UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE CIENCIAS BIOLÓGICAS

TESIS DOCTORAL

Distribución de hongos corticioides : patrones y procesos

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Sandra Nogal Prata

Directoras

María Teresa Telleria Jorge
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ÍNDICE

Resumen

Título

Distribución de hongos corticioides: patrones y procesos

Introducción

Los hongos corticioides (Agaricomycotina, Basidiomycota) son un grupo polifilético que comparte una morfología sencilla del basidioma (resupinada o efuso-refleja) y un mismo nicho ecológico (madera muerta). Por su modo de vida como descomponedores de la madera juegan un papel fundamental en los ecosistemas forestales y el ciclo del carbono, considerando además que la mayoría de sus representantes son hongos de pudrición blanca. Durante mucho tiempo se habían agrupado en una misma familia, Corticiaceae, pero gracias a la aplicación de las técnicas moleculares se ha demostrado que se distribuyen en los principales linajes de Agaricomycotina. Se han considerado organismos ampliamente distribuidos, lo que se ha explicado porque los nichos en los que se desarrollan son muy estables, las propias especies se suponen muy antiguas, permanecen más o menos invariables a lo largo del tiempo y la delimitación de las mismas se ha basado tradicionalmente en caracteres morfológicos.

Durante las últimas décadas, las aproximaciones moleculares han sido una herramienta fundamental en el estudio de diversidad de este grupo de hongos, ya que han permitido analizar complejos integrados por varias especies con patrones de distribución más restringidos, demostrando que las distribuciones inferidas dependen, en buena medida, del método de reconocimiento utilizado. Hoy en día se considera que para conseguir una delimitación de especies precisa es necesario estudiar la diversidad dentro del marco de una taxonomía integradora, que incorpore datos de diferentes fuentes de evidencia taxonómica y que minimice los sesgos que conlleva el estudio basado únicamente en datos morfológicos. Este enfoque permite delimitar con más precisión las especies e inferir sus patrones de distribución geográfica y, en consecuencia, reconocer los procesos de distribución que explican los patrones.

Aunque son pocos los estudios sobre biogeografía de hongos, estos confirman que los procesos de dispersión a larga distancia y vicarianza juegan un papel fundamental en sus patrones de distribución, como ya se había indicado para otros organismos. La dispersión de hongos corticioides se ha asociado con diferentes vectores, entre ellos el agua, el viento, los insectos y el hombre.

Objetivos y Resultados

La hipótesis de trabajo planteada para esta tesis doctoral es que los rangos de distribución geográfica inferidos para las especies de hongos corticioides dependen del método de reconocimiento utilizado. Para corroborar o refutar la hipótesis se seleccionaron tres casos distintos y se plantearon los siguientes objetivos: 1) evaluar los patrones de distribución de los hongos corticioides sobre la base de una taxonomía integradora; y 2) analizar los posibles procesos responsables de las distribuciones observadas. La tesis doctoral se estructura en cuatro capítulos, los dos primeros relacionados con el objetivo 1 y los dos siguientes con el objetivo 2, para los que se han obtenido los siguientes resultados:

En el Capítulo 1 se analizan tres especímenes recolectados en la Reserva Biológica de Santiago de Huinay (Chile) morfológicamente próximos a *Aleurodiscus limonisporus*, una especie descrita de Australia y citada también de Nueva Zelanda. A partir de los resultados moleculares y morfológicos se determina que los especímenes chilenos corresponden a una especie nueva, *Aleurodiscus patagonicus*, confirmando que *A. limonisporus* no presenta una distribución austral.

En el Capítulo 2 se aborda el estudio de *Cystostereum* s.l., un género que ha incluido un total de 11 especies, algunas con distribuciones muy amplias, y a partir del cual se segregaron *Crustomyces* y *Cystidiodontia*. Sobre los resultados morfológicos y moleculares se circunscriben los tres géneros y se delimitan seis especies en *Cystostereum*, todas ellas con un marcado patrón geográfico: *C. australe* en el Caribe, *C. kenyense* en Kenia, *C. effusum* comb. nov. en Norte América, *C. murrayi* en Cuba, *C. sirmaurense* en India y *C. tuberculosum* comb. nov. en Europa.

En el Capítulo 3 se revisa la identificación de *Amylostereum laevigatum* procedente de Japón. A partir de los resultados moleculares y morfológicos se concluye que las muestras corresponden a *Amylostereum orientale*, originalmente descrita de China.

En el Capítulo 4 se evalúa la dispersión a larga distancia mediada por el viento para explicar la distribución de *Amylostereum laevigatum*, una especie que establece simbiosis con avispas de madera, en Azores. Sobre la base de datos moleculares se determina Europa continental como origen geográfico de *A. laevigatum* en Azores. Los test estadísticos de Mantel revelaron que la matriz genética de *A. laevigatum* está correlacionada con la de conectividad por vientos y no con la matriz de distancia geográfica, apoyando la dispersión a larga distancia mediada por el viento.

Conclusiones

1— Los patrones de distribución inferidos para las especies de hongos corticioides aparecen sesgados por los criterios de reconocimiento empleados. Así, la delimitación basada exclusivamente en datos morfológicos ha sobreestimado la distribución en hongos corticioides como *Aleurodiscus limonisporus*, *Cystostereum murrayi* o *Amylostereum laevigatum*.

2— Las especies detectadas en las aproximaciones filogenéticas se han corroborado con los análisis de tipo morfológico, lo que confirma la importancia de esta fuente de evidencia taxonómica.

3— Los estudios monográficos de géneros, sobre la base de una taxonomía integradora, son necesarios para tener un conocimiento más preciso de la diversidad fúngica y desvelar los taxones que pueden permanecer ocultos en complejos de especies, así como las identificaciones erróneas.

4— No se pueden inferir los patrones de distribución de aquellas especies asociadas a pocos registros o exclusivamente al ejemplar tipo, ya que probablemente presenten patrones más amplios que son aún desconocidos.

5— A medida que se incorporan datos de diferentes fuentes de evidencia, se alcanzan inferencias más robustas que permiten interpretar el patrón de distribución. Los análisis de coste basados en datos vectoriales de viento prueban la relevancia de la dispersión a larga distancia mediada por el viento hacia los archipiélagos oceánicos.

6— Los resultados obtenidos en esta tesis doctoral evidencian que el conocimiento sobre los hongos corticioides es desigual a muchos niveles. Es especialmente notable la necesidad de exploración de las regiones desconocidas, que nos permita aumentar las colecciones y desarrollar trabajos florísticos.

7— La falta de registro fósil en hongos supone un impedimento a la hora de evaluar la diversidad y sus distribuciones dentro de un marco evolutivo.

Summary

Title

Distribution of corticioid fungi: patterns and processes

Introduction

Corticioid fungi (Agaricomycotina, Basidiomycota) are a polyphyletic group that shares a simple morphology of basidiomata (resupinate or effused-reflexed) and the same ecological niche (dead wood). As wood-decayers, they play a key role in forest ecosystems and the carbon cycle, with most members causing white rot in both hardwood and softwood. For a long time these fungi have been classified in a single family, Corticiaceae, but molecular phylogenetic analyses have shown that they are distributed among the major lineages within Agaricomycotina. Corticioid fungi have been regarded as widely distributed, which has been explained because the niches in which they develop are very stable, the species themselves are supposed to be very old, they have remained more or less unchanged over time and their delimitation has traditionally been based on morphological characters.

During the last decades, molecular approaches have usually revealed several lineages with more restricted geographic distributions under a single name, showing that the inferred distributions depend upon the method of species recognition. Nowadays, in order to achieve a precise delimitation of species, studies should be carried within the framework of an integrative taxonomy, which incorporates data from different sources of taxonomic evidence and minimizes the biases of the studies based solely on morphological data. This approach makes it possible to delimit species with more precision and to infer their geographic distribution patterns and, consequently, to recognize the processes shaping the distribution patterns.

Few studies have been focussed on fungal biogeography, although these proved that long-distance dispersal and vicariance processes play a key role in their distribution patterns, as has already been indicated for other organisms. The spread of corticioid fungi has been associated with different vectors, including water, wind, and insects, as well as human-mediated spread.

Objectives and results

Our working hypothesis is that the inferred geographic distribution ranges for the species of corticioid fungi depend on the species recognition method used. In order to corroborate or refute this hypothesis, three cases were selected and the following objectives were proposed: 1) to evaluate the distribution patterns of corticioid fungi on the basis of an integrative taxonomy; and 2) to analyse the possible processes responsible for the observed distributions. This PhD dissertation has four chapters, the first two related to objective 1 and the next two to objective 2. The following results have been obtained:

In Chapter 1 three specimens collected in the Santiago de Huinay Biological Reserve (Chile) that are morphologically close to *Aleurodiscus limonisporus*, a species described from Australia and also cited from New Zealand, are analysed. From the molecular and morphological results, it is determined that the Chilean specimens correspond to a new species, *Aleurodiscus patagonicus*, demonstrating that *A. limonisporus* does not have an austral distribution.

Chapter 2 reports the study of *Cystostereum* in a broad sense. This genus has included a total of 11 species, some of them widely distributed, and *Crustomyces* and *Cystidiodontia* were segregated from that genus. On the basis of morphological and molecular data, the three genera are circumscribed and six species are delimited in *Cystostereum*, all of them with a marked geographic distribution: *C. australe* in the Caribbean region, *C. kenyense* in Kenya, *C. effusum* comb. nov. in North America, *C. murrayi* in Cuba, *C. sirmaurense* in India and *C. tuberculosum* comb. nov. in Europe.

In Chapter 3 the identification of *Amylostereum laevigatum* from Japan is reviewed. From the molecular and morphological results it is concluded that the samples correspond to *Amylostereum orientale*, originally described from China.

In Chapter 4, wind-mediated long distance dispersal is evaluated to explain the presence of *Amylostereum laevigatum*, a symbiont of siricid woodwasps, in the Azores archipelago. On the basis of molecular data, mainland Europe is the geographical origin of *A. laevigatum* in the Azores. The Mantel statistical tests revealed that the genetic matrix of *A. laevigatum* is correlated with the wind connectivity matrix, supporting the wind-mediated long distance dispersal towards the archipelago.

Conclusions

1— The distribution range of corticioid fungi is biased by the criteria used in species recognition. Thus, species delimitation based exclusively on morphological characters led to overestimation of the distribution of corticoid fungi such as *Aleurodiscus limonisporus, Cystostereum murrayi* or *Amylostereum laevigatum*.

2— The species detected in the phylogenetic approaches have been confirmed on the basis of morphological analysis, which support the relevance of this source of evidence in species delimitation.

3— Monographic studies of genera based on an integrative taxonomy should be conducted to obtain a more precise knowledge of fungal diversity, as well as to reveal taxa that may remain hidden in species complexes and as misidentifications.

4— The distribution patterns cannot be inferred for those species that are known from only a few records or exclusively from a type specimen, since they likely present broader patterns that are still unknown.

5— As data from more sources of evidence are incorporated, more robust inferences are reached allowing us to interpret the distribution pattern. Cost analyses based on wind vectors support the relevance of wind-mediated long distance dispersal towards oceanic archipelagos.

6— The results obtained in this thesis dissertation show that knowledge about corticioid fungi is uneven at many levels. It is especially pertinent to explore unknown regions, which allows us to increase collections and to enhance floristic work.

7— The lack of fungal fossil records presents an impediment to evaluating diversity and its distribution within an evolutionary framework.

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Introducción General

Hongos corticioides: Análisis y desafíos

Los hongos son organismos eucariotas y heterótrofos, que se alimentan por absorción, se reproducen por esporas de origen tanto sexual como asexual y, por lo general, están provistos de hifas; unas estructuras somáticas filamentosas que presentan paredes celulares de quitina, que en su conjunto forman lo que denominamos micelio (Telleria 2011). En general, los hongos constituyen un grupo diverso por su gran variedad de morfologías, ciclos de vida y funciones ecosistémicas que desempeñan. De acuerdo con Wijayawardene et al. (2020), este grupo de organismos se puede clasificar en 19 filos distintos, entre los que Ascomycota es el mayoritario en número de especies, con unas 92.000, seguido de Basidiomycota, con más de 50.000 especies descritas (Catalogue of Life, http://www.catalogueoflife.org, 5/12/2021). Se encuentran ampliamente distribuidos por todo el mundo, incluyendo los hábitats más extremos, y dado su carácter heterótrofo se comportan como parásitos, saprobios o mutualistas de otros organismos como algas, vertebrados, invertebrados, plantas e incluso otros hongos. Hasta el momento, se han descrito cerca de 146.000 especies (Catalogue of Life, http://www.catalogueoflife.org, 12/10/2021), lo que apenas supone un 10% de los 1.5 millones que se estiman probables (Hawksworth 1991). Aunque esta estimación es la más aceptada, dista mucho de estar consolidada, ya que actualmente las cifras se sitúan entre los 2.2 y 3.8 millones (Hawksworth & Lücking 2017).

Los hongos corticioides son un grupo polifilético dentro de Basidiomycota que comparte una morfología sencilla del basidioma (en la mayoría de los casos resupinada o efuso-refleja), un mismo nicho ecológico (por lo general madera muerta), así como una amplia distribución geográfica. Por su modo de vida como descomponedores de la madera, desempeñan una labor fundamental en la dinámica de los ecosistemas forestales y el ciclo del carbono. La mayoría de las especies conocidas de hongos corticioides son de pudrición blanca (Hjortstam et al. 1987), esto es que son capaces de degradar la lignina gracias a enzimas como peroxidasas y lacasas, llegando a representar el 80% frente a un 5% de especies de pudrición parda (Yurchenko 2006), que son aquellas capaces de degradar la celulosa pero no la lignina. Aunque en su mayoría son saprobios, también encontramos representantes parásitos, como la especie *Pseudolagarobasidium acaciicola* que fue descrita de Sudáfrica parasitando las raíces de la leguminosa *Acacia cyclops* (Wood & Ginns 2006), y micorrizógenos, como algunas especies de los géneros *Piloderma* (Dahlberg et al. 1997) y *Tomentella* (Kõljalg et al. 2002).

Durante mucho tiempo, los hongos corticioides se habían agrupado en una única familia, Corticiaceae, dentro del orden Aphyllophorales, hasta que Donk (1964) en su intento de hacer una clasificación natural del orden, lo dividió en 21 familias distintas, manteniendo Corticiaceae. Con posterioridad, Parmasto (1968) se centró en una clasificación específica para esta familia, en la que mantuvo las especies con sistema de hifas monomítico, y describió Steccherinaceae para agrupar las especies con un sistema de hifas dimítico. En su clasificación de los niveles superiores de Basidiomycota, Jülich (1981) propuso 31 órdenes nuevos y 126 familias, considerando que Corticiaceae, al igual que otras familias como Polyporaceae y Clavariaceae, representaba un grupo muy heterogéneo, por lo que la escindió en familias más pequeñas.

Sobre la base de datos moleculares, Hibbett & Binder (2002) probaron que la simplicidad morfológica de los hongos corticioides encierra importantes desafíos taxonómicos y sistemáticos, ya que sus análisis de evolución de caracteres basados en métodos de máxima parsimonia y máxima verosimilitud confirman que no forman un grupo claramente delimitado. Los análisis filogenéticos llevados a cabo por Larsson et al. (2004) y Binder et al. (2005) demostraron que estos hongos son un grupo polifilético que se distribuye por los principales linajes de Agaricomycotina. En los análisis de Larsson (2007), basados en las subunidades 5.8S y LSU del ADN ribosómico nuclear, los 138 taxones con basidioma corticioide se distribuyeron en 41 familias distintas, situadas en los órdenes Agaricales, Atheliales, Boletales, Cantharellales, Corticiales, Gloeophyllales, Gomphales, Hymenochaetales, Polyporales, Russulales, Thelephorales y Trechisporales (Fig. 1).

Se han publicado algunos datos sobre el número de especies conocidas de este grupo de hongos, como el de la base de datos Cortbase, que incluyó 8.112 nombres de los que 2.101 eran aceptados (Parmasto et al. 2004); cifra que aumentó con 300 especies nuevas transcurridos cinco años (http://andromeda.botany.gu.se/cortbase.html). Posteriormente, Mueller et al. (2007) registran un total de 1.853 especies conocidas de hongos corticioides, indicando que representan en torno al 18.5% de las 10.023 que se estiman probables (Tabla 1). Este conocimiento no es uniforme para todas las regiones del mundo, así en Europa y Norte América se estima que se desconoce en torno al 30% de las especies, en contraste con lo poco conocido para otras, como la región tropical de América y África, para las que apenas se conoce un 40% y 32% de las especies, respectivamente (Tabla 1).

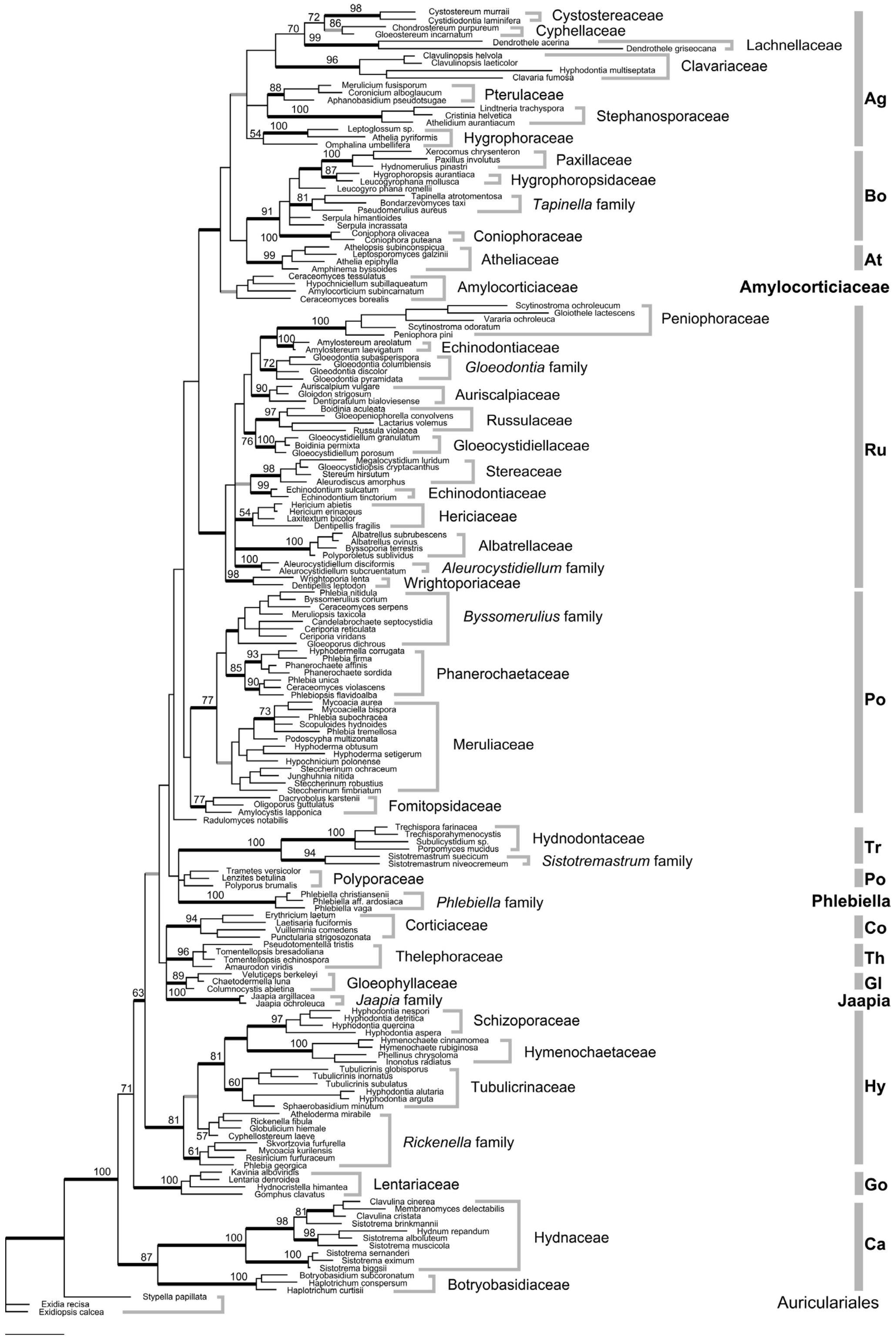

Fig. 1. Topología bayesiana inferida para las secuencias 5.8S y LSU de hongos corticioides. La longitud de las ramas indica el número de cambios, los valores de bootstrap de Máxima Parsimonia se indican en todos los nodos internos, y las probabilidades posteriores del análisis bayesiano (pp) que se encuentren entre 0.97-0.99 se indican como ramas más gruesas en gris, y los valores de 1.0 se muestran como ramas más gruesas en negro. Ag: Agaricales, At: Atheliales, Bo: Boletales, Ca: Cantharellales, Co: Corticiales, Gl: Gloeophyllales, Go: Gomphales, Hy: Hymenochaetales, Po: Polyporales, Ru: Russulales, Th: Thelephorales, Tr: Trechisporales. Fuente: Larsson (2007).

Tabla 1. Datos K-H Larsson y K. Hjortstam sobre la distribución geográfica de Corticiaceae. Adaptada de Mueller et al. (2007).

Region		Known species	% endemic to region	Estimated unknown
Polar				
	Arctic	0	0	50/100%
	Antarctic	0	0	50/100%
Western Hemisphere				
	Boreal	344	9	280/45%
	N. temperate	605	17	400/40%
	Tropical	598	31	900/60%
	S. temperate	83	28	420/83%
Eastern Hemisphere				
	Boreal	478	6	150/24%
	Europe template	611	11	240/28%
	Asian temperate	507	23	500/50%
	Northern Africa	177	4	220/56%
	Tropical Africa	483	36	1000/68%
	Madagascar	16	6	580/97%
	Temperate Africa	65	17	440/87%
	Mid East	18	6	380/95%
	Tropical Asia[1]	259	29	1250/83%
	Australasia	165	9	840/83%
	Pacific Islands	234	32	470/67%
	Total	1.853		

[1]Tropical Asia includes Indonesia, Philippines, Malasya and New Guinea.

Distribución de hongos corticioides: Patrones y procesos

Desde muy antiguo los hongos se han considerado organismos cosmopolitas que sólo dependían de las condiciones del medio para fructificar (De Candolle 1820), libres de las barreras de dispersión que afectan a otros organismos. Así, la hipótesis EisE “Everything is everywhere, but the environment selects” [`Todo está en todas partes, pero el ambiente selecciona´], originalmente propuesta por Baas-Becking (1934) para explicar el cosmopolitismo de bacterias, se extendió para explicar las amplias distribuciones de los hongos. Bisby (1943) indicó que su distribución estaba ante todo condicionada por la distribución de sus hospedantes y sustratos y, también, por factores ambientales como el clima, la radiación solar o la estacionalidad, aunque los efectos de estos eran más difíciles de evaluar.

Los análisis de diversidad basados en catálogos florísticos señalan que los hongos corticioides presentan, generalmente, distribuciones geográficas amplias. Gilbertson (1980) muestra índices de similitud de hasta el 70% entre la biota de Norte América y Europa; valor parecido al que llega Hallenberg (1981) para el norte de Irán, que señala un porcentaje de similitud del 78% con Europa, o Ghobad-Nejhad (2012) para los hongos corticioides de la región del Cáucaso, que presenta un porcentaje de similitud mayor con Europa y Norte América que con el este de Asia y la India. Telleria et al. (2009) también encontraron valores de similitud altos entre el archipiélago de las Azores y las micobiotas del suroeste de Europa continental (83%), Norte América (73%), Canarias (63%) y, aunque menor, también con la de Marruecos (39%). Para el hemisferio sur se extraen conclusiones parecidas con trabajos como el de Boidin & Gilles (1986), que señalan que el 60% de las especies de hongos corticioides de la Isla de Reunión ya eran conocidas de otras áreas tropicales, o el de Hjortstam & Ryvarden (1985), que incluyen un total de 42 especies en su checklist de Tierra de Fuego (Argentina), de las que 40 habían sido previamente descritas y citadas de diferentes partes del Hemisferio Norte. Para los Andes Patagónicos de Argentina y Chile, las checklists de Greslebin & Rajchenberg (2003) y Gorjón & Hallenberg (2013) también indican porcentajes altos de especies cosmopolitas, un 58% y casi el 50% respectivamente.

Según Hallenberg (1995), estas distribuciones amplias de los hongos corticioides se podrían explicar porque los nichos en los que se desarrollan son muy estables (madera muerta), las especies son muy antiguas y han permanecido más o menos invariables a lo largo del tiempo, a diferencia de las plantas vasculares que han sufrido importantes cambios evolutivos. Sin embargo, se debe tener en cuenta que estas distribuciones se basan en el concepto morfológico de especie, lo que es especialmente notable para este grupo de hongos dado que cuentan con una morfología sencilla con pocos caracteres diagnósticos. Por otra parte, se han encontrado excepciones que presentan distribuciones claramente más limitadas, como algunas especies de los géneros *Aleurodiscus* y *Vuilleminia*. Núñez & Ryvarden (1997) en su monografía de *Aleurodiscus* incluyeron *A. coralloides*, conocida hasta la fecha de Nueva Zelanda, *A. vitellinus* del sur de Chile y Argentina, o *A. limoniposrus*, de Australia y Nueva Zelanda, las tres especies asociadas a madera de *Nothofagus*. En el caso de *Vuilleminia* también se han encontrado distribuciones restringidas, como *V. pseudocystidiata*, una especie rara que se ha citado de Alemania, Armenia, Cerdeña, Francia y Ucrania creciendo en *Cornus* y *Crataegus*, y *V. megalospora*, conocida de Francia, Italia (incluyendo Cerdeña) e Irán creciendo en *Crataegus* y *Quercus* (Ghobad-Nejhad et al. 2010).

Los análisis de intercompatibilidad de micelios basados en el concepto biológico de especie vinieron a apoyar muchas de estas distribuciones amplias. Hallenberg (1984) analizó un total de 47 especies de hongos corticioides comunes a Norte América y Europa, de las que 34 presentaron una compatibilidad total o parcial a pesar del aislamiento geográfico, llevando a aceptar lo que previamente se había definido sobre la morfología, mientras que los especímenes europeos y norteamericanos de las otras 13 especies analizadas mostraron incompatibilidad de micelios. En un trabajo posterior, Hallenberg (1985a) probó que existía compatibilidad de micelios entre los especímenes de Canadá y Europa de otras seis especies. En el caso de *Scytinostroma galactinum*, descrita originalmente de Norte América, Boidin & Lanquetin (1987) demostraron incompatibilidad de micelios entre los especímenes euroasiáticos, africanos y norteamericanos, lo que llevó a la descripción de tres especies nuevas: *Sc. eurasiaticogalactinum*, *Sc. africanogalactinum* y *Sc. neogalactinum*. Sin embargo, estos experimentos mostraron que *Sc. eurasiaticogalactinum* y *Sc. africanogalactinum* presentaban una compatibilidad parcial.

A partir de la década de los noventa, gracias al desarrollo de las técnicas moleculares, las aproximaciones basadas en ADN comenzaron a aplicarse en el campo de la taxonomía, lo que en el caso de los hongos vino marcado por el diseño de iniciadores generales para el marcador ribosomal ITS (White et al. 1990). Los estudios moleculares que se llevaron a cabo en *Schizophyllum commune* por James et al. (1999, 2001), una especie considerada cosmopolita y que los experimentos de intercompatiblidad de micelios así parecían confirmar (Raper et al. 1958), fueron un punto de referencia al demostrar que se trataba de un complejo integrado por varias especies con distribuciones más restringidas. La incorporación de estas técnicas moleculares ha proliferado en el estudio de complejos de especies como el de *Peniophorella praetermissa*, una especie ampliamente distribuida que se resolvió como ocho clados distintos, tres de los cuales se pudieron diferenciar por caracteres morfológicos, *P. praetermissa* de Europa, *P. odontiaeformis* de África, y *P. subpraetermissa* de Asia, y todos los clados mostraron incompatibilidad de micelios excepto dos (Hallenberg et al. 2007).

Taylor et al. (2000) considerando que la delimitación de especies basada en el uso exclusivo de caracteres fenotípicos podría detectar un menor número de taxones que la realizada sobre el aislamiento reproductivo, y este a su vez un número menor que el filogenético, propusieron tres conceptos de reconocimiento de especies en hongos: el morfológico (MSR, *Morphological Species Recognition*); el biológico (BSR, *Biological Species Recognition*); y el filogenético (PSR, *Phylogenetic Species Recognition*), este último basado en la concordancia de genealogías (GCPSR, *Genealogical Concordance Phylogenetic Species Recognition*).

De acuerdo con Taylor et al. (2006), el método utilizado para el reconocimiento de las especies no solo a su número, sino también al rango de distribución inferido para las mismas. Hoy en día se considera que para conseguir una delimitación de especies precisa es necesario utilizar una taxonomía integradora, basada en datos de diferentes fuentes de evidencia taxonómica, como la morfológica, la molecular o la ecológica (Dayrat 2005). Dentro de este marco se han analizado un gran número de complejos de especies de hongos corticioides, como es el complejo de *Phanerochaete sanguinea* (Floudas & Hibbett 2015), que se resolvió como un complejo integrado por cuatro especies, una distribuida en Europa (*Ph. sanguinea* s.s.), y tres en Norte América (*Ph. citrinosanguinea*, *Ph. pseudosanguinea* y *Ph. sanguineocarnosa*); al que se ha sumado *Ph. australosanguinea*, descrita recientemente de la Patagonia chilena (Phookamsak et al. 2019). En *Lyomyces sambuci*, que ya se había anticipado por Eriksson & Ryvarden (1976) como un complejo de especies, Yurchenko et al. (2017) han descrito recientemente cuatro especies, dos para la isla de Reunión, *L. allantosporus* y *L. mascarensis*, otra para el sureste de Brasil, *L. organensis*, y la cuarta para Taiwan, *L. orientalis.* En *Xylodon raduloides*, una especie cosmopolita, se han resuelto cuatro especies con distribuciones más restringidas: *X. raduloides* se restringe a Europa, *X. laurentianus* para Norte América, *X. novozelandicus* para Australia-Nueva Zelanda, y *X. patagonicus* para la región Patagónica (Fernández-López et al. 2019). Aunque en todos estos ejemplos el estudio morfológico apoyó los resultados filogenéticos, también se han detectado especies crípticas, como es el caso de *Hyphoderma macaronesicum* e *H. paramacaronesicum* (Martín et al. 2018).

Son muy pocos los estudios que se han hecho sobre patrones y procesos de distribución de hongos, exceptuando algunos casos como el de *Cyttaria*, un género parásito de Nothofagaceae que se conoce del sur de Sudamérica, Australia y Nueva Zelanda, y que presenta unos patrones de distribución que se explican mediante una fragmentación inicial del supercontinente Gondwana y dispersiones a larga distancia más recientes de Australia a Nueva Zelanda (Peterson et al. 2010). Los análisis de reloj molecular llevados a cabo con el género *Lentinula* también apoyan la combinación de estos procesos para explicar sus distribuciones, que incluye una fragmentación de Laurasia y una dispersión a larga distancia entre Australia y Nueva Zelanda (Hibbett 2001). De acuerdo con Lumbsch et al. (2008) estos estudios prueban que los patrones de distribución de hongos resultan de un mosaico complejo de vicarianza y dispersión a larga distancia, como se había indicado para otros organismos.

En el caso concreto de la dispersión de hongos corticioides se han descrito varios mecanismos, uno de ellos es el de los cordones miceliares o rizomorfos, que posibilitan colonizar nuevos sustratos y establecerse muy próximos a su origen. Este modo se conoce para hongos corticioides como *Resinicium furfuraceum, R. bicolor, Ceraceomyces borealis* y algunas especies del género *Phanerochaete* (Boddy 1999). En cuanto a la dispersión mediada por vectores, hasta el momento se han relacionado el agua, el viento y los insectos (Edman 2003). De todos ellos, el agua es el menos frecuente, y sólo se ha probado para casos concretos como el de *Bulbillomyces farinosus*, que en su ciclo cuenta con un estado de esclerocios que actúan como diásporas flotantes, o el de *Jaapia argillacea*, que cuenta con unas esporas de grandes dimensiones que después de la descarga contraen el protoplasma haciendo que los extremos queden como cámaras de aire (Eriksson & Ryvarden 1976).

El viento ha sido considerado el principal vector en la dispersión de los hongos. Hay ejemplos que se conocen desde antiguo, como el caso de las royas, cuyas esporas ornamentadas, de paredes oscuras y gruesas se mantienen viables incluso tras la dispersión de un continente a otro, como en el caso de la roya del café *Hemileia vastatrix* (Bowden et al. 1971). Otro ejemplo es *Ganoderma*, que tiene unas esporas ornamentadas de paredes oscuras, gruesas y melanizadas (Moncalvo & Buchanan 2008), características que posibilitan su viabilidad en condiciones extremas de desecación y radiación ultravioleta (Deacon 1997). En este tipo de dispersión a larga distancia a través del viento siempre se ha pensado en los vientos de la corriente en chorro (*jet stream*). Estos se producen a gran altitud, donde las condiciones de supervivencia de las esporas o cualquier otro propágulo son extremadamente adversas. Sin embargo, Muñoz et al. (2004), utilizando datos de briófitos, helechos y líquenes, demostraron que los vientos de la parte baja de la atmósfera son los responsables de la dispersión a larga distancia de estos organismos entre territorios del hemisferio sur situados entre la costa del continente Antártico y los 30º de latitud sur.

Para la dispersión mediada por viento, las características de las esporas pueden ser un factor limitante. En el caso de los hongos corticioides, las esporas son generalmente hialinas y de paredes delgadas, características que afectan a su viabilidad en condiciones adversas (Kramer 1982), como las que puede implicar un proceso de dispersión. Además, algunos estudios estadísticos y biomecánicos, han revelado que la efectividad de la dispersión de las esporas de hongos corticioides puede ser muy limitada, ya no sólo a largas distancias, sino también a cortas (Norros et al. 2012). Otros estudios, como el Hallenberg & Kúffer (2001), han demostrado que algunos hongos corticioides, como *Laurilia sulcata* y *Peniophora aurantiaca,* podían llegar a dispersarse hasta distancias de 400 y 1.000 km. Sin embargo, esto se puede aplicar a escala continental, pero no parece ser extrapolable a aquellos casos que impliquen superar barreras como un océano.

Desde principios del siglo XX, ya se reconoce que los insectos son vectores en la dispersión de hongos (Buller 1922), llegando a considerarse como uno de los más efectivos (Stenlid &

Gustafsson 2001), lo que se ha ido confirmando a medida que se ha profundizado en el estudio de la ecología forestal y relaciones entre organismos que habitan en la madera (Jacobsen et al. 2017). Se han descrito relaciones de simbiosis entre hongos corticioides e insectos de la madera, como algunas especies del género *Amylostereum* y los sirícidos *Sirex* y *Urocerus* (Slippers et al. 2003), o entre *Phlebiopsis gigantea* (Harrington 2005), *Entomocorticium*, *Cylindrobasidium ipidophilum* (Harrington et al. 2021) y escarabajos ambrosia. Estos hongos se han aislado de las galerías excavadas en la madera por estos insectos o directamente de los micangios, unos orgánulos especializados situados en la cavidad abdominal de las hembras. En estas estrechas relaciones de simbiosis, el hongo es transportado e inoculado en la madera por los insectos, mientras que estos se benefician porque el hongo la procesa y la hace accesible para las larvas.

También se ha señalado al hombre como posible vector implicado en la dispersión de hongos corticioides, como en el caso de *Serpula lacrymans*, una especie que responsable de importante daños en construcciones de madera en las regiones templadas y que rara vez se ha citado de entornos naturales (Hallenberg 1985b). Los análisis moleculares de Kauserud et al. (2007) revelaron la existencia de dos linajes, uno no agresivo y otro agresivo, y sugieren que este último podría haber sido introducido desde Asia en Europa, Norte América y Oceanía. También son los casos de *Schizophyllum commune*, una especie introducida en Nueva Zelanda por el transporte de maderas (Fuller et al. 2013), o *Amylostereum areolatum*, una especie nativa del hemisferio norte que fue introducida en el hemisferio sur con las plantaciones de *Pinus*, convirtiéndose además en un patógeno que está produciendo grandes pérdidas económicas (Slippers et al. 2001).

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Hipótesis, objetivos y estructura de la Tesis Doctoral

La hipótesis de trabajo de este tesis doctoral es que los patrones de distribución inferidos en hongos corticioides están relacionados con el método de reconocimiento de especies utilizado. Para corroborar o refutar la hipótesis, se han propuesto los siguientes objetivos:

1— Evaluar los patrones de distribución de los hongos corticioides sobre la base de una taxonomía integradora.
2— Analizar los posibles procesos responsables de las distribuciones resultantes.

Para abordarlos, esta tesis doctoral se ha estructurado en cuatro capítulos, los dos primeros relacionados con el objetivo 1, y los otros dos capítulos con el objetivo 2.

En el **Capítulo 1**, con el fin de comprobar si *Aleurodiscus limonisporus*, una especie descrita originalmente de Australia, presenta una distribución austral, se analizan y comparan 29 especímenes procedentes de Australia y Nueva Zelanda con tres especímenes morfológicamente similares recolectados en la Reserva Biológica de Santiago de Huinay (Chile).

En el **Capítulo 2** se aborda el estudio de *Cystostereum* sensu lato, un género que ha llegado a incluir, sobre la base de caracteres morfológicos, un total de 11 especies, algunas con distribuciones geográficas muy amplias. El objetivo de este capítulo es circunscribir *Cystostereum*, así como los géneros *Crustomyces* y *Cystidiodontia* que fueron segregados a partir de él, delimitar las especies de *Cystostereum*, y conocer sus patrones de distribución utilizando datos de diferentes fuentes de evidencia taxonómica (ADN, morfología, corología y ecología).

En el **Capítulo 3** se revisa la identificación de *Amylostereum laevigatum* procedente de Japón. Para ello, se llevan a cabo análisis filogenéticos basados en nrDNA ITS y *rpb*2 y el cálculo de distancias genéticas (análisis de *barcoding*), así como una revisión morfológica de los especímenes.

En el **Capítulo 4** se evalúa la dispersión a larga distancia mediada por el viento para explicar la distribución de *Amylostereum laevigatum*, una especie que establece simbiosis con avispas de la madera, en Azores. Para ello, se analiza el origen geográfico mediante aproximaciones filogenéticas basadas en los marcadores nrDNA ITS y *rpb*2, y se calcula la correlación entre la matriz genética de *A. laevigatum* y las matrices de distancia geográfica y de conectividad por viento.

Chapter 1

Aleurodiscus patagonicus sp. nov.[*]

Sandra Nogal-Prata, M. Teresa Telleria, Margarita Dueñas, María P. Martín

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[*] Una versión de este capítulo ha sido publicada en el compendio de *Fungal diversity notes 929–1035*:

Phookamsak R, Hyde KD, Jeewon R, Bhat DJ, Jones EBG, Maharachchikumbura SSN, Raspé O, Karunarathna A, Wanasinghe DN, Hongsanan S, Doilom M, Tennakoon DS, Machado AR, Firmino AL, Ghosh A, Karunarathna A, Mešić A, Dutta AK, Thongbai B, Devadatha B, Norphanphoun Ch, Senwanna Ch, Wei D, Pem D, Ackah FK, Wang GN, Jiang HB, Madrid H, Lee HB, Goonasekara ID, Manawasinghe IS, Kušan I, Cano J, Gené J, Li J, Das K, Acharya K, Raj KNA, Latha KPD, Chethana KWT, He MQ, Dueñas M, Jadan M, Martín MP, Samarakoon MC, Dayarathne MC, Raza M, Park MS, Telleria MT, Chaiwan N, Matočec N, de Silva NI, Pereira OL, Singh PN, Manimohan P, Uniyal1 P, Shang Q-J, Bhatt RP, Perera RH, Alvarenga RLM, **Nogal-Prata S**, Singh SK, Vadthanarat S, Oh SY, Huang SK, Rana S, Konta S, Paloi S, Jayasiri1 SC, Jeon SJ, Mehmood T, Gibertoni TB, Nguyen TTT, Singh U, Thiyagaraja V, Sarma VV, Dong W, Yu XD, Lu Y-Z, Lim YW, Chen Y, Tkalčec Z, Zhang Z-F, Luo Z-L, Daranagama DA, Thambugala KM, Tibpromma S, Camporesi E, Bulgakov TS, Dissanayake AJ, Senanayake IC, Dai DQ, Tang L-Z, Khan S, Zhang H, I Promputtha I, Cai L, Chomnunti P, Zhao R-L, Lumyong S, Boonmee S, Wen T-C, Mortimer PE, Xu J (2019) Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 95(1): 1–273. https://doi.org/10.1007/s13225-019-00421-w

ABSTRACT

Aleurodiscus patagonicus sp. nov. is described on the basis of morphological and molecular evidence. Specimens of this species were collected in Huinay Reserve, in the Valdivian temperate rainforest of the Chilean Northern Patagonian region. Morphologically, it is related to *A. limonisporus*, known from Australia and New Zealand. However, *A. patagonicus* differs by having scattered clamps in paraphysoid hyphae, longer and wider basidia with basal clamp, and spores with lower length/width ratio. Phylogenetic analyses, based on the concatenated dataset of the internal transcribed spacer (ITS) and large subunit (LSU) of nuclear ribosomal DNA (nrDNA), reveal that *A. patagonicus* forms a highly supported clade, which is close to the *A. limonisporus* clade. According to our data, *A. limonisporus* has not an austral distribution.

A description of the family Stereaceae and the genus *Aleurodiscus* are also provided according to rules of *Fungal diversity notes*.

Stereaceae Pilát

The family Stereaceae was proposed by Pilát (1930) with *Stereum* Hill ex Pers. as generic type. This family is one of most widespread and diverse in the Russulales, together with Russulaceae. It includes species with basidiome appresed, effuse-reflexed or discoid, rarely stalked; pileus often zoned; monomitic or dimitic hyphal system; hymenophore smooth to tuberculate; basidia and spores hyaline, smooth, amyloid or non-amyloid. The family comprises species that grow in exposed positions, such as dead branches still attached to hardwood or conifer trees, causing white rot (Miller et al. 2006, Larsson 2007).

Aleurodiscus Rabenh. ex J. Schröt.

Aleurodiscus is one of the largest genera in Stereaceae. It was proposed by Rabenhorst (1874) without diagnosis, and it was validated by Schröter (1888), with *Peziza amorpha* Pers. (≡ *Aleurodiscus amorphus* (Pers.) J. Schröt.) as type species. Pilát (1926) included in *Aleurodiscus* corticioid fungi with large basidia and large spores, as well as variable sterile elements, and this conception was largely accepted (Bourdot & Galzin 1912, 1928; Burt 1918). Lemke (1964a, b) considered the genus to be artificial and he proposed to exclude species with inamyloid spores, and described the following new genera: *Aleurocystidiellum* Lemke, *Aleurocorticium* Lemke and *Licrostroma* Lemke.

On the basis of morphological and molecular data, Wu et al. (2000) described *Acanthofungus* Sheng H. Wu et al. with *Acanthofungus rimosus* Sheng H. Wu et al. as type species, and Wu et al. (2010) described *Neoaleurodiscus* Sheng H. Wu with *Neoaleurodiscus fujii* Sheng H. Wu as type species. From a phylogenetic perspective, Wu et al. (2001) analyzed the limits of *Aleurodiscus sensu lato* and the monophyly of previously segregated genera, concluding that *Aleurocystidiellum* and *Acanthobasidium* are monophyletic. However, subsequent studies (Larsson & Larsson 2003; Miller et al. 2006; Larsson 2007) have shown that *Aleurocystidiellum* does not belong to Stereaceae. Wu et al. (2001) set the boundaries of *Aleurodiscus sensu stricto* and confirmed that *Aleurodiscus sensu lato* is paraphyletic.

According to Wu et al. (2001), *Aleurodiscus sensu lato* includes species with highly variable characters; these are not congruent and usually overlap, so the species in *Aleurodiscus sensu lato* show all possible combinations of spore surface from smooth or ornamented, presence or absence of acanthophyses, and hyphae with clamps or simple-septate. The novel species, *Aleurodiscus patagonicus*, is introduced following the treatment in Wu et al. (2001), Dai & He (2016), and Dai et al. (2017a, b).

Aleurodiscus patagonicus Nogal, Telleria, M. Dueñas & M.P. Martín, **sp. nov.**
MycoBank number: MB 823981, *Facesoffungi number*: FoF05726 **Figs. 1, 2**

Etymology.— Named after the Chilean Northern Patagonian region where the holotype and paratypes were collected.

Holotypus.— 19609Tell., MA-Fungi 90714

Colour codes follow.— ISCC-NBS Centroid Colour Charts (Kelly & Judd 1976).

Basidiomes first discoid then confluent; margin determinate, involute; hymenophore smooth to slightly reticulate, pale orange yellow to medium orange yellow (73. p. OY – 71. m. OY). *Hyphal system monomitic*; hyphae thick-walled, 3−6 μm wide; paraphysoid hyphae cylindrical, occasionally branched, with scattered clamps, 3–5 μm wide. *Basidia* clavate, thin walled, stalked, with basal clamp, 150−190 × (22−)24−27 μm, with four sterigmata, 5–7 μm wide. Spores citriform, smooth, thin-walled, hyaline, strongly amyloid, 19−22(−24) × 14−16 μm, Q = 1.38.

Material examined.— CHILE, Palena, Comuna Hualaihué, Huinay Reserve, path to Cerro del Tambor, 42º22’44”S, 72º24’25”W, 100 m asl., on unidentified wood, 7 May 2013, M. Dueñas, M.P. Martín & M.T. Telleria, 19609Tell. (MA-Fungi 90714, holotype); idem, 14537MD (MA-Fungi 90713). Comuna Hualaihué, Base Paula, path of Geyser, on unidentified wood, 42º24’16.1”S, 72º44’0.59”W, 52 m asl., 28 May 2012, M. Dueñas, M.P. Martín & M.T. Telleria, 14080MD (MA-Fungi 90711).

Habitat and distribution.— on unidentified wood in Valdivian temperate rainforest in Chilean Northern Patagonian region.

Additional material examined.— *Aleurodiscus limonisporus*: AUSTRALIA, Victoria, Cumberland falls, on *Nothofagus cunninghamii*, 6 Jun. 1954, A. Miller (PDD 16691, **isotype**). NEW ZEALAND, Bay of Plenty, Te Urewera, Tarapounamu, west of road, on decaying branch, 19 May 2005, B. Paulus & M. Fletcher (PDD 83502); Te Urewera, Tarapounamu, east of road, on decaying wood, 17 May 2005, B. Paulus (PDD 83552); Te Urewera, Tarapounamu, west of road, on fallen branch, 19 May 2005, B. Paulus & M. Fletcher (PDD 83553); Buller, North of Reefton, Perseverance Bridge, 5 May 2001, E. Johannesen (PDD 72991); Paparoa National Park, Bullock

Creek Farm, on *Nothofagus fusca*, 26 Apr. 1987, P.K. Buchanan (PDD 55241); Paparoa Ranges, Tiropahi Walk, on *Nothofagus menziesii*, 27 Apr. 1987, P.K. Buchanan (PDD 55264); Victoria Forest Park, east of Maruia, on *Nothofagus menziesii*, 22 Apr. 1986, R.E. Beever (PDD 53413); Canterbury, Arthur's Pass National Park, Dobson Nature Walk, on *Olearia capillaris*, 20 Nov. 1988, P.K. Buchanan (PDD 55021); Jollie's Bush Reserve, Christchurch, on standing dead tree, 4 Jul. 2010, J.A. Cooper (PDD 95980); Gisborne, Moanui Conservation Area, 38º24'24.84"S, 177º23'58.92"E, on *Nothofagus*, 13 May 2013, S.R. Pennycook (PDD 97004); Hawke's Bay, Upper Mohaka River, Kaimanawa Range, on *Nothofagus fusca*, May 1953, J.M. Dingley (PDD 12600); Nelson, Abel Tasman National Park, Hardwoods Hole, on decaying branch of *Nothofagus solandri*, 15 Apr. 2008, A.J. O'Donnell & B.C. Paulus (PDD 94131); Kahurangi National Park, Flora Saddle, on decaying bark, 14 April 2008, A.J. O'Donnell & B.C. Paulus (PDD 94144); Murchison, on *Nothofagus fusca*, April 1956, S.D. & P.J. Brook (PDD 17122); Otago Lakes, Beyond Paradise, on *Nothofagus* small branch, 7 May 2016, P. Catcheside (PDD 110288); Glacier Burn Track, on *Nothofagus*, 8 May 2016, A. Chinn (PDD 109766); Southland, Porakino Valley, on bark of *Nothofagus solandri*, 7 May 2012, Lloyd (PDD 96617); Wairarapa, Tararua Forest Park, Mt Holdsworth, Gentle Annie Track, on *Nothofagus fusca*, 10 May 2007, A.J. O'Donnell (PDD 92582); Tararua Forest Park, Mt Holdsworth, Donnelly Flat track, on decaying branch, 10 May 2007, B.C. Paulus (PDD 92616); Tararua Forest Park, Mt Holdsworth track, on decaying wood, 7 May 2007, G. Gates & D. Ratkowsky (PDD 92829); Wellington, Kaimanawa Range, on *Nothofagus menziesii*, Apr. 1955, J.M. Dingley (PDD 15227); Mangatorutoru Stream, on *Nothofagus solandri* var. *cliffortioides*, Mar. 1948, J.M. Dingley (PDD 7452); Tongariro National Park, on *Nothofagus solandri* var. *cliffortioides*, Feb. 1951, G.H. Cunningham (PDD 15230); Ohakune, Lake Surprise Track, on *Nothofagus* sp., Apr. 1935, E.E. Chamberlain (PDD 15229); Whakapapa-iti Stream, Ruapehu, on *Nothofagus solandri* var. *cliffortioides*, Sep 1955, J.M. Dingley (PDD 15355): Whakapapa-iti Stream, Tongariro National Park, on *Nothofagus solandri* var. *cliffortioides*, Jan. 1951, J.M. Dingley (PDD 11188); Turangi, near Beggs Pool, Kaimanawa Range, on *Weinmannia racemosa*, 25 May 1970, J.M. Dingley (PDD 28631); Westland, Granville Forest, Orwell Creek, Ahaura, on *Nothofagus fusca*, 2 Apr. 1963, J.M. Dingley (PDD 20997).

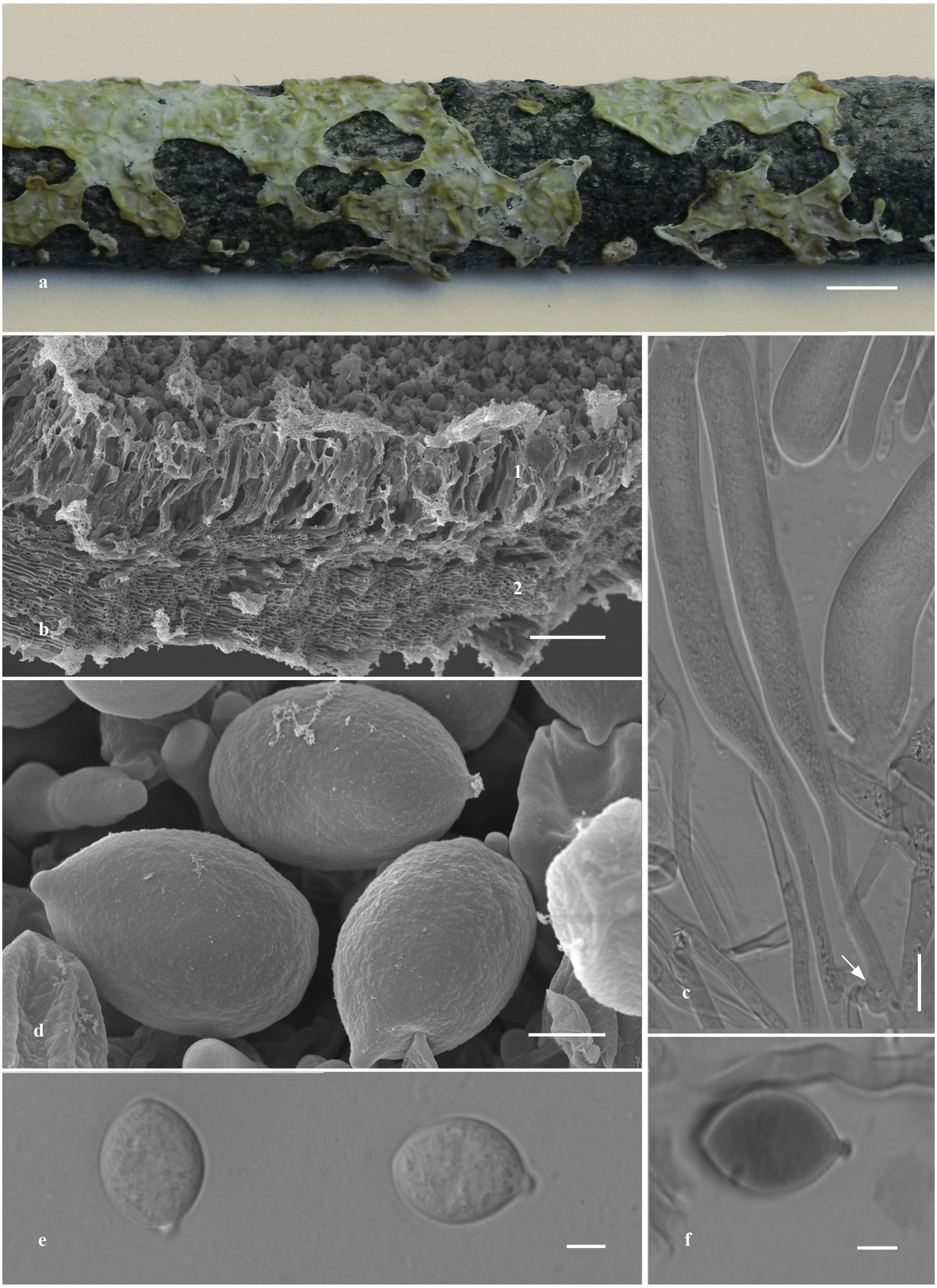

Fig. 1. *Aleurodiscus patagonicus* (19609Tell., MA-Fungi 90714, holotype) **a.** Basidiome. **b.** Section of basidiome under SEM; hymenial layer (1), basal layer (2). **c.** Probasidia with basal clamp. **d.** Spores under SEM. **e, f.** Spores. *Scale bars* a = 5 mm, b = 50 µm, c = 10 µm, d, e, f = 5 µm. Scanning electron microscope (SEM) was used after coating basidiome samples in gold with Balzers SCD 004 sputter coater with a Hitachi S-3000N SEM.

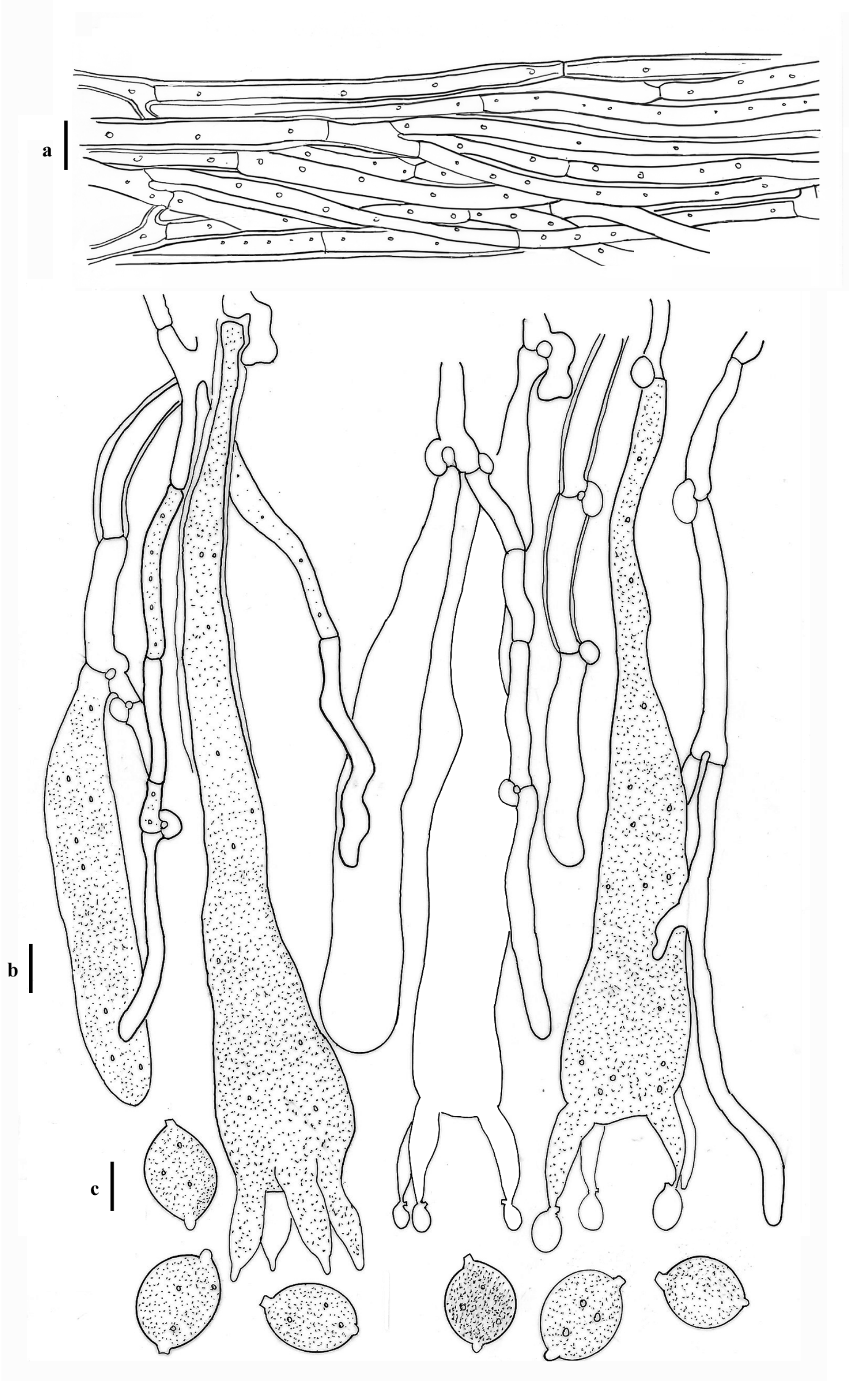

Fig. 2. *Aleurodiscus patagonicus* (19609Tell., MA-Fungi 90714, holotype) **a.** Basal layer. **b.** Hymenial layer with basidia and paraphysoid hyphae. **c.** Spores. *Scale bars* = 10 µm. Line drawings were made with a Leyca DM2500 microscope with aid of a drawing tube by M. Dueñas.

GenBank numbers.— MA-Fungi 90711 ITS: MF631175; LSU: MF631191. MA-Fungi 90713 ITS: MF631176; LSU: MF631192. MA-Fungi 90714 ITS: MF631177; LSU: MF631193. PDD 7452 ITS: MF631152; LSU: MF631178. PDD 12600 ITS: MF631153. PDD 15229 ITS: MF631154. PDD 15230 ITS: MF631155. PDD 16691 ITS: MF631156; LSU: MF631179. PDD 17122 ITS: MF631157. PDD 28631 ITS: MF631158. PDD 53413 ITS: MF631159. PDD 55021 ITS: MF631160. PDD 55241 ITS: MF631161. PDD 72991 ITS: MF631162. PDD 83502 ITS: MF631163. PDD 83552 ITS: MF631164; LSU: MF631180. PDD 92582 ITS: MF631165; LSU: MF631181. PDD 92616 ITS: MF631166; LSU: MF631182. PDD 92829 ITS: MF631167; LSU: MF631183. PDD 94131 ITS: MF631168; LSU: MF631184. PDD 94144 ITS: MF631169; LSU: MF631185. PDD 95980 ITS: MF631170; LSU: MF631186. PDD 96617 ITS: MF631171; LSU: MF631187. PDD 97004 ITS: MF631172; LSU: MF631188. PDD 109766 ITS: MF631173; LSU: MF631189. PDD 110288 ITS: MF631174; LSU: MF631190.

Notes.— Phylogenetic analyses based on a combined ITS and LSU sequence dataset (Fig. 3) shows that *Aleurodiscus patagonicus* clusters within Stereaceae, far away from the *Aleurodiscus sensu stricto* clade, which includes only *A. amorphous* (Pers.) J. Schröt. (type species) and *A. grantii* Lloyd (Wu et al. 2001). *Aleurodiscus sensu lato* is paraphyletic; sequencing shows species of apparently unrelated genera intermingled with the species of *Aleurodiscus sensu lato* (Wu et al. 2001, 2010; Larsson & Larsson 2003; Binder et al. 2005; Miller et al. 2006). In the phylogeny (Fig. 3), *A. patagonicus* groups in a highly supported clade (99% ML, 99% MP and 1.00 PP), which is sister to the *A. limonisporus* D.A. Reid clade and this relationship is also highly support (92% ML, 90% MP and 1.00 PP).

Aleurodiscus patagonicus is similar to *A. limonisporus*. Both species have a monomitic hyphal system, cylindrical paraphysoid hyphae, and characteristic citriform smooth spores. However, *A. patagonicus* differs in the following characters: clamps present in paraphysoid hyphae, longer and wider basidia, with basal clamp, and, spores with higher length/width ratio, as well as, in the geographical distribution (Table 1). *Aleurodiscus patagonicus* is reported from Chilean Northern Patagonia, while *A. limonisporus* occurs in Australia (Reid 1955) and New Zealand (McKenzie et al. 2000).

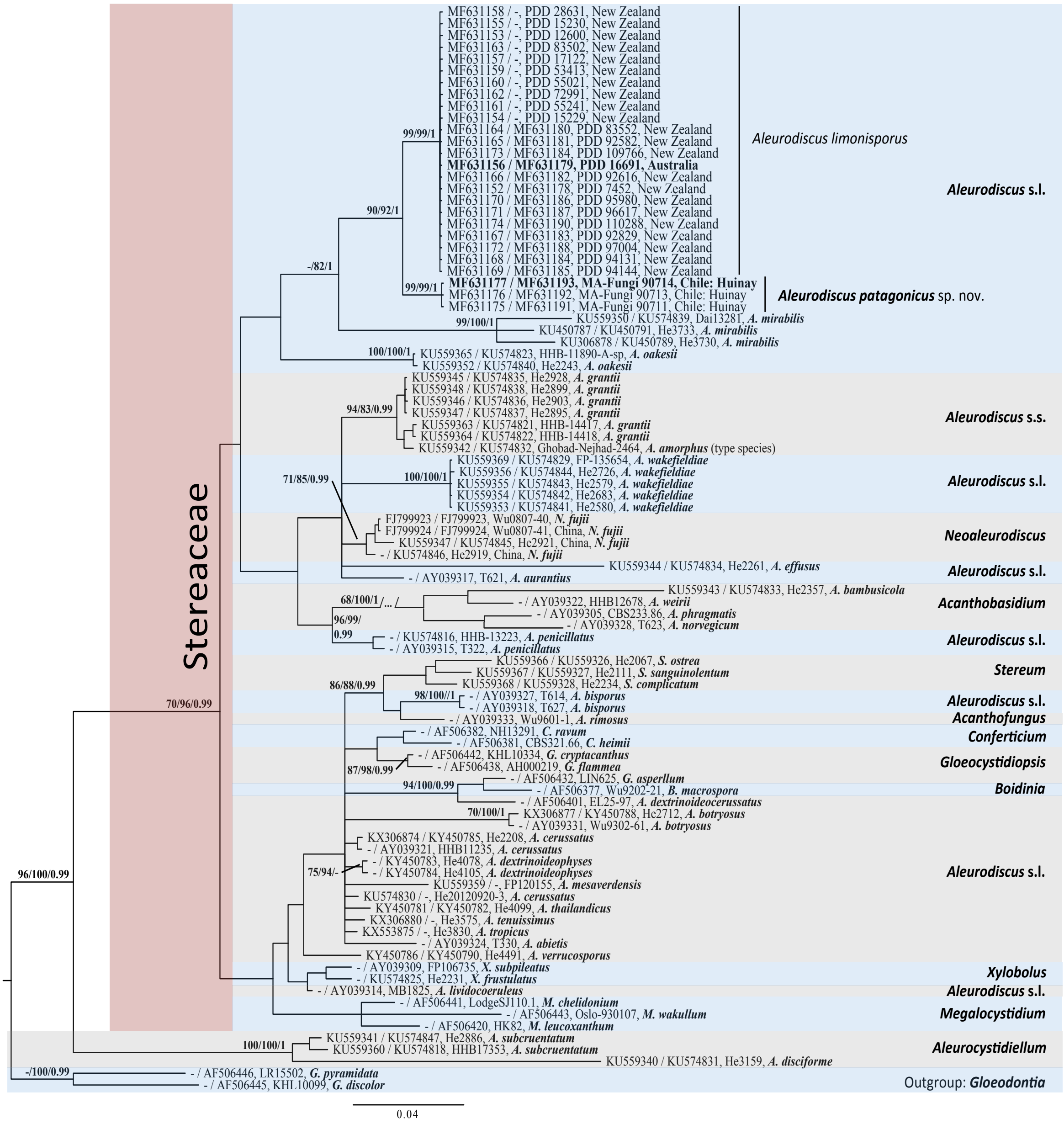

Fig. 3. Majority-rule bayesian topology based on ITS and LSU nrDNA sequence data. Two *Gloeodontia* species were included as outgroup. Parsimony bootstrap support (≥ 50 %), likelihood bootstrap support (≥ 50), and posterior probability (≥ 0.95) are indicated above branches. Newly generated sequences are marked in bold face. Scale bar indicates substitution per site. Sequences were retrieved from EMBL Nucleotide Sequence Database (Cochrane et al. 2016), and they were published in Wu et al. (2001), Larsson & Larsson (2003), Wu et al. (2010), Dai & He (2016), Dai et al. (2017a, b). *: Types.

Table 1. Comparison of morphological characters and distribution between *Aleurodiscus patagonicus* and *A. limonisporus*. ANOVA analyses were performed to assess the significance of basidia and spore morphology using the function "aov" of the stats R package v 3.2.1 (R Core Team 2015).

Morphology and distribution	***A. patagonicus***	***A. limonisporus***	**ANOVA**
Basidia lenght (μm)	150−190	130−160(−190)	**F = 31.26, p < 0.05**
Basidia width (μm)	(22−)24−27	20−25(−26)	**F = 21.45, p < 0.05**
Basal clamp in basidia	Present	Absent	-
Spores lenght, L (μm)	19−22(−24)	20−24(−26)	F = 0.808, p = 0.379
Spores width, W (μm)	14−16	12−16	F = 4.195 p = 0.0532
Q Index, L/W	1.36–1.40	1.41–1.60	**F = 10.02, p < 0.05**
Distribution	Chile: Huinay Reserve	Australia, New Zealand	-

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Chapter 2

Deciphering the phylogeny, taxonomy and distribution of the genus *Cystostereum* (Cystostereaceae, Agaricales)

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Manuscrito en preparación

ABSTRACT

Cystostereum is a genus of corticioid fungi characterized by a dimitic hyphal system, numerous gloeocystidia at various layers of the basidiome and non-amyloid basidiospores. It has included up to eleven species, of which five have been transferred to the genera *Crustomyces* and *Cystidiodontia*. In this study, the phylogenetic position and the diversity of *Cystostereum sensu lato* are examined through an integrative taxonomic approach based on morphological and molecular analyses, considering the whole known geographical range of the eleven species. The molecular analyses of four DNA regions (ITS nrDNA, LSU nrDNA, *rpb*2 and *rpb*1) were used to circumscribe the genus, and confirmed six species in *Cystostereum sensu stricto*: *C. australe, C. effusum, C. kenyense, C. murrayi, C. sirmaurense* and *C. tuberculosum*. The specimens originally identified as *C. murrayi*, the generic type and considered widely distributed, were resolved into four different lineages that belong to *Cystostereum,* and a group formed by specimens from Cuba, Jamaica and Puerto Rico that do not belong to this genus, and that are listed as *incertae sedis*. The four lineages obtained from *C. murrayi* correspond to four groups from different geographic origins: a) a singleton corresponds to the type specimen from Cuba, b) one group was formed by North American specimens; c) a second group with European specimens; and d) a third one with specimens from India. Two new combinations are proposed: *C. effusum* and *C. tuberculosum,* corresponding to two heterotypic synonyms for *C. murrayi*. *Cystostereum stratosum* was excluded from *Cystostereum,* based on molecular and morphological evidence and, the type specimen of *C. saxitas,* initially considered in the genus, was not able to be characterized by DNA sequencing.

INTRODUCTION

The genus *Cystostereum* was described by Pouzar (1959) to accommodate only one species, *Cystostereum murrayi* (Berk. & M.A. Curtis) Pouzar. According to Pouzar (1959), the genus is characterized by "*a great number of gloeocystidia, dimitic hyphal system with very scarce, light coloured skeletal hyphae, hard consistency of trama and nonamyloid spores*".

Since its description, eleven species have been placed in the genus. *Cystostereum murrayi* originally described from Cuba by Berkeley & Curtis (1869) as *Thelephora murrayi,* has been considered a common hardwood decay fungus in North America (Burt 1920, Lentz 1955, Ginns 1986); in Europe, where it is a boreal-montane species, fruiting preferably on different species of *Picea* and *Abies* (Eriksson & Ryvarden 1975); and it is also known from South Africa (Talbot 1954), New Zealand (Cunningham 1963), India and Nepal (Rattan 1977). *Cystostereum pini-canadense* described from Pennsylvania by von Schweinitz (1832) as *Radulum pini-canandense* was included in *Cystostereum* by Parmasto (1968), and seems to be frequent in the Northeastern and North Central United States and the East of Canada on conifers and hardwoods (Chamuris 1986). *Cystostereum subabruptum* was described from France by Bourdot & Galzin (1927) as *Odontia subabrupta* and combined to *Cystostereum* by Eriksson & Ryvarden (1975); it is common in central and southern Europe (Eriksson & Ryvarden 1975, Telleria & Melo 1995, Bernicchia & Gorjón 2010), and in the northwestern United States and British Columbia (Chamuris 1986). Both *C. pini-canadense* and *C. subabruptum*, differ morphologically from *C. murrayi* by having dendrohyphidia and gloeocystidia more or less moniliform with resinuous content instead of oily; along with a third species, *Cystostereum artocreas* (Berk. & M.A. Curtis ex Cooke) Hallenb. & Ryvarden, originally described from Venezuela as *Hydnum artocreas* by Berkeley & Curtis in Cooke (1891). Hallenberg & Ryvarden (1975) described *Cystostereum* subg. *Dendrohyphidium* to accomodate these three species. However, the morphological variations among those species led to their segregation into two new genera, *Crustomyces* and *Cystidiodontia*.

Cystostereum subabruptum and *C. pini-canadense* were transferred to *Crustomyces* by Jülich (1978) selecting *Crustomyces subabruptus* as the type species of the genus; Chamuris (1986) reconsidered them again in *Cystostereum* but as subspecies of *C. pini-canadense,* and later Ginns & Lefebvre (1993) transferred them back to *Crustomyces*. Also, the species *Cystostereum piceinum* (Overh.) Lindsey & Gilb., originally described from Cherry Mountain in New Hampshire as *Peniophora piceina* by Overholts (1930), was then synonymized with *Cystostereum pini-canadense* by Chamuris (1986).

On the other hand, Hjortstam (1983), based on African specimens and morphological characters such as thick-walled spores, presence of dendrohyphidia and skeletal hyphae with dextrinoid reaction, described the genus *Cystidiodontia,* with only one species *Cystidiodontia artocreas*, and considered that it was one of the most common species collected by Ryvarden in Africa. Later, Hjortstam & Ryvarden (1986) revised the African specimens considering that they were conspecific with *Kneiffia isabellina*, a species described from Ceylon by Berkeley & Broome (1875), and proposed *Cystidiodontia isabellina.* Thus, *C. artocreas* is known from Venezuela and Argentina and *C. isabellina* from Ceylon and eastern Africa.

Besides, six additional species were included in *Cystostereum*: *Cystostereum stratosum* described from Iran by Hallenberg (1978), and then reported from India by De (1995); *Cystostereum heteromorphum* described by Hallenberg (1980) from Ukraine and later transferred to *Crustomyces* by Hjortstam (1987); *C. australe* described by Nakasone (1983) and known from the southeastern USA, and Costa Rica; *C. kenyense* described from Kenya by Hjortstam (1987); *C. saxitas* (Burt) A.L. Welden, originally described by Burt (1920) from Mexico and Jamaica as *Stereum saxitas*; and, *C. sirmaurense* R. Kaur, Avn.P. Singh & Dhingra that was recently described from India (Kaur et al. 2019).

Currently and according to Index Fungorum (http://www.indexfungorum.org; 15/11/2021), eight species are accepted in the genus *Cystostereum* (*C. australe*, *C. heteromorphum*, *C. kenyense, C. murrayi, C. pini-canadense, C. saxitas, C. sirmaurense* and *C. stratosum*), and seven according to Mycobank (https://www.mycobank.org/; 15/11/2021): *C. australe*, *C. heteromorphum, C. kenyense, C. murrayi*, *C. pini-canadense, C. sirmaurense,* and *C. stratosum*).

Cystostereum was initially placed in Stereaceae by Donk (1964), later Parmasto (1968) included it in the subfamily Cystostereoideae in Steccherinaceae, and Jülich (1981) described the family Cystostereaceae, which was typified by *Cystostereum* and at that time included *Crustomyces*. Molecular phylogenetic analyses carried out by Larsson (2007), based on 5.8S and LSU nrDNA sequences, showed a well-supported family relationship between generic types *Cystostereum murrayi* and *Cystidiodontia artocreas* in Cystostereaceae, in the order Agaricales, and Song et al. (2018) confirmed that *Crustomyces subabruptus* also belongs to the family with high support.

Despite the intra- and interspecific morphological variations of the species included in *Cystostereum*, few DNA-based studies have been conducted to clarify species boundaries, as well as their geographical distributions and relationships within the segregated genera. In agreement with Taylor et al. (2006), molecular approaches have generally demonstrated that species boundaries, as well as their geographic range, depend on the method of species recognition. Thus, the scarcity of studies including molecular data and the wide distribution of type species *Cystostereum murrayi* suggests it may encompass several taxa with more restricted distribution. This led us to address the following objetives: (1) to produce a better resolution of the limits of *Cystostereum,* through morphological and phylogenetic analysis of the 11 species that have been included, at any time, in the genus (2) to clarify the systematics of *Cystostereum sensu stricto,* based on an integrative approach using morphological and molecular methods, as well as other sources of information, such as chorology and ecology, that allow us to better understand distribution patterns in the species included in the genus.

MATERIALS AND METHODS

Taxon sampling

Based on the information deposited in the GBIF biodiversity data portal (www.gbif.org) and an exhaustive bibliographic search, a database with 1473 entries was built with information about the specimens of the 11 species included in any time in *Cystostereum*. From that database, 233 specimens were selected trying to cover: 1) type specimens, including heterotypic synonyms, and 2) representative sampling of geographical distribution of each species. The specimens were requested from 16 herbaria: ARIZ, BPI, BR, CFMR, FH, GB, ICN, K(M), MA-Fungi, MEL, NY, O, PDD, TAAM, TU and UPS (herbarium acronyms are given according to the Index Herbariorum, http://sweetgum.nybg.org/science/ih, Thiers 2016). In addition, five specimens collected in Comuna San Pablo (XIV Region, Los Lagos, Chile) during fieldwork in 2017 were included.

Morphological studies

Macro- and micromorphological characters used in corticioid fungal taxonomy were observed and measured for each specimen. Macroscopic characters, such as shape and color of basidiome, hymenophore and margin, were obserserved with a Nikon SMZ645 stereo microscope. The colour codes were given following the ISCC-NBS Centroid Color Charts (Kelly & Judd 1976). Microscopic characters such as hyphal system, morphology and size of hyphae, cystidia, basidia and basidiospores were studied from sections mounted in aqueous solutions of 3% and 5%

aqueous KOH and a solution of Congo Red ammonia. In addition, sulfovainilline, Melzer reagent and Cotton Blue were used in order to determine presence of sulfocystidia, amyloid reaction and cyanophilic reaction of spores and/or other structures, respectively. In the taxonomic part, SA+ means that gloeocystidia are positive to sulfovainilline, SA- those negative; CB+ means that the walls of the cells are stained by Cotton Blue (cyanophilous) and CB– that they are not stained; and IKI– that there is no reaction to Melzer´s reagent (non amyloid). Sections were examined at magnifications up to 1000× using a Nikon Eclipse 80i. Whenever possible, the length (L) and width (W) of 10 cystidia, 10 basidia and 20 basidiospores were measured; and length/width ratio (Q) and mean values (L', W' and Q') were calculated. One-way ANOVA tests were conducted to assess the significance of differences in spore morphology between some of the clades identified from *Cystostereum murrayi* in phylogenetic analyses. These analyses were performed using the function "aov" of the stats R package v 3.2.1 (R Core Team 2015).

DNA extraction

A fragment <10mg was removed from dried basidiomes and genomic DNA was extracted using DNeasy™ Plant Mini Kit (Qiagen, Valencia, CA), following the manufacturer's instructions except in lysis buffer incubation, which was performed overnight at 60 °C.

PCR amplification and sequencing

The following DNA regions were studied: 28S nrDNA (LSU), ITS1 and ITS2 including 5.8S nrDNA (ITS), RNA polymerase II second largest subunit (*rpb*2), and conserved domains A and C of RNA polymerase II largest subunit (*rpb*1). The PCR reactions were done using general primers and eight new primers that were desgined using Primer-BLAST tool (Ye et al. 2012), and synthesized by Macrogen (Madrid, Spain). All primers used in this study are listed in Table 1, indicating the marker, direction, sequence, annealing temperature (T_m) and reference. Individual reactions were carried out to a final volume of 25 µl using Illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare, Buckinghamshire, UK) or to 20 µl using Mouse Direct PCR kit (Biotool) with a 10 pmol/µl primer concentration. Negative controls were run with each experiment for detecting any possible contamination. We consider that amplimer was obtained when bands were visualized after gel electrophoresis.

ITS nrDNA

Direct amplification of ITS was carried out using primer pair ITS5/ITS4. When direct amplifications failed, nested-PCR was done with the pair ITS1F/ITS4B to the first PCR reaction, and ITS5/ITS4 in the second in which one µl from the first amplification served as DNA template. Moreover, amplifications by parts were conducted with recalcitrant samples: the region ITS1 and the initial part of 5.8S was amplified with pairs ITS5/ITS2 or the designed ITS33Cyst combined with ITS2; and the final part of 5.8S and the region ITS2 was amplified using the pair ITS3/4B or ITS3/ITS4. Amplifications were performed with thermal cycling conditions described in Martín & Winka (2000): an initial denaturation at 94 °C for 5 min, 5 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min; 33 cycles of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 1 min; with a final extension at 72 °C for 10 min. This program was modified for combination of ITS33Cyst and ITS2, in which annealing temperature was increased to 58 ºC in the first five cycles.

Prior to sequencing, PCR products were purified using QIAquick Gel PCR Purification (QIAGEN, Valencia, California) or Illustra ExoStar-1-Step (GE Healthcare, Buckinghamshire, UK) according to the instructions indicated by manufacturers. Purification methods were selected depending on quality of bands observed after gel electrophoresis of PCR products. When multiple bands and smear were detected, gel extraction using QIAquick Gel PCR Purification was followed, while Exosap protocol was chosen when the quality of DNA was high (a unique amplicon of above 20 ng/µl concentration). Purified amplicons, with a concentration of 20 ng/µl or more, were sent to Macrogen (Madrid, Spain) for sequencing both strands with the same primers used in the amplification.

Consensus sequences were assembled using Geneious version 9.0.2 (Kearse et al. 2012). Subsequently, to check for possible contaminations, the consensus sequences were subjected to a BLAST search with megablast option to find similarity percentage with sequences in the National Center for Biotechnology Information (NCBI) nucleotide databases (Altschul et al. 1990). Newly generated sequences will be published in the EMBL/GenBank/DDBJ databases and the accession numbers will be as indicated in Table 2.

LSU nrDNA

Direct amplifications of LSU were conducted using the primer pair LR0R/LR5r from all DNA isolations from which ITS sequences were obtained. When this combination failed, seminested-

PCR was carried in two steps: first, an amplification with the primer pair LR0R/LR7r, and a second amplification with LR0R/LR5r, for which one µl from the first amplification was used as DNA template. When this strategy failed, direct PCR was conducted with the designed primer LR0R-Cyst in combination with LR5r. With the general primers, cycling parameters were: initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 60 °C (annealing) for 1 min 30 s and 72 °C for 1 min 30 s (extension), with final extension at 72 °C for 10 min; and for the combination of the designed primer LR0R-Cyst with LR5r, thermal conditions are the same except annealing temperature, 61.3 ºC. Purification and sequencing of the PCR-products followed protocols mentioned above for ITS.

RPB2 and RPB1

From those specimens with ITS and LSU sequences, amplification and sequencing of *rpb*2 and *rpb*1 markers was tried. First, nested-PCR was performed for *rpb*2 using general primers: the primer pair fRPB2-5F/bRPB2-7.1R in the first amplification, and bRPB2-6F/bRPB2-7R.2 in the second. One µl from the first amplification served as DNA template and cycling conditions were those indicated in Wilson et al. (2011). When nested-PCR failed with general primers, the designed forward primer fRPB2-Cyst1 or fRPB2-Cyst2 combined with bRPB2-7R.2 in the second amplification. The thermal cycling conditions used for amplification with general primers *rpb*2 were: an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 54 °C for 1 min and 72 °C for 1 min, with final extension at 72 °C for 10 min. For the second amplification with the designed primers fRPB2-Cyst1 or fRPB2-Cyst2, cycles and temperature were the same as indicated for LSU, except in annealing temperature, which was 59.4 ºC and 61 ºC, respectively.For *rpb*1, direct amplification using the primer pair RPB1-Af/RPB1-Cr was carried out. When direct amplification failed, a semi-nested strategy was carried out with alternative forward primers RPB1-2f, RPB1-2.1f or RPB1-2.2f in the second amplification using one µl from the first as DNA template. The touchdown protocol indicated by Floudas & Hibbett (2015) was followed: an initial denaturation at 94 ºC for 2 min, 9 cycles of denaturation at 94 ºC for 40 s, 60 ºC for 40 s (minus 1º C per cycle), 72 ºC for 2 min; 36 cycles of denaturation at 94 ºC for 45 s, 53 ºC for 1 min 30 s, 72 ºC for 2 min, with final extension at 72 °C for 10 min. If direct and semi-nested strategies failed, amplification by parts was conducted using the designed primer pairs RPB1-Cyst3/RPB1-Cyst4 and RPB1-Cyst5/RPB1-Cyst6, following amplification cycles and temperatures indicated for LSU, except in annealing temperature, which was 61 ºC and 59.9 ºC, respectively.

Table 1. Summary of PCR primers used in this study. Asterisk (*) indicates modification in the original primer.

Primer name	Direction	Primer sequence (5´-3´)	T_m (ºC)	Reference
ITSnrDNA				
ITS1F	Forward	cttggtcatttagaggaagtaa	56.6	Gardes & Bruns (1993)
ITS3	Forward	gcatcgatgaagaacgcagc	62.0	White et al. (1990)
ITS5	Forward	ggaagtaaaagtcgtaacaagg	58.4	White et al. (1990)
ITSCyst33	Forward	gtgctgtagctggccttmgt	60.5	This study
ITS2	Reverse	gctgcgttcttcatcgatgc	62.0	White et al. (1990)
ITS4	Reverse	tcctccgcttattgatatgc	56.4	White et al. (1990)
ITS4B	Reverse	caggagacttgtacacggtccag	66.4	Gardes & Bruns (1993)
LSU nrDNA				
LR0R	Forward	acccgctgaacttaagc	52.4	Cubeta et al. (1991)
LR0R-Cyst	Forward	ccgygctggacygtgtayaag	61.3	This study
LR5r	Reverse	atcctgagggaaacttcg*	58.8	Vilgalys & Hester (1990)
LR7r	Reverse	tactaccaccaagatct	47.6	Vilgalys &Hester (1990)
***rpb*2**				
fRPB2-5F	Forward	gaygaymgwgatcayttygg	56.4	Matheny (2005)
bRPB2-6F	Forward	tggggyatggtntgyccygc	60.5	Matheny (2005)
fRPB2-Cyst1	Forward	atygarttcytggaagagtggg	58.4	This study
fRPB2-Cyst2	Forward	agtggggtytbgartcgytgg	59.4	This study
bRPB2-7.1R	Reverse	cccatrgcytgyttmcccatdgc	62.9	Matheny (2005)
bRPB2-7R.2	Reverse	acytgrttrtgrtcnggraangg	57.5	Matheny et al. (2007)
***rpb*1**				
RPB1-Af	Forward	gartgyccdggdcayttygg*	56.4	Stiller & Hall (1997)
RPB1-Int2f	Forward	ttmbtctrctcgtttygcac	52.3	Frøslev et al. (2005)
RPB1-Int 2.1f	Forward	gctgaacgagsagtgc	53.3	Frøslev et al. (2005)
RPB1-Int 2.2f	Forward	cgttttcgrtcgcttgat	51.6	Binder et al. (2010)
RPB1-Cyst3	Forward	atggaacggctcctccwttg	59.9	This study
RPB1-Cyst5	Forward	gatgcaacccgacaagagga	59.9	This study
RPB1-Cr	Reverse	ccngcdatntcrttrtccatrta	55.7	Matheny et al. (2002)
RPB1-Cyst4	Reverse	gtgtagacctcggagggagt	61.9	This study
RPB1-Cyst6	Reverse	acgtcaagtcatcctcgctc	59.9	This study

Purification and sequencing of the *rpb*2 and *rpb*1 PCR-products followed protocols mentioned for ITS.

Sequence alignments and phylogenetic analyses

With the new sequences obtained for the four markers (Table 2), independent alignment matrices were built using the program Geneious. Homologous sequences were retrieved from EMBL/GenBank/DDBJ and UNITE (Nilsson et al. 2019) databases; as well as sequences of *Chondrostereum* and *Gloeostereum,* since in Larsson (2007) these genera belong to Cyphellaceae, the sister family of Cystostereaceae.

For LSU and ITS alignments, *Dendrothele griseocana* (Bres.) Bourdot & Galzin sequences were included as outgroup because in Larsson (2007) two species of this genus appear as the sister group of Cystostereaceae and Cyphellaceae families; moreover, sequences of *Duportella renispora* Boidin, Lanq. & Gilles were also incorporated since based on morphological features this species appears a suitable outgroup. On the other hand, based on preliminary results from these analyses and the availability of sequences in DNA databases, for *rpb*2 alignment the sequence AY218477 under *Chondrostereum purpureum* (Pers.) Pouzar was included as outgroup. Since no *rpb*1 sequence of *Ch. purpureum* was available, *Crustomyces* and *Cystidiodontia* sequences were selected as outgroup.

Analyses under Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) were conducted. In order to infer the generic position of all the known species that have been included in *Cystostereum*, LSU analyses were done first. Posterior analyses were performed with ITS alignment, followed by *rpb*2 and *rpb*1.

For each marker, first MP analyses were done using PAUP 4.0b10 (Swofford 2003) with the heuristic search option. Gaps were treated as missing data and branch lengths equal to zero were collapsed to polytomies. MAXTREES limit was set to 100. Non-parametric bootstrap (MPbs) support (Felsenstein 1985) for each clade was tested based on 10,000 replicates with the FAST-STEP option. Second, ML analyses were performed using RaxML (Stamatakis 2014) implemented on CIPRES Portal (CIPRES Science Gateway v.3.3, Miller et al. 2010), using the default parameters established in CIPRES; except for the best-fitting model of nucleotide substitution for each independent locus, which was estimated using jModelTest2 (Darriba et al. 2012) and selected according to Bayesian Information Criterion (BIC). As in MP analyses, non-parametric bootstrap (MLbs) support (Felsenstein 1985) was calculated for each clade, but in this case specifying 1,000 replicates. Finally, BI was performed using the software MrBayes v.3.2. (Ronquist et al. 2012), assuming the general time reversible model (Rodríguez et al. 1990) and discrete gamma distribution with six categories (GTR+I+G). A run with 2,000,000 generations starting with a random tree and employing 12 simultaneous chains was executed, sampling every 100th tree. The 50% majority-rule consensus tree and the posterior probability (pp) of the nodes were calculated from the remaining trees. Also, preliminary analyses were done with different concatenated matrices, such as LSU + ITS, *rpb*2 + *rpb*1, and LSU + ITS + *rpb*2 + *rpb*1, however, due to the differences between the number of sequences obtained for each marker, many ambigueties were introduced, and the phylogenetic trees were not well resolved.

Table 2. Specimens and sequences included in this study. Collector, country, date and substrate are indicated when available. Newly generated sequences were marked in bold face and those not obtained are indicated as (-). When cells are empty are referred to not amplification attempts or that sequences are not available in GenBank.

Species/Specimens[1], cultures	Country	Date	Substrate	GenBank/UNITE Accesion n.º			
				ITS	28S	RPB1	RPB2
I-A, *Crustomyces subabruptus* (Bourdot & Galzin) Jülich							
MA-Fungi sn, 520JFL	Chile	6 Nov 2017	*Eucalyptus* sp.	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
MA-Fungi sn, 3946 MPM	Chile	6 Nov 2017	*Eucalyptus* sp.	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
MA-Fungi sn, 3947 MPM	Chile	6 Nov 2017	*Eucalyptus* sp.	**Acc. N.**	-	**-**	**Acc. N.**
MA-Fungi sn, 15597MD	Chile	6 Nov 2017	*Eucalyptus* sp.	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
MA-Fungi sn, 21090Tell.	Chile	6 Nov 2017	*Eucalyptus* sp.	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
TAAM 2419	Estonia	23 Sep 1953	*Alnus incana* fallen trunk	**Acc. N.**	-		
BPI 266215, paratype, Galzin 27322	France	May 1922	Beech branches	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
MA-Fungi 70568, 10771IS	France	1 Nov 2006	*Fagus sylvatica*	**Acc. N.**	**Acc. N.**		-
MA-Fungi 70569, 10772IS	France	1 Nov 2006	*Carpinus betulus*	**Acc. N.**	**Acc. N.**		**Acc. N.**
MA-Fungi 70571, 10774IS	France	1 Nov 2006	*Carpinus betulus*	**Acc. N.**	**Acc. N.**		-
MA-Fungi 70599, 16725Tell.	France	1 Nov 2006	Unidentified wood	**Acc. N.**	-		
MA-Fungi 70603, 16735Tell.	France	1 Nov 2006	Unidentified wood	**Acc. N.**	**Acc. N.**		-
MA-Fungi 70648, 10783IS	France	1 Nov 2006	Unidentified wood	**Acc. N.**	**Acc. N.**		-
MA-Fungi 79280, 18288Tell.	France	28 Oct 2009	*Fagus sylvatica*	**Acc. N.**	**Acc. N.**		-
MA-Fungi 79281, 18289Tell.	France	28 Oct 2009	*Fagus sylvatica*	**Acc. N.**	**Acc. N.**		-
GB-0084458, paratype *Cystostereum heteromorphum* NH 2712	Iran	10-13 May 1978	Fallen log of *Fagus*	**Acc. N.**	**Acc. N.**		-
GB-0084474, NH 2671	Iran	10-13 May 1978	White rotted wood of *Fagus*	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
GB-0084475, NH 2802	Iran	10-13 May 1978	White rotted wood of *Fagus*	**Acc. N.**	-		
GB-0084476, NH 2887	Iran	10-13 May 1978	Fallen, white rotted, branch	**Acc. N.**	-		
GB-0084477, NH 2896	Iran	10-13 May 1978	Wood on the ground	**Acc. N.**	-		
GB-0084478, NH 2784	Iran	10-13 May 1978	Wood on the ground	**Acc. N.**	**Acc. N.**		**Acc. N.**
GB-0084479, NH 2638	Iran	10-13 May 1978	Fallen branch of *Fagus*	**Acc. N.**	**Acc. N.**		**Acc. N.**
GB-0084480, NH 2780	Iran	10-13 May 1978	Fallen log of *Fagus*	**Acc. N.**	-		
GB-0084487, NH 2711	Iran	10-13 May 1978	Fallen log of *Fagus*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
GB-0084488, NH1927	Iran	17 Jul 1976	Decayed log of *Buxus*	**Acc. N.**			
(CFMR) AS-545, A. Saitta 545	Italy	18 May 2014		**Acc. N.**	**Acc. N.**		**Acc. N.**

Table 2. (cont.).

Species/Specimens^1, cultures	**Country**	**Date**	**Substrate**	**GenBank/UNITE Accesion n.º**			
				ITS	**28S**	**RPB1**	**RPB2**
I-A, *Crustomyces subabruptus* (Bourdot & Galzin) Jülich							
TU124343, Alessandro Saitta 122	Italy	10 Jul 2012		**Acc. N.**	**Acc. N.**		-
DK 070922-7941	Poland				MK277900		
GB-0084492, NH, Hjortstam, KHL 2197	Poland	12 Sep 1973	*Abies* trunk	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
GB-0084512	Poland	28 Aug 1965	*Ad ramum emortuum*	**Acc. N.**	-		
GB-0084513	Poland	11 Aug 1965	*Ad caudicem Piceae abietis*	**Acc. N.**	-		
GB-0084489, NH 9129	Romania	16 Oct 1985	Conifer log	**Acc. N.**	-		
GB-0181551, NH 12065	Russia	8-10 Sep 1991	Fallen trunk of *Fagus*	**Acc. N.**	-		-
TAAM 8226	Russia	Sep 1959	*Betula pendula* fallen branch	**Acc. N.**	-		
TAAM 132423	Russia	Sep 1966	*Populus* fallen trunk	**Acc. N.**	-		
TAAM 132570	Russia	Aug 1966	Rotten wood *Populus*	**Acc. N.**	-		
TAAM 134106	Russia	31 Aug 1962	*Carpinus* fallen trunk	**Acc. N.**	-		
MA-Fungi 44813, M. Honrubia 2726	Spain	19 Aug 1980	Dead wood *Pinus halepensis*	**Acc. N.**	-		
GB-0084481, NH 11365	Turkey	2-4 Oct 1989	Deciduous wood	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
GB-0084482, NH 11393	Turkey	2-4 Oct 1989	Deciduous wood	**Acc. N.**	-		
GB-0084483, NH 11388	Turkey	2-4 Oct 1989	Deciduous wood	**Acc. N.**	-		
GB-0084484, NH 11295	Turkey	2-4 Oct 1989	*Picea*-branch	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
GB-0084486, NH 11280	Turkey	2-4 Oct 1989	Wood	**Acc. N.**	-		
BPI 266214	Ukraine	Jul 1934	Wood frondose	**Acc. N.**	**Acc. N.**		-
BPI 1105985, holotype *Cystostereum heteromorphum*, A. Pilát 2474	Ukraine	Jul 1930	Wood	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
GB-0084457, paratype *Cystostereum heteromorphum*, A. Pilát 685	Ukraine	Jul 1930	Wood	**Acc. N.**	**Acc. N.**		-
GB-0084496	Ukraine	Aug 1934	*Ad ligna Fagi silvaticae*	**Acc. N.**	-		
TAAM 192927, U. Kalmeti 772	Ukraine	4 Sep1972	*Fagus*, on a fallen trunk	**Acc. N.**	-		
(CFMR)FP-133011, M.J. Larsen	USA	11 Jun 1971	*Alnus* sp.	**Acc. N.**	-		
GB-0084498, J.L. Lowe 8103	USA	30 Aug 1957	*Acer macrophyllum*	**Acc. N.**	-		

Table 2. ont.).

Species/Specimens[1], cultures	Country	Date	Substrate	GenBank/UNITE Accesion n.º			
				ITS	**28S**	**RPB1**	**RPB2**
I-B, *Crustomyces pini-canadensis* (Schwein.) Jülich							
GB-0084465, NH 6558	Canada	12 Aug 1982	Fallen log of *Populus*	**Acc. N.**	**Acc. N.**		-
BPI 280098	USA	14 Sep 1937	Abies balsamea	**Acc. N.**	-		
GB-0084461, Robert L. Gilbertson 9833	USA	24 Aug 1970	*Populus tremuloides*	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
(CFMR) HHB-7991, H.H. Burdsall 7991	USA	26 Jul 1974	*Thuja occidentalis*	**Acc. N.**	-		
NY 1930080, H.H. Burdsall Jr. 7957	USA	25 Jul 1974	*Quercus rubra*	**Acc. N.**	**Acc. N.**		-
I-C, *Crustomyces* sp.							
CLZhao 685	China			MG231495			
CLZhao 4176	China			MK268878			
CLZhao 6194	China			MK404325			
CLZhao 6241	China			MK404326			
CLZhao 6243	China			MK404327			
CLZhao 6275	China			MK404329			
SWFU006289	China			MK809430			
II-A, *Cystidiodontia laminifera* (Berk. & M.A. Curtis) Hjortstam							
GB-0084732, L. Ryvarden 676	Argentina	1-5 Mar 1982	Deciduous wood	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
ICN 169015, J.M. Baltazar 1771	Brazil	27 Mar 2010		**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
ICN 169023, J.M. Baltazar 1983	Brazil	11 Jun 2010		**Acc. N.**	**Acc. N.**	-	**Acc. N.**
ICN 169027, J.M. Baltazar 2012	Brazil	21 Jun 2010		**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
GB s.n., KHL13057	Costa Rica			EU118622	EU118622		
KM 263081, Elliott 305	Sant Vicent	1 Feb 1872	Rotten wood	**Acc. N.**	**Acc. N.**	-	
II-B, *Cystidiodontia isabellina* (Berk. & Broome) Hjortstam & Ryvarden							
BR 5020034184403, J. Rammeloo 4650	Burundi	20 Sep 1974	Dead branch	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
BR 5020034186421, J. Rammeloo 4664	Burundi	20 Sep 1974	*Caropa grandiflora*	**Acc. N.**	**Acc. N.**		**Acc. N.**

Table 2. (cont.).

Species/Specimens[1], cultures	Country	Date	Substrate	GenBank/UNITE Accesion n.º			
				ITS	**28S**	**RPB1**	**RPB2**
II-B, *Cystidiodontia isabellina* (Berk. & Broome) Hjortstam & Ryvarden							
BR 5020034228848, isotype *H. grandinioides,* J. Rammeloo 4651	Burundi	Sep 1974	Decidious wood	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
(O)F-902734, L. Ryvarden 11551	Malawi			**Acc. N.**	**Acc. N.**	-	**Acc. N.**
(O)F-902725, L. Ryvarden 10350	Tanzania			**Acc. N.**	-		
NY 520001, L. Ryvarden 5159	Tanzania	17 Jan 1971		**Acc. N.**	-		
III, *Parvodontia* cf. ***albocrustacea*** (Rick) Baltazar & Rajchenb.							
ARIZ-M-AN10442, R.L.Gilbertson 22129	Hawaii	18 Nov1998	On *Myrica indica*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
IV-A, *Cystostereum tuberculosum* Nogal, Telleria, M. Dueñas, M.P. Martín **comb. nov.**							
BR 5020133531597, P. Heineman 2918	Czech Slovakia	30 Aug 1960	Above trunk of *Abies*	**Acc. N.**	-	-	-
GB-0084733	Finland	23 Aug 1978	*Picea abies*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
GB-0084789	Finland	18 Sep 1962	Fallen trunk of *Picea abies*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
MA-Fungi 11382, 7165Tell.	Norway	23 Aug 1985	*Picea abies*	**Acc. N.**	**Acc. N.**	**Acc. N.**	-
(O)F-248296	Norway	11 Oct 2014		UDB037966			
(O)F-254007	Norway	29 Aug 2014		UDB037409			
(O)F-303900	Norway	28 Jun 2012		UDB036765			
GB-0084786, Hallenberg & Larsson 707	Poland	16 Sep 1973	*Abies*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
GB-0084787, Hallenberg & Larsson 2600	Poland	15 Sep 1973	*Picea*	**Acc. N.**	**Acc. N.**	**Acc. N.**	-
GB-0084793, NH 9127	Romania	16 Oct 1985	*Picea*-log	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
TAAM 18408	Russia	20 Aug 1965	*Picea obovata* fallen trunk	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
TAAM 53168	Russia	21 Sep 1968	*Abies nordmanniana*	**Acc. N.**			
TAAM 58736	Russia	22 May 1975	*Abies nordmanniana*	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
TAAM 126341	Russia	8 Sep 1988	*Picea obovata* fallen trunk	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
F-014297	Sweden	17 Aug 1939	On bark and wood of a dry, fallen spruce-trunk	-	**Acc. N.**	-	**Acc. N.**
GB-0084738, Berif Eriksson	Sweden	10 Jul 1966	Fallen trunk of *Picea abies*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**

Table 2. (cont.).

Species/Specimens[1], cultures	**Country**	**Date**	**Substrate**	**GenBank/UNITE Accesion n.º**			
				ITS	**28S**	**RPB1**	**RPB2**
IV-A, *Cystostereum tuberculosum* Nogal, Telleria, M. Dueñas, M.P. Martín **comb. nov.**							
GB-0084766, Hjortstam & Strid	Sweden	16 Sep 1970	Fallen trunk of *Picea abies*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
KHL 12496 (GB)	Sweden			EU118623			
TUF111090	Sweden	3 Jul 2015		UDB032408			
O-86914	Sweden	15 Sep 1892		**Acc. N.**	**Acc. N.**	**Acc. N.**	-
TAAM 3933	Ukraine	17 Aug 1956	*Abies alba* fallen rotten trunk	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
TAAM 4116	Ukraine	23 Aug 1956	*Picea abies* fallen trunk	**Acc. N.**	-		
TAAM 4094	Ukraine	23 Aug 1956	*Picea abies* fallen trunk	**Acc. N.**	**Acc. N.**	**Acc. N.**	-
NFLI 2000-86/2/1				JQ358801			
IV-B, *Cystostereum effusum* Nogal, Telleria, M. Dueñas, M.P. Martín **comb. nov.**							
NY 1990991, G.W. Martin 5067	USA	22 Aug 1940	On aspen	**Acc. N.**	**Acc. N.**	**Acc. N.**	-
FH 00530129, type *Stereum pulverulentum* Peck, F.L. Harvey	USA	Oct 1892	*Betula papyracea*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
NY 1992048	USA	Aug 1907	*Fagus*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
GB-0084780, Robert L. Gilbertson 9637	USA	10 Aug 1970	*Betula papyrifera*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
NY 1781928, H.H. Burdsall Jr. 3470	USA	12 Sep 1969	*Fagus* sp.	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
FH 00607441	USA	18 Aug 1933	Hardwood log	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
NY 1992024	USA	Aug 1908	Bark of *Betula*	**Acc. N.**	-		
NY 1992004, C.T. Rogerson 1513	USA	05 Jun 1947	*Betula lutea*	**Acc. N.**	-		
NY 1992001	USA	16 Sep 1978	Trunk of living apple tree	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
NY 1992023	USA	12 Jun 1915	Old beech log	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
NY 1991000	USA	7-16 Aug 1948		**Acc. N.**			
NY 1992173	USA	20 Jun 1940		**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
FH 00530089	USA	11 May 1902	Bark wood of *Acer*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
NY 1992020	USA	24-27 Oct 1916	Standing birch trunk	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
NY 1992169	USA	25 Oct 1936	*Betula lutea*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
NY 1992046	Italy	Aug 1893	*Ad radices Abietis*	**Acc. N.**	**Acc. N.**		

Table 2. (cont.).

Species/Specimens[1], cultures	**Country**	**Date**	**Substrate**	**GenBank/UNITE Accesion n.º**			
				ITS	**28S**	**RPB1**	**RPB2**
IV-C, *Cystostereum australe* Nakasone							
(CFMR)L-12913-Sp, paratype, J.L. Lowe 12913	Costa Rica	27 Jul 1963	Bark of dead hardwood	**Acc. N.**	**Acc. N.**	-	
NY 776088, paratype *Stereum saxitas*	Jamaica	Jun 1906		**Acc. N.**	-		
(CFMR)HHB-6648, paratype, H.H. Brurdsall Jr. 6648	USA	18 Jul 1972	*Ulmus* sp.	**Acc. N.**	-	-	**Acc. N.**
(CFMR)FP-105668, holotype	USA	15 Apr 1961	Hardwood log	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
IV-D, *Cystostereum kenyense* Hjortstam							
O-F-450365, holotype, L. Ryvarden 9841	Kenya	2-3 Feb 1973		**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
IV-E, *Cystostereum sirmaurense*							
BJFC sn, 8952	India		*Cedrus deodara*	MH510035			
PUN 6606	India	20 Aug 2010	*Myrica esculenta*	KP715571			
TU113199, Sanjeev Sanyal	India	2 Sep 2011	*Myrica esculenta*	**Acc. N.**	**Acc. N.**	**Acc. N.**	**Acc. N.**
IV-F, *Cystostereum murrayi* (Berk & M.A. Curtis) Pouzar							
K(M)264815, type, C. Wright 269	Cuba			**Acc. N.**	-	**Acc. N.**	
V-A, (*Cystostereum murrayi*) – *Incertae sedis*							
NY 1990977	Jamaica	20 Oct/24 Nov 1902		**Acc. N.**	**Acc. N.**		-
NY 1990979	Jamaica			**Acc. N.**	**Acc. N.**		-
NY 1990981	Cuba	7-12 Mar 1905	Dead wood	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
NY 1990982	Cuba	19-20 Mar 1905	Dead wood	**Acc. N.**	**Acc. N.**		-
NY 1990983	Cuba	21 Mar 1905	Dead wood	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
NY 1992016	Puerto Rico	30 Apr 1975	Hardwood limb	**Acc. N.**	**Acc. N.**	-	**Acc. N.**

Table 2. (cont.).

Species/Specimens[1], cultures	Country	Date	Substrate	GenBank/UNITE Accesion n.º			
				ITS	**28S**	**RPB1**	**RPB2**
V-B (*Cystostereum stratosum*) Hallenberg **– *Incertae sedis***							
GB-0156903, holotype, NH 1925	Iran	17 Jul 1976	Fallen log	**Acc. N.**	**Acc. N.**	-	**Acc. N.**
Chondrostereum purpureum (Pers.) Pouzar							
CBS367.58			*Prunus domestica*	MH857815			
RGM2167	Chile			MK788300			
CHO3881, RGM2166	Chile	2015	*Salix babylonica*	MH270445			
CHO3902, RGM2181	Chile	2013	*Pistacia vera*	MH270449			
TUF118793	Estonia			UDB019577			
CBS350.53	France			MH857241			
Champ-111	France			KX449515			
PDD 91629	New Zealand			GQ411519			
O-F-75179, MY1-0619	Norway			UDB035815			
GB/EL59-97	USA				AY586644		
HHB-13334-Sp	USA: Alaska						AY218477
Chondrostereum sp.							
1013-SAB SA4 7	Brazil		*Diphylla ecaudata*	MT820373			
Crustomyces expallens (Bres.) Hjortstam							
HCC-2	Russia				MK277899		
Gloeostereum incarnatum S. Ito & S. Imai							
Strain 3332				AF141637			
HHC-3	Russia				MK278092		
KUC20131022-28	South Korea			KJ668540			
BCC 41461	Thailand		Unidentified wood	KY614001			

Table 2. (cont.).

Species/Specimens[1], cultures	Country	Date	Substrate	GenBank/UNITE Accesion n.º			
				ITS	**28S**	**RPB1**	**RPB2**
Gloeostereum cimrii							
CBS 145006				MT023735			
Dendrothele griseocana Boidin, Lanq. & Gilles							
LY 4941, CBS 340.66	France	4 Oct 1964	*Ulmus* sp.	MH858816	MH870455		
Duportella renispora Boidin, Lanq. & Gilles							
LY 12699, CBS 733.91, type	Reunion	2 Apr 1987	Branch	MH862319	MH873991		

[1] Abreviatures IS, JFL, KHL, MD, MPM, NH, Tell. Are referred to Isabel Salcedo, Javier Fernández-López, Karl-Henrik Larsson, Margarita Dueñas, María P. Martín, Nils Hallenberg and María T. Telleria, respectively.

Barcoding analysis

In order to delimit possible species under *Cystostereum murrayi,* a reduced ITS matrix was generated with the length of the *C. murrayi* type sequence. A barcoding approach was used calculating Kimura-2-Parameter (K2P) pairwise distances (Kimura 1980) as indicated in Schoch et al. (2012). Distances were calculated using PAUP 4.0b10.

Taxonomic treatment

The nomenclature was treated in agreement with the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2018). Since this Ph-D thesis has no ISSN or ISBN, the nomenclatural novelties included cannot be considered as validly published (Art. 30.8). They will be validly published after acceptance and publication in the corresponding scientific journal.

The description of *Crustomyces*, *Cystidiodontia* and *Cystostereum* genera were included with the following information: current name, MycoBank number, protologue, description based on material studied, information on the type species, its protologue and mycobank number; as well as some notes. For species studied that were previously included in *Cystostereum*, but do not belong to this genus, the current name, protologue, MycoBank number, basionym and synonyms are included.

Each entry for a *Cystostereum* species includes the current name, MycoBank number, figures, synonymy and description based on specimens analyzed, ecology and habitat, known distribution, material studied and notes. The information recorded in material studied is the transcription of the labels and includes country, locality, coordinates, altitude, substrate, date, collector, herbarium acronym (with number when indicated), as well as if they correspond to types. The asterisk (*) after the transcription of each label means that molecular data were obtained.

At the end of the taxonomic part the unresolved taxa are included as *incertae sedis*.

RESULTS

DNA isolation, PCR amplifications, Sequencing and Alignments

Genomic DNA was isolated from 238 dried specimens requested from 16 international herbaria.

First, preliminary ITS amplification attempts were performed with MA-Fungi and NY specimens through direct PCR using primer pairs ITS5/ITS4 or ITS5/ITS4B. Among the 35

MA-Fungi specimens, amplimers were obtained for 13; however, amplimers from the 58 NY specimens were not visualized in the gels. Since direct attempts in most cases failed, nested-PCR was performed; however, this strategy failed to obtain amplimers from both MA-Fungi and NY specimens. The next strategy used was PCR by parts. From the 22 remaining MA-Fungi specimens, only two amplimers were obtained, and of the 58 NY specimens amplimers were visualized for 21.

Based on these preliminary results, with the rest of the specimens, PCR by parts was selected as the default protocol for ITS processing of DNA isolations; that is why this strategy represents the major percentage of attempts (Table 3). However, when no amplimers were visualized after PCR by parts, direct and/or nested-PCR were also performed. An example of amplification carried out with some GB specimens, using direct and PCR by parts strategies are shown in Fig. 1. Also, as shown in Table 3, ITS sequences were obtained using general primers, except for *Thelephora murrayi* type (K(M)264815) for which the designed ITS33Cyst combined with ITS2 allowed the amplification of ITS1 plus the initial part of 5.8S region.

From 1455 individual PCR attempts of ITS (Table 3), only *ca*. 39% were visualized. After sequencing, 50.64 % of purified amplimers corresponded to target fungi; the rest of the sequences were contaminations or sequences of low quality (e.g. double peaks, low signal in the electropherograms).

Supplementary Table 1 includes the 117 specimens for which ITS sequence was not obtained, as well as five collections for which BLAST searches found similarity with other corticioid fungi not related to Cystostereaceae (*Haploporus alabamae*, *Phlebiopsis* sp., and three *Junghuhnia crustacea*). The 113 ITS sequences included in the subsequent molecular analyses are indicated in Table 2.

As mentioned in Materials and Methods, LSU amplifications were carried out from the 113 specimens with ITS sequence. In these cases, amplimers were mainly obtained by direct PCR with general primer pair LR0R/LR5r (Table 3); although, usually with a yield of less than 20 ng/µl (Fig. 2A, lines 2-8), and sequences recovered were mainly of low quality or corresponded to contaminant fungi. After seminested-PCR no amplimers were visualized; however, from the recalcitrant samples, 43.55% were successfully amplified with the designed primer LR0R-Cyst combined with LR5r (Fig. 2B), of which 98% corresponded to the target fungi by BLAST searches.

Table 3. Total of amplification attempts performed in this study from 238 specimens. The numbers and percentages of successful amplifications and PCR strategies (direct, nested or semi-nested, by parts) are indicated to each marker. *Amplimer visualized means that bands were observed after gel electrophoresis.

Strategy	**Primer pair**	**PCR attempts**	**Amplimer visualized***	**% Amplimer visualized**
ITS nrDNA				
Direct-PCR	ITS5/ITS4 or ITS5/ITS4B	191	13	6.80
Nested-PCR	1st PCR: ITS1F/ITS4B 2nd PCR: ITS5/ITS4	134	0	0
PCR by parts	ITS5/ITS2	619	310	50.08
	ITS3/ITS4 or ITS3/ITS4B	507	241	47.53
PCR by parts, designed primer	ITS33Cyst/ITS2	4	1	25.00
	Total	1455	565	38.83
LSU nrDNA				
Direct-PCR	LR0R/LR5r	428	227	53.04
Semi-nested PCR	1st PCR: LR0R/LR7r 2nd PCR: LR0R/LR5r	40	0	0
Direct-PCR, designed primer	LR0R-Cyst/LR5r	124	54	43.55
	Total	592	281	45.77
***rpb*2**				
Nested-PCR	1st PCR: fRPB2-5F/bRPB2-7.1R 2nd PCR: bRPB2-6F/bRPB2-7R.2	334	104	31.13
Nested-PCR, designed primer	1st PCR: fRPB2-5F/bRPB2-7.1R 2nd PCR: fRPB2-5F/bRPB2-Cyst1 or 2nd PCR: fRPB2-5F/bRPB2-Cyst2	191	51	26.70
	Total	525	155	29.52
***rpb*1**				
Direct-PCR	RPB1-Af/RPB1-Cr	75	20	26.67
Semi-nested PCR1	1st PCR: RPB1-Af/RPB1- Cr 2nd PCR: RPB1-2f/RPB1-Cr	24	5	20.83
Semi-nested PCR2	1st PCR: RPB1-Af/RPB1-Cr 2nd PCR: RPB1-2.1f/RPB1-Cr	20	0	0
Semi-nested PCR3	1st PCR: RPB1-Af/RPB1-Cr 2nd PCR: RPB1-2.2f/RPB1-Cr	20	0	0
PCR by parts, designed primers	RPB1-Cyst3/RPB1-Cyst4	24	24	100
	RPB1-Cyst5/RPB1-Cyst6	28	24	85.71
	Total	183	65	35.52

For *rpb*2, only a nested strategy was performed with different primer pairs in the second amplification (Table 3). Around 31% of amplimers were obtained using bRPB2-6F/bRPB2-7R.2 in the second amplification with a concentration ≥ 20 ng/µl (Fig. 3A); but sequences mainly corresponded to *Malassezia restricta* (99−100% similarity in BLAST searches). A similar percentage of amplification (26.70%) was achieved with forward designed primers in combination with the general reverse bRPB2-7R.2 (Fig. 3B, C); and 96% of high quality sequences corresponded, after BLAST searches, to target fungi.

In the case of *rpb*1, multiple bands were usually observed in electrophoresis gels, both by direct PCR with RPB1-Af/RPB1-Cr (Fig. 4A) or seminested-PCR using RPB1-2f/RPB1-Cr in the second amplification (Fig. 4B). Around 90% of attempys carried out by parts with designed primers were successfully amplified (Table 3), resulting in amplimers with ≥20 ng/µl (Fig. 4C, D), and nearly 85% corresponded to high quality sequences with BLAST search matching target fungi.

Fig. 1. Visualization on 2% Agarose gel of PCR products from amplification of ITS nrDNA. **A)** Direct PCR with primer pair ITS5/ITS4B. **B)** Amplification of ITS1 + initial 5.8S region with ITS5/ITS2. **C)** Amplification of final 5.8S + ITS2 region with ITS3/ITS4B. Lines 1-12: GB specimens from GB-0084473 to GB-0084484. Line 13 in A, B, C: Negative control, M: molecular marker 1 Kb Plus.

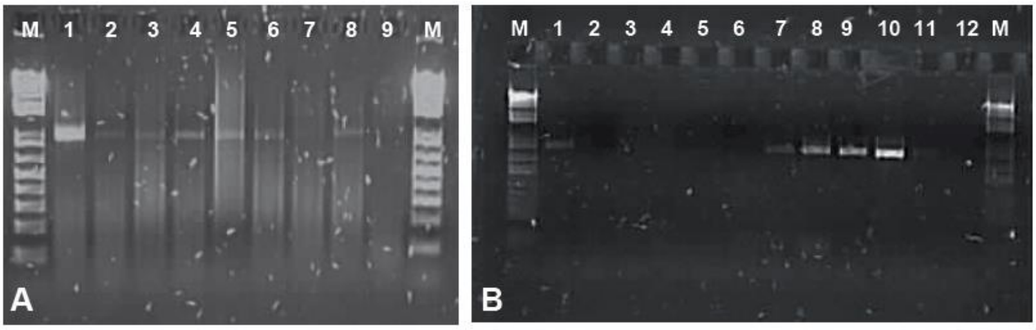


Fig. 2. Visualization on 2% Agarose gel of PCR products from amplification of LSU nrDNA. **A)** Direct PCR with LR0R/LR5r; lines 1-8: MA-Fungi 79281, NY 1992022, NY 1992024, NY 1992046, TAAM 126341, TAAM53168, TAAM 132423 and TAAM 134106, respectively. **B)** Direct PCR with the designed LR0R-Cyst combined with LR5r; lines 1 and 7-10 correspond to NY 1998708, NY 776088, GB-0084661, BR 5020034184403, (O)F-902734, respectively. Line 9 in A and line 12 in B: Negative control, M: molecular marker 1 Kb Plus.

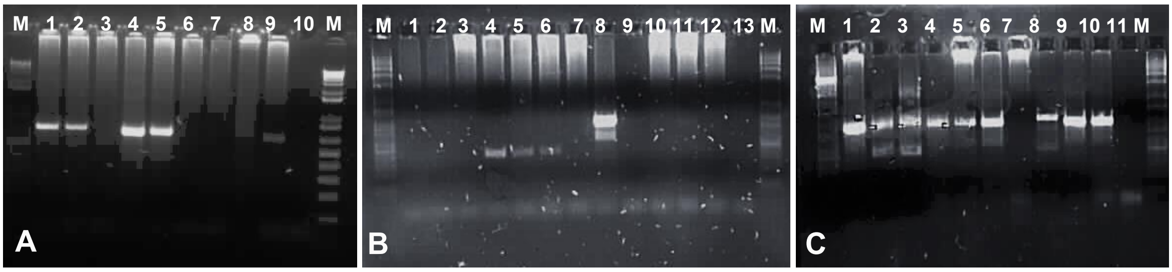


Fig. 3. Visualization on 2% Agarose gel of PCR products from second amplification of the nested strategy conducted for *rpb*2. **A)** General primer pair bRPB2-6F/bRPB2-7R.2; lines 1, 2, 4, 5 and 9 correpond to TAAM 8226, TAAM 132570, GB-0084478, GB-0084479, GB-0084465, respectively. **B)** Primer pair with designed fRPB2-Cyst1 and general bRPB2-7R.2; lines 4-6 and 8 correspond to TAAM 8226, TAAM 132570, TAAM 3933, GB-0084465, respectively. **C)** Primer pair with designed fRPB2-Cyst2 and general bRPB2-7R.2; lines 1-6 and 8-10 correspond to NY 1990977, NY 1990979, NY 1990982, NY 1990983, GB-0084461, GB-0084465, FH 00607441, FH 00530129, FH 00530089, respectively. Line 10 in A, line 13 in B and line 11 in C: Negative control, M: molecular marker 1 Kb Plus.

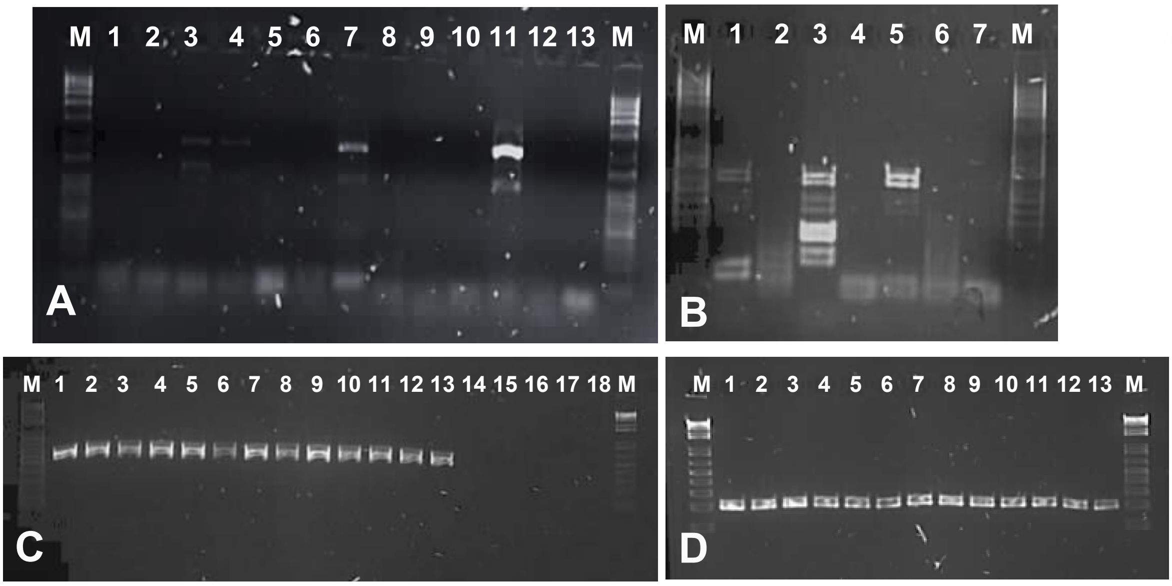
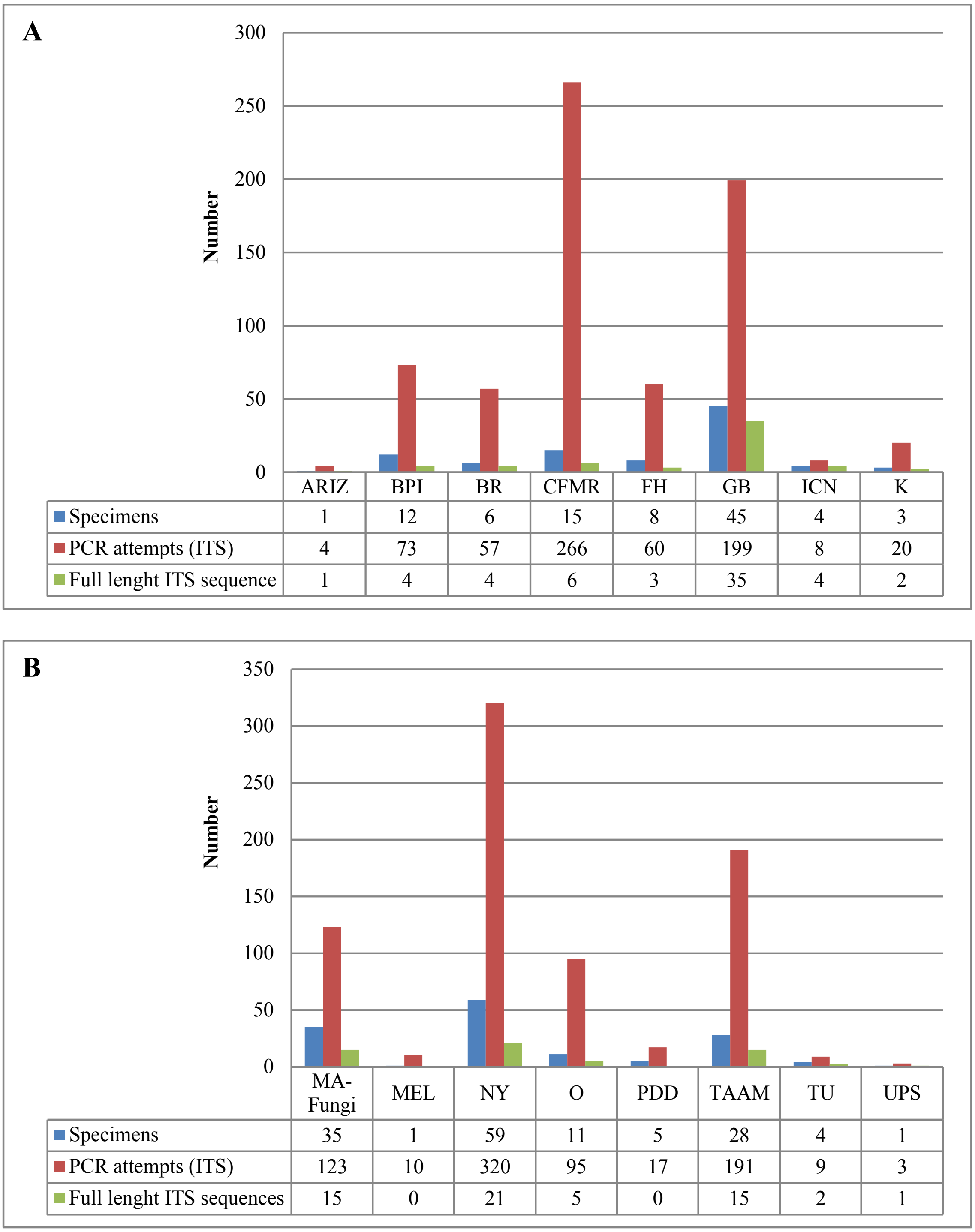


Fig. 4. Visualization on 2% Agarose gel of PCR products of *rpb*1. **A)** Direct PCR with general primer pair RPB1-Af/RPB1-Cr; lines 3, 4, 7 and 11 correpond to GB-0084481, GB-0084487, GB-0084732, GB-0156903, respectively. **B)** Second amplification of semi-nested PCR using RPB1-2f/RPB1-Cr; lines 1, 3 and 5 correspond to NY 1781928, NY 1990981, NY 1992020, respectively. **C)** Direct PCR with designed primer pair RPB1-Cyst3/RPB1-Cyst4. **D)** Direct PCR with designed primer pair RPB1-Cyst5/RPB1-Cyst6. Lines 1-13 in C and D correspond to NY 1990991, NY 1992020, NY 1992023, NY 1992048, NY 1992169, NY 1992173, TAAM 126341, TAAM 18408, TAAM 58736, F- 014297, FH 00607441, FH 00530089, O-86914, respectively. Lines 13 in A, 7 in B and 18 in C: Negative control, M: molecular marker 1 Kb Plus.

Related to the age of specimens, some of the oldest ones were successfully sequenced (Table 2), although they proved difficult to amplify, requiring multiple attempts with different PCR conditions (e.g. different DNA concentration, primer pair combinations, increasing or decreasing annealing temperatures, and so on). For example, from *Stereum pulverulentum* type (FH 00530129) and *Stereum tuberculosum* O-86914, both collected in 1892, sequences of the four markers were obtained (Table 2); as well as ITS and *rpb*1 sequences of *Thelephora murrayi* type specimen, collected around 1869 (Table 2). However, for some type specimens, such as those of *Hydnum laminiferum* (=*Cystidiodontia artocreas*) and *Stereum saxitas* (≡*Cystostereum saxitas*), sequences were not gotten, even after several attempts (Supplementary Table 1).

On the other hand, as shown in Fig. 5, success in amplification and sequencing was variable depending on herbaria. In some of them, several attempts were carried out to amplify ITS, such as with specimens located in NY, CFMR or GB, which represented some of the largest sampling included in this study (Table 2 and Supplementary Table 1). Moreover, from PPD and MEL no sequences of the target fungi were obtained.

Finally, a total of 286 sequences were included in phylogenetic analyses: 113 ITS sequences, 78 LSU, 58 for *rpb*2 and 37 for *rpb*1 (Table 2). The alignment matrices, including homologous sequences retrieved from EMBL/GenBank/DDBJ and UNITE databases, contained 147 ITS, 85 LSU and 59 *rpb*2; no *rpb*1 sequences from Cystostereaceae nor Cyphellaceae were available in any of these public repositories. The matrix length (number of positions including gaps) for each marker was 764, 914, 631 and 648, respectively. The sequences retrieved from EMBL/GenBank/DDBJ are also included in Table 2.



	ARIZ	BPI	BR	CFMR	FH	GB	ICN	K
🟦 Specimens	1	12	6	15	8	45	4	3
🟥 PCR attempts (ITS)	4	73	57	266	60	199	8	20
🟩 Full lenght ITS sequence	1	4	4	6	3	35	4	2

	MA-Fungi	MEL	NY	O	PDD	TAAM	TU	UPS
🟦 Specimens	35	1	59	11	5	28	4	1
🟥 PCR attempts (ITS)	123	10	320	95	17	191	9	3
🟩 Full lenght ITS sequences	15	0	21	5	0	15	2	1

Fig. 5. The number of requested specimens, PCR attempts of ITS barcoding region and full length ITS sequences obtained by herbaria, including the five sequences that were related to other corticioid fungi by BLAST searches. **A.** Herbaria ARIZ, BPI, BR, CFMR, FH, GB, ICN and K. **B.** Herbaria MA-Fungi, MEL, NY, O, PDD, TAAM, TU and UPS.

Phylogenetic analyses

The MP, ML and BI analyses conducted for LSU, ITS, *rpb*2 and *rpb*1 resulted in similar topologies (Figs. 6-9). Statistical values of MP analyses are summarized in Table 4. The nucleotide substitution models selected by jModelTest were TIM2 + I +G for ITS and LSU, TIM2ef + I + G for *rpb*2, and TIM2 + G for *rpb*1.

Table 4. Results of Maximum Parsimony analysis for each marker.

Locus	Total characters	Constant characters	Informative characters	Tree length	Consistency Index (CI)	Retention Index (RC)
ITS	764	287	297	1063	0.675	0.636
LSU	914	648	131	445	0.703	0.652
*rpb*2	631	357	230	646	0.621	0.579
*rpb*1	648	398	209	417	0.794	0.748

LSU nrDNA

Including *Dendrothele griseo-cana* and *Duportella renispora* as outgroup, all the sequences are distributed in two major clades, one highly supported (MPbs = 99%, MLbs = 99%, pp = 1.0) indicated as Cystostereaceae family in Fig. 6, and a second one (clade V) also well supported (MPbs = 87%, MLbs = 83%, pp = 0.99).

The clades and singletons are described in numerical order.

Clade I (MPbs = 94%, MLbs = 95%, pp = 1.0) grouped sequences from specimens under *Crustomyces heteromorphus* from Italy, and *Crustomyces subabruptus* from Poland and Turkey; *Cystostereum heteromorphum* holotype from Ukraine, and one paratype from Iran, *Cystostereum pini-canadense* from Canada and USA, and *Cystostereum subabruptum* from France, Iran and Turkey; one under *Peniophora piceina* from USA; and *Odontia subabrupta* paratype from France. Moreover, five sequences of specimens collected from Chile were grouped in this clade.

Since *Odontia subabrupta* is the type of the genus *Crustomyces*, we consider that clade I encompasses the genus *Crustomyces*.

Clade II (MPbs = 59%, MLbs = 56%, pp = 0.9) is separated into two subclades. The well-supported subclade II-A (MPbs = 88%, MLbs = 91%, pp = 1.0) grouped sequences obtained from

South American specimens under *Cystidiodontia artocreas* from Argentina, *Cystidiodontia laminifera* from Brazil and Costa Rica, and *Hydnum artocreas* from Saint Vincent island. The highly supported subclade II-B (MPbs = 96%, MLbs = 98%, pp = 1.0) includes sequences obtained from four African specimens, a specimen under *Cystidiodontia isabelina* from Malawi, two specimens from Burundi named as *Cystostereum* sp. and *Cystostereum artocreas*, and the sequence of *Hypochnicium grandinioides* Ryvarden isotype [originally described from Burundi by Ryvarden (1978), and later synonymized with *Cystidiodontia artocreas* by Hjortstam (1983)].

Cystidiodontia artocreas (≡*Hydnum artocreas*) is the type species of the genus *Cystidiodontia.* Hjortstam (1990) synonymized *C. artocreas* to *Cystidiodontia laminifera* (current name accepted in MycoBank). Since the original descriptions of both taxa were based on specimens from South America, and we have included in our analyses collections from Argentina, Brazil and Costa Rica under these names, we consider that clade II encloses the genus *Cystidiodontia.*

Singleton III is related to clade II with high support (MPbs = 80%, MLbs = 81%, pp = 1.0). It corresponds to a sequence of a Hawaiian specimen under *Crustomyces heteromorphus* (R.L. Gilbertson 22129). According to our results, this collection cannot belong to the genus *Crustomyces*, since this genus corresponds to clade I. [Based on our preliminary morphological studies, R.L. Gilbertson 22129 specimen could belong to the genus *Parvodontia*].

The type species of the genus *Cystostereum* is *Cystostereum murrayi*, which was originally described from Cuba as *Thelephora murrayi* by Berkeley & Curtis (1869). Another synonym of this species is *Stereum murrayi* (Burt 1920). Clade IV included many sequences under *Cystostereum murrayi* or *Stereum murrayi* from different countries, but none from Cuba, the location of the type species. After many attempts, it was not possible to obtain a LSU sequence from the type collection of *Thelephora murrayi* K(M)264815 (Table 2); thus, we cannot confirm that clade IV corresponds to *Cystostereum*.

As indicated above, **Clade V** appears as the sister group to the Cystostereaceae and is separated into two subclades. The strongly supported subclade V-A (MPbs = 100%, MLbs = 100%, pp = 1.0) grouped sequences of specimens from Cuba, Jamaica and Puerto Rico under *Stereum murrayi*, *Cystostereum* sp. and *Stereum tuberculosum* (according to Burt 1920 the last species is synonym of *Stereum murrayi*). The subclade V-B (MPbs = 50%, MLbs = 52%, pp = 0.96) includes three sequences under *Gloeostereum incarnatum* (MK278092), *Chondrostereum purpureum* (AY586644) and *Crustomyces expallens* (MK277899) retrieved from Genbank.

Since sequences of three specimens under *Stereum murrayi* from Cuba appear in subclade V-A, this subclade could correspond to *Cystostereum*.

Clade IV (MPbs = 65%, MLbs = 76%, pp = 0.99) is separated into three highly supported subclades (IV-A, IV-B, IV-C) and two singletons (IV-D, IV-E). The subclade IV-A (MPbs = 98%, MLbs = 96%, pp = 1.0), includes 14 sequences from European specimens under *Cystostereum murrayi,* one under *Stereum murrayi*, and *Cystostereum stratosum* holotype from Iran. The subclade IV-B (MPbs = 100%, MLbs = 99%, pp = 1.0) grouped 11 sequences of North American specimens under *Cystostereum murrayi, Stereum murrayi* and *Stereum tuberculosum,* as well as one sequence of an Italian specimen under *Stereum tuberculosum* and *Stereum pulverulentum* type from USA. The subclade IV-C (MP = 97%, ML = 98%, pp = 1.0) grouped two sequences of *Cystostereum australe,* the holotype from USA and one paratype from Costa Rica. This subclade is sister to the singleton IV-D, the *Cystostereum kenyense* holotype from Kenya. This relationship between clades IV-C and IV-D is well supported (MPbs = 78%, MLbs = 83%, pp = 1.0). Finally, the singleton IV-E that appears as the most basal sequence in clade IV, corresponds to an Indian specimen under *Cystostereum murrayi*.

ITS nrDNA

The major clades and subclades recovered by LSU analyses (Fig. 6) were also resolved by ITS nrDNA analyses (Fig. 7), both trees show similar topologies. Among others, one the most important results is the ITS sequence of *Thelephora murrayi* type K(M)264815 which grouped with clade IV.

In numerical order, the clades (and singletons) are described indicating any differences with respect to LSU phylogeny.

Clade I (*Crustomyces*) is highly supported (MPbs = 95%, MLbs = 92%, pp = 1.0), and it is separated into three subclades that could correspond to three different species. The subclade I-A (MPbs = 83%, MLbs = 77%, pp = 1.0), with 49 sequences from 11 countries, could belong to *Crustomyces subabruptus* since it includes a paratype from France of *Odontia subabrupta*. Also, this subclade includes the holotype from Ukraine and two paratypes from Iran and Ukraine, sequences of *Cystostereum heteromorphum*, so that we propose this species as a synonym of *Crustomyces subabruptus.*

The subclade I–B (MPbs = 63%, MLbs = 66%, pp = 0.74) is formed by sequences from North America under *Cystostereum pini-canadense* and *Peniophora piceina*. Although the type of *Radulum pini-canadense* was not available, this subclade could belong to this species, because it was originally described from Mauch Chunk (Pennsylvania, USA).

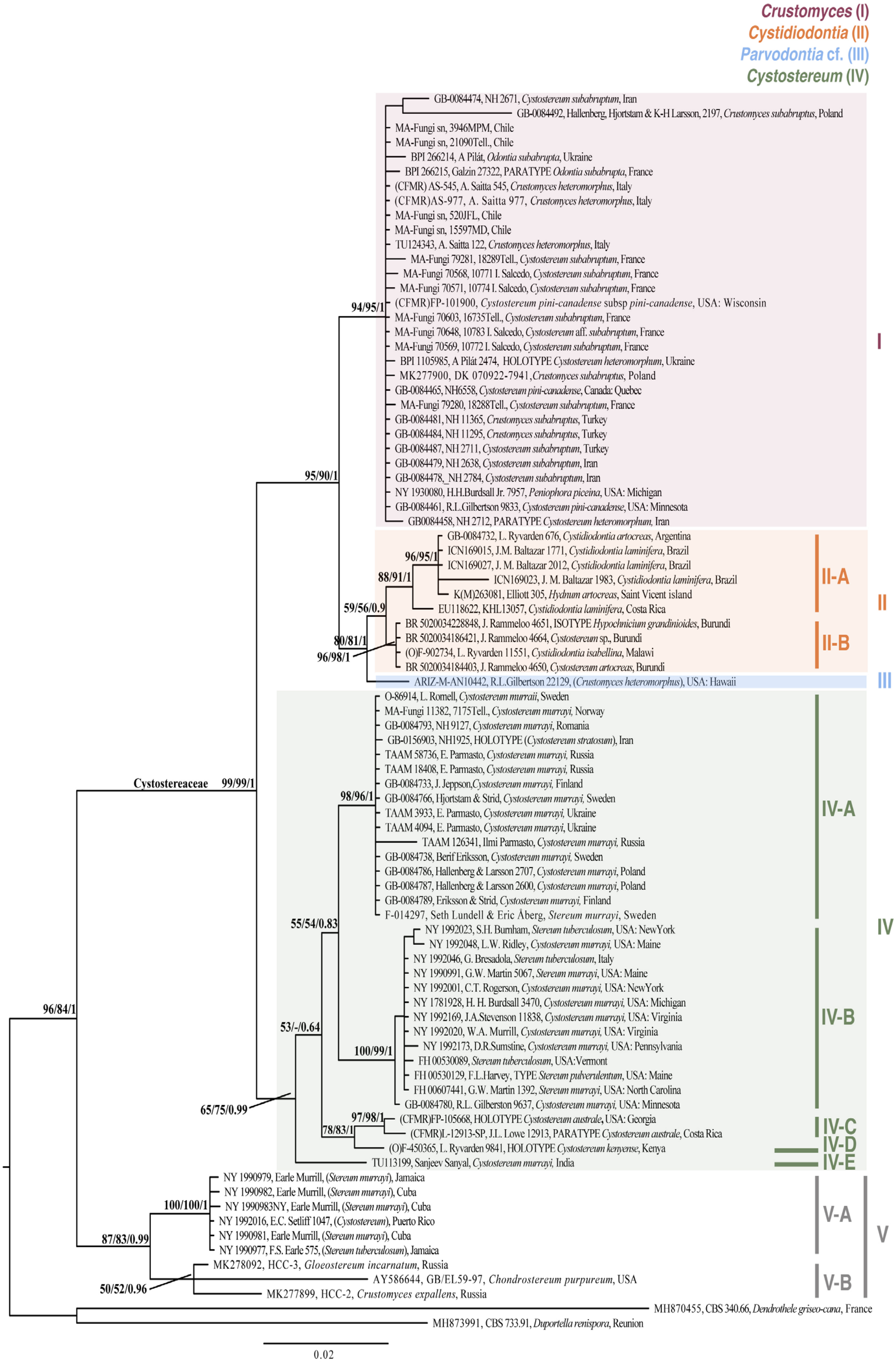


Fig. 6. Topology of LSU nrDNA obtained by Bayesian inference. One sequence of *Dendrothele griseo-cana* and *Duportella renispora* from Vu et al. (2019) were included as outgroup. The values of Parsimony bootstrap (MPbs), Maximum likelihood bootstrap (MLbs) and posterior probability (pp) are indicated above branches in this order.

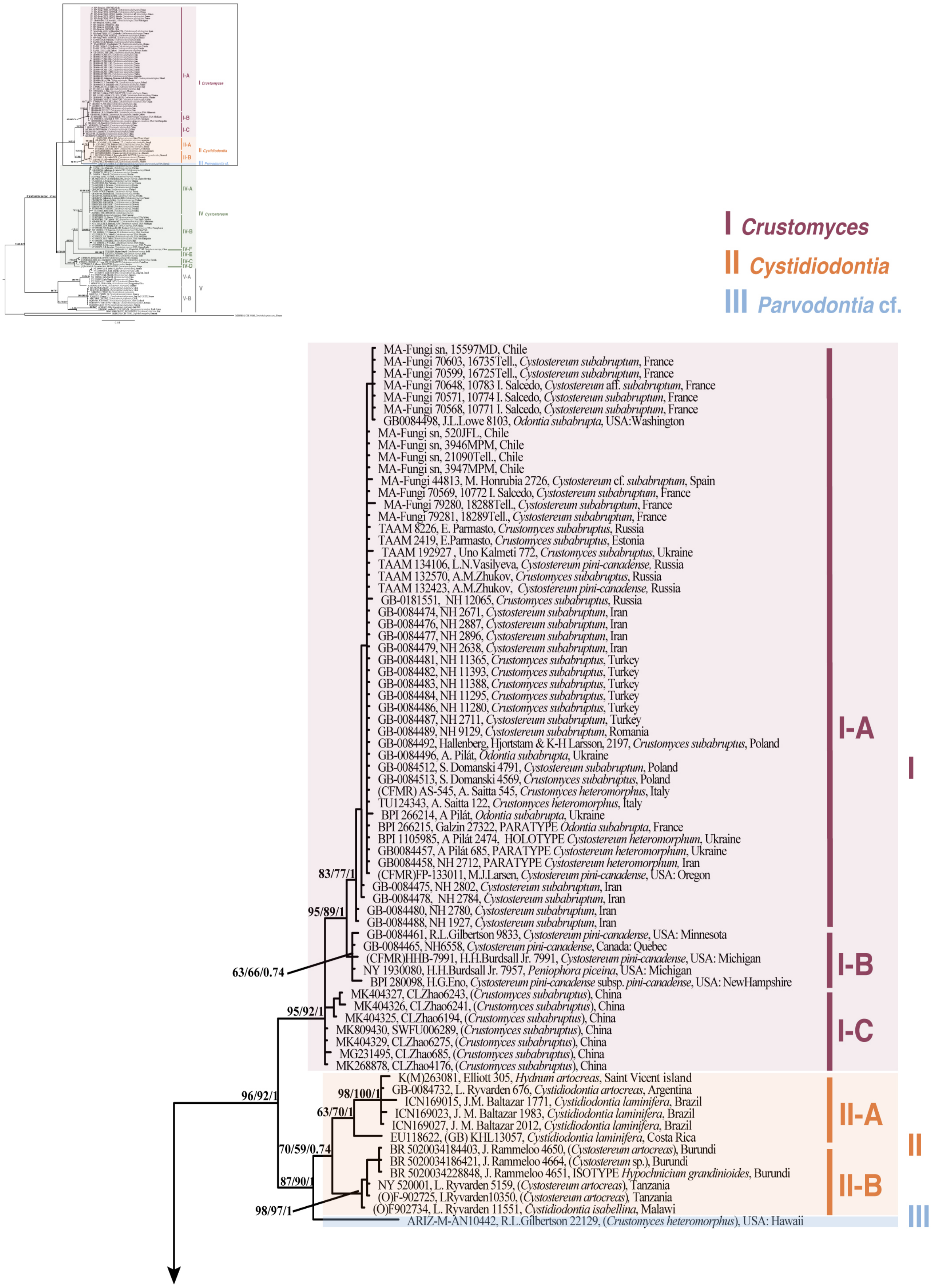


Fig. 7. Topology of ITS nrDNA obtained by Bayesian inference. One sequence of *Dendrothele griseo-cana* and *Duportella renispora* from Vu et al. (2019) were included as outgroup. The values of Parsimony bootstrap (MPbs), Maximum likelihood bootstrap (MLbs) and posterior probability (pp) are indicated above branches in this order.

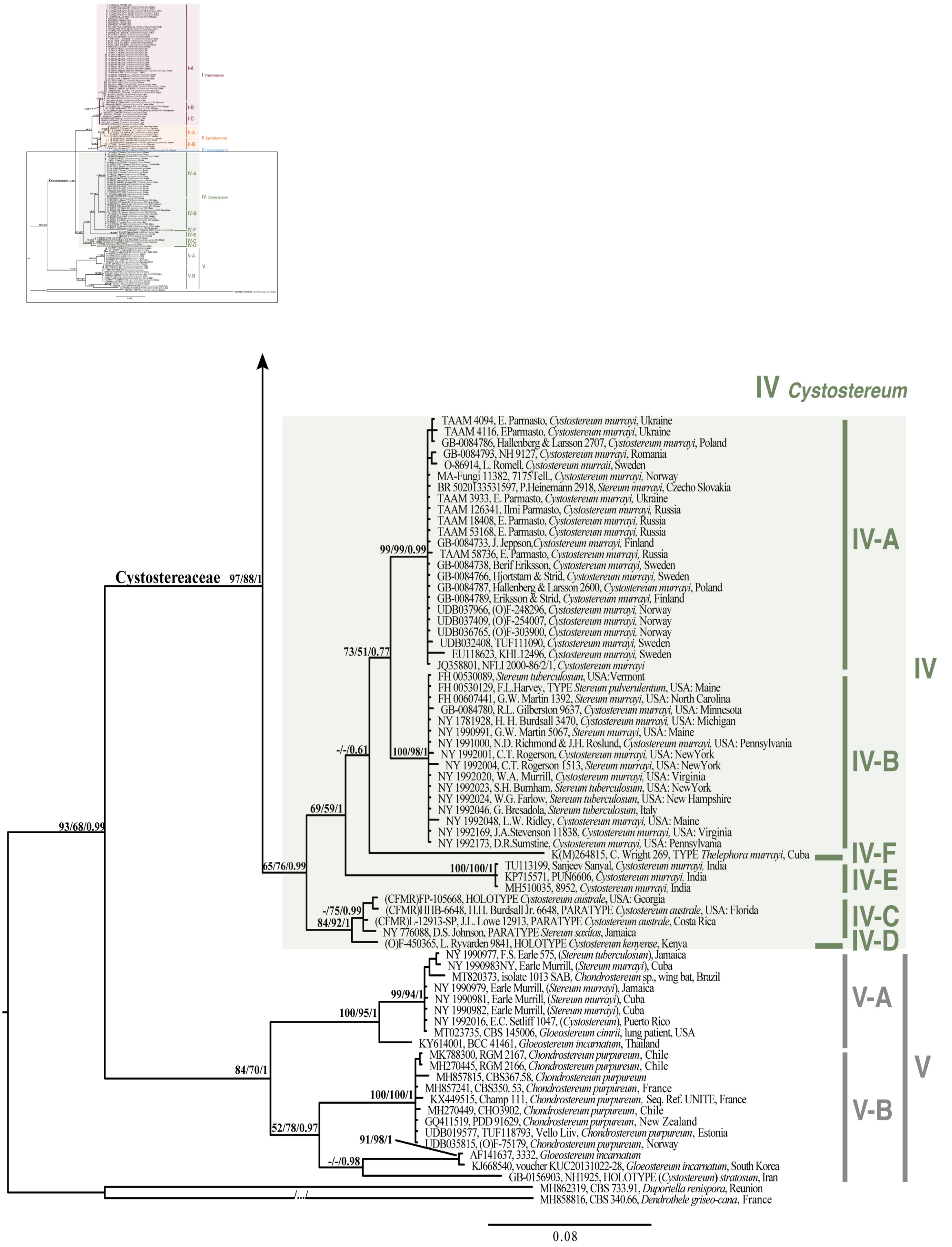


Fig. 7. (continued).

Finally, seven sequences from China under *Crustomyces subabruptus* retrieved from EMBL/GenBank/DDBJ databases are grouped in the subclade I-C; although, they were not resolved as a monophyletic group. According to the data available, these sequences were deposited by the same author, but are not linked to any published reference. These barcoding sequences are complete (ITS1 + 5.8S + ITS2), but with around 12-15 variable positions among them; this could be the explanation to this politomy. As indicated above, both in LSU and ITS phylogenies, *Crustomyces subabruptus* corresponds to subclade I-A, hence sequences from China are not correctly named.

Clade II (*Cystidiodontia*) and **Singleton III** are resolved as in the LSU analyses.

Clade IV (MPbs = 65%, MLbs = 76%, pp = 0.99), as already mentioned, includes the sequence of *Thelephora murrayi* K(M)264815 type (singleton IV-F), which supports our consideration that this clade encompasses the genus *Cystostereum*. With respect to LSU analyses few differences were detected, since subclades IV-A, IV-B, IV-C (with two more sequences, including the *Stereum saxitas* paratype from Jamaica) and IV-D were also recovered. *Cystostereum murrayi* from India corresponds to subclade IV-E (Fig. 7), along with two more Indian sequences retrieved from EMBL/GenBank/DDBJ (KP715571 and MH510035).

Since the sequence of *Thelephora murrayi* type appears as an independent singleton (IV-F), without clustering into subclades IV-A (European sequences), IV-B (North American sequences) or IV-E (Indian sequences), and based on morphological analyses, we consider that subclades IV-A, IV-B and IV-E should correspond to three independent taxa distinct from *Cystostereum murrayi*. In order to establish the limits among these three subclades and the singleton, a reduced ITS alignment was analyzed using a barcoding approach (see below).

Clade V (MPbs = 84%, MLbs = 70%, pp = 1.0) appears as the sister group to Cystostereaceae with high support (MPbs = 93%, MLbs = 68%, pp = 1.0), and also includes the subclades V-A and V-B, as recovered by the previous LSU analyses (Fig. 6). The subclade V-A includes a highly supported group (MPbs = 99%, MLbs = 94%, pp = 1.0) formed by six sequences of specimens from Cuba, Jamaica and Puerto Rico, as well as two sequences retrieved from EMBL/GenBank/DDBJ under *Chondrostereum* sp. (MT820373) and *Gloeostereum cimrii* (MT023735). The subclade V-B (MPbs = 52%, MLbs = 78%, pp = 0.97) includes nine sequences retrieved from EMBL/GenBank/DDBJ and UNITE under *Chondrostereum purpureum* forming a strongly supported cluster (MPbs = 100 %, MLbs = 100 %, pp = 1.0) that is the sister group of three sequences (MPbs < 50%, MLbs = < 50%, pp = 0.98), two under *Gloeostereum incarnatum* and the *Cystostereum stratosum* holotype.

Protein coding genes rpb2 and rpb1

Although with a lower number of sequences, the *rpb*2 analyses (Fig. 8) recovered the same number of clades as ITS and LSU markers; the phylogenetic position of *Cystostereum stratosum* holotype in clade IV-A concurs with LSU, but not with ITS.

In *rpb*1 analyses (Fig. 9), the *Thelephora murrayi* (K(M)264815) type from Cuba grouped in clade IV-A formed by European specimens, whereas in ITS phylogeny its sequence appears as a singleton.

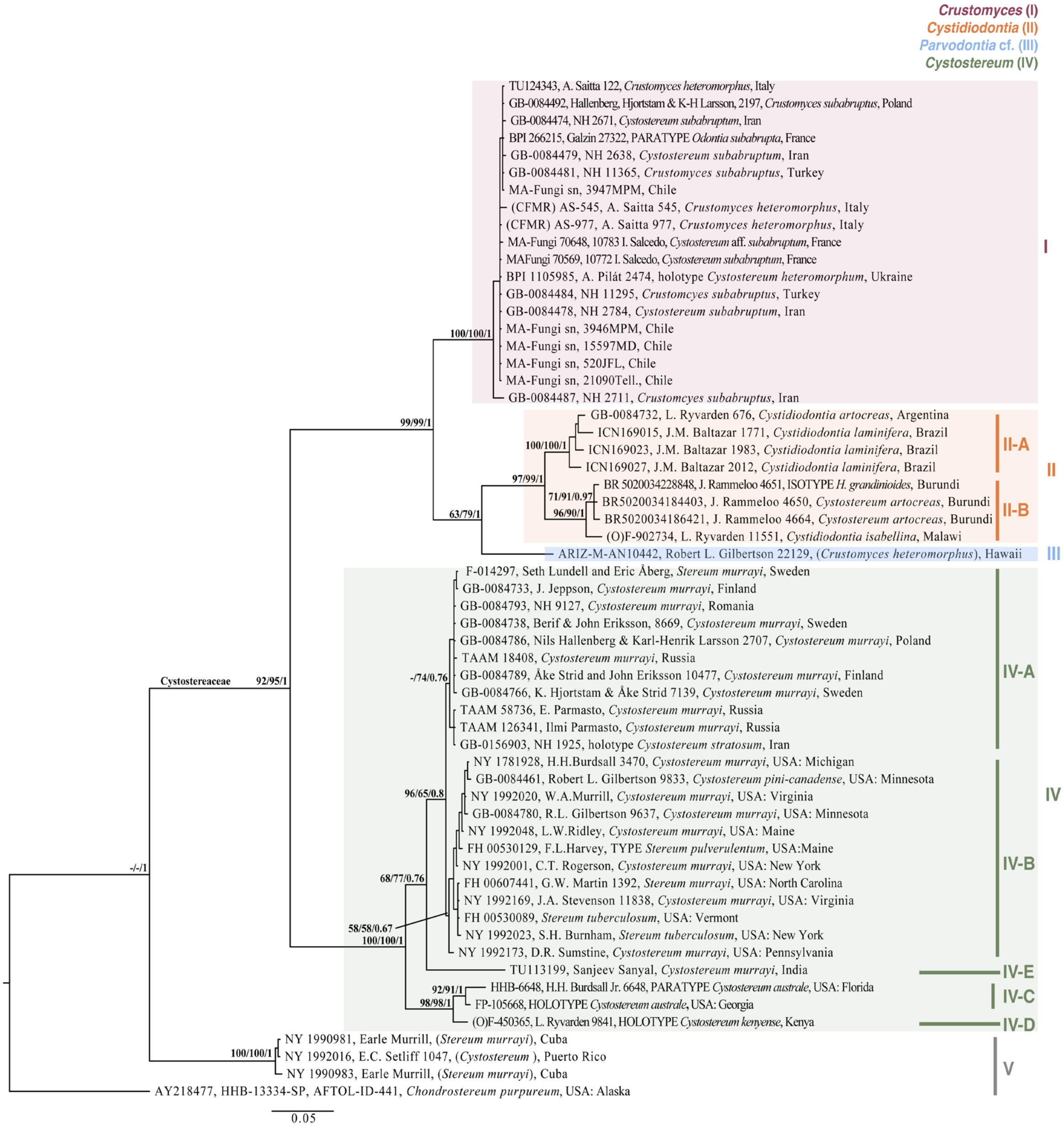


Fig. 8. Topology of *rpb*2 obtained by Bayesian inference. A sequence of *Chondrostereum purpureum* was included as outgroup. The values of Parsimony bootstrap (MPbs), Maximum likelihood bootstrap (MLbs) and posterior probability (pp) are indicated above branches in this order.

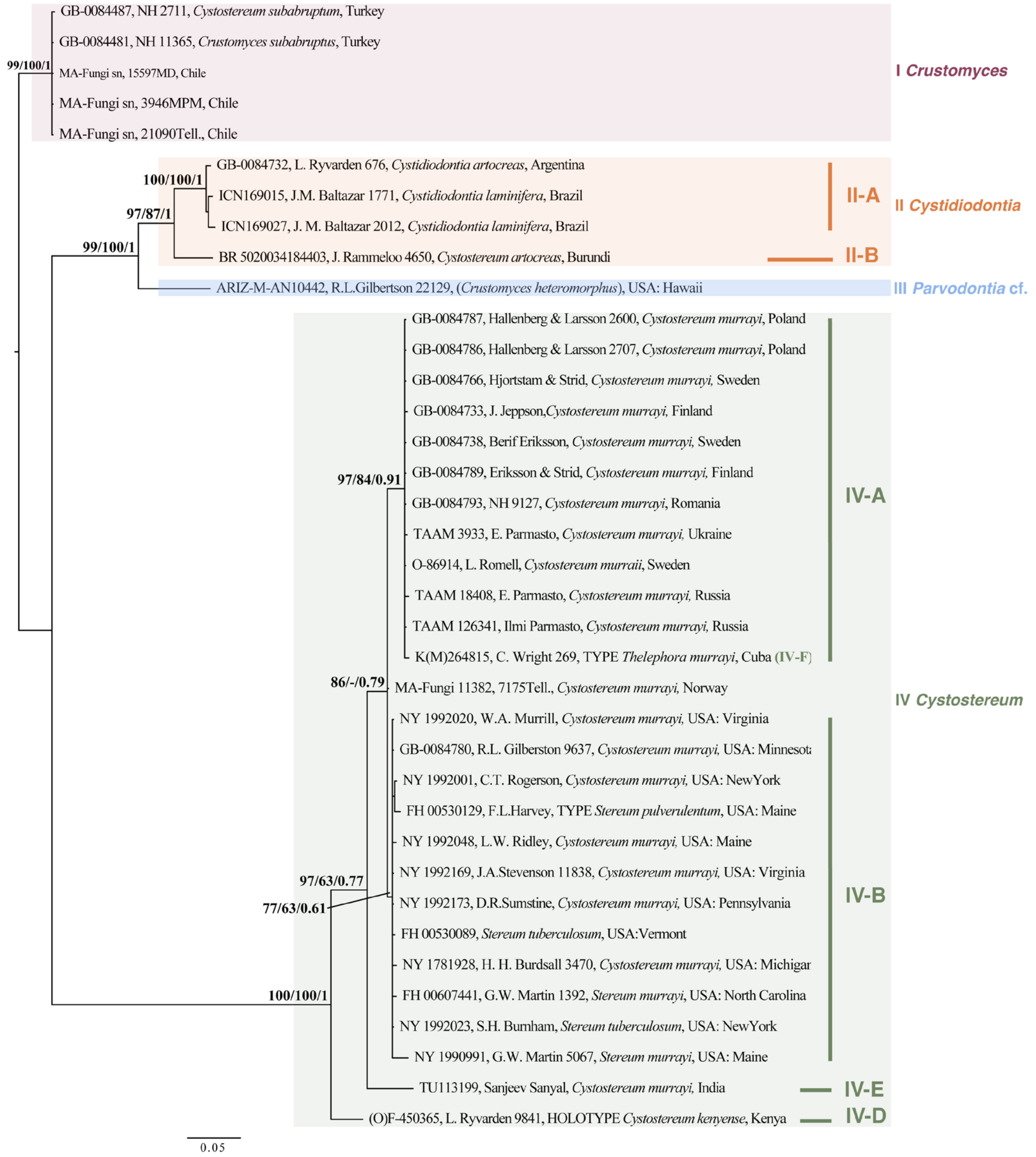


Fig. 9. Topology of *rpb*1 obtained by Bayesian inference. The values of Parsimony bootstrap (MPbs), Maximum likelihood bootstrap (MLbs) and posterior probability (pp) are indicated above branches in this order. Clades I, II and III, resolved by the three previous phylogenies, were selected as outgroup.

Barcoding analyses

The K2P genetic distance analysis was performed with the reduced ITS alignment matrix, including subclades IV-A (23 sequences), IV-B (16 sequences), IV-E (three sequences) and the singleton IV-F. The alignment length was 401 bp, to adjust the alignment to the length of *Thelephora murrayi* (K(M)264815) type sequence. In Table 5, the intervals of intraspecific and interspecific distances are summarized.

The intraspecific genetic distance among the four different taxa ranged from 0.00000 to 0.00539; whereas, the interspecific distance varied from 0.03077 to 0.13857. This analysis revealed a strong barcode gap since genetic interspecific variation was a hundred times higher than intraspecific variation among the four possible taxa, leading us to consider that four species with narrower geographical distributions have been treated under the same name.

Table 5. Ranges of inter- and intraspecific genetic divergence for ITS sequences of *Cystostereum murrayi* based on Kimura-2-Parameter (K2P) pairwise distances, in brackets the average distance.

Subclades IV	1. *C. murrayi* Europe (IV-A)	2. *C. murrayi* N. America (IV-B)	3. *C. murrayi* India (IV-E)	4. *C. murrayi* Cuba (IV-F)
1. *Cystostereum murrayi* Europe (IV-A), n = 23	0.00000-0.00271 (0.00101)			
2. *C. murrayi* N. America (IV-B), n = 16	0.03077-0.03653 (0.03160)	0.00000-0.00539 (0.00056)		
3. *C. murrayi* India (IV-E), n = 3	0.09118-0.09429 (0.09153)	0.09341-0.09657 (0.09402)	0.00000	
3. *C. murrayi* Cuba (IV-F), n = 1	0.12104-0.12110 (0.12105)	0.12445-0.12786 (0.12508)	0.13857	-

Morphological studies

Macroscopic and microscopic studies were performed for the 113 specimens included in phylogenetic analyses. Later, when characters to delimit the genera and species were clear, a morphological revision was done to 117 specimens without sequences (Table Supplementary 1). Due to the material conditions, not all diagnostic characters were observed in some of them (Table Supplementary 1).

Since the focus of this chapter is *Cystostereum* (clade IV), in the taxonomic part only the species of this genus are described; to *Crustomyces* (clade I) and *Cystidiodontia* (clade II) the description of the genus is included and the nomenclature (valid name and synonyms) of the species treated in this study.

Statistical test of morphological characters

ANOVA analyses on basidiospore length, width and length/width ratio were conducted for 24 European specimens included in subclade IV-A, and 20 North American specimens (Table 6) included in subclade IV-B. The statistical analyses detected differences in spore length ($F=25.46$, p-value < 0.05), width ($F=79.07$, p-value < 0.05), and length/width ratio ($F=27.85$, p-value < 0.05) between the European and North American samples (Fig. 10).

Table 6. Basidiospore measurements of specimens included in subclades IV-A and IV-B, as well as those without ITS sequences. The lenght (L), width (W), lenght/width ratio (Q) and mean values (L’, W’ and Q’) were indicated per specimen.

Species/Specimen	ITS sequence	L	L’	W	W’	Q	Q’
Subclade IV-A							
BR 5020133531597	Yes	4−5	4.50	2−2.5	2.10	2.00−2.50	2.15
F-014297	Yes	4−5	4.30	2−2.5	2.08	2.00−2.25	2.07
GB-0084733	Yes	(4−)4.5−5(−5.5)	4.98	(2−)2.5−3	2.63	1.67−2.00(−2.00)	1.89
GB-0084738	Yes	4−5	4.30	2−2.5	2.15	(1.80−)2.00−2.25	2.00
GB-0084766	Yes	4−5(−6)	4.80	2−2.5(−3)	2.33	(1.60−)2.00−2.50	2.06
GB-0084786	Yes	4−5	4.55	2−2.5	2.25	2.00−2.50	2.02
GB-0084787	Yes	4.5−5	4.80	2−2.5	2.10	2.00−2.50	2.29
GB-0084789	Yes	4−5(−5.5)	4.33	2−2.5(−3)	2.20	(1.60−)1.83−2.00	1.92
GB-0084793	Yes	4−5	4.55	2−2.5(−3)	2.20	(1.60−)2.00−2.50	2.07
GB-0084795	No	4−4.5(−5)	4.43	2−2.5	2.08	2.00−2.50	2.13
MA-Fungi 11382	Yes	4−5(−5.5)	4.45	2−2.5	2.10	2.00−2.25(−2.50)	2.12
NY 461646	No	4−5(−5.5)	4.60	2−2.5(−3)	2.18	1.80−2.50	2.11
NY 461649	No	4−5(−6)	4.60	2−2.5(−3)	2.25	(1.80−)2.00−2.25	2.04
NY 1992045	No	4−5	4.38	2−2.5(−3)	2.15	(1.67−)1.80−2.25	2.04
O-86914	Yes	4−5	4.60	2−2.5	2.00	2.00−2.50	2.30
TAAM 3933	Yes	4−4.5(−5)	4.25	2−2.5	2.05	2.00−2.25(−2.50)	2.07
TAAM 4094	Yes	(3.5−)4−5	4.18	2−2.5	2.05	(1.75−)2.00−2.50	2.04
TAAM 4116	Yes	-	-	-	-	-	-
TAAM 6343	No	4−5	4.43	2−2.5	2.05	2.00−2.50	2.16
TAAM 6399	No	4−5	4.60	2−2.5	2.05	2.00−2.50	2.24
TAAM 18408	Yes	4−5	4.40	2−2.5	2.08	2.00−2.50	2.12
TAAM 52247	No	4−5(−6)	4.70	2−2.5(−3)	2.43	(1.67−)1.80−2.20	1.93
TAAM 53168	Yes	4−5(−5.5)	4.63	2−2.5	2.15	2.00−2.50	2.15
TAAM 58736	Yes	4−5	4.25	2−2.5	2.05	2.00−2.25(−2.50)	2.07
TAAM 126341	Yes	4−5	4.45	2−2.5	2.25	(1.60−)1.80−2.25	1.98
Subclade IV-B							
FH 00530089	Yes	-	-	-	-	-	-
FH 00530129	Yes	4.5−5.5(−6)	5.10	2.5−3(−3.5)	2.95	1.67−1.83	1.73
FH 00607441	Yes	-	-	-	-	-	-
FH 00607436	No	(4.5−)5−5.5	5.10	3	3.00	(1.5−)1.67−1.83	1.70
GB-0084780	Yes	4.5−6	5.10	2.5−3.5	2.93	1.67−2.00	1.74
NY 461648	No	(4.5−)5−6	5.20	(2.5−)3−3.5	3.05	1.67−1.83	1.70
NY 1781928	Yes	(4.5−)5−6	5.10	2.5−3	2.85	1.67−2.00	1.79
NY 1990991	Yes	(4.5−)5−5.5	5.05	2.5−3	2.90	1.67− 1.83(−2.00)	1.74
NY 1991000	Yes	(4.5−)5−6	5.05	(2.5−)3−3.5	2.98	1.67− 1.80(−2.00)	1.69
NY 1992001	Yes	4.5−5.5	4.90	2.5−3	2.80	1.67−1.83	1.75
NY 1992002	No	5−5.5(−6)	5.18	3(−3.5)	3.03	1.67−1.83	1.71
NY 1992003	No	5−6	5.45	3−3.5	3.10	1.67−1.83(−2.00)	1.76
NY 1992004	Yes	-	-	-		-	-
NY 1992020	Yes	(4.5−)5−6	5.30	(2.5−)3−3.5	3.05	1.67−1.83	1.74
NY 1992021	No	5.5−6	5.65	3−3.5(−4)	3.20	1.67−2.00	1.77
NY 1992022	No	(4.5−)5−5.5	5.00	3−3.5	3.05	(1.43−)1.67−1.83	1.64
NY 1992023	Yes	5−6	5.55	3−3.5	3.20	1.67−1.83	1.73
NY 1992024	Yes	4.5−5.5(−6)	5.05	2.5−3(−3.5)	2.95	1.67−1.80(−1.83)	1.71
NY 1992025	No	5−6	5.50	3−3.5	3.25	1.67−1.71	1.69
NY 1992046	Yes	5−6	5.45	3−3.5	3.10	1.67−1.83	1.76
NY 1992048	Yes	(4 −)5−6	5.15	(2−)2.5−3(−3.5)	2.83	(1.6−)1.67−2.00	1.82
NY 1992169	Yes	-		-		-	-
NY 1992173	Yes	4.5−5.5(−6)	5.50	2.5−3(−3.5)	2.95	1.67−1.83	1.75
NY 1992179	No	4.5−5.5(−6)	5.18	2.5−3(−3.5)	2.95	1.67−1.83	1.76

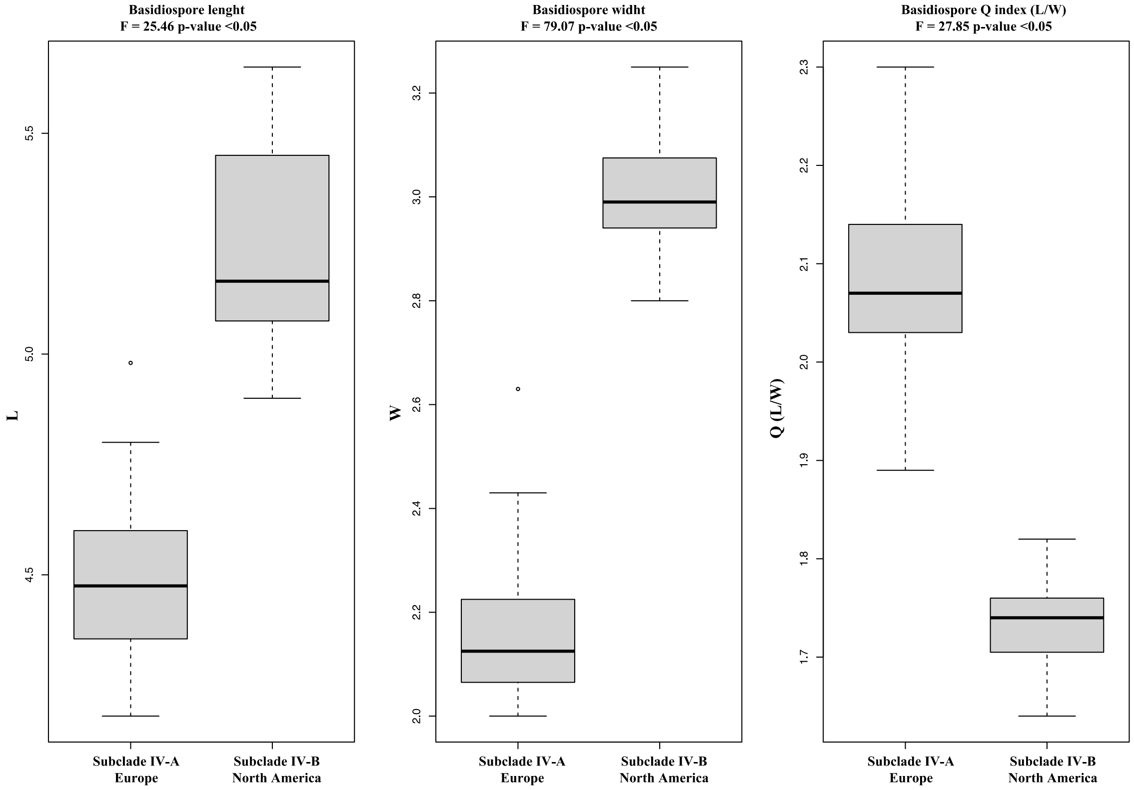


Fig. 10. ANOVA results for basidiospore morphology of subclades IV-A and IV-B. Bar plots of basidiospore length (L), width (W) and length/width ratio (Q).

TAXONOMY

Crustomyces Jülich, Persoonia 10(1): 140 (1978); MycoBank MB25476

Type.— *Crustomyces subabruptus* (Bourdot & Galzin) Jülich, Persoonia 10(1): 140 (1978); MycoBank MB312325

Basidioma resupinate, adnate, crustaceous, hymenophore tuberculate to odontoid, cracked when dried, margin determinate. Hyphal system dimitic, generative hyphae thin-walled, with clamps; skeletal hyphae thick-walled. Dendrohyphidia present or absent. Gloeocystidia thin-walled, sometimes with apical bulb, with central resinuous body and basal clamp, SA-, CB-. Basidia subclavate to clavate, sometimes constricted in the middle, with four sterigmata and basal clamp. Basidiospores ellipsoid, thin-walled, smooth, inamyloid (IKI-), cyanophilous (CB+).

Crustomyces subabruptus (Bourdot & Galzin) Jülich, Persoonia 10(1): 140 (1978); MycoBank MB312325

≡*Odontia subabrupta* Bourdot & Galzin, Hyménomycètes de France: 430 (1927); MycoBank MB254881 ≡*Cystostereum subabruptum* (Bourdot & Galzin) J. Erikss. & Ryvarden, The Corticiaceae of North Europe 3: 327 (1975); MycoBank MB312567. ≡*Cystostereum pini-canadense* subsp. *subabruptum* (Bourdot & Galzin.) Chamuris, Mycologia 78(3): 385 (1986); MycoBank MB129650 ≡*Crustomyces pini-canadensis* subsp. *subabruptus* (Bourdot & Galzin) Ginns & M.N.L. Lefebvre, Mycologia Memoirs 19: 47 (1993); MycoBank MB357995

=*Crustomyces heteromorphus* (Hallenb.) Hjortstam, Mycotaxon 28(1): 23 (1987); MycoBank MB 129761 ≡*Cystostereum heteromorphum* Hallenb., Mycotaxon 11(2): 450 (1980); MycoBank MB112893

Crustomyces pini-canadensis (Schwein.) Jülich, Persoonia 10(1): 140 (1978); MycoBank MB312324

≡*Radulum pini-canadensis* Schwein., Transactions of the American Philosophical Society 4(2): 164 (1832); MycoBank MB536418 ≡*Corticium pini-canadensis* (Schwein.) D.P. Rogers & H.S. Jacks., Farlowia 1(2): 323 (1943); MycoBank MB285653 ≡*Cystostereum pini-canadense* (Schwein.) Parmasto, Conspectus Systematics Corticiacearum: 170 (1968); MycoBank MB312565

=*Peniophora piceina* Overh., Mycologia 22(5): 238 (1930); MycoBank MB274390 ≡*Cystostereum piceinum* (Overh.) Lindsey & Gilb., Bibliotheca Mycologica 63: 203 (1978); MycoBank MB312563

Notes.— Jülich (1978) introduced *Crustomyces* with two species *C. subabruptus* and *C. pini-canadense*. Later, Chamuris (1986) considered those as subspecies of *Cystostereum pini-canadense*, which were later combined to *Crustomyces* by Ginns & Lefebvre (1993). Previous to generic description, the species *Odontia subabrupta* and *Radulum pini-canadensis* were synonymized by Gilberston & Larsen (1965), and considered as two members of the genus *Cystostereum*: *Cystostereum pini-canadensis*, and *Cystostereum subabruptum*. However, our LSU analyses (Fig. 6), confirm that *Crustomyces* and *Cystostereum* are two independent genera. In *Crustomyces* (clade I) the three subclades in Fig. 7 correspond to three different species, described below.

The first (subclade I-A) corresponds to *Crustomyces subabruptus*, since *Odontia subabrupta* paratype grouped in this subclade. In Index Fungorum, the current name of this species is correctly indicated as *Crustomyces subabruptus*, but in MycoBank it appears under *Cystostereum*. On the other hand, since sequences of the holotype and both paratypes of *Cystostereum heteromorphum* (=*Crustomyces heteromorphus*) grouped together with sequences of *Crustomyces subabruptus,* here *Crustomyces heteromorphus* is proposed as synonymous with *Crustomyces subabruptus* (see above). Hallenberg (1980) considered them as different species, since *Cystostereum heteromorphum* differs morphologically from *Cystostereum subabruptum* by lacking dendrohyphidia and having gloeocystidia with an apical bulb. However, according to Eriksson & Ryvarden (1975), dendrohyphidia were not seen in all specimens of *Cystostereum subabruptum* (=*Crustomyces subabruptus*), and on the basis of our morphological studies, gloeocystidia with apical bulb were observed not only in *Crustomyces heteromorphus* specimens, but also in specimens under *Crustomyces subabruptus*; furthermore, we observed gloeocystidia with apical bulb on *Odontia subabrupta* paratype BPI 266215.

In the subclade II-B, a specimen of *Peniophora piceina* grouped with specimens under *Cystostereum pini-canadense.* Rogers & Jackson (1943) and Chamuris (1986) considered synonyms *P. piceina* and *Radulum pini-canadensis.* Even though type specimens were not available for our study, this subclade corresponds to a unique species, *Crustomyces pini-canadensis* [Both Index Fungorum and MycoBank include this species under *Cystostereum,* even though the latter synonymy was proposed by Ginns & Lefebvre (1993)]. Further analyses should be conducted including the type specimens of *Peniophora piceina* and *Radulum pini-canadensis* to evaluate if these taxa are conspecific.

The species *Crustomyces subabruptus* and *C. pini-canadensis* are morphologically very similar; both have dimitic hyphal system, gloeocystidia with central resinuous body, sometimes with an apical bulb, and ellipsoid thin-walled basidiospores. However, they differ because specimens of *C. pini-canadense* have a tuberculate hymenophore instead of odonotioid and dendrohyphidia were not found.

The third subclade (I-C, Fig. 7) included all sequences retrieved from Genbank under *C. subabruptus* from China, and these could correspond to a unique species; however, since the specimens were not available to our study, and morphological analyses have not been recorded in any publication, we cannot confirm whether or not they belong to a species already described. Further analyses should be conducted.

Cystidiodontia Hjortstam, *Mycotaxon* 17: 571 (1983); MycoBank MB25796

Type.— Hydnum artocreas Berk. & M.A. Curtis ex Cooke, Grevillea 20(93): 1 (1891); MycoBank MB242456

Basidioma resupinate, effused, crustaceous, hymenophore hydnoid, margin determinate. Hyphal system dimitic, generative hyphae with clamps and skeletal hyphae thickwalled, strongly reddish in Congo Red solution. Dendrohyphidia present. Gloeocystidia thin-walled, sometimes with apical bulb, with central resinuous body and basal clamp, SA-, CB-. Basidia subclavate to clavate, with four sterigmata and basal clamp. Basidiospores globose to subglobose, thick-walled, smooth, IKI-, CB+.

Cystidiodontia laminifera (Berk. & M.A. Curtis) Hjortstam, Mycotaxon 39: 416 (1990); MycoBank MB127328

≡*Hydnum laminiferum* Berk. & M.A. Curtis, Journal of the Linnean Society, Botany 10(46): 325 (1869), MycoBank MB199893

=*Cystidiodontia artocreas* (Berk. & M.A. Curtis ex Cooke) Hjortstam, Mycotaxon 17: 571 (1983), MycoBank MB 108828 ≡ *Hydnum artocreas* Berk. & M.A. Curtis ex Cooke, Grevillea 20(93): 1(1891); MycoBank MB 242456 ≡ *Odontia artocreas* (Berk. & M.A. Curtis ex Cooke) Bres. Hedwigia 35: 286 (1899); MycoBank MB 470373 ≡ *Cystostereum artocreas* (Berk. & M.A. Curtis ex Cooke) Hallenb. & Ryvarden, Mycoaxon 2(1): 135 (1975); MycoBank MB 312562

Cystidiodontia isabellina (Berk. & Broome) Hjortstam & Ryvarden, Mycotaxon 25(2): 549 (1985); MycoBank MB103254

≡*Kneiffia isabellina* Berk. & Broome, Journal of the Linnean Society, Botany 14(73): 62 (1898). MycoBank MB230180

=*Hypochnicium grandinioides* Ryvarden, Bulletin du Jardin Botanique National de Belgique 48: 91 (1978); MycoBank MB315573

Notes.— On the basis of LSU analyses (Fig. 6), we confirm that *Cystidiodontia* (clade II) and *Cystostereum* are two independent genera. In *Cystidiodontia* two taxa are resolved, one from South America (subclade II-A) and one from Africa (subclade II-B).

The subclade II-A corresponds to the species *Cystidiodontia laminifera* (≡*Hydnum laminiferum*). Based on morphological studies, Hjortstam (1983) segregated the genus *Cystidiodontia* from *Cystostereum* to place *Cystostereum artocreas*, later Hjortstam (1990) synonymized *Cystidiodontia artocreas* to *Cystidiodontia laminifera,* a species described from Cuba by Berkeley & Curtis (1869) as *Hydnum laminiferum.* Unfortunately, we were not able to study the type specimen of *Hydnum artocreas* (K, Venezuela 139) since it was not available during these years. However, the type of *Hydnum laminiferum* was available and, although sequences were not obtained, morphological characters are identical to the South American specimens included in subclade II-A.

The subclade II-B corresponds to *Cystidiodontia isabellina*. It includes African specimens and the isotype of *Hypochnicium grandinioides*, a species originally described from Burundi by Ryvarden (1978), which was synonymized with *Cystidiodontia artocreas* by Hjortstam (1983).

Both species are characterized by hydnoid hymenophore, dimitic hyphal system with distinctly thick-walled skeletal hyphae that are strongly reddish in Congo Red solution, presence of dendrohyphidia, gloeocystidia with central resinuous body and the globose thick-walled basidiospores. However, according to Hallenberg & Ryvarden (1975) and Hjortstam & Ryvarden (1986), African specimens are separated from South American by their dextrinoid reaction.

Cystostereum Pouzar, Ceská Mykologie 13(1): 18 (1959); MycoBank MB17451

Type.— *Thelephora murrayi* Berk. & M.A. Curtis, Journal of the Linnean Society. Botany 10: 329 (1869); MycoBank MB225274

Basidioma perennial, resupinate to effuse-reflexed, hymenophore tuberculate, cracked when dried, darkening the substrate. Hyphal system dimitic, generative hyphae densely interwoven, with clamps, thin to slightly thick-walled and dark in deep layer, skeletal hyphae thick-walled, branched,. Gloeocystidia numerous, with oily content, variable in shape, and normally arranged in several layers, SA-, CB-. Basidia subclavate to clavate, with four sterigmata and basal clamp. Basidiospores ellipsoid to cylindrical, thin- to thick-walled, smooth, IKI-, CB+.

Notes.— Among morphological features, it is remarkable that skeletal hyphae are branched, resembling those described for *Scytinostroma* Donk by Hallenberg (1985).

Cystostereum australe Nakasone, Mycotaxon 17: 270 (1983)

MycoBank MB109457 **Fig. 11**

Type: USA, Georgia, proxime Bainbridge, hardwood log, 15 Apr. 1961, R.W. Davidson, W.A. Campbell, K. Aoshima, C.H. Driver (FP-105668-Sp! in CFMR, holotype)

Basidioma resupinate to effuse-reflexed, 1.5–3 mm thick, ligneous, upper surface between basidioma and substrate dark brown to black, hard, darkening the substrate, perennial, distinctly stratified in several layers; *hymenophore* smooth to tuberculate, cracked when dried, yellow white to pale yellow (92. y White – 89. p. Y); *margin* determinate, abrupt, mainly reflexed, concolorous with the hymenium; microscopic sections strongly yellow in 3% KOH. *Hyphal system* dimitic: generative hyphae with clamps, 1.5–2.5 µm wide, in subicular layer parallel to substratum, densely interwoven and difficult to disagregate, yellowish brown, thin to thick-walled, clearly differentiated, in subhymenial layer perpendicular to substratum, hyaline, thin-walled; skeletal hyphae thick-walled, branches numerous in subicular layer, 1–1.5 µm wide. *Hyphidia* cylindrical, 15–28 × 2–3 µm, hyaline, thin-walled, tapering toward apex, with basal clamp. *Gloeocystidia* numerous, sometimes empty but usually with yellow oily content, homogeneous in deep layers, SA-, thin-walled, generally included but sometimes projecting above hymenium up to 30 µm, variable in shape: in subicular layer cylindrical, 80–95 × (7–)10–12 µm, in subhymenial and hymenial layers fusiform, 25–45(–50) × 10–20 µm, clavate to subcylindrical, 30–46 × 7–12 µm. *Basidia* narrowly clavate to subcylindrical, sometimes constricted, 23–40 × 5–7 µm, with oil droplets, four sterigmata, and basal clamp. *Basidiospores* broadly ellipsoid, 4–5.5 × 2.5–4 µm, hyaline, smooth, thick-walled, inamyloid, indextrinoid, cyanophilous. L’ = 4.66, W’ = 3.10, Q’ = 1.50.

Ecology and habitat.— On *Carya* and *Ulmus*. According to Nakasone (1983), *C. australe* is associated with a white rot decay of hardwoods.

Known distribution.— Rare species known from Gulf of Mexico and Caribbean region.

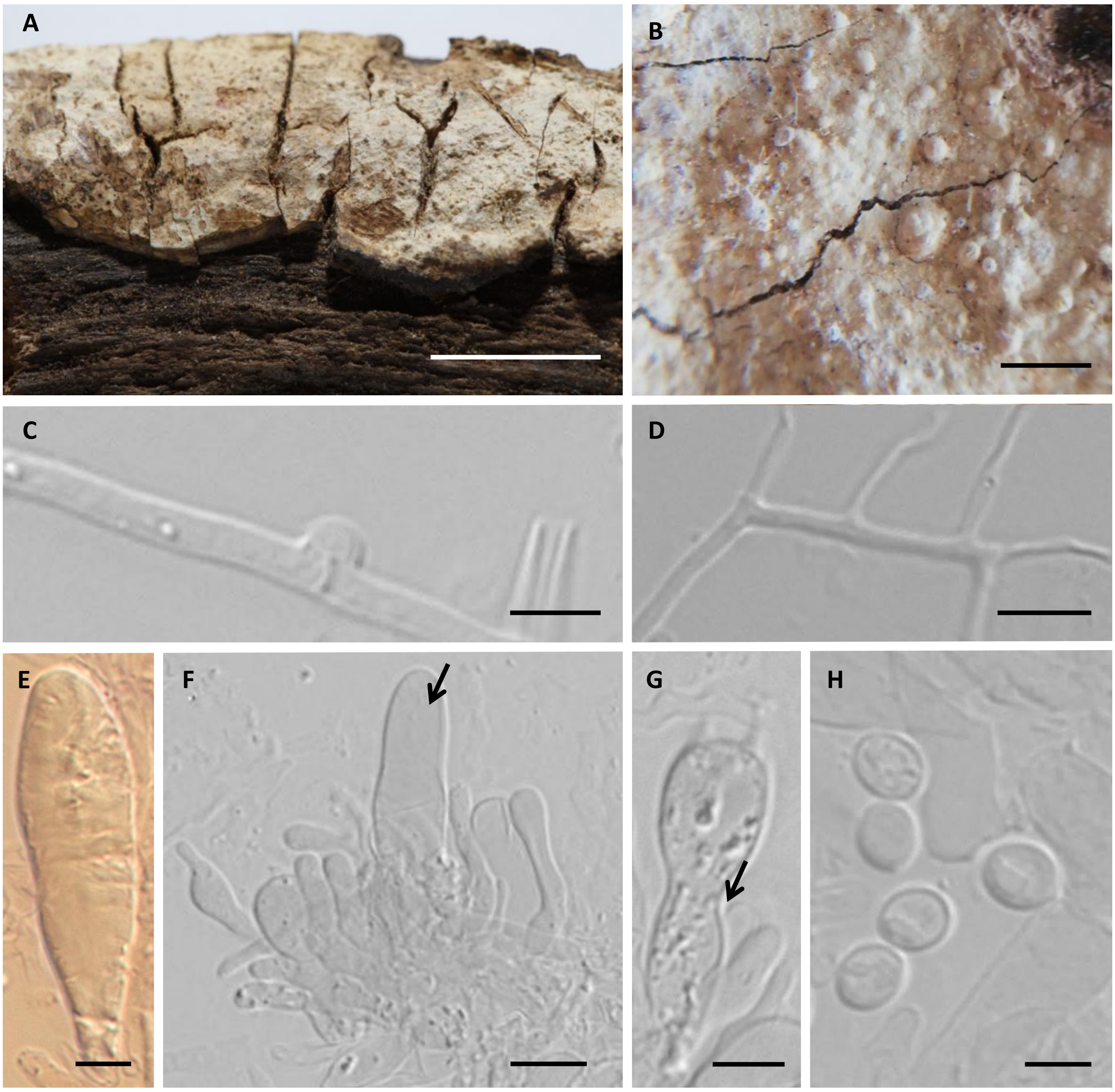


Fig. 11. ***Cystostereum australe*** Nakasone (FP-105668, **holotype**). **A.** Basidioma dry specimen. **B.** Hymenophore. **C.** Generative hyphae, with clamp. **D.** Skeletal hyphae. **E.** Gloeocystidium content. **F.** Gloeocystidium projecting above hymenial layer. **G.** Basidium constricted. **H.** Basidiospores. Scale bars: A = 1 cm; B = 1 mm; C-E = 5 µm; F = 10 µm; G, H = 5 µm.

Material examined.— Costa Rica: Heredia Province, Birri, 4700 ft., on bark of dead hardwood, 27 Jul. 1963, J.L. Lowe 12913 (CFMR*, paratype). Jamaica: John Crow Peak, May 1906, D.S. Johnson (NY 776087*, paratype of *Stereum saxitas*); USA: Alabama, Montgomery, Aug. 1916, R.P. Burke (NY 1990986); Florida, Alachua County, Devil´s Millhopper, on *Ulmus* sp., 18 Jul. 1972, H.H. Brurdsall Jr., HHB-6648 (CFMR, paratype); Florida, Hogtown Creek, Gainesville, on *Carya* sp., 27 Aug. 1952, A.S. Rhoads (FP-103021* in CFMR, paratype).

Notes.— The Jamaican specimen (NY 776087) reported by Burt (1920) as paratype of *Stereum saxitas* (≡*Cystostereum saxitas*), was revised by Chamuris and identified as *C. australe* (Chamuris 1988). On the basis of our molecular and morphological analysis (Fig. 7) we confirm that this specimen corresponds to *C. australe*.

Cystostereum effusum (Overh.) Nogal, Telleria, M. Dueñas, M.P. Martín **comb. nov.**
[MycoBank no. pending] **Fig. 12**

≡*Corticium effusum* Overh., Mycologia 22(5): 238 (1930)
=*Stereum pulverulentum* Peck, Bulletin of the Torrey Botanical Club 27: 20 (1900)
Type: USA, Maine, Penobscot Co., Orono, on *Betula papyracea*, Oct. 1892, F.L. Harvey (FH00530129!)

Non *Stereum pulverulentum* (Lév.) Mont., Annales des Sciences Naturelles. Botanique 7: 174 (1847) [=*Hymenochaete luteobadia* (Fr.) Höhn. & Litsch., Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Mathematisch-Naturwissenschaftliche Klasse. Abt. 1 116: 754 (1907)]

Type: USA, Vermont, Ferdinand, on ten year old *Acer* slash, 1 Oct. 1926, P. Spaulding 43963 (Overholts Herb. nº 11324; BPI 281097, isotype)

Basidioma resupinate to effuse-reflexed, variable in size, usually covering a large portion of substrate, 0.7−3 mm thick, suberose to ligneous, upper surface between basidioma and substrate dark brown to black, hard, darkening the substrate, perennial, distinctly stratified in several layers; *hymenophore* smooth to tuberculate, cracked when dried, light grayish yellow brown to light yellowish brown (79. l. gr. y Br − 76. l. y Br); *margin* abrupt, determinate; sections strongly yellow in 5% KOH. *Hyphal system* dimitic; generative hyphae with clamps, in subicular layer, parallel to substratum, densely interwoven, yellowish brown, thin to thick-walled, 3−4 µm wide, clearly differenciated; in subhymenial layer, perpendicular to substratum, hyaline, thin-walled, 2–3 µm wide; skeletal hyphae branched, thick-walled, 1–1.5 µm wide, mainly found in subicular layer. *Hyphydia* not projecting in the hymenium. *Gloeocystidia* numerous, included, with basal clamp, sometimes empty but usually with homogeneous yellow oily content, SA-, thin-walled, in horizontal direction in subiculum and vertical in subhymenium and hymenium, variable in shape: cylindrical in subiculum, (40–)45–87(–105) × 7–12(–13) µm, subglobose, fusiform in subhymenium, sometimes pedunculated, 20–34 × 14–20(–22) µm, and subcylindrical in the hymenium, 29–50 × 8–12 µm. *Basidia* narrowly clavate, (20–)22.5–31 × 4–5(–6) µm, four sterigmata, with basal clamp. *Basidiospores* subcylindrical, (4–)4.5–6 × 2.5–3.5(–4) µm, hyaline, thin-walled, smooth, inamyloid, indextrinoid and cyanophilous. L' = 5.23, W' = 3.01, Q' = 1.74.

Ecology and habitat.— On hardwood of *Acer saccharum, Betula* sp*., B. lutea, B. papyracea* and *Fagus* sp. The North American specimens originally under *C. murrayi* are commonly associated with white rot (Lindsey & Gilbertson 1978, Ginns 1986, Nakasone 1983) and with heart rot and steam cankers of living trees (Farr et al. 1989).

Known distribution.— Eastern North America.

Material examined.— CANADA: Ontario, Little Macaulay Lake, Algonguin Pk., on *Acer saccharum*, 24 Aug. 1940, Coll. & Det. R.F. Cain, 16431 (NY 461648). USA: Maine, Lake Millinocket, on aspen, 22 Aug. 1940, G.W.M. 5067 (NY 1990991*); Maine, Orono, on *Betula papyracea*, Oct. 1892, F.L. Harvey (FH 00530129*, type of *Stereum pulverulentum* Peck); Maine, Piscataquis Co., Loon Cove Camp (Nº. 10), a mile west of Greely´s Landing on the south shore of Sebec Lake, on birch, 9 Sep. 1905, W.A. Murrill, nº 2304 (NY 1992022); Maine, Portage, on *Fagus*, Aug. 1907, L.W. Riddle (NY 1992048*); Minnesota, Claearwater County, Lake Itasca State Park, on *Betula papyrifera*, 10 Aug. 1970, Robert L. Gilbertson 9637 (GB-0084780*); Michigan, Cheboygan Co., Colonial Pt., Burt Lake, 9 Jul. 1955, H.E. Bigelow (NY 1992179); Michigan, Luce County, Upper Tahquamenon Falls, on *Fagus* sp., 12 Sep. 1969, H.H. Burdsall Jr., HHB 3470 (NY 1781928*); North Carolina, Highlands, on hardwood log, 18 Aug. 1933, G.W. Martin 1392 (FH 00607441); New Hampshire, Chocorua, Jul. 1904, W.G. Farlow (NY 1992025); New Hampshire, on bark of *Betula*, Aug. 1908, W.G. Farlow, 368 (NY 1992024*); New York, Cayuga County, Bear Swamp, near Sempronius, on dead *Betula lutea*, 5 Jun. 1947, Coll. & Det.: C.T. Rogerson, nº 1513 (NY 1992004*); New York, Newcomb, on *Betula papyrifera*, 24 Jun. 1932, J.R. Hansbrough, J.R.H. nº 1284 (NY 1992002); New York, Lake Placid, Adirondacks, coniferous or mixed forest, 2000 ft., 17-29 Jul. 1912, W.A. Murrill & E.L.

Murrill, nº 194 (NY 1992021); New York, Tupper Lake, 2000 ft., on *Betula papyrifera*, 8 Sep. 1932, J.R. Hansbrough 1285 (NY 1992003); New York, Ulster County, near Round Pond, just south of Frost Valley, between Claryville and Oliveria, Catskill Park, on trunk of living apple tree, 16 Sep. 1978, C.T. Rogerson (NY 1992001*); New York, Washington Co., West of Tripoli, So. WFTAnn, on an old beech log, 12 Jun. 1915, S.H. Burnham (NY 1992023*); Pennsylvania, McKean County, Red Mill Brook, 4 mi. NE of Clermont, 7-16 Aug. 1948, N.D. Richmond & H. Roslund (NY 1991000*); Pennsylvania, Clarion County, 20 Jun. 1940, D.R. Sumstine (NY 1992173*); Vermont, Bristol, on bark wood of *Acer*, 11 May 1902 (FH 00530089*); Virginia, Apple Orchard Mountain, in the Blue Ridge mountains, 18 miles north of Bedford, 2500-4200 ft., on standing birch trunk, 24-27 Oct. 1916, W.A. Murrill (NY 1992020); Virginia Limberlost, White Oak Canyon, Shenandoah Nat. Park, on *Betula lutea*, 25 Oct. 1936, J.A. Stevenson, nº 11838 (NY 1992169*); Wisconsin, Ladysmith, *Betula lutea*, Sep. 1913, Humphrey (FH 00607436). Italy: in rej. Tridentina, ad radices Abietis, Aug. 1893, G. Bresadola (NY 1992046*).

Notes.— The North American specimens originally under *Cystostereum murrayi*, were grouped together in the well-supported subclade IV-B (Figs. 6-9), including type specimen of *Stereum pulverulentum* Peck., a taxon originally described from Maine by Peck (1900) that was considered by Burt (1920) as synonymous with *S. murrayi* (=*Cystostereum murrayi*). However, according to Art. 53.1 of the Shenzhen Code (Turland et al. 2018), *S. pulverulentum* Peck is an illegitimate name because it is a later homonym of *S. pulverulentum* (Lév.) Mont. (1847), which according to Lentz (1955) corresponds to *Hymenochaete luteobadia* (Fr.) Höhn. & Litsch.

Rogers & Jackson (1943) proposed the North American species *Corticium effusum* Overh. as synonym of *Cystostereum murrayi.* According to the protologue of *Corticium effusum*, basidiospores of this species are 4–5 × 2.5–3 µm; these values are according with to specimens in subclade IV-B [(4−)4.5−6 × 2.5−3.5(−4) µm], smaller than the basidiospores of *C. murrayi* s. str. [(5.5−7.5 × (3−)3.5−4 µm]. Another character that we have observed to differentiate *Cystostereum effusum* from *C. murrayi* is that *C. effusum* has a distinctly reflexed fruitbody. Nakasone (1983) studied the type of *Corticium effusum* and concluded, “it is fairly thin and appears to be a young form of *Cystostereum murrayi*”. Thus, we proposed the new combination for specimens of subclade IV-B; although, we are waiting to receive the type of *Corticium effusum* Overh. requested from BPI some months ago to perform the molecular analyses.

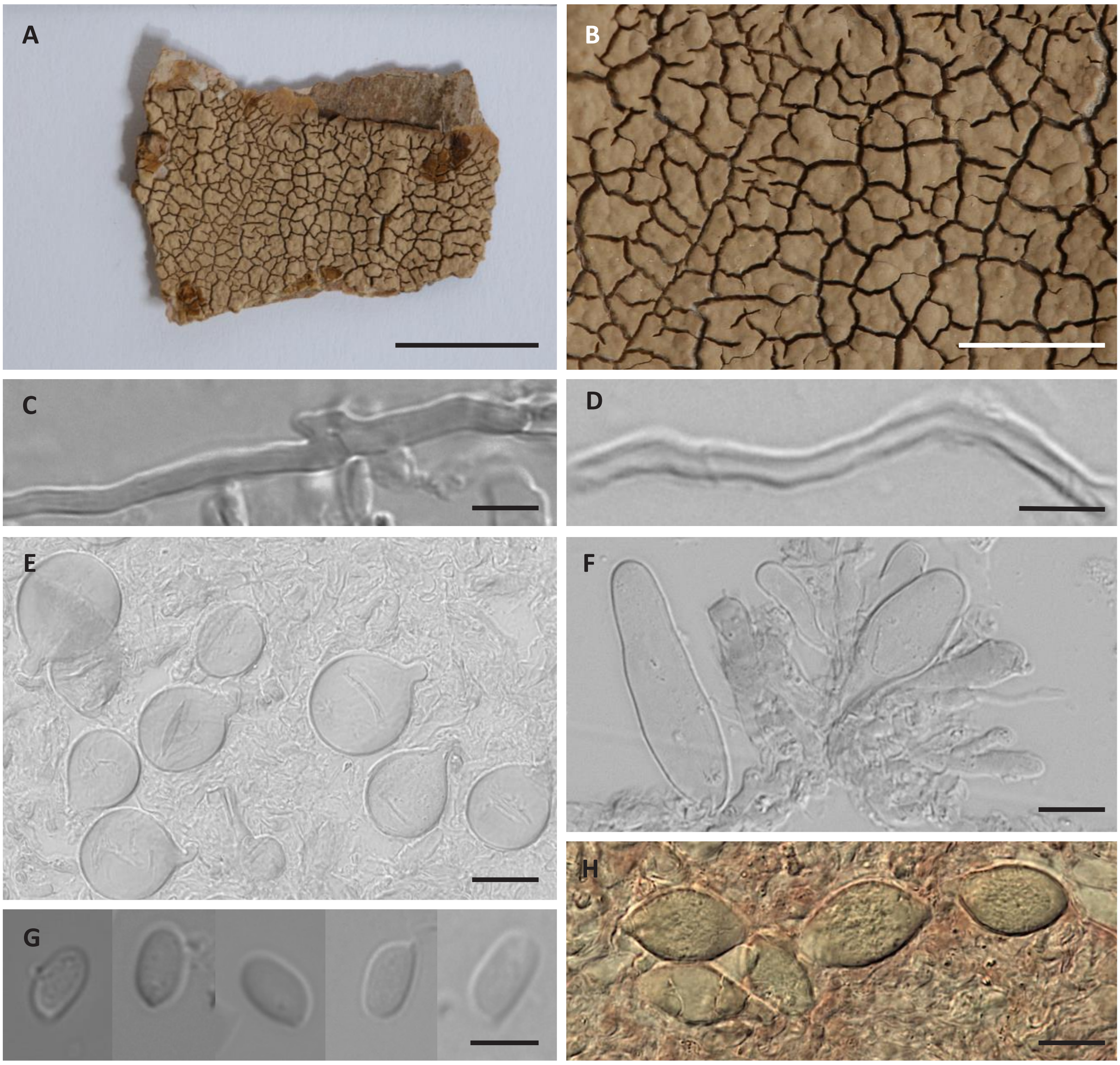


Fig. 12. ***Cystostereum effusum*** (Overh.) Nogal, Telleria, M. Dueñas, M.P. Martín (F.L. Harvey, type *Stereum pulverulentum* Peck, FH 00530129). **A.** Basidioma dry specimen, **B.** Hymenophore. **C.** Generative hyphae, with clamp. **D.** Skeletal hyphae. **E.** Gloeocystidia subglobose in subhymenium. **F.** Gloeocystidia in hymenium. **G.** Basidiospores. **H.** Gloeocystidia content. Scale bars: A = 1 cm; B = 2.5 mm; C, D = 5 µm; E, F = 10 µm; G = 5 µm; H = 10 µm.

In subclade IV-B, all collections were from Canada and USA, except the Italian specimen NY 1992046 collected in 1892 by G. Bresadola. Sequences obtained from this specimen should be undertaken with caution, since sequences from quite old collections (>100 years) are not easy to obtain; however, we have obtained sequences from the four markers at the first attempt. Normally, Bresadola′s collections have been difficult to characterize through DNA sequences. For example, Telleria et al. (2010a) obtained ITS sequence from holotype of *Hyphodermella rosae* (Bres.) Nakasone after several attempts (María P. Martín 2021, personal communication) and Telleria et al. (2015) tried unsuccessfully to obtain ITS sequences of holotype and isotype specimens of *Jaapia ochroleuca* (Bres.) Nannf. & J. Erikss. In the case of the Italian specimen NY 1992046 collected in 1892 by G. Bresadola, it looks like a young specimen, and we suspect that it could have been an interchange of material in any of the herbaria.

Cystostereum kenyense Hjortstam, Mycotaxon 28(1): 25 (1987)
MycoBank MB129764 **Fig. 13**

Type: Kenya, Eastern province, Nyeri district, Mt. Kenya S slope, Regati Forest Sta., 0º20’S 37º15’E, 2200-2300 m, 2-3 Feb. 1973, L. Ryvarden 9841 (O-F-450365! *, holotype)

Basidioma resupinate, effuse, adnate, 0.5 mm thick, crustaceous, darkening the substrate; *hymenophore* distinctly tuberculate, slightly cracked when dried, pale yellow (89. p. Y); *margin* abrupt; microscopic sections strongly yellow in 3% KOH. *Hyphal system* dimitic, generative hyphae thin-walled, with clamps, 2–3.5 µm wide, skeletal hyphae scarce, thick-walled, with a narrow lumen, branched, 1−1.5 µm wide, mainly found in subiculum. *Hyphidia* cylindrical, 20−35 × 2.5−3 µm, hyaline, thin-walled, tapering toward apex, with basal clamp. *Gloeocystidia* numerous, with basal clamp, SA-, CB-, thin-walled, in vertical direction, of two types: subcylindrical, usually pedunculated or birradicated in deep layers, 90–105(–130) × 8–13 µm; and fusiform, 50–100 × (12–)15–18(–21) µm, sometimes projecting above hymenium up to 25 µm. *Basidia* narrowly clavate, 30–32(–50) × 5 µm, with oildroplets, four sterigmata and basal clamp. *Basidiospores* broadly ellipsoid to ellipsoid, (4−)4.5−5(−5.5) × 3−4 µm, smooth, thick-walled, inamyloid, indextrinoid, cyanophilous. L’ = 4.8, W’ = 3.3, Q’ = 1.45.

Ecology and habitat.— Not known.

Known distribution.— Only known from type locality in Kenya.

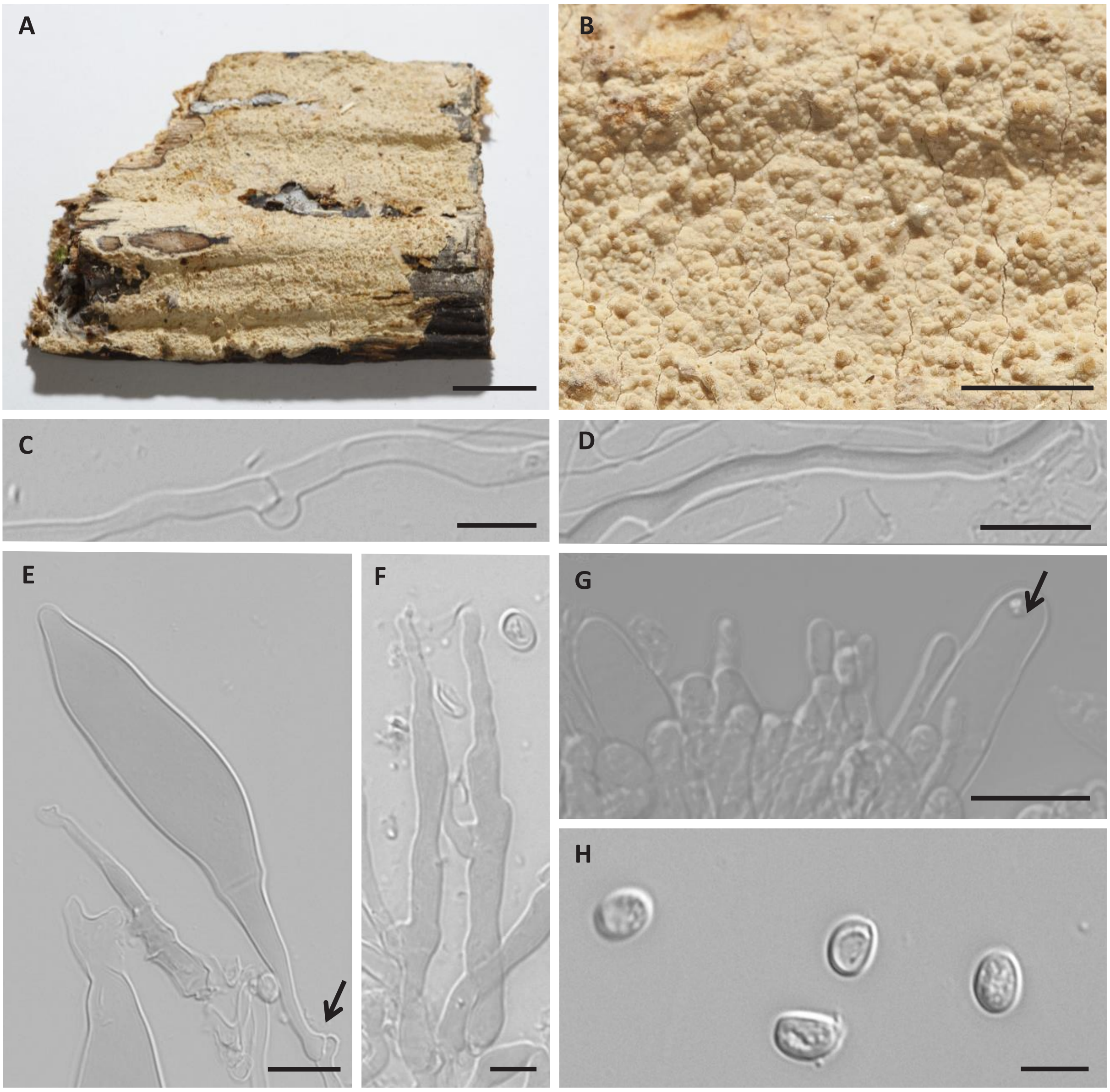


Fig. 13. ***Cystostereum kenyense*** Hjortstam (L. Ryvarden 9841, (O)F-450365, holotype). **A.** Basidioma dry specimen. **B.** Hymenophore. **C.** Generative hyphae, with clamp. **D.** Skeletal hyphae. **E.** Gloeocystidium, with basal clamp. **F.** Hyphidia. **G.** Gloeocystidium projecting above hymenium. **H.** Basidiospores. Scale bars: A = 1 cm; B = 2.5 mm; C, D = 5 µm; E = 10 µm; F = 5 µm; G = 10 µm; H = 5 µm.

Notes.— This species was originally described as monomitic (Hjortstam 1987); however we have observed skeletal hyphae (Fig. 13). This species is morphologically and phylogenetically related to *Cystostereum australe* (Figs. 6-8). Both species share the ellipsoid and thick-walled spores, and hyphidia in hymenium. However, *C. australe* has smaller gloeocystidia of different shape, and hyphae are densely interwoven in deep layers, while *C. kenyense* is characterized by having longer gloeocystidia (up to 130 µm), and hyphae are easier to disaggregate.

Cystostereum murrayi (Berk. & M.A. Curtis) Pouzar, Ceská Mykologie 13(1): 18 (1959)
MycoBank MB296367 **Figs. 14, 15**

≡*Thelephora murrayi* Berk. & M.A. Curtis, Journal of the Linnean Society. Botany 10: 329 (1869) [Described under *Thelephora murraii*, ortographic variant] ≡*Corticium murrayi* (Berk. & M.A. Curtis) Pat., Énumération Méthodique des Champignons Recueillis à la Guadeloupe et à la Martinique: 18 (1903) ≡*Stereum murrayi* (Berk. & M.A. Curtis) Burt, Annals of the Missouri Botanical Garden 7: 131 (1920)

Type: *Thelephora murrayi*, Cuba [no additional data], C. Wright 269 (FH00546382!, K(M)264815!*)

Basidioma effused, resupinate or reflexed, up to 0.7 mm thick, suberose to ligneous, upper surface between basidioma and substrate dark brown to black, hard, darkening the substrate, perennial, distinctly stratified in several layers; *hymenophore* tuberculate, with scattered tubercules, cracked when dried, light grayish yellow brown to grayish yellow brown (79. l. gr. y Br − 80. gy. Y Br); *margin* clearly differenciated, pale orange-yellow (73. p. OY); sections strongly yellow in 3% KOH. *Hyphal system* dimitic, generative hyphae with clamps, in subicular layer parallel to substratum, densely interwoven and difficult to disaggregate, yellowish brown, thin to thick-walled, 2−2.5 µm wide; in subhymenial layers, perpendicular to substratum, hyaline, thin-walled, 1−2.5 µm wide; skeletal hyphae very branched, thick-walled, 1−2 µm wide, mainly found in subicular layer. *Gloeocystidia* numerous, included, with basal clamp, sometimes empty but usually with homogeneous yellow oily content, SA-, thin-walled, in horizontal direction in subiculum and vertical in subhymenium and hymenium, variable in shape: cylindrical in subiculum, 60−100 × 10−13 µm, subglobose to fusiform in subhymenium, sometimes pedunculated, 20−48 × 12−17 µm, and subcylindrical in the hymenium, 30−60 × 7−15 µm. *Basidia* clavate, 20−30 × 5−6(−7) µm, with four sterigmata and basal clamp.

Basidiospores subcylindrical to cylindrical, 5.5−7.5 × 3−4 µm, hyaline, thin-walled, smooth, inamyloid, indextrinoid, and cyanophilous. L' = 6.47, W' = 3.55, Q' = 1.82.

Ecology and habitat.— Not known.

Known distribution.— Only known from type locality in Cuba.

Notes.— *Cystostereum murrayi* has been regarded as cosmopolitan; however, our phylogenetic and morphological results indicated that this species is restricted, at the moment, to type locality. *Thelephora murrayi* was originally described by Berkeley & Curtis (1869) in *Fungi Cubenses* with number 382 (Fig. 15A) in which they included two specimens, the first corresponds to number C. Wright 269 collected from Cuba, and the second to Murray 5809 from New England.

Burt (1920) combined *Th. murrayi* to *Stereum murrayi,* selecting a Cuban specimen as type. However, Cunningham (1963) considered the North American specimen, Murray 5809, as type of *Stereum murrayi*; later, Hjortstam (1990), after study of both collections deposited in K, mentions "specimen from New England, nº 5809, cited by Berkeley in the original description is not the same, but represents a species of *Laeticorticium* Donk".

On the other hand, *Cystostereum murrayi* differs from *C. australe* and *C. kenyense* in the basidiospores, broadly ellipsoid and thick-walled basidiospores in the latter two species; moreover, in the hymenium of *C. murrayi* hyphidia were not seen. These species also differ in geographical distribution. *Cystostereum murrayi* is currently known from Cuba, and could share distribution with *C. australe* that is a rare species restricted to the Gulf of Mexico and Caribbean region; on the other hand, *C. kenyense* is restricted to Kenya.

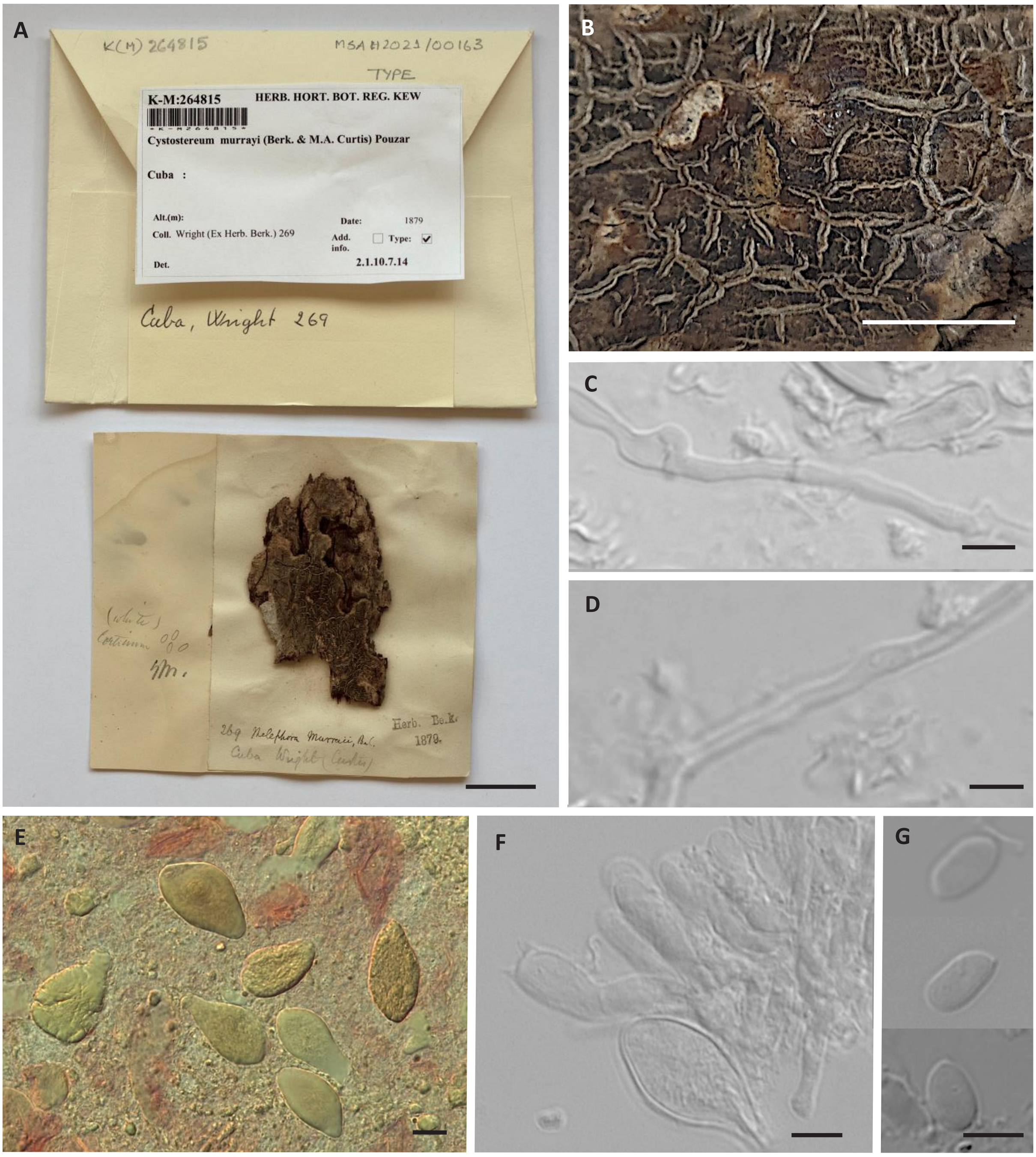
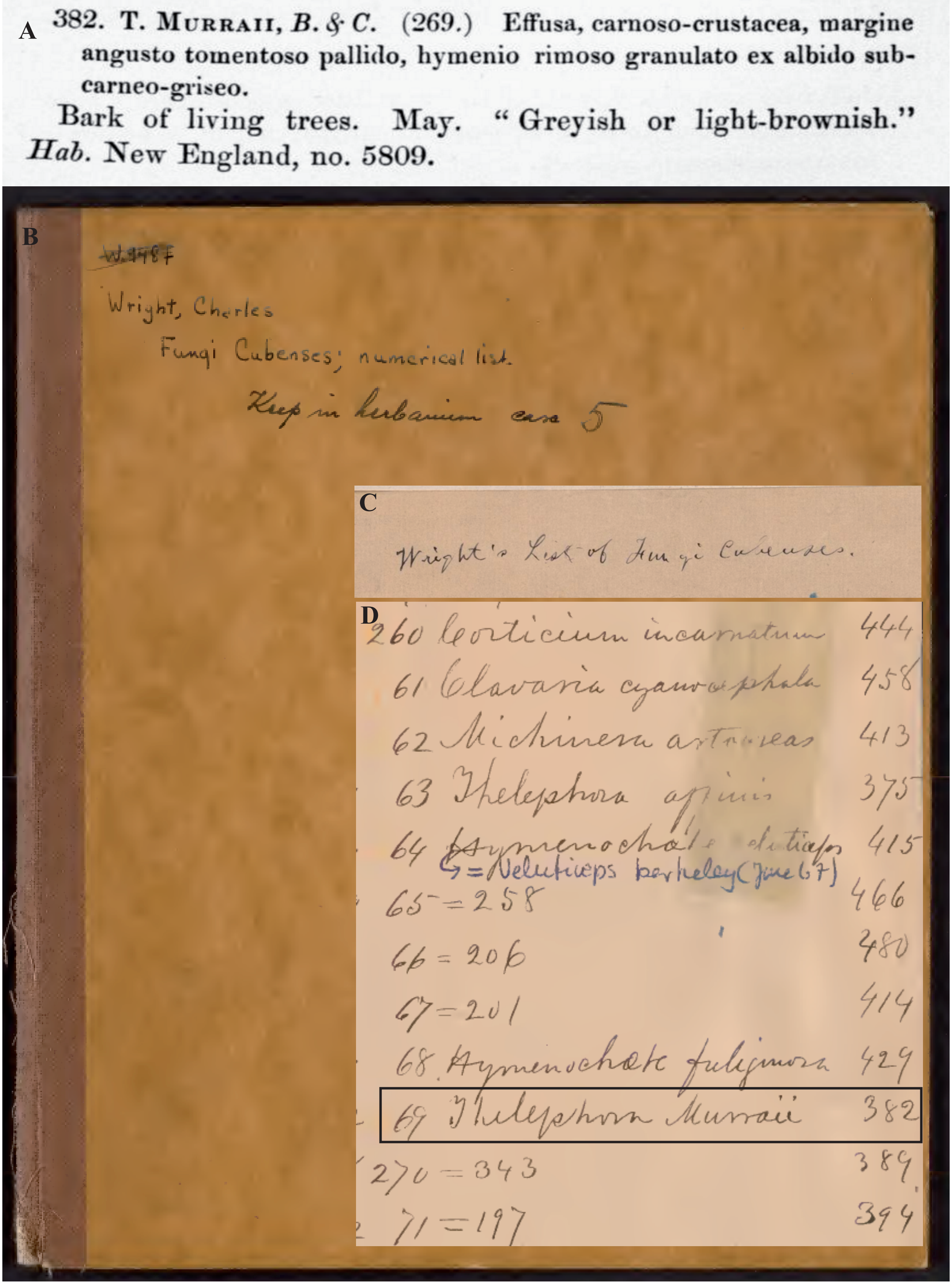


Fig. 14. ***Cystostereum murrayi*** (Berk. & M.A. Curtis) Pouzar (Charles Wright 269, K(M)264815, type). **A.** Basidioma dry specimen. **B.** Hymenophore. **C.** Generative hyphae, with clamp. **D.** Skeletal hyphae. **E.** Gloeocystidium content **F.** Hymenium. **G.** Basidiospores. Scale bars: A = 2 cm; B = 2.5 mm; C, D = 5 µm; E = 10 µm; F, G = 5 µm.



382. T. **Murraii**, *B. & C.* (269.) **Effusa, carnoso-crustacea, margine angusto tomentoso pallido, hymenio rimoso granulato ex albido subcarneo-griseo.**

Bark of living trees. May. "Greyish or light-brownish."
Hab. New England, no. 5809.

~~W.1487~~

Wright, Charles
Fungi Cubenses; numerical list.
Keep in herbarium case 5

Wright's List of Fungi Cubenses.

260	Corticium incarnatum	444
61	Clavaria cyanocephala	458
62	Michenera artocreas	413
63	Thelephora affinis	375
64	Hymenochaete [illegible] G = Veluticeps berkeley (June 67)	415
65	= 258	466
66	= 206	480
67	= 201	414
68	Hymenochaete fuliginosa	429
69	Thelephora Murraii	382
270	= 343	389
71	= 197	394

Fig. 15. A. Original description of *Thelephora murrayi*, included in *Fungi Cubenses (Hymenomycetes)*, page 329 (Berkeley & Curtis 1869). **B**. Front cover of *Fungi Cubenses: numerical list*, manuscript handwritten by W.G. Farlow. **C**. Transcription: "*Wright´s list of Fungi Cubenses*". **D**. Page not numbered from *Fungi Cubenses: numerical list* in which appears *Thelephora murrayi*; the first column left species corresponds to Wright´s numbers collected in Cuba, and the second refers to the number of the species listed in Berkeley & Curtis (1869).

Cystostereum sirmaurense R. Kaur, Avn. P. Singh & Dhingra, Mycotaxon 134: 578 (2019)
MycoBank MB829964 **Fig. 16**

Basidioma effuse, resupinate, up to 0.75 mm thick, suberose to ligneous, upper surface between basidioma and substrate dark brown to black, hard, darkening the substrate, perennial, distinctly stratified in several layers; *hymenophore* tuberculate, yellow white (92. y White), *margin* determinate; microscopic sections strongly yellow in 3% KOH. *Hyphal system* dimitic, generative hyphae with clamps, hyaline, thin to slightly thick-walled, 2.5−3 µm wide; skeletal hyphae branched, thick-walled, 2−2.5 µm wide, abundant on subicular layer. *Gloeocystidia* numerous, with basal clamp, sometimes empty but usually with homogeneous yellow oily content, SA-, thin-walled, in horizontal direction in subiculum and vertical in subhymenium and hymenium, generally included but sometimes projecting above hymenium up to 10 µm, variable in shape: cylindrical in subiculum, 43−57 × 8−13 µm, subglobose to fusiform in subhymenium, sometimes pedunculated, 30−34(−53) × 14−21(−22) µm, subcylindrical in hymenium, 30−45(−60) × 9−13 µm. *Basidia* clavate, (17.5−)20−25 × 4−6 µm with four sterigmata and basal clamp. *Basidiospores* subcylindrical to cylindrical, 4−5.5(−6) × 2−3 µm, hyaline, smooth, thin-walled, inamyloid, indextrinoid, stained in Cotton Blue. L’ = 4.83, W’ = 2.38, Q’ = 2.03.

Ecology and habitat.— On *Myrica esculenta* and, according to information from GenBank sequences (Table 2) on *Cedrus deodara*.

Known distribution.— India, Himalayan proximities.

Material examined.— INDIA: Bageshwar, Kausani, Bhedchula, 29.69754N, 79.7532E, on *Myrica esculenta*, 2 Sep. 2011, S. Sanyal, B111 (TU113199*).

Notes.— The Indian specimen originally under *Cystostereum murrayi* was grouped together with two ITS sequences of Indian specimens retrieved from GenBank, also under *C. murrayi* (Fig. 7). Morphological description is congruent with that of *C. murrayi* from India done by Rattan (1977), except for basidiospores that are smaller and ellipsoid (4−4.5 × 1.8−2.2 µm), compared to those of the specimen studied, that are subcylindrical to cylindrical [4−5.5(−6) × 2−3 µm].

Cystostereum sirmaurense R. Kaur, Avn.P. Singh & Dhingra was recently described from India on the basis of morphological characters (Kaur et al. 2019). Holotype of this species was not available for this study, and there are no sequences deposited in public databases.

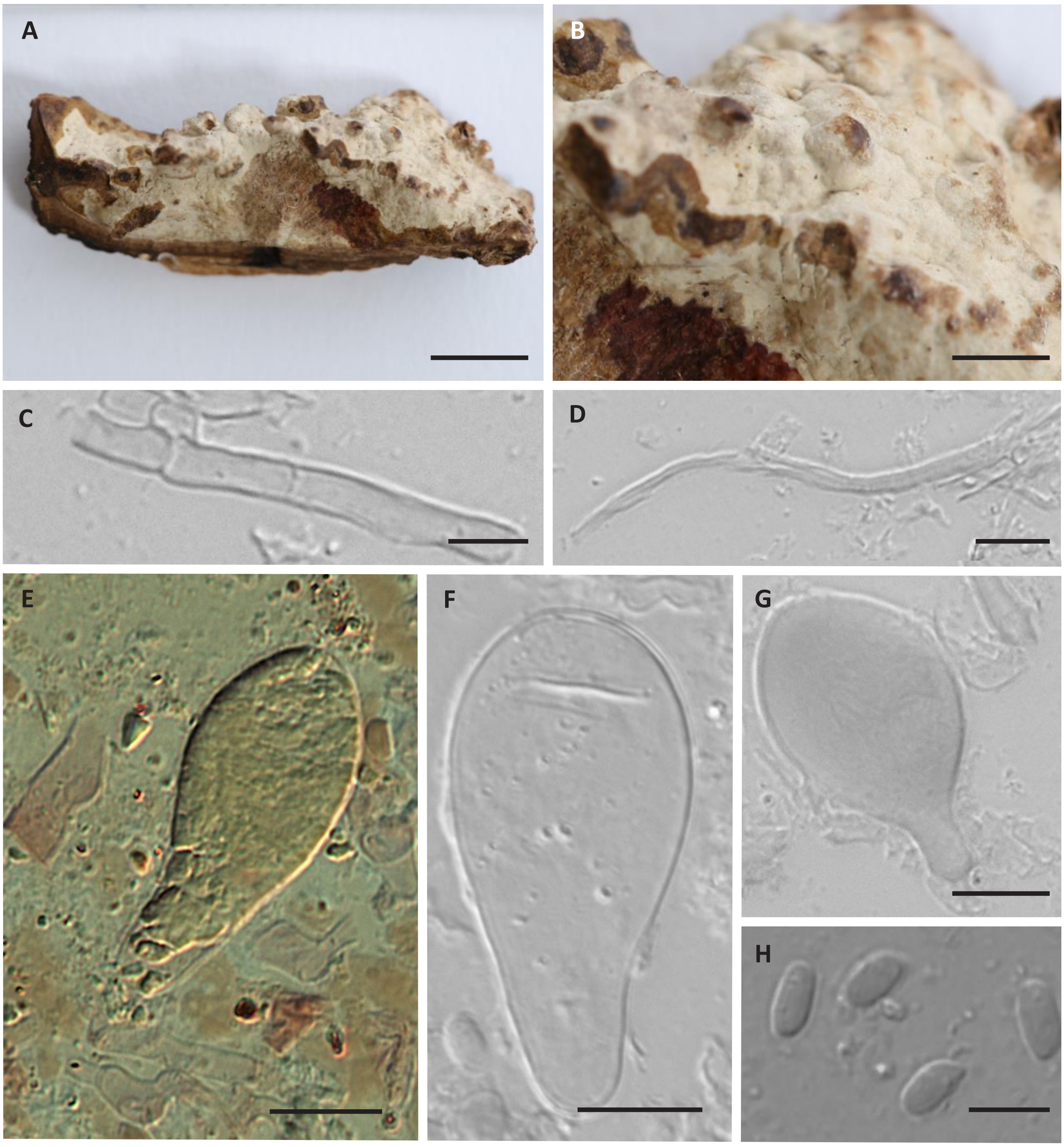


Fig. 16. ***Cystostereum sirmaurense*** R. Kaur, Avn. P. Singh & Dhingra (TU 113199). **A.** Basidioma dry specimen. **B.** Hymenophore. **C.** Generative hyphae, with clamp. **D.** Skeletal hyphae. **E.** Gloeocystidium content. **F, G.** Gloeocystidium. **H.** Basidiospores. Scale bars: A = 5 mm; B = 2.5 mm; C, D = 5 µm; E-G = 10 µm; H = 5 µm.

The specimen studied shows some differences with the description of *C. sirmaurense*: skeletal hyphae are up to 5.2 µm wide in *C. sirmaurense*, while are 2–2.5 µm in TU113199; basidia are slightly smaller in *C. sirmaurense*, 15–20 × 3.8–4.7 µm respect of (17.5–)20–25 × 4–6 µm in TU113199; and basidiospores are smaller and broadly ellipsoid in *C. sirmaurense*, 3.3–4.2 × 2.3–3.3 µm, while are cylindrical with a range 4–5.5(–6) × 2–3 µm (Q=2.03) in TU113199. Further analyses should be conducted to determine if this specimen belongs to *C. sirmaurense* or correspond to a different taxon.

Cystostereum tuberculosum (Fr.) Nogal, Telleria, M. Dueñas, M.P. Martín **comb. nov.**
[MycoBank no. pending] **Fig. 17**

≡*Stereum tuberculosum* Fr., Hymenomycetes Europaei: 644 (1874).

Non *Stereum tuberculosum* Velen., České Houby 4-5: 762 (1922) [=*Peniophora quercina* (Pers.) Cooke, Grevillea 8(45): 20 (1879)].

≡*Stereum murrayi* f. *tuberculosum* (Fr.) Pilát, Sborník Československé akademie zemědělských věd 5: 399 (1930) ≡*Stereum murrayi* var. *tuberculosum* (Fr.) Pilát, Bulletin de la Société Mycologique de France 49: 43 (1933)≡*Corticium tuberculosum* (Fr.) Rick, Brotéria, Ciências Naturais 3(4): 162 (1934).

Type: Ad cortices Norvegiae, Blytt (F-175747 in UPS).

Basidioma resupinate to effuse-reflexed, variable in size, but usually large, 1–2 mm thick, suberose to ligneous, upper surface between basidioma and substrate dark brown to black, hard, darkening the substrate, perennial, distinctly stratified in several layers; *hymenophore* tuberculate, cracked when dried, yellowish white to grayish yellow (92. y White – 90. gr. Y), *margin* thin, determinate, white; sections strongly yellow in 3% KOH. *Hyphal system* dimitic; generative hyphae with clamps, in subicular layer parallel to substrate, densely interwoven, yellowish brown, thin to thick-walled, clearly differenciated, 2–4 µm wide; in subhymenial layers perpendicular to substrate, hyaline, thin-walled, 2–3 µm wide; skeletal hyphae mainly found in subicular layer, branched, 1–3 µm wide, thick-walled. *Hyphydia* not projecting in the hymenium, no or little banched. *Gloeocystidia* numerous, included, with basal clamp, sometimes empty but usually with homogeneous yellow oily content, SA-, thin-walled, in subicular layer in horizontal direction and vertical in subhymenial layers and hymenium, variable in shape:

cylindrical in subiculum, 40−90 (−100) × 7−12 µm, subglobose, fusiform in subhymenium, sometimes pedunculated, 21−38 × (13−)15−19 µm, and subcylindrical in hymenium, 42−57(−66) × 7−10 µm. *Basidia* subclavate to clavate, (20−)22−37 × 4−5(−6) µm, four sterigmata and basal clamp. *Basidiospores* cylindrical, 4−5.5(−6) × 2−2.5(−3) µm, hyaline, thin-walled, smooth, inamyloid, indextrinoid and cyanophilous. L' = 4.50, W' = 2.17, Q' = 2.07.

Ecology and habitat.— On *Abies* spp. and *Picea*.

Known distribution.— Europe, mainly reported from northern and northeastern. Eriksson & Ryvarden (1975), after studying collections from Scandinavia, mentioned that the distribution "agrees partly with the *taiga-element*". The distribution of this species also includes Czech Republic, Germany, Poland, Romania, Russia and Slovakia. Also it has been reported from Pyrennes in Spain (Telleria & Melo 1995).

Material examined.— CZECH REPUBLIC: Boubin, sur un tronc d'*Abies*, 30 Aug. 1960, P. Heinemann 2918 (BR5020133531597*); Boubínský prales, Šumava, ad corticem *Piceae* emortuae in silva primaeva, 8 Sep. 1936, A. Hilitzer (NY 1992045). FINLAND: Kuusamo, Korvasvaara, on *Picea abies*, 23 Aug. 1978, J. Jeppson, Bohlin nº 751 (GB-0084733*); Lapland, Rovaniemi par., Pisavaara Nature Park, Sorvannulikka, on fallen trunk of *Picea abies*, 18 Sep. 1962, Å. Strid & J. Eriksson, nº 10477 (GB-0084789*). GERMANY: Bayern, Weg von der Vereinsalpe nach Mittenwald, on *Abies pectinata*, 4 Sep. 1930, V. Litschauer (GB-0084795). NORWAY: Hedmark County, Löten, Gitvola, appr. 5 km east of Gloma River, 750-850 m asl., on *Picea abies*, 23 Aug. 1985, I. Melo & M.T. Telleria, 7165Tell. (MA-Fungi 11382*). POLAND: Sucha Beskidzka, Babia Góra National Park, on *Abies*, 16 Sep. 1973, N. Hallenberg & K-H. Larsson, nº 2707 (GB-0084786*); ibid., on *Picea*, 15 Sep. 1973, N. Hallenberg & K-H. Larsson, nº 2600 (GB-0084787*). ROMANIA: Suceava, Codrul Secular Slătioara, on *Picea* log, 16 Oct. 1985, N. Hallenberg, NH 9127 (GB-0084793*). RUSSIA: Arkhangelsk, Plesetsky Co., Yemtsa, *Picea obovata* fallen trunk, 20 Aug. 1965, E. Parmasto (TAAM 18408*); Karachayevo-Cherkesiya, Baduk, Teberda Nature Reserve, 43º22'00"N 41º41'00"E, 1600-1700 m asl, *Abies nordmanniana* fallen trunk, 21 Sep. 1968, E. Parmasto (TAAM 53168*); Komi, Ust-Kulom Co., Dyakk-Yoll, on *Abies sibirica* fallen trunk on lower side, 11 Aug. 1957, E. Parmasto (TAAM 6343); Komi, Ust-Kulom, Kulom-Yu upper course, *Abies sibirica* fallen trunk on lower side, 12 Aug. 1957, E. Parmasto (TAAM 6399); Krasnodarskiy, Guzeripl, Caucasus Nature Reserve, 900 m asl., *Abies nordmanniana* fallen trunk, 22 May 1975, E. Parmasto (TAAM 58736*); Perm, Cherdynsk Co., Vizhai, *Picea obovata* fallen trunk, 8 Sep. 1988, I. Parmasto (TAAM 126341*). SLOVAKIA: Montes "Český les", silva virginea "Diana" dicta apud Sw. Kateřina (distr. Tachov), ad truncum iacentem *Abietis albae*, cca 525 msm, 15 Sep. 1964, F. Kotlaba & Z. Pouzar, nº 3137 (NY 461646); Montes "Český les", silva virginea mixta Dobro*čský* prales apud Čierny Balog, ad truncum iacentem *Picea abies*, 1 Sep. 1961, F. Kotlaba & Z. Pouzar, nº 3124 (NY 461649). Sweden: Dalarna County, Älvdalen Municipality, Idre parish, Städjan (Städjan-Nipfjället) Nature Reserve, 61.95ºN 12.85ºE, 750-800 m asl., on *Picea abies* fallen trunk, 18 Sep. 1974, E. Parmasto (TAAM 052247); Gotland, 15 Jul. 1892, L. Romell (O-86914*); Hälsingland, Hassela par., Älvåsen, in a brook valley, fallen trunk of *Picea abies*, 7 Oct. 1966, B. & J. Eriksson, nº 8669 (GB-0084738*); Uppland, Nysätra par., the coniferous wood just SW of the personage, on bark and wood of a dry, fallen spruce-trunk, 17 Aug. 1939, S. Lundell & E. Åberg (F-014297*); Västerbotten, Degerfors, Överrödå, forest reserve on Mt. Trollberget, fallen trunk of *Picea abies*, 16 Sep. 1970, K. Hjortstam & Å. Strid, nº 7139 (GB-0084766*). Ukraine: Zakarpattia, Bohdan Forest Div., SW, 1000-1200 m asl., *Abies alba* fallen rotten trunk lower side, 17 Aug. 1956, E. Parmasto (TAAM 3933*); Zakarpattia, Kvasivskoe, Velikiy Trostyanets, forest comp. nº 1, 1000-1300 m asl., *Picea abies* fallen trunk lower side, 23 Aug. 1956, E. Parmasto (TAAM 4116*); ibid., forest comp. nº 4, 1000-1300 m asl., *Picea abies* fallen trunk, 23 Aug. 1956, E. Parmasto (TAAM 4094*).

Notes.— *Stereum tuberculosum*, originally described from Norway by Fries (1874), was also reported from Sweden (Romell 1895) and Czech Republic (von Höhnel & Litschauer 1908) and synonymized with *St. murrayi* by Burt (1920). Later, Pilát (1930a) proposed *S. murrayi* f. *tuberculosum,* and Pilát (1933) raised *S. murrayi* var. *tuberculosum*. Type specimen was not available to this study, although a Norwegian specimen (7165Tell., MA-Fungi 11382), and the Swedish one cited by Romell (1895) deposited in O (O-86914), were included in our analyses. Moreover, ITS sequences UDB037966, UDB037409 and UDB036765 from UNITE database, which correspond to Norwegian specimens, were incorporated in our ITS phylogenetic analyses (Fig. 7), and were grouped in this subclade.

Cystostereum tuberculosum is morphologically similar to *C. murrayi* and *Cytostereum effusum*. All of them have a perennial fruitbody, tuberculate hymenophore, darken the substrate, dimitic hyphal system with distinctly yellowish brown generative hyphae in basal layer, and numerous gloeocystidia variable in size and shape. However, they differ in the spores: cylindrical, 4−5.5(−6) × 2−2.5(−3) µm (Q = 2.07) in *C. tuberculosum*, and subcylindrical 5.5−7.5 × 3−4 µm (Q=1.82) in *C. murrayi*, and *C. effusum*, (4−)4.5−6 × 2.5−3.5(−4) µm (Q=1.74).

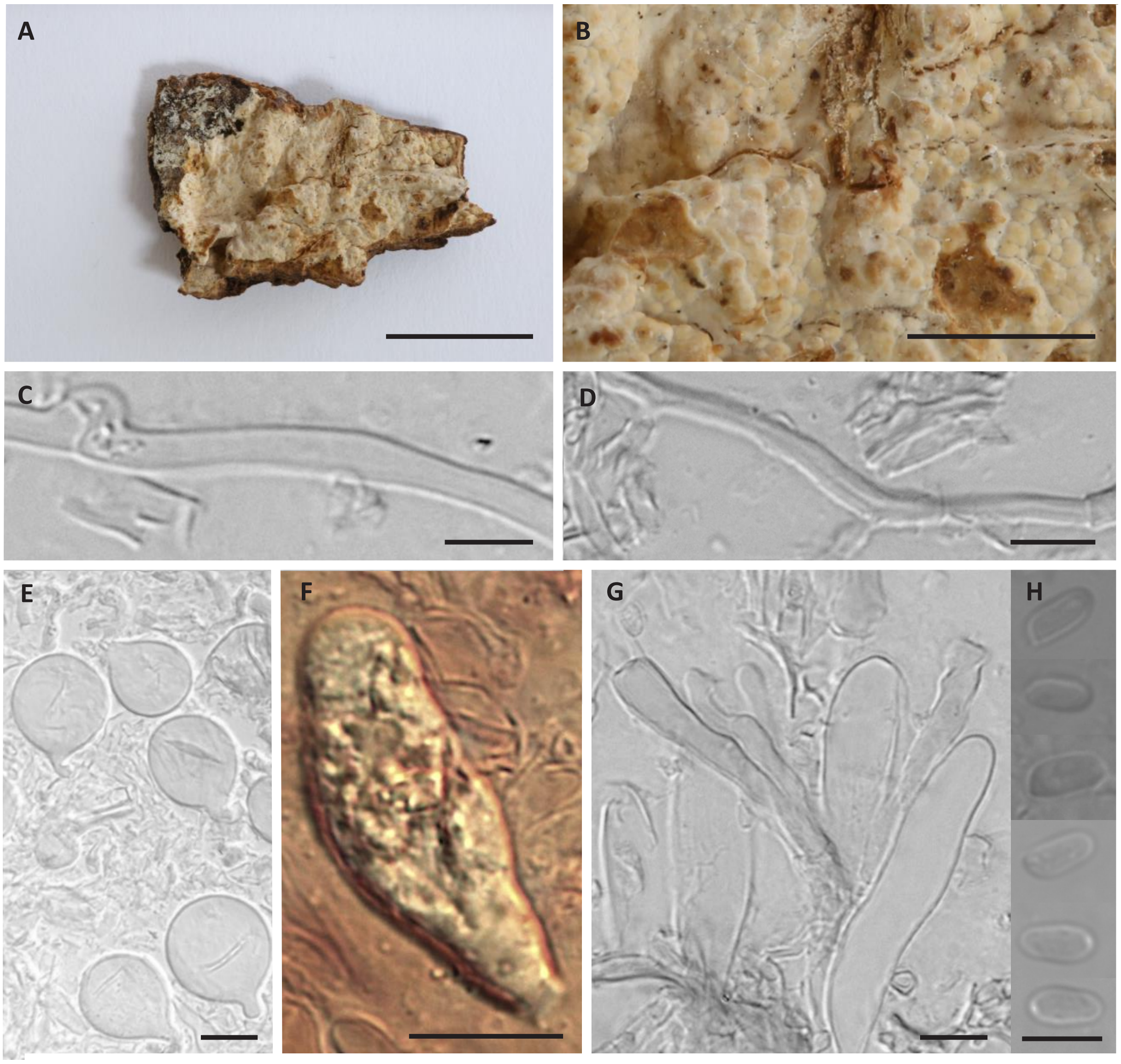


Fig. 17. ***Cystostereum tuberculosum*** (Fr.) Nogal, Telleria, M. Dueñas, M.P. Martín (7165Tell., MA-Fungi 11382). **A.** Basidioma dry specimen. **B.** Hymenophore. **C.** Generative hyphae, with clamp. **D.** Skeletal hyphae. **E.** Gloeocystidia subglobose in subhymenium. **F.** Gloeocystidia content. **G.** Gloeocystidia in hymenium. **H.** Basidiospores. Scale bars: A = 1 cm; B = 5 mm; C, D = 5 µm; E-G = 10 µm; H = 5 µm.

Differences of basidiospore measures between *C. tuberculosum* and *C. effusum* were supported by ANOVA statistical analyses (Table 6, Fig. 10). Also the three species differ in geographic distribution: *C. tuberculosum* is recorded from Europe, while *C. effusum* is known from North America, and *C. murrayi* is restricted to Cuba. Moreover, *C. tuberculosum* was found growing on conifers, while *C. effusum* was found above hardwood. Morphological differences between European and North American specimens were already noted by some authors. Bourdot & Galzin (1927) commented that North American specimens were more robust or older, with smaller gloeocystidia, and with a reflexed margin with black zone. Pilát (1930a, b) noted that *S. murrayi* (=*C. murrayi*) is extremely rare in Europe, with almost boreal distribution, and grows in conifers, while in North America is very frequent and grows above hardwood. Finally, Eriksson & Ryvarden (1975) found that European specimens have thinner fruitbody, greyish white hymenophore and spores are smaller than those from North America.

Incertae sedis

Cystostereum saxitas (Burt) A.L. Welden Flora Neotropica Monograph 106: 61 (2010)

MycoBank MB584286

≡*Stereum saxitas* Burt, Annals of the Missouri Botanical Garden 7: 134 (1920)

Type: MEXICO, Cuernavaca, 28 Dec. 1909, W.A. & E.L. Murrill, 419 (NY 776087!, holotype)

Basidioma effused, resupinate with slight reflexed margins, up to 1 mm thick, crustaceous, perennial, distinctly stratified; *hymenophore* smooth to tuberculate, scarcely cracked, pale yellow (89. p. Y); *margin* determinate, thick, reflexed**.** *Hyphal system* dimitic: generative hyphae with clamps, hyaline, thin-walled, 2.5–3 µm wide; skeletal hyphae scattered, mainly found in subicular layer, 1.5–2 µm wide, thick-walled. *Hyphidia* hyaline, cylindrical, thin-walled, with basal clamp, sinuous, tapering toward the apex, 30–35 × 2.5–3 µm. *Gloeocystidia* included, with basal clamp, sometimes empty but usually with yellow oily content, in vertical direction, SA-, CB-, thin-walled, variable in shape: subcylindrical, in all layers, 65–95 × 7–10 µm, subglobose, scarce, in hymenium and subhymenium, 30–45 × 11–14 µm. *Basidia* clavate, 24–28 × 4–6 µm, four sterigmata, with basal clamp. *Basidiospores* subglobose to broadly ellipsoid, 5.5–7 × 4–6 µm, hyaline, smooth, thin to thickening walled, inamyloid, indextrinoid, and cyanophylous. L’ = 6.05, W’ = 4.9, Q’ = 1.23.

Ecology and habitat.— Not known.

Known distribution.— This species is known only from the type locality in Mexico.

Notes.— Burt (1920) described this species as *Stereum saxitas* on the basis of two specimens, the holotype from Mexico and a paratype from Jamaica. The specimen from Jamaica was revised by Chamuris (1988), and confirmed in this study, as *Cystostereum australe*. Based on morphological characters, *Stereum saxitas* was combined to *Cystostereum* by Welden (2010).

Cystostereum stratosum Hallenb., Iranian Journal of Plant Pathology 14: 51 (1979)
MycoBank MB312566

Type: Iran, Gilan, Asalem, on a fallen log, 17 Jul. 1976, N. Hallenberg 1925 (GB-0156903! *, holotype)

Basidioma perennial, adnate, effused, resupinate with slight reflexed margins, up to 1.2 mm thick, crustaceous, upper surface between basidioma and substrate dark brown to black, darkening the substrate, distinctly stratified; *hymenophore* smooth to tuberculate, with tubercles variable in size, slightly cracked when dried, yellow white to pale yellow (92. y White − 89. p. Y); margin determinate, sometimes reflexed. *Hyphal system* monomitic, generative hyphae with clamps, in subicular layer parallel to substrate, yellowish brown, thin- to thick-walled, 2−4 µm wide, clearly differenciated; in subhymenial layer perpendicular to substrate, hyaline, thin-walled, 1.5−2 µm wide. *Gloeocystidia* numerous, in all layers, sometimes projecting above hymenium up to 20 µm, with basal clamp, with yellow oily content, SA-, CB+, thin-walled, subcylindrical to clavate, 50−80(−90) × (4−)5−8 µm. *Basidia* clavate, 27−35 × 5−6 µm, four sterigmata, with basal clamp. *Basidiospores* subcylindrical to cylindrical, 6.5−8 × (3−)3.5−4.5 µm, hyaline, smooth, thin-walled, inamyloid, indextrinoid, cyanophilous. L’ = 6.82, W’ = 3.68, Q’ = 1.85.

Ecology and habitat.— On a fallen log.

Known distribution.— This species is currently known from the type locality in Iran and from India (De 1995).

Notes.— According to Hallenberg (1978), *C. stratosum* was placed in *Cystostereum* by having tuberculate hymenophore, gloeocystidia with yellowish resinous content, densely agglutinated subhymenial hyphae, and the size and shape of basidiospores. However, on the basis of our phylogenetic results (Fig. 7), ITS sequence of *C. stratosum* did not group into the Cystostereaceae clade. However, the sequences of LSU and *rpb*2 grouped with *Cystostereum tuberculosum*. Morphologically, it is separated from *Cystostereum* by its monomitic hyphal system, longer and narrower subcylindrical cystidia that stain in Cotton Blue, and larger basidiospores. Ghobad-Nejhad & Hallenberg (2012) considered this species as synonymous with *Crustomyces expallens* (Bres.) Hjortstam (=*Laeticorticium expallens* (Bres.) J. Eriksson & Hjortstam). However, it would be necessary to study more collections of this species, both with morphological and molecular analyses to determinate its taxonomic position.

Parvodontia cf. ***albocrustacea*** (Rick) Baltazar & Rajchenb., Phytotaxa 255(2): 103 (2016)

MycoBank MB815703

Material examined.— Hawaii: South Hilo district, Scenic Drive, 18 Nov. 1998, on *Mangifera indica*, R.L. Gilberston 2219 (ARIZ-M-AN10442).

Notes.— The Hawaiian specimen under *Crustomyces heteromorphus* (ARIZ-M-AN10442, R.L. Gilberston 2219) that corresponds to the singleton III is a misidentification. The specimen studied shows an odontoid hymenophore, monomitic hyphal system, conidium-like structures in deep layer, gloeocystidia with yellowish content, 19–50 × 5.5–10 µm, small basidia 6–12.5 × 4–5 µm, and ellipsoid basidiospores, 5–6 × 2–3(–3.5) µm. These characters remind us of the description of *Parvodontia albocrustacea* (Baltazar et al. 2016).

The genus *Parvodontia* has two species, *Parvodontia luteocystidia* Hjortstam & Ryvarden and *Parvodontia albocrustacea* (Rick) Baltazar & Rajchenb. Further analyses should be conducted including the types of the two *Parvodontia* species to determine if this genus belongs to Cystostereaceae.

Unnamed Subclade V-A

Basidioma perennial, adnate, resupinate, crustaceous, up to 0.5 mm thick, upper surface between basidioma and substrate dark brown to black, hard, darkening the substrate, distinctly stratified; hymenophore smooth to tuberculate, pale orange yellow (73. p. OY); *margin* determinate. *Hyphal system* dimitic, generative hyphae with clamps, hyaline, thin-walled, branched, from 2–3 µm wide; skeletal hyphae branched, 0.5–1.5 µm, thick-walled. *Gloeocystidia* numerous, included, with basal clamp, sometimes empty but usually with yellow content, dextrinoid, SA-, variable in shape, cylindrical ventricoses, 55–170 × 7–10 µm, some of them subglobose and pedunculated 20–38 × 10–15 µm. *Basidia* subclavate to clavate, 15–23 × 3–4 µm four sterigmata and basal clamp,. *Basidiospores* subcylindrical to cylindrical, 3.5−5 × 2−3 µm, hyaline, thin-walled, smooth, inamyloid, indextrinoid, and cyanophylous. L' = 4.64, W' = 2.57, Q' = 1.81.

Ecology and habitat.— On dead wood, old trunk and hardwood limb.

Known distribution.— Cuba, Jamaica, Puerto Rico.

Material examined.— Cuba: Santiago de Cuba Province, Alto Cedro, on dead wood in low dense virgin forest, 19-20 Mar. 1905, Earle & Murrill, 491 (NY 1990982*); Puerto Principe Province, Ciego de Avila, on dead wood in low dense virgin forest, 21 March 1905, Earle & Murrill, 590 (NY 1990983*); Pinar del Rio Province, Herradura, on dead wood in dense thickets, 7-12 Mar. 1905, Coll.: Earle & Murrill, 188 (NY 1990981*). Jamaica: Constant Spring Hotel Grounds and Ravines in vicinity, 600 ft., on log, 13 Dec 1909, W.A. Murrill & E.L. Murrill (NY 1990979*); Port Antonio, old trunk, 20 Oct.-24 Nov. 1902, F.S. Earle, 575 (NY 1990977*). Puerto Rico: Route 191, km 10.3, Big Tree Trail, on a hardwood limb, 30 Apr. 1975, E.C. Setliff & A.R. Manion, 1047 (NY 1992016*).

Notes.— These specimens were included in Burt (1920) under *Cystostereum murrayi*. However, on the basis of our phylogenetic analyses, they do not belong to this genus, nor to any other in Cystostereaceae. Sequences were obtained from all specimens, but only the specimen ECS 1047 (NY 1992016) from Puerto Rico is in good condition for microscopic study, as was previously appreciated by Welden (2010). As shown in Figs. 6-8, they formed its own clade, as one of the sister clades to Cystostereaceae. BLAST searches revealed these sequences have high similarity to sequence MT023735 under *Gloeostereum cimri* (99.76 %), obtained from a culture isolated from a lung of a patient in USA, and sequence MT820373 under *Chondrostereum* sp. (99.05%), which was isolated from wing membrane of a Brazilian bat; both included in our ITS analyses (Fig. 7). Since these two sequences from GenBank were obtained from cultures, we do not have morphological characters to compare with the specimens from Cuba, Jamaica and Puerto Rico. Moreoever, the available sequence of *Gloeostereum cimri* corresponds to the type culture and the species was formally described (Ahmed et al. 2020), but it was considered an invalid name according to Art. 40.8 (Turland et al. 2018), since its protologue does not include a statement that the culture is preserved in a metabolically inactive state. On the basis of morphological and molecular data, the taxonomic identity of specimens from Cuba, Jamaica and Puerto Rico could not be interpreted, and that is why this clade is treated as *incertae sedis*.

DISCUSSION

In the present study, phylogenetic analyses based on intense sampling of *Cystostereum sensu lato*, made it possible to circumscribe not only *Cystostereum sensu stricto*, but also to confirm the genera *Crustomyces* and *Cystidiodontia*, since their type species were transferred from *Cystostereum*. On the basis of morphological characters, Jülich (1981) described the family Cystostereaceae with *Cystostereum* as generic type and at time includes *Crustomyces*; later, on the basis of molecular analysis of 5.8S and LSU nrDNA, Larsson (2007) showed that *Cystidiodontia* belongs to the family with high support in the order Agaricales. Our phylogenetic results based on four markers (ITS nrDNA, LSU nrDNA, *rpb*2 and *rpb*1) showed that the family is resolved as monophyletic with high support in all phylogenies (MPbs ≥ 92, MLbs ≥ 88, pp = 1), as was previously found by Song et al. (2008). Besides, a fourth clade with a Hawaiian specimen, ARIZ-M-AN10442 (R.L. Gilbertson 22129), originally under *Crustomyces heteromorphus* is resolved as misidentification, since it grouped as a sister of *Cystidiodontia,* and morphologically would be included in *Parvodontia,* a genus considered to be in this family (Larsson 2007).

These results are in accordance with morphological evidence; indeed, mapping of morphology over phylogenies shows strong support for relationships within genera in Cystostereaceae, emphasizing the relevance of morphological data in classification of fungal taxa, as was previously proven in other studies of corticioid fungi (Telleria et al. 2010b). According to Hjortstam (1983), *Crustomyces* and *Cystidiodontia* are more similar, since both have dendrohyphidia and gloeocystidia, more or less moniliform, with central resinuous body; also, they are genetically more closely related to each other than to *Cystostereum*, which also differs morphologically by having gloeocystidia with oily content and does not develop dendrohyphidia.

Concerning *Cystostereum*, between seven and eight species were contained in this genus when we started this study. On the basis of our molecular and morphological results, *C. australe* and *C. kenyense* are confirmed as *Cystostereum* species that are closely related. In contrast, the species *C. saxitas* and *C. stratosum*, both currently only known from type material, are listed as *incertae sedis* due to the lack of molecular data on *C. saxitas*, or because the incongruence in molecular results, such as in *C. stratosum*. In this last species, ITS analyses place it outside the Cystosteraceae family, whereas LSU and *rpb*2 analyses place *C. stratosum* in subclade IV-A; in addition, some remarkable morphological differences were found compared to the species confirmed in *Cystostereum*, such as monomitic hyphal system and longer cystidia that stained in cotton blue; this species is here considered as not belonging to *Cystostereum*.

The widely distributed species *C. murrayi* turned out to be polyphyletic, since it is resolved into five different taxa with narrower distributions, of which one does not correspond to *Cystostereum*. Apart from the type, *C. murrayi* from Cuba, three species are resolved in the genus (also supported by barcoding analyses, Table 5), two of them are proposed as new combinations of synonyms previously of *C. murrayi*: *C. effusum* from North America and *C. tuberculosum* from Europe, and a species recently described from India, *C. sirmaurense.* A fifth clade, represented by specimens from Cuba, Jamaica and Puerto Rico, previously identified as *C. murrayi*, is related to *Gloeostereum* and *Chondrostereum* sequences retrieved from GenBank; however, on the basis of the available morphological and molecular data, the taxonomic identity of these specimens could not be confirmed and they are listed as *incertae sedis*.

How species are recognized affects our knowledge about fungal diversity and, consequently, to understanding of its geographic distribution (Taylor et al. 2006). Phylogenetic results show that geographic distribution of *C. murrayi* has been overestimated on the basis of morphological approach alone. However, a second revision based on the background of phylogenetic analyses provides morphological information that supports the distinction among the resulting phylogenetic taxa with more restricted distributions. Indeed, previous authors had already noted some morphological differences between the North American and European taxa placed under *C. murrayi* (Bourdot & Galzin 1927, Eriksson & Ryvarden 1975). The main differences detected involved basidiospore dimensions, proving that spores have an important diagnostic value for distinguishing fungi at the species level (Parmasto & Parmasto 1987). Among closely related species studied under molecular approaches, spore dimensions have been the main characters that delimit the species: *Hyphodermella corrugata* and *H. rosae* (Telleria et al. 2010a), *Hypochnicium* spp. (Telleria et al. 2010b), *Jaapia* spp. (Telleria et al. 2015) or *Xylodon* spp. (Fernández-López et al. 2020). Sometimes, morphological differences cannot be found, such as between *Hyphoderma macaronesicum* and *H. paramacaronesicum* (Martín et al. 2018).

In addition to molecular and morphological evidence, the substrate provides interesting insights for distinguishing among corticioid species (Spirin et al. 2021). As was previously mentioned by Pilát (1930a, b) and Eriksson & Ryvarden (1975), it is remarkable that European specimens originally under *C. murrayi* only grow above *Abies* and *Picea*, while North American ones grow preferably above hardwood. However, a single exception was published for a North American specimen that was found growing on pine logs in Pennsylvania, USA (Sumstine 1941). In contrast to all this evidence, crossing tests did not support a distinction between these species (Hallenberg 1984), since North American and European specimens are compatible.

These "illegal matings" (Hallenberg et al. 2007), meaning that compability was found among different lineages, were previously reported for different genetic lineages in other corticioid fungi, such as in *Peniophorella praetermissa* species complex (Hallenberg et al. 2007) or *Peniophora cinerea* species complex (Hallenberg & Larsson 1992).

As mentioned, at least four species are resolved in the *C. murrayi* complex, although it is likely that others may be pending to be revealed. Specimens under *C. murrayi* collected in New Zealand were included in this study, but their sequences were not obtained and they are not in good condition for morphological study. Also, *C. murrayi* was cited from Nepal (Rattan 1977) and South Africa (Doidge 1950, Talbot 1954), but those specimens are not available as a loan to be studied. Thus, it cannot be evaluated if these reports correspond to any of the known *Cystostereum* species.

An integrative approach provides an accurate framework to obtain reliable knowledge about species diversity, as well their geographic ranges. However, obtaining DNA data from dried specimens commonly presents some challenges, such as scarce biological material or low DNA quality, which could be severely affected by the age of a fungal specimen (Brock et al. 2009) as well by how specimens were collected, dried and conserved (Leino et al. 2009). This could be especially problematic with type specimens, since they are required to solve taxonomic and systematic issues (Bruns et al. 1990). In this study, after several attempts and using designed primers, ITS and *rpb*2 sequences of *Thelephora murrayi* type specimen (K(M)264815) were obtained, whose collection date is unknown, but it is necessarily older than its original description (Berkeley & Curtis 1869). These sequences were crucial not only to resolve the generic limits of *Cystostereum*, but also to confirm boundaries of the family Cystostereaceae. On the other hand, sequences were not obtained from specimens located in MEL (Australia) and PDD (New Zealand). These specimens are not older than most of those studied, so in this case preservation conditions instead of age are likely affecting DNA quality.

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Table S1. Specimens included in this study from which molecular data was not obtained. The species name is given according to the herbarium label.

Specimen voucher	Country	Date	Substrate
Crustomyces heteromorphus (Hallenb.) Hjortstam			
TU124341 (duplicate of (CFMR)AS-545 included in Table 1)	Italy	18 May 2014	
TU124342 (duplicate of (CFMR)AS 977)	Italy	12 Apr 2013	
AS-122 (duplicate of TU124343, included in Table 1)	Italy	10 Jul 2012	
Crustomyces subabruptus (Bourdot & Galzin) Jülich			
TAAM 15331, E. Parmasto	Armenia	16 Sep 1962	*Fagus orientalis* fallen trunk
GB-0181429, NH 10654[1]	Canada	12 Aug 1988	*Populus* log
MA-Fungi 78502, 12558MD	France	3 Nov 2008	On *Acer pseudoplatanus*
MA-Fungi 10633, 6525Tell.[1]	Spain	20 Nov 1984	*Fagus sylvatica*
MA-Fungi 10634, 5701Tell.	Spain	8 May 1984	*Fagus sylvatica*
MA-Fungi 10635, 3028Tell.	Spain	14 Jun 1983	*Fagus sylvatica*
MA-Fungi 10637, 3033Tell.	Spain	14 Jun 1983	*Fagus sylvatica*
MA-Fungi 13972, 2978Tell.[1]	Spain	14 Jun 1983	*Fagus sylvatica*
MA-Fungi 26547, 8574Tell.[1]	Spain	22 Jun 1988	*Fagus sylvatica*
MA-Fungi 38006, 3193IS	Spain	7 Feb 1987	*Fagus sylvatica*
GB-0084485, NH 11556	Turkey	2-12 Oct 1989	*Corylus*-branch
Cystidiodontia isabellina (Berk. & Broome) Hjortstam & Ryvarden			
(O)F-902744, L. Ryvarden 11299	Ethiopia	8-9 Jan 1973	
(O)F-902743, L. Ryvarden 5159	Tanzania		
Cystostereum Pouzar			
MA-Fungi 70581, 10819IS[1]	France	2 Nov. 2006	On *Quercus petraea*
Cystostereum artocreas (Berk. & M.A. Curtis ex Cooke) Hallenb. & Ryvarden			
NY 520008, L. Ryvarden	Kenya	23-24 Jan 1971	
(O)F-902719, L. Ryvarden 8735	Malawi	9-10 Mar 1973	
NY 542842, J. Chavelas & F. Hartado	Mexico	4 Sep 1959	
BR 5020034185417, J. Rammeloo 4076	Rwanda	27 Jul 1974	On dead bark
(O)F-902723, L. Ryvarden 11052	Tanzania	24-26 Feb 1973	
NY 520001, L. Ryvarden	Tanzania	17 Jan 1971	
TAAM 178734, L. Ryvarden 676, duplicate GB- 0084732 included in Table 1	Argentina	1-5 Mar 1982	On deciduous wood
Cystostereum australe Nakasone			
BPI 275836, duplicate of NY 776088 included in Table 1	Jamaica	Jun 1906	
(CFMR)FP-103021, A.S. Rhoads	USA: Florida	27 Aug. 1952	On *Carya* sp.
GB- 0084783 (duplicate (CFMR) HHB-6648 included in Table 1)	USA: Florida	18 Jul 1972	On *Ulmus* sp.
NY 1990986, R.P. Burke	USA: Alabama	Aug 1916	
Cystostereum murrayi (Berk. & M.A. Curtis) Pouzar			
MEL 2310915[1]	Australia	6 Aug 1967	Dead rotten long fallen limbs

Table S1 (cont.).

Specimen voucher	Country	Date	Substrate
Cystostereum murrayi (Berk. & M.A. Curtis) Pouzar			
NY 1992158[1]	Brazil	1930	
NY 461648, R.F. Cain	Canada	24 Aug 1940	On *Acer saccharum*
NY 520021, H.S.J. [1]	Canada	16 Sep 1938	On *Betula papyrifera*
NY 1992172, HEB 5716[1]	Canada	24 Jul 1957	
O-165927, TEB 229-05[1]	Norway	18 Aug 2005	*Picea abies*
TAAM 6343, E. Parmasto	Russia	11 Aug 1957	*Abies sibirica* fallen trunk
TAAM 6399, E. Parmasto	Russia	12 Aug 1957	*Abies sibirica* fallen trunk
TAAM 16141, E. Parmasto[1]	Russia	2 Sep 1961	*Carpinus cordata* fallen branch
NY 461646, F. Kotlaba, Z. Pouzar	Slovakia	29 Aug 1961	*Ad truncum iacentem Abietis albae*
NY 461649, F. Kotlaba, Z. Pouzar	Slovakia	29 Aug 1961	*Ad truncum iacentem Picea abies*
GB-0090658, L. Ryvarden	Sweden	10 Sep 2004	*Picea abies*
TAAM 52247, E. Parmasto	Sweden	18 Sep 1974	*Picea abies* fallen trunk
NY 461650, B. Shimek	USA: Iowa	11 Aug 1928	On hard maple log
NY 1992179, HEB	USA: Michigan	9 Jul 1955	
NY 1992171, HEB 11669	USA: New Hampshire	8 Jul 1963	
Cystostereum pini-canadense (Schwein.) Parmasto			
TAAM 15508, E. Parmasto[1]	Armenia	27 Sep 1962	*Capinus caucasica* fallen trunk
TAAM 18637, E. Parmasto[1]	Belarus	27 Aug 1966	*Populus tremula* fallen trunk
NY 1992057	Brazil	1906	In ligno frondoso
TAAM 16372, E. Parmasto[1]	Georgia	19 Oct 1963	*Carpinus caucasica* fallen branch
TAAM 7714, E. Parmasto	Russia	27 Aug 1958	*Abies sibirica* fallen trunk
TAAM 4017, E. Parmasto	Ukraine	19 Aug 1956	Fallen rotten trunk
TAAM 3733, E. Parmasto	Ukraine	13 Aug 1956	*Fagus sylvatica* fallen branch
(CFMR)L-10615, J.L. Lowe	USA: California	1 Nov 1958	Redwood
(CFMR)HHB-9143, H.H. Burdsall	USA: Michigan	21 Aug 1976	*Thuja occidentalis*
GB-0084460, H.H. Burdsall Jr.11361[1]	USA: Minnesota	6 Aug 1981	On white cedar
(CFMR)RLG-5891, R.L. Gilberston	USA: Montana	29 Jun 1966	*Larix occidentalis*
(CFMR)FP-101900, Nakasone	USA: Wisconsin	16 Jul 1983	Conifer
(CFMR)FP-131171-sp, M.J. Larsen	USA: Wisconsin	8 Jun 1972	*Populus*
Cystostereum saxitas (Burt) A.L. Welden			
NY 776087 holotype, BPI 276657	Mexico	28 Dec 1909	
Cystostereum subabruptum (Bourdot & Galzin) J. Erikss. & Ryvarden			
MA-Fungi 70601, 16728Tell.[1]	France	1 Nov 2006	Unidentified wood
GB-0084473, L. & N. Hallenberg, B. Danesh-Pashuuh	Iran	26 Apr-8 May 1978	On a fallen branch
GB-0084503, A. Raitviir[1]	Kyrgyzstan	26 Aug 1965	*Lonicera*
NY 520010, S. Dománski	Poland	8 Nov 1960	
MA-Fungi 390, M.T. Telleria	Spain	4 Jul 1978	*Quercus ilex* ssp. *ilex*
MA-Fungi 413, M.T. Telleria	Spain	4 Jul 1978	*Quercus ilex* ssp. *ilex*
MA-Fungi 885, M.T. Telleria	Spain	4 Jul 1978	*Quercus ilex* ssp. *ilex*
MA-Fungi 904, 24/78 E-2 Tell.	Spain	4 Jul 1978	*Quercus ilex* ssp. *ilex*
MA-Fungi 8530, 5317Tell.	Spain	29 Mar 1984	*Quercus ilex*
MA-Fungi 10632, 5701 bis Tell.	Spain	8 May 1984	*Fagus sylvatica*
MA-Fungi 37227, 24/78Tell.[1]	Spain	4 Jul 1978	*Quercus ilex* subsp. *ilex*
MA-Fungi 37229, 28/78Tell.[1]	Spain	4 Jul 1978	*Quercus ilex* subsp. *ilex*
MA-Fungi 37230, 7/78Tell.[1]	Spain	4 Jul 1978	*Quercus ilex* subsp. *ilex*
MA-Fungi 37231, 29/78Tell.[1]	Spain	4 Jul 1978	*Quercus ilex* subsp. *ilex*

Table S1 (cont.).

Specimen voucher	Country	Date	Substrate
Hydnum artocreas Berk. & M.A. Curtis ex Cooke			
BPI 258898	Canada		
BPI 258900, Burnham? Spaulding?	USA: Missouri	Jun 1906	
BPI 258901, Spaulding	USA: Missouri	1907	*Quercus* sp.
Hydnum laminiferum Berk. & M.A. Curtis			
NY 776209, type	Cuba		
NY 776210, C. Wright, possible type	Cuba		
BPI 2596211	USA: Florida	Mar 1923	
Irpex cervinus Rick			
NY 1990976, J. Rick	Brazil	1940	On dead wood
NY 1992162, J. Rick	Brazil	1941	On dead wood
NY 1992164, J. Rick	Brazil	1936	On dead wood
NY 985662, J. Rick 6424, isolectotype	Brazil	13 Apr 2005	On dead wood
Irpex cervinus var. ***subcervinus***			
NY 985650, J. Rick 6383, syntype	Brazil		On dead wood
NY 985651, J. Rick 6386, syntype	Brazil	Oct1939	On dead wood
NY 1992161, J. Rick	Brazil	1935	On dead wood
Odontia subabrupta Bourdot & Galzin			
BPI266212, J. Rick[1]	Brazil		Wood frondose
BPI266213, Theissen? [1]	Brazil		Substrate undeterminated
Peniophora piceina Overh.			
FH 00605291, D.H. Linder	USA: Maine	20 Aug 1940	*Picea rubra*
GB-0084463, H.G. Eno[1]	USA: Connecticut	14 Oct 1939	On *Juniperus virginiana*
Radulum pini-canadensis Schwein.			
NY 1998708, R.L. Gilberston 5357	Canada	24 Aug 1965	On *Abies balsamea*
Stereum murrayi (Berk. & M.A. Curtis) Burt			
NY 1992047, V. Litschauer 27[1]	Austria	19 Oct 1931	On *Abies pectinata*
GB-0084795, V. Litschauer	Germany	4 Sep 1930	*Abies pectinata*
PDD 14427, M.E. Lancaster[1]	New Zealand	Dec 1951	On *Weinmannia silvicola*
PDD 14428, G.H. Cunningham[1]	New Zealand	Jul 1950	On *Dacrydium cupressinum*
PDD 15479, J.M. Dingley[1]	New Zealand	Sep 1953	On *Dacrydium cupressinum*
PDD 15480, J.M. Dingley[1]	New Zealand	Feb 1948	On *Phyllocladus alpinus*
PDD 39341, W. Colenso b. 342[1]	New Zealand		On bark
FH 00607442, E. Batholomew 10431[1]	USA: Kansas	13 Sep 1928	Fallen oak limb
NY 1992002, J.R. Hansbrough 1284	USA: New York	24 Jun 1932	On *Betula papyrifera*
NY 1992003, J.R. Hansbrough 1285	USA: New York	8 Sep 1932	On *Betula papyrifera*
NY 1992005, H.D. House[1]	USA: New York	11 Aug 1919	*Betula lutea*
NY 1990993, duplicate of FH 00607441 included in Table 1	USA: North Carolina	18 Aug 1933	On hardwood log
NY 1990992, J.R. Hansbrough 1286[1]	USA: Vermont	10 May 1933	On *Pyrus malus*
FH 00607436, A. Pilát	USA: Wisconsin	Sep 1913	*Betula lutea*

Table S1 (cont.).

Specimen voucher	Country	Date	Substrate
Stereum tuberculosum Fr.			
GB-0084796, V. Litschauer[1]	Austria	12 Jun 1921	
NY 1992045, [L.A. Hilitzer] [1]	Czech Republic	8 Sep 1936	*Ad corticem Piceae emortuae*
BR 5020132243323, P. Sydow[1]	Germany	16 Jul 1923	*Fagus sylvatica*
NY 1990978, J.A. Stevenson[1]	Puerto Rico	4 Dec 1915	Rotten wood
NY 1992022, W.A. Murrill	USA: Maine	9 Sep 1905	On birch
NY 1992025, W.G. Farlow	USA: New Hampshire	Jul 1904	
NY 1992021, W.A. Murrill and E.L. Murrill	USA: New York	17-29 Jul 1912	
NY 1990980, W.A. Murrill & E.L. Murrill[1]	Jamaica	4 Jan 1909	
Thelephora murrayi Berk. & M.A. Curtis			
K(M)203388, Murray 5809	USA: New England	May	Bark of living tree
FH 00546382, C. Wright	Cuba		
Excluded taxa[2]			
ICN 154775, M.A. Reck 084/09	Brazil	25 May 2009	
(CFMR) HHB-19951	China	17 Mar 2016	
TAAM 208628, L. Ryvarden 10061 (duplicate (O)F-902727)	Kenya	25 Jan 1973	On a bark of a unidentified tree
TAAM 208629, L. Ryvarden 9413 (duplicate TAAM 178734)	Tanzania	8 Feb 1973	
(O)F-902724	Kenya	25-27 Jan 1973	

[1] Collections that are not in good condition for morphological study.

[2] Collections with ITS sequence that were not included in phylogenetic analyses since BLAST searches found high similarity (94-100%) with other genera of corticioid fungi: *Haploporus alabamae* (99.28%), *Phlebiopsis* sp. (100%), *Junghuhnia crustacea* (95.06%), *Junghuhnia crustacea* (98.59%), *Junghuhnia crustacea* (94.41%), in this order.

Chapter 3

Based on DNA sequences of ITS and *rpb*2, *Amylostereum orientale* is reported for the first time in Japan[*]

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ABSTRACT

Amylostereum laevigatum has been reported from Japan growing on *Cryptomeria japonica* and *Chamaecyparis obtusa*. Using morphological and molecular data (ITS nrDNA and *rpb*2 markers), we demonstrate that some specimens under *A. laevigatum* from Japan belong to *A. orientale*, a species described from China. *Amylostereum orientale* differs from *A. laevigatum* mainly in having smaller spores. This is the first citation of *A. orientale* in Japan.

Keywords: *Amylostereum laevigatum*, Basidiomycota, China, ITS nrDNA, *rpb*2 gene

INTRODUCTION

Amylostereum Boidin (Russulales, Basidiomycota) is a genus of corticioid fungi mainly growing on conifers, with resupinate to reflexed basidiomes, and characterized by lamprocystidia and narrowly cylindrical to ellipsoid, smooth and amyloid basidiospores (Eriksson & Ryvarden 1973, Boidin & Lanquetin 1984, Telleria & Melo 1995). According to Mycobank (http://www.mycobank.org, 28/06/2016), five species are included in this genus: *A. areolatum* (Fr.) Boidin, *A. chailletii* (Pers.) Boidin (the type species), *A. ferreum* (Berk. & M.A. Curtis) Boidin & Lanq., A. *laevigatum* (Fr.) Boidin, and *A. sacratum* (G. Cunn.) Burds. (≡*Gloeopeniophorella sacrata* (G. Cunn.) Hjorstam & Ryvarden); and recently, He & Li (2013) described a new species from China, *A. orientale* S.H. He & Hai J. Li. As mentioned in Slippers et al. (2003), for many years, *A. areolatum* and *A. chailletii* were known to establish obligate mutualistic relationships with woodwasps of the genus *Sirex*; Tabata & Abe (1997) showed that specimens of *A. laevigatum* from Japan establish this relationship with the woodwasps *Urocerus antennatus* Marlatt and *U. japonicus* Smith, effectively inoculating new wood (Slippers et al. 2003).

Amylostereum laevigatum has been reported in Japan (Tabata & Abe 1997) growing on *Cryptomeria japonica* D. Don and *Chamaecyparis obtusa* Sieb. & Zucc. (Tabata et al. 2000). This species was originally described from Europe (Sweden) (Fries 1828), where it is widespread (Eriksson & Ryvarden 1973, Legon 2005). It has been reported from Macaronesia: Madeira (Telleria et al. 2008), and the Azores islands (Dennis et al. 1977, Telleria et al. 2008, Telleria et al. 2009a, b); as well as from North America (Ginns & Lefebvre 1993), and China (Maekawa et al. 2002).

Based on analysis of the internal transcribed spacer (ITS) region of nrDNA, the barcode for fungi (Schoch et al. 2012), as well as on the manganese-dependent peroxidase gene, Tabata et al. (2000) showed that specimens of *A. laevigatum* from Japan and *A. laevigatum* from France grouped separately; also, they reported differences in the spore size and in their hosts. Tabata et al. (2000), without providing spore dimensions, noted that specimens from Japan present smaller spores than those from France. In the description of *A. orientale*, He & Li (2013) reported the spore size of this species as smaller than *A. laevigatum* from Europe, but they did not include specimens from Japan to confirm the presence of *A. orientale* in that country.

Therefore, the aim of this research was to clarify the identity of *Amylostereum laevigatum* from Japan using a barcoding approach (Schoch et al. 2012), and to provide an accurate revision of the morphological characters.

MATERIALS AND METHODS

Specimens examined.— Ochidani Park, on unidentified wood, 2 Jul. 2008 leg. H. Suhara, TUMH61905 (Tottori University); Nankan-achi, on *Cryptomeria japonica*, Jun. 2004, leg. N. Maekawa, TUMH61916 (Tottori University).

Culture examined.— Japan, Ochidani Park, 2 Jul. 2008, leg. H. Suhara, TUFC11625 (isolated from TUMH61905; Tottori University); Japan, Nankan-achi, Jun. 2004, leg. N. Maekawa, TUFC12106 (isolated from TUMH61916; Tottori University); Japan, without locality, cultures B1361 and B1362 isolated from *Urocerus japonicus* mycangium (Iowa University); Japan, Motoyama, culture B1368 isolated from basidiospores obtained from basidiome growing on *Cryptomeria japonica*; Japan, B1369 from *Chamaecyparis obtusa* (Iowa University). [Data from B1361, B1362, and B1369 as indicated in Tabata et al. (2000), and provided by T. Harrington. Data from B1368 as indicated in Tabata & Abe (1999)].

The genomic DNA from cultures B1361, B1362, B1368, B1369 was extracted using the FTA® Indicating Micro Cards (Cat N°WB120211, Whatman, Maidstone, England), following Telleria et al. (2014). The ITS nrDNA and *rpb*2 markers were amplified using Illustra™ PureTaq™Ready-To-Go™ PCR Beads (GE Healthcare, Buckinghamshire, UK). The ITS nrDNA was amplified with the primer pair ITS1/ITS4 (White et al. 1990; Liu et al. 1999), following thermal cycling conditions described in Martín & Winka (2000). To amplify and sequence ribosomal polymerase two, subunit two (*rpb*2), regions 5–7, nested-PCR was performed with the following combination of primers: the primer pair RPB2-5F/RPB2-7.1R (Liu et al. 1999; Matheny 2007) was used in the first amplification, and the pair RPB2-6F/RPB2-7.2R (Matheny 2007) in the second one, using 1 µL of the first amplification as target DNA. The PCR products were purified using the QIAquick Gel Extraction (Qiagen, Hilden, Germany) as indicated by the manufacturer. All these protocols were carried out at the Real Jardín Botánico (Madrid, Spain). Purified amplimers were sent to Macrogen (Belgium/South Korea) for sequencing.

From cultures TUFC12106 and TUFC11625, the genomic DNA was isolated following Hosaka & Castellano (2008). The ITS nrDNA and *rpb*2 markers were amplified using dNTPs, KOD FX Neo (TOYOBO Co., Ltd., Osaka, Japan) and PCR Buffer for KOD FX Neo. The ITS nrDNA was amplified using the primer pair ITS5/ITS4 (White et al. 1990), following a PCR program with an initial denaturation at 94ºC (1 min) was followed by 35 cycles of 94ºC (15 s), 55ºC (30 s) and 72ºC (1 min), and final extension at 72ºC (5 min). The *rpb*2 was amplified as indicated above. The PCR products were purified using the MonoFas DNA Purification Kit (GL Sciences, Tokyo, Japan), and sequenced with ABI 3130 DNA sequencer (Applied Biosystems Inc., California, USA) in Tottori University.

Consensus sequences were assembled using Geneious Pro v9 (http://www.geneious.com; Kearse et al. 2012). Prior to the alignments, sequences generated in the present research were compared with homologous sequences in EMBL/GenBank/DDBJ (Cochrane et al. 2011) using the BLASTn algorithm (Altschul et al. 1997) to check for contamination. Two alignments, one to each marker, were generated including the new sequences and selected sequences from *Amylostereum* species from EMBL/GenBank/DDBJ. In both alignments, *Echinodontium tinctorium* (Ellis & Everh.) Ellis & Everh. sequences were included as outgroup, since in Tabata et al. (2000), the ITS sequence of this species, as well as other *Echinodotium* species closely matched the ITS sequence of *Amylostereum*.

Three analyses were performed for each alignment. First, a maximum parsimony (MP) analysis using the program PAUP 4.0a147 (Swofford 2003), as indicated in Telleria et al. (2013), with a default setting to stop the analyses at 100 trees. Exhaustive searches were conducted, and gaps treated as missing data. Tree scores, including tree length, consistency index (CI), homoplasy index (HI), and retention index (RI) were calculated from each exhaustive search; non-parametric searches (Felsenstein 1985) were used to calculate branch support (bootstrap, MPbs), performing 10,000 replicates using the fast-step option. Second, a maximum likelihood (ML) analysis was performed using the RAxML (Stamanakis 2014) in the CIPRES portal (CIPRES Science Gateway v.3.3; Miller et al. 2010), assuming a GTR model (default model); nonparametric bootstrap (MLbs) support (Felsenstein 1985) for each clade, based on 1000 replicates was tested. Finally, a Bayesian analysis (Larget & Simon 1999, Huelsenbeck et al. 2001) was performed using the software MrBayes v.3.2. (Ronquist et al. 2012), and assuming the general time reversible model (Rodríguez et al. 1990), including estimation of invariant sites and assuming a discrete gamma distribution with six categories (GTR+I+G) selected by PAUP*4.0b10; the 50% majority–rule consensus tree and the posterior probability (pp) of the nodes were calculated from the remaining trees with MrBayes. To assess whether a barcoding gap exists (Hebert et al. 2004), Kimura-2-parameter (K2P) pair-wise ITS distances were calculated using the software PAUP 4.0a147. On the basis of the widely used threshold for barcoding studies, a barcoding gap exists if the average interspecific difference is 10 times the average intraspecific difference (Hebert et al. 2004).

RESULTS AND DISCUSSION

The 12 new consensus sequences have been lodged in the EMBL/GenBank/DDBJ (Accession Numbers included in Fig. 1A, B). A matrix of 534 aligned nucleotide positions was produced for the ITS (459 characters are constant, 31 parsimony informative and 44 parsimony-uninformative). The 100 most parsimonious trees gave a length of 93 steps, CI = 0.8710, RI = 0.9481, and RC = 0.8257. The topology of the parsimony strict consensus tree (not shown) was similar to the ML (not shown) and the 50% majority rule Bayesian tree (Fig. 1A): sequences of *A. laevigatum* from Japan appeared separated from sequences of *A. laevigatum* from Europes. The sequences from Japan grouped in the *A. orientale* clade along with the *A. orientale* sequences from He & Li (2013) with very high support (MPbs = 100 %, MLbs = 98 %, pp = 1.0). For the *rpb*2 gene, an alignment with 702 characters (541 characters are constant, 113 parsimony-informative and 48 parsimony uninformative) was produced. The 100 most parsimonious trees gave a length of 246 steps, CI = 0.7510, RI = 0.9402, and RC = 0.7061. The *rpb*2 topologies obtained with each analysis were identical to each other; the 50% majority rule strict Bayesian tree is shown in Fig. 1B. Sequences of *A. laevigatum* from Japan form a clade of their own, clearly separated from *A. laevigatum* from Europe. The alignments and trees were deposited in TreeBASE (Submission ID 20108).

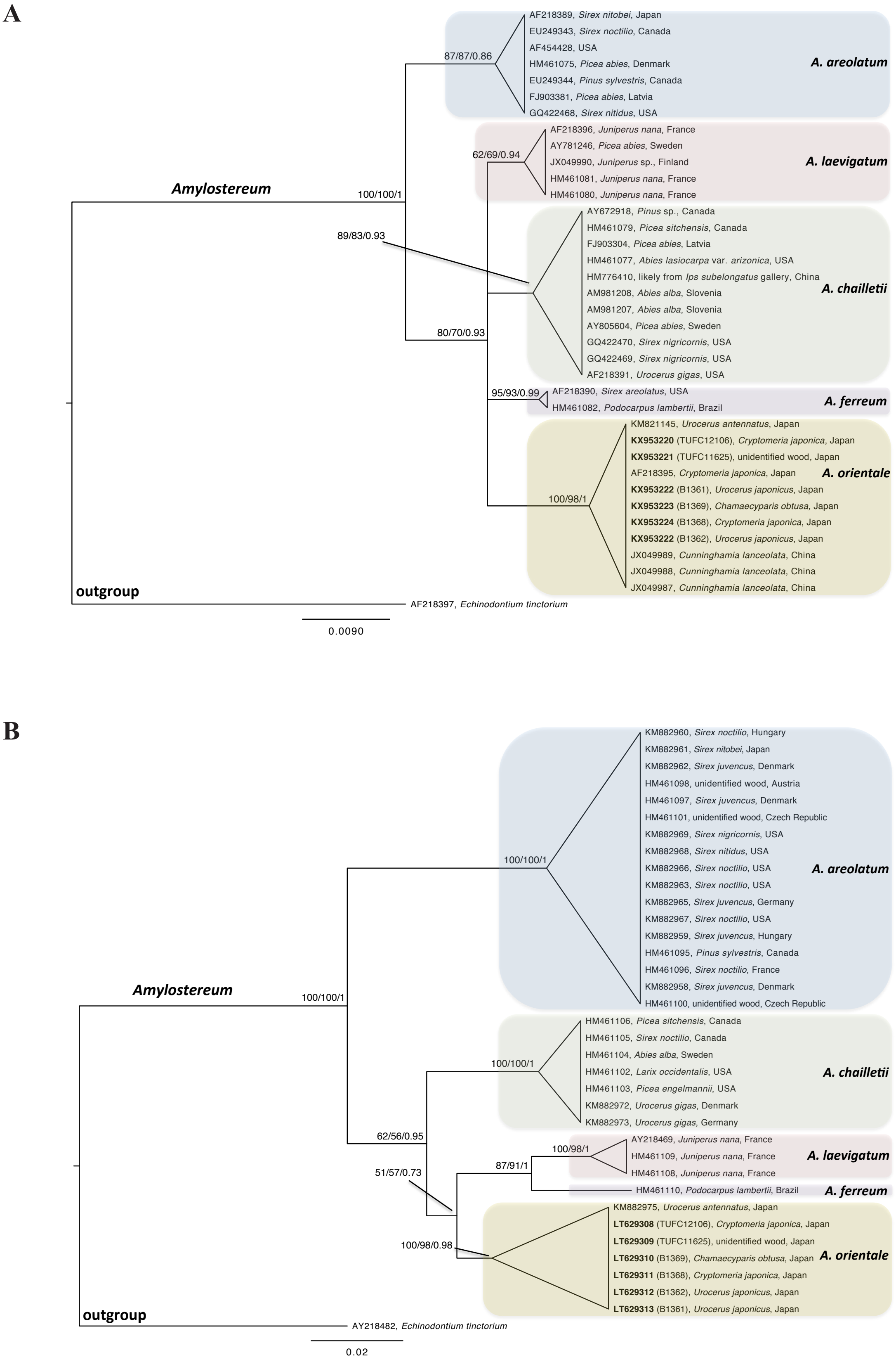

Figure 1. Phylogenetic trees obtained from Bayesian inference of sequences of *Amylostereum* spp. including *Echinodontium tinctorium* as outgroup. **A.** ITS nrDNA. **B.** *rpb*2. The host trees and localities are shown next to the accession numbers. Parsimony bootstrap support (MPbs), likelihood bootstrap support (MLbs) and posterior probability (pp) indicated above branches. New sequences obtained in this work are marked in bold face, and culture numbers are provided.

On the other hand, the K2P distances matrix (Table 1) indicates that the maximum genetic distance between ITS sequences of any two isolates of *A. laevigatum* from Japan is 0.00196, and between any two isolates of *A. laevigatum* from Europe is 0.00241; however, the large genetic distance between *A. laevigatum* from Japan and all the specimens of *A. laevigatum* from Europe (minimum 0.01894) confirms that they belong to different species. Moreover, the low value between *A. laevigatum* from Japan and *A. orientale* (maximum 0.00590), demonstrates that collections studied from Japan correspond to the species *A. orientale*. In *Amylostereum*, a barcoding gap exists in the ITS marker since the interspecific values are ten times higher than intraspecific values (Table 1), allowing identification to the species level.

Table 1. Matrix of pairwise intra- and interspecific Kimura-2 Parameter (K2P) distance among *Amylostereum* spp. ITS nrDNA sequences. The gray shading indicates the interspecific K2P among *A. orientale* and *A. laevigatum*, from Japan and Europe.

Taxa	**1**	**2**	**3**	**4**	**5**	**6**
1 *A. orientale*	0					
2 *A. laevigatum,* Japan	0.00590	0.00196				
3 *A. laevigatum,* Europe	0.01881	0.01894	0.00241			
4 *A. chailletii*	0.02189	0.01990	0.00991	0.00412		
5 *A. ferreum*	0.02556	0.02360	0.01173	0.01381	0	
6 *A. areolatum*	0.03338	0.03530	0.02547	0.03124	0.02738	0.00590

The revision of morphological features of TUMH61905 and TUMH61916 was performed following Telleria & Melo (1995) and He & Li (2013). A short description of these collections is provided. Basidiocarps resupinate, adnate and coriaceus; hymenium and context brownish with numerous lamprocystidia; hyphal system monomitic, with generative hyphae with clamps, thin-walled; lamprocystidia thick-walled, brownish, 22–26 × 5–6 μm; young cystidia subulate, hyaline, thin-walled, smooth; basidia subclavate to clavate, with a basal clamp connection and four sterigmata, 26 × 5–6 μm; basidiospores ellipsoid, hyaline, thin-walled, amyloid, 6.5 × 3.5–4.5 μm (very few observed).

As detected in Tabata et al. (2000), our molecular and morphological analyses clearly separate Japanese *A. laevigatum* from European *A. laevigatum*. The position in the ITS nrDNA tree, as well as the genetic K2P distance values obtained show that Japanese collections studied belong to *A. orientale*, instead of *A. laevigatum*. Although no *rpb*2 sequences from *A. orientale* from China are included in our analyses, the position of the Japanese collections in the *rpb*2 tree, forming a group of its own separated from *A. laevigatum,* support our conclusion. These are the first confirmed records of *A. orientale* in Japan.

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Chapter 4

Sailing with the wind: long distance dispersal explains the occurrence of *Amylostereum laevigatum* (Russulales, Basidiomycota) in Azores archipelago

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Manuscrito en preparación

ABSTRACT

Dispersal over long distances has been identified as relevant in dissemination of many organisms, especially toward oceanic archipelagos, although evidence is still limited in some groups such as corticioid fungi. The circumboreal species *Amylostereum laevigatum* has been reported from the Azorean islands of Flores, São Miguel and Terceira, fruiting on the endemic *Juniperus azorica* and introduced *Cryptomeria japonica*. Additionally, the close symbiotic relationship that different species of *Amylostereum* establish with woodwasps of the genus *Sirex* and the presence of *S. noctilio* in the Azores, suggests possible long distance dispersal (LDD) of *A. laevigatum* through insects. As active flight to remote oceanic islands appears unlikely for flighted invertebrates, wind-mediated LDD was hypothesized and evaluated from the fungal perspective. First, phylogenetic analyses based on ITS and *rpb*2 were conducted to confirm specimen identity and to determine its geographic origin; second, wind-mediated LDD was evaluated through a model-based approach. To test whether fungal genetic structure conforms better to isolation by distance or isolation by wind-mediated resistance, three different matrices were constructed: genetic, geographic and wind-mediated distance, and Mantel tests were conducted with matrix pairs (genetic-geographic, genetic-wind) to assess the correlation among them. Our results showed Europe as geographic origin of *A. laevigatum* in the Azores since North American specimens were resolved as a different *Amylostereum* species, and results showed that fungal genetic structure conforms better to the least cost pathway based on wind, supporting the wind-mediated LDD of *A. laevigatum* from Europe to Azores.

Keywords: Corticioid fungi, Mantel test, microsatellites, oceanic islands, symbiosis, woodwasps

INTRODUCTION

A high percentage of terrestrial taxa in the Azores are fungi, *ca*. 10% excluding lichens (Borges et al. 2010). Among this fungal diversity, several studies have focused on corticioid fungi (Dueñas et al. 2008, Melo et al. 2008, Dueñas et al. 2009, Telleria et al. 2009a, b, 2012, 2013), a polyphyletic group of effuse and resupinate wood decay basidiomycetes (Larsson 2007). One of the most representative corticioid species in the archipelago is *Amylostereum laevigatum* (Fr.) Boidin (Telleria et al. 2009b), the only species of the genus that has been found there. This species has been reported from Flores, San Miguel (Telleria 2009b) and Terceira (Dennis et al. 1977, Telleria et al. 2009a) islands, growing in the endemic *Juniperus brevifolia* subsp. *azorica* and the introduced *Cryptomeria japonica*.

Amylostereum laevigatum (Fr.) Boidin was originally described from mainland Europe by Fries (1828), where it is widespread (Eriksson & Ryvarden 1973, Legón 2005, Bernicchia & Gorjón 2010) and grows on gymnosperms such as *Cupressus*, *Juniperus*, *Taxus* and cultivated *Thuja*. In addition, it was also reported from Madeira (Telleria et al. 2008), North America (Ginns & Lefebvre 1993), China and Japan (Maekawa et al. 2002, Tabata & Abe 1997, 1999), where it was found fruiting on *Cryptomeria japonica* and *Chamaecyparis obtusa*. However, He & Li (2013) and Nogal-Prata et al. (2017) proved that records from China and Japan correspond to *A. orientale.* Some *Amylostereum* species are well known to be involved in symbioses with siricid woodwasps (Talbot 1977, Slippers et al. 2003). It is spread and inoculated into new wood by females during oviposition (Gilmour 1965, King 1966); the fungus rots and dries the wood, providing a suitable environment for development and survival of insect larvae (Madden & Coutts 1979). The symbiont, *Sirex noctilio*, is a woodwasp with worldwide distribution, native to Europe, the Azores, North Africa, and Asia (Smith 1978, Ciesla 2011); and it became a pest in several countries of the southern hemisphere in plantations of conifers (Ciesla 2011).

The biota of remote oceanic islands has long been recognized as a result of long distance dispersal (LDD) events (de Queiroz 2005). Although dispersal has been identified as the fundamental factor shaping the current distribution of global biodiversity (Waters & Fraser 2016), this account was mainly conceived from studies of oceanic islands, since these have never been connected to the mainland and their biota has necessarily been received by dispersal from distant areas (Cowie & Holland 2006). Furthermore, oceanic islands represent model systems to drive studies about LDD due to their discrete geographical nature, reduced gene flow by oceanic barriers and small size in comparison with continental landmasses (Emerson 2002).

Located in the North Atlantic Ocean between 36º45’- 39º43’N latitude and 24º45’- 31º17’O longitude, the Azores archipelago appears formed by nine volcanic islands, which are geologically recent (20-36 Myr). It is an isolated archipelago, separated by approximately 1300 km from Western Europe, 1600 km from Eastern North America, and 800 km from Madeira. Azorean biota is partly the result of LDD events (Borges et al. 2009), although a high percentage of introductions were reported (Silva & Smith 2004). This is consistent with the lower percentage of endemism compared with the other oceanic islands, such as Madeira or Canary islands (Borges et al. 2009). Regarding LDD, although most storms and prevailing winds come from the west (Borges et al. 2009), studies carried with flightless beetles (Borges et al. 2005), mollusks (Cameron et al. 2007), or vascular flora (Fernández-Palacios et al. 2011), suggested a dominant Palearctic/Macaronesian origin of the Azorean biota. However, there are clear exceptions to this geographic origin, as Azorean goldenrod *Solidago azorica* (Schaefer 2015) and the bryophyte *Leptoscyphus azoricus* (Vanderpoorten & Long 2006) are of American origin.

Although progress has been made in knowledge about fungal diversity, little is known about biogeography, ecology and evolution of fungi in oceanic archipelagos (Keirle et al. 2012). Research about LDD has been mainly carried out with plants or animals, because it appears difficult to address in case of microorganisms or larger organisms that produce microscopic propagules (Tesson et al. 2016), such as fungi. In this context, Holyoak et al. (2008) showed that only 1.3% of published studies about biotic movement were focused on fungi, while more than 60% were conducted with plants, mammals, birds and insects. The traditional view is that fungi are cosmopolitan organisms that do not have dispersal limitations (Bisby 1943). But challenges to this view have received lower attention because of taxonomic, empirical and methodological difficulties (Golan & Pringle 2017). However, molecular phylogenies are providing contrary evidence to wide distributions, and prevalence of LDD is being questioned in many cases. However, concerning oceanic archipelagos, it may be normally accepted that the main fungal biota of oceanic archipelagos is a result of LDD events from distant areas (Li et al. 2020), although mechanisms remain unknown.

For sessile terrestrial organisms, dispersal is defined as passive and it is mediated by either abiotic or biotic vectors (Nathan 2006). In fungi, wind has been regarded as the most important abiotic vector implied (Norros 2013), whereas water is considered to play a minor role (Edam 2003), and different animals were indicated as biotic vectors (Watkinson et al. 2015). In the particular case of wood inhabiting fungi, these have generally been supposed to be dispersed by wind (Junninen & Komonen 2011); even though insect-vectored dispersal has been also identified as relevant for mutualistic (Harrington 2005) and non-mutualistic (Jacobsen et al. 2017) wood inhabiting fungi.

Somehow the corticioid fungus *Amylostereum laevigatum* crossed an ocean distance of at least 1300 km. Symbiosis with siricid woodwasps suggests an insect-vectored dispersal; however, distance to remote islands appears difficult to solve by active dispersal in case of little fligthed invertebrates, and it resulted mainly from wind-mediated LDD (García-Olivares et al. 2017). In this study, wind-mediated LDD of the symbiont couple is hypothesized to explain the occurrence of *A. laevigatum* in the Azores. For test it, two main objectives are addressed: 1) to confirm fungal distribution and determinate the geographic origin through a phylogenetic approach, and 2) to demonstrate whether the genetic structure is better explained by geographic distance (random LDD – isolation by distance; null model) or by wind connectivity (wind mediated LDD – isolation by wind-mediated resistance) between geographic occurrences following Muñoz et al. (2004).

MATERIALS AND METHODS

Fungal sampling

A total of 37 specimens and one culture of *Amylostereum laevigatum* from three Azorean islands (Flores, San Miguel and Terceira) and Europe were analyzed: 35 specimens from MA-Fungi (Real Jardín Botánico, Madrid, Spain), two from TFC-Mic (La Laguna University, Tenerife, Spain), and one culture from ISC (Iowa, Ames, United States of America) herbaria. In addition, two North American specimens under *A. laevigatum* were included in order to prove whether it corresponds to the same identity as those from Azores and Europe: one specimen from CFMR (U.S. Forest Service, NRS, Center for Forest Mycology Research, Madison, Wisconsin, USA), and one from NY herbarium (The New York Botanical Garden, Bronx, New York, USA). Also, two specimens of *A. chailletii* from MA-Fungi were included**.** Information related to collections is indicated in Table 1.

DNA extraction, amplification and sequencing

For extraction, two protocols were followed depending on specimens or culture. From herbarium specimens, a fragment (<10 mg) was removed from basidiomes, and genomic DNA isolation was performed using DNeasyTM Plant Mini Kit (Qiagen, Valencia, CA), as indicated by the manufacturer; except in the lysis step, where incubation was done overnight at 55ºC (Whiting et al. 1997). The DNA culture extraction was done with FTA® Indicating Micro Cards (Cat Nº WB120211, Whatman, Maidstone, England) following Telleria et al. (2014).

Amplifications of two markers were performed. First, the fungal barcoding ITS nrDNA (Schoch et al. 2012) was amplified with the primer pair ITS1/ITS4 (White et al. 1990), following thermal cycling conditions described in Martín & Winka (2000). Second, protein-coding *rpb*2 was amplified following nested-PCR protocol described in Wilson et al. (2011) with primer pair fRPB2-5F/bRPB2-7.1R (Matheny 2005) in first amplification, and primer pair bRPB2-6F/bRPB2-7R.2 (Matheny 2005, Matheny et al. 2007) in the second one, using one µl of the first amplification as target DNA in the second amplification. The thermal cycling conditions for both amplifications of *rpb*2 were: an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72°C for 1 min; with final extension at 72 °C for 10 min.

Individual reactions were carried out using Illustra™ PuReTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Buckingham) with a 10 pmol µL primer concentration. Negative controls lacking fungal DNA were run for each experiment to check for contamination. PCR products were purified using a QIAquick gel PCR purification kit (QIAGEN, Valencia, California) as indicated by the manufacturer and both strands were sequenced separately by Macrogen (Belgium/South Korea).

Table 1. Specimens and sequences included in this study. Sequences newly generated in the study were marked in bold face.

Species / Specimens[1], cultures	**Country[2]**	**Collection date**	**Insect/Host**	**GenBank Accesion no.**	
				ITS	***rpb*2**
***Amylostereum areolatum* (Chaillet ex Fr.) Boidin**					
AtI19	Austria			-	HM461099
AtIII2	Austria			-	HM461098
SSMF0757011, DAOM239281	Canada		*Pinus sylvestris*	EU249343	-
SSMF0757014, DAOM239284	Canada		*Pinus sylvestris*	EU249344	HM461095
CZ3.1	Czech Rep.			-	HM461101
CZAAII9	Czech Rep.			-	HM461100
DAA545	Denmark		*Sirex juvencus*	HM461075	HM461097
RMK11-001	Denmark		*Sirex juvencus*	-	KM882958
RMK11-006	Denmark		*Sirex juvencus*	-	KM882962
CBS 655.93	Denmark		*Picea abies*	HM461074	-
CBS 334.66, LY 4922	France	18 Sep 1964		AF454428	-
CMW3309	France		*Sirex noctilio*	-	HM461096
B1352	Germany		*Picea abies*	-	KM882964
B1385	Germany		*Sirex juvencus*	-	KM882965
Ecogrow	Hungary		*Sirex juvencus*	GQ422466	-
RMK11-011	Hungary		*Sirex juvencus*	-	KM882959
RMK11-022	Hungary		*Sirex noctilio*	-	KM882960
B1350	Japan		*Pinus densiflora*	AF218389	-
B1395	Japan		*Sirex nitobei*	-	KM882961
N36	Latvia		*Picea abies*	FJ903375	-
T1	Latvia		*Picea abies*	FJ903381	-
B1/1/1/4/A1	Slovenia	28 Nov 2006	*Abies alba*	AM981216	-
olrim368	Sweden		*Picea abies*	AY781245	-
AH1-01	USA	16 Jul 2007	*Sirex noctilio*	GQ422464	-
AH1-17	USA		*Sirex noctilio*	-	KM882966
ADW-1050701	USA		*Pinus resinosa*	FJ040860	-
GR94	USA		*Sirex noctilio*	-	KM882963
OtisAa	USA	Jan 2006	*Sirex noctilio*	GQ422465	KM882967
SedDF	USA		*Sirex edwardsii*	-	KM882969
S. ed DF-9/18	USA	18 Sep 2007	*Sirex edwardsii*	GQ422467	-
S. cy ME-9/10	USA	10 Sep 2007	*Sirex* sp.` *nitidus*´	GQ422468	KM882968
Ecogrow nematode culture				HM461073	-
***Amylostereum chailletii* (Pers.) Boidin**					
MA-Fungi 61760	Andorra	17 Oct 2002	*Juniperus communis*	**KX953262**	**LT629332**
B1355	Canada		*Abies balsamea*	AF218393	-
DAOM:21498	Canada		*Picea sitchensis*	HM461079	HM461105
DAOM:X-549	Canada		Conifer wood	HM461076	-
DAOM X-589	Canada			-	HM461107
MY28AW2	Canada		*Pinus* sp.	AY672918	-
NMG_5	China		*Ips subelongatus*	HM776410	-
DK546	Denmark		*Urocerus gigas*	-	KM882972
DK538	Denmark		*Urocerus gigas*	-	KM882971
DK537	Denmark		*Urocerus gigas*	-	KM882970
B1387	Germany		*Urocerus gigas*	AF218392	KM882973
B32	Latvia		*Picea abies*	FJ903304	-
MA-Fungi 23924, 9631Tell.	Portugal	30 Apr 1989	Unidentified wood	**KX953261**	-
B3/1/2/3/A1	Slovenia		*Abies alba*	AM981245	-
B2/3/1/3/A2	Slovenia	28 Nov 2006	*Abies alba*	AM981219	-
B3/2/1/5/A1	Slovenia	28 Nov 2006	*Abies alba*	AM981212	-
B3/2/1/2/B2	Slovenia	28 Nov 2006	*Abies alba*	AM981208	-
B3/2/1/2/A2	Slovenia	28 Nov 2006	*Abies alba*	AM981207	-
LY 8950, CBS:632.84	Sweden	31 Oct 1978	*Abies alba*	HM461078	HM461104
R.Vasiliauskas olrim536	Sweden		*Picea abies*	AY805604	-
B1354	United Kingdom		*Urocerus gigas*	AF218391	-
DWAch2	USA	2 Oct 2007	*Sirex nigricornis*	GQ422470	-
FP100271	USA	1952	*Picea engelmanii*	-	HM461103
FP105519	USA	1960	*Larix occidentalis*	-	HM461102
GFSed	USA		*Sirex nigricornis*	-	KM882974
RLG8273	USA		*Abies lasiocarpa*	HM461077	-
SAC203	USA	2009	*Sirex californicus*	-	KT337460
Sni-DF-09/21-2	USA	21 Sep 2007	*Sirex. nigricornis*	GQ422469	-

Table 1 (cont.).

Species / Specimens^1, cultures	**Country^2**	**Collection date**	**Insect/Host**	**GenBank Accesion no.**	
				ITS	***rpb*2**
Amylostereum ferreum **(Berk. & M.A. Curtis) Boidin & Lanq.**					
B1359	USA		*Sirex areolatus*	AF218390	-
LY 8931, CBS 637.84	Brazil	4 Oct 1978	*Podocarpus lambertii*	HM461082	HM461110
Amylostereum laevigatum **(Fr.) Boidin**					
Kotiranta 20108	Finland		*Thuja* sp.	JX049991	-
Kotiranta 20661	Finland		*Juniperus* sp.	JX049990	-
B1372, CBS 625.84	France		*Juniperus nana*	**KX953227**	**LT629330**
MA-Fungi 48382, 9423MD	France	30 Oct 2000	*Juniperus communis*	**KX953230**	-
MA-Fungi 48385, 9426MD	France	30 Oct 2000	*Juniperus communis*	**KX953229**	-
MA-Fungi 57708, 15664Tell	France	5 Sep 2003	*Juniperus communis*	-	**LT629322**
MA-Fungi 60609, 10307MD	France	28 Oct 2004	*Juniperus communis*	-	**-**
MA-Fungi 60598, 10294MD	France	28 Oct 2004	*Juniperus communis*	**KX953256**	-
MA-Fungi 60687, 15911Tell.	France	28 Oct 2004	*Pinus nigra*	**-**	-
MA-Fungi 63289, 10395IS	France	26 Oct 2005	*Juniperus* sp.	**KX953257**	-
MA-Fungi 63293, 10399IS	France	26 Oct 2005	*Juniperus* sp.	**KX953258**	**LT629325**
MA-Fungi 35441	Norway	13 Sep 1990	*Juniperus communis*	**KX953231**	-
MA-Fungi 75910, 17410Tell.	Portugal, Azores (F)	26 Mar 2007	*Juniperus brevifolia*	**KX953237**	**LT629317**
MA-Fungi 75911, 17413Tell.	Portugal, Azores (F)	26 Mar 2007	*Juniperus brevifolia*	**KX953238**	-
MA-Fungi 76032, 17448Tell.	Portugal, Azores (F)	27 Mar 2007	*Cryptomeria japonica*	**KX953244**	**LT629324**
MA-Fungi 76039, 11782MD	Portugal, Azores (F)	26 Mar 2007	*Cryptomeria japonica*	**KX953245**	**LT629315**
MA-Fungi 76269, 17400Tell.	Portugal, Azores (F)	26 Mar 2007	*Cryptomeria japonica*	**KX953252**	**LT629319**
MA-Fungi 76000, 17403Tell.	Portugal, Azores (F)	26 Mar 2007	*Cryptomeria japonica*	**KX953243**	-
TFC-Mic 18309	Portugal, Azores (F)	26 Mar 2007	*Cryptomeria japonica*	**KX953232**	-
TFC-Mic 18356	Portugal, Azores (F)	27 Mar 2007	*Juniperus brevifolia*	**KX953233**	**LT629326**
MA-Fungi 75909, 17565Tell.	Portugal, Azores (SM)	30 Mar 2007	*Cryptomeria japonica*	**KX953236**	**LT629329**
MA-Fungi 75924, 11976MD	Portugal, Azores (SM)	4 Apr 2007	*Cryptomeria japonica*	**KX953239**	-
MA-Fungi 75930, 17571Tell.	Portugal, Azores (SM)	31 Mar 2007	*Cryptomeria japonica*	**KX953240**	**LT629323**
MA-Fungi 75932, 11927MD	Portugal, Azores (SM)	30 Mar 2007	*Cryptomeria japonica*	**KX953241**	-
MA-Fungi 75973, 11958MD	Portugal, Azores (SM)	31 Mar 2007	*Erica azorica*/*Juniperus*	**KX953242**	-
MA-Fungi 76072, 17607Tell.	Portugal, Azores (SM)	4 Apr 2007	*Cryptomeria japonica*	**KX953246**	-
MA-Fungi 76088, 17567Tell.	Portugal, Azores (SM)	30 Mar 2007	*Cryptomeria japonica*	**KX953249**	**LT629321**
MA-Fungi 90384, 11893MD	Portugal, Azores (SM)	29 Mar 2007	*Pittosporum undulatum*	**KX953234**	-
MA-Fungi 90386, 11929MD	Portugal, Azores (SM)	30 Mar 2007	*Cryptomeria japonica*	**KX953235**	**LT629316**
MA-Fungi 76073, 10625MD	Portugal, Azores (T)	1 Mar 2005	Unidentified wood	**KX953247**	**LT629314**
MA-Fungi 76075, 10624MD	Portugal, Azores (T)	1 Mar 2007	Unidentified wood	**KX953248**	**LT629318**
MA-Fungi 76089, 10595MD	Portugal, Azores (T)	28 Feb 2005	*Cryptomeria japonica*	-	**LT629327**
MA-Fungi 76129, 16213Tell.	Portugal, Azores (T)	1 Mar 2005	*Erica azorica*	**KX953250**	-
MA-Fungi 76137, 16225Tell.	Portugal, Azores (T)	2 Mar 2005	*Juniperus brevifolia*	**KX953251**	**LT629328**
MA-Fungi 76161, 16195Tell.	Portugal, Azores (T)	28 Feb 2005	*Cryptomeria japonica*	**KX953253**	-
MA-Fungi 76175, 16194Tell.	Portugal, Azores (T)	28 Feb 2005	*Cryptomeria japonica*	**KX953254**	-
MA-Fungi 24171-2,3408Tell.	Spain	9 Oct 1983	*Larix* sp.	**KX953259**	-
MA-Fungi 37945, 3624IS	Spain	17 Apr 1987	*Juniperus communis*	**KX953260**	-
MA-Fungi 41603, 6468MD	Spain	25 Jun 1992	*Juniperus communis*	**KX953255**	
MA-Fungi 90385, 20913Tell.	Spain	26 May 2016	*Taxus baccata*	**KX953228**	**LT629320**
olrim409	Sweden		*Picea abies*	AY781246	**-**
Amylostereum orientale **S.H. He & Hai J. Li**					
(BJFC) He 479, holotype	China	22 Oct 2010	*Cunninghamia lanceolata*	JX049987	-
(BJFC) He 480, paratype	China	22 Oct 2010	*Cunninghamia lanceolata*	JX049988	-
(BJFC) He 468, paratype	China	22 Oct 2010	*Cunninghamia lanceolata*	JX049989	-
B1361	Japan		*Urocerus japonicus*	KX953222	LT629313
B1362	Japan		*Urocerus japonicus*	KX953225	LT629312
B1364	Japan		*Urocerus antennatus*	KM821145	KM882975
B1368	Japan		*Cryptomeria japonica*	KX953224	LT629311
B1369	Japan		*Chamaecyparis obtusa*	KX953223	LT629310
TUMH 61916, TUFC-12106	Japan	Jun 2004	*Cryptomeria japonica*	KX953220	LT629308
TUMH 61905, TUFC-11625	Japan	2 jul 2008	Unidentified wood	KX953221	LT629309

Table 1 (cont.).

Species / Specimens[1], cultures	Country[2]	Collection date	Insect/Host	GenBank Accesion no.	
				ITS	*rpb*2
***Amylostereum* sp.**					
NY 1982233, H.S. Jackson	Canada	2 Sep 1946	*Thuja*	**OK171106**	-
CCFC010375, B1393	USA		*Sirex areolatus*	AF218394	-
HHB3624, H.H. Burdsall Jr.	USA	16 Sep 1969	*Thuja*	**OK171107**	-
Outgroup: ***Echinodontium tinctorium* (Ellis & Everh.) Ellis & Everh.**					
DAOM-16666, AFTOL 455				AY854088	AY218482
B1122	USA		*Tsuga* sp.	AF218397	-

[1]: Abreviatures IS, MD and Tell. are referred to Isabel Salcedo, Margarita Dueñas and M. Teresa Telleria, respectively.
[2]: Abreviatures F, SM and T in Portugal, Azores are referred to Flores, San Miguel and Terceira islands, respectively.

Sequence alignments and phylogenetic analyses

Sequences were checked and edited manually using the software Geneious Pro v9 (http://www.geneious.com, Kearse et al. 2012) and were submitted to EMBL/GenBank/DDBJ database with the accession numbers specified in Table 1**.** Consensus ITS and *rpb*2 sequences were aligned together with sequences retrieved from EMBL/GenBank/DDBJ using Geneious Pro v9. In both alignments, *Echinodontium tinctorium* (Ellis & Everh.) Ellis & Everh. was included as outgroup following Larsson (2007).

Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed to confirm if all Azorean specimens belong to *A. laevigatum*. First, ML analyses were run using the RAxML 7.2.8 (Stamatakis 2014) through the Cyberinfrastructure for Phylogenetic Research Science Gateway portal (CIPRES Science Gateway v.3.3; Miller et al. 2010), assuming a GTR+G model (default model), nonparametric bootstrap (MLbs) support (Felsenstein 1985) for each clade, based on 1000 replicates. Bayesian inference was conducted using the software package BEAST v 2.4.3 (Drummond & Rambaut 2007), assuming the best model of nucleotide substitution implemented in JMODELTEST 0.1.1 (Posada 2008), according to Bayesian information criterion (BIC; Schwarz 1978). An uncorrelated lognormal relaxed clock was employed, and as tree prior the coalescent constant size was chosen. Other priors were set to default models. MCMC chain length was defined for 50 million generations. The majority-rule consensus tree was summarized in TreeAnnotator v 2.4.3. where 10 % was discarded as burn-in.

Anisotropic Cost Analysis

Genetic distance matrix — Minisatellites, which are hypervariable and repetitive (Meyer et al. 1993) short DNA regions distributed around numerous loci (Weising et al. 1995), have been amplified to access DNA *fingerprinting,* which is useful to detect genetic variability among even closely related individuals (Nybom et al. 1990, Meyer et al. 1991). In this study, amplification of minisatellite—M13 (Karlsson 1994) was carried out from specimens and cultures available from the Azores (Flores, San Miguel and Terceira islands), France, Norway and Spain. This amplification was performed with an initial denaturation at 94ºC for five minutes, 44 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s and extension at 72ºC for 1 min, final extension at 72 °C for 10 min, and 4 °C soak. The PCR products were subjected to electrophoresis on a 2 % (w/v) SYBR Safe - High Resolution Agarose (Agarose MS-8 Molecular Screen) gel in a 1xTBE buffer system during 45 minutes at 120 V. The images of electrophoresis gels were registered in a documentation of gels system G:BOX EF (SynGene) to perform a gel interpretation using the software Taxotron (Dr. Grimont, Institute Pasteur) and obtain the base pairs values of the bands. Next, presence-absence matrix was generated and it was transformed into a genetic distance matrix (*mean character difference*) using PAUP* 4.0b10a for Macintosh (Swofford 2003).

Geographic and wind-mediated distance matrices — For specimens amplified in the previous step, latitude and longitude were obtained from herbarium labels or collection information. Wind data for the whole study area was downloaded from the Global Forecast System (GFS) of the USA´s National Weather Service (NWS) using the R package rWind 0.1.7. (Férnandez-López & Schliep 2019). The package facilitates the download of daily measurements of U and V component of the wind vector (ugrd10m, vgrd10m), and their conversion into wind azimuth direction and speed over surface. These data were aggregated from every five days into the three months from early or mid-summer to early autumn (July, August, September), when the *Sirex noctilio* adults emerge (Ryan and Hurley 2012), and eight daily hours (00.00 - 3.00 - 6.00 - 9.00 - 12.00 - 15.00 - 18.00 - 21.00 - 00.00) for the period within 2011-2016. Also, rWind package was used to transform raw data (longitude, latitude, direction and speed) into raster layers.

To calculate the cost based on wind azimuth direction and speed, anisotropic cost analyses were performed for each wind dataset to create a friction surface (González-Solís et al. 2009). These analyses estimate the minimum accumulative cost of passively moving from a cell to an adjacent orthogonal or diagonal cell (Moore´s neighbourhood) over a friction surface based on a raster layer. Anisotropic wind friction surface was calculated as a function of the inverse of the speed multiplied by the *Horizontal Factor* (HF) that penalizes angular deviations from the wind direction (Felicísimo et al. 2008). These have been carried out through the R function *flow.dispersion* implemented in the rWind package. Wind-based cost values were obtained for each cell-to-cell transition in the study area (McRae et al. 2008) and were stored as transition matrices, one per each wind dataset. For each sample location, least-cost distance function over cost transition matrices was performed through Dijkstra´s algorithm (Dijkstra 1959) implemented in the *gdistance* package (van Etten 2017). As the function to calculate anisotropic wind cost values is non-commutative, $f(i, j) \neq f(j, i)$, asymmetric cost matrices were obtained.

Geographic distances were calculated as the minimum Cartesian distance between two geometries using R package *raster* (Hijmans 2015), and they were stored into a symmetric geographic distance matrix.

Mantel tests among distance matrices — To test the proposed models, one based on geographic distances and the other based on wind connectivity among record locations, Mantel based tests were implemented. This statistical method calculates the correlation between pairs of distance matrices (in landscape genetics, between the genetic distances matrix and the matrices of landscape variables: genetic-geographic distance matrices, genetic-wind distance matrices). Before performing the Mantel test, the asymmetric wind cost matrix was transformed into a symmetric matrix by averaging the lower and higher triangular portions. Posterior permutation tests using one-tailed Mantel test were performed in the R framework using R package ade4 (Dray & Dufour 2007) to obtain p-values (999 permutations).

RESULTS

Phylogenetic analyses

A total of 56 newly generated sequences (38 ITS and 18 *rpb*2) were lodged in EMBL/GenBank/DDBJ with the accession numbers indicated in Table 1. For all vouchers, complete ITS sequences including ITS1-5.8S-ITS2 region were obtained, except for NY 1982233, for which only ITS1 region was amplified and sequenced. Moreover, *rpb*2 sequences from North American specimens were not obtained. Alignment matrices contained 92 ITS sequences for a dataset length of 537 nucleotide positions, and 57 *rpb*2 sequences for a dataset length of 702 nucleotide positions. For ITS, TrNef+G model was selected as the best substitution model (BIC = 3916.4578) and trees obtained from ML (not shown) and Bayesian inference (Fig. 1) showed the same topology. Also for *rpb*2, TrNef+G model was selected (BIC = 5155.7256) and topological differences between ML (not shown) and Bayesian (Fig. 2) trees were not revealed.

In both analyses (Figs. 1 and 2), sequences under *A. areolatum*, *A. chailletii*, *A. ferreum* and *A. orientale* formed their own clades with high support. Sequences obtained from specimens from the Azores grouped in the same clade that those from European mainland, which is poorly supported in ITS (MLbs =60, pp =0.71) and highly supported in *rpb*2 (MLbs = 99, pp = 1.0). The ITS sequences obtained from North American specimens originally under *A. laevigatum* formed their own clade together with a sequence retrieved from GenBank (AF218394, *Amylostereum* sp., USA); this grouping is well supported by Bayesian analysis (MLbs = 54, pp = 0.99), and they are the sister group of *A. laevigatum* with this relationship well supported by Bayesian analysis (MLbs = 56, pp = 0.99).

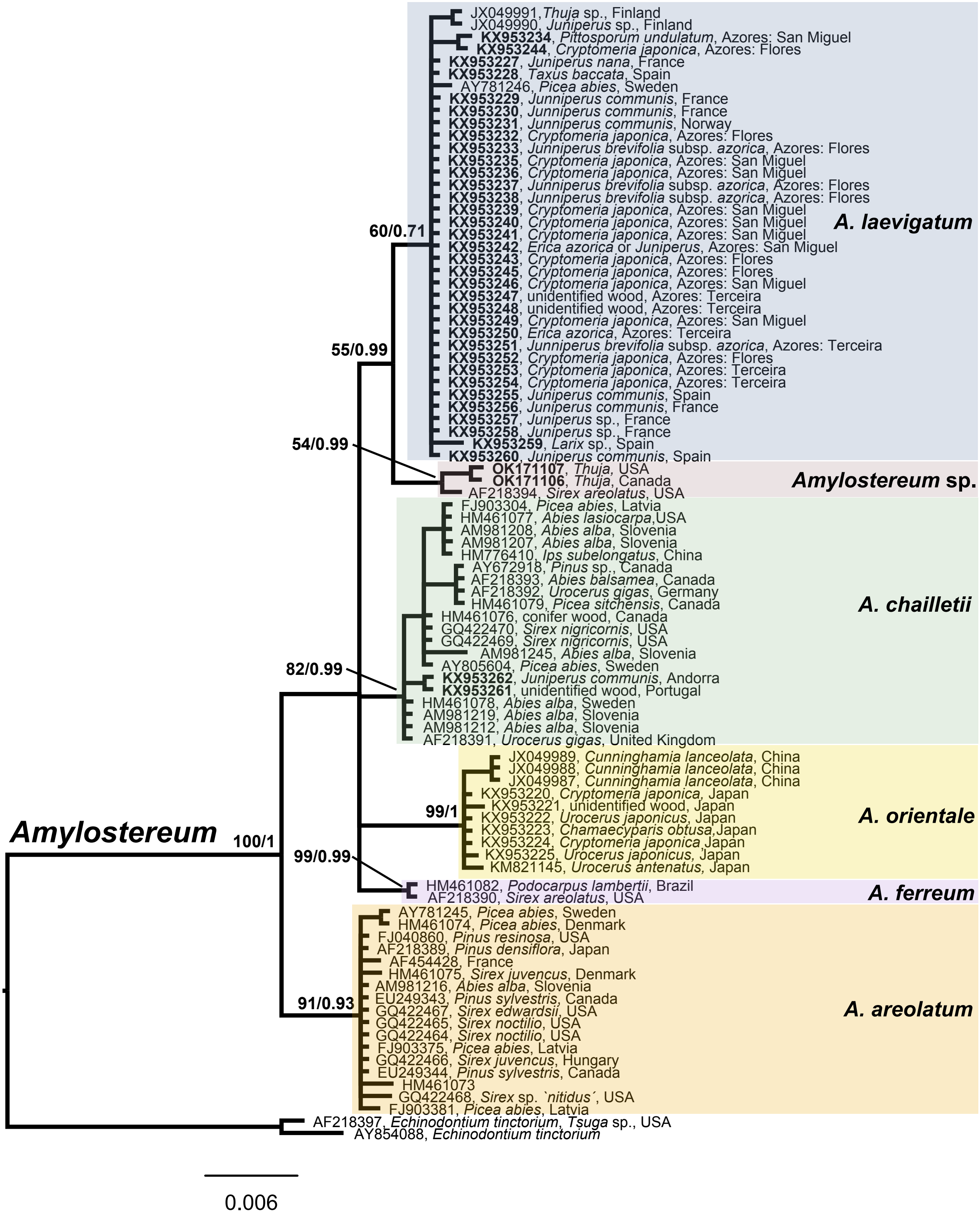

Fig. 1. Bayesian majority rule consensus topology based on ITS sequence dataset. Two *Echinodontium tinctorium* sequences were included as outgroup. Host and country are shown next to the accession numbers. Likelihood bootstrap value (%) and posterior probability are indicated below the branches in this order. Newly generated sequences are marked in bold.

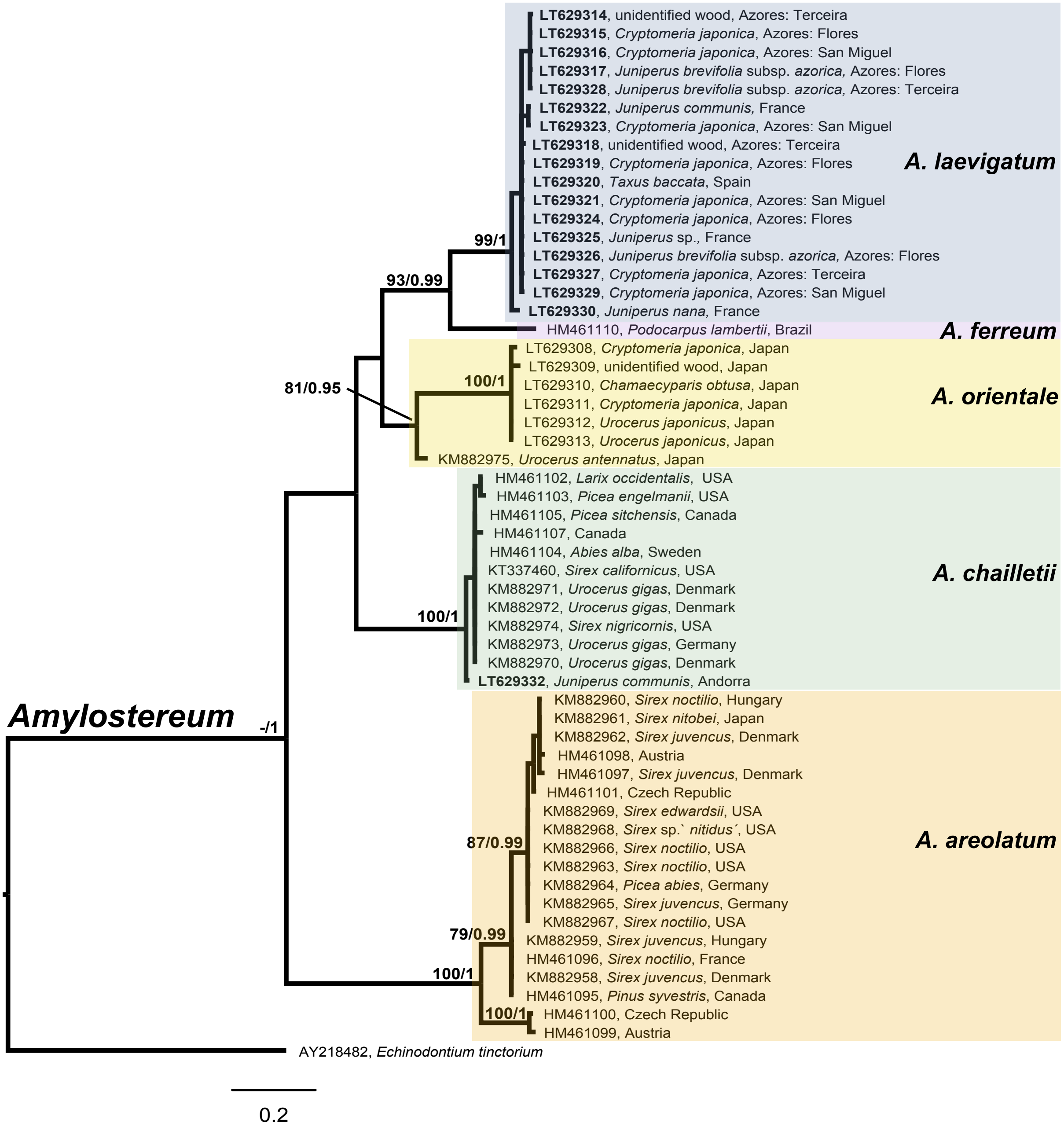

Fig. 2. Bayesian majority rule consensus topology based on *rpb*2 sequences dataset. One *Echinodontium tinctorium* sequence was included as outgroup. Host and country are shown next to the accession numbers. Likelihood bootstrap value (%) and posterior probability are indicated above the branches. Newly generated sequences are marked in bold.

Anisotropic Cost Analysis

Genetic, geographic and wind-mediated distance matrices — Two symmetric distance matrices (genetic and geographic) and one asymmetric (winds) with 34 entries (corresponding to the number of vouchers) were generated. These are provided in the Supporting Information as S1 Table, S2 Table and S3 Table, respectively. Related to wind data, a total of 108 wind datasets were downloaded and averaged in order to obtain a mean of the dispersion cost between sampled locations, which was summarized in a single matrix. Data collected about azimuth direction and speed revealed the wind anisotropic property (Fig. 3). Generally, wind connectivity from Europe mainland towards Azores was stronger than from the archipelago to the continent, indicating that the cost of moving from Europe to Azores is lower than from islands to continent. Wind connectivity is stronger within France and Spain localities, and Norway appears as the most isolated location with respect to the rest of the European mainland and the Azores archipelago. Moreover, wind connectivity is strong between the three islands.

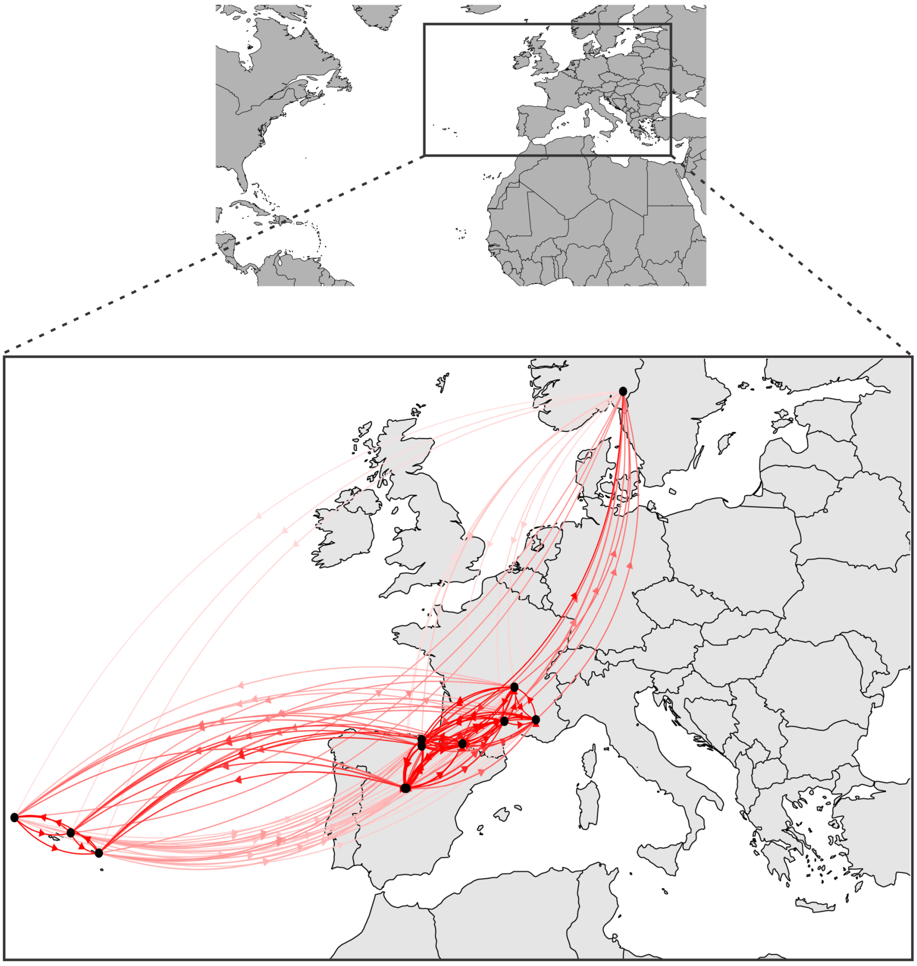

Fig. 3. Wind anisotropic connectivity between geographic distributions of the *Amylostereum laevigatum* specimens studied.

Mantel tests among distance matrices — The one-tailed Mantel-based tests showed a non-significant correlation (R = 0.065 and p-value = 0.061) between genetic and geographic distance matrices (Fig. 4, Model 1). However, significant correlation (p-value < 0.05, R = 0.197) between genetic distance matrix and cost matrix based on wind azimuth direction and speed was detected (Fig. 4, Model 2), suggesting that the genetic and wind cost matrix entries are positively associated.

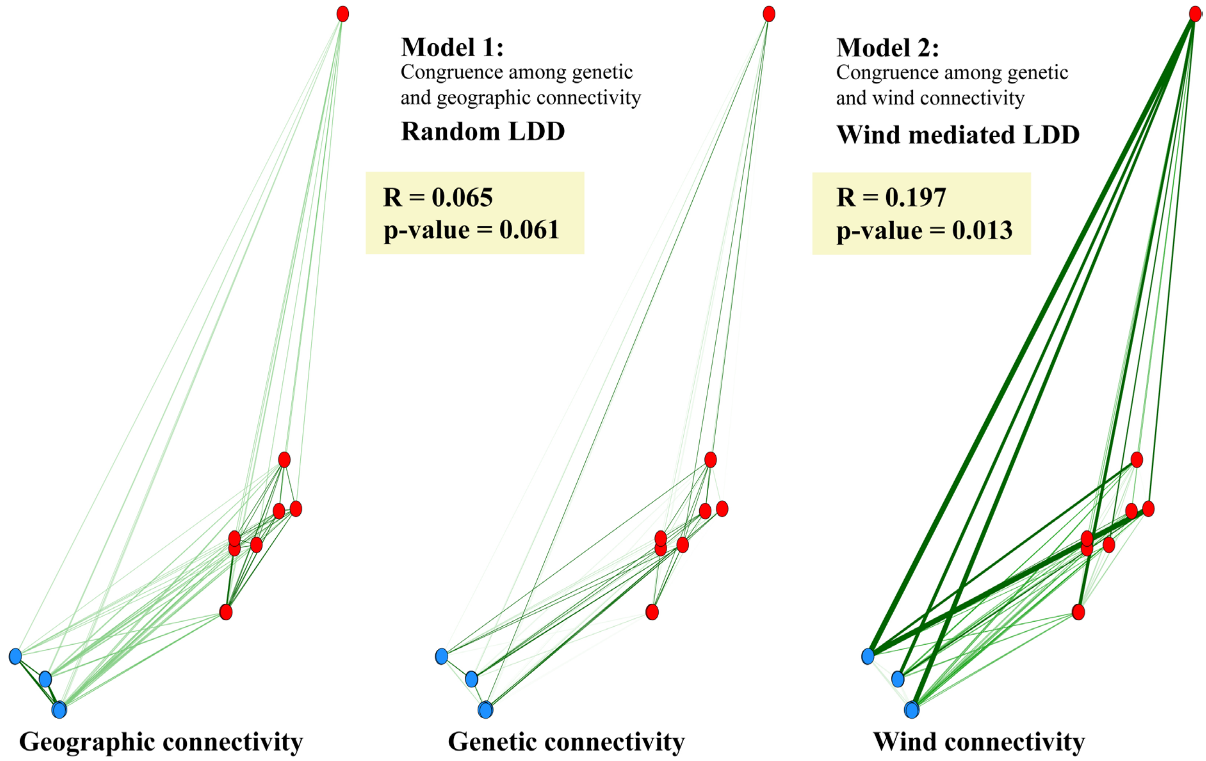

Fig. 4. Connectivity plots obtained from the three symmetric distance matrices (cost distance matrix based on wind, genetic distance matrix and geographic distance matrix). Azores islands (San Miguel, Terceira and Flores) and European mainland (Norway, France and Spain) were represented in blue and red, respectively. The green branches show the relationship between the different locations included, and their intensity reflect the level of relation. Mantel test correlations (R) and the significance of these (p-value) obtained among pairs of matrices (**Model 1:** genetic distance and geographic distance; **Model 2:** genetic distance and wind mediated distance) are indicated.

DISCUSSION

Amylostereum laevigatum was initially defined as a species with a wide geographic distribution in the Northern Hemisphere, including Europe, North America and the Azorean islands of Flores, San Miguel and Terceira. Our phylogenetic analyses based on ITS and *rpb*2 confirm that records from Azores correspond to *A. laevigatum*, and show that North American specimens do not belong to any of the known *Amylostereum* species. These findings are consistent with the previous available reports of *A. laevigatum*: it was largely reported from Europe and the Azores, while few reports were given from North America, suggesting that it is a rare or even an absent species there. Other *Amylostereum* species, such as *A. areolatum, A. chailletii* and *A. orientale*, form strongly supported clades in both phylogenies, as was previously indicated by Tabata et al. (2000), Slippers et al. (2002), He & Li (2013) and Nogal-Prata et al. (2017).

The specimens of *A. laevigatum* from the Azores were collected growing on the endemic *Juniperus brevifolia* subsp. *azorica* and the introduced *Pittosporum undulatum* and *Cryptomeria japonica*, which has become progressively dominant on Azorean islands (Borges et al. 2009). Introduction of the latter from Japan may also indicate a fungal introduction, since it is a substrate of *A. laevigatum* in the archipelago. There are previous reports of corticioid basidiomycetes that have been suggested to be spread by human-mediated movement of timber or plants (Kauserud et al. 2007, Linzer et al. 2008); however, introduction in Azores was rejected since it was shown that species growing in *C. japonica* in Japan correspond to *A. orientale* (Nogal-Prata et al. 2017), while in the Azores it is *A. laevigatum* that grows on *C. japonica*.

Research about fungal LDD faces important methodological challenges (Golan & Pringle 2017). Usually, LDD is addressed through three major methodologies: (1) direct tracking of individuals or propagules, (2) genetic inference and (3) mathematical models (Nathan 2001). Fungal LDD studies were mainly conducted through genetic inferences (Moncalvo & Buchanan 2008, Matheny et al. 2009, Peterson et al. 2010), also for corticioid fungi. For example, multilocus analyses in Carlsen et al. (2011) showed five lineages within the widespread morphospecies *Serpula himantioides*, among which one has wide distribution and little phylogeographical structure, suggesting frequent and recent LDD. Also, studies about direct tracking conducted with corticioid fungi have provided evidence of dispersal mainly over short distances (Norros et al. 2012), but also over long distances. The study conducted by Hallenberg & Kúffer (2001) provided evidence about an efficient LDD by spores of corticioid species *Peniophora* *aurantiaca*, which are dispersed even up to 1000 km; although this study was conducted across a continental landmass.

In our case, genetic and mathematical approaches were combined. First, on the basis of the phylogenetic results, European mainland was proven to be the geographic origin of fungal LDD to the Azores, emphasizing the rigorous delimitation of fungal taxa as a basic requirement to test hypotheses about biogeography (Lumbsch et al. 2008). How species are delimitated affects our understanding about taxonomy and geographic distribution, and consequently could bias our hypotheses about biogeography and lead us to mistaken conclusions. In this case, wind-mediated LDD analysis from Europe to Azores was conducted, since phylogenetic species recognition (PSR, Taylor et al. 2000) shows that *A. laevigatum* occurs in European mainland and Azorean islands but not in North America.

The wind anisotropic cost data indicate a minimum resistance to the movement from European mainland to the Azores (Fig. 3), suggesting the significance of wind currents on LDD toward the archipelago. In addition, wind connectivity and genetic matrices reveal a relationship between Flores, San Miguel and Terceira (Fig. 4), which could be indicating an inter-island wind dispersal. In this line, fieldwork will be required for Graciosa and São Jorge islands to find out if *A. laevigatum* is also distributed there, since strong wind connectivity was recovered across them (Fig. 3); also for Corvo and Santa María, although the wind data included in this study did not cover these outlying islands. Concerning Faial and Pico, some localities were extensively studied by Telleria et al. (2009a), but *A. laevigatum* was not reported from there.

Mantel tests revealed a correlation between wind connectivity and fungal genetics (Fig. 4), providing indirect evidence for wind mediated LDD of *A. laevigatum* in symbiosis with siricid woodwasps. Traditionally, it was considered that LDD events depend on the geographic distance between source and islands (Nathan 2006); however, no correlation between fungal genetics and geographic distance was revealed (Fig. 4) indicating that genetic similarities are not explained by the shortest pathway. Moreover, correlation with wind connectivity and not correlation with geographic distance indicate that nearest distributions do not correspond to minimum wind cost, sometimes even the furthest locations have the least cost values based on wind currents to transitioning between adjacent cells (Fig. 4). Although fungal genetic structure was revealed to be correlated with wind connectivity, this correlation does not imply causation and other environmental variables could be correlated as well to the observed genetic structure.

In contrast to our results, those obtained for the saprotrophic fungus *Rhodocollybia laulaha* in the oceanic archipelago of Hawaii showed a limited genetic structure within island populations, which is mostly correlated with geographic distance (Keirle et al. 2012). However, this case should not be treated in the same way, since the species *R. laulaha* is considered as a Hawaiian putative endemic and the dispersal mechanism is unclear (Keirle et al. 2010), while speciation is not recognized in the Azores and a particular LDD mechanism could be evaluated because of the symbiosis between *A. laevigatum* and siricid woodwasps. Also, Forsythe et al. (2021) found evidence for gene flow among North American populations of the ascomycete *Pseudogymnoascus destructans* and it was specifically compared to landscape features proposing the same null model, in which geographic distance was hypothesized as the main contributor to genetic distance. They evaluated wind conductance as an additional abiotic factor; however, it was shown that genetic connectivity among *P. destructans* populations is not consistent with prevailing wind currents. On the other hand, our results are in accordance with those obtained by Muñoz et al. (2004), who found that floristic similarities of ferns, mosses and lichens in the Southern Hemisphere are better explained by wind connectivity than by geographic proximities, providing indirect evidence of wind LDD.

This study underlines the relevance of having a broad geographic sample of corticioid species, as well as showing that symbiosis and wind are important factors involved in fungal dispersal. Moreover, to our knowledge, it introduces a novelty for corticioid fungi: LDD mechanism was inferred through a model-based approach. Our data support a wind-mediated LDD hypothesis from a fungal perspective, but do not reveal information related to LDD and colonization of the Azores by the symbiotic couple. Further co-dispersal analyses should be conducted to examine population structure and to prove whether gene flow is maintained toward and between islands, even from archipelago to continent, and if the presence of *A. laevigatum* in Azores may be a result of recent colonization. Also, this may be evaluated in order to know how woodwasp dispersal could be affecting fungal dispersal. Recently, Matthews et al. (2021) demonstrated a male-biased LDD in the fungus-gardening ant *Trachymyrmex septentrionalis*, indicating more limited female dispersal capabilities than males. This case resembles the *Amylostereum–Sirex* symbiosis, since in both females carry the fungus.

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Discusión General

Con los resultados obtenidos en la presente tesis doctoral, corroboramos la hipótesis de trabajo que postula que los rangos de distribución inferidos para los hongos corticioides dependen del método de reconocimiento utilizado, apoyando la necesidad de una taxonomía integradora (Dayrat 2005) que incorpore datos de diferentes fuentes de evidencia taxonómica.

En los casos de *Cystostereum murrayi* (capítulo 2) y *Amylostereum laevigatum* (capítulos 3 y 4), dos especies que se habían considerado ampliamente distribuidas en el hemisferio norte, se han detectado varias especies con patrones de distribución más restringidos. *Cystostereum murrayi* se ha resuelto como un complejo de cuatro especies con un marcado patrón geográfico: *C. murrayi* de Cuba, *Cystostereum effusum* de Norte América, *Cystostereum tuberculosum* de Europa, y *Cystostereum sirmaurense* de India; y *Amylostereum laevigatum* se confirma para Europa continental y el archipiélago de Azores, mientras que los especímenes japoneses corresponden a la especie *Amylostereum orientale*, descrita originalmente de China (He & Li 2013), y los norteamericanos no corresponden a ninguna de las cinco especies conocidas del género. Estos patrones de distribución amplia en el hemisferio norte, se han resuelto de manera similar en otros hongos corticioides como *Hyphoderma setigerum*, que consiste en un complejo integrado por nueve especies con distribuciones más restringidas (Nilsson et al. 2003), al que además se ha sumado *H. australosetigerum,* una especie descrita y conocida hasta el momento de la región Patagónica en Chile (Boonmee et al. 2021), o *Xylodon raduloides*, una especie cosmopolita que se ha resuelto como un complejo integrado por cuatro especies, dos de ellas distribuidas en el hemisferio norte, *X. raduloides* en Europa y *X. laurentianus* en Norte América (Fernández-López et al. 2019) y otras dos en el Hemisferio Sur: *X. patagonicus* y *X. novozelandicus*, de Chile y Australia-Nueva Zelanda respectivamente.

La separación de una especie europea y otro norteamericana, que se encuentran filogenética y morfológicamente relacionadas, como *C. tuberculosum* y *C. effusum* o *Amylostereum laevigatum* y una especie de *Amylostereum* aún por describir, se puede interpretar como una especiación alopátrida, ya que aparecen como el resultado de un aislamiento geográfico, lo que ya se había señalado para algunos hongos corticioides utilizando el concepto morfológico y biológico de especie (Hallenberg 1991). En el capítulo 2, se detecta un caso similar pero dentro de un mismo continente: *Crustomyces pini-canadensis*, una especie distribuida en la parte este y medio-oeste de Norte América, que está relacionada genética y morfológicamente con *Crustomyces subabruptus*. Esta última, a su vez, se confirma con una distribución amplia en el hemisferio norte que incluye la costa noroccidental de Norte América y Eurasia, a la que además se suman cinco especímenes recolectados en una plantación de *Eucalyptus* en Chile, registros que se podrían explicar por la falta de muestreo, o por el transporte de maderas, ya que según los estudios que se están llevando a cabo sobre la diversidad de hongos corticioides en el bosque húmedo valdiviano, solo se ha localizado fructificando en repoblaciones de *Eucalyptus* y no en el bosque autóctono. Este patrón circumboreal también se ha confirmado sobre la base de datos moleculares para otros casos, como el de *Basidioradulum radula*, una especie que se distribuye en Europa, el norte de Estados Unidos, China y Korea del Sur (Viner et al. 2021), o el poliporáceo *Skeletocutis semipileata*, una de las 11 especies que integran el complejo de *S. nivea* (Korhonen et al. 2018) que se distribuye a lo largo de la región templada del hemisferio norte incluyendo Europa y Norte América.

A partir de los resultados obtenidos en el capítulo 3, se descartó la posible introducción de *Amylostereum laevigatum* desde Japón por la importación de *Cryptomeria japonica* como proceso para explicar su presencia en Azores, y se propuso evaluar la dispersión a larga distancia considerando dos vectores, por un lado avispas de las madera con las que establece relaciones de simbiosis, y por otro el viento, ya que resulta improbable que estos insectos se dispersen a largas distancias por vuelo activo (capítulo 4). Los modelos de coste basados en Muñoz et al. (2004), probaron que la matriz genética de *A. laevigatum* está correlacionada con la matriz de conectividad por vientos desde Europa a Azores. Estos resultados parecen indicar que *A. laevigatum* habría colonizado la madera de *Juniperus azorica*, y de esta habría pasado a *C. japonica*. La dispersión a larga distancia medida por el viento se ha señalado como uno de los principales mecanismos de dispersión, y está bien estudiado en organismos como las plantas (Nathan et al. 2002). Sin embargo, las evidencias son escasas para los hongos en general, y los corticioides en particular, y se han desarrollado a escala continental (Hallenberg & Kúffer 2001, Norros et al. 2012). Nuestros resultados de conectividad por viento, sumados a otros como el de Jacobsen et al. (2017), que demostraron que los escarabajos de la madera son importantes vectores de dispersión de hongos con los que establecen simbiosis, así como de otros con los que a priori no establecen ningún tipo de relación, señalan la necesidad de profundizar en el estudio conjunto de avispas y hongos de la madera para probar si podría ser un mecanismo clave en la colonización de islas oceánicas por parte de este grupo de hongos.

En el caso tratado del hemisferio sur (capítulo 1), sobre la base de datos moleculares y morfológicos se han resuelto dos especies a partir del patrón austral de *Aleurodiscus limonisporus*, una de ellas con un patrón templado-meridional (*Aleurodiscus patagonicus* sp. nov.), y otra con un rango australasiático (*A. limonisporus*). En las checklists de hongos corticioides disponibles para la región de los Andes de Argentina (Greslebin & Rajchenberg 2003) y Chile (Gorjón & Hallenberg 2013), siete de las 168 listadas de Argentina presentan un patrón austral y tres de las 93 citadas para Chile. Este es el caso *Hyphodontia australis* (=*Xylodon australis),* una especie originalmente descrita de Australia como *Grandinia australis* Berk. (Hooker 1860) y conocida también de Nueva Zelanda y Argentina (Greslebin et al. 2000). Tras el estudio de Fernández-López et al. (2020), también se confirma que integra dos especies distintas, *X. australis* que se distribuye en Australia y Nueva Zelanda y *X. magallanesii* en Chile y Argentina. Esta separación de dos especies muy similares morfológicamente y que están relacionadas filogenéticamente, se puede asociar con un evento de vicarianza por la separación del supercontinente de Gondwana. Por otra parte, dado que los especímenes colectados en Australia y Nueva Zelanda se confirman como la misma especie, esto podría indicar un flujo genético continuo que se mantiene gracias a la dispersión a larga distancia. Esta combinación de vicarianza y dispersión a larga distancia ha sido generalmente aceptada para plantas y animales en el hemisferio sur (Sanmartín & Ronquist 2004), y también para hongos como *Cyttaria*, un género estrechamente relacionado con *Nothofagus* y *Lophozonia* (Nothofagaceae), para el que su distribución transantártica que se explica alternando los dos procesos: vicarianza por la separación de Gondwana y eventos reciente de dispersión a larga distancia de Australia a Nueva Zelanda (Peterson et al. 2010).

Frente a los resultados en los que se resuelven dos o más especies a partir de distribuciones amplias, se han confirmado los patrones que eran más restringidos, como los de *Cystostereum australe* y *C. kenyense* (capítulo 2), especies conocidas hasta el momento del Caribe y Kenia, respectivamente. Estas distribuciones posiblemente sean resultado del escaso conocimiento que existe sobre estas regiones en comparación con otras que son mejor conocidas, como Europa o Norte América (Mueller et al. 2007). A partir de los patrones detectados en esta memoria, *C. kenyense* y *C. murrayi* se pueden definir como especies huérfanas (Feuerer & Hawksworth 2007), ya que sólo se conocen sobre su material tipo. Casos similares son los de *Phanerochaete aluticolor*, una especie descrita originalmente de Madeira como *Peniophora aluticolor* (Torrend 1912) que no se ha vuelto a localizar (Melo et al. 2012), o *Efibula clarkii*, una especie que solamente se conoce sobre su espécimen tipo colectado en Worcester, Massachusetts (Floudas & Hibbett 2015).

Los estudios incluidos en esta tesis doctoral apoyan la importancia de las colecciones de herbario en los análisis sobre diversidad de hongos (Osmundson et al. 2013). Sin embargo, debemos insistir en los problemas que plantea el uso de estos materiales, ya que la edad de los especímenes o los tratamientos utilizados para su conservación influyen en la calidad del ADN

(Brock et al. 2009, Leino et al. 2009), lo que dificulta la obtención de datos moleculares para analizar las especies en el marco de una taxonomía integradora. Esto se ha hecho patente en el capítulo 2, en el que cabe destacar que la obtención de la secuencia ITS del tipo de *C. murrayi*, especie tipo del género, recolectada en el siglo XIX, ha sido fundamental para circunscribir el mismo, así como de la familia Cystostereaceae, ya que otros especímenes identificados como *C. murrayi*, también de Cuba, resultaron pertenecer a un género, incluso a una familia distinta. En casos en los que no ha sido posible obtener datos morfológicos y moleculares a partir de materiales tipo, con el fin de aclarar la taxonomía y sistemática de grupos concretos (p.e. complejos de especies) se han propuesto epitificaciones, como en el caso *Pseudotomentella tristis* (Svantesson et al. 2019) o *Peniophorella pratermissa* s.s. (Hallenberg et al. 2007). Para resolver este tipo de problemas se están desarrollando otras herramientas, como la función *locate.yeti* del paquete de R *phytools* desarrollada por Revell (2012), que implementa una aproximación basada en análisis de máxima verosimilitud que integra datos morfológicos y filogenéticos en un mismo análisis. Esta metodología fue aplicada por primera vez en hongos en el caso de *Xylodon australis* (Fernández-López et al. 2020), cuyo material tipo se logra ubicar en la filogenia integrando las medidas de esporas y basidios, ya que por su valor histórico no se permite la extracción ADN para estudios moleculares. Esta aproximación también podría considerarse para aquellas colecciones que han sido tratadas químicamente para su conservación, lo que habría influido en la calidad de su ADN.

Limitaciones del estudio y perspectivas futuras

En esta tesis se hace patente que los métodos utilizados en la delimitación de las especies afecta al conocimiento sobre la diversidad fúngica y los patrones de distribución geográfica y, en consecuencia, influye en las hipótesis sobre los procesos responsables de dichos patrones (Lumbsch et al. 2008). Gracias al avance en las técnicas moleculares se han incrementado los estudios sobre biogeografía, aunque continúan siendo escasos en comparación con otros organismos (Skrede et al. 2011). De hecho, los hongos no se encuentran tratados en textos clásicos de biogeografía (Lomolino et al. 2006), lo que se puede atribuir a: (1) una taxonomía y sistemática incompletas o que requieren revisión (Lodge et al. 2004); (2) el conocimiento limitado sobre la distribución de las especies (Yang 2011); (3) muchas regiones permanecen inexploradas para los hongos (Mueller et al. 2007); y (4) un registro fósil particularmente pobre y difícil de interpretar, que imposibilita proponer y desarrollar análisis biogeográficos dentro de un marco evolutivo (Berbee & Taylor 2010).

Todos estos puntos se han hecho patentes con los casos abordados en esta memoria, especialmente del (1) al (3). Una de las principales limitaciones que se plantean para los estudios de distribución de hongos corticioides es que la mayoría de las especies se han descrito sobre la base de datos morfológicos, por lo que deben ser revisadas dentro de un marco polifásico e integrador como paso previo al de abordar análisis sobre patrones y procesos de distribución.

Por otra parte, se hacen evidentes los sesgos de muestreo, que se han señalado como uno de los principales problemas a la hora de abordar estudios sobre distribución de hongos (Sato et al. 2012). En esta tesis doctoral, mientras que un buen número de colecciones ha estado disponible para Europa, que está bien estudiada, para otras zonas como África el número de muestras ha sido muy limitado, bien porque no se encuentra material depositado en los herbarios o porque no se han hecho prospecciones. Para eliminar esos sesgos, así como para seguir completando el inventario y conocimiento de la diversidad fúngica, que se estima como una de las más numerosas (Blackwell 2011), el muestreo de esas regiones inexploradas es una tarea urgente. Sumado a esto, debemos considerar que la pérdida de diversidad se está dando a gran escala, por lo que si no avanzamos en el estudio de esas regiones, probablemente no lleguemos a conocer buena parte de toda la diversidad pendiente por descubrir.

En este sentido, se han desarrollado importantes proyectos y programas para el reconocimiento automático de las especies basado en secuencias de ADN, que han contribuido de manera notable a acelerar el descubrimiento y descripción de la diversidad fúngica. Es el caso de UNITE, una plataforma para la identificación automática de hongos a partir de su secuencia ITS nrDNA (*barcode* de hongos), que se basa en la asignación de "hipótesis de especies" (SH) y a la designación para cada SH de una secuencia de referencia que, a su vez, suele estar asociada a un espécimen. Sin embargo, hay secuencias que no tienen material asociado, como aquellas procedentes de micorrizas o muestras de suelo. Esta iniciativa definió umbrales de similitud del 97-99% para discriminar entre los diferentes SH a nivel de especie (Kõljalg et al. 2013). En el caso de los géneros tratados en esta memoria, se ha incluido la secuencia ITS de *Gloeostereum cimrii* (capítulo 2), una especie nueva que no se considera válida al no tener material de referencia asociado.

Como ya indicaban Hibbett et al. (2011), el número de MOTUs (*Molecular operational taxonomic units*) que se descubren anualmente a partir de muestras ambientales es muy alto; sin embargo, de acuerdo con el Código Internacional de Nomenclatura para algas, hongos y plantas

(ICN), no se les puede acuñar un binomio. Hawksworth et al. (2016) proponían emendar el ICN para permitir que esas secuencias sin especímenes físicos asociados sirvieran como tipos para taxones de hongos, lo que aceleraría la descripción de toda la diversidad pendiente. Sin embargo, esta propuesta fue rechazada y, en opinión de autores como Thines et al. (2018), estos cambios pueden conllevar fatales consecuencias como una ciencia irreproducible o la tipificación de datos que son resultado de artefactos, entre otros. Es innegable que todas estas contribuciones han supuesto un avance en el conocimiento de la diversidad, aunque deben continuar en desarrollo y evaluación hasta que podamos establecer metodologías y aproximaciones que nos permitan acelerar la descripción de toda esa diversidad fúngica, pero asegurando delimitaciones precisas.

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Conclusiones/Conclusions

Conclusiones

1— Los patrones de distribución inferidos para las especies de hongos corticioides aparecen sesgados por los criterios de reconocimiento empleados. Así, la delimitación basada exclusivamente en datos morfológicos ha sobreestimado la distribución en hongos corticioides como *Aleurodiscus limonisporus*, *Cystostereum murrayi* o *Amylostereum laevigatum*.

2— Las especies detectadas en las aproximaciones filogenéticas se han corroborado con los análisis de tipo morfológico, lo que confirma la importancia de esta fuente de evidencia taxonómica.

3— Los estudios monográficos de géneros, sobre la base de una taxonomía integradora, son necesarios para tener un conocimiento más preciso de la diversidad fúngica y desvelar los taxones que pueden permanecer ocultos en complejos de especies, así como las identificaciones erróneas.

4— No se pueden inferir los patrones de distribución de aquellas especies asociadas a pocos registros o exclusivamente al ejemplar tipo, ya que probablemente presenten patrones más amplios que son aún desconocidos.

5— A medida que se incorporan datos de diferentes fuentes de evidencia, se alcanzan inferencias más robustas que permiten interpretar el patrón de distribución. Los análisis de coste basados en datos vectoriales de viento prueban la relevancia de la dispersión a larga distancia mediada por el viento hacia los archipiélagos oceánicos.

6— Los resultados obtenidos en esta tesis doctoral evidencian que el conocimiento sobre los hongos corticioides es desigual a muchos niveles. Es especialmente notable la necesidad de exploración de las regiones desconocidas, que nos permita aumentar las colecciones y desarrollar trabajos florísticos.

7— La falta de registro fósil en hongos supone un impedimento a la hora de evaluar la diversidad y sus distribuciones dentro de un marco evolutivo.

Conclusions

1— The distribution range of corticioid fungi is biased by the criteria used in species recognition. Thus, species delimitation based exclusively on morphological characters led to overestimation of the distribution of corticoid fungi such as *Aleurodiscus limonisporus, Cystostereum murrayi* or *Amylostereum laevigatum*.

2— The species detected in the phylogenetic approaches have been confirmed on the basis of morphological analysis, which support the relevance of this source of evidence in species delimitation.

3— Monographic studies of genera based on an integrative taxonomy should be conducted to obtain a more precise knowledge of fungal diversity, as well as to reveal taxa that may remain hidden in species complexes and as misidentifications.

4— The distribution patterns cannot be inferred for those species that are known from only a few records or exclusively from a type specimen, since they likely present broader patterns that are still unknown.

5— As data from more sources of evidence are incorporated, more robust inferences are reached allowing us to interpret the distribution pattern. Cost analyses based on wind vectors support the relevance of wind-mediated long distance dispersal towards oceanic archipelagos.

6— The results obtained in this thesis dissertation show that knowledge about corticioid fungi is uneven at many levels. It is especially pertinent to explore unknown regions, which allows us to increase collections and to enhance floristic work.

7— The lack of fungal fossil records presents an impediment to evaluating diversity and its distribution within an evolutionary framework.