

Deadly mushrooms of the genus *Galerina* found in Antarctica colonized the continent as early as the Pleistocene

ISAAC GARRIDO-BENAVENT ¹, ROBERT A. BLANCHETTE ² and ASUNCIÓN DE LOS RÍOS ³

¹*Departament de Botànica i Geologia, Facultat de Ciències Biològiques, Universitat de València, C/Doctor Moliner 50, E-46100 Burjassot, València, Spain*

²*Department of Plant Pathology, 1991 Upper Buford Circle, 495 Borlaug Hall, University of Minnesota, St. Paul, MN 55108-6030, USA*

³*Department of Biogeochemistry and Microbial Ecology, National Museum of Natural Sciences (MNCN), CSIC, E-28006 Madrid, Spain*
Isaac.Garrido@uv.es

Abstract: Fungi are probably the most diverse group of eukaryotic organisms in the Antarctic continent and nearby archipelagos, and they dominate communities in either mild or harsh habitats. However, our knowledge of their global distribution ranges and the temporal origins of their Antarctic populations is rather limited or almost absent, especially for species that do not lichenize. We focused for the first time on elucidating the taxonomic identity and phylogenetic relationships of several Antarctic collections of the deadly fungal Basidiomycota genus *Galerina*. By using molecular sequence data from the universal fungal barcode and a dataset encompassing 178 specimens, the inferred phylogeny showed that the Antarctic specimens corresponded with the sub-cosmopolitan species *Galerina marginata*, *Galerina badipes* and *Galerina fallax*, and their most closely related intraspecific genetic lineages were from northern Europe and North America. We found that these species probably host Antarctic-endemic intraspecific lineages. Furthermore, our dating analyses indicated that their Antarctic populations originated in the Pleistocene, a temporal frame that agrees with that proposed for the Antarctic colonization of plants such as the grass *Deschampsia antarctica*, mosses and some amphitropical lichens. Altogether, these findings converge on the same temporal scenario for the assembly of the most conspicuous terrestrial Antarctic plant and fungal communities.

Received 8 November 2022, accepted 29 April 2023

Key words: biogeography, dating analysis, fungal endemism, *Galerina marginata*, long-distance dispersal, non-lichenized fungi

Introduction

Fungi are probably the most widespread and diverse group of eukaryotic organisms inhabiting Antarctica, with a known fossil record dating back to the Permian period (White Jr & Taylor 1991, Harper *et al.* 2016). They are involved in key processes in terrestrial ecosystems, such as decomposition and symbiotic mutualism (Treseder & Lennon 2015, Asplund & Wardle 2017), and therefore they contribute greatly to biogeochemical cycles in otherwise low-nutrient habitats. The number of known species in territories south of 60°S and archipelagos at lower latitudes, such as South Georgia, is ~1500 (Øvstedal & Lewis Smith 2001, 2011, Onofri *et al.* 2005, Bridge *et al.* 2008, Bridge & Spooner 2012). Almost a third of them associate symbiotically with eukaryotic algae (chlorophytes) or cyanobacteria, forming macroscopic lichen thalli. Lichens are in fact one of the most conspicuous elements of Antarctic terrestrial habitats, and their communities develop profusely in

maritime areas (Søchting *et al.* 2004, Peat *et al.* 2007), and even in rocky outcrops at harsher locations in the continent (Kappen *et al.* 1981, Broady & Weinstein 1998, Pérez-Ortega *et al.* 2012). On the other hand, non-lichenized fungi generally remain unnoticeable because either they are unicellular (e.g. yeasts) or they form unseen mycelia and small reproductive structures. Microfungi are the most abundant in Antarctic soils (Vishniac & Hempfling 1979, Ruisi *et al.* 2007, Arenz & Blanchette 2011, Arenz *et al.* 2014), and many Ascomycota and even some Basidiomycota have been reported on wood brought to Antarctica (Arenz & Blanchette 2009, Blanchette *et al.* 2010, Arenz *et al.* 2011, Held & Blanchette 2017). Additional research using next-generation sequencing techniques has also revealed a high diversity of fungi even in the most unexpected habitats (Coleine *et al.* 2018, Garrido-Benavent *et al.* 2020, Rosa *et al.* 2021), with similarities in species diversity and community composition to the Arctic (Cox *et al.* 2016).

Macrofungi (i.e. non-lichenized species that form relatively large fruiting bodies or 'mushrooms') are infrequently reported in Antarctica, with a few dozen species growing in the climatically milder sub-Antarctic, Maritime Antarctica and occasionally in the western Antarctic Peninsula, often occurring on large carpets of mosses and vascular plants (Pegler *et al.* 1980, Gumińska *et al.* 1994, Bridge *et al.* 2008, Putzke *et al.* 2012, Held & Blanchette 2017, Newsham *et al.* 2021). Bridge & Spooner (2012) and Newsham *et al.* (2021) suggested that the general absence of large land animals and higher plants with woody components in Antarctica is a limiting factor for the development of these fungi. From a taxonomic and biogeographical viewpoint, the scarcity of available collections of mushroom-forming fungi from the Antarctic has so far impeded detailed comparisons with species that are known from elsewhere. More specifically, the lack of genetic data has prevented the deciphering of the most probable temporal and spatial origins of the Antarctic populations of certain species. In fact, despite being one of the most diverse groups in the whole continent, there is still a general lack of knowledge as to whether non-lichenized fungi, and particularly the Antarctic macrofungi, form specific populations of cosmopolitan species or constitute true endemic species (Bridge & Spooner 2012, Arenz *et al.* 2014).

To provide an answer to this question, the present work uses recent collections of fruiting bodies of *Galerina* species to assess their taxonomic identity and estimate a date for their Antarctic origin within a phylogenetic framework. This genus of basidiomycetous fungi encompasses ~300 species worldwide (Horak 1994, Gulden *et al.* 2005), which form relatively small, yellowish to reddish-brown fruiting bodies with campanulate, convex to flat pilei and slender stipes. Several *Galerina* species are well known for posing a poisoning risk due to the production of deadly amatoxins (Landry *et al.* 2021). The genus shows a broad distribution in Mediterranean, temperate and boreal regions in the Northern Hemisphere (GBIF 2022), where saprotrophic species generally grow on dead parts of bryophytes in peat bogs or are associated with woody remnants or other plant debris in forests, on which this genus degrades wood cell wall components (Gulden *et al.* 2005, Grzesiak & Wolski 2015, Kohler *et al.* 2015). In the Antarctic continent and nearby archipelagos, the number of *Galerina* species reported is ~11, with *Galerina antarctica* Singer, *Galerina glebarum* (Berk.) Singer and *Galerina perrara* Singer originally being described, and these are known only from these regions (Fig. 1; Berkeley 1847, Singer & Corte 1962, Pegler *et al.* 1980, Bridge *et al.* 2008). Based on the inferred phylogeny, we aim to ascertain whether the sequenced Antarctic specimens belong to geographically

restricted, species-level lineages (i.e. putative endemic species) or conform to particular intraspecific lineages of cosmopolitan *Galerina* (non-endemic species). In lichenized fungi, Antarctic endemic species have been shown to have a relictual, pre-Pleistocene origin, whereas Antarctic populations of amphitropical lichens are generally much younger, dating back from the Pleistocene onwards (Fernández-Mendoza & Printzen 2013, Garrido-Benavent *et al.* 2016, 2018, 2021). The temporal frame estimated with the time-calibrated *Galerina* phylogeny will further help us to discern whether their evolution in Antarctica conforms to either of these two scenarios.

Material and methods

Fieldwork and morpho-anatomical study of fruiting bodies

Several fruiting bodies of *Galerina* growing in a localized area, and therefore probably corresponding to a single mycelium, were collected in March 2018 from Livingston Island (South Shetland Islands) and more specifically in Punta Hannah (62°39'15.37" S, 60°36'27.44" W, 377 m above sea level), which is the second largest island in the South Shetland Islands, a mountainous archipelago located in Maritime Antarctica. These fruiting bodies grew abundantly on soil, with a profuse development of cryptogams (mosses and the chlorophyte macroalgae *Prasiola*) and the Antarctic hair grass *Deschampsia antarctica* Desv. (Fig. 2). Sampling permit no. CPE-2017-3 was obtained through the Spanish Polar Committee. Specimens were frozen until further processing at the laboratory, where they were observed under a Leica S8APO dissecting microscope equipped with a Leica EC3 image capture system. Handmade sections of lamellae were rehydrated in distilled H₂O to describe anatomical characteristics. Microscopic observations were made using a Zeiss Axioplan 2 microscope fitted with 'Nomarski' differential interference contrast, and photographs were taken with a Zeiss AxioCam digital camera. Microscopic measurements were made by means of the Zeiss Axiovision 4.8 imaging system. Reported data are averages followed by standard deviations, and the maximum and minimum values are given in parentheses.

DNA extraction and polymerase chain reaction amplification

The isolation of genomic DNA from a single *Galerina* basidioma (pl. basidiomata; i.e. basidiomycete fruiting bodies) was done from a piece of lamellae and using the Speed Tools DNA Extraction Kit (Biotools, Madrid, Spain), following the manufacturer's recommendations. The extracted DNA was eluted in a final volume of 60 µl

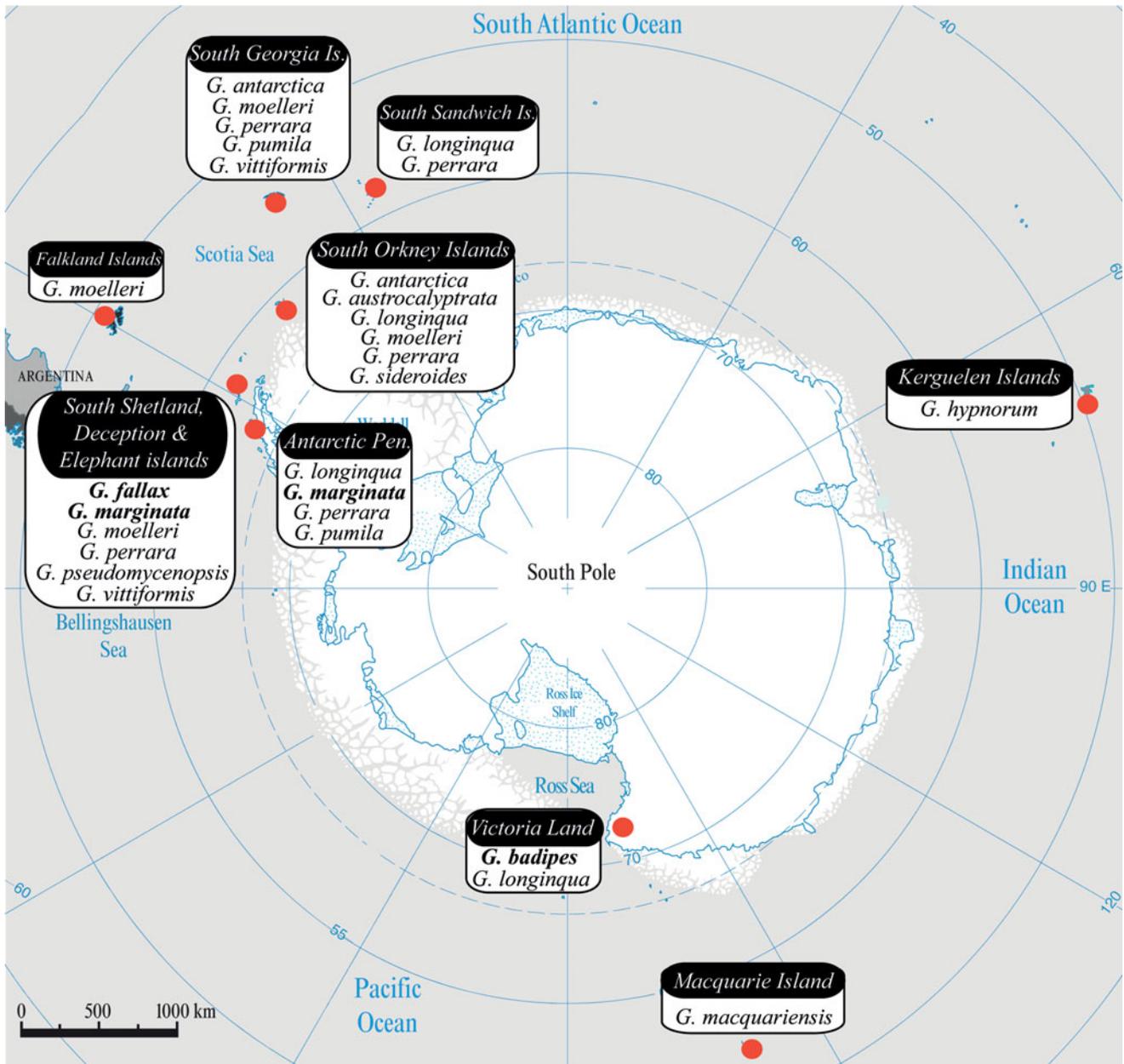


Fig. 1. Diversity and distribution of *Galerina* species in Antarctica based on collection data provided by Bridge *et al.* (2008), Arenz *et al.* (2014), Krishnan *et al.* (2016) and Canini *et al.* (2020). Species for which samples have been included in the present work are in bold.

with sterile purified water (SIGMA). Sequence data of the internal transcribed spacer of the nuclear ribosomal DNA (the so-called fungal barcode marker; Schoch *et al.* 2012) was amplified using the primer pair ITS1F-KYO2 and ITS4-KYO2 (Toju *et al.* 2012). Polymerase chain reaction (PCR) experiments were performed in a total volume of 10 μ l, containing 1 μ l of reaction buffer (Biotools[®]), 2 μ l of dNTPs (1 mM), 0.5 μ l of each primer (10 μ M), 0.2 U of DNA polymerase (Biotools[®]) and 1.5 μ l of the genomic DNA elution; the final volume was reached by adding distilled water (SIGMA). The following PCR temperature profile was employed:

5 min at 95°C, then 30 cycles of 30 s at 95°C, 1 min at 52°C and 1.5 min at 72°C, with a final extension of 10 min at 72°C. The PCR experiments were visualized on 1% agarose gel stained with PRONASAFE nucleic acid stain solution (CONDA Laboratories). The PCR products were purified and cleaned using the UltraClean PCR Clean-Up Kit (MOBIO Laboratories, Inc.). Both complementary DNA strands were sequenced at Macrogen Europe (Spain) using the same primer set as for the initial amplification. Electropherograms were checked and assembled using *SeqManII* v.5.07[®] (DNASTAR, Inc.).

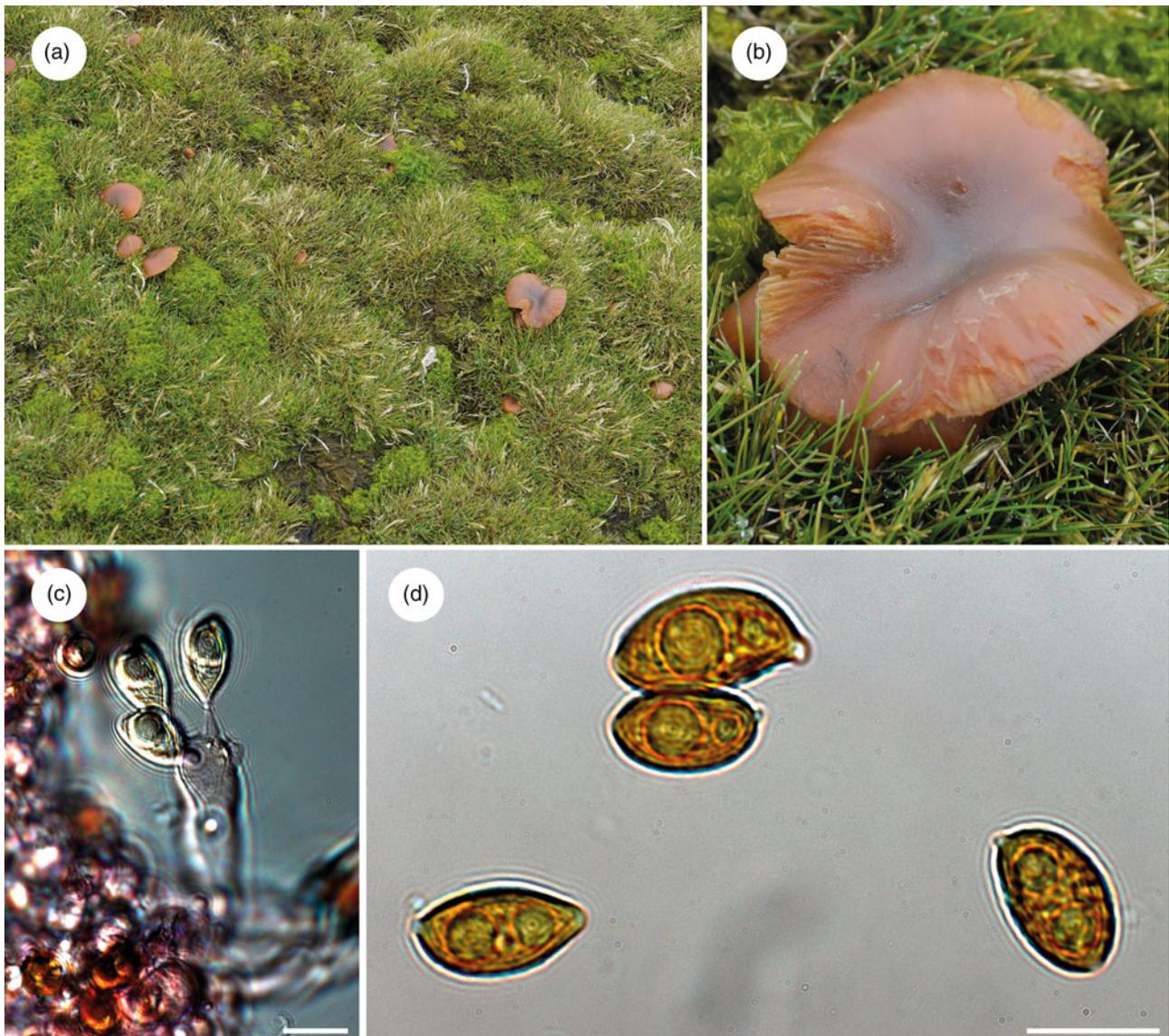


Fig. 2. *Galerina marginata*: **a.** fruiting bodies growing on a carpet of *Deschampsia antarctica* and *Prasiola* sp. in Punta Hannah (Livingston Island), **b.** a detail of the fruiting body pileus, **c.** a basidium (i.e. basidiomycete sporangium) with developing spores and **d.** spores. Scale bars: 10 μ m.

Compilation of the specimen-based nrITS dataset and sequence alignment

The newly produced sequence was submitted to the BLAST online tool (Altschul *et al.* 1990) to check for possible PCR product contamination and to identify and retrieve available, highly similar nrITS sequences. To this purpose, the GenBank (<http://www.ncbi.nlm.nih.gov/>), UNITE (Nilsson *et al.* 2019) and BOLD (Ratnasingham & Hebert 2007) nucleotide databases were used as references. A total of 118 sequences (97 GenBank, 13 UNITE and 8 BOLD) spanning a 97–100% similarity range were downloaded. Most were accessions labelled with the species name *Galerina marginata*

(Batsch) Kühner. A closely related nrITS sequence of a *Galerina* collection from Amsler Island (Antarctic Peninsula) was included as well. This was also collected from an area where mosses were growing under permit ACA-2012-013. DNA extraction and sequencing were done using methods previously described (Blanchette *et al.* 2016). An additional search in public databases was conducted to select and retrieve any available nrITS data for other Antarctic *Galerina* collections. Two sequences obtained from soil isolates were found: MK537266, which was generated in a study by Canini *et al.* (2020) from Victoria Land; and MF692967, from King George Island (Krishnan *et al.* 2018). BLAST searches against the GenBank database revealed a close

match of these accessions to nrITS sequences labelled with the species names *Galerina badipes* (Pers.) Kühner and *Galerina fallax* A.H. Sm. & Singer. Twenty-five sequences hosted in GenBank belonging to these two species were downloaded and incorporated into the dataset as well. Finally, the dataset was completed by including additional *Galerina* species based on works by Gulden *et al.* (2005) and Latha *et al.* (2015), which provided two of the most comprehensive *Galerina* phylogenies published to date. The final nrITS consisted of 178 sequences. We followed Gulden *et al.* (2005) in selecting adequate outgroup taxa for our phylogenetic analyses. Although the genus *Galerina* was revealed to be polyphyletic by these authors, they considered a group of taxa referred to 'tubariopsis' to be a suitable outgroup. In our dataset, this is represented by the following species: *Galerina arctica* (Singer) Nezdójm., *Galerina clavata* (Velen.) Kühner, *Galerina discreta* E. Horak, Senn-Irlet, M. Curti & Musumeci, *Galerina laevis* Singer, *Galerina pseudocerina* A.H. Sm. & Singer and *Galerina stordalii* A.H. Sm.

The program *MAFFT* v.7.308 (Katoh & Standley 2013) was used to generate a multiple-sequence alignment with the following parameters: the FFT-NS-I x1000 algorithm, the 200PAM/ $k=2$ scoring matrix, a gap open penalty of 1.5 and an offset value of 0.123. The resulting alignment was manually optimized in *Geneious* v.9.0.2 to 1) trim alignment ends of longer sequences that included part of the 18S–28S ribosomal subunits, 2) replace gaps at the ends of shorter sequences with an International Union of Pure and Applied Chemistry (IUPAC) base representing any base ('N') and 3) replace doubtful base calls at the extremes with 'N'. The software *GBlocks* 0.91b (Castresana 2000) was subsequently used to automatically deal with ambiguously aligned regions, implementing the least stringent parameters but allowing gaps in 50% of the sequences. Alignments were deposited in FigShare (DOI: 10.6084/m9.figshare.22219546).

Maximum-likelihood phylogenetic analyses

The online version of RAxML-HPC2 hosted at the CIPRES Science Gateway (Stamatakis 2006, Stamatakis *et al.* 2008, Miller *et al.* 2010) was used to estimate two maximum-likelihood (ML) phylogenies based on the *GBlocks*-trimmed (GB) and untrimmed (ORG) alignments. This approach would allow us to evaluate the effect of alignment uncertainty on the inferred nodal support. The analyses used the GTRGAMMA nucleotide substitution model for the two delimited partitions within the nrITS (ITS1+2, 5.8S), and nodal support was evaluated by conducting 1000 rapid bootstrap pseudoreplicates. The resulting phylogenetic trees were visualized in *FigTree* v.1.4 (<http://tree.bio.ed.ac.uk/software/tracer/>), and

Adobe Illustrator CS5 was used for artwork. Tree nodes with bootstrap support (BS) values $\geq 70\%$ were regarded as significantly supported.

Haplotype networks, DNA polymorphism and neutrality tests

The genealogical relationships among specimens included in the *G. marginata* clade were calculated under a statistical parsimony framework in *PopART* v.1.7 (Leigh & Bryant 2015) using the method of Templeton *et al.* (1992). To this purpose, a sub-alignment of 120 sequences was extracted from the *GBlocks*-untrimmed, original alignment. Because the inference of haplotype networks is sensitive to ambiguous base calls and missing data (Joly *et al.* 2007), the sub-alignment was edited to remove 16 sequences with a high proportion of missing data at their extremes and 34 sequences with ambiguous base calls occurring at polymorphic positions. Haplotypes were subsequently inferred with *DnaSP* v.5.10 (Librado & Rozas 2009) considering sites with alignment gaps and removing invariable sites. The network was artistically edited in *Adobe Illustrator CS5* and haplotypes were labelled according to their geographical origin. DNA polymorphism in the 70 remaining sequences was evaluated with the software *DnaSP* v.5.10 (Librado & Rozas 2009). The computed indices were the number of segregating sites (s), the number of haplotypes (h), haplotype diversity (Hd) calculated without considering gap positions and the nucleotide diversity (π) using the Jukes & Cantor (1969) correction. Deviations from neutrality with Tajima's D and Fu's F_s statistics were also assessed to infer past population size changes. The tests were carried out in *DnaSP* v.5.10 using the number of segregating sites, and their significance was assessed based on 10^4 coalescent simulations. We did not infer haplotype networks, nor do we evaluate DNA polymorphism for *G. badipes* and *G. fallax* because of the few sequences these species encompassed and due to the substantial amount of missing data in sequences masking the existing polymorphism.

Dating analyses

The inference of a time frame for the global evolutionary history of *Galerina* was conducted under a Bayesian framework with *BEAST* 1.8.1 (Drummond *et al.* 2012). Because this Basidiomycota genus lacks a suitable fossil record, the dating analysis used a secondary calibration imposed on the nrITS substitution rate. Hence, the BEAST analysis implemented the average rate of 4.61×10^{-3} substitutions per site per million years (s/s/Ma) inferred for the genus *Phaeocollybia* R. Heim in Ryberg & Matheny (2012), because this genus and *Galerina* belong into the family Hymenogastraceae (Matheny *et al.*

2015). This analysis was referred to as Dating A. To take into account the uncertainty associated with that rate, we re-ran analyses using the estimates representing the minimum (2.92×10^{-3} s/s/Ma, Dating B) and maximum (6.45×10^{-3} s/s/Ma, Dating C) values of the rate's 95% credibility interval provided by Ryberg & Matheny (2012). The analyses were conducted with the two alignment versions (GB and ORG) to learn about the impact on age estimates of keeping ambiguously aligned positions in the alignment. Redundant sequences were removed from the alignments using the *FaBox* v.1.41 online toolbox (Villesen 2007). *PartitionFinder* 1.1.1 (Lanfear *et al.* 2012) was used to infer optimal substitution models for the two nrITS partitions considering a model with linked branch lengths and the Bayesian information criterion. This analysis favoured the GTR+ Γ model for the ITS1+ITS2 partition and the K80 model for the 5.8S partition. We conducted preliminary Bayes factor comparisons (Kass & Raftery 1995) of ML estimates (MLEs) calculated with path sampling and stepping-stone approaches (Lartillot & Philippe 2006, Xie *et al.* 2011) to choose among different *BEAST* tree priors and molecular clocks. The use of an uncorrelated lognormal relaxed molecular clock over the strict clock was strongly supported for the GB and ORG datasets (Tables SI & SII). As for the tree priors, models incorporating the coalescent-constant size produced substantially higher MLE values than models using the birth-death and Yule process priors. Runs using chain lengths of 1.5×10^8 steps were implemented, and parameters were logged every 1.5×10^4 steps. Resulting log files were checked in *Tracer* 1.7 to ensure that all parameters had effective sample sizes > 200 after removing the first 20% of saved trees as burn-in. Then, the median heights of the 1×10^4 post-burn-in tree samples were annotated with *TreeAnnotator* 1.8.1, and the chronograms were drawn with *FigTree* 1.4. *Tracer* 1.7 and *TreeAnnotator* 1.8.1 are available at <http://tree.bio.ed.ac.uk/>. We set the value of Bayesian posterior probabilities (PPs) at a minimum of 0.97 for considering tree nodes to be well supported.

Results

Specimen study

The *G. marginata* specimens studied morphologically and phylogenetically showed relatively small pilei of up to 4 cm in diameter, first convex and then turning flat (Fig. 2). Basidia were tetrasporic and produced ellipsoid to broadly amygdaloid, brownish and verrucose spores, with a rounded apex and a visible hilar appendix and containing one to two guttules. The size (length \times width) of 25 spores measured in water was (11.2) 13.0 ± 1.0 (15.8) \times (6.2) 7.4 ± 0.8 (9.9) μm , and the length/width

ratio was (1.4) 1.8 ± 0.1 (2.0). Care must be taken when comparing the size of pilei and microscopic characteristics with literature data because specimens were brought back from Antarctica frozen and were examined after melting, a process that might have affected the structure of these characteristics.

Alignments and phylogenies under ML

The original alignment (ORG) done with *MAFFT* consisted of 178 *Galerina* nrITS sequences and 665 positions, of which 279 were variable and 82 corresponded to singleton sites. After processing the alignment with *GBLOCKS* (GB), 603 positions (90% of the original alignment) were retained in 29 selected blocks; 255 positions were variable and 73 were singleton sites. The ML analyses in *RAxML* estimated phylogenies with $\ln L = -5168.2$ (GB) and $\ln L = -5493.96$ (ORG). Although the topologies inferred based on the two alignments were not identical, they showed no supported conflicts (Supplemental Figs 1 & 2). In general, sister relationships among the different *Galerina* species included in the ingroup lacked support. Bootstrap values > 70% were obtained for the crown nodes encompassing all *G. marginata*, *G. badipes* and *G. fallax* sequences, where the data obtained from Antarctic material are placed in both topologies. It must be highlighted that the *G. marginata* clade, hereinafter referred to as *G. marginata* s.l., included sequences from specimens originally labelled as *Galerina autumnalis*, *G. hygrophila*, *G. pseudomycesopsis*, *G. unicolor* and *G. venenata* (including its type sequence, MH827070). Furthermore, BS values > 78% were found for the sister relationship of *G. marginata* s.l. and *G. badipes*, *G. minima* and *G. atkinsoniana*, *G. pseudobadipes* and *G. stylifera*, and *G. cephalotricha* and *G. mniophila*. The sister relationship of *G. jaapii* with the clade containing *G. marginata* s.l. and *G. badipes* received a BS value of 82% (GB), whereas the clade containing the latter three species along with *G. indica* had a BS value of 81%. Within *G. marginata* s.l., the newly generated sequence from Livingston Island (GenBank accession OQ569484) was located at the bottom and close to three other sequences from Antarctica: OP795715, which was obtained from a basidioma collected at Norsel Point on Amsler Island and differed by one nucleotide; KU559684, an environmental sequence co-occurring in Antarctica and the Arctic (Cox *et al.* 2016), which is shorter than the other sequences and therefore was composed of a number of missing nucleotides; and KT990212, labelled as '*Arrhenia antarctica*' and collected by Halina Galera from an uncertain location within Antarctica that showed missing and ambiguous positions together with one diverging nucleotide. Two sequences labelled as *G. pseudomycesopsis* (AJ585503 and GU234057) collected

in the USA and Svalbard were closely related as well and differed, in general, by fewer than five alignment positions.

Genetic diversity in *G. marginata* s.l.

Forty-two haplotypes, producing a haplotype diversity (Hd) of 0.930, were recovered from the 70 analysed sequences of *G. marginata* s.l. (Fig. 3). The haplotype network revealed a close relationship between the two Antarctic haplotypes and others obtained from Northern Hemisphere specimens, including either North America (USA and Canada) or northern Europe (Scandinavia, Baltic countries and the UK). Single mutations segregated these haplotypes. Furthermore, the haplotype network showed two star-like sub-networks separated by just one mutation. The most evident sub-network was composed of a central haplotype with a wide distribution in North America (especially in Canada) that also occurred in Switzerland (central Europe). Connected to this central haplotype by just one or two mutations were several minor haplotypes from North America and northern Europe. In contrast, the second star-like sub-network had a central haplotype distributed in

Europe overall and the Caucasus mountainous region, and this was linked to minor haplotypes distributed in Europe as well. At the bottom of the network in Fig. 3, a number of haplotypes from Asia (Altay Republic, China, South Korea and Japan) were connected by one or up to four mutations to haplotypes occurring in North America and Mexico. A couple of these haplotypes were shared by regions on both sides of the Pacific Ocean. Finally, Tajima's and Fu's neutrality tests estimated negative values of D (-1.46358, $P > 0.10$; not significant) and F_s (-24.755, $P < 0.001$; significant). The negative values of D and F_s indicate an excess of low-frequency polymorphisms relative to expectation and an excess of the number of alleles, respectively. Collectively, the results from these two tests suggest a population or demographic expansion in *G. marginata* s.l.

BEAST phylogenies and age estimates

The average effective sample sizes were > 200 for all parameters in the Bayesian dating analyses conducted with *BEAST* based on the two alignments (GB and ORG). No supported topological conflicts were observed between the two *BEAST* topologies (Fig. 4) or between

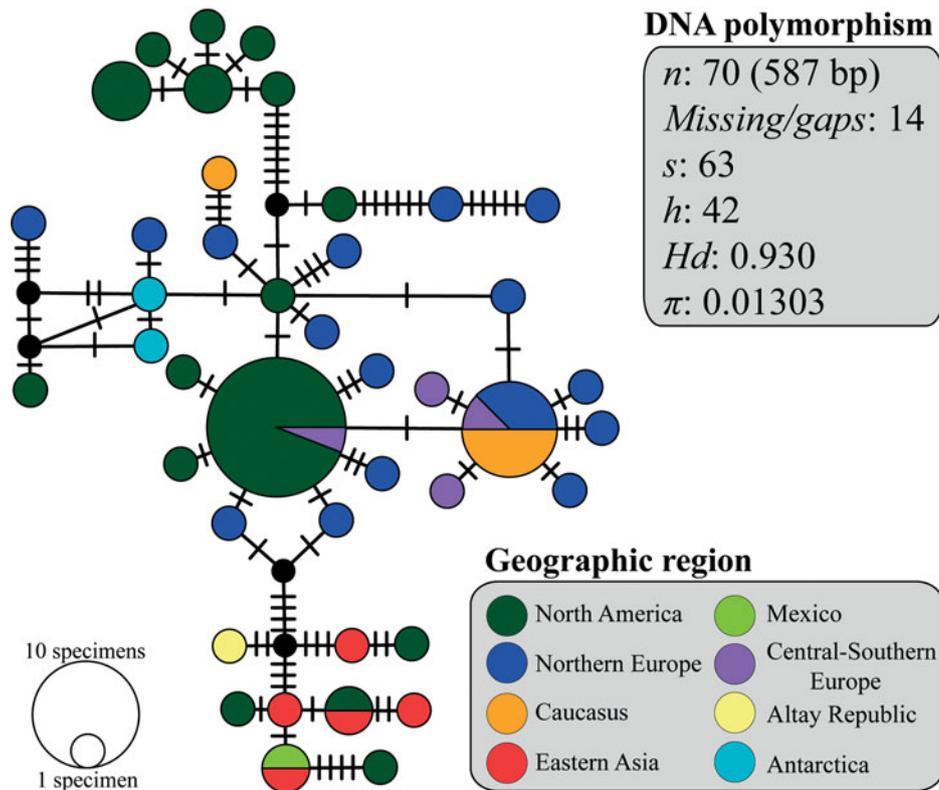


Fig. 3. Statistical parsimony networks connecting *Galerina marginata* haplotypes and summary of DNA polymorphism indices.

Haplotypes were coloured according to the geographical origin of samples. The sizes of the circles in the networks are proportional to the numbers of individuals bearing the haplotype; black-filled smaller circles indicate missing haplotypes. Mutations are shown as hatch marks. S = segregating sites; h = number of haplotypes; Hd = haplotype diversity; π = nucleotide diversity.

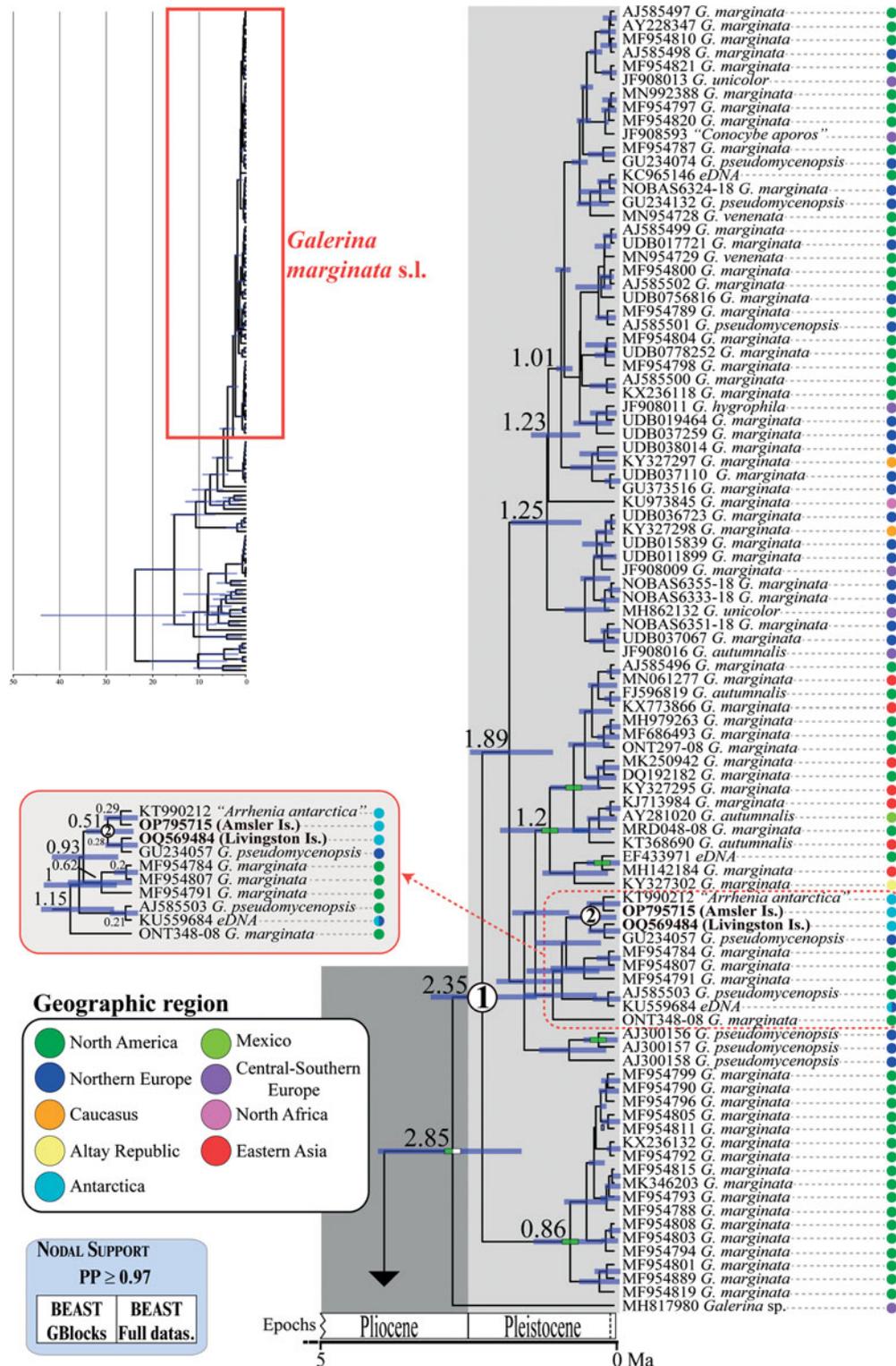


Fig. 4. Chronogram obtained with *BEAST* based on nrITS data depicting the evolutionary history of *Galerina* species. Dashed red rectangles highlight the clades where the Antarctic collections are included. The mean age estimate for the divergence of selected nodes is provided in million years ago (Ma). For each terminal in the tree, the GenBank, UNITE or BOLD nrITS accession number, the taxonomic identity as originally deposited in these databases and the geographical origin are given. Green-filled rectangles indicate nodal support (posterior probability (PP) ≥ 0.97) in analyses using the two versions of the nrITS alignment (GB and ORG). The newly produced nrITS sequences with the corresponding GenBank codes are highlighted in bold. Numbers 1–4 in white circles indicate phylogenetic clades where sequenced specimens of Antarctic *Galerina* are placed: 1–2 = *Galerina marginata*, 3 = *Galerina badipes*) and 4 = *Galerina fallax*.

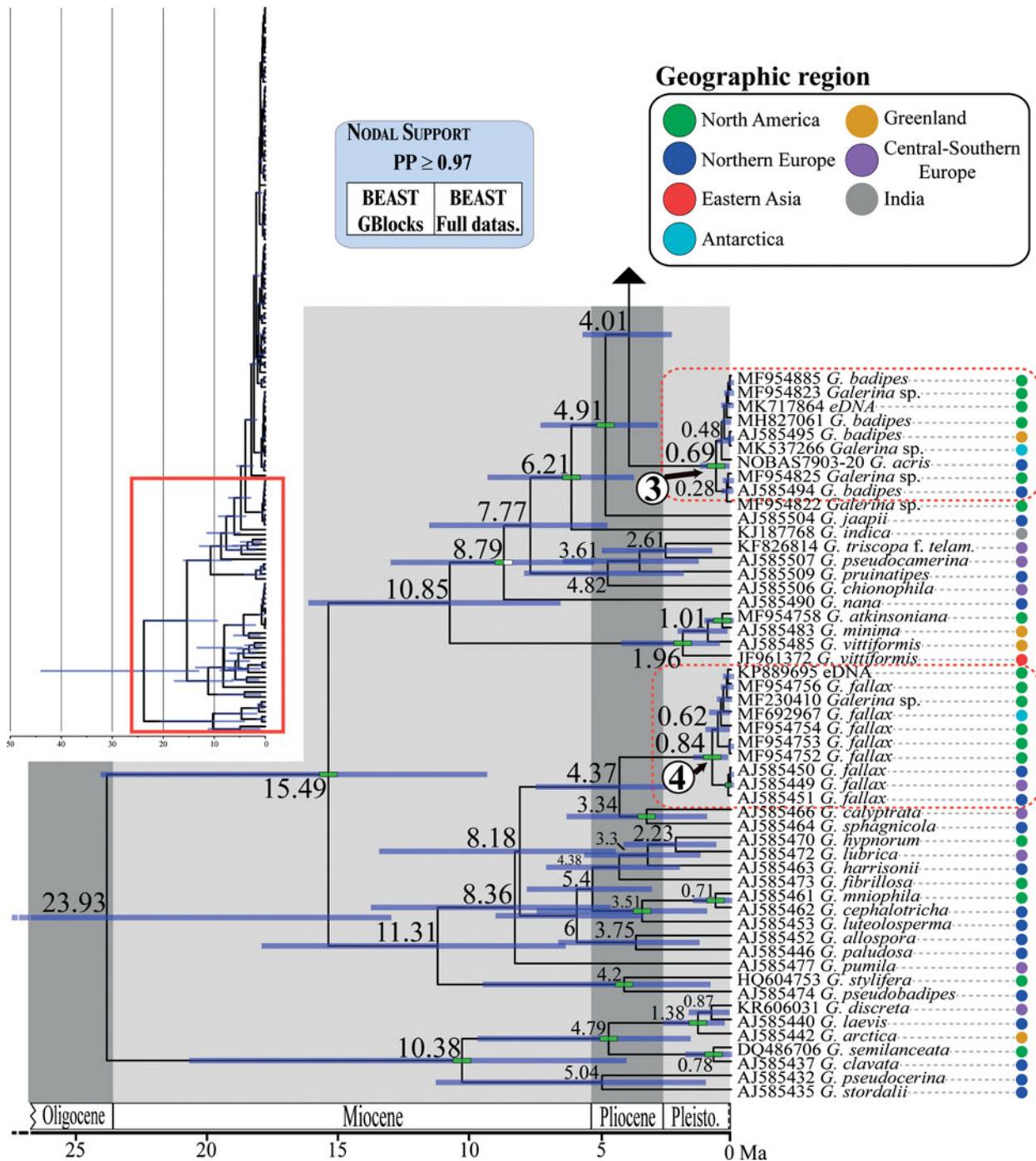


Fig. 4. Continued.

them and those obtained in *RAxML* under ML (Supplemental Figs 1 & 2). However, the *BEAST* analysis using the GB alignment found support ($PP > 0.97$) for a sister relationship between a *Galerina* sp. collected from the Balearic Islands (MH817980) and the bulk of *G. marginata* s.l. sequences. Within *G. marginata* s.l., sequences from Antarctica were phylogenetically close to the same sequences reported in the *RAxML*

analyses. Moreover, two highly supported clades were revealed: one encompassing several sequences from North America and Asia, which correspond with the haplotypes shown at the bottom of the haplotype network (Fig. 3); and a second clade containing several sequences obtained from North American as well as one from Switzerland. This clade is represented by the most evident star-like sub-network in Fig. 3. High support

Table I. Estimated divergence ages for the selected crown nodes in Fig. 4 representing *Galerina* species with Antarctic populations. The dating analyses in *BEAST* used alternative nrITS alignment versions (GB vs ORG). For each of these versions, the mean age value and the corresponding 95% highest posterior density intervals are provided in million years ago (Ma) considering the mean, minimum or maximum values of the nrITS substitution rate inferred for the genus *Phaeocollybia* in Ryberg & Matheny (2012), which was used here for calibration purposes. The time frame proposed in the 'Epoch' column considered the six mean ages estimated in each row.

	GBlocks-trimmed alignment (GB)			Original alignment (ORG)			Epoch
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
(1) Crown <i>Galerina marginata</i> s.l.	2.35 (3.22–1.42)	3.91 (5.03–2.22)	1.61 (2.30–1.04)	3.09 (4.44–1.74)	5.47 (7.00–2.87)	1.76 (2.38–1.01)	Pliocene-Pleistocene
(2) Crown of clade with Antarctic <i>Galerina marginata</i> s.l.	0.51 (0.88–0.07)	1.1 (1.63–0.14)	0.33 (0.72–0.08)	0.57 (0.90–0.13)	2.04 (2.16–0.49)	0.55 (0.83–0.23)	Pleistocene
(3) Crown <i>Galerina badipes</i>	0.69 (1.31–0.17)	1.33 (2.07–0.24)	0.43 (0.92–0.12)	0.79 (1.38–0.16)	1.78 (2.14–0.26)	0.55 (1.00–0.10)	Pleistocene
(4) Crown <i>Galerina fallax</i>	0.84 (1.56–0.23)	1.55 (2.42–0.35)	0.54 (1.10–0.19)	0.95 (1.63–0.24)	2.04 (2.55–0.39)	0.67 (1.16–0.17)	Pleistocene

was also revealed for a clade containing *G. marginata* s.l. and *G. badipes* together with *G. jaapii*, *G. indica*, *G. triscopa* f. *telamonioides*, *G. pseudocamerina*, *G. pruinatipes*, *G. chionophila* and *G. nana*. Supported sister relationships were also found for *G. calyptrata*, *G. sphagnicola* and *G. luteolosperra* and for the pair *G. mniophila-cephalotricha*.

The dating analysis based on the GB alignment generated slightly lower age estimates than the analysis that used the ORG alignment (Table I). However, the inferred 95% highest posterior density (HPD) intervals obtained with the two alternative analyses overlapped to a considerable extent (Table I). For example, when the average nrITS substitution rate of 4.61×10^{-3} s/s/Ma was employed (Dating A), the crown node of *G. marginata* s.l. was dated back to 2.35 Ma (3.22–1.42 Ma, 95% HPD; GB alignment) and 3.09 Ma (4.44–1.74 Ma, 95% HPD; ORG alignment), a time interval at the transition from the Pliocene to the Pleistocene. For simplicity, the discussion below is based on the chronogram estimated with the GB alignment (Fig. 4) because it did not include potentially misaligned (ambiguous) regions. This chronogram reveals that major diversification events in *Galerina* took place since the Miocene epoch (*ca.* 23.03 to 5.30 Ma) and extended into the Pliocene (*ca.* 5.30 to 2.58 Ma). Speciation occurred also in the Pleistocene (*ca.* 2.58 Ma to 11 700 years ago), as is observed between the species *G. mniophila* and *G. cephalotricha* and between *G. minima* and *G. atkinsoniana*. Intraspecific diversification in *G. marginata* s.l., *G. badipes* and *G. fallax*, which include sequences obtained from Antarctic material, occurred mainly in the Pleistocene, with the Antarctic haplotypes originating during the last 500 000 years on average (Fig. 4). Finally, it should be highlighted that Dating B and C, which used the minimum and maximum values of the rate's 95% credibility interval provided by Ryberg & Matheny (2012), produced older and younger age estimates, respectively, compared with results using the

average rate value. Table I summarizes the age estimates and corresponding 95% HPD intervals for selected nodes (see Fig. 4) based on the three dating analyses (Dating A, B and C).

Discussion

The present study validated by means of molecular phylogenetics the existence in Antarctica of populations of *G. marginata*, *G. badipes* and *G. fallax*. The former species (and probably *G. badipes* too) is well known for producing amatoxins, which can have dramatic consequences for human ingestion (Landry *et al.* 2021). The samples of *Galerina* collected from Amsler Island, Antarctica, were also found to contain alpha-amanitin (unpublished data 2013, analyses completed by Jonathan Walton, University of Michigan). These three *Galerina* species, *G. marginata*, *G. badipes* and *G. fallax*, represent relatively common macrofungi in Mediterranean and Temperate-Arctic ecosystems in the Northern Hemisphere (GBIF 2022). The closest genetic lineages to sequenced Antarctic *Galerina* were in fact collected in northern Europe (*G. marginata*), Greenland (*G. badipes*) and North America (*G. fallax*), according to the inferred phylogenies. Therefore, their distribution is here shown to be potentially sub-cosmopolitan or amphitropical, given the few occurrences in tropical regions (GBIF 2022). This biogeographical interpretation of our results supports the opinion of Pegler *et al.* (1980), who used morphological evidence to suggest a close similarity between macrofungal species from the sub-Antarctic and the Temperate-Arctic regions of the Northern Hemisphere. The distribution patterns of the studied *Galerina* match to a great extent with the global geographical distribution of some non-lichenized Antarctic microfungi (Bridge & Newsham 2009, Bridge & Spooner 2012, Cox *et al.* 2016), but, most interestingly, they also match with the amphitropical

distribution pattern displayed by a significant proportion of lichenized fungi, which in Antarctica account for almost 40% of lichens (Øvstedal & Lewis Smith 2001). The existence of nearly identical global distribution patterns in various lichenized and non-lichenized Antarctic fungi makes us hypothesize that, at the geological time scale, these species overcame similar ecological and geographical filters to acquire their current distribution. To accumulate evidence for supporting or rejecting this hypothesis, other Antarctic species of *Galerina* and members of additional non-lichenized macrofungi genera should be surveyed and studied phylogenetically.

The phylogenetic and haplotype network analyses indicated that the studied intraspecific genetic lineages of the Antarctic *Galerina* might be geographically restricted and therefore endemic to this polar region. The fact that the considered species produced fruiting bodies in the surveyed Antarctic localities indicates that these macrofungi have established permanent populations, and therefore they are not transient visitors. Basidiomata formation represents the last step in the life cycle of Basidiomycota fungi. Briefly, it starts with spore germination and mycelium growth once abiotic and biotic requirements are met, followed usually by mating of two compatible, distinct mycelia, and, as a result, basidiomata develop and spores are produced and released after meiosis. We suggest that the three *Galerina* species have been established in this region long enough for mutations to accumulate in the studied genetic locus, the nrITS, which is known to evolve at a higher rate compared to other commonly used fungal molecular markers (Schoch *et al.* 2012). Moreover, the existence of Antarctic-endemic intraspecific lineages of these fungi is of the utmost importance for designing conservation policies that consider a broad spectrum of eukaryotic organisms and not only plants and animals.

However, assessing endemism in fungi poses some risks, even at the intraspecific genetic level. In fact, the existence or not of true Antarctic-endemic non-lichenized fungal lineages has been hotly debated (e.g. Bridge & Spooner 2012, Arenz *et al.* 2014) because of the obvious difficulties in observing and/or isolating macro- and microfungi in Antarctica, or elsewhere, due to their complex life cycles, ecologies and/or sizes. In this sense, Bridge & Spooner (2012) mentioned that some of the allegedly Antarctic-endemic species reported by Onofri *et al.* (2005) were found later elsewhere. Assessing endemism in lichenized fungi is comparatively more straightforward because they usually form macroscopic and enduring lichen thalli (but see Hale *et al.* 2019). Hence, the proportion of Antarctic-endemic lichens has been estimated at ~30% (Øvstedal & Lewis Smith 2001). In our opinion, the analysis of biogeographical patterns in non-lichenized Antarctic fungi may be more accurate

if approached phylogenetically as long as extensive specimen and molecular datasets are compiled. For example, genotypes restricted to Antarctica were revealed for widespread fungi, such as the ascomycete *Thelebolus microsporus* (Berk. & Broome) Kimbr. and other microfungi (de Hoog *et al.* 2005, Bridge & Newsham 2009, Bridge & Spooner 2012, Gonçalves *et al.* 2017). Even so, the lack of availability of sequence data and collections from as yet unexplored areas in the Southern Hemisphere makes it difficult to assess endemism in Antarctic fungi. Although we compiled a large specimen dataset in the present *Galerina* study, it lacked sequence data associated with reports of *G. marginata* and *G. badipes* from Australia and New Zealand (GBIF 2022). Because of the geographical proximity of these austral regions to Antarctica, it would be worth checking whether Antarctic, Australian and New Zealand populations of *Galerina* share the same genotype or are at least closely related. In this way, endemism or colonization routes would be judged more correctly. Furthermore, DNA sequence length and quality are crucial for precise interpretations of biogeographical patterns. This is of particular importance today due to the abundance of short sequences from DNA metabarcoding studies. If short and long sequences are combined in datasets, the resulting biogeographical interpretations could be misleading to some extent, as unrealistic phylogenetic affinities could be inferred. For example, we used a short nrITS sequence of *G. marginata* that co-occurred in Antarctica and the Arctic (KU559684; Cox *et al.* 2016). Despite the existence of identical DNA sequences in individuals from both poles having been observed in, for example, the lichenized fungus *Pseudephebe minuscula* (Arnold) Brodo & D. Hawksw. (Garrido-Benavent *et al.* 2021), it cannot be ruled out that comparison of the *Galerina* sequences along their entire length would reveal some genetic differences.

To the best of our knowledge, the present work is the first examining the temporal origins of Antarctic macrofungal populations. Thus, the Antarctic lineages of *Galerina* probably diverged from their Northern Hemisphere relatives during the Pleistocene, based on a consensus estimate of divergence times inferred with the various dating strategies implemented in the present study. In *G. marginata*, the divergence was probably linked to a demographic expansion, as revealed by the calculated neutrality tests. Moreover, the calculated divergence time intervals for the three *Galerina* agree with the inferred Pleistocene origin of Antarctic populations of amphitropical lichens (Fernández-Mendoza & Printzen 2013, Garrido-Benavent *et al.* 2021). In the lichenized fungi *Cetraria aculeata* (Schreb.) Fr. and *P. minuscula*, a close genetic affinity of Maritime Antarctica specimens to South American (Chilean) collections suggested a

colonization route through the Sea of Hoces (Drake Passage). In addition, continental specimens of the second species were genetically close to Svalbard (Northern Hemisphere) specimens, which indicated an independent colonization route. The closest relatives of *G. marginata*, *G. badipes* and *G. fallax*, based on the inferred phylogenies, grew in North America and northern Europe, so that a direct, long-distance dispersal across the tropics and ending in the establishment of Antarctic *Galerina* populations might be assumed. However, the possibility that these species colonized the Antarctic in a series of stepping-stone movements from other territories in the Southern Hemisphere, for which neither specimen nor sequence data are yet available, must not be ruled out. South America was in fact the region from which the vascular plant *D. antarctica* is believed to have colonized the Antarctic region, also during the Pleistocene (Fasanella *et al.* 2017). It is worth recalling that the studied *Galerina* species grew in tight association with carpets of this plant as well as mosses, where these fungi behave as saprophytes. Even some common populations of Antarctic mosses had a Pleistocene origin (Pisa *et al.* 2014, Biersma *et al.* 2017, 2018). The overlapping temporal frameworks for the origins of these plants and fungi that coexist in the same Antarctic terrestrial communities further support a relatively recent Antarctic colonization of *Galerina*. The meiotic spores produced by their basidiomata, which in general are ellipsoidal or amygdaliform and < 15 µm in length, constitute the expected mode of dispersal, and either wind currents or migratory birds could be involved in such transoceanic dispersals (Muñoz *et al.* 2004, Viana *et al.* 2016). For example, Biersma *et al.* (2018) inferred aerial models that indicated local wind patterns as the most probable transfer mechanisms from southern South America to the northern Maritime Antarctic. A greater research effort is needed to corroborate these means of dispersal.

The biogeographical history of the studied Antarctic *Galerina* has been interpreted on the basis of time trees inferred using a secondary calibration (i.e. nrITS substitution rate). Although this approach would be expected to lead to more inaccurate dating results than phylograms calibrated using fossil data (Schenk 2016), the divergence times calculated for the whole *Galerina* phylogeny in this study are largely coherent with those reported for the divergence of species in other Basidiomycota genera (e.g. *Amanita*, *Heterobasidion*, *Russula*) that used different calibration strategies and more extensive molecular sequence datasets (Chen *et al.* 2015, Sánchez-Ramírez *et al.* 2015, Looney *et al.* 2020). Furthermore, the diversification events within the three Antarctic *Galerina* species also agree with the inferred colonization events of *D. antarctica* and mosses that, together with these fungi, form typical terrestrial habitats in Antarctica.

Acknowledgements

The UTM-CSIC technicians are thanked for their assistance during Antarctic fieldwork. The authors also acknowledge Esther Rodríguez (MNCN) for technical assistance in the laboratory; Alexandra Isern, Director Antarctic Earth Sciences, US National Science Foundation, Carolyn Lipke and other personnel at Palmer Station, Polar Programs; the US National Science Foundation for their help collecting *Galerina* samples from the Antarctic Peninsula; and Benjamin Held, University of Minnesota, for laboratory assistance. This research was part of POLARCSIC activities. Two anonymous peer reviewers are thanked for their feedback.

Funding

This work was supported by grants CTM2017-84441-R (MINECO/FEDER, UE) and PID2019-105469RB-C22 (MICINN, AEI).

Author contributions

IG-B: conceived the project, conducted the analyses and wrote the first draft of the manuscript. RAB: contributed to the molecular dataset and editing of the final manuscript. AdIR: obtained funding and field resources, conducted fieldwork (including specimen collection) and contributed to the editing of the final manuscript.

Supplemental material

A supplemental table will be found at <https://doi.org/10.1017/S0954102023000196>.

References

- ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W. & LIPMAN, D.J. 1990. Basic Local Alignment Search Tool. *Journal of Molecular Biology*, **215**, 403–410.
- ARENZ, B.E. & BLANCHETTE R.A. 2009. Investigations of fungal diversity in wooden structures and soils at historic sites on the Antarctic Peninsula. *Canadian Journal of Microbiology*, **55**, 46–56.
- ARENZ, B.E. & BLANCHETTE R.A. 2011. Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea region and McMurdo Dry Valleys. *Soil Biology and Biochemistry*, **43**, 308–315.
- ARENZ, B.E., BLANCHETTE, R.A. & FARRELL, R.L. 2014. Fungal diversity in Antarctic soils. In COWAN, D., ed. *Antarctic terrestrial microbiology*. Berlin, Springer, 35–53.
- ARENZ, B.E., HELD, B.W., JURGENS, J.A. & BLANCHETTE R.A. 2011. Fungal colonization of exotic substrates in Antarctica. *Fungal Diversity*, **49**, 13–22.
- ASPLUND, J. & WARDLE, D.A. 2017. How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*, **92**, 1720–1738.
- BERKELEY, M.J. 1847. Fungi. In HOOKER J.D., ed. *The botany of the Antarctic voyage of H.M. Discovery ships Erebus and Terror in the years 1839–1843. Part 2. Flora Antarctica. Botany of Fuegia, The Falklands, Kerguelen's Land, etc.* London, Reeve Brothers, 447–454.

- 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**, 599–610.
- BIERSMA, E.M., JACKSON, J.A., HYVÖNEN, J., KOSKINEN, S., LINSE, K., GRIFFITHS, H. & CONVEY, P. 2017. Global biogeographic patterns in bipolar moss species. *Royal Society Open Science*, **4**, 170147.
- BLANCHETTE, R.A., HELD, B.W., HELLMANN, L., MILLMAN, L. & BÜNTGEN, U. 2016. Arctic driftwood reveals unexpectedly rich fungal diversity. *Fungal Ecology*, **23**, 58–65.
- BLANCHETTE, R.A., HELD, B.W., ARENZ, B.E. JURGENS, J.A., BALTES, N.J., DUNCAN, S.M. & FARRELL, R.L. 2010. An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. *Microbial Ecology*, **60**, 29–38.
- BRIDGE, P.D. & NEWSHAM K.K. 2009. Soil fungal community of composition at Mars Oasis, a southern Maritime Antarctic site, assessed by PCR amplification and cloning. *Fungal Ecology*, **2**, 66–74.
- BRIDGE, P.D. & SPOONER, B.M. 2012. Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? *Fungal Ecology*, **5**, 381–394.
- BRIDGE, P.D., SPOONER, B.M. & ROBERTS, P.J. 2008. List of Non-Lichenized Fungi from the Antarctic Region. Version 2.3.3. Retrieved from https://legacy.bas.ac.uk/bas_research/data/access/fungi/ (accessed 13 September 2022).
- BROADY, P.A. & WEINSTEIN, R.N. 1998. Algae, lichens and fungi in La Gorce Mountains, Antarctica. *Antarctic Science*, **10**, 376–385.
- CANINI, F., GEML, J., D'ACQUI, L.P., SELBMANN, L., ONOFRI, S., VENTURA, S. & ZUCCONI, L. 2020. Exchangeable cations and pH drive diversity and functionality of fungal communities in biological soil crusts from coastal sites of Victoria Land, Antarctica. *Fungal Ecology*, **45**, 100923.
- CASTRESANA, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, **17**, 540–552.
- CHEN, J.J., CUI, B.K., ZHOU, L.W., KORHONEN, K. & DAI, Y.C. 2015. Phylogeny, divergence time estimation, and biogeography of the genus *Heterobasidion* (Basidiomycota, Russulales). *Fungal Diversity*, **71**, 185–200.
- COLEINE, C., STAJICH, J.E., ZUCCONI, L., ONOFRI, S., POMBUBPA, N., EGIDI, E., *et al.* 2018. Antarctic cryptoendolithic fungal communities are highly adapted and dominated by Lecanoromycetes and Dothideomycetes. *Frontiers in Microbiology*, **9**, 1392.
- COX, F., NEWSHAM, K.K., BOL, R., DUNGAIT, J.A. & ROBINSON, C.H. 2016. Not poles apart: Antarctic soil fungal communities show similarities to those of the distant Arctic. *Ecology Letters*, **19**, 528–536.
- DE HOOG, G.S., GOTTLICH, E., PLATAS, G., GENILLOU, O., LEOTTA, G. & VAN BRUMMELEN, J. 2005. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. *Studies in Mycology*, **51**, 33–76.
- DRUMMOND, A.J., SUCHARD, M.A., XIE, D. & RAMBAUT, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.
- FASANELLA, M., PREMOLI, A.C., URDAMPILLETA, J.D., GONZÁLEZ, M.L. & CHIAPELLA, J.O. 2017. How did a grass reach Antarctica? The Patagonian connection of *Deschampsia antarctica* (Poaceae). *Botanical Journal of the Linnean Society*, **185**, 511–524.
- FERNÁNDEZ-MENDOZA, F. & PRINTZEN, C. 2013. Pleistocene expansion of the bipolar lichen *Cetraria aculeata* into the Southern hemisphere. *Molecular Ecology*, **22**, 1961–1983.
- GARRIDO-BENAVENT, I., DE LOS RÍOS, A., FERNÁNDEZ-MENDOZA, F. & PÉREZ-ORTEGA, S. 2018. No need for stepping stones: direct, joint dispersal of the lichen-forming fungus *Mastodia tessellata* (Ascomycota) and its photobiont explains their bipolar distribution. *Journal of Biogeography*, **45**, 213–224.
- GARRIDO-BENAVENT, I., PÉREZ-ORTEGA, S., DE LOS RÍOS, A., MAYRHOFER, H. & FERNÁNDEZ-MENDOZA, F. 2021. Neogene speciation and Pleistocene expansion of the genus *Pseudophebe* (Parmeliaceae, lichenized fungi) involving multiple colonizations of Antarctica. *Molecular Phylogenetics and Evolution*, **155**, 107020.
- GARRIDO-BENAVENT, I., SÖCHTING, U., DE LOS RÍOS MURILLO, A. & PÉREZ-ORTEGA, S. 2016. *Shackletonia cryodesertorum* (Teloschistaceae, Ascomycota), a new species from the McMurdo Dry Valleys (Antarctica) with notes on the biogeography of the genus *Shackletonia*. *Mycological Progress*, **15**, 743–754.
- GARRIDO-BENAVENT, I., PÉREZ-ORTEGA, S., DURÁN, J., ASCASO, C., POINTING, S.B., RODRÍGUEZ-CIELOS, R., *et al.* 2020. Differential colonization and succession of microbial communities in rock and soil substrates on a Maritime Antarctic glacier forefield. *Frontiers in Microbiology*, **11**, 126.
- GBIF. 2022. *Galerina marginata* (Batsch) Kühner. In GBIF Secretariat (2021), GBIF Backbone Taxonomy. Checklist dataset. Retrieved from <https://doi.org/10.15468/39omei> (accessed 15 September 2022).
- GONÇALVES, V.N., VITORELI, G.A., DE MENEZES, G.C., MENDES, C.R., SECCHI, E.R., ROSA, C.A. & ROSA, L.H. 2017. Taxonomy, phylogeny and ecology of cultivable fungi present in seawater gradients across the Northern Antarctica Peninsula. *Extremophiles*, **21**, 1005–1015.
- GRZESIAK, B. & WOLSKI, G.J. 2015. Bryophilous species of the genus *Galerina* in peat bogs of Central Poland. *Herzogia*, **28**, 607–623.
- GULDEN, G., STENSURUD, Ø., SHALCHIAN-TABRIZI, K. & KAUSERUD, H. 2005. *Galerina* Earle: a polyphyletic genus in the consortium of dark-spored agarics. *Mycologia*, **97**, 823–837.
- GUMIŃSKA, B., HEINRICH, Z. & OLECH, M. 1994. Macromycetes of the South Shetland Islands (Antarctica). *Polish Polar Research*, **15**, 103–109.
- HALE, E., FISHER, M.L., KEULER, R., SMITH, B. & LEAVITT, S.D. 2019. A biogeographic connection between Antarctica and montane regions of western North America highlights the need for further study of leicideoid lichens. *The Bryologist*, **122**, 315–324.
- HARPER, C.J., TAYLOR, T.N., KRINGS, M. & TAYLOR, E.L. 2016. Structurally preserved fungi from Antarctica: diversity and interactions in late Palaeozoic and Mesozoic polar forest ecosystems. *Antarctic Science*, **28**, 153–173.
- HELD, B.W. & BLANCHETTE R.A. 2017. Deception Island Antarctica harbors a diverse assemblage of wood decay fungi. *Fungal Biology*, **121**, 145–157.
- HORAK, E. 1994. Addenda ad Galerinam. 1. *Galerina robertii* sp. n., eine neue Art aus den französischen Alpen. *Zeitschrift für Mykologie*, **60**, 85–90.
- JOLY, S., STEVENS, M.I. & VAN VUUREN, B.J. 2007. Haplotype networks can be misleading in the presence of missing data. *Systematic Biology*, **56**, 857–862.
- JUKES, T.H. & CANTOR, C.R. 1969. Evolution of protein molecules. *Mammalian Protein Metabolism*, **3**, 21–132.
- KAPPEN, L., FRIEDMANN, E.I. & GARTY, J. 1981. Ecophysiology of lichens in the dry valleys of Southern Victoria Land, Antarctica I. Microclimate of the cryptoendolithic lichen habitat. *Flora*, **171**, 216–235.
- KASS, R.E. & RAFTERY, A.E. 1995. Bayes factors. *Journal of the American Statistical Association*, **90**, 773–795.
- KATO, K. & STANDLEY, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**, 772–780.
- KOHLER, A., KUO, A., NAGY, L.G., MORIN, E., BARRY, K.W., BUSCOT, F., *et al.* 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics*, **47**, 410–415.
- KRISHNAN, A., CONVEY, P., GONZÁLEZ-ROCHA, G. & ALIAS, S.A. 2016. Production of extracellular hydrolase enzymes by fungi from King George Island. *Polar Biology*, **39**, 65–76.
- KRISHNAN, A., CONVEY, P., GONZÁLEZ, M., SMYKLA, J. & ALIAS, S.A. 2018. Effects of temperature on extracellular hydrolase enzymes from soil microfungi. *Polar Biology*, **41**, 537–551.
- LANDRY, B., WHITTON, J., BAZZICALUPO, A.L., CESKA, O. & BERBEE, M.L. 2021. Phylogenetic analysis of the distribution of deadly amatoxins

- among the little brown mushrooms of the genus *Galerina*. *PLoS ONE*, **16**, e0246575.
- LANFEAR, R., CALCOTT, B., HO, S.Y. & GUINDON, S. 2012. *PartitionFinder*: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**, 1695–1701.
- LARTILLOT, N. & PHILIPPE, H. 2006. Computing Bayes factors using thermodynamic integration. *Systematic Biology*, **55**, 195–207.
- LATHA, K.D., RAJ, K.A., PARAMBAN, R. & MANIMOHAN, P. 2015. Two new bryophilous agarics from India. *Mycoscience*, **56**, 75–80.
- LEIGH, J.W. & BRYANT, D. 2015. *PopART*: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, **6**, 1110–1116.
- LIBRADO, P. & ROZAS, J. 2009. *DnaSP v5*: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.
- LOONEY, B.P., ADAMČIK, S. & MATHENY, P.B. 2020. Coalescent-based delimitation and species-tree estimations reveal Appalachian origin and Neogene diversification in *Russula* subsection *Roseinae*. *Molecular Phylogenetics and Evolution*, **147**, 106787.
- MATHENY, P.B., MOREAU, P.A., VIZZINI, A., HARROWER, E., DE HAAN, A., CONTU, M. & CURTI, M. 2015. *Crassisporium* and *Romagnesiella*: two new genera of dark-spored Agaricales. *Systematics and Biodiversity*, **13**, 28–41.
- MILLER, M.A., PFEIFFER, W. & SCHWARTZ, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, 14 November 2010*. Red Hook, NY: Curran Associates, 1–8.
- MUÑOZ, J., FELICISIMO, A.M., CABEZAS, F., BURGAS, A.R. & MARTÍNEZ, I. 2004. Wind as a long-distance dispersal vehicle in the Southern Hemisphere. *Science*, **304**, 1144–1147.
- NEWSHAM, K.K., DAVEY, M.L., HOPKINS, D.W. & DENNIS, P.G. 2021. Regional diversity of Maritime Antarctic soil fungi and predicted responses of guilds and growth forms to climate change. *Frontiers in Microbiology*, **11**, 615659.
- NILSSON, R.H., LARSSON, K.H., TAYLOR, A.F.S., BENGTSSON-PALME, J., JEPPESEN, T.S., SCHIGEL, D., *et al.* 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, **47**, D259–D264.
- ONOFRI, S., SELBMANN, L., ZUCCONI, L., TOSI, S. & DE HOOG, G.S. 2005. The mycota of continental Antarctica. *Terra Antarctic Reports*, **11**, 37–42.
- ØVSTEDAL, D.O. & LEWIS SMITH, R.I. 2001. *Lichens of Antarctica and South Georgia: a guide to their identification and ecology*. Cambridge University Press, Cambridge, UK, 424 pp.
- ØVSTEDAL, D.O. & LEWIS SMITH, R.I. 2011. Four additional lichens from the Antarctic and South Georgia, including a new *Leciophysma* species. *Folia Cryptogamica Estonica*, **48**, 65–68.
- PEAT, H.J., CLARKE, A. & CONVEY, P. 2007. Diversity and biogeography of the Antarctic flora. *Journal of Biogeography*, **34**, 132–146.
- PEGLER, D.N., SPOONER, B.M. & LEWIS SMITH, R.I. 1980. Higher fungi of Antarctica, the subantarctic zone and Falkland Islands. *Kew Bulletin*, **35**, 499–562.
- PÉREZ-ORTEGA, S., ORTIZ-ÁLVAREZ, R., GREEN, T.G.A. & DE LOS RÍOS, A. 2012. Lichen myco- and photobiont diversity and their relationships at the edge of life (McMurdo Dry Valleys, Antarctica). *FEMS Microbiology Ecology*, **82**, 429–448.
- PISA, S., BIERMA, E.M., CONVEY, P., PATIÑO, J., VANDERPOORTEN, A., WERNER, O. & ROS, R.M. 2014. The cosmopolitan moss *Bryum argenteum* in Antarctica: recent colonisation or in situ survival? *Polar Biology*, **37**, 1469–1477.
- PUTZKE, J., PUTZKE, M.T.L., PEREIRA, A.B. & ALBUQUERQUE, M.P. 2012. *Agaricales* (Basidiomycota) fungi in the South Shetland Islands, Antarctica. *INCT-APA Annual Activity Report*. Retrieved from <https://doi.editoracubo.com.br/10.4322/apa.2014.065> (accessed 15 June 2023).
- RATNASINGHAM, S. & HEBERT, P.D. 2007. BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes*, **7**, 355–364.
- ROSA, L.H., PINTO, O.H.B., CONVEY, P., CARVALHO-SILVA, M., ROSA, C.A. & CÂMARA, P.E.A.S. 2021. DNA metabarcoding to assess the diversity of airborne fungi present over Keller Peninsula, King George Island, Antarctica. *Microbial Ecology*, **82**, 165–172.
- RUISE, S., BARRECA, D., SELBMANN, L., ZUCCONI, L. & ONOFRI, S. 2007. Fungi in Antarctica. *Reviews in Environmental Science and Biotechnology*, **6**, 127–141.
- RYBERG, M. & MATHENY, P.B. 2012. Asynchronous origins of ectomycorrhizal clades of Agaricales. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2003–2011.
- SÁNCHEZ-RAMÍREZ, S., TULLOSS, R.E., AMALFI, M. & MONCALVO, J.M. 2015. Palaeotropical origins, boreotropical distribution and increased rates of diversification in a clade of edible ectomycorrhizal mushrooms (*Amanita* section *Caesareae*). *Journal of Biogeography*, **42**, 351–363.
- SCHENK, J.J. 2016. Consequences of secondary calibrations on divergence time estimates. *PLoS ONE*, **11**, e0148228.
- SCHOCH, C.L., SEIFERT, K.A., HUHDORF, S., ROBERT, V., SPOUGE, J.L., LEVESQUE, C.A., *et al.* 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceeding of the National Academy of Sciences of the United States of America*, **109**, 6241–6246.
- SINGER, R. & CORTE, A. 1962. Estudio sobre los Basidiomycetes antárticos. *Contribuciones científicas del Instituto Antártico Argentino*, **71**, 1–43.
- SØCHTING, U., ØVSTEDAL, D.O. & SANCHO, L.G. 2004. The lichens of Hurd Peninsula, Livingston Island, South Shetlands, Antarctica. *Bibliotheca Lichenologica*, **88**, 607–658.
- STAMATAKIS, A. 2006. *RAXML-VI-HPC*: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.
- STAMATAKIS, A., HOOVER, P. & ROUGEMONT, J. 2008. A rapid bootstrap algorithm for the *RAXML* web-servers. *Systematic Biology*, **57**, 758–771.
- TEMPLETON, A.R., CRANDALL, K.A. & SING, C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.
- TOJU, H., TANABE, A.S., YAMAMOTO, S. & SATO, H. 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS ONE*, **7**, e40863.
- TRESEDER, K.K. & LENNON, J.T. 2015. Fungal traits that drive ecosystem dynamics on land. *Microbiology and Molecular Biology Reviews*, **79**, 243–262.
- VIANA, D.S., SANTAMARÍA, L. & FIGUEROLA, J. 2016. Migratory birds as global dispersal vectors. *Trends in Ecology and Evolution*, **31**, 763–775.
- VILLESEN, P. 2007. *FaBox*: an online toolbox for fasta sequences. *Molecular Ecology Notes*, **7**, 965–968.
- VISHNIAC, H.S. & HEMPFING, W.P. 1979. Evidence of an indigenous microbiota (yeast) in the dry valleys of Antarctica. *Microbiology*, **112**, 301–314.
- WHITE JR, J.F. & TAYLOR, T.N. 1991. Fungal sporocarps from Triassic peat deposits in Antarctica. *Review of Palaeobotany and Palynology*, **67**, 229–236.
- XIE, W., LEWIS, P.O., FAN, Y., KUO, L. & CHEN, M.H. 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology*, **60**, 150–160.