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To cite this article: Nicolás Pastor, Jorge Chiapella, Francisco Kuhar, Alija Bajro Mujic, Esteban M. Crespo & Eduardo R. Nouhra (2019): Unveiling new sequestrate *Cortinarius* species from northern Patagonian Nothofagaceae forests based on molecular and morphological data, *Mycologia*, DOI: [10.1080/00275514.2018.1537350](https://doi.org/10.1080/00275514.2018.1537350)

To link to this article: <https://doi.org/10.1080/00275514.2018.1537350>



Published online: 24 Jan 2019.







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## Unveiling new sequestrate *Cortinarius* species from northern Patagonian Nothofagaceae forests based on molecular and morphological data

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### ABSTRACT

Because of systematic sampling campaigns in the northern Patagonian Nothofagaceae forests of Argentina, several specimens of sequestrate fungi were collected. Some of those collections showed phylogenetic affinities and morphological similarities to members of the formerly recognized sequestrate genus *Thaxterogaster*, currently a synonym of *Cortinarius* on the basis of molecular data. Comparisons of macro- and micromorphological features and sequences of nuc rDNA internal transcribed spacer (ITS) regions have revealed that these collections belong to formerly undescribed species. The sequences of the four new taxa presented here, *Cortinarius flavopurpureus*, *C. translucidus*, *C. nahuelhuapensis*, and *C. infrequens*, were combined into a data set including additional sequences generated from herbarium collections and retrieved from public gene databases and analyzed by maximum likelihood and Bayesian inference methods. The four new species were resolved as distinct clades with strong support; at the same time, they showed unique morphological characteristics (hypogeous to subhypogeous habit, complete gasteromycetation, and spore shape and ornamentation) that separate them from previously described *Cortinarius* species. In addition, several undescribed and/or not previously sequenced species from these forests were detected through phylogenetic analysis of ectomycorrhizal root tip sequences. A key of characters to identify the sequestrate *Cortinarius* from Patagonia is provided.

### ARTICLE HISTORY

Received 18 December 2017  
Accepted 15 October 2018

### KEYWORDS

Argentina; gasteromycete;  
*Lophozonia*; *Nothofagus*;  
*Thaxterogaster*; truffle-like  
fungi; 4 new taxa

## INTRODUCTION

*Cortinarius* is one of the largest and taxonomically most complex genera in the order Agaricales (Basidiomycota), with more than 2000 species described (Peintner et al. 2004; Garnica et al. 2005; Frøslev et al. 2007; Danks et al. 2010). The enormous diversity of morphological and ecological forms within *Cortinarius* and related genera has resulted in a confusing infrageneric classification system that includes an array of formerly recognized groups that are no longer valid and several recently proposed clades based on phylogenetic studies (Peintner et al. 2001, 2004; Frøslev et al. 2005; Garnica et al. 2005; Danks et al. 2010; Gasparini 2014). For instance, it is difficult to define, beyond molecular identification, the relationship of a newly described species to an infrageneric clade within the *Cortinarius* genus as it is currently accepted (Harrower et al. 2011). In addition, *Thaxterogaster*, *Protoglossum*, and various other sequestrate genera previously considered to be related to *Cortinarius* have been shown to result from independent events of sequestration within *Cortinarius* on the basis of molecular evidence (Peintner et al. 2001, 2002).

The Nothofagaceae forests in Patagonia harbor a broad diversity of *Cortinarius* spp., and this genus is one of the largest and most widely distributed genera of ectomycorrhizal fungi (ECM) in the region (Horak and Moser 1965; Garrido 1986; Garnica et al. 2002). According to a recent checklist, the number of *Cortinarius* species cited for these forests is 240 (Romano and Lechner 2014), a relatively large number considering that these ECM fungi locally associate only with *Nothofagus* and *Lophozonia* spp. (Garnica et al. 2003a). In addition, Truong et al. (2017) also recovered high species diversity of *Cortinarius* for the region, counting a total of 147 OTUs (operational taxonomic units). Nevertheless, few studies using molecular approaches focused on the South American “cortinarioid” fungi. Due to the lack of studies, our understanding of the current classification, diversity, and ecology of the sequestrate taxa in *Cortinarius* remains obscure. Several recent, single- and multigene studies on “cortinarioid” fungi have increased the number of available sequences on public databases for several European and North American *Cortinarius* subgenera (Frøslev et al. 2005; Garnica et al.

2009; Niskanen et al. 2009, 2011, 2013a, 2013b; Liimatainen et al. 2014). Furthermore, a recent review on South American hypogeous sequestrate fungi reports that *Cortinarius* (including ex *Thaxterogaster*) is the most common and abundant hypogeous genus in the region (Sulzbacher et al. 2017); however, many new species remains undescribed.

The aim of this study is to describe the morphological features of four new hypogeous sequestrate *Cortinarius* species. In addition, we explored the diversity of *Cortinarius* taxa inhabiting the region by analyzing the available sequence information of *Cortinarius* from Patagonia.

## MATERIALS AND METHODS

**Basidiomes sampling.**—The basidiomes were collected on several collecting campaigns in Apr 2001, Nov and Apr 2006–2009, May 2013–2014, and Nov 2015, in the Nothofagaceae forests (*Lophozonia alpina*, *L. obliqua*, *Nothofagus dombeyi*, *N. pumilio*) of northern Patagonia Andean range (Nahuel Huapi and Lanín national parks). Specimens were preserved and deposited in the herbarium of the Museo Botánico de Córdoba, Universidad Nacional de Córdoba (CORD).

**Morphological analysis.**—Size, shape, color, habit, and detailed anatomy of the basidiomes were recorded from fresh collections. Color names for fresh and dry specimens are given in parentheses according to the color chart by Ridgway (1912). To describe microscopic characters, freehand tissue sections were mounted in water, 5% KOH, phloxine, Melzer's reagent, and Congo red, following Garnica et al. (2003b) and Niskanen et al. (2012). Spore dimensions, including ornamentations, are based on at least 30 basidiospores from 4–8 basidiomes. Microscopic characters were observed and photographed with a Nikon Eclipse E200 light microscope (Tokyo, Japan) at 200–1000× magnifications. Scanning electron microscopy (SEM) photographs were made with a Zeiss LEO 1450VP microscope (Oberkochen, Germany) in order to observe and describe in detail the spore ornamentations (ornamentation type, ornamentation structure, hilar appendage).

A key to the sequestrate *Cortinarius* species from the Nothofagaceae Patagonian forest was constructed mostly on the basis of diagnostic characters. In this regard, we refer to sequestrate fungi in general terms, with the concepts of “gasteroid” and “secotioid” morphologies as defined by Peintner et al. (2001), regarding the types of basidiomes morphology occurring in *Cortinarius*. Indeed, we differentiate the gasteroid morphology as the

*Protoglossum*-like morphology, which has a peridium that fully encloses a loculated gleba lacking a stipitate columella, from the secotioid *Thaxterogaster*-like morphology, in which the margin of the pileus remains appressed to the stipe. For instance, we included in the key those *Cortinarius* species with loculated unexposed gleba, enclosed by a peridium, having a highly reduced stipe or a small sterile basal attachment, with hypogeous to subhypogeous habit, and having globose to subglobose basidiospores.

**Molecular analysis.**—DNA was extracted from dehydrated glebal tissue by the cetyltrimethylammonium bromide (CTAB)-chloroform method (Rogers and Bendich 1994), with a few modifications, including 1–3 cycles of isopropanol and sodium acetate solution rinses to improve DNA precipitation. The nuc rDNA internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) barcode region was amplified via polymerase chain reaction (PCR) with different primers pairs: ITS1, ITS1-F, ITS4, ITS4-B and ITS5 (White et al. 1990; Gardes and Bruns 1993). PCR reactions were performed in 25- $\mu$ L reaction tubes with 1.1× Reddy Mix PCR Master Mix (2.5 mM MgCl<sub>2</sub>) (ABGene, Thermo Fisher Scientific, Epsom, UK) according to the manufacturer's instructions. Cycling conditions consisted of 2 min at 94 C, followed by 35 cycles of 45 s at 94 C, 30 s at 50 C, and 60 s (+ 1 s/cycle) at 72 C, and a 10-min final extension at 72 C. The PCR products were checked for positive amplification on 1% agarose gels and amplified products were sent to Macrogen (Seoul, South Korea) for purification and sequencing on an ABI 3730 XL automatic sequencer (Applied Biosystems, Carlsbad, California).

**Phylogenetic analysis.**—An ITS matrix for phylogenetic analyses was built through a similarity search on publicly available sequences of cortinarioid fungi. For this, we extracted the Cortinariaceae sequences from the complete UNITE public fungi database version 7 (including all sequences from the National Center for Biotechnology Information [NCBI] and UNITE), to use these as the target for a similarity search using USEARCH (Edgar 2010). The ITS sequence from the type specimens presented here was used as query, and the search was conducted with a USEARCH\_GLOBAL strategy, with a 0.9 identity threshold. The best 50 hits for each query were retained, and after excluding duplicates sequence (i.e., sequences that were retrieved because of their similarity with more than one query), 127 sequences of Cortinariaceae fungi were retained. To this data set, additional sequences from the species described here and novel sequences obtained from herbarium specimens identified as “*Thaxterogaster*” from Patagonia were

added. Two sequences of *Hebeloma* were included as outgroups, following Danks et al. (2010). After an initial analysis, several sequences with duplicate entries on the UNITE and NCBI databases were detected, and only one was retained. The final ITS data set used to compute the trees comprised 133 sequences (TABLE 1). Alignment of the ITS data set was performed with MUSCLE (Edgar 2004), whereas manually corrections were done in BioEdit 7.0.5.3 (Hall 1999). Maximum likelihood (ML) analysis was performed using PhyML 3.0 (Guindon et al. 2010) under the TN+G+I substitution model, previously determined as the best-fit model with jModelTest 2.0 (Posada 2008). Bootstrap analyses were run with 1000 replicates. Bayesian inference (BI) analyses were conducted in MrBayes 3.2.2 (Ronquist et al. 2012) with four incrementally heated simultaneous Monte Carlo Markov chains over 10 million generations under the GTR+G+I model of DNA substitution, since the TN model cannot be implemented. Random trees were used as the starting point, and the sample frequency was 1000 generations, resulting in 10 000 sampled trees. A majority rule consensus tree was computed using all the trees remaining after discarding the “burn-in” fraction. To confirm that the analysis has reached a stationary state and to estimate the burn-in fraction, the trace files were visualized in Tracer 1.6 (Rambaut et al. 2014).

In order to assess the diversity of “cortinarioid” taxa, we used the species hypothesis (SH) based on a threshold dissimilarity value of 0.5% (Köljalg et al. 2013) to improve the accuracy and ease the comparison among studies. Garnica et al. (2016) considered that a value of 1% is suitable for distinguishing species in *Cortinarius* using the complete ITS region (Garnica et al. 2016).

## RESULTS

**Phylogenetic analyses.**—The data set comprised *Cortinarius* representatives from Europe, Oceania, North America, and South America, which were distributed across the whole tree. The hypogeous *Cortinarius* specimens analyzed were resolved as four individual clades at the species level, showing good support in both maximum likelihood (bootstrap [BS] >75) and Bayesian (posterior probability [PP] >0.95) analyses. However, basal relationships of the clades were poorly resolved, as expected due the low power of the ITS region to determine basal relationships within *Cortinarius* (Frøslev et al. 2005). Of the four new species resolved with strong support, three of them nested with a number of unidentified ECM sequences obtained from Patagonian Nothofagaceae root tips.

The analysis of the ITS data set allowed us to define eight clades belonging to either previously unsequenced or undescribed species within *Cortinarius* (FIG. 1) that were known by previously generated ECM root tip sequences (Nouhra et al. 2013). The majority of these clades received high support values, with the exception of clades 2 and 3, which correspond to single-sequence clades. Moreover, the sequences in each of the eight groups formed shown a unique SH code within each clade that depicts the independent nature of these taxa, and for which no other sequences are available.

## TAXONOMY

*Cortinarius flavopurpureus* Pastor & Nouhra, sp. nov. FIGS. 2a, 3a–b, 4a–b

MycoBank MB805356

**Diagnosis:** Basidiomata hypogeous, gasteroid, globose to subglobose in shape, enclosed. Peridium bright yellow, opaque. Gleba loculated to labyrinthiform, vinaceous-brown. Basidiospores mostly globose, ornamented with anastomosed irregular rounded small warts.

**Typification:** ARGENTINA. NEUQUÉN: Departamento (Dpto.) Lacar, Lanín National Park, route to Huam Hum (40°08'14.6"S, 71°28'2.6"W), 26 Nov 2008, E. Nouhra (**holotype** CORDC00006037).

**Etymology:** Referring to the yellowish coloration of the peridium surface and the lilac tints of the gleba (Latin: *flavus* = yellow; *purpureus* = lilac, violaceous).

Basidiome gasteroid, 25 mm diam × 15 mm high. Globose to subglobose in shape, irregular, with depressions on the peridium surface that retain soil particles. Small, white, sometimes translucent, sterile base not protruding from the surface, percurrent through the gleba as a columella, occasionally absent. Basal mycelium attached to the base. Odor sweetish, somewhat rancid, yeasty, very strong. Peridium light yellow-brown (chamois to cinnamon-buff). Peridial context white to cream-colored (white to ivory yellow), waxy in appearance. Gleba loculated to labyrinthiform, vinaceous-brown, brown-pink to light brown (vinaceous-cinnamon to vinaceous-fawn). Chambers elongated, 0.14–0.55 mm. Peridiopellis 110–460 µm thick, 3-layered. Suprapellis of yellow-brown, gelatinized, collapsed hyphae, 1.5–2 µm diam. Mediopellis of hyaline, irregular, clamped hyphae, tightly intermingled, 3–12 µm diam. Subpellis with hyaline, somewhat cylindrical hyphae, septa inflated, 3–9 µm diam. Basidia with 4 sterigmata, 30–35 × 8–10 µm, sterigmata 5–7.5 × 1–2 µm, acutely conic to bottle-shaped with a narrowing short neck. Basidiospores globose to subglobose, rarely ellipsoidal, cinnamon brown to caramel, 9.5–14.5 ×

**Table 1.** ITS sequences used in the phylogenetic analyses.

Sequence name	Herbarium	Accession no.	SH (07FU)	Origin	Source
<i>Cortinarius achrous</i>	PDD 107695	KT875174	SH304195	OC	BSD
<i>C. albocanus</i>	CORDC00006081	MG554214	—	SA	BSD
<i>C. albocanus</i>	EN27 (CORD)	JX983156	SH305032	SA	BSD
<i>C. austrovenetus</i>	MEL2089663	JX679099	SH304074	OC	BSD
<i>C. balteatocumatilis</i>	KS CO1393	KJ421057	SH304033	EU	BSD
<i>C. balteatocumatilis</i>	TUB 019792	KJ421047	SH304033	EU	BSD
<i>C. balteatocumatilis</i>	TUB 011440	AY174801	SH304033	EU	BSD
<i>C. balteatocumatilis</i>	TAAM128845	UDB016235	SH304033	EU	BSD
<i>C. cf. fuegianus</i>	CORDC00006080	MG554215	—	SA	BSD
<i>C. cycneus</i>	PDD 88966	GU222272	SH304205	OC	BSD
<i>C. cycneus</i>	OTA 60178	JX178610	SH304205	OC	BSD
<i>C. cycneus</i>	PDD 89071	GU222297	SH304205	OC	BSD
<i>C. dibaphoides</i>	CORDC00006034	MG554223	—	SA	BSD
<i>C. dibaphoides</i>	CORDC00006048	MG554225	—	SA	BSD
<i>C. elaiops</i>	PDD 107732	KT875181	SH304937	OC	BSD
<i>C. elaiops</i> (T)	PDD 88271	JX000369	SH304937	OC	BSD
<b><i>C. flavopurpureus</i>, sp. nov.</b> (T)	CORDC00006037	JX983147	SH304137	SA	BSD
<b><i>C. flavopurpureus</i>, sp. nov.</b>	CORDC00006038	JX983148	SH304137	SA	BSD
<b><i>C. flavopurpureus</i>, sp. nov.</b>	CORDC00006039	JX983149	SH304137	SA	BSD
<b><i>C. flavopurpureus</i>, sp. nov.</b>	CORDC00006046	JX983150	SH304137	SA	BSD
<i>C. fuegianus</i>	EN165(CORD)	JX983158	SH304808	SA	BSD
<i>C. glaucopus</i>	UP21	DQ658854	—	EU	BSD
<i>C. holojanthinus</i>	CORDC00006051	MG554219	—	SA	BSD
<i>C. holojanthinus</i>	EN166(CORD)	JX983159	SH304056	SA	BSD
<i>C. indotatus</i>	KS CO1624	KJ421110	SH575022	OC	BSD
<b><i>C. infrequens</i>, sp. nov.</b> (T)	CORDC00006049	JX983155	SH304100	SA	BSD
<b><i>C. infrequens</i>, sp. nov.</b>	CORDC00006031	KY462276	—	SA	BSD
<i>C. ionomataius</i>	PDD 107698	KT875177	SH304326	OC	BSD
<i>C. ionomataius</i>	PDD 89089	GU222303	SH304326	OC	BSD
<b><i>C. nahuelhuapensis</i>, sp. nov.</b> (T)	CORDC00006044	JX983153	SH287082	SA	BSD
<b><i>C. nahuelhuapensis</i>, sp. nov.</b>	CORDC00006043	JX983154	SH287082	SA	BSD
<i>C. napivelatus</i>	PDD 103876	KF727356	SH304323	OC	BSD
<i>C. nothofagi</i>	CORDC00006052	MG554218	—	SA	BSD
<i>C. orixanthus</i>	PDD 107703	KT875185	SH304228	OC	BSD
<i>C. orixanthus</i>	PDD 107709	KT875186	SH304228	OC	BSD
<i>C. paradoxus</i>	CHI 70221	AF389132	SH287100	SA	BSD
<i>C. paradoxus</i>	IB 19650506	AY033108	SH287102	SA	BSD
<i>C. paradoxus</i>	CHI 7022	AY033107	SH287102	SA	BSD
<i>C. psudolargus</i> (T)	PC:R. Henry 70218	KF732395	SH304011	EU	BSD
<i>C. saturniorum</i> (H)	PDD 67176	GU233337	SH304109	OC	BSD
<i>C. sphaerocephalus</i>	CORDC00006082	MG554216	—	SA	BSD
<i>C. sphaerocephalus</i>	CORDC00006050	MG554217	—	SA	BSD
<i>C. subcastanellus</i>	CO 1256	AY669623	SH304082	OC	BSD
<i>C. subcastanellus</i>	NZ8503	AY033110	SH304301	OC	BSD
<b><i>C. translucidus</i>, sp. nov.</b> (T)	CORDC00006045	JX983151	SH304078	SA	BSD
<b><i>C. translucidus</i>, sp. nov.</b>	CORDC00006079	MG554224	—	SA	BSD
<b><i>C. translucidus</i>, sp. nov.</b>	CORDC00006042	JX983152	SH304078	SA	BSD
<i>C. tympanicus</i>	CORDC00006032	MG554220	—	SA	BSD
<i>C. tympanicus</i>	CORDC00006036	MG554221	—	SA	BSD
<i>C. tympanicus</i>	EN76 (CORD)	JX983157	SH304716	SA	BSD
<i>C. variosimilis</i>	EH33 (CPRD)	FJ717597	SH304111	NA	BSD
<i>C. variosimilis</i>	F16580	GQ159915	SH304111	NA	BSD
<i>C. variosimilis</i>	IB 19890493	KF732468	SH304111	NA	BSD
<i>C. variosimilis</i>	VMS 26	FJ717596	SH304111	NA	BSD
<i>C. walkeri</i>	HO A20528A0	AY669632	SH304074	OC	BSD
<i>C. xenosoma</i>	PDD 73149	JX000358	SH304246	OC	BSD
<i>C. xenosoma</i>	PDD 88274	KJ635229	SH304246	OC	BSD
<i>C. xenosoma</i>	PDD 101822	KJ635207	SH304246	OC	BSD
<i>Cortinarius</i> sp.	BH2022F	JF960673	SH304074	OC	BSD

(Continued)

Table 1. (Continued).

Sequence name	Herbarium	Accession no.	SH (07FU)	Origin	Source
<i>Cortinarius</i> sp.	CORDC00006035	MG554222	—	SA	BSD
<i>Cortinarius</i> sp.	F31303	UDB024893	SH304094	NA	BSD
<i>Cortinarius</i> sp.	JLF 2069	JX415335	SH305029	NA	BSD
<i>Cortinarius</i> sp.	PDD 97543	KJ635242	SH304082	OC	BSD
<i>Cortinarius</i> sp.	PDD 103668	KF727362	SH304861	OC	BSD
<i>Cortinarius</i> sp.	PDD 103887	KF727361	SH304964	OC	BSD
<i>Cortinarius</i> sp.	PDD 107717	KT875183	SH304861	OC	BSD
<i>Cortinarius</i> sp.	PDD 103689	KF727357	SH304946	OC	BSD
<i>Cortinarius</i> sp.	T1172	JF960721	SH482408	OC	BSD
<i>Dermocybe austronanceiensis</i>	IB19930363	AF389161	—	SA	BSD
<i>D. purpurata</i>	PDD 103883	KF727360	SH304945	OC	BSD
<i>Hebeloma circinans</i>	DKA d638	AF124699	SH298076	EU	BSD
<i>H. fastibile</i>	IB 19940036	AF325643	SH298050	EU	BSD
<i>Thaxterogaster albocanus</i>	Halling 583	AF325599	SH304637	SA	BSD
<i>T. fragile</i>	Trappe18313	AF325559	SH303987	OC	BSD
<i>T. leucocephalus</i>	MEL2057558	DQ328103	SH304386	OC	BSD
<i>T. levisporus</i>	MEL2057536	DQ328148	SH304234	OC	BSD
<i>T. violaceum</i>	DAOM198883	AF325556	SH304056	SA	BSD
Uncult. <i>Cortinarius</i> clone_Kunzea_21_82	—	JX178498	—	OC	SL
Uncult. <i>Cortinarius</i> clone_Nothofagus_44_166	—	JX178562	—	OC	SL
Uncult. <i>Cortinarius</i> clone_Nothofagus_44_167	—	JX178563	—	OC	SL
Uncult. <i>Cortinarius</i> clone_Nothofagus_46_171	—	JX178567	—	OC	SL
Uncult. <i>Cortinarius</i> clone_Nothofagus_46_172	—	JX178568	—	OC	SL
Uncult. <i>Cortinarius</i> clone_Nothofagus_48_175	—	JX178571	—	OC	SL
Uncult. <i>Cortinarius</i> clone_Nothofagus_48_181	—	JX178577	—	OC	SL
Uncult. <i>Cortinarius</i> clone_Nothofagus_49_182	—	JX178578	—	OC	SL
Uncult. <i>Cortinarius</i> clone_Nothofagus_50_184	—	JX178580	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_33_36	—	JX178531	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_37_136	—	JX178540	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_38_144	—	JX178547	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Nothofagus_51_38	—	JX178583	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Nothofagus_52_42	—	JX178587	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Nothofagus_48_179	—	JX178575	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Pinus_1_25	—	JX178453	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Pinus_6_58	—	JX178477	—	OC	SL
Uncultured <i>Cortinarius</i> clone_60FS	—	KJ701304	SH304100	SA	ECM ( <i>N. nervosa</i> )
Uncultured <i>Cortinarius</i> clone_Kunzea_21_84	—	JX178499	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_25_96	—	JX178513	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_26_98	—	JX178515	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_31_116	—	JX178525	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_32_32	—	JX178527	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_32_34	—	JX178528	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_38_141	—	JX178545	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Kunzea_38_143	—	JX178546	—	OC	SL
Uncultured <i>Cortinarius</i> clone_MorphIS	—	JQ791125	—	OC	ECM ( <i>Pinus</i> sp.)
Uncultured <i>Cortinarius</i> clone_Nothofagus_46_170	—	JX178566	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Nothofagus_57_190	—	JX178600	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Pinus_4_54	—	JX178472	—	OC	SL
Uncultured <i>Cortinarius</i> clone_Pinus_7_66	—	JX178482	—	OC	SL
Uncultured <i>Cortinarius</i> clone_11NF	—	KJ701302	SH303986	SA	ECM ( <i>N. nervosa</i> )
Uncultured fungus clone_C6B10.12	—	JX316249	SH304064	SA	ECM ( <i>N. dombeyi</i> )
Uncultured fungus clone_C8M3.1	—	JX316275	—	SA	ECM ( <i>N. dombeyi</i> )
Uncultured fungus clone_C8M8.27	—	JX316288	SH287082	SA	ECM ( <i>N. dombeyi</i> )
Uncultured fungus clone_CH7.6	—	UDB014322	SH304299	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_OT1.22	—	UDB014285	SH304100	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_OT6.1X	—	UDB014290	SH304681	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_OTT13	—	JX316448	SH304116	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_OTTO1.1	—	UDB014304	SH304047	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_OTTO3.1T	—	UDB014295	SH304078	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_P5M4.1	—	JX316299	—	SA	ECM ( <i>L. obliqua</i> )

(Continued)

**Table 1.** (Continued).

Sequence name	Herbarium	Accession no.	SH (07FU)	Origin	Source
Uncultured fungus clone_P6M3.1	—	JX316312	SH303986	SA	ECM ( <i>L. obliqua</i> )
Uncultured fungus clone_P7B2.1	—	JX316333	SH303986	SA	ECM ( <i>L. obliqua</i> )
Uncultured fungus clone_P7B4.20	—	JX316341	SH304116	SA	ECM ( <i>L. obliqua</i> )
Uncultured fungus clone_R4M2.1	—	JX316363	SH304047	SA	ECM ( <i>L. alpina</i> )
Uncultured fungus clone_R4M7.1	—	JX316370	SH303986	SA	ECM ( <i>L. alpina</i> )
Uncultured fungus clone_R8M1.1	—	UDB007191	SH304100	SA	ECM ( <i>L. alpina</i> )
Uncultured fungus clone_R8M3.1	—	JX316419	SH304290	SA	ECM ( <i>L. alpina</i> )
Uncultured fungus clone_R8M5.1	—	JX316427	SH303986	SA	ECM ( <i>L. alpina</i> )
Uncultured fungus clone_R8M8.1	—	UDB007213	—	SA	ECM ( <i>L. alpina</i> )
Uncultured fungus clone_TR1.6	—	UDB014297	SH304711	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_TR6.14	—	UDB014330	SH287118	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_TRO01	—	JX316449	SH304047	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_TRO01YB	—	JX316450	SH304078	SA	ECM ( <i>N. pumilio</i> )
Uncultured fungus clone_TRON1.2	—	UDB014312	SH304047	SA	ECM ( <i>N. pumilio</i> )

Note. Voucher numbers, GenBank or UNITE accession numbers, and origins and sources of the DNA are listed. Whenever available, the species hypothesis (SH) number according to Kõljalg et al. (2013) corresponding to a 0.5% distance threshold and the know host is given. T = sequence from type; H = sequences from holotype; EU = Europe; NA = North America; OC = Oceania; SA = South America; BSD = basidiome; ECM = ectomycorrhiza; SL = soil.

8–14.5 µm including the ornamentation (length/width ratio (Qr) = 1.12). Ornamentation darker and constituted by conical warts isolated or in groups, showing a blunt apex, 1–2 µm high. Hilar appendix usually conspicuous in the mature basidiospores, sometimes very prominent; inconspicuous when immature.

**Habitat and distribution:** Hypogeous, in organic soil and litter, under *Nothofagus dombeyi* and *Lophozonia obliqua*. Spring and autumn in northern Patagonia.

**Specimens examined:** ARGENTINA. RÍO NEGRO: Dpto. Bariloche, Nahuel Huapi National Park: Mascardi sector, Laguna Los Moscos (41°20'23.2"S, 71°36'8.2"W), 27 Nov 2006, *E. Nouhra*, CORDC00006038; *ibed.*, CORDC00006039; *ibed.*, CORDC00006046; Los Notros Creek, Colonia Suiza (41°04'59.7"S, 71°33'02.6"W), 7 May 2006, *E. Nouhra*, CORDC00006040; *ibid.*, 30 Nov 2005, *E. Nouhra*, CORDC00006047.

**Notes:** In our phylogenetic analyses, *Cortinarius flavo-purpureus* appeared as a monophyletic group and is the only species presented here that does not cluster with available ECM sequences from Nothofagaceae root tips. Considering that these species are difficult to differentiate based only on their anatomical characters, this species can be separated roughly from the other hypogeous gasteroid *Cortinarius* species in South America on the basis of the peridium coloration and structure, constituted by three distinct layers, as well as the smaller size of the basidiospores and ornamentations.

***Cortinarius translucidus*** Pastor & Nouhra, sp. nov. **FIGS. 2b, 3c–d, 4c–d**  
MycoBank MB805357

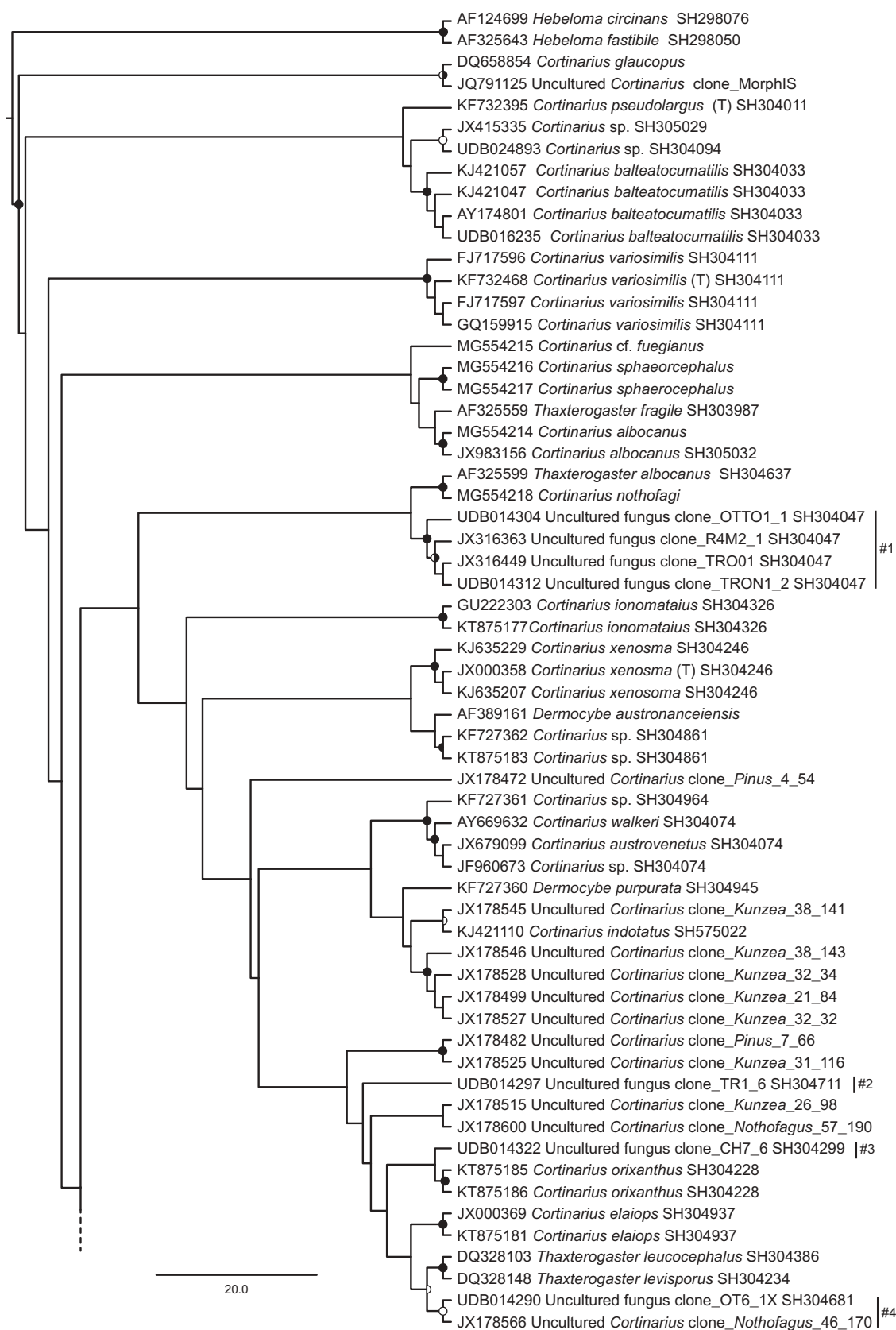
**Diagnosis:** Basidiomata hypogeous, gasteroid, subglobose in shape, fully enclosed in a yellow to yellowish

white translucent peridium. Columella and sterile base present. Gleba labyrinthiform, brown to purple-brown. Basidiospores globose to subglobose, ornamented with big, irregular subconical warts.

**Typification:** ARGENTINA. RÍO NEGRO: Dpto. Bariloche, Nahuel Huapi National Park, Los Notros Creek, near Punto Panorámico (41°20'48"S, 71°36'05.5"W), 30 Nov 2005, *G. Daniele* (**holotype** CORDC00006045).

**Etymology:** Referring to the translucent peridial surface (Latin: *translucidus* = translucent).

Basidiome gasteroid, 20–35 mm diam × 30–50 mm high. Subglobose in shape, lobed, irregular with depressions on the peridium surface that retain soil particles. Reduced sterile base present, barely protruding from the peridium surface. Odor intense, sweetish, rancid, reminiscent of rotten fruits. Peridium white-cream (pale gull gray) to white-yellow (Naples yellow) in the apical part, with lilac tints, somewhat waxy and translucent. On immature individuals the peridium is yellow-brown with lilac tints and waxy appearance. Gleba labyrinthiform, with irregular chambers, purple-brown to vinaceous-brown (fawn-colored to army brown), light brown in immature specimens. Columella arising from the basal attachment, variable in shape and length. Peridiopellis 125–430 µm thick, 2-layered. Suprapellis 60–230 µm thick, clamped, hyaline, cylindrical hyphae, loosely arranged, intermingled, disposed to the surface, tortuously branched, septa inflated, clamped, 1.5–7 µm diam. Subpellis 65–200 µm thick of cylindrical, irregular, hyaline hyphae, forming a tightly intermingled pseudoparenchyma, some hyphae with thickened walls, simple septa, 2–16 µm diam. Basidia with 4 sterigmata, 13–17 × 18–30 µm, protruding from the hymenium, sterigmata



**Figure 1.** Maximum likelihood phylogram inferred from the ITS data set. Bootstrap values >50 and Bayesian posterior probabilities >0.5 are indicated in the nodes by the symbols: ● = BS > 75, PP > 0.95; ◐ = BS > 75, PP < 0.95; ◑ = BS < 75, PP > 0.95; ○ = BS < 75, PP < 0.95. New taxa presented here are indicated in boldface and a gray box. # symbols indicate taxa known only from ECM sequences. SH values correspond to version 7.0 of the UNITE database.



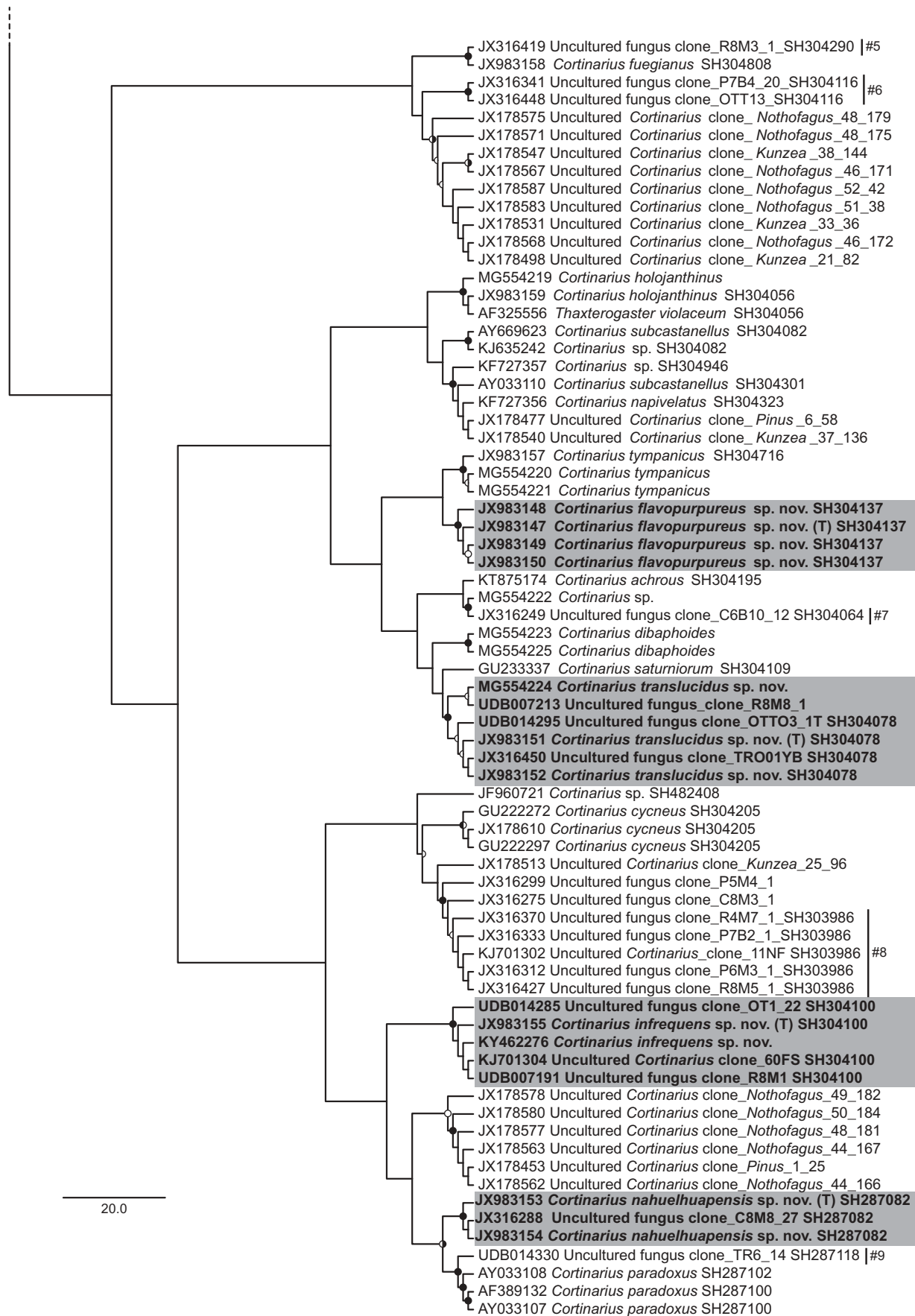
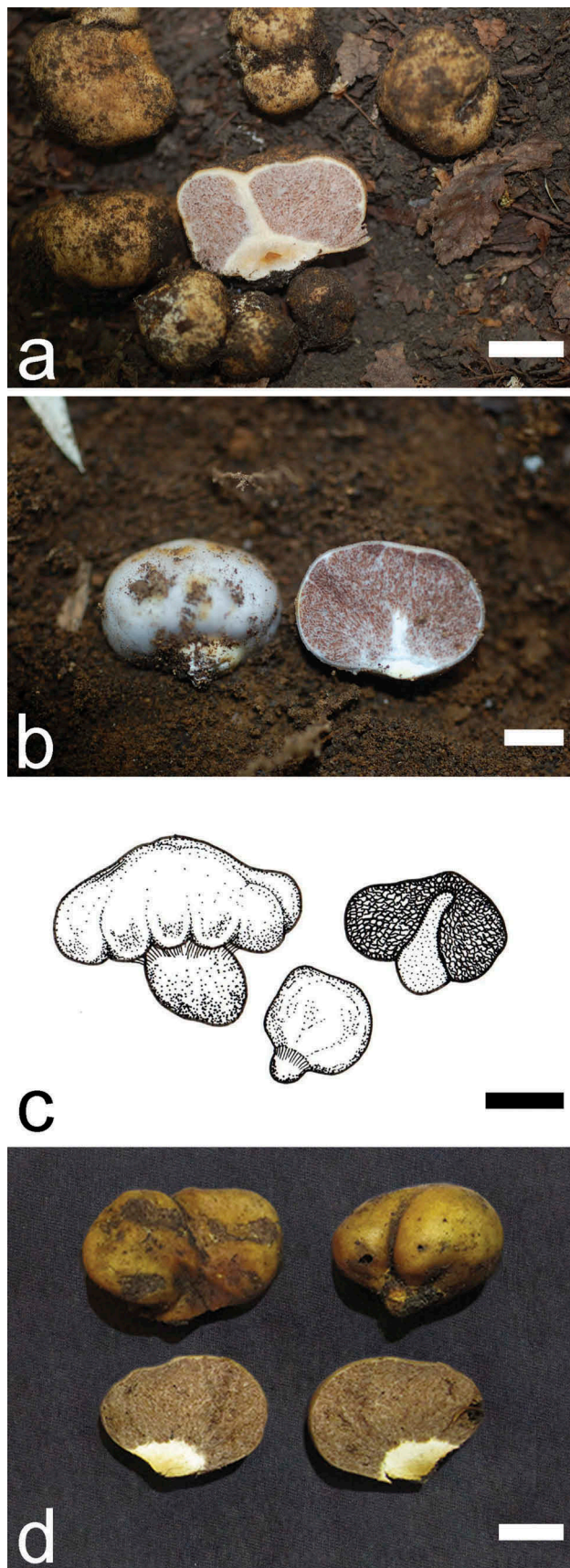
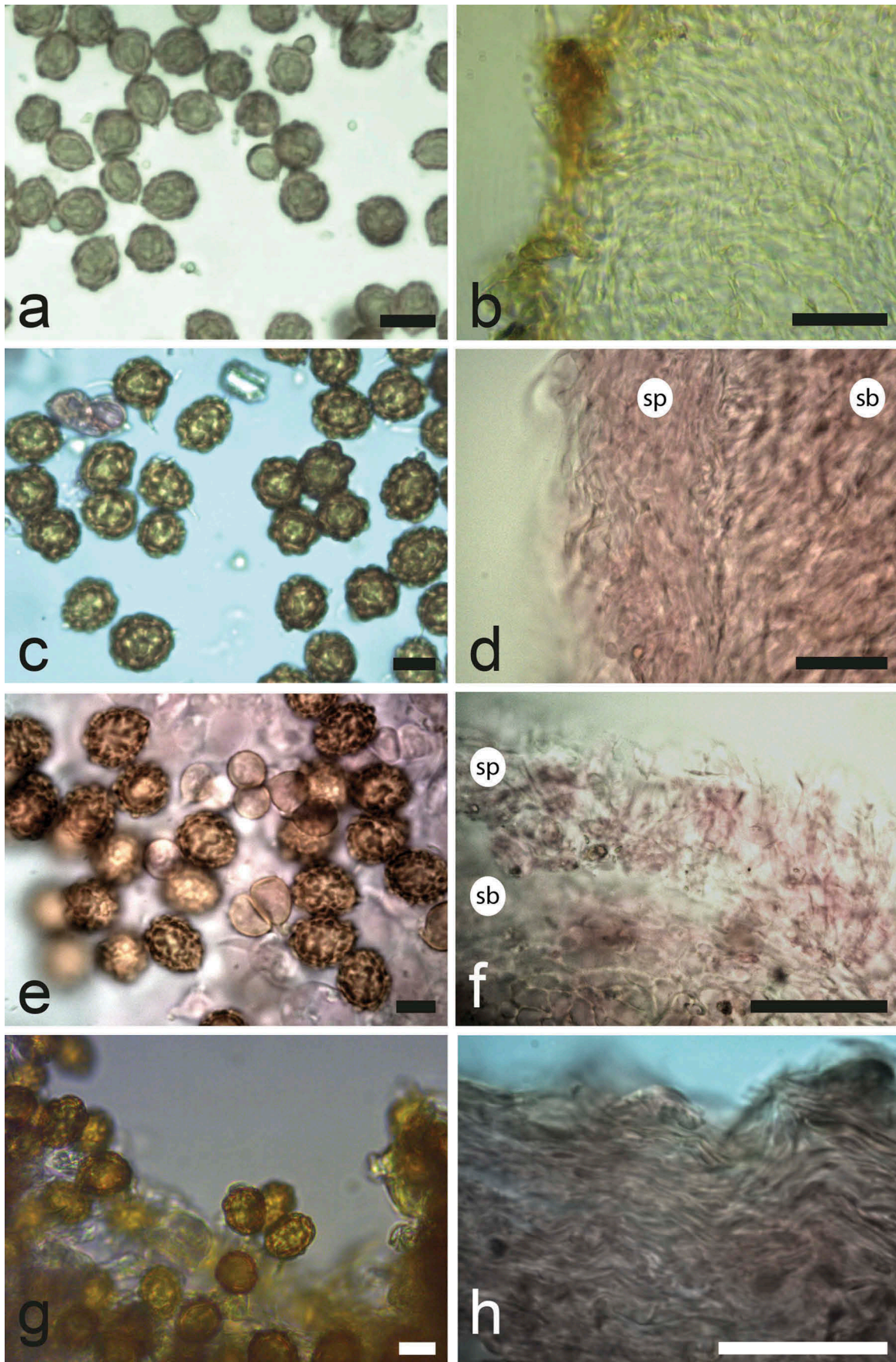


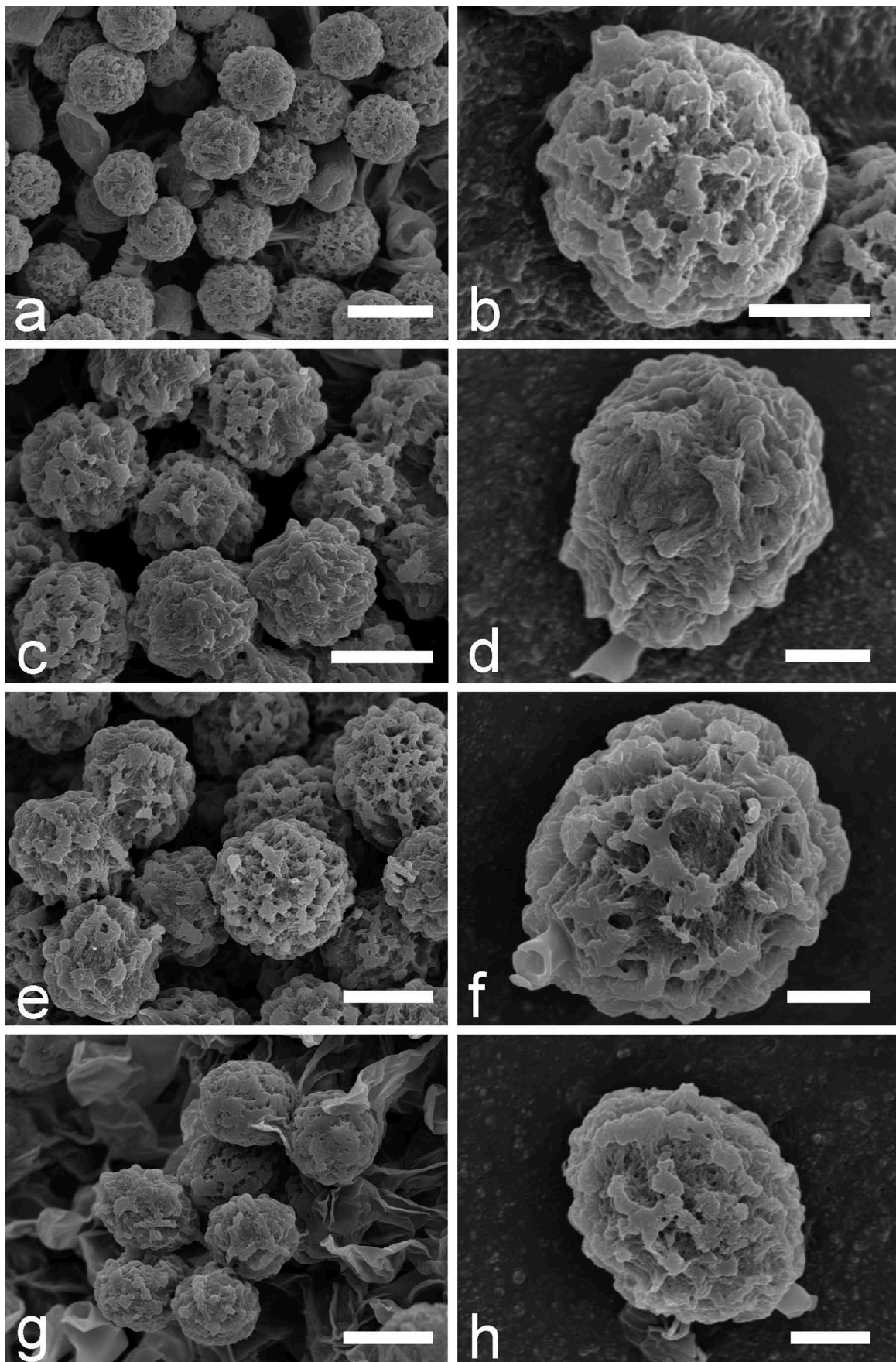
Figure 1. Continued.



**Figure 2.** Basidiomes of the new species. a. *Cortinarius flavopurpureus*. b. *C. translucidus*. c. *C. nahuelhuapensis*. d. *C. infrequens*. Bars = 1 cm.



**Figure 3.** Basidiospores and peridiopellis of the new species. a–b. *Cortinarius flavopurpureus*. c–d. *C. translucidus*. e–f. *C. nahuelhuapensis*. g–h. *C. infrequens*. sp = suprapellis; sb = subpellis. Bars: a, c, e, g = 10 μm; b, h = 40 μm; d, f = 100 μm.



**Figure 4.** Scanning electron microphotographs of the basidiospores of the new species. a–b. *Cortinarius flavopurpureus*. c–d. *C. translucidus*. e–f. *C. nahuelhuapensis*. g–h. *C. infrequens*. Bars: a, c, e, g = 10 μm; b, d, f, h = 4 μm.

straight, bottle-shaped, 8–12  $\mu\text{m}$  long, with a basal cylindrical portion 3–5  $\mu\text{m}$  wide and a long neck 1–2  $\mu\text{m}$  wide. Basidiospores globose to subglobose, (13–)14.5–19.5  $\times$  13–17  $\mu\text{m}$ , including the ornamentation ( $Q_r = 1.08$ ). Dark brown to honey-colored in 5% KOH. Ornamentation constituted by irregular groups of conical warts, with a blunt apex, 2–4.5  $\mu\text{m}$  high. Hilar appendix conspicuous in the mature basidiospores, absent in immature basidiospores.

*Habitat and distribution:* Hypogeous, in organic soil and litter, under *Nothofagus dombeyi*, *N. antarctica*, and *Lophozonia alpina*. Spring and autumn in northern Patagonia.

*Specimens examined:* ARGENTINA. RÍO NEGRO: Dpto. Bariloche, Nahuel Huapi National Park, Cerro Tronador, Ventisquero Negro (41°11'58"S, 71°49'42.9"W), 3 Dec 2005, *E. Nouhra*, CORDC00006033; NEUQUÉN: Dpto. Los Lagos, Nahuel Huapi National Park, RN 231, 9.3 km east of the Chilean border (40°40'40.32"S, 71°44'51.84"W), 29 Apr 2001, *J. Trappe*, CORDC00006053, *Trappe26340* (OSC); Dpto. Lacar, Lanín National Park, road to Cascada Chachín, Don Bartolo Campground (40°07'56.4"S, 71°39'26.2"W), 26 Nov 2008, *E. Nouhra*, CORDC00006041; *ibed.*, CORDC00006042; CHUBUT: Dpto. Futaleufú, Cañadon Huemules (42°46'39"S, 71°27'49"W), 24 Apr 2013, CORDC00006079.

*Notes:* In the ITS data set analysis, *Cortinarius translucidus* nested together with ECM sequences from *Nothofagus pumilio* and *N. alpina*. The fully enclosed gasteroid hypogeous habit differentiates this species from previously described taxa in the region. In addition, the whitish coloration and translucent appearance of the peridium, as well as 2-layered peridiopellis instead of 3-layered or single-layered, respectively, separates this species from the other gasteroid hypogeous *C. flavopurpureus* and *C. infrequens* described in this study.

***Cortinarius nahuelhuapensis*** Pastor & Nouhra, sp. nov. FIGS. 2c, 3e–f, 4e–f

MycoBank MB805358

*Diagnosis:* Basidiomata subhypogeous, secotioid, dorsoventrally compressed, with a reduced stipe at the base. Gray to brown slightly translucent peridium. Partial veil persistent. Columella cartilaginous. Gleba loculated, dark brown. Basidiospores oval-shaped to subglobose. Ornamentation constituted by anastomosed groups of blunt warts.

*Typification:* ARGENTINA. RÍO NEGRO: Dpto. Bariloche, Nahuel Huapi National Park, Mascardi sector, Laguna Los Moscos (41°20'52.2"S, 71°36'00.8"W), 9 May 2006, *E. Nouhra* (**holotype** CORDC00006044).

*Etymology:* Referring to the type locality, Nahuel Huapi National Park.

Basidiome secotioid, 32 mm diam  $\times$  30 mm high. Globose in shape, irregular, dorsoventrally compressed, with a reduced undeveloped stipe protruding from the base. Odor intense, fruity. Peridium vinaceous-brown (army brown), variable, when immature, gray to light brown (dark olive-buff) with water-green (water green) tints, somewhat translucent, with soil particles attached. Gleba loculated, dark brown. Columella present, cartilaginous, white to cream, with light brown tints. Partial veil persistent, fibrillose, cream, somewhat lilac. Stipe short, barely protruding downward, white to cream, to light brown with lilac tints (fawn color), translucent at the base. Peridiopellis 85–260  $\mu\text{m}$  thick, 2-layered. Suprapellis 65–180  $\mu\text{m}$  thick, hyaline, cylindrical, clamped, branched, and thin-walled hyphae, loosely intermingled, 1.5–5  $\mu\text{m}$  diam. Subpellis 20–80  $\mu\text{m}$  thick of irregular hyphae with inflated septa, 6–20  $\mu\text{m}$  diam, forming a prosenchyma. In immature specimens, the peridiopellis presents a discontinuous and evanescent surface layer, formed by polygonal hyphal segments 19–28  $\times$  13–20  $\mu\text{m}$ , and constituting a pseudoparenchymatous tissue, 110–180  $\mu\text{m}$  wide. Basidia with 4 sterigmata, 15–24  $\times$  10–14  $\mu\text{m}$ , widening toward the apical portion, sterigmata straight, reaching 10  $\mu\text{m}$ , bottle-shaped to acute, never wider than 3  $\mu\text{m}$  in the base. Basidiospores oval-shaped, subglobose, sometimes globose, ferruginous, honey-cinnamon in 5% KOH, 13–17.5(–21)  $\times$  10–17.5(–19)  $\mu\text{m}$  including the ornamentation ( $Q_r = 1.20$ ). Warty ornamentation with blunt warts composed of anastomosed columnar processes, some isolated, 1–3.5  $\mu\text{m}$ . Hilar appendix conspicuous in the mature basidiospores.

*Habitat and distribution:* Hypogeous to subhypogeous, in organic soil and litter, under *Nothofagus dombeyi* and *N. pumilio*. Autumn in northern Patagonia.

*Specimen examined:* ARGENTINA. RÍO NEGRO: Dpto. Bariloche, Nahuel Huapi National Park, Cerro Tronador, Ventisquero Negro (41°11'58"S, 71°49'42.9"W), 8 May 2008, *E. Nouhra*, CORDC00006043.

*Notes:* On the basis of the phylogenetic analysis of the ITS sequences, *Cortinarius nahuelhuapensis* sequences grouped together with a *Nothofagus pumilio* root tip ECM sequence. This clade appears as a sister taxon of *C. paradoxus* M.M. Moser & Horak, another *Cortinarius* representative from Nothofagaceae forests in South America, with a strong support in the BI analysis (PP > 0.95) and a medium support in the ML analysis (75 > BS > 50). Morphologically, *C. nahuelhuapensis* is similar to *C. holojanthinus*, a previously described species from Patagonia. Some obvious morphological differences between these two species are their coloration, being lilac-white in the latter and vinaceous-brown in the former,

and also the fact that *C. holojanthinus* has ovoid to ellipsoidal basidiospores, which are finely ornamented, whereas *C. nahuelhuapensis* has globose to subglobose basidiospores, with coarsely warty ornamentations. *Cortinarius nahuelhuapensis* can also be separated from a hypogeous stipitate species described from Argentina in 1960 by Singer, as *Thaxterogaster brevisporus*, currently transferred as *C. brachyspermus* Peintner & M.M. Moser. The main differences between these two species at the morphological scale are the grayish white viscid peridium in *T. brevisporus* and bigger and globose basidiospores on average (16.5–18.5 × 14.5–16.5 µm), characterized by small and blunt warts, often merging into crested short ridges and connected by thin, vein-like anastomoses, as described and illustrated in Singer (1960). *Cortinarius nahuelhuapensis* has smaller subglobose basidiospores with much coarser warted ornamentation. We have not been able to study additional collections of *T. brevisporus*; the only known collection is the holotype, which is deposited at the Lillo herbarium (LIL). This specimen showed basidiospores similar in shape (but not size) to those of *C. nahuelhuapensis*; however, other relevant structures of the basidiome in the holotype are missing, precluding us to further characterize this specimen. We have also not succeeded in getting DNA samples.

***Cortinarius infrequens*** Pastor & Nouhra, sp. nov.

FIGS. 2d, 3g–h, 4g–h

MycoBank MB805359

**Diagnosis:** Basidiomata hypogeous, gasteroid, dorsoventrally compressed. Peridium opaque, brownish orange, glabrous. Columella arising from a small base, occasionally absent. Gleba loculated, pale brown. Basidiospores globose to subglobose, some oval-shaped. Ornamentation constituted by irregular anastomosed ridges and warts with a blunt apex.

**Typification:** ARGENTINA. RÍO NEGRO: Dpto. Bariloche, Estacada Agreste Camping, RN 231, 48.5 KM from Bariloche (40°50'16"S, 71°32'15"W), 30 Apr 2001, L. Domínguez (**holotype** CORDC00006049). Isotype Trappe26352 (OSC).

**Etymology:** Referring to the scarcity of the species.

Basidiome gasteroid, 32 mm diam × 17 mm high. Ellipsoidal in shape, dorsoventrally compressed, irregular, with depressions on the peridium surface that retain soil particles. Odor mild. Peridium brown-orange (orange-cinnamon to Mikado brown), glabrous. Gleba loculated, cinnamon (cinnamon) to pale brown; tramal plates narrow, gray. Spore mass cinnamon. Columella white to light brown, arising from a broadened base, percurrent; occasionally absent. Peridiopellis 25–65 µm thick, of tightly intermingled, irregular to cylindrical, hyaline or yellowish, gelatinized, collapsed hyphae, some of them with

thickened walls, simple septa, 3–8 µm diam. Basidia with 4 sterigmata, 23–31 × 7–11 µm, sterigmata straight to slightly curved, 5–8 × 1–2 µm, bottle-shaped with a narrowing short neck. Basidiospores globose to subglobose, some oval-shaped, cinnamon brown to honey-colored, 11–17.5 × 9–13.5 µm including the ornamentation (Qr = 1.31). Ornamentation formed by irregular anastomosed ridges and warts with a blunt apex, 1–5(–6) µm high. Hilar appendix sometimes conspicuous in mature basidiospores.

**Habitat and distribution:** Hypogeous, in organic soil and litter, under *Nothofagus dombeyi*. Autumn and spring in northern Patagonia.

**Specimen examined:** ARGENTINA. RÍO NEGRO: Dpto. Bariloche, Nahuel Huapi National Park, Mascardi sector, near park ranger guard house (41°21'12.9"S, 71°30'54.89"W), 13 Nov 2015, A. Mujic, CORDC00006031.

**Notes:** The phylogenetic analysis of the ITS showed that the *Cortinarius infrequens* sequence clustered and shared an SH code with ECM root tip sequences from *Nothofagus pumilio*, *N. nervosa*, and *Lophozonia alpina*. This species might be morphologically confused with *C. flavopurpureus* due to peridium coloration and shape, yet it differentiates from that species by having a single-layered peridiopellis instead of a 3-layered peridiopellis. A recent work found this species to be the closest relative to *Cortinarius majesticus* (Anderson and Orlovich 2016), although the sequence of this specimen was not recovered in our search.

#### KEY TO SEQUESTRATE *CORTINARIUS* SPECIES FROM PATAGONIA WITH DISTINCTLY GLOBOSE-SUBGLOBOSE BASIDIOSPORES

1. Basidiome enclosed, stipe not reduced to a sterile base, gleba not exposed..... 2
- 1'. Basidiome enclosed without stipe or having a small basal attachment ..... 3
2. Peridium grayish white and viscid, basidiospores globose, 16.5–18.5 × 13.5–16.5 µm, heavily verrucose, forming ridges and anastomoses.....  
.....*C. brachyspermus* Peintner & M.M. Moser
- 2'. Peridium vinaceous-brown, basidiospores globose to subglobose, 13–17.5 × 10–17.5 µm, with warty ornamentation, blunt warts composed of anastomosed columnar processes.....*C. nahuelhuapensis*
3. Peridium translucent, white to whitish yellow with lilac tints, somewhat waxy in appearance, sterile base reduced and percurrent as a columella, variable in shape.....*C. translucidus*
- 3'. Peridium opaque, pale yellow, yellowish brown to brownish orange, sometimes with lilac tints, never translucent ..... 4

4. Peridium light yellow-brown. Peridiopellis constituted by three distinct layers, sterile base reduced, not protruding downward, but forming a well-developed columella, occasionally absent.....  
..... *C. flavopurpureus*
- 4'. Peridium brown-orange. Peridiopellis constituted by a single layer of tightly intermingled, irregular to cylindrical hyphae, with a columella sometimes present, arising from a broadened base.....  
..... *C. infrequens*

## DISCUSSION

The four newly described taxa were resolved with strong support at the species level in both ML and BI analyses with the ITS data set, which was also true for several species across the whole tree. This confirms that a single-locus ITS analysis is useful for differentiating species within *Cortinarius*, even when they share high sequence identity, as previously shown by Garnica et al. (2016). A detailed discussion of individual aspects related to each species analyzed is given in the previous paragraphs.

Our analysis could not resolve the basal relationships within the data set, and the new taxa described here cannot be assigned to any particular infrageneric group within *Cortinarius*. However, we observed that the loss of gross morphological characters in sequestrate basidiomes can make the placement of taxa difficult within broader sectional groupings, as suggested by Danks et al. (2010). Fortunately, an upcoming work attempting to span the genus *Cortinarius* on the scale of global supraspecific structure, which proposes new sections on the basis of molecular data, is in preparation (Soop, pers. comm.). This study will include some of the new species presented here, which may bring some new light to their infrageneric placement.

In summary, four new sequestrate species of *Cortinarius* from the Nothofagaceae forests of Argentina were described on the basis of their morphology and phylogenetic affinities. The exploration of new and cryptic morphological characters within this group, such as those evidenced by SEM photography (shape and distribution of spore ornamentations), provided additional traits for species characterization. Additionally, environmental sequences were linked to *C. fuegianus* Peintner & M.M. Moser (JX983158) and *C. paradoxus* (AF389132 and AY033108) voucher materials, which corroborates the ECM nature of these species. In addition, at least nine clades were also detected from ECM sequences from Nothofagaceae in Patagonia. These ECM sequences correspond to a previous study (Nouhra et al. 2013), and

they should be considered potentially new taxa. Further studies are necessary, to provide data from voucher material and environmental sequences from the vast Nothofagaceae forests of South America and further explore the diversity of “cortinarioid” taxa in Patagonia.

## ACKNOWLEDGMENTS

We would like to thank B. Dima, S. Garnica, J. Geml, and G. Robledo for their important contributions to the quality of the manuscript, and to J. Trappe, L. Domínguez, and G. Daniele for their assistance in the sampling campaigns. We also thank the Laboratorio de Microscopía Electrónica y Microanálisis (LABMEM) of the Universidad Nacional de San Luis, Argentina, for the SEM photomicrographs.

## FUNDING

This study was supported by CONICET, PIP 6193 and SECYT 214/10. Participation for A. B. Mujic was supported by the US National Science Foundation grant DEB 1354802 (to M. E. Smith).

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