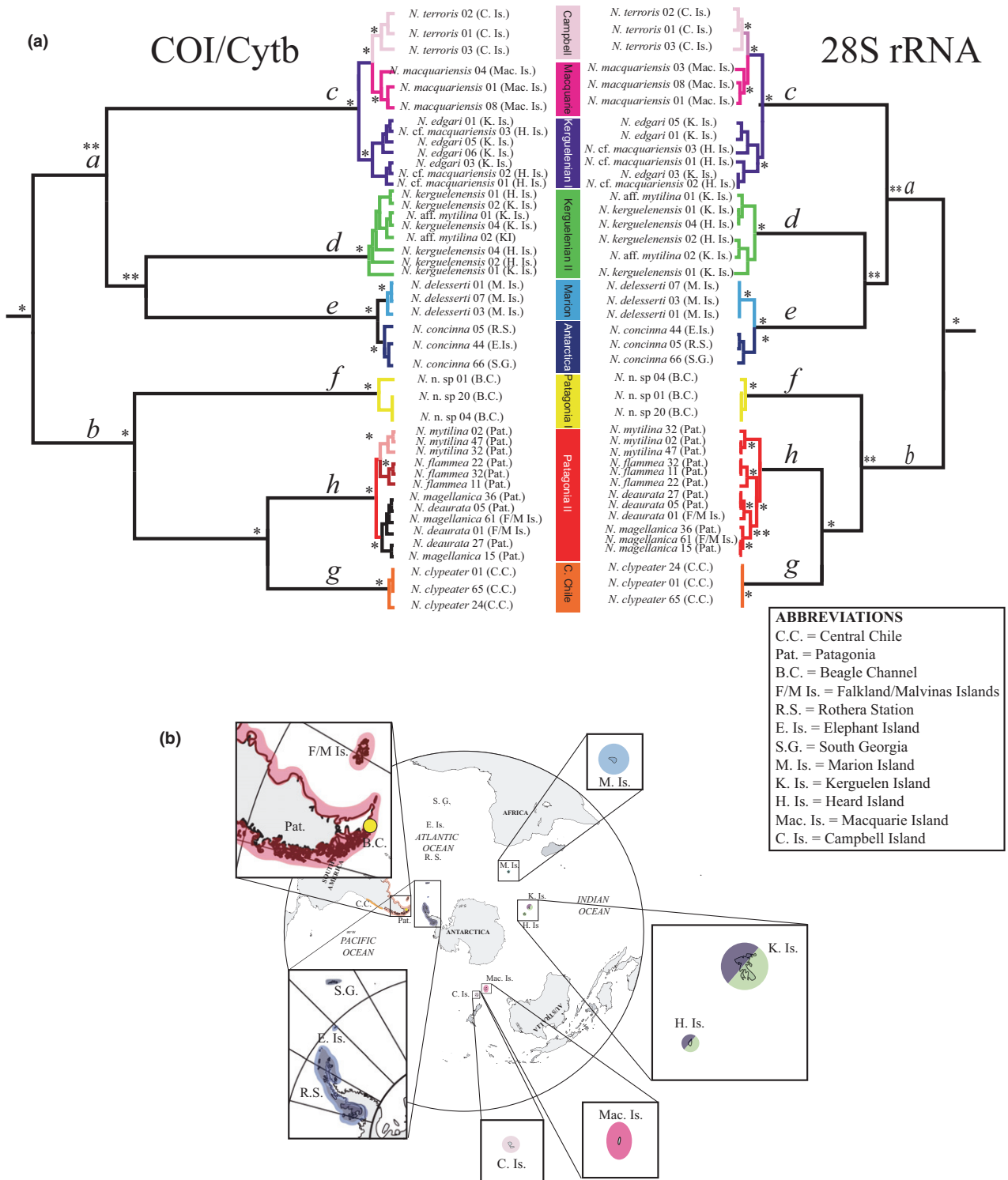
Figure 2 (a) Bayesian phylogenetic MCMC reconstructions based on mtDNA (COI + Cytb) and nucDNA (28S rRNA) datasets. BPP: * > 0.95 to ≥ 0.95, ** > 0.75. (b) The map shows strongly supported lineages within *Nacella*.

(*N.* aff. *mytilina*) from Kerguelen. The latter fell within the intraspecific variation of *N. kerguelenensis*, and therefore, they simply represent morphological variants. Hence, the broader distribution ascribed to the Patagonian *N. mytilina* (Powell, 1973) is rejected.

Within lineage *e*, all the reconstructions recognized the reciprocal monophyly and the sister relationship between *N. concinna* from Antarctica (dark blue, Fig. 2) and *N. delesserti* from sub-Antarctic Marion Island (light blue, Fig 2).

Lineage *f* (Patagonia I, yellow, Fig. 2) of individuals from the Beagle Channel clearly represented a well-resolved, reciprocally monophyletic clade and corresponds to a distinct species. Lineage *g* (Central Chile, orange, Fig. 2) includes *N. clypeater* from the Pacific margin of South America. Finally, subclade *h* (Patagonia II, red, Fig. 2) represents the most morphologically diverse lineage and includes four taxa. Mitochondrial DNA reconstructions recognized the reciprocal monophyly of *N. mytilina* and *N. flammea*, but failed to recover that of *N. deaurata* and *N. magellanica*. However, 28S rRNA sequences recovered the reciprocal monophyly of all four species.

The separation between *Nacella* and *Cellana* occurred *c.* 32 Ma (25–42 Ma). Within *Nacella*, the separation between South-American taxa and Antarctic/sub-Antarctic ones occurred *c.* 12 Ma (18 to 9.5 Ma; Fig. 3). Following this, the different lineages of *Nacella* diversified during the late Miocene in the different provinces of the Southern Ocean (maritime Antarctica, the Kerguelen Plateau and South America). The Patagonian diversification of *Nacella* is very recent, no more than *c.* 1 Ma (1.8 to 0.5 Ma; Fig. 3a). The separation between *N. edgari* (Kerguelen and Heard Islands) and *N. macquariensis* (Macquarie Island) + *N. terroris* (Campbell Island) occurred *c.* 0.6 Ma (Fig. 3a). Similarly, the separation between *N. concinna* (Antarctic Peninsula) and *N. delesserti* (Marion Island) occurred *c.* 0.35 Ma and that between *N. macquariensis* (Macquarie Island) and *N. terroris* (Campbell Island) just *c.* 0.25 Ma (Fig. 3a).

The LTT plot and diversification rate-variation recorded two pulses of diversification with Yule2rates as the best-fit model (Table 1). A first diversification round occurred at the end of the Miocene (Fig. 3c) in different provinces of the Southern Ocean (Fig. 3b). Estimates of the ratio of variance of the mean in divergence times ($\Omega = 0.00$; posterior density > 400) and the number of possible divergence times ($\psi = 1.00$; pp > 0.9) suggest a single synchronous diversification event, rather than a gradual accumulation of species/lineages. Accordingly, five lineages: (1) *N. concinna* + *N. delesserti*; (2) *N. kerguelenensis*; (3) *N. edgari* + *N. terroris* + *N. macquariensis*; (4) *N.* n. sp.; (5) *N. clypeater* + *N. magellanica* + *N. deaurata* + *N. mytilina* + *N. flammea* arose almost simultaneously from a single ancestral population. A second diversification pulse included (1) the colonization of sub-Antarctic islands followed by local differentiation and (2) the recent radiation in Patagonia (Fig. 3b).

DISCUSSION

This study constitutes the first integrative biogeographical approach at the scale of the whole Southern Ocean to elucidate evolutionary relationships, divergence times and diversification rates in the circum-Antarctic limpet genus *Nacella*. Molecular data suggest that the tmrca of *Cellana* and *Nacella* occurred *c.* 32 Ma (25–42 Ma), close to the Eocene-Oligocene boundary (Fig. 3a). This period represented one of the

Earth's most pronounced cooling events, a shift from a 'Greenhouse' to an 'Icehouse' world (Zachos *et al.*, 2001; DeConto & Pollard, 2003; Fig. 4). This transition from an unglaciated to a glaciated planet was largely due to the combined effect of a gradual northward movement of continental landmasses and the closure of the Thethys Sea. At the same time, major oceanic gateways openings in the Southern Ocean initiated the Antarctic Circumpolar Current (ACC) and isolated Antarctica (Barker & Thomas, 2004; Pfuhl & McCave, 2005; Scher & Martin, 2006; Convey *et al.*, 2009; Scher *et al.*, 2015). Here, we argue that the separation between tropical/temperate *Cellana* and its Antarctic/sub-Antarctic sister genus, *Nacella*, coincides with the late-Eocene climate shift to glacial conditions in the Southern Ocean (Fig. 4; Liu *et al.*, 2009).

The subsequent diversification of *Nacella* occurred long after the physical separation of the continental landmasses. Our estimate of the tmrca of the extant lineages of *Nacella* was ~12.5 Ma (ranging from 18 to 10 Ma; Fig. 3b), close to the Middle Miocene Climatic Transition (MMCT). The MMCT was a period of major oceanographical and climatic change in the Southern Ocean associated with intensification of the ACC and the re-establishment of a continental Antarctic ice sheet (Zachos *et al.*, 2001; DeConto & Pollard, 2003; Mackensen, 2004; Lewis *et al.*, 2008; Verducci *et al.*, 2009). Major oceanic circulation shifts were probably related to the full development of a deep ACC, a process that was only achieved during the late Miocene *c.* 12 Ma (Dalziel *et al.*, 2013). In the case of *Nacella*, fluctuations in latitudinal positioning and in strengthening of the ACC during the Miocene may have favoured the colonization of geographical distant provinces of the region. Alternatively, and in accord with our diversification rates analyses, the establishment of a strong, deep ACC acting as an effective oceanographical barrier may explain the almost simultaneous diversification of *Nacella* in different areas of the Southern Ocean.

Divergence-time estimation and LTT plots within *Nacella* recovered two pulses of diversification (Yule-2rates model; Fig. 3c; Table 1). The first one encompassed the simultaneous diversification of *Nacella* in three biogeographical provinces of the Southern Ocean (8.0 to 5.5 Ma). This event is consistent with molecular-based analyses of Southern Ocean fish and benthic invertebrates. For instance, most of the extant nothothenioid species-rich clades diversified 11.6–5.3 Ma, more than 10 Ma after their origin (Near *et al.*, 2012). Similarly, the levels of genetic divergence between amphi-ACC octocoral populations indicate that the separation occurred during the Middle Miocene 12.6 Ma (Dueñas *et al.*, 2016). Levels of mtDNA divergence between Antarctic and South-American marine invertebrates (González-Wevar *et al.*, 2012; Poulin *et al.*, 2014), including echinoderms of the genera *Sterechinus* (Díaz *et al.*, 2011), *Astrotoma* (Hunter & Halanych, 2008) and *Odontaster* (Janosik *et al.*, 2011), bivalves like *Limatula* (Page & Linse, 2002) and *Yoldia* (González-Wevar *et al.*, 2012), suggest that their respective radiations occurred no more than 8 Ma. A second pulse of
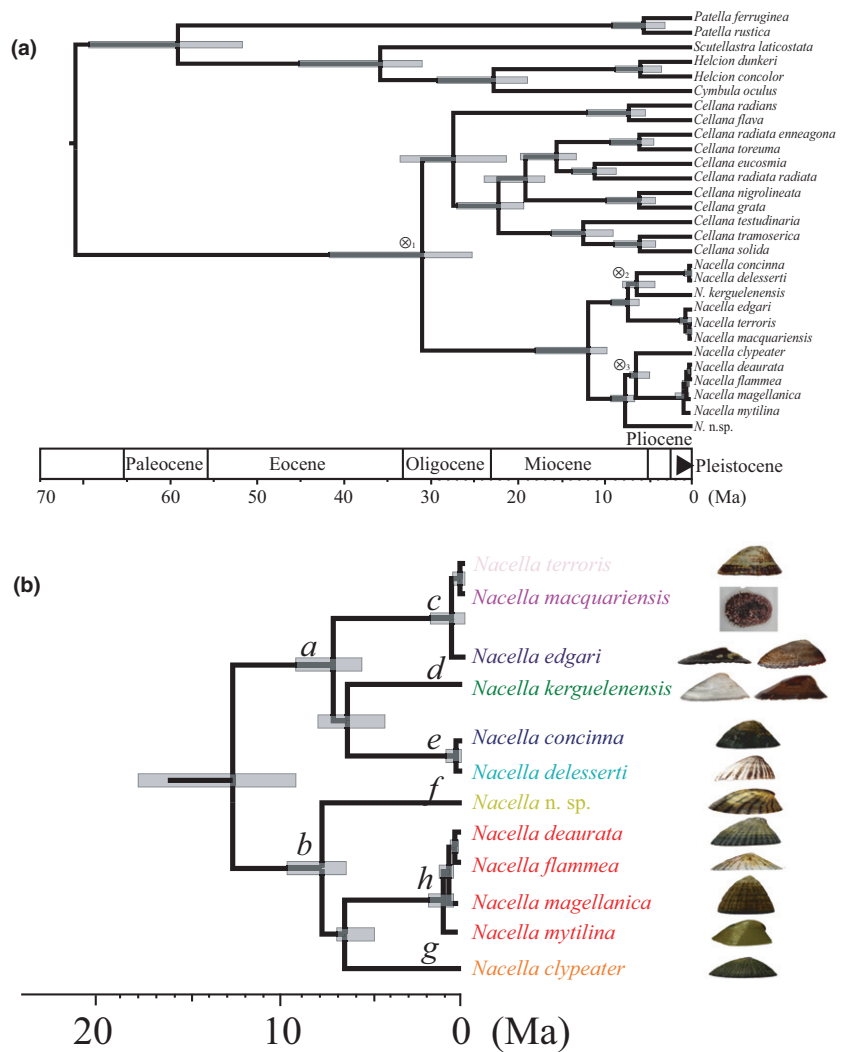
Figure 3 Most probable evolutionary tree relating (a) different groups of patellogastropods and (b) within *Nacella*. Calibration fossils within Nacellidae and *Nacella* are showed: $\otimes_1$ = *Cellana ampla* (Lindberg & Hickman, 1986); $\otimes_2$ = oldest *Nacella concinna* fossil from Cockburn Island (Jonkers, 1998); $\otimes_3$ *N. clypeater*-like fossil from southern Peru (DeVries, 2009). Grey boxed regions depict 95% Bayesian credible intervals (BCIs) for relative divergence times. Representative shells of each *Nacella* species are illustrated. (c) Lineage-through-time plots in the diversification of *Nacella* across the Southern Ocean. Rounds of diversification recorded in *Nacella* are shown: D_1 = 8.5 to 7 Ma; D_2 = > 1.5 to 0.5 Ma.

diversification during the Pleistocene involved long-distance dispersal events and the recent radiation in Patagonia (Fig. 3c). Dispersal from the Kerguelen Plateau to the Australia/New Zealand province occurred between 0.8 and 0.2 Ma, which agrees with the estimated geologic age of Macquarie Island (0.7 Ma; Chown *et al.*, 2008). Considering the absence of *Nacella* in other sub-Antarctic New Zealand islands (i.e. Auckland, Snares, Bounty and Antipodes Islands), the colonization of Campbell Island was probably reliant on the prior establishment at Macquarie Island. A second dispersal led to the colonization and differentiation of *N. delesserti* on Marion Island between 0.65 and 0.3 Ma. Again, this timing matches with the estimated geologic age of Marion Island around *c.* 0.45 Ma (Chown *et al.*, 2008).

Table 1 Comparison between constant-rate diversification models (pure birth, birth–death) and variable-rate models (Exponential, lineal density-dependent model and two-rate Yule model) using Maximum likelihood ΔAIC test statistics (ΔAICrc = 2.685). Where: R[1] and R[2] = net diversification rates; a = extinction rates; xp = exponential rates; k = maximum number of lineages in a group; st = shift-times; AIC = Akaike information criterion for each model and ΔAIC = the difference in AIC scores between the model (i) and the overall of the best-fit model.

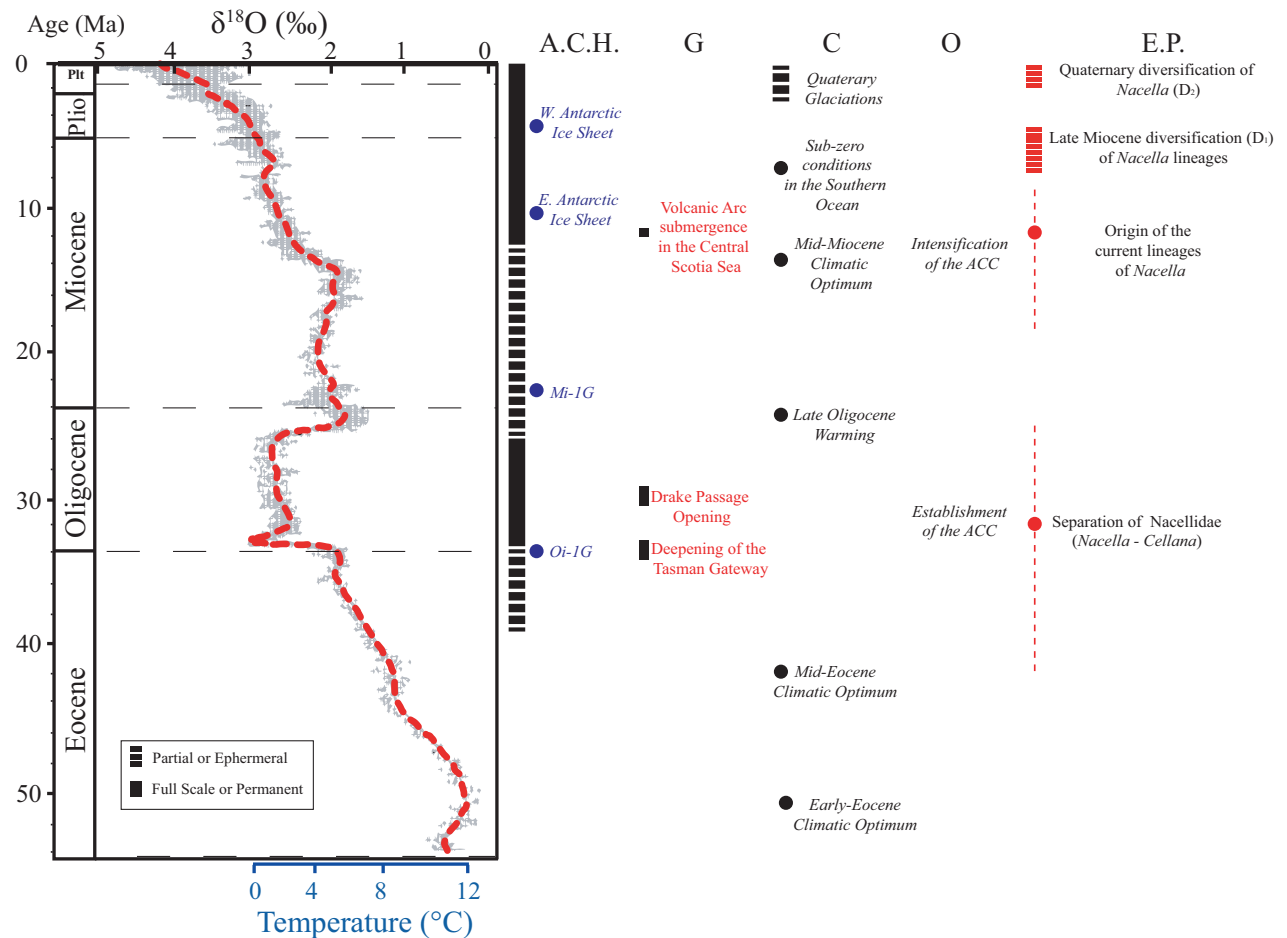| Type	Model	lnLH	R^1	R^2	a	xp	k	st	AIC	ΔAIC
Constant rates	pureBirth	34.13	22.53	–	–	–	–	–	−66.27	2.68
	bd	34.61	6.46	–	0.84	–	–	–	−65.24	3.72
Rate variable	DDL	34.15	27.49	–	–	0.12	–	–	−64.30	4.66
	DDX	34.14	22.53	–	–	–	187385	–	−64.27	4.68
	yule2rate	37.48	12.11	72.22	–	–	–	0.007	−68.96	0

Figure 4 Global deep-sea oxygen isotope and temperature records during the last 50 Ma (Zachos *et al.*, 2001) showing the Antarctic Cryosphere History (A.C.H.) major geologic (G), climatic (C), oceanographic (O) events, and the evolutionary patterns (E.P.) recorded in *Nacella* across the Southern Ocean.

Nacella delesserti is sister to the Antarctic limpet *N. concinna* in our phylogeny, even though the Polar Front has constituted a barrier to effective dispersal between Antarctic and sub-Antarctic provinces since the late Miocene (Thornhill *et al.*, 2008; Wilson *et al.*, 2009; González-Wevar *et al.*, 2012; Poulin *et al.*, 2014). González-Wevar *et al.* (2016) have suggested that the dispersal of *Nacella* from maritime Antarctica and/or peri-Antarctic islands towards Marion Island could have been promoted by the northward shift of the Polar Front during one of the coolest glacial events.

We identified a Pleistocene radiation of *Nacella* in Patagonia generating four closely related species. Increased glaciation and sea ice could have resulted in the local extinction or isolation in small refugia, while subsequent ice retreats would then have allowed recolonization of vacant areas. Repeated Pleistocene glacial events at higher latitudes may have enhanced geographical isolation and speciation (Schluter, 2000; González-Wevar *et al.*, 2011; Fraser *et al.*, 2012; Near *et al.*, 2012). A number of recent molecular studies have shown the significance of the Quaternary in
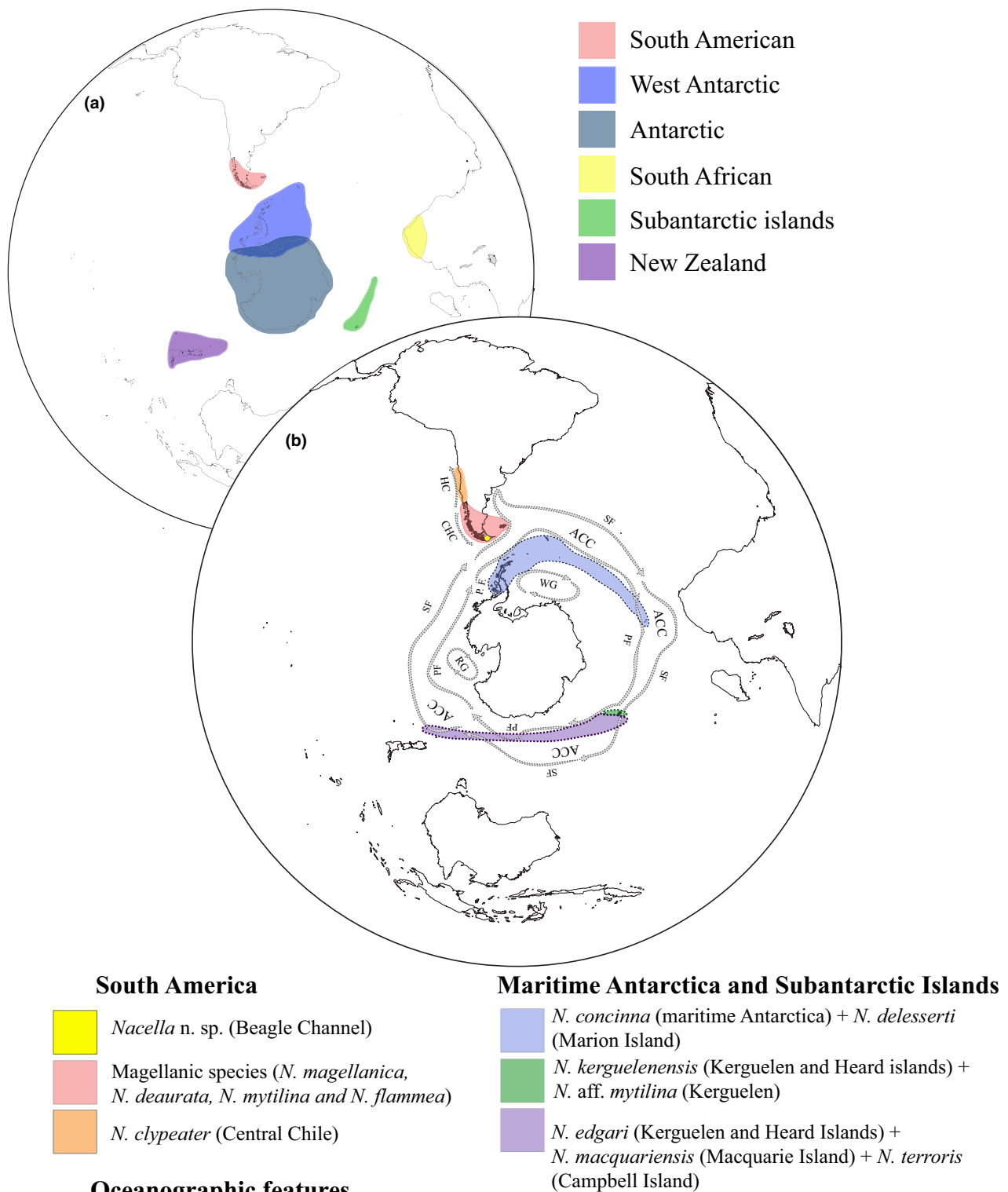
Figure 5 (a) Large-scale faunal patterns described for Southern Ocean gastropods (Griffiths *et al.*, 2009). (b) Molecular-based biogeographical affinities within *Nacella* showing the phylogenetic relationships among species from different provinces of the Southern Ocean, as well as main oceanographical features in the region.

diversification processes as well as cryptic speciation in Southern Ocean fishes (Near *et al.*, 2012; Hüne *et al.*, 2015), invertebrates (Goldstien *et al.*, 2006; Wilson *et al.*, 2009; Janosik *et al.*, 2011; Reisser *et al.*, 2011) and protists (Darling *et al.*, 2014).

Divergence-time estimations are in basic agreement with the scarce fossil record of *Nacella* (Gordillo, 1999; Gordillo *et al.*, 2005; DeVries, 2009). However, older fossils of *Nacella* include a late Oligocene species (*N. reichae*) from tropical latitudes of southern Peru and a late Miocene taxon from southern Chile (*N. nielseni*; DeVries, 2009). If these latter fossil species are true representatives of *Nacella*, they could correspond to low-latitude South-American ancestors of the current Antarctic/sub-Antarctic clades. These fossils could also be evidence that the ecological range of *Nacella* once overlapped that of its warm-water sister genus *Cellana* and that the modern lineages of the genus might have had low-latitude ancestors (DeVries, 2009).

New biogeographical approaches in the Southern Ocean

The distribution of *Nacella*'s evolutionary lineages is in basic agreement with the large-scale faunal pattern described for Southern Ocean gastropods (Fig. 5a). However, from an evolutionary perspective (Fig. 5b), sub-Antarctic provinces (Ekman, 1953; Knox, 1960; Powell, 1960; Hedgpeth, 1969; Dell, 1972) do not constitute a natural, self-contained biogeographical unit but shares common elements with other provinces. For instance, species from New Zealand, *N. macquariensis* and *N. terroris*, constitute recent offshoots from the Kerguelen and Heard Islands (Fig. 5b). Similarly, Marion Island's *N. delesserti* is derived from the Antarctic *Nacella* lineage. Finally, in South America, there are at least three well-resolved lineages of *Nacella* instead of a single Magellanic faunal province.

New biogeographical analyses of marine benthic invertebrates of the Southern Ocean, especially molluscs (Gérard *et al.*, 2008; González-Wevar *et al.*, 2010, 2016; Williams *et al.*, 2010; Barco *et al.*, 2012; Cumming *et al.*, 2014), are showing that the patterns of lineage distribution are more complex than previously thought and cannot be generalized across particular taxonomic groups. For instance, Southern Ocean lineages of the muricid subfamily Trophoninae (Barco *et al.*, 2012) as well as our case study *Nacella* (González-Wevar *et al.*, 2010; this study) are clearly differentiated across the main provinces of the region. In contrast, the brooder pulmonate *Onchidella marginata* is widely distributed across geographically distant provinces of the Southern Ocean (Cumming *et al.*, 2014). Similar patterns have been recorded in mytilids (Gérard *et al.*, 2008), echinoderms (Díaz *et al.*, 2011) and crustaceans (Nikula *et al.*, 2010; Pérez-Barros *et al.*, 2014). Hence, geographical distributions of related genetic lineages across the Southern Ocean are not reflective of taxonomy but rather of life-history traits, including bathymetric ranges, developmental modes, larval lifespans and the stochasticity of long-distance dispersal by rafting.

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SUPPORTING INFORMATION

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

Appendix S1 Patellogastropod species, individuals and sampling localities.

Appendix S2 Phylogenetic reconstructions in *Nacella* using mtDNA and nucDNA sequences.

Appendix S3 Concatenated (mtDNA and nucDNA) MCMC reconstructions in *Nacella*.

BIOSKETCH

Claudio González-Wevar is a molecular ecologist and a marine biogeographer at the Universidad de Magallanes, Chile. He is interested in understanding biogeographical patterns in the Southern Ocean near-shore marine benthic fauna and is currently working on several groups of marine molluscs using molecular markers at different geographical and temporal scales. He is the South-American representative of the SCAR (Scientific Committee on Antarctic Research) scientific research programme AntEco (State of the Antarctic Ecosystem).

Author contributions: C.A.G-W., T.N., E.P. and H.G.S. conceived the ideas; C.A.G-W., H.G.S., A.M., T.S., G.J. and E.P. collected *Nacella* material in different provinces of the Southern Ocean. C.A.G-W. performed laboratory work supervised by E.P., T.N. and H.G.S.; C.A.G-W., M.H. and N.I.S. performed phylogenetic reconstructions and analysed the data; C.A.G-W., T.N., E.P, S.C., T.S. and H.G.S. interpreted the data; and C.A.G-W, E.P. S.C. and H.G.S. led the writing of the manuscript.

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