

Diversity of the genus *Ramaria* in the Patagonian Andes Forests of Argentina

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Abstract

Within the wide diversity of fungal species of the Patagonian Andes forests, species of the genus *Ramaria* are some of the most attractive. Its macro and micro-morphological characters are so diverse that the species cannot be readily separated based on morphological characters alone. In Patagonia (Argentina and Chile) *Ramaria* has been reported associated with Nothofagaceae; however, their diversity and phylogenetic relationships are largely unknown. Currently, 18 species are reported for the region. In this study, a detailed account of *Ramaria* in Patagonia is presented based on an integrative analysis of phylogenetic, macro- and micro-morphological features. Internal transcribed spacer (ITS) and nuclear 28S rDNA (LSU) genes were analyzed to identify specimens from Argentina and to examine their phylogenetic relationships with other *Ramaria* species. The phylogenetic tree revealed that the sequences represent six *Ramaria* species found in Argentinian Patagonia: *R. patagonica*, *R. botrytis*, *R. inedulilis*, *R. stricta*, and two new phylogenetic groups characterized as new species: *R. flavinedulis* and *R. dendrophora*. *Ramaria flavinedulis* produces brightly colored yellow or yellow-orange basidioma with a fused, twisted compound stipe, basidia not clamped, and basidiospores ellipsoid to cylindrical. *Ramaria dendrophora* exhibits pale yellow to pale rose basidioma, basidiospores ornamented with conspicuous and irregular warts. This study increased our knowledge of the phylogenetic diversity and taxonomy in *Ramaria* from the Patagonian Andes Forests compared with those found elsewhere.

Introduction

Ramaria Fr. ex Bonord. (Gomphaceae, Gomphales) is a widespread non-gilled basidiomycete genus (Marr & Stuntz 1973, Petersen 1981, Humpert et al. 2001) which includes a remarkable number of cosmopolitan species, with different lifestyles. Many species have been confirmed as presenting an ectomycorrhizal lifestyle, and it has been suggested that this is shared by all terricolous *Ramaria* spp. (Humpert et al. 2001). Other species are saprobic, lignicolous or humicolous (Marr & Stuntz 1973, Exeter et al. 2006). *Ramaria* was initially treated as a subgenus within *Clavaria* (Coker 1923, Doty 1944) until Corner (1970) elevated it to genus rank. With more than 230 species described worldwide, it is the most diverse genus within the Gomphales, *R. botrytis* (Pers.) Ricken is the type species.

Different studies based on morphological and molecular data (Humpert et al. 2001, Hosaka et al. 2006, Giachini et al. 2010) agree on the paraphyletic state of *Ramaria*. Its macro- and micro-morphological characters are so diverse that the species cannot be readily separated based on morphological characters alone (Nouhra et al. 2005). However, the genus shares the following typical features: highly branched basidiomata; mono- to dimitic hyphal systems with clamped or simple-septate generative hyphae, mostly guttulate; yellow to ochraceous or brown colored basidiospores with smooth walls or with echinulate, verrucose-reticulate or striate ornamentation (Corner 1950, Marr & Stuntz 1973, Petersen 1981, Humpert et al. 2001). Currently, the genus is divided into four subgenera (Marr & Stuntz 1973, Exeter et al. 2006, Humpert et al. 2001, Knudson 2012): (I) *Ramaria* subg. *Ramaria*, (II) *Ramaria* subg. *Laeticolora* Marr & D.E. Stuntz, (III) *Ramaria* subg. *Lentoramaria* Corner, and (IV) *Ramaria* subg. *Echinoramaria* Corner (Marr & Stuntz 1973, Humpert et al. 2001, Exeter et al. 2006, Hanif et al. 2019). *Ramaria* subg. *Ramaria* contains large species that grow from the soil and that often present a cauliflower appearance, the generative hyphae are clamped and the spores have striate ornamentation (Humpert et al. 2001, Knudson 2012). Species of *R.* subg. *Laeticolora* are large and terricolous, with or without clamps, and smooth or warty spores (Corner 1950, Humpert et al. 2001, Knudson 2012). Both subgenera often have brightly colored fruit bodies (Exeter et al. 2006). *Lentoramaria* species grow on rotting wood, have smooth or verrucose ornamentation but consistently have clamps. The subgenus *Echinoramaria* is the most easily distinguishable based on the echinulated spore ornamentation, by always presenting clamped generative hyphae, and by the relatively small basidiomata (Humpert et al. 2001.) *Lentoramaria* and *Echinoramaria* fruiting bodies have neutral colors, in browns and creams (Exeter et al. 2006).

In Patagonia (Argentina and Chile) *Ramaria* has been reported associated with Nothofagaceae (Spegazzini 1929, Singer 1969), but their diversity and phylogenetic relationships are largely unknown. Currently, 18 species are reported for the region: *R. stricta* (Pers.) Quél., *R. flava* (Schaeff.) Quél., *R. flaccida* (Fr.) Bourdot, *R. aurea* (Schaeff.) Quél., *R. aurantiaca* Corner, *R. subaurantiaca* Corner, *R. holrubella* (G.F. Atk.) Corner, *R. flavobrunnescens* (G.F. Atk.) Corner, *R. zipelli* (Lév.) Corner, *R. strasseri* (Bres.) Corner, *R. subtilis* (Coker) Schild, *R. moelleriana* (Bres. & Roum.) Corner, *R. obtusissima* (Peck) Corner, *R. acutissima* (Berk.) Corner, *R. botrytis* (Pers.) Ricken, *R. valdiviana* Singer, *R. patagonica* (Speg.) Corner and *R. inedulilis* Singer, the last three considered endemic. *Ramaria patagonica* is traditionally appreciated by the local communities because of its flavor and texture and is commonly named 'changle' (Molares et al. 2020). Biochemical studies have revealed it is low in fat, rich in proteins and carbohydrates, and has high antioxidant activity, highlighting its potential as a functional food and a source of bioactive compounds (Toledo et al. 2016).

The aims of this study were to analyze the Patagonian taxa of *Ramaria* from Nothofagaceae forests from a phylogenetic perspective and to describe two new species. We collected specimens and examined their diversity using molecular and morphological data. Given the edibility of several *Ramaria* species, intraspecific morphological and phylogenetic variability detection is an important contribution to the correct analysis of their use and properties.

Materials And Methods

Specimens studied

A total of 49 basidiomata of *Ramaria* spp. were collected and GPS-referenced during the autumn of 2019 and 2020 in the Patagonian provinces of Chubut, Río Negro, Neuquén and Tierra del Fuego (Fig. 1). Most specimens were photographed *in situ* or in the laboratory while fresh. Each collection site was characterized by the surrounding dominating tree species. Basidiomata were air-dried for subsequent analysis, and all collections were deposited at the Herbarium Centro de Investigación y Extensión Forestal Andino Patagónico (HCFC; Thiers, continuously updated).

Morphological studies

Specimens examined are presented in Table 1, including specimens kept at the Institute of Botany Carlos Spegazzini (LPS) and the Herbarium of Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BAFC). Morphology (stipe, branching type and apex), color, size and shape were recorded. The positive test reaction of ferric sulfate (FSW) on hymenial surfaces (Exeter et al. 2006) were registered. Shape and size of basidiospores and basidia, and presence/absence of clamp connections were recorded. Basidiospore measurements are expressed as L x W (L = mean basidiospore length as the arithmetic average of all basidiospores \pm SD, W = mean spore width as the arithmetic average of all basidiospores \pm SD), Q as the mean variation in the L/W ratios between the specimens studied and n/s = number of basidiospores measured from a given number of specimens. Furthermore, SEM micrographs were obtained using a scanning electron microscope (FEI Quanta 200) at the Materials Characterization Laboratory of the Balseiro Institute (San Carlos de Bariloche, Argentina).

Table 1

List of *Ramaria* specimens analyzed morphologically, including the collector, country of origin, collection year, and herbarium voucher

Taxon	Collector(s)	Origin	Year	Specimen voucher
<i>Ramaria patagonica</i>	G. González & M. Rugolo	Argentina, LANP	2019	GM19016 (HCFC 5584)
<i>Ramaria patagonica</i>	G. González & M. Rugolo	Argentina, LANP	2019	GM19017 (HCFC 5585)
<i>Ramaria patagonica</i>	G. González & M. Rugolo	Argentina, LANP	2019	GM19026 (HCFC 5586)
<i>Ramaria patagonica</i>	G. González & M. Rugolo	Argentina, LANP	2019	GM19030 (HCFC 5587)
<i>Ramaria patagonica</i>	G. González & M. Rugolo	Argentina, LANP	2019	GM19032 (HCFC 5588)
<i>Ramaria patagonica</i>	G. González & M. Rugolo	Argentina, NHNP	2019	GM19042 (HCFC 5589)
<i>Ramaria patagonica</i>	G. González & M. Rugolo	Argentina, LANP	2019	GM19064 (HCFC 5590)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19087 (HCFC 5591)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19084 (HCFC 5592)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19095 (HCFC 5593)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19085 (HCFC 5594)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19088 (HCFC 5595)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19102 (HCFC 5596)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19103 (HCFC 5597)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19104 (HCFC 5598)
<i>Ramaria patagonica</i>	G. González	Argentina, LNP	2019	GM19106 (HCFC 5599)
<i>Ramaria patagonica</i>	C. Toledo	Chile	2013	403 (HCFC 5600)
<i>Ramaria patagonica</i>	M. Rajchenberg	Chile, Collao market*	2019	12659 (HCFC 5601)
<i>Ramaria patagonica</i>	M. Rajchenberg	Chile, Collao market*	2019	12645 (HCFC 5602)
<i>Ramaria patagonica</i>	M. Rajchenberg	Chile, Collao market*	2019	12647 (HCFC 5603)
<i>Ramaria patagonica</i>	G. González	Argentina, NHNP	2019	GM19129 (HCFC 5605)
<i>Ramaria patagonica</i> (co-type)	Spegazzini	Argentina, Ushuaia	1951	LPS 2235
<i>Ramaria flavinedulis</i>	G. González & M. Rugolo	Argentina, NHNP	2019	GM19035 (HCFC 5604)
<i>Ramaria flavinedulis</i>	G. González	Argentina, LNP	2019	GM19111 (HCFC 5606)
<i>Ramaria flavinedulis</i>	G. González	Argentina, LNP	2019	GM19112 (HCFC 5607)
<i>Ramaria flavinedulis</i>	G. González	Argentina, LNP	2019	GM19113 (HCFC 5608)
<i>Ramaria flavinedulis</i>	G. González	Argentina, LNP	2019	GM19114 (HCFC 5609)
<i>Ramaria flavinedulis</i>	G. González	Argentina, LNP	2019	GM19117 (HCFC 5610)
<i>Ramaria flavinedulis</i>	M. Rajchenberg	Chile, Collao market*	2019	12649 (HCFC 5611)
<i>Ramaria flavinedulis</i>	G. González	Argentina, NHNP	2019	GM19125 (HCFC 5612)
<i>Ramaria flavinedulis</i>	G. González	Argentina, NHNP	2019	GM19126 (HCFC 5613)
<i>Ramaria flavinedulis</i>	G. González	Argentina, TDF	2019	1U (HCFC 5614)
<i>Ramaria flavinedulis</i>	G. González	Argentina, TDF	2019	3U (HCFC 5615)
<i>Ramaria dendrophora</i>	M. Rugolo	Argentina, LANP	2020	GG20020 (HCFC 5616)
<i>Ramaria dendrophora</i>	G. González	Argentina, LNP	2019	GM19094 (HCFC 5617)

* Material from Angol – LNP: Lanin National Park; NHNP: Nahuel Huapi National Park; LPNT: Lago Puelo National Park; LANP: Los Alerces National Park.

Taxon	Collector(s)	Origin	Year	Specimen voucher
<i>Ramaria botrytis</i>	G. González & M. Rugolo	Argentina, LANP	2019	GM19013 (HCFC 5618)
<i>Ramaria botrytis</i>	G. González & M. Rugolo	Argentina, LANP	2019	GM19020 (HCFC 5619)
<i>Ramaria botrytis</i>	G. González & M. Rugolo	Argentina, NHNP	2019	GM19044 (HCFC 5620)
<i>Ramaria botrytis</i>	G. González & M. Rugolo	Argentina, NHNP	2019	GM19046 (HCFC 5621)
<i>Ramaria botrytis</i>	G. González	Argentina, NHNP	2019	GM19124 (HCFC 5622)
<i>Ramaria botrytis</i>	G. González	Argentina, NHNP	2019	GM19127 (HCFC 5623)
<i>Ramaria botrytis</i>	M. Rajchenberg	Chile, Collao market*	2019	12646 (HCFC 5624)
<i>Ramaria inedulilis</i>	G. González & M. Rugolo	Argentina, NHNP	2019	GM19047 (HCFC 5625)
<i>Ramaria inedulilis</i>	M. Rajchenberg	Chile, Collao market*	2019	12648 (HCFC 5626)
<i>Ramaria inedulilis</i>	Singer	Argentina, NHNP	1969	BAFC 50087
<i>Ramaria stricta</i>	G. González	Argentina, LNP	2019	GM19082 (HCFC 5627)
<i>Ramaria stricta</i>	G. González	Argentina, LANP	2022	GG22001 (HCFC 5628)
<i>Ramaria stricta</i>	G. González	Argentina, LANP	2022	GG22002 (HCFC 5629)

* Material from Angol – LNP: Lanin National Park; NHNP: Nahuel Huapi National Park; LPNT: Lago Puelo National Park; LANP: Los Alerces National Park.

DNA extraction and PCR conditions

DNA extraction followed a CTAB (Cetyl Trimethyl Ammonium Bromide) protocol modified from Doyle & Doyle (1990). DNA pellets were air dried and resuspended in 70 μ L ultrapure water. DNA integrity was examined in 0.8% agarose gels. Two gene regions were analyzed: the primer pairs LROR-LR7 (Vilgalys & Hester 1990) and ITS1-ITS4 (Gardes & Bruns 1993) were used to amplify the partial 28S rDNA and the full ITS region (i.e., ITS1, ITS2 and the intervening 5.8S RNA gene), respectively. Polymerase chain reaction (PCR) mixtures for amplification of both regions included 12.5 μ L of MasterMix (Promega, Madison, Wisconsin); 0.5 μ L of each primer (10 μ M); 1 μ L DNA was used as a template and sterilized H₂O to complete the volume. The final reaction volume was 25 μ L. The PCR were performed in a thermal cycler (MyCycler™, BioRad) following the methods of Dutta et al. (2015) for the ITS region and Humpert et al. (2001) for the 28S region. To check the concentration of PCR products, a Multiskan™ SkyHigh μ Drop plate was used (Thermo Fisher Scientific, USA). PCR products were purified and sequenced by Macrogen Corporation (Seoul, Korea). All sequences analyzed in this study were deposited at GenBank (Table 2).

Table 2

Gomphales specimens used in the phylogenetic analyses, including herbarium vouchers, country of origin and National Center for Biotechnology Information (NCBI) GenBank accession numbers.

Species name	Specimen voucher	Location	GenBank Accession no.	
			ITS	LSU
<i>Clavariadelphus mucronatus</i>	OSC_1064138	USA	EU526000	EU526000
<i>Clavariadelphus pistillaris</i>	AR09762	Mexico	KT874979	KT874979
<i>Gallacea eburnea</i>	PDD:95772	New Zealand	HQ533040	HQ533040
<i>Ganoderma resinaceum</i>	KX371982	China	KX371982	-
<i>Gautieria caudata</i>	OSC41323S	USA	AF377057	-
<i>Gautieria graveolens</i>	16988	Italy	JF908017	JF908017
<i>Gautieria parksiana</i>	SNF236USA	USA	AF377059	-
<i>Gloeocantharellus aculeatus</i>	FLOR_47977	Brazil	KU884895	KU884895
<i>Gloeocantharellus echinosporus</i>	CGE_16041	Solomon Islands	KU884899	KU884899
<i>Gomphus clavatus</i>	MA-Fungi 48085	Spain	AJ292292	-
<i>Gomphus ludovicianus</i>	TFB14476	USA	KJ655570	KJ655570
<i>Hysterangium setchellii</i>	29.3	USA	DQ365634	DQ365634
<i>Kavinia alboviridis</i>	CFMR:DLL2011-131	USA	KJ140634	-
<i>Kavinia himantia</i>	CFMR:DLL2011-079	USA	KJ140598	-
<i>Lactarius</i> sp.	PDD:113066	New Zealand	MW683864	MW683864
<i>Lentaria byssiseda</i>	TENN61159	USA	FJ596788	FJ596788
<i>Lentaria micheneri</i>	RRD6 (TENN)	USA	MF773634	MF773634
<i>Phaeoclavulina argentea</i>	AGK_042	USA	JQ408234	JQ408234
<i>Ramaria abetonensis</i>	MCVE:28638	Italy	NR155721	-
<i>Ramaria abietina</i>	MA-Fungi 48119	Spain	AJ408383	-
<i>Ramaria acrisiccescens</i>	OSC87692	USA	AY102858	-
<i>Ramaria admiratia</i>	TENN_691	USA	NR137862	-
<i>Ramaria amyloidea</i>	OSC_67046	USA	KP658114	-
<i>Ramaria apiculata</i>	17678	Italy	MK801295	-
<i>Ramaria apiculata</i> var. <i>brunnea</i>	CBS_148.74	USA	MH860839	-
<i>Ramaria araiospora</i>	OSC 108707	Germany	EU846298	EU846298
<i>Ramaria aurea</i>	AGK_025	USA	JQ408226	JQ408226
<i>Ramaria aurea</i>	MA-Fungi 48120	Germany	AJ408387	-
<i>Ramaria boreimaxima</i>	H:l. Kytovuori 96–525	Finland	NR158898	-
<i>Ramaria botrytis</i>	AMB n. 18201	Italy	KY626151	-
<i>Ramaria botrytis</i>	MA-Fungi 47951	Spain	AJ2922942	-
<i>Ramaria botrytis</i>	snf213	USA	AF377055	-
<i>Ramaria botrytis</i>	GM19044	Argentina	OP177707	OP177871
<i>Ramaria botrytis</i>	GM19124	Argentina	OP177708	OP177872
<i>Ramaria botrytis</i>	12646	Chile	OP177709	OP177873

				GenBank Accession no.	
<i>Ramaria botrytis f. musicolor</i>	ZT Myc 57160	Italy	KY626144	-	
<i>Ramaria botrytis</i> var. <i>aurantiiramosa</i>	WTU-F-043053	USA	KX574471	KX574471	
<i>Ramaria botrytis</i> var. <i>aurantiiramosa</i>	OSC 140667	USA	JX310410	JX310410	
<i>Ramaria calvodistalis</i>	TENN_69095	USA	NR137861	-	
<i>Ramaria celerivirescens</i>	OSC 140471	USA	JX310392	JX310392	
<i>Ramaria celerivirescens</i>	OSC_140471	USA	JX310392	JX310392	
<i>Ramaria claviramulata</i>	WTU-F-043055	USA	KX574472	KX574472	
<i>Ramaria claviramulata</i>	WTU-F-043055	USA	KX574472	KX574472	
<i>Ramaria conjunctipes</i>	OSC:110613	USA	KC346861	-	
<i>Ramaria coulterae</i>	OSC 69929	USA	EU669320	EU669320	
<i>Ramaria curta</i>	MA-Fungi 48081	Spain	AJ408359	-	
<i>Ramaria curta</i>	MA-Fungi 48029	Spain	AJ408358	-	
<i>Ramaria cystidiophora</i>	UBCOGTR0419s	Canada	EU597077	EU597077	
<i>Ramaria dendrophora</i>	GM19094	Argentina	OP177715	OP177879	
<i>Ramaria dendrophora</i>	GM20020	Argentina	OP177716	OP177880	
<i>Ramaria fennica</i>	AMB n. 17522	Italy	MK682678	-	
<i>Ramaria fennica</i>	AMB n. 17522	Italy	MK682678	-	
<i>Ramaria flaccida</i>	MA-Fungi 48076	Spain	AJ408371	-	
<i>Ramaria flava</i>	AMB 17393	Italy	MK493035	-	
<i>Ramaria flava</i>	JLH MyCoPortal 6604751	USA	MK578702	MK578702	
<i>Ramaria flavescens</i>	AMB 17404	Italy	MK493036	-	
<i>Ramaria flavinedulis</i>	GM19056	Argentina	OP177717	OP177881	
<i>Ramaria flavinedulis</i>	GM19117	Argentina	OP177718	OP177882	
<i>Ramaria flavinedulis</i>	GM19042	Argentina	OP177719	OP177883	
<i>Ramaria flavinedulis</i>	GM19035	Argentina	OP177720	OP177884	
<i>Ramaria flavinedulis</i>	GM19105	Argentina	OP177721	OP177885	
<i>Ramaria flavobrunnescens</i>	MA-Fungi 48059	Spain	AJ408379	-	
<i>Ramaria foetida</i>	AGK_058	USA	JQ408239	JQ408239	
<i>Ramaria formosa</i>	17542	Italy	MK723992	MK723992	
<i>Ramaria fumosiavellanea</i>	WTU-F-063048	USA	MK169345	-	
<i>Ramaria gelatiniaurantia</i>	OSC_65737	USA	KP658144	-	
<i>Ramaria gracilis</i>	17580	Italy	MK791724	-	
<i>Ramaria inedulius</i>	GM19047	Argentina	OP177722	OP177886	
<i>Ramaria inedulius</i>	12648	Chile	OP177723	OP177887	
<i>Ramaria largentii</i>	AH:48019	Spain	MF564302	MF564302	
<i>Ramaria luteovernalis</i>	MCVE_28637	Italy	NR155720	-	
<i>Ramaria magnipes</i>	WTU-F-063057	USA	MK169351	MK169351	
<i>Ramaria obtusissima</i>	TFB14473	USA	KJ655554	KJ655554	

			GenBank Accession no.	
<i>Ramaria patagonica</i>	403	Argentina	OP177710	OP177874
<i>Ramaria patagonica</i>	GM19026	Argentina	OP177711	OP177875
<i>Ramaria patagonica</i>	GM19032	Argentina	OP177712	OP177876
<i>Ramaria patagonica</i>	GM19106	Argentina	OP177713	OP177877
<i>Ramaria patagonica</i>	GM19095	Argentina	OP177714	OP177878
<i>Ramaria pseudoflava</i>	AMB_17392	Italy	MK493046	MK493046
<i>Ramaria rainierensis</i>	KA12-1702	South Korea	KR673634	-
<i>Ramaria rasilisporoides</i>	WTU-F-043029	USA	MK169346	-
<i>Ramaria rubella</i>	OSC_115946	USA	EU669317	-
<i>Ramaria rubribrunnescens</i>	OSC 119676	USA	EU652352	EU652352
<i>Ramaria rubribrunnescens</i>	OSC 66051	USA	KY354750	KY354750
<i>Ramaria spinulosa</i>	M 0127250	Germany	MF288926	-
<i>Ramaria stricta</i>	CBS_165.48	Germany	MH856299	MH856299
<i>Ramaria stricta</i>	GM19082	Argentina	-	OP177888
<i>Ramaria stuntzii</i>	OSC_73315	USA	KP658122	KP658122
<i>Ramaria subtilis</i>	AH:48020	Spain	MF564300	MF564300
<i>Ramaria suecica</i>	OSC_115933	USA	KP658148	-
<i>Ramaria thiersii</i>	OSC_112045	USA	KY354761	-
<i>Ramaria velocimutans</i>	WTU-F-063045	USA	KX574487	KX574487
<i>Ramaria verlotensis</i>	WTU-F-063047	USA	KX574480	KX574480
<i>Ramaricium polyporoideum</i>	TENN:065654	USA	MF992160	MF992160
<i>Turbinellus floccosus</i>	PKSR1	India	KJ411951	KJ411951
<i>Turbinellus floccosus</i>	OSC 70574	USA	EU846244	EU846244

Sequence and phylogenetic analyses

Sequence data generated in this study were manually edited with MEGA X (Kumar et al. 2018) and additional sequences were retrieved from GenBank. Members of Russulales (*Lactarius* sp. MW6838641) and Ganodermataceae (*Ganoderma resinaceum* KX3719821) which remain clearly outside the Gomphales/Phallales clade (Hosaka et al. 2006) were selected for the outgroup. Alignment of the 28S and ITS sequence datasets were performed automatically with Multiple Sequence MAFFT (Kato & Standley 2013) and were visually inspected and manually adjusted with MEGA V.X

Phylogenetic relationships were inferred with maximum likelihood (ML) and Bayesian (BA) optimality criteria for both datasets. The best-fit models of evolution were determined with AIC (Akaike 1974) implemented in jModelTest (Darriba et al. 2012), and these were used for both the ML and BA analyses. Branch support was determined with nonparametric bootstrapping implemented in RAxML 7.2.8 (Stamatakis et al. 2014), using the default parameters, executed on the CIPRES (cyberinfrastructure for phylogenetic research) Science Gateway 3.1 (Miller et al. 2010; www.phylo.org/sub_sections/portal) with bootstrap statistics calculated from 1000 bootstrap replicates. Bayesian phylogenetic analyses were performed with Mr Bayes 3.2.6 (Ronquist et al. 2012) for 10,000,000 generations, with four chains and trees sampled every 100 generations. Branch support was assessed using posterior probabilities calculated from the posterior set of trees after stationarity was reached.

Results

Molecular phylogeny

The phylogenetic analyses of *Ramaria* are presented in Fig. 2. The combined datasets of ITS and 28S included 97 sequences and comprised 1028 characters including gaps, of which 183 characters were constant. The best model for the combined ITS and 28S partition was a GTR+I+G model. The average standard deviation of split frequencies of BI was 0.015439. Both BI and ML analyses yielded the same tree topology. Therefore, only the Bayesian trees with both BPP (≥ 0.95) and MLB values $\geq 65\%$ are shown. The phylogenetic tree revealed that the sequences represent six *Ramaria* species present in Argentinian Patagonia, which group within the subgenera *Ramaria*, *Laeticolora* and *Lentoramaria*.

Despite forming a well-supported group (BPP: 1.00 MLB: 100%), *Ramaria botrytis* collections showed substructure within the clade. The collections from South America, North America, and Europe identified as *Ramaria botrytis* fell within a gradient of small clades with strong statistical support. Also, this species formed a well-supported group with the gasteroid (non-ramarioid) *Gautieria* (BPP: 0.99 MLB: 93%). *Ramaria patagonica* is located inside subg. *Laeticolora* for its phylogenetic position (Fig. 2). The phylogenetic analysis also confirmed the position of *R. dendrophora* under *R.* subgen. *Laeticolora* in a monophyletic group (BPP: 1.00 MLB: 100%) with species from Europe and the USA such as *R. aurea* (Schaeff.) Quél., *R. largentii* Marr & D.E. Stuntz and *R. admiratia* R.H. Petersen. *Ramaria inedulis* and the novel species *R. flavinedulis* form a new lineage entirely represented by Patagonian collections within subg. *Laeticolora* (BPP: 0.99 MLB: 94%). *Ramaria flavinedulis* formed a well-supported group (BPP: 1.00 MLB: 100%). The Patagonian collections of *Ramaria stricta* formed a well-supported lineage (BPP: 0.99 MLB: 96%) with collections of this species from other parts of the world.

Taxonomy

Ramaria dendrophora G.C. González, Barroetaveña & Pildain sp. nov. Fig. 3.

Mycobank no.: 846327

GenBank no.: OP177716 - OP177880.

Etymology: *dendrophora* in reference to its appearance of a tree.

Diagnosis: This taxon is morphologically characterized by pale yellow to pale rose basidioma with 8–10 cm high \times 7–10 cm wide at the widest point. Basidiospores (10–)11–2(–13) \times (4–)4.8–5, ornamented with conspicuous and irregular warts. Context hymenium immediately turns green with ferric sulfate.

Holotype: ARGENTINA. Chubut, Lago Puelo, Río Azul, -42.10298 - 71.80094, alt 230 m, on the ground in *Nothofagus dombeyi* forest with *Diostea juncea* and presence of *Pinus* spp. May 2020, leg. M. Rugolo GM20020 (HCFC 5616).

Other specimens studied: ARGENTINA. Neuquén, Lanín National Park, Queñi Lake -40.145763 -71.617588, 15.5.2019. leg. G. González GM19094 (HCFC 5617).

Description: Basidiomata solitary to gregarious, with 8–10 cm high \times 7–10 cm wide at the widest point, repeatedly branched dichotomously, in 5–6 ranks, pastel yellow when young, becoming paler with maturity, apices acute to subacute, yellow when young, becoming pale yellow after bruising or at maturity. Stipe ≥ 2 (–3) cm high, compound to fasciculate in groups of 2–5, emerging from a common base. Taste mild, odor fungoid. Context opaque, turning slightly greenish with FeSO_4 , IKI–. Hyphal system monomitic, generative hyphae simple-septate, branched, walls smooth and hyaline; basal stem with tramal hyphae 5–7 μm wide and inflated ones up to 10 μm , branched, thin-walled, compactly arranged in parallel, hyaline; tramal hyphae of branches 2.5–6 μm wide; gloeoplerous hyphae present, up to 8 μm wide, compactly interwoven, thin-walled, hyaline, deeply stained with phloxine. Hymenium all along the basidiomata.

Basidia 34–65(–70) \times 8–11 μm , clavate, simple-septate, slightly grainy in contents, four-spored; sterigmata 5–7 μm long. Basidioles 30–60 \times 7–10 μm , elongated clavate, smooth, hyaline, contents homogeneous. Basidiospores ellipsoid, (10–)11–12(–13) \times (4–)4.8–5 μm , densely warted, with 1–several guttulae.

Ecology: Growing under *Nothofagus dombeyi*, *Austrocedrus chilensis* and *Diostea juncea* mixed forest or near introduced *Pinus* sp., occurring in the rainy season during autumn.

Ramaria flavinedulis G.C. González, Barroetaveña & Pildain sp. nov. Fig. 4.

Mycobank no.: 846326

GenBank no.: OP177720 - OP177885.

Etymology: *flavinedulis*, in reference to its yellow color (*flavi* = yellow in Latin) and its morphological similarity and phylogenetic proximity to *Ramaria inedulis*.

Diagnosis: This species produces brightly colored yellow or yellow-orange basidiomata with a fused, twisted compound stipe, both stipe and hymenium context reaction with ferric sulfate is positive. Apices concolorous with branches or brighter ('maize' or 'sunflower') yellow. Basidia not clamped. Basidiospores ellipsoid to cylindrical (10–)11–12(–15) × (4–)4.8–5(–6).

Holotype: ARGENTINA. Río Negro, Nahuel Huapi National Park, Puerto Blest, -41.030435 -71.804487, alt 810 m, on the ground in *Nothofagus dombeyi* forest with *Chusquea culeou* and *Ribes magellanicum*, May 2019, GM19035 (HCFC 5604).

Other specimens studied: Argentina, Lanín National Park, Quillén Lake, -39.390542 -71.257217, 15.5.2019. leg. G. González GM19111 (HCFC 5606); *ibid.* -39.390598 -71.257488 15.5.2019 leg. G. González GM19112 (HCFC 5607); *ibid.* 15.5.2019. leg. G. González GM19113 (HCFC 5308); *ibid.* 18.5.2019 leg. G. González GM19114 (HCFC 5309); *ibid.* -39.36823 -71.233141 18.5.2019. leg. G. González GM19117 (HCFC 5610). Nahuel Huapi National Park, El Manso, -41.567509 -71.771487 11.6.2019. leg. G. González GM19125 (HCFC 5612); *ibid.* 11.6.2019. leg. G. González GM19126 (HCFC 5613). Tierra del Fuego, Ushuaia, -54.715102 -68.016357 15.6.2019. leg. G. González, GM1U (HCFC 5614); *ibid.* -54.7242 -68.0169 15.6.2019. leg. G. González GM3U (HCFC 5615). Chile, Bío Bío, Concepción, El Collao market, 5.2019 leg. G. González 12649 (HCFC 5611).

Description: Basidiomata solitary or gregarious, up to 7 cm high, waxy, repeatedly branched, dichotomous, coralloid. Stipe ≥3(–4) cm high, compound to fasciculate in groups of 2–5; sometimes tortuous, with the appearance of several strongly welded stipes. Branches in 3–4 ranks, from pastel to bright yellow in color when young, becoming pale with maturity. Apices rounded to sharp, concolorous. Taste mild, odor fungoid. Context opaque, turning greenish with FeSO₄.

Hyphal system monomitic, generative hyphae simple-septate, branched, smooth, hyaline; tramal hyphae of basal stem 4–6 μm wide, branched, thin-walled, parallel and compactly arranged, hyaline; gloeopleurous hyphae scattered in the subhymenium up to 5 μm wide; tramal hyphae of branches 7–10 μm wide, compactly interwoven. Gloeopleurous system represented by short hyphae with occasional swellings up to 11 μm in diam., thin-walled, hyaline. Hymenium amphigenous all along the basidiomata.

Basidia 55–65(–70) × 10–14 μm, clavate, simple-septate, multiguttulate, four spored; sterigmata 7–10 μm long. Basidioles 30–50 × 8–10 μm, elongated clavate, smooth, hyaline. Basidiospores ellipsoid to cylindrical, (10–)11–12(–15) × (4–)4.8–5(–6) μm, cyanophilous, with densely warty wall ornamentation.

Ecology: Growing under *Nothofagus dombeyi*, *N. pumilio*, *N. antarctica* (G. Forst.) Oerst., *Lophozonia obliqua* and *L. alpina* in subalpine forests. Fruiting in the rainy season during autumn.

Ramaria botrytis (Pers.) Ricken, *Vademecum für Pilzfreunde*: 253 (1918). Figs 5 and 6.

Descriptions: Exeter et al. 2006.

Specimens studied: Argentina, Los Alerces National Park, -42.671467 -71.696326 16.4.2019 leg. G. González GM19013 (HCFC 5618); *ibid.* -42.671284 -71.696159 16.4.2019 leg. G. González GM19020 (HCFC XX); *ibid.* GM19044 (HCFC 5620) leg. G. González. Nahuel Huapi National Park, Blest Harbor, -41.014611 -71.820895 19.4.2019 leg. G. González GM19046 (HCFC 5621); *ibid.* -41.566491 -71.772655 GM19124 (HCFC 5622); *ibid.* -41.56608 -71.77379 GM19127 (HCFC 5623).

Ecology: *Ramaria botrytis* is commonly known as the 'pink cauliflower'. Specimens from Patagonia were collected from stands dominated by *Nothofagus dombeyi* (Mirb.) Oerst., *Chusquea culeou* E. Desv., *Aristotelia chilensis* (Molina) Stuntz and *Berberis darwinii* Hook. In addition, some specimens were collected from spots with abundant ferns.

Remarks: This taxon presents robust basidiomata (12–)10–9(10) cm high × (10)8–7(6) cm wide. Stipe is opaque, white to pink white with a massive base and the context is white, both being fleshy fibrous to firm when fresh. Branches in 4–5 ranks, primary branch pale flesh, and ultimate branchlet 'pink tint' to pale red, compact and cauliflower-like. Rounded apices with a more intense color than the branches (reddish white to magenta) and concolorous at maturity. Another characteristic of this species is that the incipient basidiomata contain adherent soil particles. Basidiospores are elliptical, apiculate, guttulate, (10)12–4(15) × (4)5–5.5(6) μm, strongly cyanophilic, thin-walled, with warty ornamentation aligned to form longitudinal groove. Basidia 20–30 × 4–10 μm, clamped, claviform, tetrasporated, sterigmata 8–10 μm long. Contextual hymenium immediately turns green with ferric sulfate.

Ramaria inedulis Singer, *Beihefte zur Nova Hedwigia* 29: 384 (1969). Figs 5 and 6.

Description: Singer (1969)

Specimens studied: Argentina, Nahuel Huapi National Park, Blest Harbor, -41.014493 -71.820832 19.4.2019 leg. G. González GM19046 (HCFC XX). Chile, Bío Bío, Concepción, El Collao market, 5.2019 leg. G. González 12649 (HCFC XX).

Ecology: This is a rare species, collected on the ground in a humid spot within an adult forest matrix dominated by *N. dombeyi* (coihue) in addition to *Saxegothaea conspicua* (mañío), *Weinmannia trichosperma* Cav. (tineo), *Raukaua laetevirens* (Gay) Frodin (devil's elder) and *Chusquea culeou*.

Remarks: Basidioma is coralloid and oblong, grey or purple when mature and light gray when young, and turns white towards the base of the stipe. Stipe is smooth and radiant towards the base. Long and fine tips with dichotomic and trichotomic branching. Basidiospores are cylindrical to ellipsoid, 11–12.2(15) × 4.95–5(6), ornamented with warts. Basidia are simple septated. Stipe and hymenium context reaction with ferric sulfate is negative.

Ramaria patagonica (Speg.) Corner. Figs 5 and 6.

Descriptions: Spegazzini (1887); Corner (1957).

Specimens studied: Argentina, Los Alerces National Park, -42.671467 -71.696326 16.4.2019 leg. G. González GM19016 (HCFC 5584); *ibid.* -42.668245 -71.693692 leg. G. González 16.4.2019 GM19017 (HCFC 5585); *ibid.* -42.661578 -71.696027 leg. G. González 16.4.2019 GM19026 (HCFC 5586); *ibid.* -42.8976561 -71.725332 leg. G. González; *ibid.* -42.897661 -71.725332 leg. G. González 16.4.2019 GM19064 (HCFC 5586). Nahuel Huapi National Park, Blest Harbor, -41.029108 -71.810438 19.4.2019 leg. G. González GM19030 (HCFC 5587); *ibid.* -41.030832 -71.809927 leg. G. González 16.4.2019 GM19032 (HCFC 5588); *ibid.* -41.564123 -71.776667 leg. G. González 11.6.2019 GM19129 (HCFC 5605). Lanín National Park, Queñi Lake, -40.14673 -71.719567 leg. G. González 16.5.2019 GM19084 (HCFC 5592); *ibid.* -40.13613 -71.707702 16.5.2019 leg. G. González GM19087 (HCFC 5591); *ibid.* -40.144993 -71.618112 16.5.2019 leg. G. González GM19095 (HCFC 5593); *ibid.* -40.145051 -71.618210 16.5.2019 leg. G. González GM19088 (HCFC 5595); *ibid.* -39.370488 -71.234857 18.5.2019 leg. G. González GM19102 (HCFC 5596); *ibid.* -39.377074 -71.234413 18.5.2019 leg. G. González GM19103 (HCFC 5597); *ibid.* -39.377075 -71.234413 18.5.2019 leg. G. González GM19104 (HCFC 5598); *ibid.* -39.370795 -71.234415 18.5.2019 leg. G. González GM19106 (HCFC 5599). Chile, Bío Bío, Concepción, El Collao market, 5.2019 leg. M. Rajchenberg 12659 (HCFC 5601).

Ecology: Fruiting in autumn, on wet soil, gregarious and rarely solitary, close to native forest species including *Nothofagus dombeyi*, *N. pumilio*, *N. antarctica*, *Lophozonia alpina* and *L. obliqua*, mixed with *Chusquea culeou*, *Ribes magellanicum* and *Raukaua laetevirens*.

Remarks: Morphologically it is distinguished by a coralloid, robust/massive basidioma, 8.5–12 cm high × 5–9 cm wide, soft to pale yellow, with 3 to 4 branching strata; stipe 4–8 cm high, smooth, radiant and unified towards the base, densely branched towards the apex. Rounded apices, cuspidate, slightly inflated, concolourous, with trichotomous or more tip. White, solid, non-gelatinous context. Basidiospores were ellipsoidal to cylindrical, (9)10–12(13) × 4–5 μm, cyanophilic, apiculate, ornamented with irregularly arranged warts. Spores measured in this study are larger than in the original description of the species: 6.5–8 × 4–5 μm (Spegazzini 1921), verified reviewing the type material deposited in LPS. Nevertheless, this divergence could be because we did not collect materials from Tierra del Fuego Province (from where the type herbarium material comes). Since we could not obtain DNA from the type herbarium material, molecular comparison was not possible. The description given here is a revised concept of the species.

Ramaria stricta (Pers.) Quél. Fig. 5.

Descriptions: Corner (1957); Exeter et al. (2006).

Specimens studied: Lanín National Park, Queñi Lake, -40.134847 -71.70817 leg. G. González 16.5.2019 GM19082 (HCFC 5627). Los Alerces National Park, -42.771216 -71.730788; *ibid.* -42.661578 -71.696027 leg. G. González 16.4.2019 GM22001 (HCFC 5628); *ibid.* -42.661578 -71.696027 leg. G. González 16.4.2019 GM22002 (HCFC 5629).

Ecology: This species has a cosmopolitan distribution, a lignicolous habit morphologically (Exeter et al. 2006). Patagonian collections came from native forest composed mainly of *Nothofagus* sp. or *Lophozonia* sp., *Lomatia hirsuta* and *Chusquea culeou*, fruiting gregariously on wood in an advanced state of decomposition, buried underground near the surface.

Remarks: Basidioma small, up to 6 cm long and 4 cm thick, pale yellow to cinnamon-brown, fine ramifications, context opaque, turning immediately greenish with ferric sulfate. Branches yellowish toward the tips, some shade of cinnamon below; long, fine and dichotomous apices. Basidiospores warty (8)9.66–10 × (4)4.44–5 μm, similar to was reported by Exeter et al. (2006), Marr and Stuntz (1973).

Discussion

This study provided the first estimation of phylogenetic relationship of *changles* from southern South America. The neat distinction of *Ramaria* species from Patagonia is of high relevance, given the abundance of these fungi, their role in the ecology Nothofagaceae forests and the edibility of some of them. Six *Ramaria* species were sampled and studied (Table 3), including the description of two new species; 49 records were revised; and related genera with similar morphology are critically discussed. This study supports previous research (Humpert 2001, Giachini 2004, Giachini et al. 2010) indicating that *Ramaria* is a paraphyletic taxon.

Table 3 - Summary of macro- and microscopical features of *Ramaria* species of PATAGONIA.

Taxon	Basidioma appearance	Apices	Ferric sulfate reaction	Clamps	Basidiospore size	Basidiospores (shape / ornamentation)
<i>R. botrytis</i>	Ramaroid, robust/massive, branched cauliflower, pink	Rounded – reddish to magenta	+++	Present	(10)12–14(15)×(4)5–5.5(6)	Elliptical, striate
<i>R. patagonica</i>	Ramaroid, robust/massive, densely branched, pale yellow	Rounded, cuspidate, concolorous, trichotomous or more tip	++	Present	(9)10–12(13)×4–5	Ellipsoidal to cylindrical warty
<i>R. dendrophora</i>	Ramaroid branched, yellow when young, then, pale pink	Thick, short and rounded, concolorous, trichotomic or more branched	-	Absent	(10)11.3–12×(4)4.3–5	Ellipsoidal to cylindrical densely warty
<i>R. flavinedulis</i>	Ramaroid branched, oblong, fused twisted compound stipe, brightly yellow or orange	Thin, dichotomic, concolorous or with brighter branches	+	Absent	(10)12.5(15)×4–4.8(5)	Ellipsoidal, rough
<i>R. inedulis</i>	Oblong, grey/ purple	Long, thin, concolorous, dichotomic, trichotomic	-	Absent	11–12.2(15)×4.95–5(6)	Cylindrical to ellipsoidal warty
<i>R. stricta</i>	Ramaroid, oblong, pale yellow to cinnamon brown	Thin, concolorous ,dichotomic	+++	Present	(8–)9.6–10 × (4–)4.4–5	Ellipsoidal, warty

Ramaria and related genera

Phaeoclavulina, which is morphologically similar to *Ramaria*, forms a distinct clade in the phylogenetic tree (MLBS: 93; PP: 0.99). It is not related to the *Ramaria* species described in this study, agreeing with the findings of Giachini (2004), Giachini et al. (2010) and Acharya et al. (2016). This genus contains coral-like, pileate basidiomata, a monomitic hyphal system with clamped generative hyphae and echinulate to verrucose spores (Giachini 2004, Giachini et al. 2010, Knudson 2012). *Phaeoclavulina flaccida* (Fr.) Giachini (= *Ramaria flaccida* var. *chilensis*) has been registered in Chile (Lazo 2001), although it has not been found on the eastern slope of the Andes surveyed in this study. It differs morphologically from *Ramaria* by presenting more elongated and very slender ramifications that often collapse because of their own weight, and echinulated spores (Petersen 1981, Exeter et al. 2006). *Lentaria* is morphologically similar and closely related to *Ramaria* (Villegas et al. 2005); however, their species can be distinguished by their smooth, hyaline and thin-walled spores (Corner 1950, Knudson 2012, Liu et al. 2017). In Patagonia, *Lentaria rionegrensis* Petersen develops small basidiomata (up to 6 cm high) arising from buried wood, with very tough, off-white flesh, and basidiospores (11.2–)15.2–7.6(–18.4) × 4.8–5.6 μm (Petersen 2000).

Notes on Patagonian *Ramaria* species

We detected three already-described *Ramaria* species for Argentina in the surveyed area within the eastern side of the Patagonian Andes: *R. patagonica*, *R. aurantiaca* and *R. inedulis* (Spegazzini 1887, Corner 1957; Singer 1969). Regarding *R. patagonica*, the original description reported smaller spores (Spegazzini 1921) than this study, verified when reviewing the type material at LPS. Our results indicated that this endemic species is one of the most frequent (23 collections), abundant and conspicuous species of *Ramaria* in the Nothofagaceae forests of Argentina, widely distributed in different forest sites associated with several Nothofagaceae species (Barroetaveña & Toledo 2020,

Barroetaveña et al. 2019, Toledo et al. 2014). This species is not only considered an edible fungus (Barroetaveña & Toledo 2020) of high nutritional quality, but is also renowned for its antioxidant activity and high content of phenolic compounds (Toledo et al. 2016). *Ramaria aurantiaca* Corner (1957) was described from Neuquén and Tierra del Fuego, and later considered as *R. subaurantiaca* Corner var. *aurantiaca* (Corner) Singer (Singer 1969). Its distinctive characteristic is orange basidioma, although no sequences are available. Our specimens do not match the original description; moreover, some of its morphological characteristics (simple septate hyphae, spore size) are shared with *R. flavinedulis*. Therefore, we speculate it may be another species endemic to the Patagonian Andean forests. Future molecular analysis should include specimens of this species, to define its phylogenetic position.

Ramaria inedulis is distinguished morphologically by its grey- to purple-colored branches with bright fine apices. This species was only found in soils with high humidity, and our findings coincide with the Valdivian Forest distribution. Although it was described as inedible (Singer 1969), according to popular comments it is edible and can be found in the markets in Chile (Gomez 2015).

Other species reported from Argentina, as *R. aurea*, *R. flava*, *R. strasserii* and *R. moelleriana*, (Spegazzini 1921, Singer 1969, Lazo 2001) were recorded but were not found in our surveys. The records of these species are on a morphological basis and our specimens do not link to the description of this species. It remains to be determined whether these species, described for North America or Europe, are truly present in Patagonia. Some of these species were recorded from single collections and have not been found again. The case of *Ramaria flava*, a highly cited species especially in Chile (Lazo 2001, Deschamps 2002, Furci 2007), did not group with any of the collections analyzed in this work; however, it is morphologically similar to *R. flavinedulis*. This could be due to many taxa with yellow basidiomata having been identified as *R. flava* in the past, as was the case in Europe (Petersen 1988), or the fact that this species occurs only in the Valdivian forests. Further surveys and research are needed to unravel the presence of *R. flava* in Patagonia, whether it is a misidentification or an ectomycorrhizal species introduction that has switched hosts, as is the case for *Amanita muscaria* in Nothofagaceae forests of Australia and Argentina (Dunk et al. 2012, Giles et al. 2021).

Regarding species with cosmopolitan distribution that are detected in Patagonia, *Ramaria stricta* is a species complex growing on decayed wood, and presenting dimitic rhizomorphs (Marr & Stuntz 1973, Humpert et al. 2001). Specimens from Patagonia were found on mixed forests dominated by *Nothofagus dombeyi*, *Lophozonia obliqua*, *Lomatia hirsuta* and *Chusquea culeou*, on dead wood, stumps or trunks, presenting yellow/cinnamon basidiomata, similar to specimens collected from the USA (Exeter et al. 2006), although with longer spores.

All collections of *Ramaria botrytis* from South America grouped in one cluster that formed a sister group with specimens from Europe. Results suggest a biogeographic isolation between collections of this species and the existence of a possible, not yet complete, allopatric speciation process. The Patagonian collections were found fruiting in high humidity associated with *Nothofagus dombeyi* and *N. antarctica*, but not with *Lophozonia* forests. Morphologically, they agree with the description of *R. botrytis* by Corner (1950) and are phylogenetically concordant with the Northern Hemisphere specimens. The presence of longitudinally ridged spores of *Gautieria* provide a shared character with subg. *Ramaria* (Humpert et al. 2001), which are also known mycorrhizal associates.

Molecular data, associated vegetation, basidioma shape and geographic distribution demonstrate that the new species *Ramaria flavinedulis* is native to the Patagonian forests, is morphologically and phylogenetically distinct from other species in the genus, and is phylogenetically allied to *R. inedulis* (also described from Argentina). Both species share similarities in morphological characteristics such as an oblong basidioma, white-colored flesh, simple-septate hyphae and basidiospores size (12.5–15 µm). Based on these results and the evidence that this clade is composed only of Patagonian collections (BPP: 0.99 MLB: 94), it is likely that these two species diverged from a single ancestor on Nothofagaceae exclusive to the Southern Hemisphere. In this sense, the Southern Hemisphere harbors many unique fungal lineages that are absent from the Northern Hemisphere (Tedersoo & Smith 2013, Tedersoo et al. 2014).

This study shows that the new species *Ramaria dendrophora* is closely related to *Ramaria* species of the Northern Hemisphere based on phylogeny (BPP: 1.00 MLB: 100%). *Ramaria aurea* (reported from Patagonia) and *R. largentii* have morphological similarities such as compact basidiomata, long warty basidiospores and clamped hyphae. However, *R. dendrophora* differs by having yellow basidiomata when young that turn pale pink upon maturity, and by presenting an immediate reaction to ferric sulfate.

Ecology for *Ramaria* species

Ramaria species perform two important and distinct ecological roles: terricolous or saprobic habits (Knudson 2012). It is suggested that all terricolous species are mycorrhizal (Humpert et al. 2001, Knudson 2012) and the saprobic species including the lignicolous habit may be ancestral to the Gomphales (Humpert et al. 2001). *Ramaria botrytis*, *R. flavinedulis*, *R. inedulis*, *R. patagonica* and *R. dendrophora* are terricolous and we assumed that these species are mycorrhizal. Future studies of soil sampling, examination of tree roots for fungal partners, and genetic sequencing could confirm this hypothesis.

The biogeographical places within which Argentina and Chile are located comprise the southern temperate areas of southern South America, South Africa, Australia, New Zealand, New Guinea, New Caledonia, and Antarctica (Morrone 2018). Among the plant families endemic to the Austral kingdom, Nothofagaceae is one of the most interesting owing to the austral distribution of living species and extensive fossil remnants (Torres et al. 2009, Vento and Agraín 2018). An example of the current distribution patterns of ancestral Australasian biota is the coevolution of *Cyttaria* spp. (Ascomycota) and *Nothofagus* spp. (Peterson & Pfister. 2010). In addition, Agaricales (González et al. 2021) and polypores (Pildain et al. 2009; Pildain & Rajchenberg 2013) species belonging to the Southern Hemisphere are clustered in one strongly supported monophyletic lineage. However, we did not find any evidence of a connection between *Ramaria* from the Southern Hemisphere. The clavarioid fungi of New Zealand are mainly known through the work of Petersen (1988), who recorded that all four subgenera of *Ramaria* are represented in New Zealand, and some species appear under Nothofagaceae forests. However, the overall distribution of some species is not known and more data are needed to support this connection.

Ramaria species have been reported as non-host specific, and host shifting between Pinaceae and broadleaves trees occurs frequently (Petersen et al. 2014). This is the case with several taxa such as *R. botrytis*, *R. aurea*, *R. flava*, *R. formosa* (Pers.) Quél, *R. fumigata* (Peck) Corner, *R. largentii* and *R. ochraceovirens* (Jungh.) Donk, which form mycorrhizae with species of *Abies*, *Cedrus*, *Fagus*, *Larix*, *Picea*, *Pinus*, and *Quercus* (Corner 1950, Trappe 1962, Baier et al. 2006, Exeter et al. 2006, Scattolin et al. 2008, Knudson 2012). *Ramaria* species registered in Patagonia follow this same pattern, as our results did not indicate any species-specific association with their tree hosts. Our results also suggest that the ectomycorrhizal *Ramaria* species from Patagonia are generalists within the Nothofagaceae. Specimens were largely found under forests dominated by the evergreen *N. dombeyi*, agreeing with findings by Singer (1969), Lazo (2001) and Valenzuela (2003) and, to a lesser extent, were associated with *N. antarctica*, *N. pumilio* and *Lophozonia* species. Barroetaveña et al. (2019) showed that *N. dombeyi* presents the highest number of ectomycorrhizal species, including *Ramaria* species, probably because of its wide distribution (Donoso Zegers 2006), mostly in areas with high humidity, such as proximity to lakes and river margins that have favorable conditions for fungal fruiting. In addition to the dominant forest cover, fruiting bodies were often found near *Chusquea* spp., agreeing with records by Valenzuela (2003) and Gómez (2015). *Nothofagus antarctica* shares *N. pumilio*'s distribution but, because of its resistance to drought, it is mostly present in the forest-steppe area. *Lophozonia obliqua* and *L. alpina* have more restricted distributions, growing from 30 to 41°S and 35 to 40.5°S, respectively, both in Chile and Argentina. One outstanding phylogeographic pattern from our study concerns the presence of well-known and frequent species of the Northern Hemisphere in southern South American forests. Regarding the evidently complex history of biotic exchanges and continental drift, we suggest that *R. stricta* and *R. botrytis* may have colonized South America from North America, and their occurrence could be due to:

1. A migration through the Isthmus of Panama land bridge that triggered the Great American Biotic Interchange at the end of the Tertiary period, about 3.1–2.8 Ma (Hoffmeister & Pino 2016). Some evidence has shown that the same geographic events that affect the distribution of plants and animals also have a great effect on the distribution of fungi (Lumbsch et al. 2008, Peay et al. 2010, Skrede et al. 2011).
2. *Ramaria* could have originated in the North Hemisphere 65 Mya (He et al. 2019) and later dispersed to the tropics and the South Hemisphere, with the occurrence of radiation and diversification events. Previous research shows that Long Distance Dispersal plays a significant role in the biogeographic history of fungi in the Southern Hemisphere (Moncalvo & Buchanan 2008, Du et al. 2012, Pildain et al. 2014).

In conclusion, we have established a comprehensive phylogeny that shows the relationships and disposition of *Ramaria* taxa from the Patagonian Andes Forests with those found around the world. Two new *Ramaria* species from southern Argentina are described, and other specimens examined from this work, together with specimens from previous studies, indicate that there are various new species to be discovered in this genus. Despite incursions of Valdivian forest elements in some areas of southern Argentina, it is likely that *Ramaria* species in Chile will most probably be far richer than those in Argentina. Taxonomic and ecological information about these species can lay the groundwork to develop myco-silviculture management options to increase their productivity in natural forests. In addition, these are essential tools for planning and executing fungal conservation and survey programs, especially for endemic species with restricted distributions. Further research needs to be focused on underrepresented areas such as southern Chile, to clarify the biogeographical patterns of *Ramaria* taxa in southern South America and to provide a more complete framework of *Ramaria* evolution and the phylogenetic relationships with related genera.

Declarations

Conflict of interest: The authors declare no competing interests.

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Author contribution

Conceptualization: Gabriela González, Carolina Barroetaveña, and Maria Belén Pildain; methodology: Gabriela González, Carolina Barroetaveña, and Maria Belén Pildain; formal analysis and investigation: Gabriela González; writing—original draft preparation: Gabriela González; writing – review and editing: Carolina Barroetaveña, Mario Rajchenberg, and Maria Belén Pildain; funding acquisition: Carolina Barroetaveña and Maria Belén Pildain; resources: Carolina Barroetaveña, Sandra B. Visnovsky, and Maria Belén Pildain; supervision: Carolina Barroetaveña, Mario Rajchenberg, and Maria Belén Pildain.

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Data Availability

All data generated or analyzed during this study are included in this published article.

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Figures

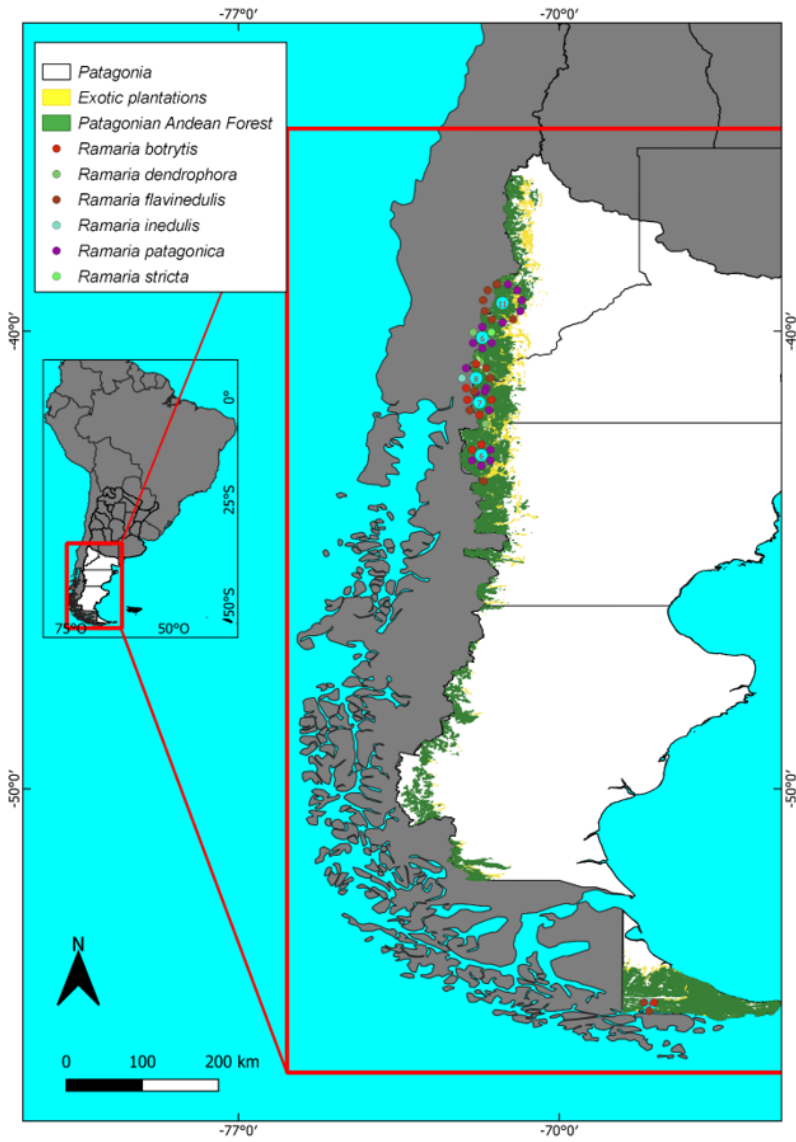


Figure 1

Study area in southern Argentina. Map showing the forest types from where the specimens of *Ramaria* were collected.

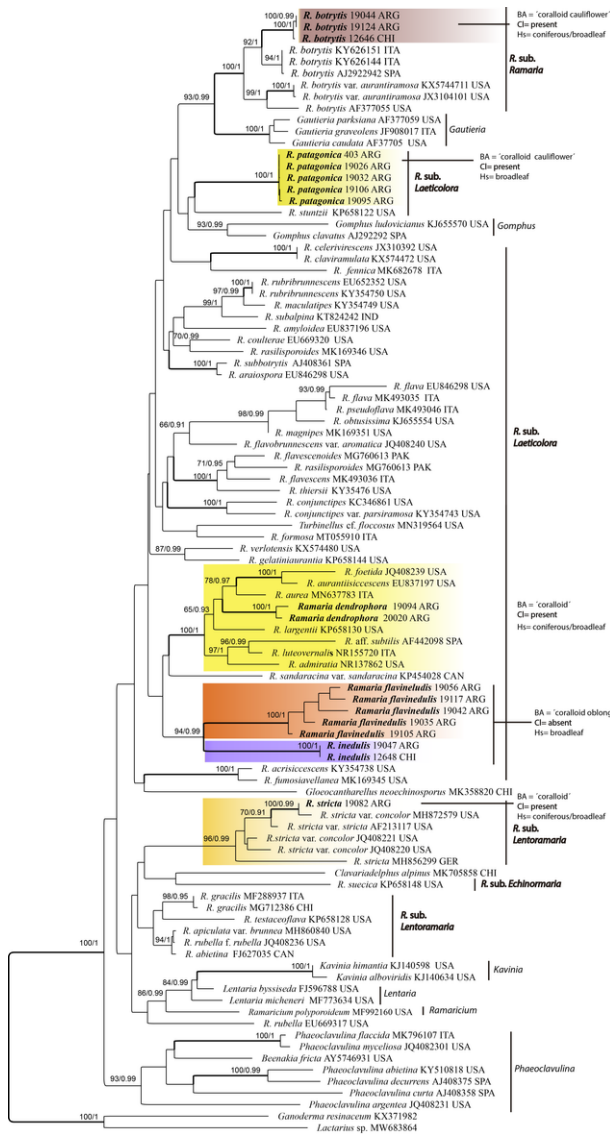


Figure 2

Phylogenetic position of the Patagonian collections of *Ramaria* based on ITS and 28S combined datasets, using maximum likelihood (ML) and Bayesian analyses. Bootstrap values lower than 75% and posterior probability values lower than 0.95 are not indicated.

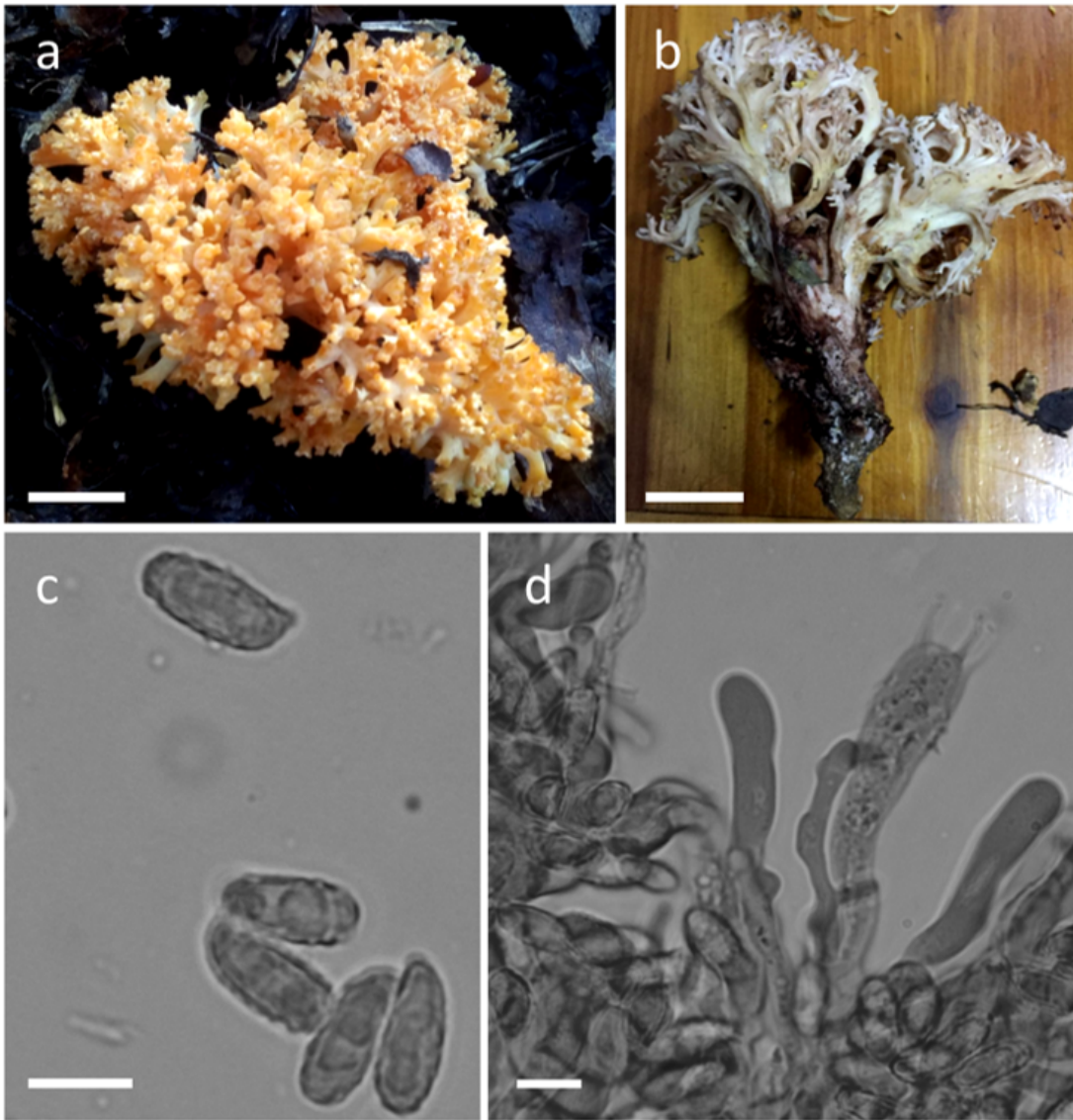


Figure 3

Ramaria dendrophora sp. nov., morphological features (Holotype HCFC 5617). a and b: Basidiomata; c: basidiospores; d: basidia. Scale bars: a = 1, cm b = 2 cm, c – d = 10 μ m.

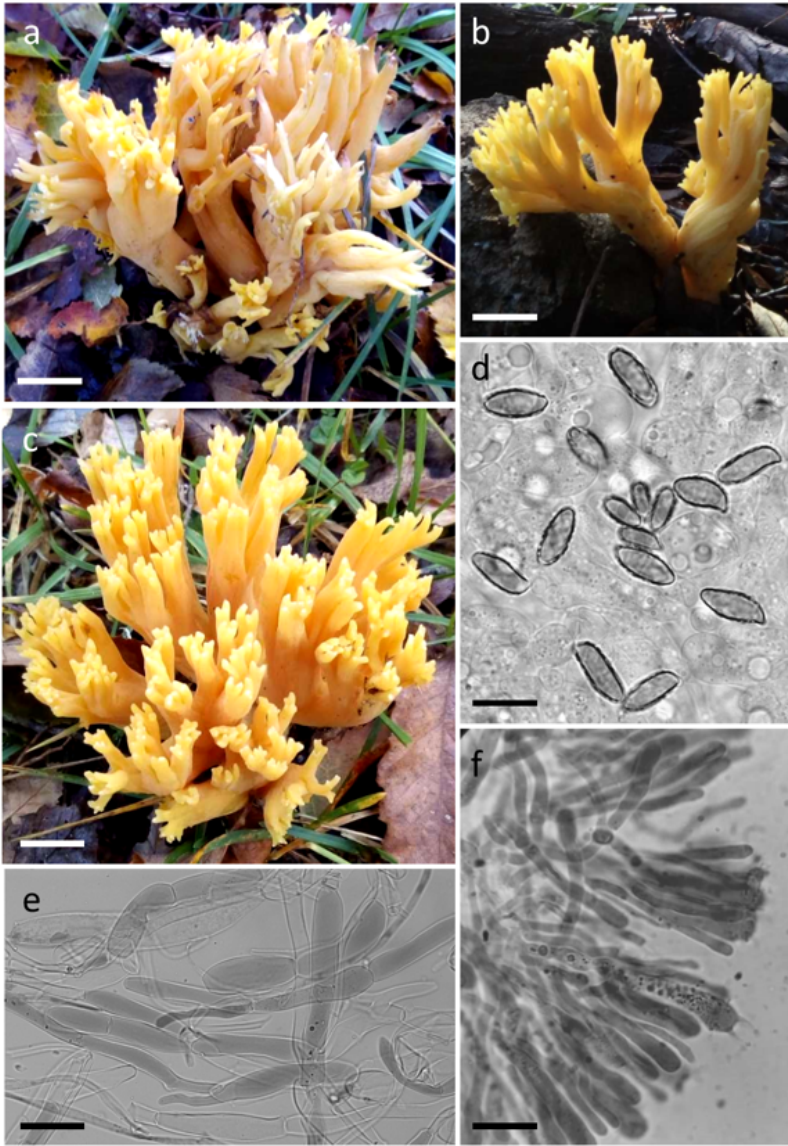


Figure 4

Ramaria flavinedulis sp. nov., morphological features (Holotype HCFC 5604). a–c: Basidiomata; d: basidiospores; e: gloeopleurous system; f: basidia. Scale bars: a = 1 cm, b = 2 cm, c = 1 cm, d = 12 μ m, e = 20 μ m, f = 15 μ m.



Figure 5

Basidiomas of *Ramaria* species from Patagonia. a and b: *R. stricta*; c and f: *R. botrytis*; d and e: *R. patagonica*; g: *R. inedulis*. Scale bar: a–c = 1 cm, d–f = 2 cm, g = 1 cm.

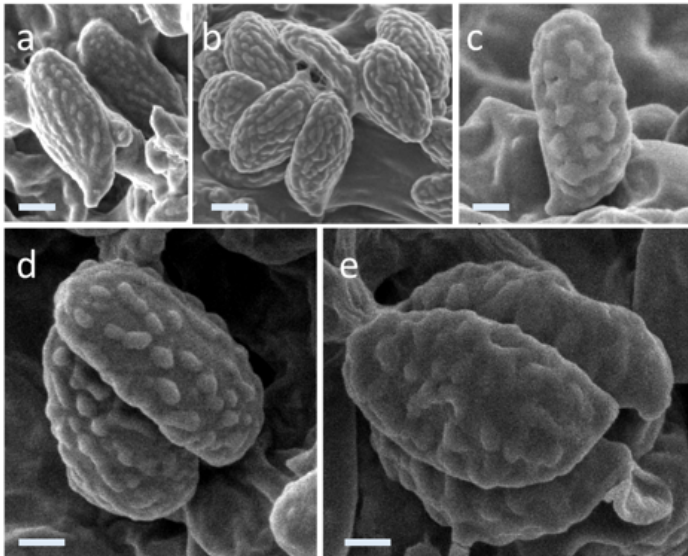


Figure 6

Scanning electron microscope (SEM) photographs of basidiospores. a: *R. botrytis*; b: *R. inedulis*; c: *R. patagonica*; d: *R. dendrophora*; e: *R. flavinedulis*. Scale bars: a = 2 μm , b = 3 μm , c = 1 μm ; d and e = 2 μm .