Check for updates

Received: 25 January 2021 | Revised: 21 July 2021 | Accepted: 9 August 2021

DOI: 10.1111/ddi.13405

RESEARCH ARTICLE

Diversity and Distributions WILEY

Historical biogeography of the Gondwanan freshwater genus *Boeckella* (Crustacea): Timing and modes of speciation in the Southern Hemisphere

Claudia S. Maturana[1,2,3] | Sebastián Rosenfeld[1,2,4] | Elisabeth M. Biersma[3,5] | Nicolás I. Segovia[2,6] | Claudio A. González-Wevar[2,7,8] | Angie Díaz[2,9] | Javier Naretto[1,10] | Ian C. Duggan[11] | Ian D. Hogg[11,12] | Elie Poulin[1,2] | Peter Convey[3] | Jennifer A. Jackson[3]

[1]Laboratorio de Ecología Molecular, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Ñuñoa, Chile

[2]Instituto de Ecología y Biodiversidad (IEB), Ñuñoa, Chile

[3]British Antarctic Survey (BAS), Natural Environment Research Council, Cambridge, UK

[4]Laboratorio de Ecosistemas Marinos Antárticos y Subantárticos, Universidad de Magallanes, Punta Arenas, Chile

[5]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

[6]Laboratorio de Diversidad Molecular, Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile

[7]Instituto de Ciencias Marinas y Limnológicas (ICML), Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

[8]Centro FONDAP de Investigaciones en Dinámicas de Ecosistemas Marinos de Altas Latitudes, Universidad Austral de Chile, Valdivia, Chile

[9]Laboratorio de Ecología Molecular Marina, Departamento de Zoología, Universidad de Concepción, Concepción, Chile

[10]Costa Humboldt, Peñalolén, Chile

[11]School of Science, University of Waikato, Hamilton, New Zealand

[12]Polar Knowledge Canada, Canadian High Arctic Research Station, Cambridge Bay, Vic., Canada

Correspondence
Claudia S. Maturana, Laboratorio de Ecología Molecular, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras # 3425, Ñuñoa, Santiago, Chile.
Email: claudiamaturana@uchile.cl

Funding information
British Antarctic Survey; Comisión Nacional de Investigación Científica y Tecnológica, Grant/Award Number: NERC-CONICYT grant NE/P003079/1, PIA APOYO CCTE AFB170008, PIA CONICYT ACT172065, Ph.D. Grant 21140632 and Ph.D. Grant 21150317; Universidad de Magallanes, Grant/Award Number: Program PR-06-CRN-18; Carlsbergfondet, Grant/Award Number: CF18-0267; Institut chilien de l'Antarctique, Grant/Award Number: DG_03-16 and DT_04-16

Editor: Zhonge Hou

Abstract

Aim: We investigated evolutionary relationships and biogeographical patterns within the genus *Boeckella* to evaluate (1) whether its current widespread distribution in the Southern Hemisphere is due to recent long-distance dispersal or long-term diversification; and (2) the age and origin of sub-Antarctic and Antarctic *Boeckella* species, with particular focus on the most widely distributed species: *Boeckella poppei*.

Location: South America, sub-Antarctic islands, maritime Antarctica, continental Antarctica and Australasia.

Methods: To reconstruct phylogenetic patterns of *Boeckella*, we used molecular sequence data collected from 12 regions and applied Bayesian and Maximum Likelihood analyses using multiple loci. We also estimated divergence times and reconstructed ancestral ranges using two different models of species evolution.

Results: Phylogenetic analyses and divergence time estimates suggested that *Boeckella* originated on the Gondwanan supercontinent and initially split into two main clades during the late Cretaceous (*ca*. 80 Ma). The first clade diversified in Australasia, and the second clade is currently distributed in South America, various

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2021 The Authors. *Diversity and Distributions* published by John Wiley & Sons Ltd.

sub-Antarctic islands and Antarctica. Dispersal from South America to the Kerguelen and Crozet archipelagos occurred during the Eocene/Oligocene (*B. vallentini*) and in the late Pliocene (*B. brevicaudata*), while South Georgia and the maritime Antarctic were likely colonized during the late Pleistocene (*B. poppei*).

Main conclusions: *Boeckella* has a Gondwanan origin, with further diversifications after the physical separation of the continental landmasses. Extant populations of *Boeckella* from the Scotia Arc islands and Antarctic Peninsula originated from South America during the Pleistocene, suggesting that original Antarctic Gondwanan lineages did not survive repeated glacial cycles during the Quaternary ice ages. A continuous decline in the species accumulation rate is apparent within the genus as the early Eocene, suggesting that *Boeckella* diversification may have decreased due to progressive cooling throughout the Cenozoic era.

KEYWORDS

28S rRNA, Antarctica, Australasia, biogeography, *cox*1, DNA barcoding, Gondwana, *ITS*, South America

1 | INTRODUCTION

In the Southern Hemisphere, clades with widely distributed taxa have long represented fascinating models to study historical biogeography and diversification patterns. Indeed, the break-up of the mega-continent Gondwana (*ca*. 1,000 Ma) and the later formation of isolated volcanic oceanic islands represents an outstanding opportunity to disentangle the respective roles of vicariance and long-distance dispersal and establishment (LDDE) involved in the diversification process. On the one hand, vicariance is a process by which a previously continuous geographic distribution is physically disrupted into fragmented elements (Rosen, 1978). It is the key biogeographic process underlying the currently disjointed distributions of multiple taxa across the Southern Hemisphere, including the tree genus *Nothofagus* (Swenson et al., 2001), liverworts (Vanderpoorten et al., 2010), moss bugs (Burckhardt, 2009) and midges (Cranston et al., 2010). On the other hand, LDDE (Crisp et al., 2011; de Queiroz, 2005; Waters, 2008) is a process by which organisms or their propagules overcome large geographical barriers to gene flow *via* rare dispersal events (Biersma et al., 2017). This can occur with the assistance of vectors, which can be either biotic (with fauna or flora), abiotic (atmospheric and oceanic phenomena) or, most recently, anthropogenic (with human assistance).

The genus *Boeckella* is the most widespread group of freshwater calanoid copepods in the Southern Hemisphere (Adamowicz et al., 2007; Bayly, 1992b; Maturana et al., 2019; Menu-Marque et al., 2000). Its contemporary distribution includes Australia, New Zealand, Tasmania, New Caledonia, South America, maritime and continental Antarctica, and various sub-Antarctic (South Georgia, Marion and Prince Edward Islands, Crozet Islands, Kerguelen Islands, Heard Island, Macquarie Island) and cool temperate islands (Falkland/Malvinas Islands, Campbell Island, Amsterdam Island) (see Table S1). The lack of records from South Africa of *Boeckella* specifically and, more broadly, of Centropagidae has been previously discussed by Bayly (1992b). Most *Boeckella* species inhabit permanent or temporary fresh to brackish water bodies (Boxshall & Jaume, 2000; Quinlan & Bayly, 2017). The only exception is *B. poopoensis*, which exclusively inhabits saline water bodies from 43° to 15°S in South America (Menu-Marque et al., 2000; Samanez & López, 2014). Resistant eggs have been reported in at least three species of *Boeckella* in maritime Antarctica and Australia (*B. poppei*, *B. triarticulata* and *B. hamata*). This physiological feature allows a period of dormancy through periods of harsh environmental conditions such as desiccation, extreme temperature, low oxygen levels or pollutants, with revival once conditions ameliorate (Brendonck & de Meester, 2003; Couch et al., 2001; Hall & Burns, 2001; Jiang et al., 2012; Reed et al., 2021).

The species *B. poppei* (Mrázek, 1901) has the broadest distribution and is the only representative of the genus in Antarctica today (Bayly et al., 2003; Díaz et al., 2019; Maturana et al., 2019; Menu-Marque et al., 2000), where it is restricted to freshwater bodies in seasonally or permanently snow- or ice-free areas, on glacier surfaces and epishelf lakes (Laybourn-Parry & Pearce, 2007). It has been reported in lakes in continental Antarctica (King Charles Mountains, Bayly et al., 2003; Gibson & Bayly, 2007), Alexander Island, the Antarctic Peninsula, the South Shetland and South Orkney Islands in maritime Antarctica and sub-Antarctic South Georgia, as well as in southern South America and the Falkland/Malvinas Islands (Bayly et al., 2003; Maturana et al., 2019; Menu-Marque et al., 2000). This unique distribution spanning the three main Antarctic biogeographic regions (sub-Antarctic islands, continental and maritime Antarctic) catalysed a long debate about the true origin of Antarctic populations, in particular whether the isolated populations in continental Antarctica are the result of anthropogenic introduction or

persistence of populations through multiple glacial cycles (Convey et al., 2009; Gibson & Bayly, 2007; Pugh et al., 2002).

Currently, the World of Copepods database (WoRMS—Copepoda; http://www.marinespecies.org/copepoda) validates 52 species of *Boeckella* (see Walter & Boxshall, 2019). However, this includes species previously considered synonyms (Bayly, 1992b). As Bayly's (1992b) comprehensive work on the non-marine Centropagidae, there have been several additions to the genus: *Boeckella timmsi* from a turbid claypan in Queensland (Bayly, 1998), *B. pilkililli*, from a single turbid freshwater claypan in Western Australia (Quinlan & Bayly, 2017), *B. spinogibba*, endemic to New Caledonia (Defaye, 1998), *B. diamantina* from an Argentinean Andean lake (Menu-Marque & Zuñiga, 1994) and *B. antiqua* from a single ephemeral pond in the Patagonian steppe (Menu-Marque & Balseiro, 2000). The definitive number of species within the genus has been challenged from genetic (Adamowicz et al., 2007, 2010), morphological and ecological (Bayly & Boxshall, 2009) or biogeographical (Maturana et al., 2019) perspectives. Adamowicz et al. (2007, 2010) showed the value of complementing standard taxonomy with genetic analyses, confirming a close concordance between both approaches in Argentinean species of *Boeckella*. They grouped the different specimens available based on morphological taxonomy and then associated them with different clades. This pioneering and—until today—only integrated study advanced understanding of the pattern of evolution in part of the genus. However, as the study only included individuals from the grassland ecoregion in Argentinian Patagonia, it provided only a limited contribution to the evolutionary history of the genus as a whole. Across the Southern Hemisphere, the evolutionary history of *Boeckella* is therefore still poorly understood, and studies with broader sampling across the genus' geographic range are required to provide a robust assessment of its biogeographic history.

The current study aimed to significantly improve understanding of the evolutionary history, diversity and biogeography of *Boeckella* across the Southern Hemisphere. We performed multi-locus phylogenetic, divergence time and ancestral range analyses, including material from southern South America, maritime Antarctica, sub-Antarctic islands and Australasia. As representatives of *Boeckella* occur in both major continental landmasses and younger oceanic islands across the Southern Hemisphere, this allowed us to assess the relative contribution of vicariance and LDDE, and therefore to disentangle the evolutionary history of diversification. This study provides new information on the biogeography and evolution of higher latitude freshwater biota, whose ecosystems are particularly sensitive to environmental change (Convey & Peck, 2019).

2 | METHODS

2.1 | Sampling and taxonomic identification

Boeckella individuals were collected from the shoreline of lakes, ponds and small pools using a zooplankton net (200 μm pore diameter) in sub-Antarctic islands (Crozet, Kerguelen and South Georgia), maritime Antarctica (southern Antarctic Peninsula, South Shetland and South Orkney Islands) and southern South America including the Falkland/Malvinas Islands and Cape Horn (see Table S2, and for more detail see Maturana et al. (2019)). Samples from Antarctica were collected during the Antarctic Expeditions of the Chilean Antarctic Institute (INACH) and British Antarctic Survey (BAS) in the austral summers of 2016/17 and 2017/18. Finally, individuals from Kerguelen and Crozet Islands were collected in 2017 through the PROTEKER project of the French Polar Institute Paul Emile Victor (IPEV).

All specimens were morphologically identified under a stereomicroscope at 3.5× magnification following Bayly (1992b). For species determination, we examined the structure of the endopodites and exopodites, the presence of spines or teeth attached to proximal and/or distal segments in the exopodite, and the inner distal portion of the left distal protopodite segment, under an inverted microscope at 10× and 20× magnification. These observations were performed on the male fifth leg, which represents the most important identification character for *Boeckella* (Bayly, 1992a, 1992b).

2.2 | Data collation and geographic coverage

For phylogenetic and ancestral geographic range reconstruction, we added another 17 *Boeckella* species for which DNA sequences are available, giving a total of 22 species from five biogeographic regions (see Table S1): maritime Antarctica (1 species), sub-Antarctic islands (4 species), southern South America including the Falkland/Malvinas Islands (12 species), Australasia (12 species) and continental Antarctica (1 species). Of the 22 species, we examined and photographed specimens of 12 species across the five biogeographic regions considered here (Figure 1 and Table S1).

The species of *Boeckella* from South America, sub-Antarctic islands, maritime and continental Antarctica were validated using all the available taxonomic, distribution and phylogenetic data (Adamowicz et al., 2007; Bayly, 1992b; Maturana et al., 2019; Menu-Marque et al., 2000; Walter & Boxshall, 2019). The Australasian taxonomic species list was updated to exclude erroneous identifications, rule out possible synonymies and to update current taxonomy. We reviewed *Boeckella* records from the scientific literature (Quinlan & Bayly, 2017, and references therein), including those available from Monash University, the Barcode Of Life Database (BOLD) Systems and Global Biodiversity Information Facility (GBIF). All records were checked for mismatches between reported geographic location and the associated metadata, and taxonomically dubious records were excluded from further analyses. Only records including the geographic location (coordinates) or approximate (identifiable) location of reported samples were incorporated in the final dataset. Biogeographic definitions of continental, maritime and sub-Antarctic regions are as described for terrestrial ecosystems by Convey (2017).

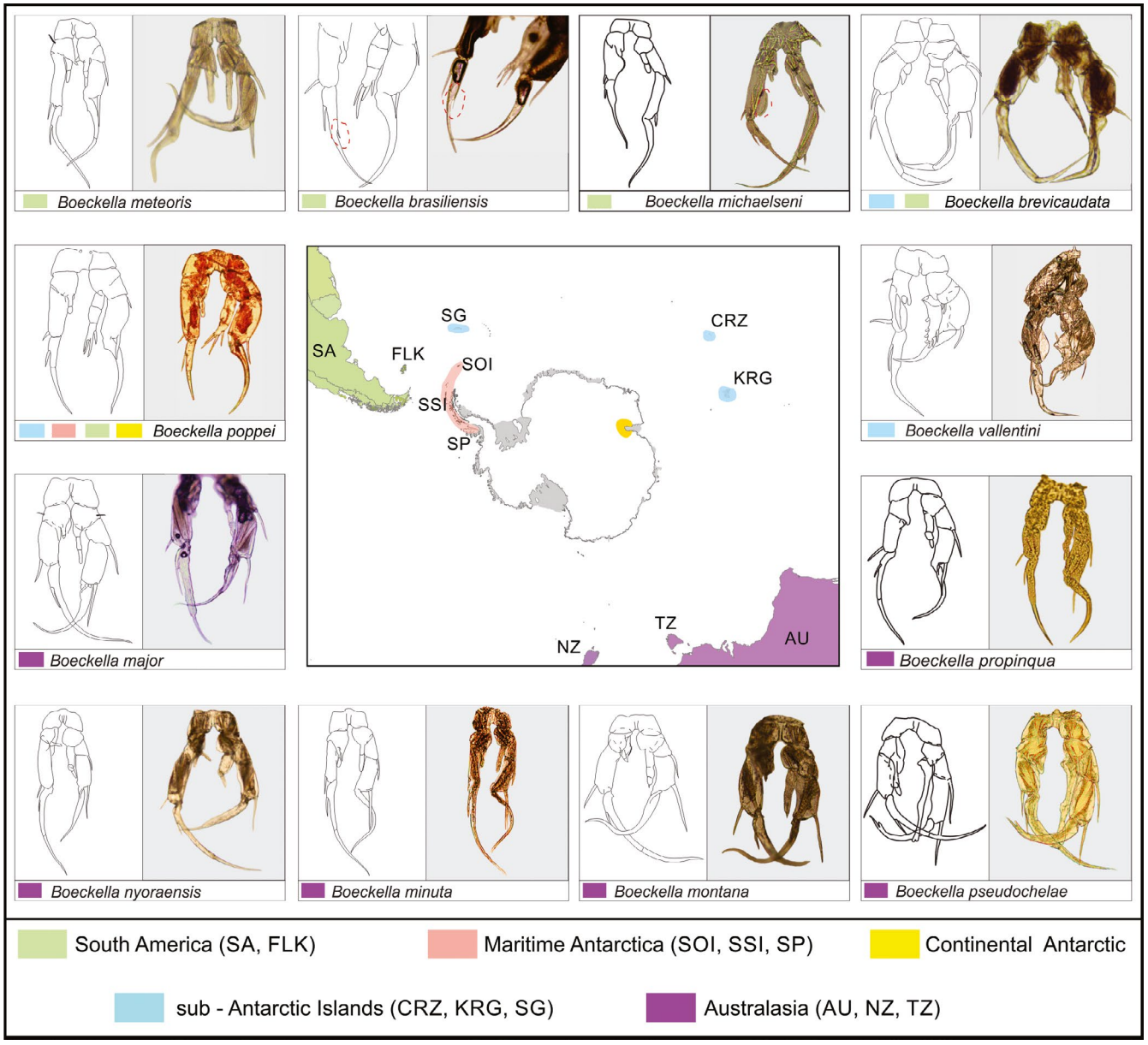

FIGURE 1 Map of the distribution of *Boeckella* species in the Southern Hemisphere included in this study. The rectangles include diagrams (left) and photographs (right) of the male 5th leg from 12 species of *Boeckella*. Drawings of the fifth male leg are modified from Bayly (1992b). SA: South America, FLK: Falkland Is/Malvinas, SOI: South Orkney Islands, SSI: South Shetland Islands, SP: South Peninsula, CRZ: Crozet Island, KRG: Kerguelen Island, AU: Australia, TZ: Tasmania, NZ: New Zealand

2.3 | DNA extraction and sequencing

We extracted DNA from entire individuals using the DNeasy Blood & Tissue Kit (Qiagen), with a modified protocol for small amounts of tissue (Appendix S1 in Data Source). Three different loci were amplified including a fragment of the mitochondrial cytochrome c oxidase subunit I (*cox*1) gene, the nuclear 28S rRNA gene and a segment spanning the Internal Transcribed Spacers 1 and 2 (*ITS* hereafter). For primer details and PCR amplification settings, see Appendix S2 and S3. PCR amplicons were purified and sequenced in both directions by Macrogen (Korea). Forward and reverse sequences were manually examined using Phred scores to ensure all sequenced bases matched and were of good quality. Contigs were assembled using GENEIOUS 10.2.2 (Kearse et al., 2012) and independently aligned using MUSCLE (Edgar, 2004) with standard settings. Stems and loops in RNA sequences were analysed in LocARNA using the default parameters (Will et al., 2012). Numbers of variable and parsimony-informative (PI) sites, and genetic distances were calculated using the Kimura 2-parameter model (K2P) implemented in MEGA X (Kumar et al., 2018). Mitochondrial DNA codon usage was determined using the effective number of codon values (ECN, Wright, 1990) in DNASP 5.0 (Librado & Rozas, 2009).

2.4 | Phylogenetic reconstruction

Boeckella cox1 and 28S rDNA sequences available from Argentinian Patagonia (Adamowicz et al., 2007), Australia and New Zealand through GenBank and BOLD Systems (Table S1) were aligned with the *cox1* and 28S rRNA datasets obtained from this study using MUSCLE criteria with default parameters (Edgar, 2004) implemented in GENEIOUS 10.2.2 and then manually checked. Specimens from continental Antarctica were only available for 28S rDNA (Bissett et al., 2005). For analyses of *cox1*, 28S and *ITS*, the tree was rooted with *Calamoecia ampulla* and/or *Parabroteas sarsi*, based on the only available phylogenetic analyses including the genus *Boeckella* within members of Centropagidae (Adamowicz et al., 2007; Scheihing et al., 2009). A DNA saturation analysis was performed in DAMBE 5 (Xia, 2013) following Xia et al. (2003). The datasets of *cox*1 + 28S and *cox*1 + 28S + *ITS* were combined in SEQUENCEMATRIX v1.8 (Vaidya et al., 2011). To assess for topological incongruence among phylogenies derived from the cpDNA and nrDNA partitions, we used >70% bootstrap (BS) and >95% posterior probability support (PP) thresholds. Optimal nucleotide substitution models and partitioning scheme were selected for each marker separately using the corrected Akaike information criteria (AIC_c) in PARTITIONFINDER v2.1, allowing for *cox1* codon partitioning (Lanfear et al., 2017).

Phylogenetic analyses were conducted on individual loci and concatenated datasets using maximum likelihood (ML) in MEGA X (Kumar et al., 2018) and Bayesian analyses (BA) in MRBAYES v3.2.6 (Ronquist & Huelsenbeck, 2003). For ML analyses, bootstrap support (BS) was assessed with the heuristic search option and 1,000 pseudoreplicates. Bayesian inference PPs were estimated using the Markov chain Monte Carlo algorithm (MCMC), running four chains for 50×10^6 generations, with trees sampled every 500 generations, with the initial 10% of parameter values discarded as "burn-in." Convergence was assessed by checking that split frequencies had an average standard deviation below 0.01 and all parameters had effective sample sizes (ESS) >200 using TRACER v.1.6 (Rambaut et al., 2014). Maximum clade credibility trees were generated using TREEANNOTATOR 2.4.7 (http://beast.bio.ed.ac.uk/TreeAnnotator) and visualized with FIGTREE 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree).

Genetic support for the monophyly of specimens currently assigned to *B. poppei* was assessed by rerunning the analyses with topological constraints. For ML trees, Shimodaira-Hasegawa (SH, Shimodaira & Hasegawa, 1999), Kishino-Hasegawa (KH, Kishino & Hasegawa, 1989) and the approximately unbiased (AU, Shimodaira, 2002) tests implemented in IQ-TREE were performed with 10,000 RELL replicated. These tests assume a null hypothesis that there are no differences between the unconstrained and the constrained trees, and thus, a phylogenetic hypothesis is rejected if its *p*-value <.05.

A *B. poppei*-specific ML haplotype network analysis was also conducted using the only available short 28S rRNA sequences (399 bp) from continental Antarctica (ancient and modern DNA, Bissett et al., 2005) aligned to the *B. poppei* dataset. Genealogical relationship reconstruction was performed in MEGA and visualized in Hapview (http://www.cibic.at/~greg/haploviewer).

2.5 | Divergence time estimation

Divergence time analyses were performed in BEAST v.2.4.6 (Bouckaert et al., 2014) using the *cox*1 dataset and applying the relaxed lognormal molecular clock, a Yule model speciation prior and GTR+I+Γ substitution model. A thinned dataset of one individual per taxon or biogeographic region (e.g. for *B. poppei* and *B. brevicaudata*, we used individuals from different biogeographic regions to recover their geographic ancestry) was used to improve accuracy and computation time. Four chains were run independently three times for 50×10^6 generations, and trees were sampled every 5,000 generations. Based on the absence of fossil records for *Boeckella* and no direct estimates of mutation rates for *cox*1 being available for copepods, the time of the most recent common ancestor (tMRCA) was estimated using a conservative *cox*1 mutation rate of 1.8% per site per million years, corresponding to a *cox*1 rate estimated for limnic amphipods (Copilaş-Ciocianu et al., 2019), in accordance with other estimates for crustaceans (Lessios, 2008). Tracer was used to ensure convergence of MCMC and check that all ESS values were >200. A maximum clade credibility with median node heights was generated using Treeannotator and visualized with FigTree.

2.6 | Diversification rates

Net diversification rate within *Boeckella* was estimated using "geiger" (Harmon et al., 2008) in R v.3.6.0 (R Core Team, 2019) using the *cox*1 dataset. To evaluate temporal variation in diversification rates, the AIC scores of constant and variable rate models were compared using "laser" (Rabosky, 2006). To evaluate the best-fit model, we performed a statistical test for constancy of diversification rates using the fitdAIC.rc function based on DAIC (differences of the Akaike information criterion). This function considers score differences between AICRC (best-fit constant rate model) and AICRV (best-fit rate-variable model, Rabosky, 2006) scores. A test of significance of DAIC was conducted using 10,000 simulations. A lineage-through-time (LTT) plot tracks the number of species that have descendants in the present through time (Xu & Etienne, 2018). This was generated using "APE" (Paradis et al., 2004) in the R environment using a birth–death model and several extinction rates to assess model robustness. The pure birth (pb) model was determined from the branching times, and the expected number of lineages was estimated under a constant model (i.e. pure birth) using 1,000 simulations in "PHYTOOLS" (Revell, 2012). We calculated the γ-statistic in order to detect acceleration or deceleration in the rate of cladogenesis (Pybus & Harvey, 2000). This statistic measures the density of ordered inter-node distance on a phylogeny, to determine if they are evenly distributed ($\gamma = 0$, pure birth), clustered early ($\gamma < 0$; early burst) or clustered late ($\gamma > 0$; late burst or high early extinction).

2.7 | Ancestral range distribution

We used the package BIOGEOBEARS in R (Matzke, 2014) to estimate the ML of the ancestral geographical range and dispersal hypotheses along the time-calibrated phylogeny generated in BEAST using the *cox*1 dataset. As members of *Boeckella* are distributed on sub-Antarctic islands with a volcanic and post-Gondwanan origin, we assumed founder event speciation was likely to be a highly relevant biogeographic process (Cowie & Holland, 2006; Matzke, 2013). We therefore also explored the influence of founder event speciation by additionally analysing the influence of the "+J" parameter in our analyses. Descriptions of the assumptions and limitations of the models tested in BIOGEOBEARS are given by Matzke (2013, 2014). We selected the model that fitted best using the AIC and the likelihood ratio test. The maximum number of areas per node was set to four (i.e. South America, sub-Antarctic islands, maritime Antarctic and Australasia).

3 | RESULTS

3.1 | Geographic and taxonomic coverage

We established a list of 42 validated species within *Boeckella*, of which 22 were associated with genetic sequences in the current study (Table S2). A total of 541 records were published in GBIF, updating the previous *Boeckella* database (GBIF; Maturana et al., 2018). Among the newly collected samples, we identified *B. vallentini* from Kerguelen (KRG) and Crozet Islands (CRZ), *B. brasiliensis* from southern South America (SA), *B. brevicaudata* from Kerguelen and southern South America, *B. meteoris* from southern South America and *B. poppei* from southern South America, South Georgia (SG), Antarctic Peninsula (AP), South Orkney Islands (SOI) and South Shetland Islands (SSI), the latter three locations being within maritime Antarctica (MA) (Figure 1).

3.2 | Phylogenetic patterns in *Boeckella*

New sequences from five sampled species (*B. poppei* = 68; *B. brasiliensis* = 32; *B. meteoris* = 24; *B. brevicaudata* = 16; *B. vallentini* = 10) were included in our analyses (Tables S2 and S3). Fragment sizes of the analysed markers were 417, 649 and 727 bp for *cox*1, 28S rRNA and *ITS*, respectively. The length of the concatenated dataset of *cox*1 + 28S rRNA was 1,080 bp, and that of all markers combined was 1,793 bp. The *cox*1 marker coded for 140 amino acids and included no insertion/deletion or stop codons. No evidence for codon bias was detected (ENC = 37.44). The total *cox*1, 28S rRNA and *ITS* datasets included 182, 56 and 115 variable sites and 170, 27 and 46 PI sites, respectively. Mitochondrial and nuclear sequences were not saturated at any position. The GTR+I+Γ model was selected for all markers, including each codon position in *cox*1.

ML and BI analyses of *cox*1 + 28S rRNA, all markers combined, and individual markers (data not shown) retained the topology of the two major clades. The *cox*1 + 28S rRNA analysis (Figure 2a) revealed an early division into two clades from different biogeographic regions. Clade A comprised lineages from Antarctica, South America and the sub-Antarctic islands, and clade B included lineages from Australia and New Zealand ("Australasia" hereafter). ML and BI analyses placed representatives of the species *B. brevicaudata*, *B. meteoris*, *B. vallentini* and *B. brasiliensis* as monophyletic groups within clade A. Clade B contained all the Australasian species, with each forming a monophyletic group (Figure 2a).

3.3 | Morphological and molecular discrepancy within *Boeckella poppei*

Each of the markers, as well as the combined *cox*1 + 28S + *ITS* dataset (Figures S1–S4), placed different representatives of *B. poppei* into three polyphyletic clades within the genus *Boeckella*. When *B. poppei* was constrained to be monophyletic, the topology slightly decreased in log-likelihood ($logL_{const}$: −5,465.86 vs. $logL_{unconst}$: −5,447.20) relative to the unconstrained topology. Moreover, the constrained topology was marginally rejected ($p = .05$) in all tests (Table 1). Even though all *B. poppei* specimens were morphologically identified as such, they exhibited high levels of mtDNA genetic divergence (K2P genetic distance) ranging between 11% and 19% (clade *i* vs. *ii* = 11%, clade *i* vs. *iii* = 19%, clade *ii* vs. *iii* = 16%). The first clade (i) was highly supported (pp = 1, bs = 100; Figure 2a) and included populations from the maritime Antarctic, southern South America and South Georgia (the latter being the species' type locality) (Poppe & Mrázek, 1895; Mrázek, 1901). A second strongly supported clade (ii) (pp = 1.0, bs = 98%; Figure 2a) included specimens from Chilean and Argentinean Patagonia (GenBank accession number DQ356568, Adamowicz et al., 2007) and specimens from the Falkland/Malvinas Islands (Figure 2a). Finally, a third strongly supported clade (iii) (pp = 1, bs = 100; Figure 2a) included two specimens from Hornos Island (IH) in the Magellanic sub-Antarctic Ecoregion (Figure 2a).

The 28S rRNA ML network analyses including ancient and modern sequences of the 28S rRNA marker for *B. poppei* populations from clade (i) and from continental Antarctica revealed three haplotypes separated by one mutational step (Figure 2b). One solely comprised ancient specimens from continental Antarctic lake sediment (GenBank accession number AY997794, 3,430 years before present, YBP; Bissett et al., 2005). The second included only South American individuals. The third, and dominant, haplotype included continental Antarctic material (AY997795 and AY997791, modern and ancient DNA, respectively (Bissett et al., 2005)), and the remaining *B. poppei* populations from clade (i) (i.e. Patagonia, the maritime Antarctic and the type location of South Georgia).

3.4 | Divergence time estimation and ancestral range probabilities

The ancestral range reconstruction and molecular dating analysis of the *cox*1 dataset provided estimates of the timing of diversification and spatial origin of *Boeckella* in the Southern Hemisphere (Figure 3).

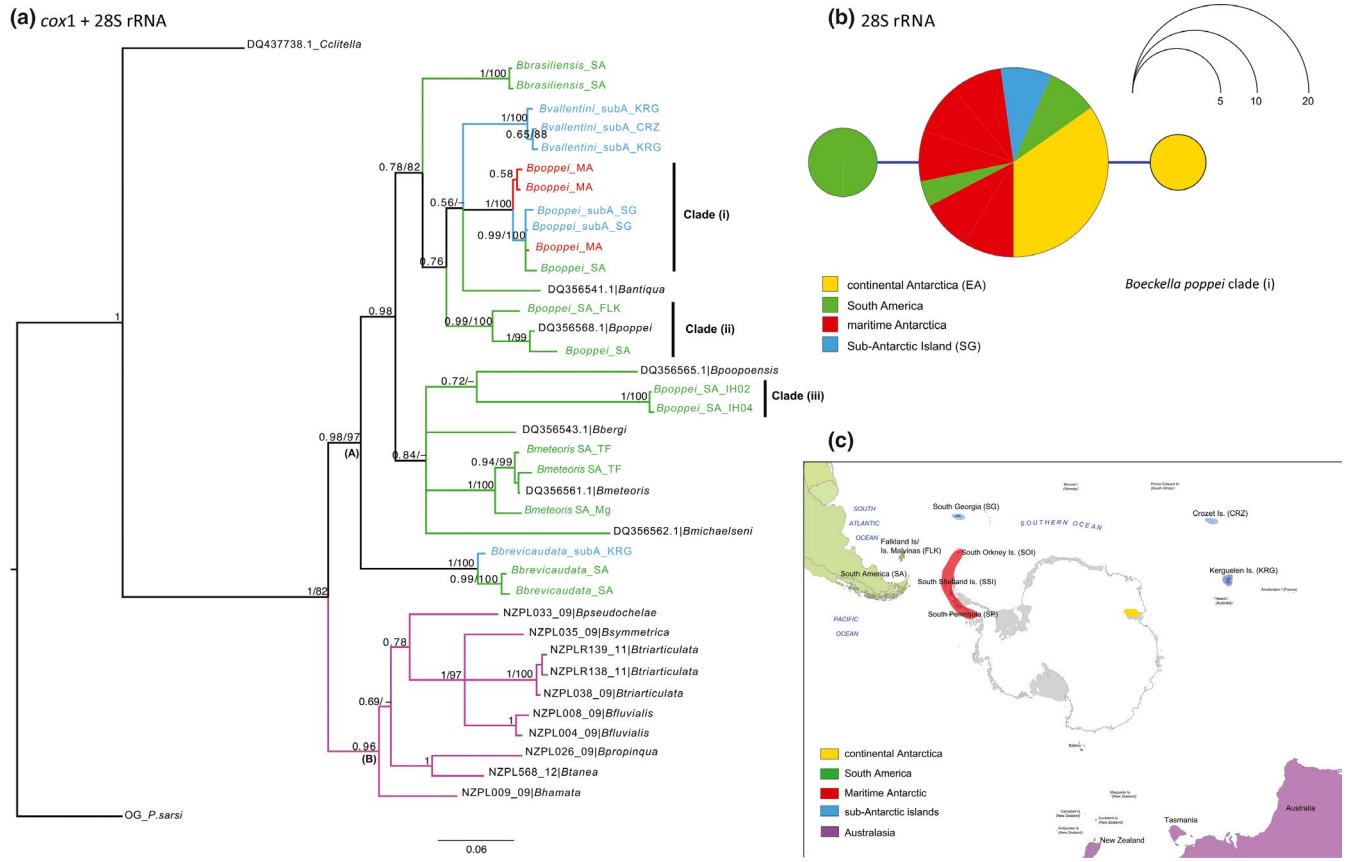

FIGURE 2 Phylogenetic reconstructions of *Boeckella* in the Southern Hemisphere. (a) Bayesian inference using *cox*1 and 28S rRNA sequence data of all species studied; node values represent the posterior probability and bootstrap support values, respectively; colours reflect the biogeographic regions shown in (c). The main clades are represented by (A) and (B). The polyphyletic *B. poppei* clades are indicated by (i), (ii) and (iii). (b) Haplotype network of 28S rDNA of *B. poppei* populations from clade (i) including ancient and modern DNA from East Antarctica (EA) in continental Antarctica. c) Distribution map of the species with the colours representing the biogeographic regions of the species

TABLE 1 Test of constrained tree

Constraint	p-SH	p-KH	p-AU	Log-Likelihood
Unconstrained				−5,447.199
Monophyly of *Boeckella poppei*	.0514	.0514	.0536	−5,465.862

Note: Tests trees from unconstrained versus constrained search. p-SH: *p*-value for multiple testing of Shimodaira–Hasegawa test; p-KH: *p*-value of one sided Kishino–Hasegawa test; p-AU: *p*-value of approximately unbiased test.

Ancestral range estimation favoured the DEC+J (LnL = −24.75, AIC = 55.49) model of species evolution (Dispersal-Extinction-Cladogenesis, with a founder event component +J), closely followed by the DEC model (LnL = −24.94, AIC = 53.87). The ancestral area reconstruction and divergence time of the earliest linages at 99.70 Mya (95% highest posterior density intervals (HPD95): 62.87–148.70 My) suggested a Gondwanan origin for *Boeckella*. At that time, the genus split into two main clades. The first (Clade A) diversified in South America during the Paleogene (85.39 Mya, HPD95: 53.36–123.78 Mya) and also colonized the sub-Antarctic Kerguelen Islands and South Georgia, and the maritime Antarctic. Historical dispersal from South America to Antarctica and the sub-Antarctic Islands occurred on different timescales. While *B. vallentini* likely dispersed to the Kerguelen Islands during the Eocene (37.48 Mya, HPD95: 22.67–58.88 Mya), *B. brevicaudata* likely arrived during the Pliocene (3.05 Mya, HPD95: 1.28–5.96 Mya). Finally, *B. poppei* dispersed from South America to the maritime Antarctic and sub-Antarctic South Georgia, during the mid-Pleistocene (1.3 Mya, HPD95: 0.45–2.37 Mya). The second clade (B) diversified at the beginning of the Paleocene (67.31 Mya, HPD95: 40.78–102.69 Mya), giving rise to all contemporary Australasian *Boeckella* species (Figure 3).

3.5 | Diversification rates

The best-fitting model was density-dependent linear diversification (DDL, AIC 114.97, see Table 2), with a Pybus & Harvey γ-value of −2.05 (Figure 4). This was significantly different from the null

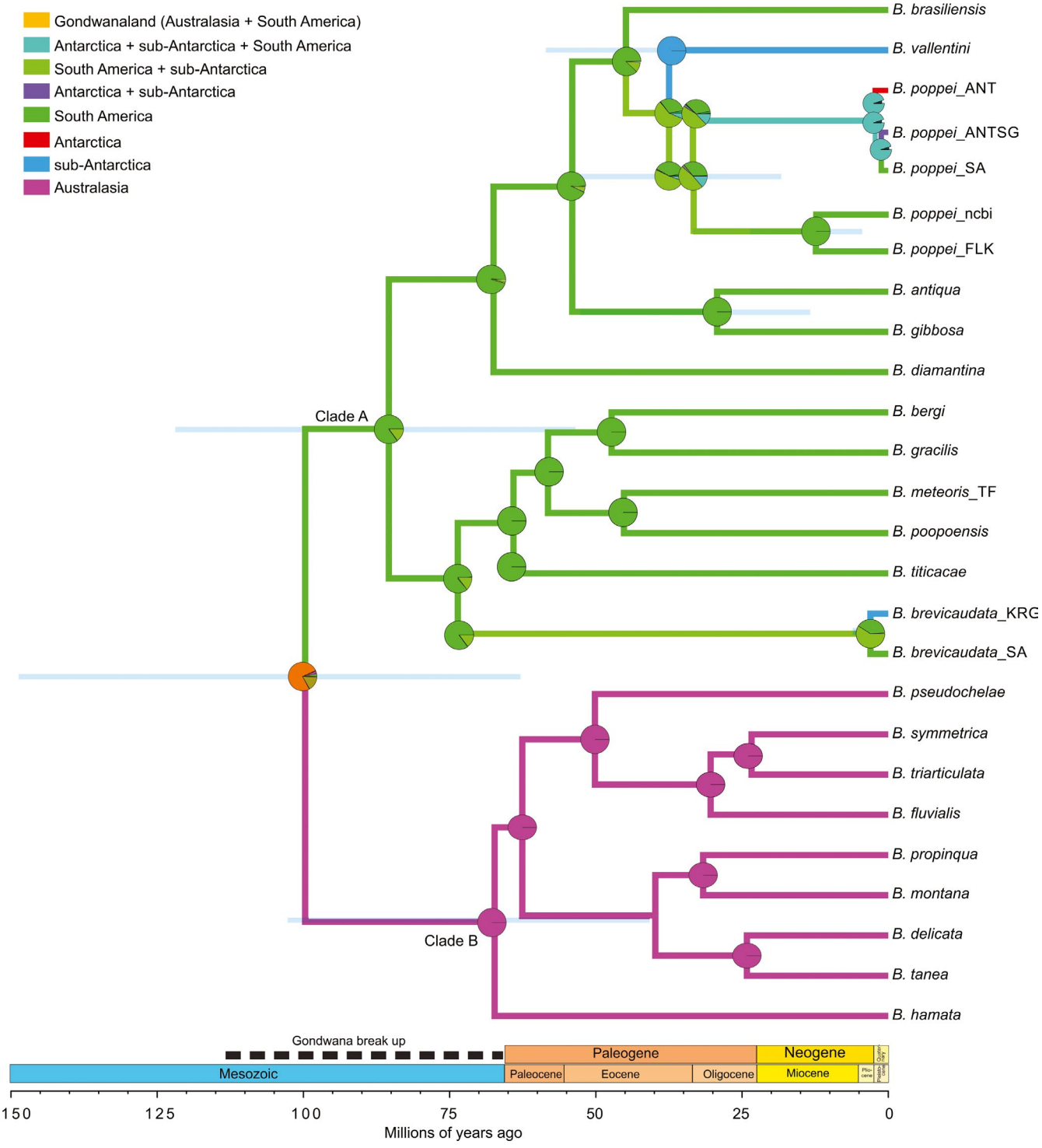

FIGURE 3 Historical biogeography and timing of divergence of *Boeckella* in the Southern Hemisphere using *cox*1. The maximum clade credibility tree shows the median divergence time estimates with 95% height posterior distribution. The bars were displayed on the nodes that exhibited more than .7 posterior probability. The coloured pie chart represents ancestral range probabilities at each node as recovered by BioGeoBEARS. Different colours represent different regions and geographical ranges (see Figure 1)

hypothesis that per-lineage speciation and extinction rates remained constant through time (p-value = .04). This negative γ-value indicates that the rate of *Boeckella* species accumulation has slowed over evolutionary time. Additionally, we observed three different rates of cladogenesis (a, b and c, Figure 4), representing different speciation rates through time. The first encompassed the rapid diversification of *Boeckella* in South America and Australasia until *ca.* 55 Mya. The second showed a slight reduction in the rate of cladogenesis during

TABLE 2 Temporal shifts in diversification rates

Type	Model	lnLH	R1	R2	*a*	xp	*k*	st	AIC	ΔAIC
Constant rates	Pure birth	−58.41	0.02		118.82				118.82	3.85
	bd	−58.41	0.02		0.00				120.82	5.85
Variable rates	DDX	−55.90	0.12		115.80	0.61			115.80	0.83
	DDL	−55.48	0.05				33.27		114.97	0.00
	yule2rate	−54.58	0.02	0.06				1.20	115.15	0.18

Note: Comparison between constant rate diversification models (pure birth, birth-death) and variable rate models (exponential, density-dependent linear model and two-rate Yule model) using maximum likelihood ΔAIC test statistic. 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 and the overall of the best-fit model.

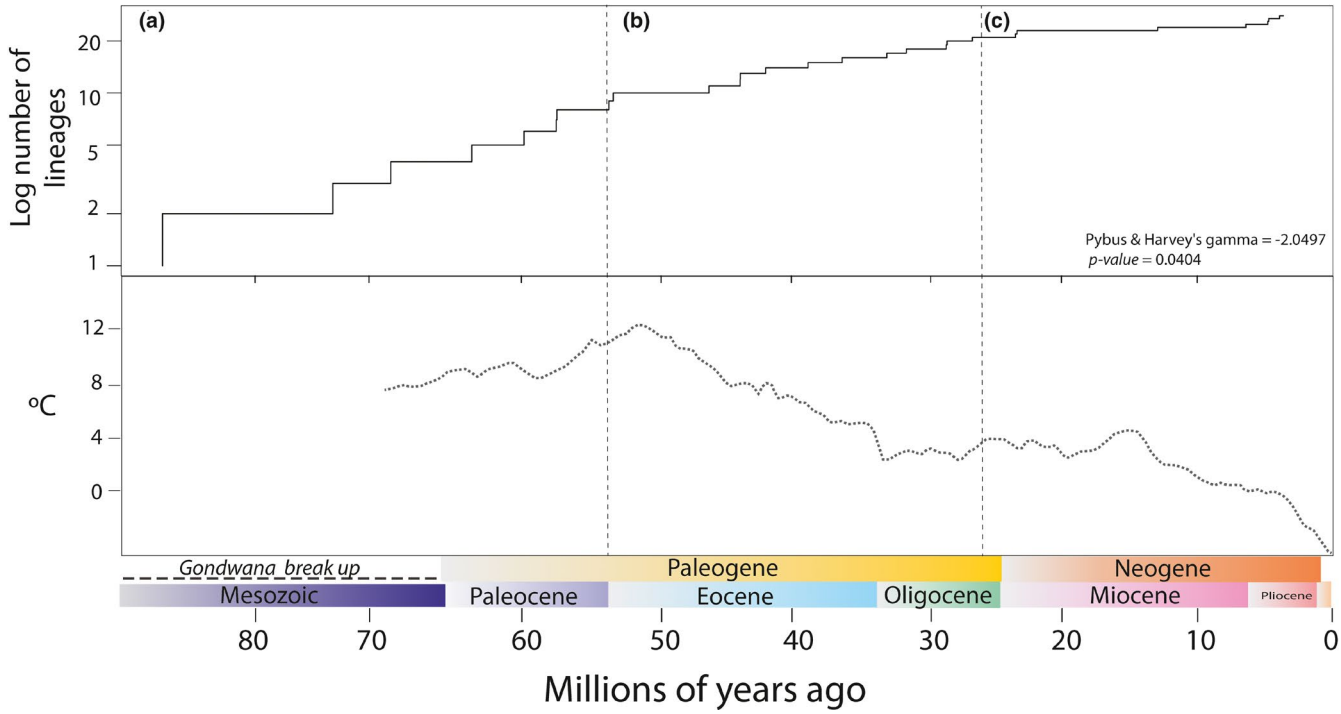

FIGURE 4 Lineage through time plot and γ-statistics within *Boeckella*. Upper panel displays the number of lineages through time, with three different periods of diversification a, b and c. Each period matches with the long-term variation pattern of deep sea paleotemperature (Crame, 2018; Zachos et al., 2001)

the Oligocene-Miocene (50–25 Mya) and the third a subsequent strong decrease in the speciation rate (20 Mya to present).

4 | DISCUSSION

4.1 | Gondwanan origin of *Boeckella*

We provide the first integrated biogeographic analyses of the freshwater copepod genus *Boeckella* at the scale of the Southern Hemisphere, as well as the first global phylogeographic study of freshwater copepods. Our analyses revealed that the genus likely had a Gondwanan origin (99.70 Mya; HPD95: 62.87–148.70 Mya), with an early separation into two well-supported clades currently found in (A) South America, the sub-Antarctic islands (South Georgia, Crozet and Kerguelen) and Antarctica, and (B) Australasia (Figure 2a). Although the Gondwanan origin of the genus was previously hypothesized given its geographic distribution (Adamowicz et al., 2010; Bayly, 1992b; Pugh et al., 2002), our analyses confirm a deep-time evolutionary history for the genus best explained by the break-up of the Gondwana supercontinent, including both vicariance and long-distance dispersal processes. As no appropriate copepod fossils are available to assist our molecular clock calibration, we used a conservative molecular phylogenetic rate estimated for related crustacean species. The divergences presented here must therefore be considered approximate as they are not based on direct inference of evolutionary rates for this genus.

Divergence time estimation and LTT plots within *Boeckella* detected a rapid speciation rate after the origin of the genus, which subsequently reduced (Figure 4). Three different phases of diversification

within *Boeckella* were identified based on speciation rates, which are consistent with key stages in the evolution of climate worldwide, and particularly of the Southern Ocean region (Crame, 2018). First, rapid diversification occurred during the Paleocene and Eocene (Figure 4a) close to the Early Eocene Climatic Optimum (*ca*. 50 Mya). At this time, the southern continental landmasses were tightly clustered, including a land bridge between South America and Antarctica (Livermore et al., 2007) and slow seafloor spreading between Australia and Antarctica (Blakey, 2008). Terrestrial faunal and floral fossil evidence indicates that intercontinental dispersal and vicariance events may have occurred during the onset of this climatic optimum (58.5–58.6 My, Reguero et al., 2002), including major evolutionary radiations in some Antarctic marine groups (Crame, 2018). After this period, there was a slight decline in lineage accumulation close to the late Eocene-Oligocene (Figure 4b), plausibly associated with rapid reduction in temperature across the Southern Hemisphere and the first appearances of small and ephemeral Antarctic ice sheets (Zachos et al., 2001). Finally, there was a pronounced decrease in the diversification rate in the last 15 My (Figure 4c), coincident with the onset of the Middle Miocene Climatic Transition (MMCT) at *ca*. 14 My, which marks the second steepest decline in global ocean temperatures in the entire Cenozoic (Zachos et al., 2001). Additionally, at this time major expansion of both the East and West Antarctic ice sheets occurred, with an uninterrupted polar desert climate which appears to underlie the final near-complete extinction in the Miocene (Convey et al., 2020; Lewis et al., 2008).

4.2 | Long-distance dispersal events in *Boeckella*

We found evidence of several LDDE events from southern South America to sub-Antarctic islands and Antarctica, suggesting this process has played an important role in establishing the current geographic distribution of the genus. *Boeckella vallentini* likely colonized the Kerguelen Islands from South America during the Eocene, soon after the emergence of this volcanic sub-Antarctic archipelago around 40 Mya (Van der Putten et al., 2010). As the arrival of *B. vallentini* predates the emergence of Crozet Island (8 Mya), the species' contemporary occurrence in the latter island is likely to be the result of at least one dispersal event from the Kerguelen Islands. *Boeckella brevicaudata* likely dispersed from South America to the Kerguelen Islands during the Pliocene (3.05 Mya, HPD95: 1.28–5.96 Mya). Finally, *B. poppei* likely dispersed to maritime and continental Antarctica and South Georgia from South America during the mid-Pleistocene to late Pleistocene (1.3 Mya, HPD95: 0.45–2.37 Mya). Based on the geographic range reconstruction, this species' current distribution is likely to be the result of one or more colonization events, mediated by LDDE from southern South America.

The vectors involved in these LDDE events are not known, but wind and birds have repeatedly been suggested as the two most likely primary vectors for LDDE of terrestrial and freshwater organisms in the Southern Hemisphere (Biersma et al., 2017, 2018; Gillespie et al., 2012; Nogales et al., 2012). Dispersing propagules are physiologically more resistant to harsh environments, such as extreme temperature, low oxygen and pollution, than are their active forms (Brendonck & de Meester, 2003). Therefore, the resistant eggs of zooplankton may contribute to the effectiveness of these two vectors as dispersal agents. In the case of wind, landscape genetic analyses of Antarctic terrestrial invertebrates have supported the hypothesis that wind facilitates dispersal (McGaughran et al., 2019). Moreover, simple aerial modelling studies have also revealed that small particles (e.g. spores) transported in regional air masses can potentially cover the distances between southern South America and the northern Antarctic Peninsula and Scotia Arc archipelagoes (Biersma et al., 2017). Migratory birds may also be an effective biotic transport mechanism, as they move over broad spatial scales and stop at sites with similar habitat characteristics along their migratory routes (Nogales et al., 2012; Viana et al., 2016).

4.3 | Evidence of cryptic speciation within *Boeckella poppei*

The non-monophyletic clades detected within specimens morphologically identified as *B. poppei* (clades i–iii in Figure 2a) imply the presence of cryptic species and highlight the need for detailed taxonomic revision of this species, including re-examination of previous records of *B. poppei*. We suggest that the nominal species, *B. poppei*, corresponds to clade i, which includes specimens collected at the type locality, South Georgia (Poppe & Mrázek, 1895). Based on this new molecular evidence, the *B. poppei* lineage reported from Argentinean Patagonia (Adamowicz et al., 2007) requires reclassification. This clade (ii), and the one restricted to Hornos Island (iii), was placed in different parts of the phylogeny from the type locality clade (i) and now require formal description.

The likely presence of cryptic species and the need for taxonomic revision identified in the genus *Boeckella* are consistent with studies of other Centropagidae lineages (e.g. *Calamoecia tasmanica subattenuata*, *B. michaelseni*; Adamowicz et al., 2010), as well other Antarctic terrestrial invertebrate lineages (Carapelli et al., 2020; Stevens et al., 2021). It is notable that the strong congruence between taxonomic and molecular data that has characterized most of the *Boeckella* species examined (Adamowicz et al., 2007, 2010; present study) was almost exclusively absent in *B. poppei*, the species with the largest geographic distribution in the genus. This highlights the need for further studies including a wider biogeographic scope, using both molecular and morphological data along with niche modelling, both within the genus *Boeckella* and more widely across other understudied freshwater invertebrate groups.

4.4 | Timing of *Boeckella* in Antarctica and Patagonia

The molecular dating analyses based on *cox*1 sequences suggested that the nominal species *B. poppei* (clade i) had an estimated origin 1.2 Mya (1.3 Mya, HPD95: 0.45–2.37 Mya). We also compared

the few available shorter sequences (28S rRNA, 399 bp) from ancient (3,430 and 9,950 YBP) and modern DNA recovered from lake sediments in the Prince Charles Mountains, continental Antarctica (Bissett et al., 2005) with the other sampled locations (Figure 2b). Haplotype network analysis was used in this part of our study, as this method allows simple reconstruction of phylogenies based on intraspecific molecular data with limited genetic divergence (Posada & Crandall, 2001). The haplotype network generated revealed three closely related haplotypes. Although genetic differentiation was very limited due to the low species-level resolution of 28S rRNA, we suggest that the continental Antarctic sequences are more closely related to *B. poppei* from South America and the sub-Antarctic and maritime Antarctic than to other *Boeckella* species.

While the overall patterns identified here suggest that no ancient Antarctic Gondwanan lineage(s) survived the major Quaternary ice ages on the continent in situ, we cannot conclude with confidence the precise age of extant populations of *B. poppei* in Antarctica. While our analyses suggest a mid-Pleistocene origin, this estimate is necessarily based on the use of a rate derived from limnic amphipods, which are distant relations within the Crustacea. If correct, this would require the presence of at least one within-region refugium throughout the last glacial maximum (LGM) and several previous Pleistocene glacial cycles. Antarctica and southern high latitude regions experienced eight distinct glacial events in the last million years (EPICA, 2004), including the Great Patagonian Glaciation and Wisconsinian last glacial cycle, with its termination in the LGM (21 ka BP, LGM sensu stricto, Denton et al., 2010). The current broad regional distribution of *B. poppei* could therefore be the result of the rapid and effective colonization of newly available, suitable ice-free terrestrial habitats from such in situ refugia during the intervening interglacial periods. Various studies have highlighted the importance of refugia for enabling the historical persistence of contemporary Antarctic terrestrial biota, although in most cases specific refugial locations are yet to be unequivocally identified (Convey et al., 2020; Fraser et al., 2014).

The long-term persistence of several *Boeckella* species in southern South American regions that are also thought to have been extensively ice-covered throughout several Quaternary glaciations similarly implies local survival in Patagonia (Sersic et al., 2011). This could apply to the 14 species reported at higher southern latitudes in South America (Maturana et al., 2019), as well as the new lineages of *Boeckella* identified here that were restricted to Hornos Island and the Falkland/Malvinas Islands + southern South America. However, further geographic sampling is required to evaluate different biogeographic scenarios for each *Boeckella* species.

5 | CONCLUDING REMARKS

This study provides the first evidence of the timing and modes of speciation in the Gondwanan freshwater copepod genus *Boeckella*. The evolutionary history of this genus is consistent with the sequential break-up of southern Gondwana from the late Cretaceous and explains its current wide distribution in the Southern Hemisphere. Our evidence supports radiation within *Boeckella* generating multiple species in South America and Australasia, with migration from South America to the sub-Antarctic islands and maritime Antarctic (and possibly the continental Antarctic) via long-distance dispersal events in the Oligocene, Pliocene and Pleistocene.

ACKNOWLEDGEMENTS

Funding was obtained from PIA CONICYT ACT172065 to CSM, SR, JN, EP, CGW and AD. CONICYT PIA APOYO CCTE AFB170008 from Institute of Ecology and Biodiversity (IEB). CONICYT Ph.D. Grant 21150317, INACH DT_04-16 and FONDECYT Postdoctorado 3210063 to CSM, CONICYT Ph.D. Grant 21140632 and INACH DG_03-16 to JN, Direction of Research of the Universidad de Magallanes (Programme PR-06-CRN-18) to SR. NERC core funding to the BAS "Biodiversity, Evolution and Adaptation" and "Ecosystems" Teams to JJ, PC and EMB. EMB was funded by NERC-CONICYT grant NE/P003079/1 and Carlsberg Foundation grant CF18-0267. Financial support for the Australasian samples was provided by the New Zealand Ministry of Business, Innovation and Employment through contract UOW0505. Field access facilities to Crozet and Kerguelen islands were supported by the French Polar Institute-IPEV (programme No. 1044 PROTEKER). Relevant fieldworks permissions were obtained from the competent institutions (Magellanic Region: Rx N° 711; Cape Horn Rx N° :679/2018; Falkland Islands: Rx N°R19/2017; Antarctica: S7-15-2017, 528/2017, 99/106, 1058/1059 and 1060; South Georgia: RAP2017-018; Crozet and Kerguelen: TAAF/A-2017-117. CSM thanks BAS station staff on Signy Island, King Edward Point and Bird Island on South Georgia and staff at the South Atlantic Environment Research Institute (Falkland Is.) for their support and assistance. Additionally, we thank to Francisca Campos for assistance with laboratory work, and the Chilean Navy, in particular the crews of the vessels AP-41 *Aquiles* and AP-46 *Almirante Oscar Viel*. Finally, I would like to thank my mother for her infinite inspiration and love.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ddi.13405.

DATA AVAILABILITY STATEMENT

All taxonomic and genetic data sources used in this study are available at DataDryad (https://doi.org/10.5061/dryad.w9ghx3fpv). New sequences were deposited in GenBank and BOLD System, the former includes a list of individual collected specimens with exact location, photographs and the sequences obtained from the three loci. Sequences were deposited in GenBank under the following accession numbers: cox1 (MZ757026–MZ757095), 28S

rRNA (MZ772976–MZ773003) and ITS (MZ826889–MZ826980). Additionally, the occurrence dataset of Boeckella across the Southern Hemisphere is available from the GBIF repository: https://www.gbif.org/es/dataset/474ee54a-9eae-4b67-b79b-d8fc2d9ec884

ORCID

Claudia S. Maturana https://orcid.org/0000-0002-4427-8093

REFERENCES

Adamowicz, S. J., Menu-Marque, S., Halse, S. A., Topan, J. C., Zemlak, T. S., Hebert, P. D., & Witt, J. D. (2010). The evolutionary diversification of the Centropagidae (Crustacea, Calanoida): A history of habitat shifts. *Molecular Phylogenetics and Evolution*, *55*(2), 418–430. https://doi.org/10.1016/j.ympev.2009.12.008

Adamowicz, S. J., Menu-Marque, S., Hebert, P. D., & Purvis, A. (2007). Molecular systematics and patterns of morphological evolution in the Centropagidae (Copepoda: Calanoida) of Argentina. *Biological Journal of the Linnean Society*, *90*(2), 279–292. https://doi.org/10.1111/j.1095-8312.2007.00723.x

Bayly, I. A. E. (1992a). Fusion of the genera *Boeckella* and *Pseudoboeckella* (Copepoda) and revision of their species from South America and sub-Antarctic islands. *Revista Chilena de Historia Natural*, *65*(1), 17–63.

Bayly, I. A. E. (Ed.). (1992b). The non-marine Centropagidae: (Copepoda: Calanoida) of the World. In *Guides to the identification of the microinvertebrates of the Continental waters of the world* (Vol. *III*, pp. 1–27). SPB Academic Publishing.

Bayly, I. A. E. (1998). New species of Calamoecia and Boeckella (freshwater Copepoda: Calanoida) from Western Australia and Queensland. *Journal of the Royal Society of Western Australia*, *81*, 177–182.

Bayly, I. A. E., & Boxshall, G. A. (2009). An all-conquering ecological journey: From the sea, calanoid copepods mastered brackish, fresh and athalassic saline waters. *Hydriobiologia*, *630*, 39–47. https://doi.org/10.1007/s10750-009-9797-6

Bayly, I. A. E., Gibson, J. A. E., Wagner, B., & Swadling, K. M. (2003). Taxonomy, ecology and zoogeography of two east Antarctic freshwater calanoid copepod species: *Boeckella poppei* and *Gladioferens antarcticus*. *Antarctic Science*, *15*(4), 439–448.

Biersma, E. M., Jackson, J. A., Bracegirdle, T. J., Griffiths, H., Linse, K., & Convey, P. (2018). Low genetic variation between South American and Antarctic populations of the bank-forming moss *Chorisodontium aciphyllum* (Dicranaceae). *Polar Biology*, *41*(4), 599–610. https://doi.org/10.1007/s00300-017-2221-1

Biersma, E. M., Jackson, J. A., Hyvonen, J., Koskinen, S., Linse, K., Griffiths, H., & Convey, P. (2017). Global biogeographic patterns in bipolar moss species. *Royal Society Open Science*, *4*(7), 170147. https://doi.org/10.1098/rsos.170147

Bissett, A., Gibson, J. A. E., Jarman, S. N., Swadling, K. M., & Cromer, L. (2005). Isolation, amplification, and identification of ancient copepod DNA from lake sediments. *Limnology and Oceanography: Methods*, *3*(12), 533–542. https://doi.org/10.4319/lom.2005.3.533

Blakey, R. C. (2008). Gondwana paleogeography from assembly to breakup—A 500 my odyssey. *Geological Society of America Special Papers*, *441*, 1–28.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *10*(4), e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Boxshall, G. A., & Jaume, D. (2000). Making waves: The repeated colonization of freshwater by copepod crustaceans. *Advances in Ecological Research*, *31*, 61–79.

Brendonck, L., & de Meester, L. (2003). Egg banks in freshwater zooplankton: Evolutionary and ecological archives in the sediment. *Hydrobiologia*, *491*, 65–84. https://doi.org/10.1023/A:1024454905119

Burckhardt, D. (2009). Taxonomy and phylogeny of the Gondwanan moss bugs or Peloridiidae (Hemiptera, Coleorrhyncha). *Deutsche Entomologische Zeitschrift*, *56*(2), 173–235. https://doi.org/10.1002/mmnd.200900019

Carapelli, A., Greenslade, P., Nardi, F., Leo, C., Convey, P., Frati, F., & Fanciulli, P. P. (2020). Evidence for cryptic diversity in the "Pan-Antarctic" Springtail *Friesea antarctica* and the description of two new species. *Insects*, *11*(3), 141. https://doi.org/10.3390/insects11030141

Convey, P. (2017). Antarctic ecosystems. In *Reference module in life sciences* (pp. 1–13). Elsevier.

Convey, P., Biersma, E. M., Casanova-Katny, A., & Maturana, C. S. (2020). Refuges of Antarctic diversity. In M. Oliva & J. Ruiz-Fernández (Eds.), *Past Antarctica* (pp. 181–200). Elsevier.

Convey, P., & Peck, L. S. (2019). Antarctic environmental change and biological responses. *Science Advances Review*, *11*, 1–16. https://doi.org/10.1126/sciadv.aaz0888

Convey, P., Stevens, M. I., Hodgson, D. A., Smellie, J. L., Hillenbrand, C.-D., Barnes, D. K. A., Clarke, A., Pugh, P. J. A., Linse, K., & Cary, S. C. (2009). Exploring biological constraints on the glacial history of Antarctica. *Quaternary Science Reviews*, *28*(27), 3035–3048. https://doi.org/10.1016/j.quascirev.2009.08.015

Copilaş-Ciocianu, D., Sidorov, D., & Gontcharov, A. (2019). Adrift across tectonic plates: Molecular phylogenetics supports the ancient Laurasian origin of old limnic crangonyctid amphipods. *Organisms Diversity & Evolution*, *19*(2), 191–207. https://doi.org/10.1007/s13127-019-00401-7

Couch, K. M., Downes, M., & Burns, C. W. (2001). Morphological differences between subitaneous and diapause eggs of *Boeckella triarticulata* (Copepoda: Calanoida). *Freshwater Biology*, *46*, 925–933.

Cowie, R. H., & Holland, B. S. (2006). Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. *Journal of Biogeography*, *33*(2), 193–198. https://doi.org/10.1111/j.1365-2699.2005.01383.x

Crame, J. A. (2018). Key stages in the evolution of the Antarctic marine fauna. *Journal of Biogeography*, *45*(5), 986–994. https://doi.org/10.1111/jbi.13208

Cranston, P. S., Hardy, N. B., Morse, G. E., Puslednik, L., & McCluen, S. R. (2010). When molecules and morphology concur: The 'Gondwanan' midges (Diptera: Chironomidae). *Systematic Entomology*, *35*(4), 636–648. https://doi.org/10.1111/j.1365-3113.2010.00531.x

Crisp, M. D., Trewick, S. A., & Cook, L. G. (2011). Hypothesis testing in biogeography. *Trends in Ecology & Evolution*, *26*(2), 66–72. https://doi.org/10.1016/j.tree.2010.11.005

de Queiroz, A. (2005). The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology & Evolution*, *20*(2), 68–73. https://doi.org/10.1016/j.tree.2004.11.006

Defaye, D. (1998). Description of the first *Boeckella* (Copepoda, Calanoida, Centropagidae) from New Caledonia. *Crustaceana*, *71*(6), 686–699. https://doi.org/10.1163/156854098X00680

Denton, G. H., Anderson, R. F., Toggweiler, J. R., Edwards, R. L., Schaefer, J. M., & Putnam, A. E. (2010). The last glacial termination. *Science*, *328*(5986), 1652–1656. https://doi.org/10.1126/science.1184119

Díaz, A., Maturana, C. S., Boyero, L., De Los Ríos Escalante, P., Tonin, A. M., & Correa-Araneda, F. (2019). Spatial distribution of freshwater crustaceans in Antarctic and Subantarctic lakes. *Scientific Reports*, *9*(1), 7928. https://doi.org/10.1038/s41598-019-44290-4

Edgar, R. C. (2004). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, *5*, 1–19. https://doi.org/10.1186/1471-2105-5-113

EPICA (2004). Eight glacial cycles from an Antarctic ice core. *Nature*, *429*, 623–628.

Fraser, C. I., Terauds, A., Smellie, J., Convey, P., & Chown, S. L. (2014). Geothermal activity helps life survive glacial cycles. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(15), 5634–5639. https://doi.org/10.1073/pnas.1321437111

Gibson, J. A. E., & Bayly, I. A. E. (2007). New insights into the origins of crustaceans of Antarctic lakes. *Antarctic Science*, *19*(2), 157–163. https://doi.org/10.1017/S0954102007000235

Gillespie, R. G., Baldwin, B. G., Waters, J. M., Fraser, C. I., Nikula, R., & Roderick, G. K. (2012). Long-distance dispersal: A framework for hypothesis testing. *Trends in Ecology & Evolution*, *27*(1), 47–56. https://doi.org/10.1016/j.tree.2011.08.009

Hall, C. J., & Burns, C. W. (2001). Hatching of *Boeckella hamata* (Copepoda: Calanoida) resting eggs from sediments of a tidally influenced lake. *New Zealand Journal of Marine and Freshwater Research*, *35*(2), 235–238. https://doi.org/10.1080/00288330.2001.9516994

Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E., & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics*, *24*(1), 129–131. https://doi.org/10.1093/bioinformatics/btm538

Jiang, X., Zhao, S., Xu, Z., Wang, G., He, J., & Cai, M. (2012). Abundance and age of viable resting eggs of the calanoid copepod *Boeckella poppei* Mrázek in sediments: Evidence of egg banks in two Antarctic maritime lakes. *Polar Biology*, *35*(10), 1525–1531. https://doi.org/10.1007/s00300-012-1192-5

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kishino, H., & Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in hominoidea. *Journal of Molecular Evolution*, *29*(2), 170–179. https://doi.org/10.1007/BF02100115

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., & Battistuzzi, F. U. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2017). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, *34*(3), 772–773. https://doi.org/10.1093/molbev/msw260

Laybourn-Parry, J., & Pearce, D. A. (2007). The biodiversity and ecology of Antarctic lakes: Models for evolution. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *362*(1488), 2273–2289. https://doi.org/10.1098/rstb.2006.1945

Lessios, H. A. (2008). The Great American Schism: Divergence of marine organisms after the rise of Central American Isthmus. *Annual Review of Ecology Evolution and Systematics*, *39*, 63–91.

Lewis, A. R., Marchant, D. R., Ashworth, A. C., Hedenas, L., Hemming, S. R., Johnson, J. V., Leng, M. J., Machlus, M. L., Newton, A. E., Raine, J. I., Willenbring, J. K., Williams, M., & Wolfe, A. P. (2008). Mid-Miocene cooling and the extinction of tundra in continental Antarctica. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(31), 10676–10680. https://doi.org/10.1073/pnas.0802501105

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Livermore, R., Hillenbrand, C.-D., Meredith, M., & Eagles, G. (2007). Drake Passage and Cenozoic climate: An open and shut case? *Geochemistry, Geophysics, Geosystems*, *8*(1), 4–11. https://doi.org/10.1029/2005gc001224

Maturana, C., Rosenfeld, S., Naretto, J., Convey, P., & Poulin, E. (2019). Distribution of the genus *Boeckella* (Crustacea, Copepoda, Calanoida, Centropagidae) at high latitudes in South America and the main Antarctic biogeographic regions. *ZooKeys*, *854*, 1–15. https://doi.org/10.3897/zookeys.854.29614

Maturana, C., Rosenfeld, S., Naretto, J., Convey, P., Poulin, E., Lopez, Z., Gerard, K., Contador, T., Rendoll, J., Jackson, J. A., Hogg, I. D., Duggan, I. C., & Bahamonde, F. (2018, October). Occurrences of Boeckella at high latitudes in the Southern Hemisphere. Version 1.1. 2018. https://doi.org/10.15468/zc6y59

Matzke, N. J. (2013). Probabilistic historical biogeography: New models for founder- event speciation, imperfect detection, and fossils allow im- proved accuracy and model-testing. *Frontiers of Biogeography*, *5*(4), 242. https://doi.org/10.21425/F55419694

Matzke, N. J. (2014). Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology*, *63*(6), 951–970. https://doi.org/10.1093/sysbio/syu056

McGaughran, A., Terauds, A., Convey, P., & Fraser, C. I. (2019). Genome-wide SNP data reveal improved evidence for Antarctic glacial refugia and dispersal of terrestrial invertebrates. *Molecular Ecology*, *28*(22), 4941–4957. https://doi.org/10.1111/mec.15269

Menu-Marque, S., & Balseiro, E. (2000). *Boeckella antiqua* n.sp. (Copepoda, Calanoida, Contropagidae) from Patagonia. *Hydrobiologia*, *429*(1–3), 1–7.

Menu-Marque, S., Morrone, J. J., & de Mitrovic, C. L. (2000). Distributional pattern of the South American species of *Boeckella* (Copepoda: Centropagidae): A track analysis. *Journal of Crustacean Biology*, *20*(2), 262–272. https://doi.org/10.1163/20021975-99990038

Menu-Marque, S., & Zuñiga, L. R. (1994). *Boeckella diamantina* n.sp (Calanoia, Centropagidae), from a high Andean lake in Mendoza, Argentina. *Hydrobiologia*, *292*(293), 81–87.

Nogales, M., Heleno, R., Traveset, A., & Vargas, P. (2012). Evidence for overlooked mechanisms of long-distance seed dispersal to and between oceanic islands. *New Phytologist*, *194*, 313–317. https://doi.org/10.1111/j.1469-8137.2011.04051.x

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Poppe, S., & Mrázek, A. (1895). Entomostraken des Naturhistorischen Museum in Hamburg. 2. Entomostraken von Süd-Georgien, 2. Jahrbuch. *Hamburgischen Wissenschaftlichen Anstalten*, *12*, 135–138.

Posada, D., & Crandall, K. A. (2001). Intraspecific gene genealogies: Trees grafting into networks. *Trends in Ecology & Evolution*, *16*(1), 37–45. https://doi.org/10.1016/s0169-5347(00)02026-7

Pugh, P. J. A., Dartnall, H. J. G., & Mcinnes, S. J. (2002). The non-marine Crustacea of Antarctica and the Islands of the Southern Ocean: Biodiversity and biogeography. *Journal of Natural History*, *36*(9), 1047–1103. https://doi.org/10.1080/00222930110039602

Pybus, O. G., & Harvey, P. H. (2000). Testing macro-evolutionary models using incomplete molecular phylogenies. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1459), 2267–2272. https://doi.org/10.1098/rspb.2000.1278

Quinlan, K., & Bayly, I. A. E. (2017). A new species of *Boeckella* (Copepoda: Calanoida) from arid Western Australia, an updated key, and aspects of claypan ecology. *Records of the Western Australian Museum*, *32*(2), 191–206. https://doi.org/10.18195/issn.0312-3162.32(2).2017.191-206

R Core Team (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rabosky, D. L. (2006). LASER: A maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. *Evolutionary Bioinformatics Online*, *2*, 247–250. https://doi.org/10.1177/117693430600200024

Rambaut, A., Suchard, M. A., Xie, D., & Drummond, A. J. (2014). Tracer v1.6. Retrieved from http://beast.bio.ed.ac.uk/Tracerhttp://beast.bio.ed.ac.uk/Tracer

Reed, K. A., Lee, S. G., Lee, J. H., Park, H., & Covi, J. A. (2021). The ultrastructure of resurrection: Post-diapause development in an Antarctic freshwater copepod. *Journal of Structural Biology*, *213*(1), 107705. https://doi.org/10.1016/j.jsb.2021.107705

Reguero, M. A., Marenssi, S. A., & Santillana, S. N. (2002). Antarctic Peninsula and South America (Patagonia) Paleogene terrestrial faunas and environments: Biogeographic relationships. *Palaeogeography,*

Palaeoclimatology, Palaeoecology, *179*, 182–210. https://doi.org/10.1016/S0031-0182(01)00417-5

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*(2), 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*(12), 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rosen, D. E. (1978). Vicariant patterns and historical explanation in biogeography. *Systematic Biology*, *27*(2), 159–188. https://doi.org/10.2307/2412970

Samanez, I., & López, D. (2014). Distribución geográfica de *Boeckella* y *Neoboeckella* (Calanoida: Centropagidae) en el Perú. *Revista Peruana de Biologia*, *21*(3), 223–228. https://doi.org/10.15381/rpb.v21i3.10895

Scheihing, R., Cardenas, L., Nespolo, R. F., Krall, P., Walz, K., Kohshima, S., & Labarca, P. (2009). Morphological and molecular analysis of centropagids from the high Andean plateau (Copepoda: Calanoidea). *Hydrobiologia*, *637*(1), 45–52. https://doi.org/10.1007/s10750-009-9983-6

Sérsic, A. N., Cosacov, A., Cocucci, A. A., Johnson, L. A., Pozner, R., Avila, L. J., SITES Jr., J. W., & Morando, M. (2011). Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biological Journal of the Linnean Society*, *103*(2), 475–494. https://doi.org/10.1111/j.1095-8312.2011.01656.x

Shimodaira, H. (2002). An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, *51*(3), 492–508. https://doi.org/10.1080/10635150290069913

Shimodaira, H., & Hasegawa, M. (1999). Multiple comparisons of Log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, *16*(8), 1114–1116. https://doi.org/10.1093/oxfordjournals.molbev.a026201

Stevens, M. I., Greenslade, P., & D'Haese, C. A. (2021). Species diversity in *Friesea* (Neanuridae) reveals similar biogeographic patterns among Antarctic Collembola. *Zoologica Scripta*, *50*(5), 647–657. https://doi.org/10.1111/zsc.12490

Swenson, U., Hill, R. S., & McLoughlin, S. (2001). Biogeography of Nothofagus supports the sequence of Gondwana break-up. *Taxon*, *50*, 1025–1041.

Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: Concatenation software for the fast assembly of multi-gene dataset with character set and codon information. *Cladistics*, *27*, 171–180.

Van der Putten, N., Verbruggen, C., Ochyra, R., Verleyen, E., & Frenot, Y. (2010). Subantarctic flowering plants: Pre-glacial survivors or post-glacial immigrants? *Journal of Biogeography*, *37*(3), 582–592. https://doi.org/10.1111/j.1365-2699.2009.02217.x

Vanderpoorten, A., Gradstein, S. R., Carine, M. A., & Devos, N. (2010). The ghosts of Gondwana and Laurasia in modern liverwort distributions. *Biological Reviews of the Cambridge Philosophical Society*, *85*(3), 471–487. https://doi.org/10.1111/j.1469-185X.2009.00111.x

Viana, D. S., Gangoso, L., Bouten, W., & Figuerola, J. (2016). Overseas seed dispersal by migratory birds. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1822), 20152406. https://doi.org/10.1098/rspb.2015.2406

Walter, T., & Boxshall, G. (2019). World of Copepods database. *World Register of Marine Species. Retrieved on May 3rd*

Waters, J. M. (2008). Driven by the West Wind Drift? A synthesis of southern temperate marine biogeography, with new directions for dispersalism. *Journal of Biogeography*, *35*(3), 417–427. https://doi.org/10.1111/j.1365-2699.2007.01724.x

Will, S., Joshi, T., Hofacker, I. L., Stadler, P. F., & Backofen, R. (2012). LocARNA-P: Accurate boundary prediction and improved detection of structural RNAs. *RNA*, *18*(5), 900–914. https://doi.org/10.1261/rna.029041.111

Wright, F. (1990). The "effective number of codons" used in a gene. *Gene*, *87*, 23–29. https://doi.org/10.1016/0378-1119(90)90491-9

Xia, X. (2013). DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution*, *30*(7), 1720–1728. https://doi.org/10.1093/molbev/mst064

Xia, X., Xie, Z., Salemi, M., Chen, L., & Wang, Y. (2003). An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution*, *26*, 1–7. https://doi.org/10.1016/S1055-7903(02)00326-3

Xu, L., & Etienne, R. S. (2018). Detecting local diversity-dependence in diversification. *Evolution*, *72*(6), 1294–1305. https://doi.org/10.1111/evo.13482

Zachos, J., Pagani, M., Sloan, L., Thomas, E., & Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, *292*(5517), 686–693. https://doi.org/10.1126/science.1059412

BIOSKETCH

Claudia S. Maturana is an evolutionary biologist interested in biogeography, evolutionary history and ecology of high latitude organisms, in particular freshwater and marine invertebrates. Her PhD focussed on understanding the macro- and micro-evolutionary processes that underlie the current distribution of freshwater biota in Antarctic and sub-Antarctic regions. https://www.researchgate.net/profile/Claudia_Maturana2

Author contributions: C.S.M., E.P., J.A.J. and P.C. conceived the idea and designed the analyses. C.S.M. and S.R. performed the taxonomic revision. C.S.M. conducted the molecular work, phylogenetic and range reconstruction analyses. C.S.M., S.R., J.N., E.P., P.C., J.A.J., E.M.B., conducted fieldwork. I.C.D and I.D.H provided genetic and georeferenced data. C.S.M., S.R. and J.N. reviewed and updated the database in GBIF. N.I.S. performed the diversification rates model analyses. C.S.M., N.S. and S.R. designed and edit figures and maps. E.M.B., A.D. and C.A.G.-W., J.A.J. contributed to every analytical step regarding the interpretation of results and in preparing the manuscript. C.S.M. wrote the paper, but E.P., J.A.J., P.C., E.M.B., S.R., C.A.G.-W., I.D.H., and A.D. discussed the results and contributed to the final manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Maturana, C. S., Rosenfeld, S., Biersma, E. M., Segovia, N. I., González-Wevar, C. A., Díaz, A., Naretto, J., Duggan, I. C., Hogg, I. D., Poulin, E., Convey, P., & Jackson, J. A. (2021). Historical biogeography of the Gondwanan freshwater genus *Boeckella* (Crustacea): Timing and modes of speciation in the Southern Hemisphere. *Diversity and Distributions*, 27, 2330–2343. https://doi.org/10.1111/ddi.13405