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Vibrisseaceous fungi from the southern hemisphere, including *Chlorovibrissea chilensis* (Helotiales, incertae sedis) sp. nov.

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Abstract: The discovery of *Chlorovibrissea chilensis* sp. nov. expands the distribution of *Chlorovibrissea* from Australasia to include South America. *C. chilensis*, phylogenetically distinct from other species in the genus, is also characterized morphologically by its ascoma with emerald green stalk and pale orange-brown head, budding paraphyses and 5–6-septate ascospores. Based on the phylogenetic analysis, the Australasian species *Vibrissea albofusca* is recombined in *Chlorovibrissea*, despite the fact it lacks the distinctive green pigmentation of other species in this genus. In addition, the genus *Vibrissea* in a strict phylogenetic sense is confirmed from the southern hemisphere for the first time; *Vibrissea truncorum* is reported from Chile and *V. flavovirens* from New Zealand.

Key words: Ascomycota, Chile, New Zealand, *Nothofagus*, phylogeny, *Vibrissea*

Introduction

Mycological exploration in southern Chile revealed three interesting and beautiful collections of vibrisseaceous fungi. One of them, comprising many apothecial ascomata with emerald green stalks and pale orange-brown heads, was macromorphologically typical of *Chlorovibrissea* species whereas the other two collections matched *Vibrissea.*

Chlorovibrissea was erected by Kohn (1989) for *Vibrissea* species from Australia and New Zealand that were characterized by having apothecia with green pigmentation and an incomplete apical ascal ring that turns intensely blue in Melzer's reagent. Kohn proposed a new species from New Zealand (*C. phialophora* Samuels & L.M. Kohn) and transferred three species, *C. tasmania* (Rodway) L.M. Kohn, *C. melanochlora* (Beaton & Weste) L.M. Kohn and *C. bicolor* (Beaton & Weste) L.M. Kohn, from *Vibrissea* to the new genus. Kohn thus narrowed the circumscription of *Vibrissea* from that of Sanchez and Korf (1966) and Beaton and Weste (1976), distinguished *Vibrissea* from *Chlorovibrissea* on the basis of the lack of green pigmentation in its stipitate or sessile ascomata, a less broad amyloid apical ring. *Vibrissea* in this sense had a distribution mainly in the northern hemisphere, with the unique exception of *V. albofusca* G.W. Beaton, that was known only from the southern hemisphere.

In this article we contribute to the knowledge of vibrisseaceous fungi based on morphological and molecular analyses of new collections from South America and New Zealand.

Materials and methods

Collections.—The samples were collected and processed according to Rossman et al. (1998). Microscopic preparations were made in water, Melzer's reagent or 5% KOH and floxine with optical microscopy and in calcofluor (Romero and Minter 1988) with epifluorescence microscopy (EPM). Vertical sections were cut to 20 μm thick with a freezing microtome and mounted in lactic acid. Cultures were grown from ascospores shot upward onto water agar plates from freshly collected apothecia and stored in liquid N. Voucher specimens have been kept at the BAFC, SGO and PDD herbaria and living cultures have been deposited in the ICMP culture collection (International Collection of Microorganisms from Plants, maintained by Landcare Research, Auckland, New Zealand, www.landcareresearch.co.nz/science/plants-animals-fungi/fungi/collections-and-databases). Herbarium acronyms follow Thiers (2013).

Molecular techniques.—DNA was isolated from mycelium grown in culture in a liquid medium containing 2% Difco malt extract or from dried apothecia taken from fungarium specimens. For DNA extraction, dried mycelium harvested from the liquid cultures or apothecia from fungarium specimens were ground in 400 μL lysis buffer (QIAGEN,

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TABLE I. New ITS sequences generated as part of this project

Species	Country	Voucher No.	GenBank No.
Chlorovibrissea albofusca	New Zealand	PDD 88457	JN809648
Chlorovibrissea bicolor	Australia	ICMP 19895	KF924737
Chlorovibrissea chilensis	Chile	PDD 99891	KF429258
Chlorovibrissea phialophora	New Zealand	PDD 83226	KF429261
Vibrissea flavovirens	New Zealand	ICMP 19442	KF429257
Vibrissea truncorum	Chile	PDD 99892	KF429259
Vibrissea truncorum	Chile	PDD 99893	KF429260

USA) with a plastic pestle followed by incubation for 2 h at 55 C; then 220 μL lysed solution was loaded into the QIAxtractor Robot (QIAGEN, USA) and DNA extraction was performed with a QIAGEN DX reagent pack and tissue extraction protocol. Amplification primers were ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993).

Phylogenetic analyses.—The dataset included newly generated ITS sequences from recent collections from Australia, Chile and New Zealand (TABLE I), together with sequences from representative taxa from Wang et al. (2006) that were closely related to the *Vibrissea* and southern *Vibrissea* clades as recognized by Johnston et al. (2010) and Hustad and Miller (2011), with *Mitrula* as outgroup.

Thirty-three sequences were aligned with Clustal X with default settings (Thompson et al. 1997). Alignments and phylogenetic analyses for ITS are deposited in TreeBASE under accession number S15893. Phylogenetic analysis of the sequence data was performed with maximum parsimony on NONA 2.0 (Goloboff 2005) with all characters equally weighted and gaps scored as missing data. Overall 3.5% of the datamatrix cells were scored as gaps. To determine support for each clade, a bootstrap analysis was performed with 1000 replications.

Bayesian inference was calculated with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) under the GTR+I+I model, which was selected as the optimal model with Modeltest 3.5 (Posada and Crandall 1998). Five chains were run from random starting trees for 5 000 000 generations and sampled every 250 generations. The first 5000 generations were discarded as burn-in. Bayesian posterior probabilities were obtained from the 50% majority-rule consensus of the remaining trees, and clades with posterior probabilities ≥ 0.70 were considered to be significantly supported.

RESULTS

Molecular phylogeny.—The trees from Bayesian and maximum parsimony analyses showed no difference in the position of the *Chlorovibrissea* and *Vibrissea* clades. The Bayesian tree is illustrated (FIG. 1). Maximum parsimony analysis yielded one tree of 1263 steps with a consistency index (CI) 0.43 and a retention index (RI) = 0.59 from 267 informative characters.

C. chilensis (GenBank accession number KF429258) is placed within the ''southern hemisphere *Vibrissea* group'' of Wang et al. (2006), close to the specimen PDD 88457 (JN809648) and the reference sequence of *V. albofusca* AY789384. The two specimens from which these sequences were obtained are accepted here as *Chlorovibrissea albofusca* and form a sister relationship to a clade containing an unidentified *Chlorovibrissea* sp. (DQ257353) from New Zealand, *C. bicolor* (KF924737) from Australia and *C. phialophora* (KF429261) from New Zealand.

The two *Vibrissea* species, *V. flavovirens* and *V. truncorum*, represented by specimens from North America (AY34713) and New Zealand (KF429257) and from Europe (EU434855) and Chile (KF429259, KF429260) respectively, form a separate clade in a different part of the tree far from the *Chlorovibrissea* group and are accepted here as *V. truncorum* and *V. flavovirens* (see notes in TAXONOMY).

TAXONOMY

Chlorovibrissea chilensis Sandoval-Leiva, A.I. Romero & P.R. Johnst., sp. nov. FIGS. 2A–D, 3A–J, 4A–B, D. MycoBank MB808576

Ascomata apothecial, stipitate-capitate, erect, fleshy-cartilaginous, gregarious or sometimes in cluster of 2–3 from rising on surface of bark from wood, up to 17 mm high; fertile portion subglobose to cerebroid, usually slightly compressed on top, up to 6 mm high and 9 mm diam, light pinkish beige tinged with orange when fresh and orange to dark gray when dry. Stalk cylindrical up to 1.9 mm thick but somewhat bulbous in the base and thicker on the union with fertile portion up 3.7 mm, longitudinally furrowed and light green darkening to emerald green at the base; darker when dried at the base; generally simple, occasionally bifurcate. Ascomata leaching a green pigment in 5% KOH. Inner excipulum composed of textura intricata, hyphae μm 3–4 diam, continuous with medulla of stipe. External excipulum thin, 15–20 μm, hyphae parallel to the excipular surface; cells cylindrical, external cells producing free

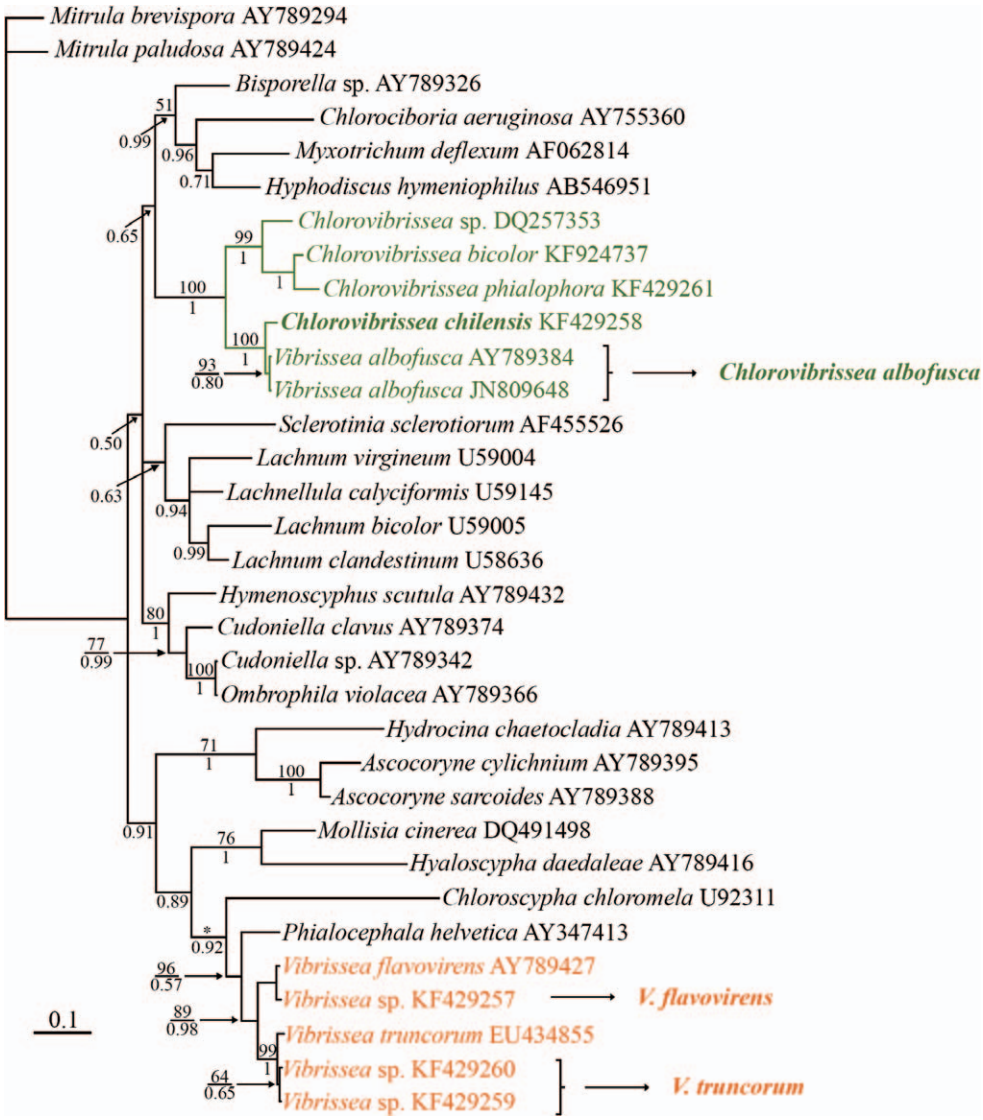

FIG. 1. Tree obtained from nrDNA ITS sequences using Bayesian inference (the BPP are shown below the line and bootstrap values above the line; less than 50% are not shown). Clade marked with an asterisk corresponds to the only branch not resolved in the maximum parsimony analysis.

hyphal tips. Asci eight-spored, 185–200 × 7–8 µm, cylindrical, apical ring J+ in Melzer's reagent. Paraphyses extending beyond asci by ca. 10 µm, hyaline, unbranched, filiform, spathulate or globose, 4–5 µm broad, septate, multiple budding, sometimes monilioid. Ascopores 90–115 × 1.5–2 µm, multiseriate, hyaline, filiform, 5–6-septate.

Holotype: CHILE. AISÉN: comuna Puerto Aisén, Fjord Aisén, Cuervo's river mouth, 45°20′59.79″S, 73°2′55.09″W, on fallen trunk in a mixed forest of *Nothofagus nitida* and *Laureliopsis philipiana*, 10 Jul 2008, *P. Sandoval-Leiva 203* (SGO 163165). *Isotypes* BAFC 52324 and PDD 99891.

Etymology: named after Chile.

Known distribution: type locality.

Notes: The description of the new species was based on one abundant collection comprising at least 50 ascomata growing on a fallen, very wet trunk of an unidentified tree, from a mixed forest of mainly *Nothofagus nitida* (Phil.) Krasser and *Laureliopsis philippiana* (Looser) Schodde, close to the mouth of the Cuervo River, obtained during a trip to Aisén Fjord, southern Chile (FIG. 5B). According to Gajardo's Chilean vegetation classification system (Gajardo 1994), the collection site is within the region of evergreen forest and the bogs and, according to Köeppen's classification system (Kottek et al. 2006), the site has a humid, warm-temperate climate with a high, evenly distributed rainfall throughout the year (over 3000 mm/y in nearby Puerto Chacabuco).

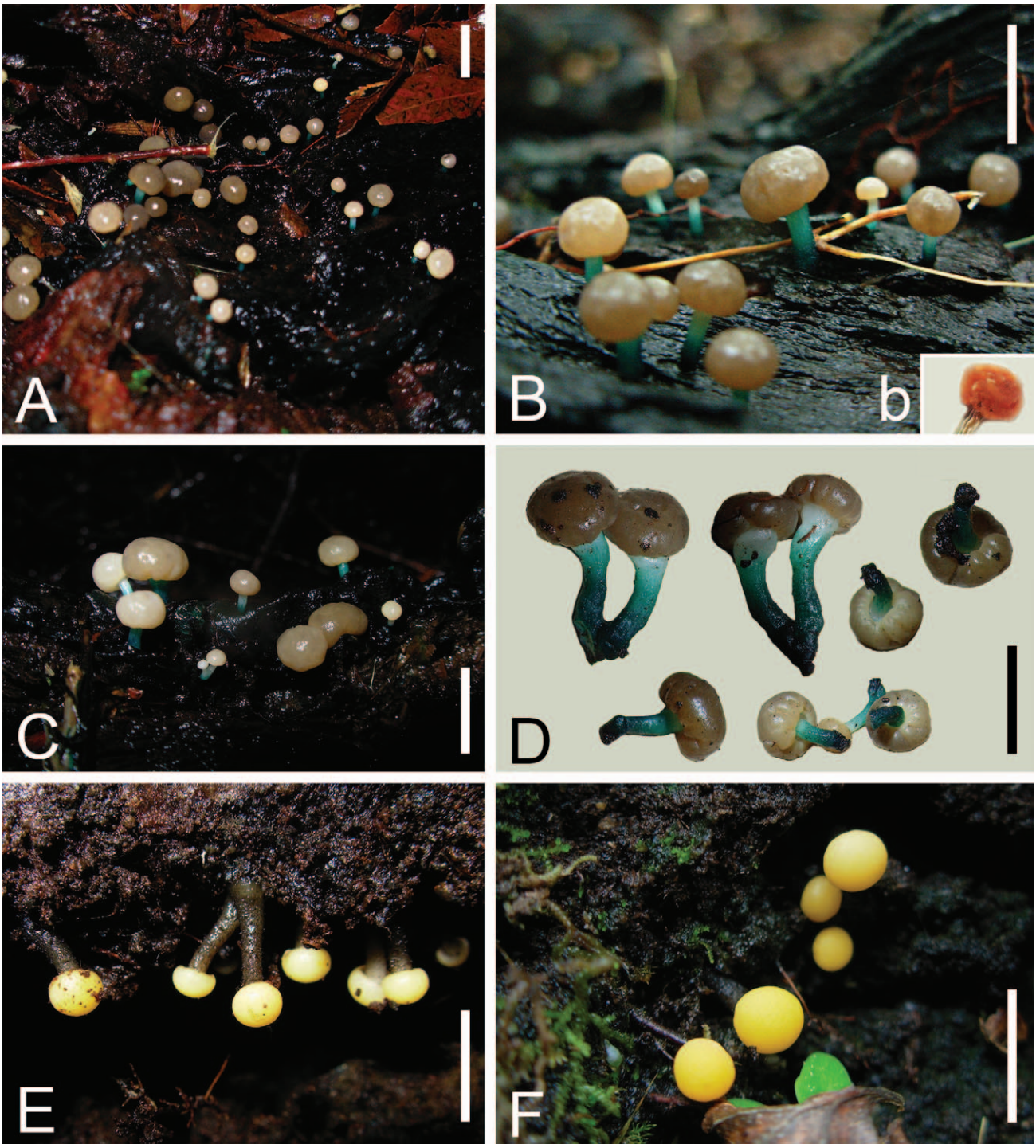

FIG. 2. General aspect of ascomata of Chilean collections of Vibrisseaceous fungi. A–D. *Chlorovibrissea chilensis*. B. Orange in dry specimen. E, F. *Vibrissea truncorum*. Bars: A–D = 1 cm.

Chlorovibrissea chilensis differs from the other four known species, *C. bicolor*, *C. tasmanica*, *C. melanochlora* and *C. phialophora*, in having a striking emerald green stipe and multiple budding (FIG. 3A, F, G) and sometimes monilioid (FIG. 3D, H) paraphyses; in addition buds from ascospores have been observed (FIG. 3I). *Chlorovibrissea phialophora* is easily recognized by the production of phialides and ascoconidia while still in the asci (Kohn 1989). *Chlorovibrissea bicolor* also has a green stalk but this

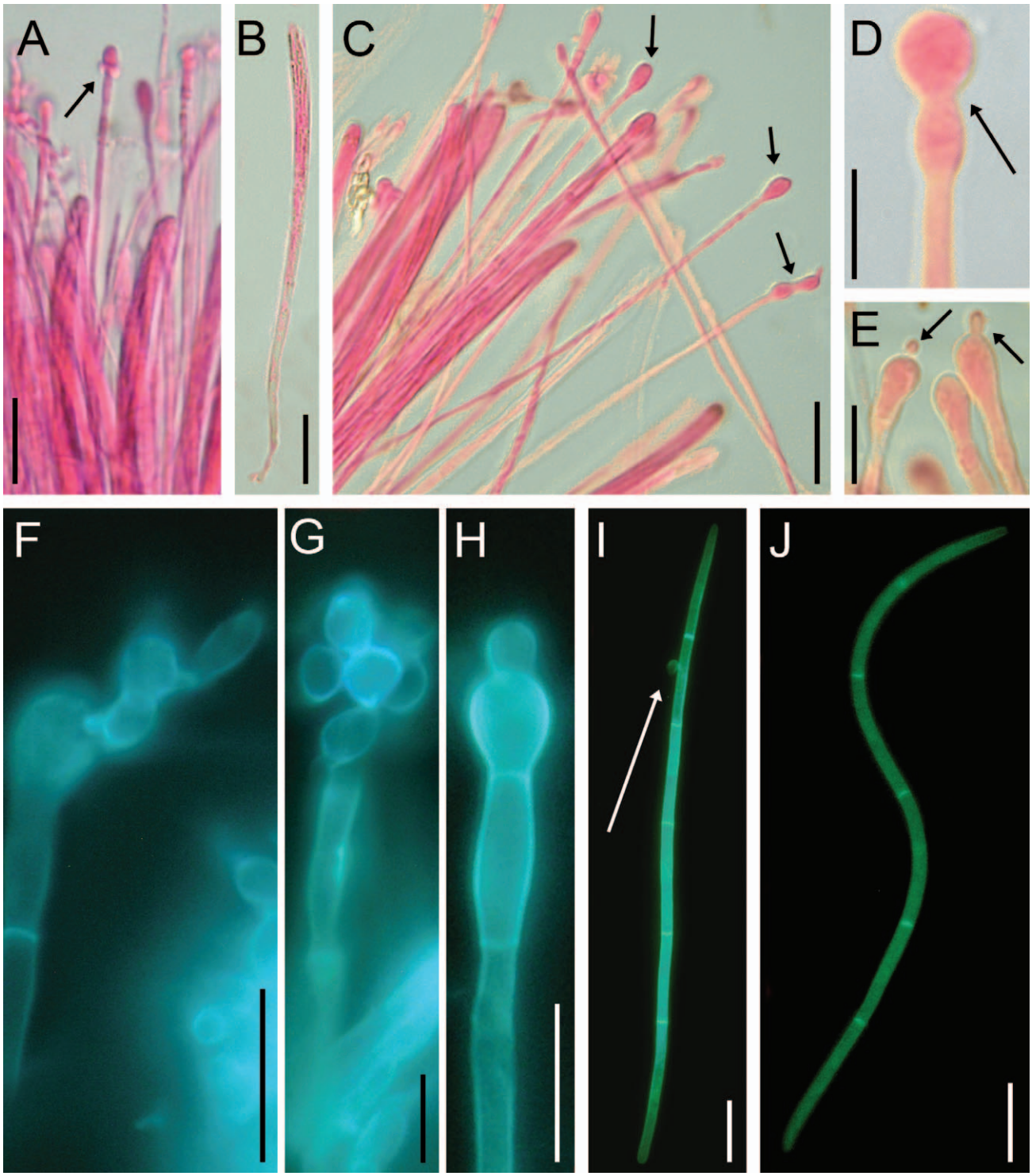

FIG. 3. *Chlorovibrissea chilensis*. A–D. Detail of asci and paraphyses. A. Hymenium. Note paraphysis with several buds (arrow). B. Ascus. C. Spathuliform paraphyses (two arrows from left) and one paraphysis budding in chain (arrow to the right). D–E. Paraphyses with young buds. F–H. Budding paraphyses (EFM; F, G multiple buds). F, G. Different view of same paraphysis. I, J. Ascospores (EFM). I. Budding ascospore (arrow). Bars: A–C = 30 µm, D–J = 10 µm.

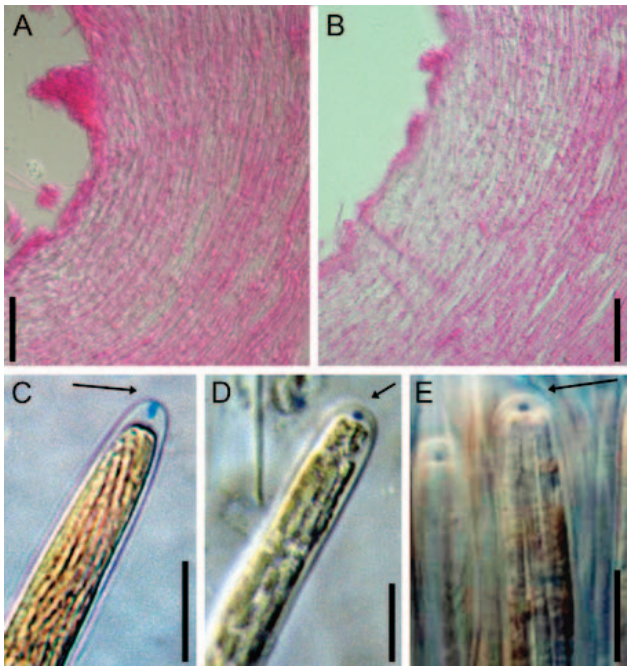

FIG. 4. Microscopic features. A, B. Exipule of *Chlorovibrissea chilensis*. C, D. Positive reaction of apical apparatus of asci in Melzer's reagent (arrow). C. *V. truncorum*. D. *C. chilensis*. E. *C. albofusca*. Bars: A, B = 80 µm; C–E = 10 µm.

species produces nonseptate ascospores apically coiled inside the ascus (Beaton and Weste 1977). In *C. melanochlora* the whole ascoma is green, a green pigment is released in KOH, the ascospores have nine septa and the paraphyses have uniform, thick tips (Beaton and Weste 1976). *Chlorovibrissea tasmanica* has a pale green ascoma head and the stem is covered with a dense layer of hairs (Beaton and Weste 1976).

Chlorovibrissea albofusca (G.W. Beaton) Sandoval-Leiva, A.I. Romero & P.R. Johnst., comb. nov. FIG. 4E

MycoBank MB808577

Basionym: Vibrissea albofusca G.W. Beaton, in Beaton & Weste, N.Z. J Bot. 21:281 (1983).

Material examined: NEW ZEALAND. AUCKLAND: Hunua Ranges, Mangatangi Dam, 30 Jul 1980, *G.J. Samuels, P.R. Johnston* (PDD 42916 – Holotype). WESTLAND: Okura, May 2002, *C. Shirley* (PDD 75692). TAUPO: Rangitoto Station, Native Forests Track, on *Beilschmiedia tawa* rotten wood, 16 May 2006, *P.R. Johnston, B.C. Paulus* (PDD 88457).

Notes: The phylogenetic results support those of Wang et al. (2006) in placing their sequence AY789384 (PDD 75692, cited by Wang et al. as *V. albofusca*) with the *Chlorovibrissea* spp. DNA sequences from a second specimen morphologically typical of *V. albofusca* (PDD 88457; JN809648) match exactly those reported by Wang et al. (2006), leading us to recombine this species in *Chlorovibrissea*. Despite the lack of green pigment, the excipular tissues and the apical apparatus of the asci in the specimens examined were consistent with those described for *Chlorovibrissea* species (FIG. 4E). See also notes in DISCUSSION.

Vibrissea truncorum (Alb. & Schwein.) Fr., Syst. mycol. 2:51 (1822). FIGS. 2E, F, 4C

Material examined: CHILE. REGIÓN DE AISÉN: comuna Puerto Aisén, mountains next to Condor Lake, on branches of *Nothofagus antarctica* half-buried in the snow, 45°32′47.59″S, 72°55′2746″W, 16 Dec 2009, *P. Sandoval-Leiva 392* (SGO 163163; BAFC 52325; PDD 99892). REGIÓN DE LOS RÍOS: Comuna Futrono, Mountain next to Maihue Lake, on fallen branches in a *Nothofagus pumilio* forest, 40°7′37″S, 71°56′39″W, 31 Jan 2010, *P. Sandoval-Leiva 407* (SGO 163164; BAFC 52326; PDD 99893). GERMANY. Westfalen, Jul 1920, *A. Ludwig*, Mycotheca Germanica No. 2363 (PDD 42250).

Notes: The *Vibrissea* collections are from southern Chile. In one of them, ascomata were growing on branches of *Nothofagus antarctica* (G. Forst.) Oerst., half-buried in the snow in a forest of the same species in mountains, close to Condor Lake and next to Puerto Chacabuco (FIG. 5B). In the other collection ascomata were growing on fallen branches in a *Nothofagus pumilio* (Poepp. & Endl.) Krasser forest in mountains next to Maihue Lake and Futrono (FIG. 5A). Both collection sites are within region of Andean Patagonian forest with similar climate features and classification as the *Chlorovibrissea* collection site, according to Gajardo (1994).

The ITS sequence was the same for both specimens examined (KF429259, KF429260), and this matches closely that deposited in GenBank as EU434855 and accepted as *V. truncorum* by Grünig et al. (2009). The microscopic features of the Chilean collections agree with the description by Beaton and Weste (1980) and with Mycotheca Germanica exsiccata specimen 2363.

This is the first record of a *Vibrissea* species from southern South America; the genus was not recorded by Gamundí in her extensive studies from Chile and southern Argentina (Gamundí 1962, 2003; Gamundí and Romero 1998; Gamundí et al. 2004).

Vibrissea flavovirens (Pers.) Korf & Dixon, Mycotaxon 1:134 (1974).

Material examined: NEW ZEALAND. FIORDLAND: Fiordland National Park, 2 km south of Lake Gunn, on saturated fallen wood adjacent to stream, 44°54′35″S, 168°3′05″W, 3 Mar 1992, *P.R. Johnston* D722 (PDD59998, living culture ICMP 19442).

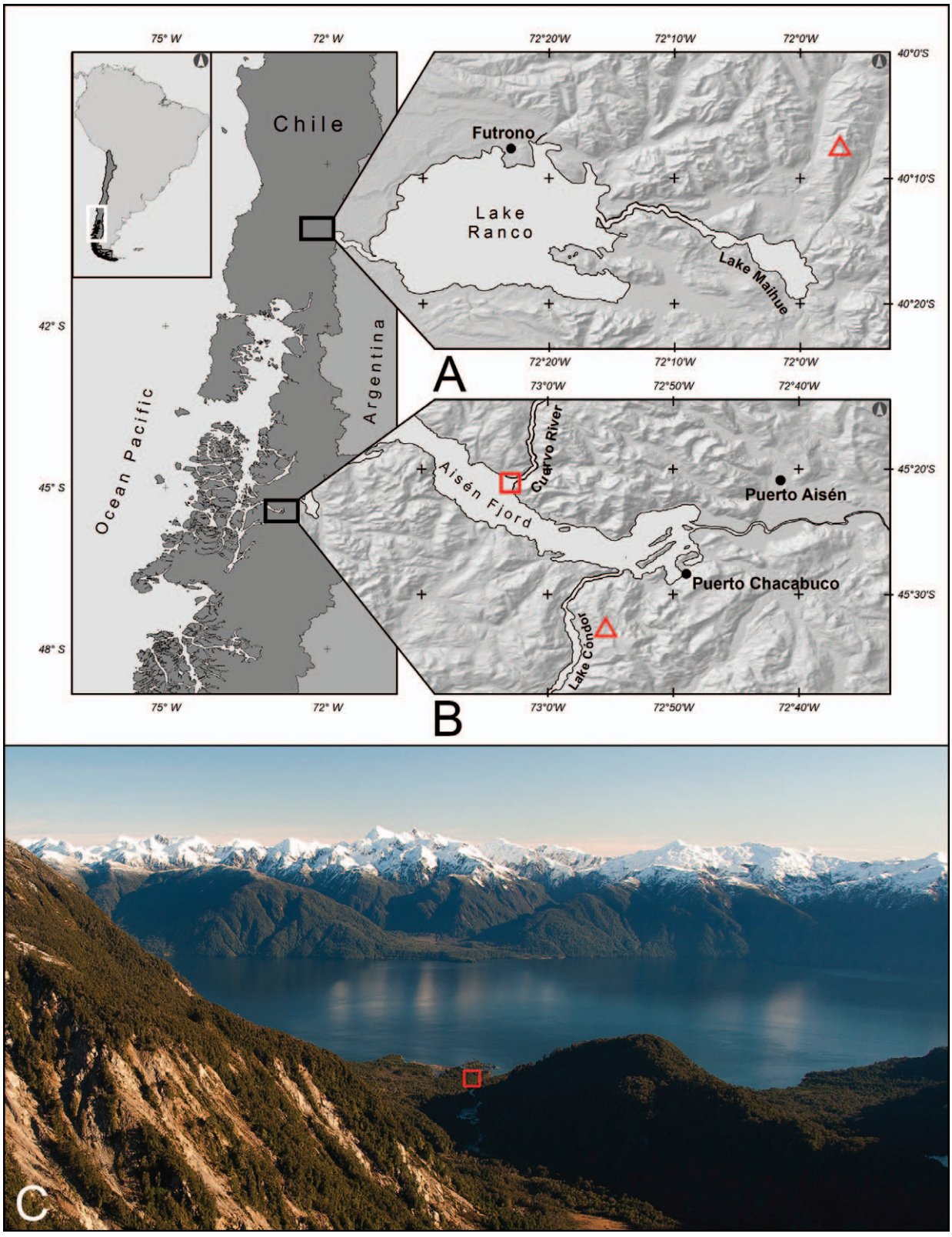

FIG. 5. Collecting sites of Vibrisseaceous fungi in Chile. A, B. Enlargement of each site. *Chlorovibrissea chilensis* (square) and *Vibrissea truncorum* (triangle). C. Landscape of Cuervo's river mouth in the Aisén fjord, at the collecting site (square) of the holotype of *C. chilensis*.

Notes: The ITS sequence from a culture grown from germinated ascospores from PDD 59998 matches closely that deposited in GenBank as AY789427, accepted as *V. flavovirens* by Wang et al. (2005). Morphologically it matches recent descriptions of *V. flavovirens* (e.g. Graddon 1965 as *Apostemidium torrenticola* and Sánchez and Korf 1966 as *Vibrissea pezizoides*). Both *A. torrenticola* and *V. pezizoides* were accepted as synonyms of *V. flavovirens* by Korf (1974).

This is the first genetic confirmation of a *Vibrissea* sensu stricto species from New Zealand.

DISCUSSION

As with the Australasian *Chlorovibrissea* species, the *C. chilensis* collection from Chile was found on a fallen, water-saturated wood (FIG. 2A–C). All known species of this genus share an aquatic habitat, either totally or partially immersed in running water (Beaton and Weste 1976, 1977; Kohn 1989; Wang et al. 2006).

This is the first record of *Chlorovibrissea* in South America. This finding confirms the observations of Kohn (1989) that, in addition to their distinctive morphology, the green vibrisseaceous fungi are characterized by a southern hemisphere distribution. Such a distribution suggests a possible Gondwanan origin for these fungi.

The new record of *V. truncorum* from Chile and *V. flavovirens* from New Zealand confirms the presence of true *Vibrissea* species in both the southern and northern hemispheres. Wang et al. (2006) showed that the family Vibrisseaceae, at that time containing both *Vibrissea* and *Chlorovibrissea*, was not monophyletic. They pointed out that ''the Vibrisseaceae'' is characterized by morphological features associated with an aquatic habit and the origin of the southern hemisphere vibrisseaceous fungi could be independent from the northern hemisphere representative''.

The results are similar to those of Wang et al. (2006), placing the *Chlorovibrissea* species and *Vibrissea* species that have been sampled genetically in separate clades. One morphological difference between the two genera appears to be the features of the excipular tissues. In *Chlorovibrissea* species, the excipular cells in the receptacle are brick-shaped to cylindrical, with a well developed outer layer of long, cylindrical, more or less free, hair-like cells, parallel to the receptacle surface. In the *Vibrissea* specimens the excipular cells are more or less globose to angular, with a poorly developed outer layer of appressed, short, cylindrical to globose cells, a difference also noted by Kohn (1989). Differences also are observed in the ascus. The ascal apex of *Chlorovibrissea* species is round to somewhat truncate, broader than the ascal apex of *Vibrissea* species, and with the apical ring broader and placed subapically. The ascal apex in *Vibrissea* species is acute, and the apical apparatus is placed at the tip. The apothecia anatomy, the apical ring, plus its position in the cladogram, support the new combination for *C. albofusca*. Based on the description of Beaton and Weste (1976), the Australian species *V. dura* also matches *Chlorovibrissea* in its apothecial anatomy, and when DNA sequences become available for this species they will provide a test for the phylogenetic significance of the differences we noted in apothecial structure. However, note also that the northern hemisphere species, *Leucovibrissea obconica* (Kanouse) Korf, matching *V. dura* in having a pale apothecium, is reported to have excipular tissue arranged in the same way as *Chlorovibrissea*, more or less parallel with the apothecia surface (Sánchez and Korf 1966, as *Vibrissea obconica*), and it is possible that future studies will show that these pale *Vibrissea* species form another phylogenetically separate group.

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