**Supplementary Table S1.** Occurrence of *Boeckella poppei* across maritime Antarctic, sub-Antarctic islands and South America. Samples from SA were collected from multiple locations between Katalalixar Reserve (S48°21’; W75°33’) to Cape Horn (S55°57’; W67°14’) and Diego Ramirez Island (S56°31’; W68°43’). 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.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sampling Site** | **Georeference** | **Sampling location** | **Sampling Area** | **Biogeographic Region** | **Clade** |
|  |
| Arctowski Base | 62°09.937'S, 58°28.210'W | King George Is. | South  Shetland  Islands | Maritime Antarctica | A,B |  |
| Corazón Pond | 62˚09'56.0"S, 58˚27'45.2"W | King George Is. | A |  |
| Laguna Baja | 62˚11''39.8'S, 58˚59'0.7''W | King George Is. | B |  |
| Great Wall Base | 62°13.000'S, 58°57.910'W | King George Is. | B |  |
| Laguna Hidrográfica | 62°12.198'S, 58°58.048'W | King George Is. | B |  |
| INACH lake | 62°12.101'S, 58°57.161'W | King George Is. | A,B |  |
| Langer Lake | 62°12.198'S, 58°58.048'W | King George Is. | B |  |
| Monolito Lake | 62°12.283'S, 58°57.658'W | King George Is. | B |  |
| Laguna Glaciar | 62°10.088'S, 58°27.640'W | King George Is. | A |  |
| Artigas Base | 62°11.003'S, 58°54.303'W | King George Is. | B |  |
| AlfaQuebec | 62°22.782'S, 59°41.762'W | Robert Is. | A |  |
| Lobo Pond | 62°22.738'S, 59°41.893'W | Robert Is. | A,B |  |
| Prat Base | 62°28.900'S, 59°37.817'W | Greenwich Is. | A |  |
| Somero Lake | 62°39.947'S, 61°06.737'W | Livingston Is. | B |  |
| Turbio Lake | 62°37.587'S, 61°5.365'W | Livingston Is. | B |  |
| Zapatilla Lake | 62°59.028'S, 60°40.507'W | Deception Is. | A |  |
| Irizar Lake | 62°58.796'S, 60°42.332'W | Deception Is. | A |  |
| Cráter Lake | 62°59.062'S, 60°40.358'W | Deception Is. | A |  |
| Avian Lake | 67°46.582'S, 68°53.534'W | Avian Island | Southern  Antarctic  Peninsula | B |  |
| Horseshoe Lake | 67°49.128'S, 67°18.492'W | Horseshoe Is. | B |  |
| Laguna Fango | 67˚49'04.4"S, 67˚18'51.9"W | Horseshoe Is. | B |  |
| Fossil Bluff | 71°19.983'S, 68°16.667'W | Alexander Is. | B |  |

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| --- | --- | --- | --- | --- | --- | --- |
| **Sampling Site** | **Georeference** | **Sampling location** | **Sampling Area** | **Biogeographic Region** | **Clade** |  |
| Changing Lake | 60°41.518'S, 45°37.151'W | Signy Island | South  Orkney  Islands | Maritime Antarctica | A |  |
| Sombre Lake | 60°41.195'S, 45°36.747'W | Signy Island | A |  |
| Heywood Lake | 60°41.330'S, 45°36.465'W | Signy Island | A |  |
| Pumphouse Lake | 60°42.055'S, 45°36.831'W | Signy Island | A |  |
| Tranquil Lake | 60°42.250'S, 45°38.732'W | Signy Island | A |  |
| Light Lake | 60°41.915'S, 45°38.884'W | Signy Island | A |  |
| Twisted Lake | 60°43.282'S, 45°39.582'W | Signy Island | A |  |
| Gull Lake | 54°17.217'S, 36°30.649'W | Thatjer Peninsula | South  Georgia | sub-Antactica Islands | B |  |
| Bird Island | 54°00.530'S, 38°04.041'W | Bird Island | B |  |
| Tierra del Fuego TF1 | 52°33.665'S, 69°25.166'W | Primera Angostura | South  America | South  America | B |  |
| Tierra del Fuego TF4 | 52°25.960'S, 69°38.197'W | Tierra del Fuego | B |  |
| Punta Delgada | 52°50.761'S, 69°51.897'W | Brunswick Peninsula | B |  |

**Supplementary Table S2.** Diversity indices of *cox*1, ITS AND 28S rRNA in *Boeckella poppei* sampled across the five sampling areas included in this study. *n* = number of sequences; S = Segregation Sites;  Mean number of pairwise differences; ****Nucleotide diversity; Hd=Haplotype Diversity; K = Number of Haplotypes; p.a. = private alleles. \* p < 0.01

***Cox*1**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Biogeographic Regions** | **Locations** | **Clade** | **Code** | **n** | **S** | **** | **** | **Hd** | **K** | **p.a** | **Tajima's D** | **Fu’s Fs** |
| **South America** | South America | B | SA | 68 | 17 | 3.003 | 0.007 | 0.813 | 11 | 10 | -0.459 | -0.369 |
| **sub-Antarctic** | South Georgia | B | SG | 37 | 11 | 0.889 | 0.002 | 0.647 | 13 | 10 | -2.815\* | -12.492\* |
| **Maritime Antarctic** | South Orkney Islands | A | SOI | 84 | 17 | 1.836 | 0.004 | 0.816 | 20 | 19 | -1.308 | -12.156\* |
| South Shetland Islands | A | SSI1 | 93 | 7 | 0.672 | 0.002 | 0.418 | 7 | 6 | -1.210 | -2.696 |
| B | SSI2 | 117 | 14 | 0.873 | 0.002 | 0.503 | 14 | 11 | -1.945\* | -9.912 |
| Antarctic Peninsula | B | AP | 39 | 14 | 1.614 | 0.004 | 0.812 | 16 | 15 | -1.625 | -12.105\* |

**ITS**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Biogeographic Regions | Areas | Code | *2n* | S | P | Hd | K | p |
| South America | Brunswick Peninsula and Tierra del Fuego | SA | 10 | 3 | 0,600 | 0,378 | 3 | 0,002 |
| sub-Antarctic | South Georgia | SG | 16 | 0 | 0 | 0 | 1 | 0 |
| Maritime Antarctic | South Orkney Island | SOI | 114 | 0 | 0 | 0 | 1 | 0 |
| South Shetland Islands | SSI | 112 | 2 | 0,314 | 0,311 | 3 | 0,0006 |
| Antarctic Peninsula | AP | 62 | 1 | 0,155 | 0,151 | 2 | 0,0003 |

**28S rRNA**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Biogeographic Regions | Areas | Code | *2n* | S | P | Hd | K | p |
| South Smerica | Brunswick Peninsula and Tierra del Fuego | SA | 30 | 1 | 0,041 | 0,041 | 2 | 0,0001 |
| Sub-antarctic | South Georgia | SG | 28 | 0 | 0 | 0 | 1 | 0 |
| Maritime antarctic | South Orkney Island | SOI | 80 | 0 | 0 | 0 | 1 | 0 |
| South Shetland Islands | SSI | 40 | 0 | 0 | 0 | 1 | 0 |
| Antarctic Peninsula | AP | 16 | 0 | 0 | 0 | 1 | 0 |

**Supplementary Fig. S3.** Haplotype network for *Boeckella poppei* using cox1 dataset from South Shetland Islands sampling sites (n=210).

**Gráfico, Diagrama, Esquemático

Descripción generada automáticamente con confianza media**

**Supplementary Fig. S4.** Haplotype network for *Boeckella poppei*, including nucDNA 28S rRNA and ITS1/ITS2 sequences obtained across the species’ distribution. Neighbour-Joining network illustrating the distribution of haplotypes in each surveyed lake in southern South America, the South Shetland Islands, the Antarctic Peninsula, the South Orkney Islands, and sub-Antarctic South Georgia. Circle sizes are proportional to the frequency of haplotypes. The colour key indicates the sampling area.

**![Chart, pie chart

Description automatically generated]()**

**Supplementary Material Table S5.** Pairwise differences values calculated among the studied locations for *Boeckella poppei* using the *cox*1 dataset*.* Computing conventional F-Statistics from haplotype frequencies(FST)shown below the diagonal and from pairwise difference based on Kimura 2P (ST) above the diagonal. \* p < 0.001 after testing multiple comparisons (False Discovery Rate, FDR).

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| --- | --- | --- | --- | --- | --- | --- |
|  | **South Orkney Islands** | **South Shetland Islands (SSI1)** | **South Shetland Islands (SSI2)** | **Antarctic Peninsula** | **South Georgia** | **South America** |
| **South Orkney Islands** | – | 0.462\* | 0.881\* | 0.807\* | 0.819\* | 0.746\* |
| **South Shetland Islands (SSI1)** | 0.357\* | – | 0.926\* | 0.900\* | 0.917\* | 0.830\* |
| **South Shetland Islands (SSI2)** | 0.358\* | 0.549\* | – | 0.222\* | 0.049\* | 0.517\* |
| **Antarctic Peninsula** | 0.186\* | 0.426\* | 0.101\* | – | 0.152\* | 0.409\* |
| **South Georgia** | 0.259\* | 0.498\* | 0.027 | 0.035 | – | 0.403\* |
| **South America** | 0.188\* | 0.405\* | 0.369\* | 0.190\* | 0.266\* | – |

**Supplementary Fig. S6:** Multi-locus Bayesian phylogenetic reconstruction framework using *cox*1 + *ITS +* 28S rRNA dataset. Tree node support values are shown for posterior probability Bayesian Inference (BI) and bootstrap Maximum Likelihood (ML) frameworks, respectively.

**Diagrama, Esquemático

Descripción generada automáticamente**

**Supplementary Material Fig. S7.** Historical models constructed under DIYABC software. Setting for different scenarios is shown for each clade. Clade A Scenario 1: Refugia in SOI and bottleneck in SSI1 with a population expansion in t1; and Scenario 2: Refugia in SSI1 and bottleneck in SOI with a population expansion in t1. Clade B Scenario 1: Refugia in SG, SSI2 and AP with a subsequent population expansion in t1; and Scenario 2: Colonization of SG, SSI2 and AP from SA during t2. First Colonized Area Scenario 1: First colonized area from SA to SSI2; Scenario 2: First colonized area from SA to SG and Scenario 3: First colonized area from SA to AP. t1: population expansion, t2: deglaciation starting time, t3: glaciation starting time, t2iv – t2i < t2 are times when colonization and population expansion occur. N1–N4 effective population size, Nb: Reduced population size.

Chart, line chart

Description automatically generated

**Supplementary Table S8.** Posterior probabilities of demographic scenarios in *Boeckella poppei* using the *cox*1 dataset. Demographic scenarios for Clade A: Scenario 1 refuge in SOI; Scenario 2 refuge in SSI1. Demographic scenarios for Clade B: Scenario 1 refuges in SG, SSI and AP; Scenario 2 colonization to SG, SSI2 and AP from SA. Colonization area: Scenario 1: SSI2 first colonized area; Scenario 2: SG first colonized area; Scenario 3: AP first colonized. Direct and logistic approaches with their respective 95% confidence interval (95% CI) are shown.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Posterior Probabilities of Scenarios | | | | |
|  |  | **Direct** | **95% CI** | **Logistic** | **95% CI** |
| Refuge | SOI – Scenario 1 | 0.984 | 0.874 – 1.000 | 0.976 | 0.956 – 0.997 |
| Clade A | SSI1 – Scenario 2 | 0.016 | 0.000 – 0.126 | 0.024 | 0.003 – 0.044 |
| Refuge | MA, SG – Scenario 1 | 0.000 | 0.000 – 0.000 | 0.000 | 0.000 – 0.000 |
| Clade B | SA – Scenario 2 | 1.000 | 1.000 – 1.000 | 0.999 | 0.999 – 1.000 |
| First colonized  area | SSI2 – Scenario 1 | 0.114 | 0.000 – 0.393 | 0.319 | 0.216 – 0.422 |
| SG – Scenario 2 | 0.198 | 0.000 – 0.547 | 0.342 | 0.244 – 0.440 |
| PA – Scenario 3 | 0.688 | 0.282 – 1.000 | 0.339 | 0.247 – 0.430 |

**Appendix 1. Setting different historical scenarios**

The prior distributions for time-related parameters (t1: population expansion, t2: deglaciation starting time and t3: glaciation starting time) were set based on published literature on the timing of LGM in maritime Antarctica, South Georgia (Hodgson et al., 2014), and southern South America (Hulton et al., 2002; Rabassa et al., 2000). Each scenario involved a variation in population size (i.e. bottleneck), which is modelled with the parameters N (effective population size) and Nb (effective population size after the bottleneck). Posterior probabilities of scenarios were estimated using historical, demographic and mutational parameters drawn from the prior distributions. For each scenario 2,000,000 datasets were simulated and the relative likelihoods of scenarios were compared using a logistic regression on the 1% of simulated data closest to the observed dataset. A principal component analysis (PCA) was applied to visualize how similar the simulated and observed statistics were for each scenario. Using the logistic regression (taking the highest posterior probability as indicating the most likely scenario) and PCA estimates, the most likely scenario in the analysis can be selected. The overall most likely scenario was then evaluated under each of the quality control-based options in DIYABC to assess the goodness of fit between the prior-posterior combination of each model parameter and the observed dataset (Cornuet et al., 2010).

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