UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Araucaria (*Araucaria araucana*) canker disease in Chile: etiology and fungal diversity

by

Felipe Alejandro Balocchi Schalchli

Submitted in fulfilment of the requirements for the degree

Philosophiae Doctor

In the Faculty of Natural & Agricultural Sciences

Department of Plant and Soil Sciences

University of Pretoria

Pretoria

August 2022

Primary Supervisor

Prof. Irene Barnes

Co-supervisors

Prof. Michael J. Wingfield

Dr. Rodrigo Ahumada

DECLARATION

I, **Felipe Alejandro Balocchi Schalchli** declare that the thesis/dissertation, which I hereby submit for the degree **Philosophiae Doctor** at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Felipe A. Balocchi S.

August 2022

TABLE OF CONTENTS

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

ACKNOWLEDGMENTS

As I have progressed in my life and carrer, the task of acknowledging people has become increasingly complicated. There are so many people that have played a role in my life, and people that have influenced my life so much, that everything that I have, that I have accomplished, and who I am to my very core are results of multiple influences. In short, I can't say less than that I have had a priviledged life. This acknowledgments should, however, focus exclusively on people who played a role in me reaching the point of completing this PhD, which is not in every case such an obvious division.

First, my family. Family is all, and mine has never been less. I was blessed by being raised by caring and supportive parents, to whom I owe everything and more. I was blessed (or cursed?) with no less than three sisters (who carry around two tiny dogs), who are full of life, energy, positive vibes and my full admiration (not talking about the dogs). I have the most wonderful son, who came to give sense to my life, and to whom I dedicate this study. I have been surrounded by wonderful cousins, uncles, aunts, and others, resulting in a huge warm hearted family that has shaped my life.

In terms of this PhD, I can't start anywhere else than by thanking my supervisors. I thank Rodrigo, who gave me this amazing opportunity and trusted me with a project in good faith. I thank Irene and Mike for receiving me with open arms having no idea who or what I was, teaching me patiently, and supporting me consistently in an amazing manner. I can't posibly express how much I value the consistent and unfailable smile with which I was received ever single time we met, and which everytime would end up been transmitted to me as well. THANK YOU.

I would like to thank as well my 'extended' supervisors, who taught me and keep teaching me patiently, sharing with me their absolutely genuine passion for what they do. With this I refer to Seonju, Cobus and Trudy, who receive(d) me everytime with the best posible disposition.

I would like to thank my labmates at Lab 2-9, all with whom I have overlapped, for making the lab a neat place to work, but as well, a generally nice place to be around. Special thanks to Ariska, our former lab manager (and a friend), who mentored me when I arrived, showed me around, taught me (and keeps teaching me) loads of things, and who kept our lab running making everyones life easier. I also would like to thank Cheyenne, who took over managing the lab, but also for her friendship and cathartic conversations.

The first months in a foreign country were not easy, and there were numerous people that helped me get established. Seems mandatory to me to start by thanking Ginna and Marinus, who like teaching a toddler to give the first steps, they took care of me and helped me get on my feet. Thanks to Duccio, Tanay and Trudy, who welcomed me in that old house, which we made an international experience, and a continuous cooking contest in which I always lost (so much nice food though).

Thanks to the friends I made during these years. In no specific order, Ginna, Marinus, Trudy, Quentin, Josephine, Ariska, Redzuan, Darryl, Joey, Allan, Nam, Runlei, Godfrey, María and Victor, and so many others with whom we have shared a piece of where we belong, a nice conversation, genuine laughter, a braai or a coffee. Special thanks to Trudy, my ‘yunta’, someone that has played so many roles in my life since I got here, for whom I feel the deepest respect and admiration, and has become part of ‘the family we choose’.

Finally, I would like to thank the friends that have accompained me throughout my life, with whom I grew in different ways, and who gave me support whenever and in whatever way I needed. Manolín, Hernandito, Carlitos, Gustavin, Marielopsis, Diego; ha sido un privilegio tenerlos en mi vida.

Quite obviously, I failed to mention everyone. But my heart felt warm thinking of all the incredible people that surronds me, that I have got to meet, and that have been there for me. Gracias totales.

ABSTRACT

The thesis titled ‘Araucaria (*Araucaria araucana*) canker disease in Chile: etiology and fungal diversity’ considered a serious disease that has recently emerged on *Araucaria araucana*, an iconic conifer species endemic to the mountain ranges of Chile and Argentina. The main objective of the study was to describe the main symptoms of the disease and to determine its causal agent, as well as to consider associated organisms. The first chapter of the thesis provides an extensive literature review on diseases of the *Araucariaceae*. This review highlighted the scarcity of studies and information available regarding the health of this mostly endangered family of trees, and the threats posed by invasive alien organisms and climate change. The first experimental research chapter in this study described the symptomatology associated with the cankers found on *A. araucana*. Through systematic sampling, isolations and pathogenicity tests, it was shown that the causal agent of the disease was a fungus in the *Coryneliaceae.* Morphological and phylogenetic analyses demonstrated that the fungus resides in a novel genus and species, described in the study as *Pewenomyces kutranfy*. In the second experimental chapter, additional *Pewenomyces* species present on the diseased *A. araucana* samples were characterised. Phylogenetic and morphological analyses, including an examination of relevant herbarium specimens for two *Caliciopsis* species (*C. brevipes* and *C. cochlearis*) previously described from the same host, revealed the presence of three new distinct species of *Pewenomyces*. These were described as *Pewenomyces lalenivora*, *P. tapulicola* and *P. kalosus*, none of which appeared to be pathogenic. The last experimental chapter of the thesis described two fungal species in the genus *Resinogalea* (*R. araucana* and *R. tapulicola*) found growing on the resin released from cankers on branches of *A. araucana*. This rare fungal species has only one close relative species, found on resin patches on branches of *Araucaria humboldtensis* in New Caledonia, and it resides in a recently described subclass of fungi, the *Cryptocaliciomycetidae*. All the fungi discovered during this thesis are rare species and seem to have a close relationship with *A. araucana* and the environmental conditions where they occur. This supports the hypothesis that these trees have a large biodiversity associated with them that and they will have coevolved after the continental drift.

PREFACE

Araucaria (*Araucaria araucana*), also commonly known as pewén or the monkey puzzle, is an ancient tree species endemic to the South-central mountain ranges in Chile and Argentina. These trees are widely recongnized based on their uncommon and robust crown structure, their large size, and their edible seeds. *Araucaria araucana* has colonised high elevation environments, where it has adapted to conditions experiencing snow and has become a core species in a unique environment (araucaria forests). The edibility of *A. araucana* seeds has made these trees a valuable resource for indigenous communities in these difficult enviromnets (Mapuche – Pewenche, formerly referred to in Spanish as Araucanos), becoming essential to their culture and economy. Consequently, *A. araucana* is considered a Chilean indigenous symbol, now recognized as the national tree of the country.

Between late 2015 and early 2016, *Araucaria* trees in most of their natural distribution started displaying severe crown die-back symptoms, and in rare ocassions mortality. Early investigations of this disease syndrome revealed a diverse range of secondary symptoms and organisms, however, the primary cause of the damage remained unresolved. Further surveys led to the discovery of cankers consistently on the branches and occasionally stems of symptomatic trees. These cankers appeared to slowly girdle the branches, ultimately killing them, and it was suspected that they were caused by a fungus. This view was based on the consistent presence of fruiting structures characteristic of *Caliciopsis* species (*Coryneliaceae*), a genus containing conifer canker pathogens, on the diseased tissues. However, there was no literature referring to these symptoms on *A. araucana* or its close relatives in the *Araucariaceae*. Consequently, this unknown canker disease became the focus of the project on which this thesis was based.

The first chapter of this thesis provides a review of the literature available on diseases of the *Araucariaceae*. This was motivated by the fact that there was very little literature available on this topic either pertaining to *A. araucana*, or the *Araucariaceae* as a whole. Considering that most of the *Araucariaceae* have limited geographical distributions and several species have been declared to be at a risk of extintion, the occurrence of diseases on these trees represents a serious threat to their existence. The aim of this chapter was to synthesise the available literature, making it visible, and raise the potential consequences of what a lack of a solid foundation on diseases can have in a predominantly endangered group of species.

The second chapter of this thesis focuses on the canker disease found on *Araucaria araucana*, with the aim of studying the symptomatology and etiology of the disease. The work in this chapter included selecting study sites in the natural distribution of *A. araucana*, monitoring of disease symptom progression and systematic collection of samples and isolation of fungi presumed to cause the disease.

The fungi are characterised using both a morphological approach and by sequencing of different DNA gene regions and pathogenicity tests are included for the canker causal agent candidates. Emphasis is given to fungi residing in the *Coryneliaceae* and this informed the structure of subsequent studies.

The third chapter focuses on the identity of a group of fungal isolates belonging in the *Coryneliaceae* that were obtained from samples of *A. araucana* and remained unresolved from Chapter 2. These isolates potentially represented new species in *Pewenomyces*, a novel genus resembling *Caliciopsis* only known from *A. araucana*. Morphological studies and DNA sequence comparisons are used to determine the identity of the isolates, including also herbarium specimens and a new collection of samples to represent two *Caliciopsis* species previously known from *A. araucana*.

The fourth chapter focuses on a rare calicioid fungus that was found emerging from dry resin patches on *A. araucana* branch samples bearing cankers. The ascomata found on the samples have an uncommon morphology, although somewhat similar to those produced by a fungus on resin on branches of *Araucaria humboldtensis* in New Caledonia, i.e., *Resinogalea humboldtensis*. However, *R. humboldtensis* does not have DNA sequence data or living cultures available for comparisons to be made, and thus, its taxonomic possition has remained uncertain. The research in this chapter considers the identity of the fungus found on the resin of *A. araucana*, including additional isolates obtained from plant tissues covered with resin, using DNA sequence data and morphological comparisons.

This thesis consists of four chapters, of which the first is a literature review, and the following are research chapters. Each chapter is composed as a stand-alone study that either has been published (see section on publications) or is in the process of being submitted to ISI-rated scientific journals. Additionally, the results of this thesis were used in an interational collaboration, resulting in a publication that considered native diseases and their environmental drivers. A summary of the thesis is provided at the end of the document.

Illustrations at the beginning of each chapter were created using the Dream by Wombo platform (http://wombo.art) by introducing keywords and/or images from each chapter.

PUBLICATIONS

1. PUBLICATIONS IN SCIENTIFIC JOURNALS

Chapter 1 (literature review):

Balocchi F, Wingfield MJ, Paap T, Ahumada R, Barnes I (2022) Pathogens of the Araucariaceae: How much do we know? Current Forestry Reports 8:1–24. https://doi.org/10.1007/s40725-022-00164-z

Chapter 2:

Balocchi F, Wingfield MJ, Ahumada R, Barnes I (2021) *Pewenomyces kutranfy* gen nov. et sp. nov. causal agent of an important canker disease on *Araucaria araucana* in Chile. Plant Pathology 70:1243–1259. https://doi.org/10.1111/ppa.13353

International collaboration:

Burgess TI, Oliva J, Sapsford SJ, Sakalidis ML, Balocchi F, Paap T (2022) Anthropogenic disturbances and the emergence of native diseases; a threat to forest health. Current Forestry Reports 8:111–123. https://doi.org/10.1007/s40725-022-00163-0

2. CHAPTERS SUBMITTED FOR PUBLICATION

Chapter 3:

Balocchi F, Marincowitz S, Wingfield MJ, Ahumada R, Barnes I (June 2022) Three new species of *Pewenomyces* (Coryneliaceae) from *Araucaria araucana* in Chile. Mycological Progress. In review.

2. PRESENTATIONS AT CONGRESSES AND CONFERENCES

Balocchi F, Marincowitz S, Wingfield MJ, Ahumada R, Barnes I (2022) *Pewenomyces kutranfy*, causal agent of an important canker disease on *Araucaria araucana* in Chile. *Oral presentation* at the 52nd Congress of the Southern African Society for Plant Pathology. Pretoria, South Africa.

Balocchi F, Marincowitz S, Wingfield MJ, Ahumada R, Barnes I (2022) Three new species of *Pewenomyces* (*Coryneliaceae*) from *Araucaria araucana* in Chile. *Poster presentation* at the 52nd Congress of the Southern African Society for Plant Pathology. Pretoria, South Africa.

Balocchi F (2022) Araucaria (*Araucaria araucana*) canker disease in Chile. *Oral presentation* at the TPCP 33rd annual meeting of the Tree Protection Co-operative Programme (TPCP) and the DSI NRF Centre of Excellence in Plant Health Biotechnology (CPHB). Pretoria, South Africa.

Balocchi F, Ahumada R, Wingfield MJ, Barnes I (2020) A new genus and species of Coryneliaceae (Coryneliales) causing a serious canker disease on *Araucaria araucana* in Chile (Poster). The Mycological Society of America: MSA 2020: Mycology from the Cloud. DOI: 10.13140/RG.2.2.34372.96642

CHAPTER 1

Pathogens of the Araucariaceae: How much do we know?

Felipe Balocchi
Michael J. Wingfield
Trudy Paap
Rodrigo Ahumada
Irene Barnes

Current Forestry Reports
https://doi.org/10.1007/s40725-022-00164-z

Pathogens of the Araucariaceae: How Much Do We Know?

Felipe Balocchi[1] · **Michael J. Wingfield**[2] · **Trudy Paap**[2] · **Rodrigo Ahumada**[3] · **Irene Barnes**[2]

Accepted: 10 February 2022

Abstract

Purpose of Review The Araucariaceae is a family of ancient conifers containing iconic tree species from diverse parts of the world. Thirty-eight extant species are present in three genera. Extensive reduction of natural populations has occurred for many species of Araucariaceae, largely due to anthropogenic disturbances. This has occurred to the extent where most species are classified at some level of extinction risk. In recent decades, several diseases have emerged on trees in the family, which has highlighted a general lack of knowledge regarding the factors impacting the health of Araucariaceae. We addressed this by compiling all available literature regarding pathogens and diseases of the Araucariaceae. Insights are given into how globalization and climate change may have, and will potentially, play a role in the emergence of current and future disease threats. These threats are considered from both an ecological and economic perspective.

Recent Findings A total of 227 disease reports were found for the family (58 for *Agathis*, 161 for *Araucaria* and eight for *Wollemia*), of which 88% related to only eight tree species. Consequently, there was a considerable number of species in the Araucariaceae for which no disease reports were found. The most prevalent pathogens reported were species of *Phytophthora*, root rotting basidiomycetes such as *Phellinus* or *Armillaria*, and pathogens within the Botryosphaeriaceae. However, only 25% of the pathogens found have had their pathogenicity confirmed through tests, and only 22% have had their identity confirmed through DNA sequencing, making evident the limited amount of research carried out on this topic.

Summary There is a general lack of baseline information on diseases for trees in the Araucariaceae. The effects that pathogens have had, and may have in the future, in this iconic family of trees are concerning as most of the species have been declared at some level of risk of preservation. Both globalization and climate change have indicated the potential effects they can have, and how unpredictable they can be. This lack of a solid baseline understanding may become an important constraint on attempts to preserve these species, and thus, it is evident that research efforts on these topics are much needed.

Keywords *Agathis* · Anthropogenic activities · *Araucaria* · Climate change · Emerging pathogens · Forest diseases · Invasive forest pathogens · *Wollemia*

The submitted work has not been published and is not under consideration for publication in any other journal or book.

This article is part of the Topical Collection on *Forest Pathology*

✉ Irene Barnes
irene.barnes@fabi.up.ac.za

Felipe Balocchi
felipe.balocchi@fabi.up.ac.za

Michael J. Wingfield
mike.wingfield@fabi.up.ac.za

Trudy Paap
trudy.paap@fabi.up.ac.za

Rodrigo Ahumada
rodrigo.ahumada@arauco.com

[1] Forestry and Agricultural Biotechnology Institute (FABI), Department of Plant and Soil Sciences, University of Pretoria, Pretoria 0002, South Africa

[2] FABI, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria 0002, South Africa

[3] Bioforest S.A, Silviculture and Phytosanitary Protection Division, Concepcion, Chile

Introduction

Tree diseases are among the main factors causing forest disturbances globally [1]. Pathogens have been responsible for severe economic losses in plantation forests [2] and have led to catastrophic tree death and damage to natural woody ecosystems [3–5]. This has primarily been driven by globalization and climate change; two factors placing increasing pressure on forests, resulting in an increasingly regular emergence of new diseases and declines [6, 7•].

Recently, an outbreak of a dieback and canker disease on *Araucaria araucana* (Araucariaceae) has emerged in these iconic trees within their native range in Chile and Argentina [8, 9]. During studies carried out to determine the aetiology of this canker disease, we considered the availability and status of literature regarding fungal diseases of *A. araucana* and its relatives. Abundant literature was encountered referring to the negative impact that human activity has had on these trees; these included issues such as intensive logging [10, 11], changes in fire regimes [12, 13] and cattle ranching [14]. However, most literature regarding fungal diseases and pathogens on *A. araucana* was fragmented, outdated and/or difficult to access.

An increasing number of cases of dieback have been reported on species within the Araucariaceae. These include those on *A. cunninghamii* and *A. bidwillii* in Australia [15], *Araucaria heterophylla* in Norfolk Island [16, 17] and *Agathis montana* in New Caledonia [18•, 19]. Importantly, information on fungal diseases and pathogens of these tree species was at best scarce and/or difficult to access. Such a lack of accessible information limits the ability to identify damaging fungi and/or to comprehend symptoms that these trees may exhibit in the case of a disease outbreak. This may also result in resources being unnecessarily expended on duplicated research.

In order to address a distinct lack of knowledge regarding diseases of the Araucariaceae and considering the global importance of these trees, this study sought to access available information on this topic. The overall aim was to provide a comprehensive compilation of the fungal diseases occurring on species of the Araucariaceae, including their status regarding taxonomy, pathogenicity, and relative importance. Perspectives are provided on the threats these diseases pose to Araucariaceae species and how globalization and climate change have had, and could have, an impact on these trees in the future.

The Hosts: Araucariaceae: Description, Phylogeny, Distribution and Socio-economic Importance

Description

The Araucariaceae is a family of mostly robust tree species, of which several reach sizes of up to 50 m tall and 2–3 m in diameter. Species in the family are evergreen, mainly monoecious (with only two dioecious species) [20–22], and contain long living species (documented cases of trees over a thousand years of age) [23–26]. They undergo episodic recruitment, which is highly dependent on gap creation following natural disturbances [27]. The family includes three genera, namely *Agathis* Salisb. (17 species), *Araucaria* Juss. (20 species) and *Wollemia* W.G. Jones (one species), which are easily differentiated from each other by basic morphological features such as shape and organization of the leaves or ornamentation of the seeds.

Phylogeny

The Araucariaceae is a monophyletic clade sister to Podocarpaceae, which collectively make up the Araucariales [28, 29•]. Within the Araucariaceae, *Agathis* species form a monophyletic clade, which along with *Wollemia* (*Wollemia nobilis*) form the "Agathoid clade" sister to the monophyletic *Araucaria* clade (Fig. 1). The genus *Araucaria* is sub-divided into four sections: *Araucaria* (two species), *Bunya* (one species), *Eutacta* (16 species) and *Intermedia* (1 species), defined by their morphology [30, 31] and further supported with molecular data [29•, 32]. The largest of these sections, *Eutacta*, almost exclusively includes species endemic to New Caledonia and forms a unique clade within *Araucaria* sister to a clade that accommodates the other three sections [29•, 33]. Section *Araucaria* accommodates the South American species, *A. araucana* and *A. angustifolia*. Sections *Intermedia* and *Bunya* each include the single species, *A. hunsteinii* and *A. bidwillii*, respectively.

Geographical Distribution

The current geographical distribution of the extant species in the Araucariaceae is almost completely restricted to the southeast Asia–Pacific regions except for two species occurring in South America (Fig. 2). Most of the species grow exclusively in the Southern Hemisphere, except for a few *Agathis* species that occur in peninsular Malaysia, northern Borneo and the Philippines. The current hotspot of Araucariaceae diversity is New Caledonia, with 14 *Araucaria* species and four *Agathis* endemic to this island [32, 34].

Most species of Araucariaceae grow in rainforests, *Agathis* in tropical to sub-tropical environments, while most *Araucaria* species in sub-tropical to temperate environments. Some species of *Araucaria* have adapted to grow in rough environments; this includes, for example, a number of New Caledonian species that grow in the ultramafic

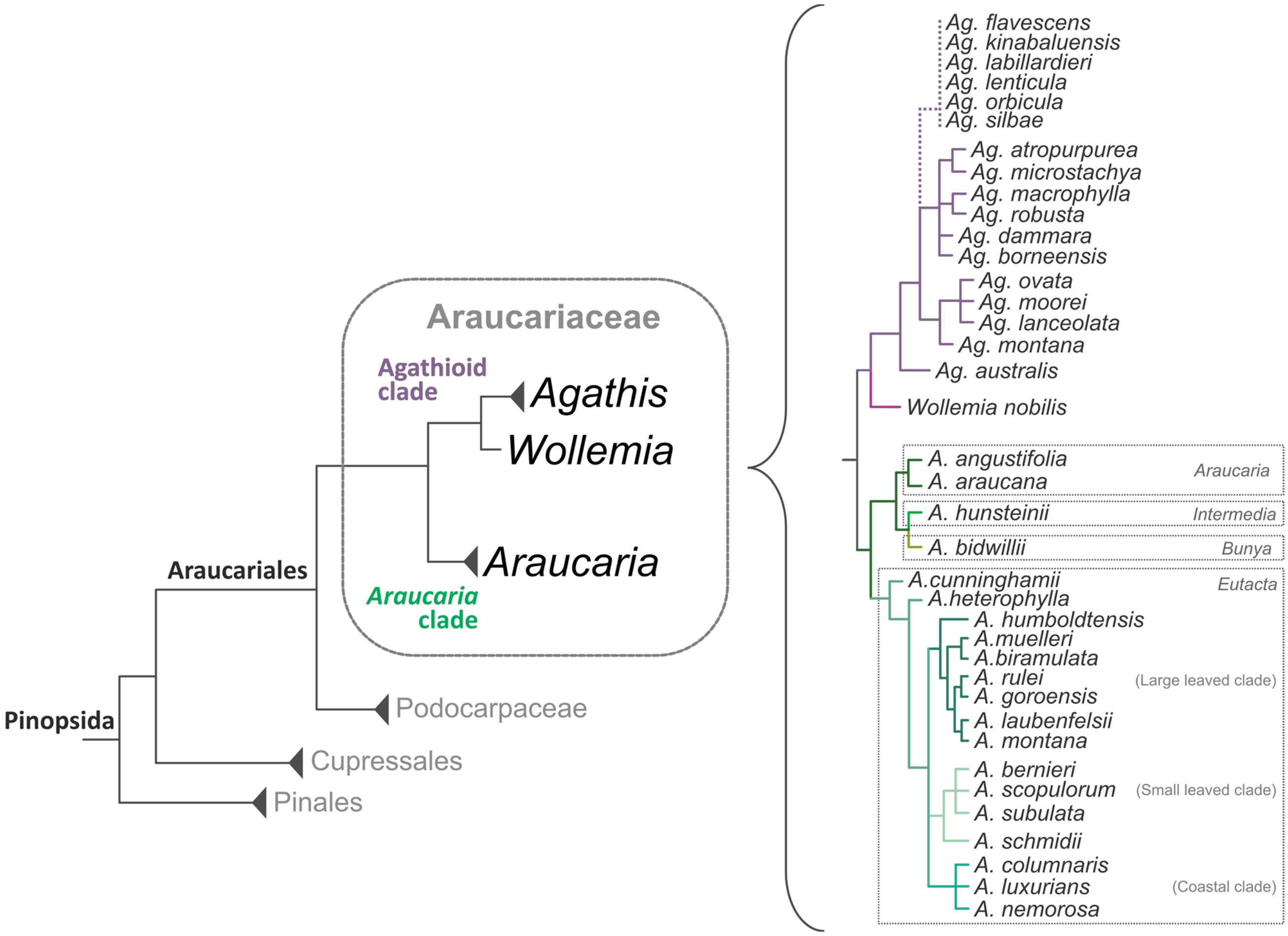


Fig. 1 Theoretical phylogeny for the Araucariaceae constructed based on Farjon (2010), Escapa and Catalano (2013), Ruhsam et al. (2015) and Mill et al. (2017)

maquis, and the South American *A. araucana* that grows in the Andes mountain range on volcanic derived soil and which has adapted to conditions with snow [21, 35]. The monotypic genus *Wollemia* includes only the relatively recently discovered species, *W. nobilis*. This species was discovered in 1994 and has a very restricted distribution in the Blue Mountains of Australia [22, 36].

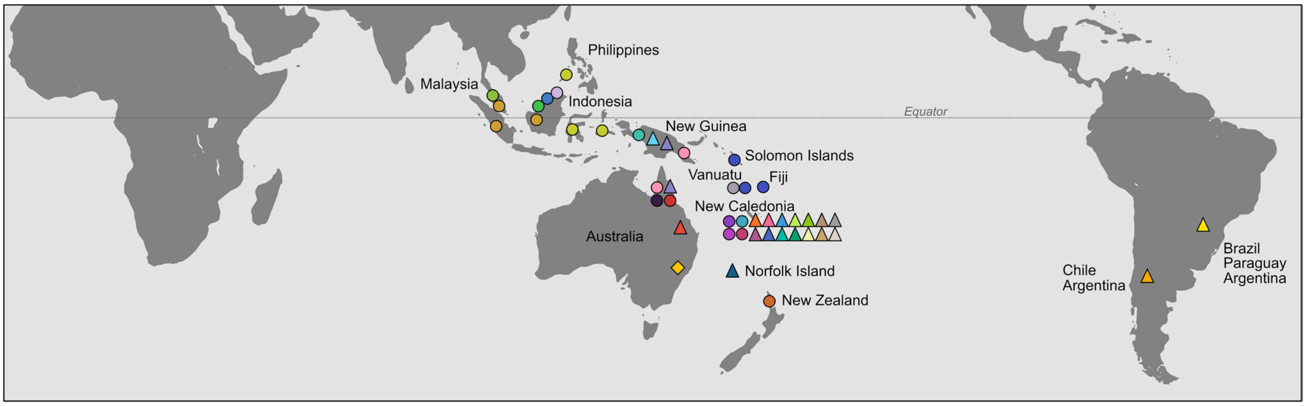
The Araucariaceae has a rich fossil record [35, 37] from which several species have been described from countries in both the Northern and the Southern Hemispheres [38–45]. This has led to the conclusion that the current distribution of the family is relictual of a broad expansion that occurred during the late-Mesozoic and early-Cenozoic eras [35].

Socio-economic Importance

Most species in the Araucariaceae are not well known, with their occurrence restricted to their natural ranges. However, a number of species are recognized for their cultural importance and/or economic value. For example, *Araucaria araucana*, the type species of the family and genus, derives its name from Arauco, the homeland of the Mapuche Pewenche people who consider this tree sacred [46]. Similarly, *Agathis australis*, commonly referred to as kauri, is of symbolic importance for the Maori people of New Zealand [47], as is *A. bidwillii*, the Bunya pine, to the indigenous people of southern Queensland and northern New South Wales in Australia [48, 49]. Species including *Ag. australis* [50], *A. cunninghamii* [51], *A. angustifolia* [52, 53] and *A. hunsteinii* [54] have been planted for silvicultural purposes, while *A. heterophylla* (Norfolk Island pine) [55] and *A. columnaris* [56] are commercialized as ornamentals in many parts of the world.

Diseases of the Araucariaceae

Generally, species in the Araucariaceae have been described as not very susceptible to diseases [57], and greater attention has been given to their insect pests. Only a small number of species in the family have been the subject of studies on



Species	Altitude range	Species	Altitude range	Species	Altitude range
Agathis		● *Ag. orbicula*	450 - 1050	▲ *A. humboldtensis*	750 - 1600
● *Ag. atropurpurea*	900 - 1500	● *Ag. ovata*	150 - 1150	▲ *A. hunsteinii*	600 - 2000
● *Ag. australis*	0 - 630	● *Ag. robusta*	5 - 1900	▲ *A. laubenfelsii*	400 - 1400
● *Ag. borneensis*	1 - 2400	● *Ag. silbae*	450 - 760	▲ *A. luxurians*	0 - 1000
● *Ag. dammara*	0 - 2200	***Araucaria***		▲ *A. montana*	500 - 1400
● *Ag. flavescens*	1200 - 1350	▲ *A. angustifolia*	500 - 2300	▲ *A. muelleri*	550 - 1000
● *Ag. kinabaluensis*	1500 - 2400	▲ *A. araucana*	600 - 1800	▲ *A. nemorosa*	20 - 185
● *Ag. labillardieri*	50 - 2000	▲ *A. bernieri*	100 - 500	▲ *A. rulei*	400 - 800
● *Ag. lanceolata*	200 - 1100	▲ *A. bidwillii*	150 - 1200	▲ *A. schmidii*	1400 - 1630
● *Ag. lenticula*	1050 - 1700	▲ *A. biramulata*	190 - 1150	▲ *A. scopulorum*	5 - 600
● *Ag. macrophylla*	75 - 900	▲ *A. columnaris*	1 - 100	▲ *A. subulata*	150 - 1070
● *Ag. microstachya*	400 - 1100	▲ *A. cunninghamii*	15 - 3355	***Wollemia***	
● *Ag. montana*	1000 - 1629	▲ *A. goroensis*	150 - 550	◆ *W. nobilis*	670 - 780
● *Ag. moorei*	250 - 1000	▲ *A. heterophylla*	5 - 316		

Fig. 2 Natural distribution of current extant Araucariacea species. Constructed based on IUCN (2021), Palmer et al. (2006) and Silba (1983)

diseases and for which the contributing pathogens have been well described.

Published Literature on Araucariaceae Diseases

Multiple searches using the Google scholar browser (https://scholar.google.es/) with a diverse combination of terms was used to compile all reports of diseases occurring on the Araucariaceae. These included (i) the host taxon names for the family, genera and each species and (ii) keywords such as "disease", "pathogen", "decline", "dieback", "damage" or a specific genus or species of pathogen. The disease reports found using these searches were grouped by pathogen-host-environment combinations, and for each of these, the methods used for assessing the identity of the causal agent and pathogenicity, and the abundance of literature available about it were recorded and ranked into categories.

A total of 227 disease reports were found for the Araucariaceae (Fig. 3). Of these, 58 were for *Agathis* (Table 1), 161 were for *Araucaria* (Table 2) and 8 for *Wollemia* (Table 3). Eighty-eight per cent of the reports found for the family related to only eight species (*Ag. australis*, *Ag. macrophylla*, *A. angustifolia*, *A. araucana*, *A. bidwillii*, *A. cunninghamii*, *A. heterophylla* and *W. nobilis*), all of which have been used for commercial purposes. It was consequently clear that there remains a large number of species for which information is extremely limited. These include 12 *Araucaria* spp. and nine *Agathis* spp. for which no reports of diseases were found.

The environment from which the reports originated were linked to the main use of the host species (Fig. 4). For example, diseases reported on *Ag. australis* and *A. angustifolia*, two tree species exploited and planted for timber in their countries of origin, were mostly from natural forests, plantations and nurseries in their countries of origin. This was also the case for *A. araucana*, a tree of ethnobotanical, conservation and ornamental interest, for which most of the disease reports were from natural forests in Chile and Argentina. *Araucaria cunninghamii*, which has been used extensively within, and outside its natural range for timber plantations and ornamental plantings, had a larger geographical range in the origins of disease reports. These reports included those from its countries of origin (Australia and Papua New Guinea) and countries where it has been established as an exotic. In contrast, *A. heterophylla*, used widely as an ornamental in several countries of the world, had 26 reports of diseases but only one of which was from its country of origin (Norfolk Island).

Most Common Pathogens of the Araucariaceae

A total of 205 host–pathogen combinations were found for species in the Araucariaceae, irrespective of the environment in which the trees occurred. These included a total of 111

Fig. 3 Diseases on species in the Araucariaceae. **A** Dieback of ornamental *Araucaria columnaris* caused by fungi in the Botryosphaeriaceae in South Africa. **B** Rust disease of *Araucaria araucana* caused by *Mikronegeria fagi* in Chile. **C–D** Dieback of *Araucaria araucana* associated to cankers caused by *Pewenomyces kutranfy* in Chile. **E** Potted plant of *Wollemia nobilis* wilting after inoculation with *Phytophthora cinnamomi* in Australia. **F** Dieback of *Agathis australis* associated to *Phytophthora agathidicida* in New Zealand (photo credits: A: Trudy Paap; B, D: Felipe Balocchi; C: Rodrigo Ahumada; E: Edward Liew; F: Peter Scott)

fungal and oomycete pathogens species. However, this number could be misleading, as species identity was confirmed using molecular tools for only 22% of these host–pathogen combinations. In addition, for 17% of the host–pathogen combinations, the associated organisms had only been identified to the genus level (Table 4).

Importantly, confirmation of the host–pathogen–symptom interactions following Koch's postulates was only fulfilled for 51 of the 205 (25%) reported cases. However, while lacking pathogenicity studies, several pathogens had consistent in-field observations (mostly root rotting basidiomycetes) or were obligate biotrophs (e.g., rusts diseases), providing some support to the presumption of pathogenicity. Overall, despite the uncertainties surrounding the available disease reports and the reliability for the identification of the causal organisms, the most prevalent pathogens for tree species in the Araucariaceae are those residing in the Oomycete genus *Phytophthora*, root rotting basidiomycetes such as *Phellinus* and *Armillaria*, and fungi residing in the Botryosphaeriaceae (Tables 1, 2 and 3).

Table 1 Diseases on *Agathis* spp.

Disease / Pathogen	Host	Location	Environment [a]	Path. [b]	Tax. [c]	Lit. [d]	Symptoms	Observations	Ref.
Root rots									
Armillaria limonea	*Ag. australis*	New Zealand	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md iS** dS	DR 1s **2+**	root rot on young trees		[47, 58-60]
A. novae-zelandiae	*Ag. australis*	New Zealand	**Nf Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR 1s **2+**	root rot on young trees		[47, 59-61]
Corticium kauri	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	brown rot of stumps		[62, 63]
Erythricium salmonicolor	*Ag. macrophylla*	Pacific Islands	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	DR **1s** 2+	root rot	"Pink disease" (=*Corticum salmonicolor*).	[64, 65]
	Ag. dammara	Indonesia	Nf **Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	DR 1s **2+**	dieback, death of trees		[23]
Ganoderma spp.	*Ag. dammara*	Indonesia	Nf **Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	root rot and death of trees, rizomorphs under bark	Five potential species have been found, one suggested as *G. applanatum* based on the fruiting bodies morphology.	[66, 67]
Phellinus noxius	*Ag. macrophylla*	Pacific Islands	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root rot, death of trees		[65, 68]
Phytophthora agathidicida	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR 1s **2+**	root and collar rot, girdles the trunks, dieback and death of trees	(= *Phytophthora* taxon Agathis; PTA) Originally reported as *P. heveae.*	[69-71]
P. cinnamomi	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As** Is **Pt**	**Md** iS **dS**	DR 1s **2+**	root rot associated to tree death in poor drained stands	Pathogenicity tests show low aggressivity.	[70, 72, 73]
P. cryptogea	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	root rot associated to diseased seedlings and soil	Pathogenicity tests show low aggressivity.	[70, 74]
P. kernoviae	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As** Is **Pt**	**Md iS** dS	DR **1s** 2+	from soil, not associated with symptoms	Detected in the soil in natural environments, a unique report from soil under a dead adult tree, inoculations indicate low aggressivity.	[75, 76]
P. multivora	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR 1s **2+**	weak pathogen detected from soil		[70, 72]
P. nicotianae	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	As **Is** Pt	**Md** iS dS	DR **1s** 2+	bleeding cankers on trunk	A unique report from a bleeding lesion on a young tree.	[75, 77]
Pyrrhoderma lamaënse	*Ag. moorei*	New Caledonia	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	basidiocarps on diseased trees	Epithet with several variations: *lamaoense, lamaense* or *lamaensis.*	[78, 79]
	Ag. ovata	New Caledonia	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	basidiocarps on diseased trees	(=*Phellinus lamaoense, Phellinidium lamaoense*)	[78, 79]
Wood rots and stains									
Fomes hemitephrus	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md iS** dS	DR 1s **2+**	white heart rot	(=*Fomitopsis hemitephra*)(=*Pilatoporus hemitephrus*)	[47]
Fuscoporia contigua	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	white rot	(=*Phellinus contiguus*)	[62]
Ganoderma applanatum	*Ag. australis*	New Zealand	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md iS** dS	**DR** 1s 2+	white heart rot extending to the sapwood	(=*Elfvingia applanata*)	[47, 80]
Heterobasidion araucariae	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR 1s **2+**	minor sapwood and heartwood decay	Originally recorded as *Heterobasidion annosum* (=*Fomes annosum*).	[47, 62, 81, 82]
	Ag. macrophylla	Fiji	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md iS** dS	DR **1s** 2+	basidiocarps on stumps or dead standing and fallen wood		[64, 81, 83]
Phaeolus schweinitzii	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	red-brown cubical butt rot on adult trees	Taxonomic revision of New Zealand's material is required.	[62, 84]
Phellinus gilvus	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	white rot	Authors indicate it occurs mostly on fallen wood.	[62]
Canker diseases									
Acremonium charticola	*Ag. macrophylla*	Malaysia	Nf **Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	associated with stem cankers	Ahmad (1986) cites Ivory (1975) incorrectly mentioning this pathogen on *Ag. dammara.*	[85, 86]
Fusarium sp.	*Ag. macrophylla*	Malaysia	Nf **Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	associated with stem cankers	Ahmad (1986) cites (Ivory 1975) incorrectly mentioning this pathogen on *Ag. dammara.*	[85, 86]
Lasiodiplodia theobromae	*Ag. macrophylla*	Malaysia	Nf **Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	associated with stem cankers	Ahmad (1986) cites Ivory (1975) incorrectly mentioning this pathogen on *Ag. dammara.*	[85, 86]
Neofusicoccum mangiferae	*Ag. robusta*	Australia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	infecting leaves and causing twig dieback	(=*Hendersonula agathi*)	[87, 88]
Rusts and Smuts									
Araucariomyces balansae	*Agathis* sp.	New Caledonia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	hypertrophies to shoot blight	(=*Aecidium balansae*) Believed to be endemic to New Caledonia.	[89]
	Ag. moorei	New Caledonia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	hypertrophies to shoot blight		[89, 90]
	Ag. ovata	New Caledonia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	hypertrophies to shoot blight		[89, 90]
A. fragiforme	*Agathis* sp.	Malaysia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	hypertrophies to shoot blight	(=*Aecidium fragiforme*)	[86, 89, 90]
	Agathis spp.	Pacific Islands	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	DR 1s **2+**	hypertrophies to shoot blight		[91]
Araucariomyces fragiforme	*Ag. borneensis*	Malaysia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	hypertrophies to shoot blight	(=*Ag. alba*, =*Ag. dammara*)	[86, 89, 90]
	Ag. labillardieri	New Guinea	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	hypertrophies to shoot blight		[89]
	Ag. macrophylla	Fiji	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	hypertrophies to shoot blight	(=*Ag. vitiensis*)	[89, 91]
	Ag. robusta	Australia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR 1s **2+**	hypertrophies to shoot blight	(=*Ag. palmerstonii*)	[89, 92]
Leaf spots and blights									
Calonectria pteridis	*Ag. macrophylla*	Pacific Islands	**Nf** Pl Nu Or Sb ?	As **Is** Pt	**Md** iS dS	**DR** 1s 2+	foliage blight	(=*Cylindrocladium macrosporum*)	[65]
Colletotrichum sp.	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	As **Is** Pt	**Md** iS dS	**DR** 1s 2+	isolated from dead and dying seedlings	Only *C. aotearoa* has been identified from *Ag. australis* [93].	[62]
	Ag. moorei	New Caledonia	Nf Pl Nu Or Sb **?**	As **Is** Pt	**Md** iS dS	**DR** 1s 2+	not specified		[78]
	Ag. ovata	New Caledonia	Nf Pl Nu Or Sb **?**	As **Is** Pt	**Md** iS dS	**DR** 1s 2+	anthracnose	Mentions *C. gloeosporioides.*	[78]
C. gloeosporioides	*Agathis* spp.	Pacific Islands	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf cast, can ultimately kill plants		[91]
Diaporthe sp.	*Ag. australis*	New Zealand	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	leaf spots		[62]
Lophodermium agathidis	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	As **Is** Pt	**Md** iS **dS**	DR **1s** 2+	fruiting bodies have been found only in dead leaves		[94-96]
L. mahuianum	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	As **Is** Pt	**Md** iS dS	**DR** 1s 2+	fruiting bodies have been found only in dead leaves		[97]
Phyllosticta sp.	*Ag. australis*	New Zealand	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	isolated from living leaves	Also reported *Guignardia* sp.	[62]
	Ag. macrophylla	Vanuatu	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf spots	(=*Guignardia pini)*	[64]
Setameliola agathidis	*Ag. robusta*	Australia	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf spots	(=*Meliola agathidis*) (=*Ag. palmerstonii)*	[98]
Trichothallus niger	*Ag. australis*	New Zealand	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	sooty mould	(=*Trichopeltheca asiatica*)	[62]
Vizella tunicata	*Ag. australis*	New Zealand	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf spots		[62]
Damping off and nursery diseases									
Botrytis cinerea	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	As **Is** Pt	**Md** iS dS	**DR** 1s 2+	grey mould, on living leaves and leaf litter.		[62]

Table 1 (continued)

Disease / Pathogen	Host	Location	Environment [a]	Path. [b]	Tax. [c]	Lit. [d]	Symptoms	Observations	Ref.
Clonostachys candelabrum	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	As **Is Pt**	**Md** iS dS	**DR** 1s 2+	not specified, inoculations showed pathogenicity	(=*Sesquicillium candelabrum*)	[62]
Fusarium sp.	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is Pt**	**Md** iS dS	DR **1s** 2+	isolated from dead and dying seedlings		[62]
Pestalotiopsis sp.	*Ag. australis*	New Zealand	**Nf** Pl **Nu** Or Sb ?	**As Is Pt**	**Md** iS dS	**DR** 1s 2+	death of recently emerged seedlings	Authors indicate is a secondary pathogen.	[47, 62]
P. funerea	*Ag. australis*	New Zealand	**Nf** Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	death of recently emerged seedlings	Authors indicate is a secondary pathogen.	[47, 62]
Pyrenochaeta sp.	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	isolated from dead and dying seedlings		[62]
Pythium irregulare	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	damping-off		[99]
P. ultimum	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	damping-off		[62,100]
Thanatephorus cucumeris	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	damping-off		[62]
Verticillium sp.	*Ag. australis*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	isolated from dead and dying seedlings		[62]

[a] Environment: **Nf**: natural forests, **Pl:** plantations, **Nu:** nurseries, **Or:** ornamental, **Sb:** seed batches, **?**: not specified.

[b] Path: Pathogenicity **As:** associated to diseased trees, **Is:** isolated from diseased tissues, **Pt:** pathogenicity confirmed through inoculations.

[c] Tax: Taxonomic position: **Md:** identified through morphology, **iS:** indirectly sequenced (e.g., isolates from same or close environments but different hosts or obsolete techniques), **dS:** directly sequenced.

[d] Lit: Literature abundance: **DR:** disease records, brief or no description of symptoms and methods, **1s:** a unique report, with descriptions of methods, **2+:** two or more reports.

Phytophthora species cause the most extensive and damaging diseases recorded for the Araucariaceae. Multiple species of *Phytophthora* have been associated with severe symptoms in hosts residing in all three genera in the family, including important diseases within natural forests. Iconic cases include *Phytophthora agathidicida* (=*Phytophthora* taxon *Agathis*, PTA) causing kauri dieback (*Ag. australis*) in its natural range in New Zealand [69, 75] (Fig. 3F), and *P. cinnamomi* causing serious severe root rot and dieback of *Wollemia nobilis* within its limited natural range in Australia [204, 205] (Fig. 3E) and on *A. angustifolia* in Brazil in areas reforested with the species [118]. There are also numerous reports of *Phytophthora* species causing disease on *Araucaria* species in nurseries within and outside their countries of origin [119, 121, 125–127].

Root rotting basidiomycetes have commonly been associated with trees displaying disease symptoms in natural stands and plantations. Among these, *Phellinus noxius* is one of the most serious root rot pathogens of *Agathis* and *Araucaria* [68, 114], among which *A. cunninghamii* trees, especially those in plantations in Australia and Papua New Guinea, have been the most seriously affected [112]. *Armillaria* species have also been associated with severe root rots and resination on species of *Agathis* and *Araucaria*, both in plantations and natural stands [47, 101, 103, 107]. *Agathis* species in the southeast Asia–Pacific region are known to be susceptible to root rots caused by other pathogenic basidiomycetes such as *Ganoderma* spp. [66], the crust fungus *Corticium kauri* [62] and *Erythricium salmonicolor*, the causal agent of “pink disease” [23, 64].

Several fungal species residing in different genera in the Botryosphaeriaceae, a family accommodating well-studied endophytic latent pathogens [208], have been associated with dieback, canker diseases, leaf blights and damping-off on tree species of all genera of the Araucariaceae [85, 87, 152, 155, 162, 163, 166, 205]. Among the pathogens of Araucariaceae, the Botryosphaeriaceae have the widest global distribution, with reports from trees planted as ornamentals or in botanical gardens in many parts of the world [125, 151, 157, 159–161] (Fig. 3A).

Some other pathogens of the Araucariaceae that have received attention are those host-specific and unique to the family. These include, for example, the rust pathogens, *Araucariomyces balansae* (=*Aecidium balansae*) and *A. fragiforme* (=*Aecidium fragiforme*) that cause leaf deformation on *Agathis* species, mostly in their native range [89], and *Mikronegeria fagi* that produces its haplont phase on *Araucaria araucana*, causing severe leaf symptoms in Chile and Argentina [168] (Fig. 3B). Similarly, the smut fungus *Uleiella* includes two species that cause diseases on *Araucaria* spp. These include *U. chilensis* that infects the female cones of *A. araucana* and *U. paradoxa* that rots the male cones of *A. angustifolia* [169, 170].

Most recently, the ascomycete *Pewenomyces kutranfy*, residing in the Coryneliaceae, has been described causing girdling cankers on branches of *A. araucana* in its natural range [8] (Fig. 3D). To date, this fungal species is known only from that host and location. Additionally, a group of pathogenic fungi has been described from unique hosts in the Araucariaceae, causing in most cases only mild leaf diseases. These fungi include species such as *Araucasphaeria foliorum*, *Mycosphaerella wollemiae*, *Kalmusia araucariae*, *Phyllosticta acicola*, *Setameliola agathidis* and *S. araucariae* [98, 173, 185, 191, 207].

Species of Araucariaceae cultivated in nurseries have also been reported as susceptible to diseases typical of these environments. Among these are several reports of diseases caused by pathogens residing in typically wide host range genera such as *Fusarium* [62, 85, 102, 179, 205], *Calonectria*, *Botrytis* [62, 196], *Pythium* [99, 100, 102], *Rhizoctonia* [102, 111, 198], *Colletotrichum* [179] and in the Botryosphaeriaceae [111, 151, 162, 163, 205]. These disease reports arise from diverse locations and species in the family. All these pathogens are known to cause similar symptoms on a wide range of other hosts, suggesting that their association with Araucariaceae is likely driven by the exposure of these tree species to nursery environments.

Table 2 Diseases on *Araucaria* spp

Disease / Pathogen	Host	Location	Environment [a]	Path. [b]	Tax. [c]	Lit. [d]	Symptoms	Observations	Ref.
Root rots									
Armillaria sp.	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root rot on trees of all ages	Originally as *A. mellea.*	[101, 102]
A. fuscipes	*A. cunninghamii*	Zimbabwe	Nf **Pl** Nu Or Sb ?	As **Is** Pt	**Md** iS **dS**	DR **1s** 2+	not specified	In some reports as *A. heimii.*	[103, 104]
A. mellea	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root rot	Taxonomy needs revision.	[105]
	A. heterophylla	Spain	Nf Pl Nu **Or** Sb ?	As **Is** Pt	**Md iS** dS	DR **1s** 2+	not specified	Detected on screening isolations from soil, identified by RFLP.	[106]
A. novae-zelandiae	*A. cunninghamii*	Australia	Nf **Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	root rot, commonly found on stumps	Identification supported by interfertility tests.	[107]
Calonectria brassicae	*A. angustifolia*	Brazil	**Nf Pl Nu** Or Sb ?	**As Is Pt**	**Md iS** dS	DR 1s **2+**	root rot, resination, leaf blight	(=*Cylindrocladium clavatum*)	[102, 108, 109]
Cylindrocladiella sp.	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	decline and death of trees		[101]
Desarmillaria tabescens	*A. araucana*	Mexico	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	root rot and tree death	(=*Armillaria tabescens*). On a single tree.	[110]
Hendersonula sp.	*A. angustifolia*	Brazil	Nf **Pl Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	collar and root rot of seedlings, rotting of male cones	Original record inaccessible.	[101, 102]
Helicobasidium longisporum	*A. cunninghamii*	Australia	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	root rot and tree death	(=*Helicobasidium compactum*) Similarity with *H. purpureum* has led to incorrect records. Original record inaccessible.	[111]
Mortierella alpina	*A. araucana*	Argentina	**Nf** Pl Nu Or Sb ?	As **Is Pt**	**Md** iS **dS**	DR **1s** 2+	isolated from necrotic tissues in stems and collars	A unique pathogenic isolate has been reported.	[9]
Mortierella aff. *basiparvispora*	*A. araucana*	Argentina	**Nf** Pl Nu Or Sb ?	As **Is Pt**	**Md** iS **dS**	DR **1s** 2+	isolated from necrotic tissues in stems and collars	A unique pathogenic isolate has been reported.	[9]
Phellinus sp.	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root and collar rot, death of trees		[101]
P. noxius	*A. cunninghamii*	Australia	Nf **Pl** Nu Or Sb ?	**As Is Pt**	**Md** iS dS	DR 1s **2+**	root rot and butt rot that leads to the death of trees	(=*Fomes*, =*Phellinidium*), =*Pyrrhoderma*? [79]	[112, 113]
		Taiwan	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	brown rot on declining trees		[114-116]
	A. heterophylla	Taiwan	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	DR **1s** 2+	brown rot on declining trees		[114]
Pyrrhoderma lamaënse	*A. columnaris*	New Caledonia	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	basidiocarps on diseased trees	Epithet with several variations: *lamaoense, lamaense* or *lamaensis* (=*Phellinus lamaoense, Phellinidium lamaoense*).	[78, 79]
Phytophthora castanae	*Araucaria* sp.	Papua New Guinea	Nf Pl Nu Or Sb **?**	**As** Is Pt	**Md iS** dS	**DR** 1s 2+	no description of symptoms	(=*Phytophthora katsurae*)	[117]
P. cinnamomi	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	As **Is Pt**	**Md** iS **dS**	DR **1s** 2+	root rot, severe crown symptoms and death of trees		[118]
		Spain	Nf Pl Nu **Or** Sb ?	**As** Is Pt	**Md** iS **dS**	DR **1s** 2+	obtained from soil, no association to symptoms		[106]
		South Africa	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md iS** dS	**DR** 1s 2+	associated to diseased plants in nurseries, root rot		[119]
	A. araucana	Chile	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root and collar rot		[120]
		New Zealand	Nf Pl **Nu** Or Sb ?	As Is **Pt**	**Md** iS dS	DR **1s** 2+	inoculated seedlings show reduction in growth	Inoculations were done with a mixture of isolates obtained from other hosts.	[121]
	A. bidwillii	Spain	Nf Pl Nu **Or** Sb ?	**As** Is Pt	**Md** iS **dS**	DR **1s** 2+	obtained from soil, no association to symptoms		[106]
	A. cunninghamii	Papua New Guinea	Nf **Pl** Nu Or Sb ?	**As** Is Pt	**Md iS** dS	DR **1s** 2+	associated to chlorotic trees	Species identity confirmed by sequencing isolates obtained from other hosts.	[122, 123]
	A. heterophylla	Hawaii	Nf Pl Nu Or Sb **?**	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	seedling root rot and tree decline	Original record inaccessible.	[124]
		New Zealand	Nf Pl **Nu** Or Sb ?	As Is **Pt**	**Md** iS dS	DR **1s** 2+	inoculated seedlings stunted and with needle chlorosis	Seedlings inoculated with a mixture of *P. cinnamomi* isolates.	[121]
P. citrophthora	*A. angustifolia*	Argentina	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root rot		[125]
	A. araucana	Chile	Nf Pl **Nu** Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	seedlings with decay, chlorosis, dieback, root rot.		[126]
P. cryptogea	*A. araucana*	United Kingdom	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS **dS**	DR **1s** 2+	no description of symptoms		[127]
P. multivora	*A. araucana*	Chile	Nf Pl **Nu** Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	seedlings with decay, chlorosis, dieback, root rot		[126]
P. nicotianae	*A. araucana*	Iran	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+			[128]
	A. heterophylla	Perú	Nf Pl Nu **Or** Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	stem cankers and branch dieback	(=*Phytophthora parasitica*) on (=*Araucaria excelsa*)	[125]
Rosellinia bunodes	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is **Pt**	**Md iS** dS	DR 1s **2+**	black root rot, death of trees	Inoculations and sequencing done with isolates obtained from poplar plantations.	[102, 129]
Wood rots and stains									
Aureobasidium pullulans	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	root and collar rot		[101]
Fomitopsis nivosa	*A. cunninghamii*	Australia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	brown wood rot	(=*Trametes hispidans*)	[130, 131]
Fusarium angustifolium	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	staining of wood	No other record of this fungal species could be found in any database.	[102]
Ganoderma sp.	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	staining of wood	(=*Elfwingia*)	[102]
G. australe	*A. angustifolia*	Argentina	Nf Pl Nu Or Sb **?**	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	white heart rot		[132, 133]
G. applanatum	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	wood rot on field		[102]
G. mexicanum	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	DR **1s** 2+	not specified		[134]
Heterobasidion araucariae	*A. bidwillii*	Australia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md iS** dS	DR **1s** 2+	fruiting bodies on a log		[83, 135]
	A. cunninghamii	Australia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR 1s **2+**	on stumps or dead standing and fallen wood		[81, 83, 135, 136]
		Papua New Guinea	**Nf Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR 1s **2+**	basidiocarps isolated from trunk of dead trees	Originally recorded as *Heterobasidion annosum* (=*Fomes annosum*).	[84, 137, 138]
Huntiella moniliformis	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md iS** dS	DR **1s** 2+	blue stain in sapwood and in the edge of logged trunks	(=*Ceratocystis moniliforme*)	[105, 139]
Lentinus villosus	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	staining of wood	.	[102]
Mycoenterolobium platysporum	*A. heterophylla*	United States (Hawaii)	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	described from decaying wood	Genus and species described from a specimen on wood.	[124, 140]
Ophiostoma araucariae	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	blue stain in sapwood and in the edge of logged trunks	Staining ability was confirmed by inoculating fresh sterile wood.	[141-143]

Table 2 (continued)

Disease / Pathogen	Host	Location	Environment [a]	Path. [b]	Tax. [c]	Lit. [d]	Symptoms	Observations	Ref.
O. triangulosporum	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	blue stain in sapwood and in the edge of logged trunks		[144, 145]
Phellinus swieteniae	*A. angustifolia*	Argentina	Nf Pl Nu Or Sb **?**	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	white heart rot on a dead standing tree	Authors indicate doubtful identification.	[133]
Phomopsis sp.	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	staining of wood		[102]
Picnoporus cinnabarinus	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	staining of wood		[102]
Pleurotus ostreatus	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	wood rot on field		[102]
Rigidoporus microporus	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md iS** dS	**DR** 1s 2+	staining of wood	(=*Fomes lignosus*)	[102]
R. ulmarius	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	DR **1s** 2+	collected from bark of living trees		[146]
R. vinctus	*A. cunninghamii*	Australia	Nf **Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	root and butt rot, tree death	(=*Junghuhnia vincta*; =*Chaetoporus radulus*)	[147-149]
		Kenya	Nf **Pl** Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	root and butt rot	(=*Poria vincta*)	[150]
Trametes versicolor	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	wood rot on field	(=*Coriolus versicolor*)	[102]
	A. araucana	Chile	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md iS** dS	**DR** 1s 2+	rotting stumps and logs		[105]
Canker diseases									
Botryosphaeria dothidea	*A. cunninghamii*	Taiwan	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	associated to declining trees		[151]
	A. rulei	New Caledonia	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	fruiting bodies on female cones (anthracnose)		[125]
Diplodia africana	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	associated to twig death	Isolated from a single tree.	[152]
D. mutila	*A. araucana*	Chile	Nf Pl Nu **Or** Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	associated to bleeding cankers		[153]
D. sapinea	*A. angustifolia*	Brazil	**Nf Pl Nu** Or Sb ?	**As Is Pt**	**Md** iS dS	DR 1s **2+**	dieback, damping-off	(=*Sphaeropsis sapinea*) (=*Diplodia pinea*)	[102, 154]
	A. cunninghamii	Australia	Nf **Pl Nu** Or Sb ?	**As Is Pt**	**Md iS** dS	DR 1s **2+**	dieback, death of trees staining of wood, damping-off	(=*Diplodia pinea*)	[111, 125]
D. seriata	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	gummy cankers on branches		[155]
	A. heterophylla	Australia	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS **dS**	**DR** 1s 2+	not specified		[156]
Dothiorella sp.	*A. heterophylla*	Hawaii	Nf Pl Nu Or Sb **?**	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	associated to bleeding cankers	Original record inaccessible.	[124]
Fusicoccum araucariae	*A. araucana*	Italy	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	collar rot and stem cankers	(=*Cryptosporella araucariae*; perfect state)	[157, 158]
Lasiodiplodia theobromae	*A. cunninghamii*	Australia	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	rotting the base of stems and damping-off		[111]
		Malaysia	Nf **Pl Nu** Or Sb ?	**As Is Pt**	**Md** iS dS	DR **1s** 2+	dieback of weakened trees, damping-off	Original record innaccessible	[85]
		Taiwan	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	dieback		[151]
	A. heterophylla	Taiwan	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	dieback		[151]
		Hawaii	Nf Pl Nu **Or** Sb ?	**As Is Pt**	**Md** iS dS	DR **1s** 2+	associated to bleeding cankers		[159]
		Ethiopia	Nf Pl Nu **Or** Sb ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	stem cankers and needle blight		[160]
Neofusicoccum hongkongense	*A. cunninghamii*	China	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	not described		[161]
Neofusicoccum mangiferae	*A. heterophylla*	Taiwan	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	associataed to tree decline		[151]
N. nonquaesitum	*A. araucana*	Chile	Nf Pl **Nu** Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	dieback and death of trees		[162]
N. occulatum	*A. cunninghamii*	Australia	Nf Pl **Nu** Or Sb ?	As **Is** Pt	**Md** iS **dS**	DR **1s** 2+	not specified		[163, 164]
N. parvum	*A. heterophylla*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	associated to dieback		[163, 165]
	A. heterophylla	Australia	Nf Pl Nu **Or** Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	dieback associated to drought		[166, 167]
N. ribis	*A. araucana*	Mauritius	Nf Pl Nu **Or** Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	dieback		[125]
	A. cunninghamii	Mauritius	Nf Pl Nu **Or** Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	dieback		[125]
		Australia	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	stem cankers		[111]
Pewenomyces kutranfy	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	girdling cankers on branches and stems		[8]
Rusts and Smuts									
Mikronegeria fagi	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As** Is **Pt**	**Md iS** dS	DR 1s **2+**	leaf damage, death of weak seedlings	(=*Caeoma sanctae-crucis*)	[90, 105, 168]
		Argentina	**Nf** Pl Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	leaf damage, death of weak seedlings		[90, 105, 168]
Uleiella chilensis	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR 1s **2+**	smut fungus that rots female cones and seeds	In some reports identified as *U. paradoxa*, considered synonym.	[105, 169]
U. paradoxa	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	smut fungus that rots male cones and seeds	Some authors wrongly mention this pathogen on *A. imbricata* (=*A. araucana*).	[170, 171]
Leaf diseases									
Appendiculella araucariae	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	parasitic on leaves	Species described and reported uniquely from this host.	[172]
Araucasphaeria foliorum	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	leaf spots		[173]
Atotospora araucariae	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	associated to spots diseased leaves	Referred as a weak pathogen.	[105, 174]
Caliciopsis brevipes	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	Leaf galls	Similar symptoms have also been attributed to mite damage [175].	[105, 176]
Calonectria spathulata	*A. angustifolia*	Brazil	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	no description	Description based on leaf spots on *Eucalyptus* spp.	[108, 177]
		Colombia	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	no description	Description based on leaf spots on *Eucalyptus* spp.	[108, 177]
Cirsosiopsis violacescens	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	necrotic spots and discoloration in the base of the leaves	Monotypic genus described from *A. angustifolia.*	[172, 178]
Coccomyces araucariae	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	associated to spots on diseased leaves	Referred as a weak pathogen.	[172]
Colletotrichum sp.	*A. hunsteinii*	Papua New Guinea	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	leaf spots, bud blight on seedlings		[179]
Cycloschizon araucariae	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf spots		[172]
Didymella araucariae	*A. araucana*	Italy	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	needle blight	(=*Araucaria imbricata*)	[157, 180]
Episphaerella araucariae	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf spots	Species description from this host is the only report.	[172]
E. serialis	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf spots	Species description from this host is the only report.	[172]
Gloeosporium araucariae	*A. rulei*	New Caledonia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	emerging from female cones and leaves	Two separate species descriptions with the same name.	[181-184]
	Araucaria sp.	USA (Hawaii)	Nf Pl Nu Or Sb **?**	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	not specified		[123]
Kalmusia araucariae	*A. bidwillii*	USA	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	**DR** 1s 2+	associated to leaf blight		[185]
Neocucurbitaria cava	*A. araucana*	USA	Nf Pl Nu **Or** Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	needle blight	(=*Phyllosticta araucariae*)	[186, 187]
	A. bidwillii	Italy	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	sporulating on leaves	(=*Phyllosticta araucariae*, =*araucariecola*)	[187, 188]

Table 2 (continued)

Disease / Pathogen	Host	Location	Environment	Path.	Tax.	Lit.	Symptoms	Observations	Ref.
Pestalotia sp.	*A. angustifolia*	Brazil	**Nf Pl** Nu Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	rotting of female cones		[101]
Phaeocryptopus araucariae	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	weak or secondary leaf blight		[105, 189]
Phyllosticta acicola	*A. angustifolia*	Brazil	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	severe leaf spots	(=*Phyllosticta brasiliensis*)	[125]
	A. heterophylla	Japan	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	brown needle blight	(=*Phyllosticta drummondii*)	[190]
		USA (Hawaii)	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	lesions on cones		[191]
	Araucaria sp.	Canada	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	needle blight, dieback and lesions on cones		[191]
Phyllosticta concentrica	*A. heterophylla*	Norfolk Island	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	lesions on cone scales		[191]
		New Zealand	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	lesions on cone scales		[191]
Pseudomeliola brasiliensis	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	circular, light brown and raised leaf spots	Species description from this host is the only report.	[172]
Rhizothyrium parasiticum	*A. araucana*	Chile	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf spots	Species description from this host is the only report.	[192]
Seiridium sp.	*A. angustifolia*	Brazil	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	kills old leaves of seedlings	(=*Pestalozzia* sp.)	[102]
Setameliola araucariae	*A. cunninghamii*	Australia	Nf Pl Nu Or Sb **?**	**As Is** Pt	**Md** iS dS	DR **1s** 2+	leaf spots, may cause the death of leaves and branchlets	(=*Meliola araucariae*)	[98]
Xenomeris acicola	*A. angustifolia*	Brazil	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	necrotic discolouration and spots on leaf surface	Species described and reported only once, only from this host.	[172]
Damping-off									
Athelia rolfsii	*A. bidwillii*	Brazil	Nf Pl **Nu** Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	collar rot and damping off		[193]
	A. cunninghamii	Australia	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	collar rot	(=*Sclerotium rolfsii*)	[111]
		Papua New Guinea	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	collar rot	(=*Sclerotium rolfsii*)	[194]
	Araucaria sp.	Brazil	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	collar rot	(=*Sclerotium rolfsii*)	[195]
Botrytis cinerea	*A. araucana*	New Zealand	Nf Pl Nu Or Sb **?**	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	not specified		[196]
Calonectria cylindrospora	*A. angustifolia*	Brazi	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	damping-off and stem cankers	(=*Cylindrocladium scoparium*)	[102]
C. pacifica	*A. heterophylla*	Hawaii	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS **dS**	**DR** 1s 2+	collar rot	(=*Cylindrocladium kyotensis;* =*Cylindrocladium floridanum*)	[124, 197]
Fusarium sp.	*A. angustifolia*	Brazil	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	DR 1s **2+**	damping-off		[101, 102]
		Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off		[198]
	A. cunninghamii	Malaysia	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	DR **1s** 2+	root rot		[85]
		Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off		[198]
	A. araucana	Chile	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root a collar rot		[120]
	A. hunsteinii	Papua New Guinea	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	wilting and death of seedlings		[179]
	A. heterophylla	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off		[198]
F. lateritium	*A. angustifolia*	Brazil	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	stem cankers with resin exudation		[102]
Fusarium robustum	*A. angustifolia*	Argentina	Nf Pl **Nu** Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR 1s **2+**	collar rot on seedlings		[199-201]
Fusarium oxysporum	*A. angustifolia*	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. bidwillii	Egypt	Nf Pl Nu Or **Sb** ?	As **Is** Pt	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. cunninghamii	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. heterophylla	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198, 202]
Glomerella cingulata	*A. cunninghamii*	Malaysia	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root rot on six-month-old seedlings		[85]
Macrophomina phaseolina	*A. araucana*	Chile	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	root a collar rot		[120]
Neocosmospora solani	*A. angustifolia*	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot	(=*Fusarium solani*)	[198]
	A. bidwillii	Egypt	Nf Pl Nu Or **Sb** ?	As **Is** Pt	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. cunninghamii	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. heterophylla	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198, 202]
Phytophthora spp.	*A. angustifolia*	Brazil	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	damping-off		[102]
Pythium sp.	*A. angustifolia*	Brazil	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	damping-off		[102]
		Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. cunninghamii	Australia	Nf Pl **Nu** Or Sb ?	As **Is** Pt	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[111]
		Egypt	Nf Pl Nu Or **Sb** ?	As **Is** Pt	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. heterophylla	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	seed rot		[198, 202]
		USA	Nf Pl **Nu** Or Sb ?	As **Is** Pt	**Md** iS dS	**DR** 1s 2+	not specified		[203]
P. irregulare	*A. heterophylla*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	damping-off		[99]
P. ultimum	*A. columnaris*	New Zealand	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	damping-off		[62,100]
Rhizoctonia sp.	*A. cunninghamii*	Australia	Nf Pl **Nu** Or Sb ?	As **Is** Pt	**Md** iS dS	DR **1s** 2+	damping-off		[111]
	A. angustifolia	Brazil	Nf Pl **Nu** Or Sb ?	**As** Is Pt	**Md** iS dS	**DR** 1s 2+	damping-off		[101]
Rhizoctonia solani	*A. angustifolia*	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. bidwillii	Egypt	Nf Pl Nu Or **Sb** ?	As **Is** Pt	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. cunninghamii	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198]
	A. heterophylla	Egypt	Nf Pl Nu Or **Sb** ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	damping-off and seed rot		[198, 202]

[a] Environment: **Nf**: natural forests, **Pl:** plantations, **Nu:** nurseries, **Or:** ornamental, **Sb:** seed batches, **?**: not specified.
[b] Path: Pathogenicity **As:** associated to diseased trees, **Is:** isolated from diseased tissues, **Pt:** pathogenicity confirmed through inoculations.
[c] Tax: Taxonomic position: **Md:** identified through morphology, **iS:** indirectly sequenced (e.g., isolates from same or close environments but different hosts or obsolete techniques), **dS:** directly sequenced.
[d] Lit: Literature abundance: **DR:** disease records, brief or no description of symptoms and methods, **1s:** a unique report, with descriptions of methods, **2+:** two or more reports.

Disease Threats

Tree pathogens represent a serious threat to the species in the Araucariaceae. The impact of tree pathogens is often quantified in terms of economic losses for plantation species. Although this could be applied for some species of Araucariaceae, it is not as concerning as the potential impact pathogens may have from an ecological perspective. This is in light of most of the species having a reduced natural distribution, to the extent where many have been declared at some level of risk by the

Table 3 Diseases on *Wollemia nobilis*

Disease / Pathogen	Host	Location	Environment [a]	Path. [b]	Tax. [c]	Lit. [d]	Symptoms	Observations	Ref.
Root rots									
Phytophthora cinnamomi	*W. nobilis*	Australia	**Nf** Pl Nu Or Sb ?	**As** Is **Pt**	**Md** iS **dS**	DR 1s **2+**	root rot associated to tree decline and death		[204-206]
P. multivora	*W. nobilis*	Australia	**Nf** Pl Nu Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	root rot associated to tree decline		[206]
Canker diseases									
Botryosphaeria sp.	*W. nobilis*	Australia	Nf Pl **Nu** Or Sb ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	not specified	Pathogenicity tests showed high aggressivity.	[205]
Fusarium oxysporum	*W. nobilis*	Australia	Nf Pl **Nu** Or Sb ?	As **Is Pt**	**Md** iS dS	DR **1s** 2+	root rot	Two out of nine inoculated plants showed symptoms.	[205]
Neofusicoccum australe	*W. nobilis*	Australia	Nf Pl **Nu** Or Sb ?	**As Is** Pt	**Md** iS **dS**	DR **1s** 2+	dieback		[163]
N. occulatum	*W. nobilis*	Australia	Nf Pl **Nu** Or Sb ?	**As Is Pt**	**Md** iS **dS**	DR **1s** 2+	dieback		[163, 164]
N. parvum	*W. nobilis*	Taiwan	Nf Pl Nu **Or** Sb ?	**As Is** Pt	**Md** iS **dS**	DR 1s **2+**	dieback	Isolates caused symptoms to *A. cunninghamii* and *A. heterophylla* when inoculated.	[151]
Leaf spots and blights									
Mycosphaerella wollemiae	*W. nobilis*	Australia	**Nf** Pl Nu Or Sb ?	**As Is** Pt	**Md** iS dS	**DR** 1s 2+	leaf spots		[207]

[a] Environment: **Nf:** natural forests, **Pl:** plantations, **Nu:** nurseries, **Or:** ornamental, **Sb:** seed batches, **?**: not specified.
[b] Path: Pathogenicity **As:** associated to diseased trees, **Is:** isolated from diseased tissues, **Pt:** pathogenicity confirmed through inoculations.
[c] Tax: Taxonomic position: **Md:** identified through morphology, **iS:** indirectly sequenced (e.g., isolates from same or close environments but different hosts or obsolete techniques), **dS:** directly sequenced.
[d] Lit: Literature abundance: **DR:** disease records, brief or no description of symptoms and methods, **1s:** a unique report, with descriptions of methods, **2+:** two or more reports.

IUCN [209]. Along with threats posed by fragmentation, climate change, altered fire regimes and anthropogenic activities such as cattle grazing, logging and seed collection, diseases can have devastating effects on the preservation of species.

Ecological Threats

The threat of *Phytophthora* species is among the most concerning of the disease risks to Araucariaceae. For example, in Australia, *P. cinnamomi* and *P. multivora* have been detected within the extremely limited native range of *W. nobilis* [204, 206]. This tree species is known to be highly susceptible to both pathogens [206], which is even more concerning for a tree species that represents a single population with low genetic diversity [210] and a limited number of trees at a reproductively mature age [211]. Similarly, *P. agathidicida*, the causal agent of kauri dieback (*Ag. australis*) in New Zealand, is an aggressive primary pathogen that can spread rapidly and can girdle trunks and kill trees of all ages [212••]. This disease has had a significant impact on the kauri population by decreasing the number of individuals, which will also translate into a negative effect on the reproduction rate of the species.

Less aggressive diseases caused by *Phytophthora* species can also represent relevant threats to Araucariaceae forests. For example, *P. cinnamomi*, *P. multivora* and *P. cryptogea* have frequently been detected within the natural range of kauri [72] and pathogenicity to kauri has been demonstrated [70]. Although these *Phytophthora* species are not considered to cause severe disease symptoms, there is evidence to suggest that *P. cinnamomi* may have an important negative impact in the regeneration of the species by causing pre- and post-emergence damping-off to seedlings [75]. Differences in susceptibility to these *Phytophthora* species among the accompanying species, as observed with *P. agathidicida* [212••], are presumed to reduce the regeneration of kauri forests species, favouring their replacement and thus altering a unique indigenous ecosystem [24].

Another threat that diseases pose on natural forests is the disturbance of sexual reproduction. There are pathogens known to directly disturb this process on some Araucariaceae growing under natural conditions. These include, for example, *Uleiella* spp. that rot male and female cones of the South American species *A. angustifolia* and *A. araucana* respectively [105, 169, 170]. Other pathogens may disturb this process indirectly, for example, by reducing the abundance of cones by infecting and killing branches of sexually mature trees. This could be the case for canker pathogens such as *P. kutranfy* on *A. araucana* [8] and Botryosphaeriaceae pathogens that occur on diverse Araucariaceae species [87, 88, 152, 154, 155]. The slow nature of the reproduction cycle observed on the long-lived species in the Araucariaceae [213], including long time frames for trees to reach sexual maturity [214–217], relatively long time frames for cones to mature [94, 215, 218] and high mortality rates of natural regeneration [214, 217–219], make these trees especially sensitive to these disturbances. The impact of pathogens on the reproduction of Araucariaceae has not been quantified; however, it is likely underestimated and should be taken seriously.

Pathogens can limit opportunities to preserve species using approaches such as restoration or commercialization. Diseases caused by pathogenic fungi are a common problem during tree production activities, and Araucariaceae are not an exception. As mentioned previously, a diverse range of fungal and oomycete pathogens have been reported from Araucariaceae in nurseries [62, 102, 111, 126, 163, 205]. Although most of these pathogens are common in nursery environments, in some cases, *Araucaria* species have shown higher levels of susceptibility than has been reported for other hosts. Examples include *Athelia rolfsii* on *A. bidwillii* in Brazil [193], *Fusarium robustum* on *A. angustifolia* in

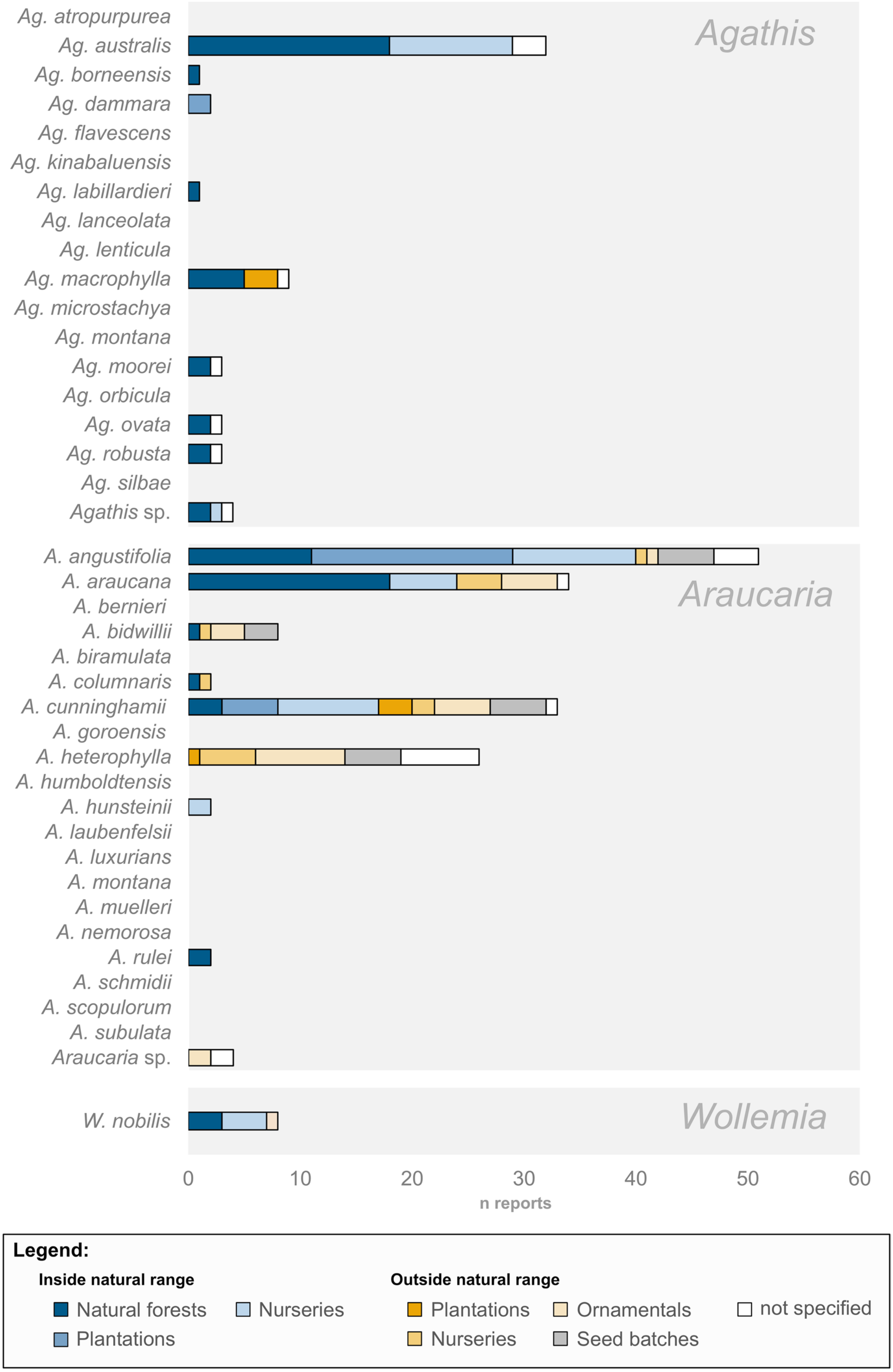


Fig. 4 Number of pathogen reports found in literature for species within Araucariaceae. Reports are categorized as inside of the host's natural range (tones of blue), outside (tones of yellow), seed batches (grey) and not specified (white)

Table 4 Statistics for the disease reports by host genera within Araucariaceae, including number of host–pathogen combinations, confirmation of pathogenicity and level of identification of the causal agent

Host genus	Disease reports[a]			Pathogenicity		Causal agent identification[b]		
	N	Hosts	Countries	Host–pathogen combinations	Pathogenicity confirmed	Species–sequenced	Species–Morphology	Genus–morphology
Agathis	58	8/17	9	58	8 (14%)	8 (14%)	38 (66%)	12 (21%)
Araucaria	161	8/20	28	139	38 (27%)	33 (24%)	85 (61%)	21 (15%)
Wollemia	8	1/1	2	8	5 (63%)	5 (63%)	2 (25%)	1 (13%)
Total	227	17/38	33 [c]	205	51 (25%)	46 (35 [c])	125 (85 [c])	34 (20 [c])

[a] Pathogen-host-location combination

[b] Percentages calculated from the total of host–pathogen combinations

[c] sum of values, excludes repetition among host genera or species

Argentina [199], and *Phytophthora multivora* and *P. citrophthora* on *A. araucana* in Chile [126]. Additionally, where Araucariaceae have been planted in the field for restoration or commercial purposes, they have frequently encountered disease problems. For example, where *A. angustifolia* has been planted in its natural range, in Brazil, it has been damaged by several pathogens including *P. cinnamomi* [118], *Diplodia sapinea* [154], *Calonectria brassicae* (=*Cylindrocladium clavatum*), *Armillaria* sp., *Rosellinia* sp. [102] and *Phellinus* sp. [101]. Given that information for most Araucariaceae is limited, non-recorded (or novel) diseases will likely continue to emerge should they start being propagated in higher quantities, impacting the success of such efforts.

Economic Threats

Diseases represent a significant threat to the potential economic contribution of the Araucariaceae. For example, kauri dieback (caused by *Phytophthora agathidicida*) has had an impact on New Zealand's tourism [212••, 220] and has been considered a potential threat if this species was to be planted for commercial purposes [221]. There was a concern of a similar situation in Chile, when *Araucaria araucana*, a species important to the economy of indigenous people and local tourism [222] began to show dieback symptoms in 2015 [8].

Pathogens can result in significant losses in the production and plantation of species in the Araucariaceae. Examples of pathogens that have been associated with losses in plantations of silvicultural purposes include *E. salmonicolor* (Pink disease) on *Ag. dammara* in Indonesia [23], *P. noxius* on *A. cunninghamii* in Australia [112] and the previously mentioned diverse range of pathogens on *A. angustifolia* in Brazil, including an additional diversity of wood-staining fungi known to occur on this tree [102]. The production of these trees in countries outside their natural range for commercialization as ornamentals, among which the most common are *A. heterophylla*, *A. cunninghamii*, *A. bidwillii* and *A. araucana*, has also been negatively affected by diseases. This is especially by pathogens in the Botryosphaeriaceae, *Phytophthora*, *Pythium* and *Fusarium* [85, 99, 100, 119, 125, 163, 203].

The economic relevance of the Araucariaceae deserves careful consideration. Economic interests are one of the main drivers known to promote monitoring and research on trees in general and, especially tree health. Yet at the same time, economic interests carry with them common triggers for the emergence of diseases. These include movement of plant material, tourism activities or as a direct consequence of planting higher densities of trees [212••, 223••, 224, 225]. These potential risks and benefits must be carefully considered and balanced for any Araucariaceae species before any economic use of these trees should be encouraged or implemented.

Globalization and Climate Change Effects on Araucariaceae Diseases

Among the effects that human activity has had on natural forests, the most damaging and concerning are those related to globalization and climate change [6, 7•]. Among the major drivers for the emergence of forest diseases are the movement of plant material around the world, resulting in accidental introductions of pest and pathogens [226, 227] and the accelerated change in climatic conditions, favouring the biology of pathogens and/or predisposing trees in different manners [7•, 228, 229]. Concerning the Araucariaceae, there are cases of emerging diseases where evidence suggests these are the result of globalization and climate change [8, 19, 108]. However, more research is required to understand the often-complex suite of abiotic and biotic factors driving disease emergence.

Globalization and Anthropogenic Activities as Drivers of Disease

Biological invasions and the movement of pathogens are emerging as a substantial threat to the Araucariaceae. This is well illustrated by the accidental introduction of *Phytophthora* spp. into the native range of *W. nobilis*, now considered among the greatest threats to the preservation of this species [206, 218]. Even though the risk of introduction was recognised [205], nothing could be done to prevent it.

Invasions by introduced mammals have been linked to the dispersal of Araucariaceae pathogens. For example, feral pigs in New Zealand have been shown to be capable of moving *Phytophthora agathidicida* propagules [230]. The introduction of mammals have also caused higher levels of seed predation and seedling browsing and trampling in Chile and Argentina, resulting in reduced reproductive success of *A. araucana* [231, 232].

The planting of foreign tree species in areas where there are native Araucariaceae has been linked to the appearance of unrecorded diseases. In Brazil, *Cylindrocladium clavatum* (= *Calonectria brassicae*) was described causing severe root rot of planted 12 to 15-year-old *A. angustifolia*, and was subsequently found causing even more severe symptoms on neighbouring *Pinus* spp. plantations [109]. Given that *A. angustifolia* is the only native host in Brazil, and that the pathogen has been described on other hosts and locations around the world [108], it is reasonable to assume the disease emerged as a result of an anthropogenic introduction. Similarly, there is abundant evidence of Araucariaceae species being susceptible to pathogens of commercially planted species of *Pinus* and *Eucalyptus* [58, 107, 108, 111, 118, 129, 151, 154, 233]. These non-native species are being planted in several countries where Araucariaceae have native representatives and may carry pathogens that the Araucariaceae have not coevolved with. This, together with the use of Araucariaceae as ornamentals around the world (including countries where other Araucariaceae species are native), leaves a considerable opportunity for the occurrence of future pathogen introductions and host-jumps.

At a local scale, human activities within or near the native range of Araucariaceae can indirectly impact the spread and severity of damage by pathogens. For example, changes in the use of soil (replacing kauri forests with plantations) enhance the sporulation of *P. agathidicida*, creating potential inoculum reservoirs [234]. Activities such as tourism and livestock production are known to move contaminated material and disturb soils on kauri stands [72, 220, 235]. Additionally, cattle ranching and overgrazing within the natural range of some Araucariaceae have negatively impacted the general health of forests and regeneration. Cases include *A. araucana* in Chile and Argentina, including zones within protected areas [14, 236], and *A. heterophylla* in Norfolk Island [16]. Attention needs to be given to how local communities (society) interact with these forests in their native range [237], as their activities may pose risks of direct exposure to novel pathogens, or may result in a reduction in tree vigour leading to a loss of resilience capacity.

Effects of Climate Change on Araucariaceae Diseases

Climate change has been associated with emergent diseases of Araucariaceae. Most reports of emerging dieback diseases on species in the family cite changes in climatic conditions as being among, or the main driver. For example, dieback of *Ag. montana* in its natural range in New Caledonia has been positively correlated with drier years and a trend in rising temperatures [19], although, no biological agent has been consistently found in association with the symptoms. A similar situation was observed in Chile, with *A. araucana* dieback. Climatic conditions were observed to be outside the normal range around the time when symptoms emerged in 2015 [238, 239] and cankers caused by *P. kutranfy* were discovered [8]. In Australia, the emergence of dieback symptoms on ornamental *A. heterophylla* trees associated with infections by fungi in the Botryosphaeriaceae has occurred in two time-independent events, both coinciding with drought/heat periods acting as predisposing factors [166, 167].

There are various means by which climate change can influence diseases in Araucariaceae forests. The most common scenarios are linked to drought stress and increases in temperature, either directly due to the tree's physiological limits being exceeded, or as non-lethal levels of stress predisposing trees to infection by secondary pathogens [240–242]. The previously mentioned cases on *Ag. montana* and *A. heterophylla* serve as examples, as do most cases that involve pathogens within the Botryosphaeriaceae [208]. Studies have shown that the Araucariaceae have ancestral mechanisms for coping with water stress, which places them among the conifers with the least tolerance to water deficits [243, 244•]. Furthermore, climatic predictions indicate that some of the areas naturally suitable for the growth of the Araucariaceae are being reduced [245•, 246, 247], indicating that stress scenarios will become more common with time.

Current trends in increasing temperatures may benefit pathogens of the Araucariaceae in different manners. For example, environments where Araucariaceae occur, such as in high altitude regions, may become more susceptible to biological invasions, including forest pathogens [248, 249]. *Phytophthora cinnamomi*, a common pathogen on several Araucariaceae, has low tolerance to colder temperatures, but based on current climatic projections, it will have the ability to expand its geographical range [4]. The range and impact of native pathogens may also be extended based on the same principle. For example, Swiss needle cast disease of *Pseudotsuga menziesii* in North America, caused by the fungus *Phaeocryptopus gaeumanii*, has led to a substantial increase in disease severity and spread through the years strongly correlated to a sustained rise in winter temperatures and spring precipitation [250]. A similar scenario is hypothesized for the emergence of *P. kutranfy* canker disease on *A. araucana* in Chile [8].

Even mild changes in climatic conditions may impact several factors influencing the extent of diseases. These include, for example, the life cycle of pathogens (including survival and production of inoculum) and the fitness and phenology of the hosts and pathogens when the interaction occurs. Additionally, native disease systems may be influenced indirectly through alterations in their surrounding environment where factors such as abundance or behaviour of insects or other organisms that may act as vectors, sources of mechanical damage or inoculum reservoirs may have been modified [228, 240, 241, 251••, 252].

It is difficult to predict how globalization and climate change interact, and how this will impact forest diseases of the Araucariaceae in the future. As previously stated, both factors may interact in diverse ways with pathogens and/or hosts, but they are not exclusive to each other. Management strategies, both broad and specific, have been proposed to diminish the risks of these uncertain scenarios [237, 240, 241], and some have already been applied for iconic species within Araucariaceae [212••, 253]. However, the applicability of such strategies usually requires a baseline of information or knowledge, which represents an important constraint for most species in the Araucariaceae.

Limitations to Current Knowledge

Knowledge regarding diseases of the Araucariaceae is relatively scarce and the topic remains largely unexplored. In summary, there are currently 21 species in Araucariaceae for which there are no disease reports. Moreover, for many of the species for which diseases have been reported, these are commonly only for a single or less than five diseases (Fig. 4, Table 4). This illustrates either a lack of research on these species, including a lack of tree health monitoring and/or information is inaccessible (e.g. grey literature). The recent emergence of dieback diseases in some Araucariaceae has highlighted a lack of knowledge regarding pathogens associated with these trees and a more general lack of knowledge of the microbes associated with these trees regarding their identity and role in causing disease.

Most disease reports for the Araucariaceae are several decades old and where pathogen descriptions were based solely on morphology. The implication is that several of these were recognised based on records of pathogens of planted tree species, especially *Pinus* species. There have thus been assumptions that the associated fungi are pathogens of the Araucariaceae without pathogenicity ever being tested. For example, *Heterobasidion araucariae*, a wood rotting basidiomycete was, for a long time, confused with *H. annosum*, a severe root rot pathogen of plantation-grown *Pinus* spp. [81]. Concern was raised regarding the detection of this supposed *H. annosum* on stumps of *Ag. australis* (New Zealand), *Ag. macrophylla* (Fiji), *A. bidwillii* (Australia) and *A. cunninghamii* (Australia and Papua New Guinea) [81], all in natural forests. However, when the fungus found on these native trees was properly studied, it turned out that this was a different and considerably less aggressive species, described as *H. araucariae* [81]. Similar cases have arisen for *Armillaria* species (as *A. mellea*) [59, 102, 105], *Ganoderma* spp. (as *G. applanatum*) [66, 67], *Pyrrhoderma* spp. (as *Phellinus* species) [78, 79] and *Rigidoporus* spp. (=*Junghuhnia vincta*) [147, 148], where identification was based on pathogens of other hosts not in the Araucariaceae. In several instances, the identification of the pathogen has not yet been confirmed, and their role as pathogens has simply been assumed.

There are some pathogens described as being unique to the Araucariaceae that remain obscure, present only in the early literature. Access to these reports is difficult given that they are not found in accessible databases, and some are in less commonly encountered languages. These reports of presumed pathogens mostly represent rare fungal species studied primarily from a mycological rather than plant pathology perspective, and where their role as pathogens has only been assumed. They for example include *Lophodermium agathidis* on *Agathis australis* [95]; *Appendiculella araucariae*, *Cirsosiopsis violacescens*, *Coccomyces araucariae*, *Cycloschizon araucariae*, *Episphaerella araucariae*, *E. serialis*, *Pseudomeliola brasiliensis* and *Xenomeris acicola*, all on *A. angustifolia* [172]; and *Atotospora araucariae* [174]; *Phaeocryptopus araucariae* [189] and *Rhizothyrium parasiticum* [192], on *A. araucana* (Fig. 5). These fungi may be easily overlooked, as they appear in single articles, and lack the DNA sequence data to allow for their accurate identification. Negative consequences of this situation include potentially duplicated species descriptions and a lack of tools for opportune detection. Most of the fungi mentioned above are associated with mild leaf symptoms in natural forests. Nevertheless, as so little is known about them, caution should be applied to prevent or minimise their accidental movement to new environments where congener species of Araucariaceae could be susceptible. This would be similar to the situation with, for example, many pathogens of *Eucalyptus* spp. that have caused more severe disease in areas where they have been introduced [223••].

Most of the known diseases (host–pathogen combinations) for Araucariaceae lack complete or recent studies. As mentioned above, of the 205 host–pathogen combinations found in accessible literature, only 51 (25%) have been subjected to pathogenicity tests, and only 46 (22%) have had the identity of the causal pathogen confirmed using DNA sequence data. There are only a limited number of diseases of the Araucariaceae where both DNA sequencing for the causal agent and

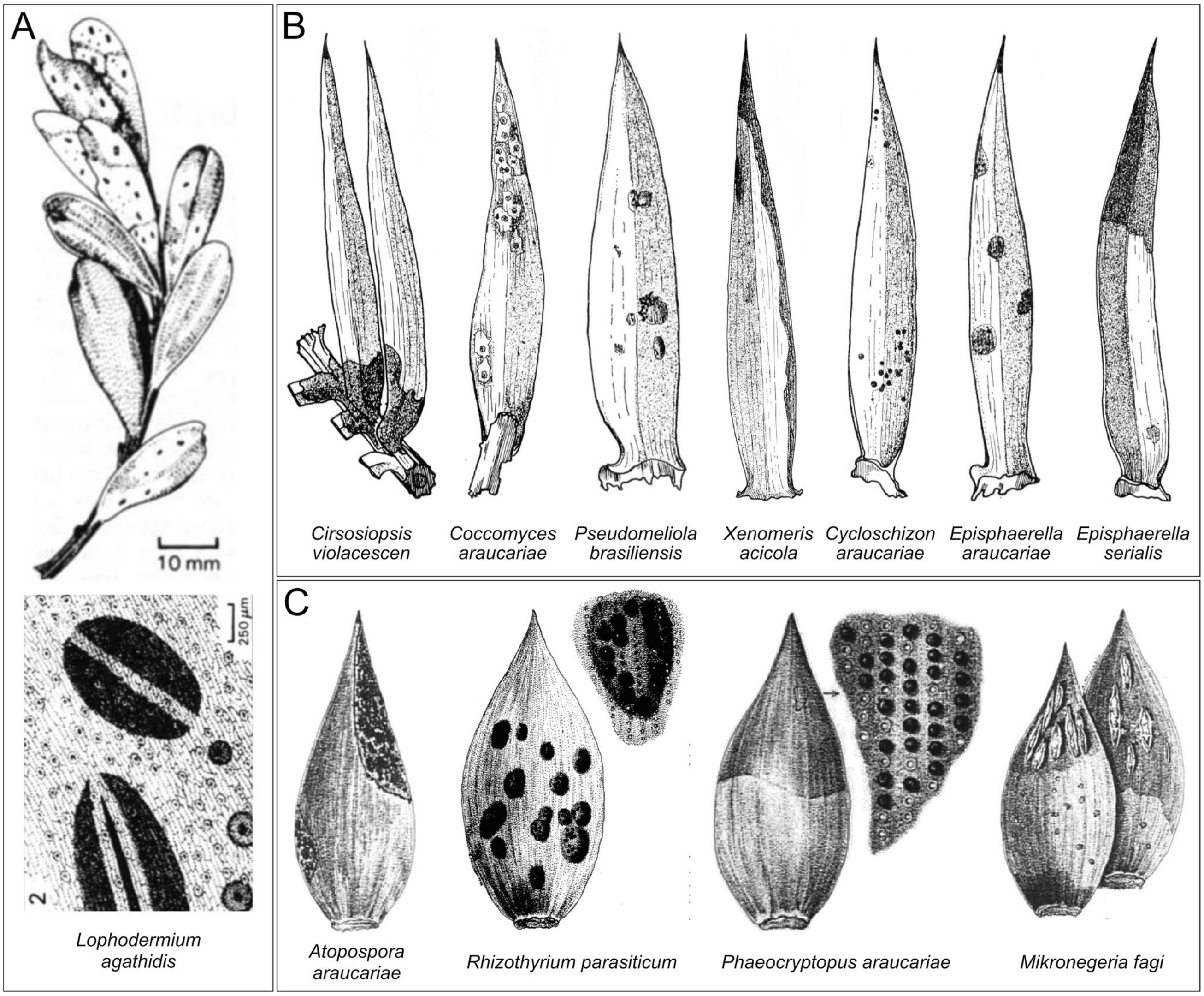


Fig. 5 Diagrams of leaf pathogens described from some species inside Araucariaceae. **A** *Lophodermium agathidis* described from *Agathis australis* in New Zealand (Minter and Hetiige 1983). **B** Leaf pathogens described on *Araucaria angustifolia* from Brazil (Butin and Speer 1978). **C** Leaf pathogens described on *Araucaria araucana* from Chile (Butin 1986; Butin and Peredo 1986)

inoculation experiments have been conducted. These include four for *Agathis* [70, 72, 75], 16 for *Araucaria* [8, 9, 118, 126, 141, 142, 152, 153, 155, 162, 193, 200, 201] and three for *Wollemia* [163, 164, 205, 206]. These low numbers are particularly concerning, as confirmation of both pathogenicity and identity of the causal agent represents the most basic knowledge regarding any disease.

Recent research on emerging or persistent diseases of Araucariaceae has highlighted the problems resulting from the lack of baseline, or in fact any information [8, 16, 17, 19, 254]. This has also demonstrated the challenges in determining the origin of cryptogenic species [255], and the difficulty in answering more advanced questions regarding abiotic and biotic drivers involved in these complex diseases. In this regard, lack of information on pathogens and diseases of the Araucariaceae should not be considered "knowledge gaps" as commonly referred to in science, but rather "knowledge islands", where nothing is known in most cases.

General Conclusions

Knowledge regarding diseases of Araucariaceae is an uneven and gap-filled territory. Except for a few species, for which some diseases have been relatively well studied, most species in the family lack any information regarding this important topic. This void, which is clearly influenced by the socioeconomic importance of each tree species, may result from a lack of monitoring and/or information accessibility. Additionally, disease outbreaks and forest declines, including Araucariaceae, are increasing, and these appear to be driven by globalization and climate change. There are numerous ways in which these factors can interact and impact on Araucariaceae species and the occurrence of diseases. The outcomes of these events are highly unpredictable, and in most cases, may be unmanageable due to the lack of baseline information. This presents a concerning scenario, given the current poor conservation status of most species of the Araucariaceae.

There is an obvious and substantial need to conduct research on the Araucariaceae, and the future of these iconic trees rests firmly on this requirement. New technologies provide many robust opportunities to strengthen the currently weak base knowledge and fulfil existing gaps. This would for example include the sequencing of old herbarium specimens and/or collection of fresh material for correct identification, or epitypification of poorly studied pathogens [256]. Also, multidisciplinary perspectives should be applied to the study of diseases affecting Araucariaceae. This can be done by applying recently proposed frameworks and strategies, such as assessing their impact on the ecological functionality of the host species [257•], and furthermore, to adequately assess the effect of climate on the diseases of these trees [7•] and including invasion biology perspectives to assess forest pathogens [258, 259]. Alternative monitoring strategies such as sentinel plantings and/or including trees in botanical gardens, arboreta and urban areas for early detection should also be considered [260, 261].

Robust research can lead to more efficient monitoring, more adequate regulations, and a greater capacity to respond in the event of a disease emergence. Efforts must also be made to ensure that well-intended actions do not result in harmful situations, e.g., the introduction of pathogens from nurseries to natural environments through restoration programmes [262]. Given the current poor status of knowledge, efforts should focus on protecting these species in their native ranges, through more active monitoring and giving special consideration to limiting the movement of material that could result in the accidental introduction of pathogens or other events that would help spread them.

Acknowledgements We thank Brett Summerell, Edward Liew and Peter Scott for providing us with some of the disease images used in Figure 3.

Funding This work was supported by the Tree Protection Cooperative Programme (TPCP), the Forestry and Agricultural Biotechnology Institute (FABI) and the University of Pretoria in South Africa; and the Chilean National Forest Corporation (Corporación Nacional Forestal, CONAF) and Bioforest S.A. Arauco in Chile.

Declarations

Human and Animal Rights This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest Felipe Balocchi, Michael J. Wingfield, Trudy Paap, Rodrigo Ahumada and Irene Barnes declare that they have no conflict of interest.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. van Lierop P, Lindquist E, Sathyapala S, Franceschini G. Global forest area disturbance from fire, insect pests, diseases and severe weather events. For Ecol Manage. 2015;352:78–88.
2. Kliejunas JT. A risk assessment of climate change and the impact of forest diseases on forest ecosystems in the Western United States and Canada. General Technical Report PSW-GTR-236. Albany, CA: USDA-Forest Service, Pacific Southwest Research Station. 2011;236:1–70.
3. Enderle R, Stenlid J, Vasaitis R. An overview of ash (*Fraxinus spp.*) and the ash dieback disease in Europe. CAB Rev. 2019;14:1–12.
4. Burgess TI, Scott JK, Mcdougall KL, Stukely MJ, Crane C, Dunstan WA, et al. Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. Glob Change Biol. 2017;23:1661–74.
5. Rizzo DM, Garbelotto M. Sudden oak death: endangering California and Oregon forest ecosystems. Front Ecol Environ. 2003;1:197–204.
6. Wingfield M, Brockerhoff E, Wingfield BD, Slippers B. Planted forest health: the need for a global strategy. Science. 2015;349:832–6.
7.• Hennon PE, Frankel SJ, Woods AJ, Worrall JJ, Norlander D, Zambino PJ, et al. A framework to evaluate climate effects on forest tree diseases. Forest Pathology. 2020;50:e12649. **This review presents a conceptual framework on how to adequately assess the effects of climate on diseases of forest at different temporal/spatial scales and explains the most common scenarios in which climate drives the emergence of disease.**
8. Balocchi F, Wingfield MJ, Ahumada R, Barnes I. *Pewenomyces kutranfy* gen nov. et sp. nov. causal agent of an important canker disease on Araucaria araucana in Chile. Plant Pathol. 2021;70:1243–59.
9. Vélez ML, Marfetán JA, Salgado Salomón ME, Taccari LE. *Mortierella* species from declining *Araucaria araucana* trees in Patagonia, Argentina. For Pathol. 2020;50:e12591.
10. Veblen TT, Delmastro R. The *Araucaria araucana* gene resource in Chile. For Genet Resour Inf. 1976;5:2–6.
11. Rodriguez R, Matthei S, Quezada MM. Flora arbórea de Chile. Santiago de Chile: Editorial de la Universidad de Concepción; 1983.
12. Mundo IA, Kitzberger T, Juñent FR, Villalba R, Barrera MD. Fire history in the *Araucaria araucana* forests of Argentina: human and climate influences. Int J Wildland Fire. 2013;22:194–206.
13. González ME, Veblen TT, Sibold JS. Fire history of *Araucaria-Nothofagus* forests in Villarrica National Park. Chile J Biogeogr. 2005;32:1187–202.
14. Zamorano-Elgueta C, Cayuela L, González-Espinosa M, Lara A, Parra-Vázquez MR. Impacts of cattle on the South American

temperate forests: challenges for the conservation of the endangered monkey puzzle tree (*Araucaria araucana*) in Chile. Biol Cons. 2012;152:110–8.

15. Shuey LS, Pegg K, Dodd S, Manners AG, White D, Burgess TI, et al. *Araucaria* dieback-a threat to native and plantation forests. In: Edwards J, editor., et al., Australasian Plant Pathology Society Conference APPS 2019 Conference handbook. Melbourne: ICMS Australasia; 2019. p. 274.
16. Benson M. Dieback of Norfolk Island pine in its natural environment. Aust For. 1980;43:245–52.
17. Francis JK. *Araucaria heterophylla* (Salisb.) Franco Araucaria, Norfolk Island-pine. In: Francis JK, Lowe CA, editors. Bioecología de Arboles Nativos y Exóticos de Puerto Rico y las Indias Occidentales, General Technical Report IITF-15. Río Piedras, Puerto Rico: International Institute of Tropical Forestry; 2000. pp. 49–52.
18.• Mazur MA, Tron F, Mille C. *Pactola kuscheli* sp. nov. (Coleoptera: Curculionidae), a potential cause for the decline of the threatened New Caledonian conifer, *Agathis montana* de Laubenfels 1969 (Araucariaceae). Austral Entomol. 2017;56:268–76. **This article includes a concise description of the decline disease that emerged on *Agathis montana* and the factors that play a role in the poor status of these trees.**
19. Casola JH, Tron FM. Interannual precipitation and temperature variability near Mt. Panié Wilderness Reserve and its connection to Kauri (*Agathis montana*) die-back. In: Tron F, Franquet R, Larsen T, Cassan J, editors. Evaluation rapide de la biodiversité du massif du Panié et des Roches de la Ouaième, province Nord, Nouvelle-Calédonie. Arlington: Conservation International; 2013. pp. 139–45.
20. Silba J. An international census of the Coniferae, I. Phytologia Memoirs VII. Plainfield, NJ: H.N & A.L Moldenke; 1984.
21. Farjon A. A handbook of the world's conifers, vol. 1. Leiden: Brill Academic Publishers; 2010.
22. Farjon A. A handbook of the world's conifers, vol. 2. Leiden: Brill Academic Publishers; 2010.
23. Whitmore TC (British Ministry of Overseas Development). A first look at *Agathis*. Oxford: Tropical Forestry Papers. Department of Forestry Oxford University; 1977. Report No.: R2881.
24. Ecroyd C. Biological flora of New Zealand 8. *Agathis australis* (D. Don) Lindl. (Araucariaceae) Kauri. N Z J Bot. 1982;20:17–36.
25. Aguilera-Betti I, Muñoz AA, Stahle D, Figueroa G, Duarte F, González-Reyes Á, et al. The first millennium-age *Araucaria araucana* in Patagonia. Tree-Ring Res. 2017;73:53–6.
26. Palmer J, Lorrey A, Turney CSM, Hogg A, Baillie M, Fifield K, et al. Extension of New Zealand kauri (*Agathis australis*) tree-ring chronologies into oxygen isotope stage (OIS) 3. J Quat Sci. 2006;21:779–87.
27. Enright NJ, Ogden J. The southern conifers - a synthesis. In: Enright NJ, Hill RS, editors. Ecology of the southern conifers. Carlton: Melbourne University Press; 1995. p. 271–87.
28. Christenhusz MJ, Reveal JL, Farjon A, Gardner MF, Mill RR, Chase MW. A new classification and linear sequence of extant gymnosperms. Phytotaxa. 2011;19:55–70.
29.• Escapa IH, Catalano SA. Phylogenetic analysis of Araucariaceae: integrating molecules, morphology, and fossils. International Journal of Plant Sciences. 2013;174:1153–70. **This paper deals with the taxonomic arrangement of the Araucariaceae using DNA sequence, morphological traits and fossils giving a robust phylogeny for the family.**
30. Endlicher SFL. Synopsis coniferarum. Sangalli: Apud Scheittin et Zollikofer; 1847.
31. Wilde MH, Eames AJ. The ovule and 'seed' of *Araucaria bidwilli* with discussion of the taxonomy of the genus II. Taxonomy. Ann Bot. 1952;16:28–49.
32. Setoguchi H, Asakawa Osawa T, Pintaud JC, Veillon JM. Phylogenetic relationships within Araucariaceae based on *rbcL* gene sequences. Am J Bot. 1998;85:1507–16.
33. Ruhsam M, Rai HS, Mathews S, Ross TG, Graham SW, Raubeson LA, et al. Does complete plastid genome sequencing improve species discrimination and phylogenetic resolution in *Araucaria*? Mol Ecol Resour. 2015;15:1067–78.
34. Mill R, Ruhsam M, Thomas P, Gardner M, Hollingsworth P. *Araucaria goroensis* (Araucariaceae), a new monkey puzzle from New Caledonia, and nomenclatural notes on *Araucaria muelleri*. Edinb J Bot. 2017;74:123–39.
35. Kershaw P, Wagstaff B. The southern conifer family Araucariaceae: history, status, and value for paleoenvironmental reconstruction. Annu Rev Ecol Syst. 2001;32:397–414.
36. Jones W, Hill K, Allen J. *Wollemia nobilis*, a new living Australian genus and species in the Araucariaceae. Telopea. 1995;6:173–6.
37. Stockey RA. Mesozoic Araucariaceae: morphology and systematic relationships. J Plant Res. 1994;107:493–502.
38. Stockey RA. Seeds and embryos of *Araucaria mirabilis*. Am J Bot. 1975;62:856–68.
39. Stockey RA. Anatomy and morphology of *Araucaria sphaerocarpa* Carruthers from the Jurassic inferior oolite of Bruton, Somerset. Bot Gaz. 1980;141:116–24.
40. Stockey RA. Jurassic araucarian cone from southern England. Palaeontology. 1980;23:657–66.
41. Stockey RA, Nishida H, Nishida M. Upper Cretaceous Araucarian cones from Hokkaido: *Araucaria nihongii* sp. nov. Rev Palaeobot Palynol. 1992;72:27–40.
42. Stockey RA, Nishida H, Nishida M. Upper Cretaceous Araucarian cones from Hokkaido and Saghalien: *Araucaria nipponensis* sp. nov. Int J Plant Sci. 1994;155:806–15.
43. Ohsawa T, Nishida H, Nishida M. *Yezonia*, a new section of *Araucaria* (Araucariaceae) based on permineralized vegetative and reproductive organs of *A. vulgaris* comb. nov. from the upper cretaceous of Hokkaido, Japan. J Plant Res. 1995;108:25–39.
44. Andruchow-Colombo A, Escapa IH, Cúneo NR, Gandolfo MA. *Araucaria lefipanensis* (Araucariaceae), a new species with dimorphic leaves from the Late Cretaceous of Patagonia, Argentina. Am J Bot. 2018;105:1067–87.
45. Shi G, Li H, Leslie AB, Zhou Z. *Araucaria* bract-scale complex and associated foliage from the early-middle Eocene of Antarctica and their implications for Gondwanan biogeography. Hist Biol. 2020;32:164–73.
46. Herrmann TM. Indigenous knowledge and management of *Araucaria araucana* forest in the Chilean Andes: implications for native forest conservation. Biodivers Conserv. 2006;15:647–62.
47. Steward GA, Beveridge AE. A review of New Zealand kauri (*Agathis australis* (D. Don) Lindl.): its ecology, history, growth and potential for management for timber. N Z J For Sci. 2010;40:33–59.
48. Dettmann ME, Clifford HT. Biogeography of araucariaceae. In: Dargavel J, editor. Australia and New Zealand forest histories: Araucarian Forests. Australian Forest History Society Inc. Occasional Publication 2. Kingston, Australia: Australian Forest History Society; 2005. pp. 1–9
49. Huth J. Introducing the Bunya Pine–a noble denizen of the scrub. Queensland Review. 2002;9:7–20.
50. Steward GA, Kimberley MO, Mason EG, Dungey HS. Growth and productivity of New Zealand kauri (*Agathis australis* (D. Don) Lindl.) in planted forests. N Z J For Sci. 2014;44:1–13.
51. DAFF (Department of Agriculture, Fisheries and Forestry). Hoop pine - *Araucaria* (plantations). Queensland, Australia: State of Queensland; 2013. Report No.: DAFF:3931. Available at: http://era.daf.qld.gov.au/id/eprint/3931/. [cited: 12th of June 2020]. Available from: http://era.daf.qld.gov.au/id/eprint/3931/.

52. ABRAF (Associação Brasileira de Produtores de Florestas Plantadas). Anuário estatístico ABRAF 2013 ano base 2012. Brasilia: ABRAF; 2013. Report No.: CDD – 634.9568105. Available from: http://www.bibliotecaflorestal.ufv.br/handle/123456789/3910.
53. Cardoso DJ, Rosot MAD, Garrastazú MC, Rosot NC, Toniolo L Jr, de Oliveira KA. Recommended thinning regimes for *Araucaria angustifolia* plantations on small properties in southern Brazil: a case study. Adv For Sci. 2017;4:211–8.
54. Zuhaidi YA, Hashim M. *Araucaria hunsteinii*: has research helped us to arrive at a decision? J Trop For Sci. 2007;19:64–6.
55. Nasir SS, Nikfarjam H. Investigating growth of *Araucaria excelsa* L. in different methods of fertilization. J Ornamental Plants. 2017;7:257–62.
56. Johns JW, Yost JM, Nicolle D, Igic B, Ritter MK. Worldwide hemisphere-dependent lean in Cook pines. Ecology. 2017;98:2482–4.
57. De Laubenfels D. Araucariaceae. In: van Steenis C, van Steenis-Kruseman M, editors. Flora Malesiana-Series 1, Spermatophyta. Djakarta: Noordhoff-Kolff; 1984. pp. 419–42.
58. Coetzee MPA, Wingfield BD, Bloomer P, Ridley GS, Kile GA, Wingfield MJ. Phylogenetic relationships of Australian and New Zealand *Armillaria* species. Mycologia. 2001;93:887–96.
59. van der Pas JB, Hood IA, MacKenzie M. Armillaria root disease. [Internet]. Farm Forestry New Zealand. Forest Pathology in New Zealand No. 4; 2008 [cited 02 Feb 2021]. Available from: https://www.nzffa.org.nz/farm-forestry-model/the-essentials/forest-health-pests-and-diseases/forestry-diseases/Armillaria/ArmillariaPath4/.
60. Dick MA. Resinosis on *Agathis australis* (Kauri). Scion Forest Health News. 2009;201:1-2.
61. Dodd SL, Ramsfield TD, Marshall JW. PCR primers to distinguish *Armillaria* species found in New Zealand. Australas Plant Pathol. 2010;39:536–43.
62. McKenzie EHC, Buchanan PK, Johnston PR. Checklist of fungi on kauri (*Agathis australis*) in New Zealand. NZ J Bot. 2002;40:269–96.
63. Cunningham GH. Thelephoraceae of New Zealand Part III. The genus *Corticium*. Trans R Soc N Z. 1954;82:271–327.
64. Ramsden M, McDonald J, Wylie FR. Forest pests in the South Pacific region: a review of the major causal agents of tree disorders. Queensland, Australia: Department of Primary Industries, Agency for Food and Fibre Sciences, Forestry Research; 2002. Report No.: ACIAR Project FST/2001/045.
65. Thomson LA. *Agathis macrophylla* (Pacific kauri), v1.2. In: Elevitch CR, editor. Species profiles for Pacific Island Agroforestry. Holualoa: Permanent Agriculture Resources; 2006. pp. 1–13. https://agroforestry.org/free-publications/traditional-tree-profiles.
66. Herliyana EN. Early report of red root rot of *Ganoderma* sp. on *Agathis* sp. (Damar) in Mount Walat Education Forest, Sukabumi, West Java. Jurnal Silvikultur Tropika. 2012;3:102–7.
67. Herliyana EN, Permatasari DP. Area of damage and distribution of occurrence the red root disease in Gunung Walat University Forest. Sukabumi Jurnal Silvikultur Tropika. 2016;7:24–31.
68. Neil PE. Root disease (*Phellinus noxius* (Corner) GH Cunn.) of *Cordia alliodora* in Vanuatu. Commonw For Rev. 1988;67:363–72.
69. Weir BS, Paderes EP, Anand N, Uchida JY, Pennycook SR, Bellgard SE, et al. A taxonomic revision of *Phytophthora* Clade 5 including two new species, *Phytophthora agathidicida* and *P. cocois*. Phytotaxa. 2015;205:21–38.
70. Horner IJ, Hough EG. Pathogenicity of four *Phytophthora* species on kauri: in vitro and glasshouse trials. N Z Plant Prot. 2014;67:54–9.
71. Gadgil PD. *Phytophthora heveae*, a pathogen of kauri. NZ J Forest Sci. 1974;4:59–63.
72. Waipara NW, Hill S, Hill LMW, Hough EG, Horner IJ. Surveillance methods to determine tree health, distribution of kauri dieback disease and associated pathogens. N Z Plant Prot. 2013;66:235–41.
73. Podger FD, Newhook FJ. *Phytophthora cinnamomi* in indigenous plant communities in New Zealand. NZ J Bot. 1971;9:625–38.
74. Newhook FJ. The association of *Phytophthora* spp. with mortality of *Pinus radiata* and other conifers: I Symptoms and epidemiology in shelterbelts. N Z J Agric Res. 1959;2:808–43.
75. Beever RE, Waipara NW, Ramsfield TD, Dick MA, Horner IJ. Kauri (*Agathis australis*) under threat from *Phytophthora*? In: Goheen E, Frankel S, editors. Proceedings of the fourth meeting of the International Union of Forest Research Organizations (IUFRO) Working Party S07.02.09: Phytophthoras in forests and natural ecosystems. Gen. Tech. Rep. PSW-GTR-221. Albany: United States Department of Agriculture; 2009. pp. 74–85.
76. Ramsfield TD, Dick MA, Beever RE, Horner IJ, McAlonan MJ, Hill CF. *Phytophthora kernoviae* in New Zealand. in Proceedings of the fourth meeting of the International Union of Forest Research Organizations (IUFRO) Working Party S07.02.09: Phytophthoras in forests and natural ecosystems. 2009. Monterey, California.
77. Brien RM, Dingley JM. Fourth supplement to “A revised list of plant diseases recorded in New Zealand”, 1957–1958. N Z J Agric Res. 1959;2:406–13.
78. Kohler F. Agents pathogènes et maladies physiologiques des plantes cultivées en Nouvelle-Calédonie et aux Iles Wallis et Futuna. Catalogues Sciences de la Vie, Phytopathologie. Vol. 1. Bondy: Orstom; 1987.
79. Zhou L-W, Ji X-H, Vlasák J, Dai Y-C. Taxonomy and phylogeny of *Pyrrhoderma*: a redefinition, the segregation of *Fulvoderma*, gen. nov., and identifying four new species. Mycologia. 2018;110:872–89.
80. Moncalvo J-M, Buchanan PK. Molecular evidence for long distance dispersal across the Southern Hemisphere in the *Ganoderma applanatum-australe* species complex (Basidiomycota). Mycol Res. 2008;112:425–36.
81. Buchanan PK. A new species of *Heterobasidion* (Polyporaceae) from Australasia. Mycotaxon. 1988;32:325–37.
82. Hood IA. Tree decays. Forest pathology in New Zealand No. 17 [Internet]. Scion Digital Library; 2009 [cited 20 March 2021]. Available from: https://scion.contentdm.oclc.org/digital/collection/p20044coll10/id/2/rec/1.
83. Ota Y, Tokuda S, Buchanan PK, Hattori T. Phylogenetic relationships of Japanese species of *Heterobasidion—H. annosum sensu lato* and an undetermined *Heterobasidion* sp. Mycologia. 2006;98:717–25.
84. Buchanan PK, Rwarden L. An annotated checklist of polypore and polypore-like fungi recorded from New Zealand. NZ J Bot. 2000;38:265–323.
85. Ivory MH. The pathology of more forest tree species in West Malaysia. Commonw For Rev. 1975;54:64–8.
86. Ahmad N. Mycological research in Malaysia. Proceedings: Plant Sciences. 1986;96:363–71.
87. Heather NW, Schaumberg JB. Plantation problems of kauri pine in south east Queensland. Aust For. 1966;30:12–9.
88. Hyland BPM. A revision of the genus *Agathis* (Araucariaceae) in Australia. Brunonia. 1977;1:103–15.
89. Punithalingam E, Jones D. *Aecidium* species on *Agathis*. Trans Br Mycol Soc. 1971;57:325–31.
90. Peterson RS. Rust fungi on Araucariaceae. Mycopathologia et mycologia applicata. 1968;34:17–26.

91. Kohler F, Pellegrin F, Jackson G, McKenzie EHC. Diseases of cultivated crops in Pacific Islands countries. Noumea, New Caledonia: South Pacific Comission; 1997.
92. Aime MC, McTaggart AR. A higher-rank classification for rust fungi, with notes on genera. Fungal Syst Evol. 2021;7:21.
93. Weir BS, Johnston PR, Damm U. The *Colletotrichum gloeosporioides* species complex. Stud Mycol. 2012;73:115–80.
94. Bowen M, Whitmore T. A second look at *Agathis*. Oxford, UK: Tropical Forestry Papers. Department of Forestry Oxford University; 1980. Report No.: 0141–8181.
95. Minter DW, Hetiige G. *Lophodermium agathidis* and *Meloderma richeae*, two members of the Rhytismataceae from Australasia. NZ J Bot. 1983;21:39–48.
96. Lantieri A, Johnston PR, Park D, Lantz H, Medardi G. *Hypoderma siculum* sp. nov. from Italy. Mycotaxon. 2012;118:393–401.
97. Johnston PR. Rhytismataceae in New Zealand 2. The genus *Lophodermium* on indigenous plants. N Z J Bot. 1989;27:243–74.
98. Ellis JP. Some thalloid *Meliolas*. Trans Br Mycol Soc. 1974;63:93–8.
99. Robertson GI. Occurrence of *Pythium* spp. in New Zealand soils, sands, pumices, and peat, and on roots of container-grown plants. N Z J Agric Res. 1973;16:357–65.
100. Robertson GI. The genus *Pythium* in New Zealand. NZ J Bot. 1980;18:73–102.
101. Auer CG, Grigoletti Jr. A. Doenças registradas em *Araucaria angustifolia* e *Pinus* spp. nos estados do Paraná e de Santa Catarina. Brazil: Embrapa, Ministério da Agricultura e do Abastecimiento; 1997. Report No.: 0104–5903.
102. de Oliveira OdS. Fungos causadores de danos em *Araucaria angustifolia* (Bert.) O. Ktze. Floresta. 1981;12:23–7.
103. Mwenje E, Ride JP. Morphological and biochemical characterization of *Armillaria* isolates from Zimbabwe. Plant Pathol. 1996;45:1036–51.
104. Mwenje E, Wingfield BD, Coetzee MP, Wingfield MJ. Molecular characterisation of *Armillaria* species from Zimbabwe. Mycol Res. 2003;107:291–6.
105. Butin H, Peredo HL. Hongos parásitos en coníferas de América del Sur, con especial referencia a Chile. Bibliotheca Mycologica. Berlin-Stuttgart: J. Cramer; 1986.
106. Vazquez JP. Diagnóstico de especies de *Armillaria* y *Phytophthora* en suelo como estrategia de conservación de árboles singulares en Galicia. In: Montero González G, Guijarro Guzmán M, editors. Actas 6° Congreso Forestal Español CD-Rom. 6CFE01–410. Pontevedra: Sociedad Española de Ciencias Forestales; 2013. pp. 1–9.
107. Kile GA, Watling R. Identification and occurrence of Australian *Armillaria* species, including *A pallidula* sp. nov. and comparative studies between them and non-Australian tropical and Indian *Armillaria*. Trans Br Mycol Soc. 1988;91:305–15.
108. Lombard L, Rodas CA, Crous PW, Wingfield BD, Wingfield MJ. *Calonectria* (*Cylindrocladium*) species associated with dying *Pinus* cuttings. Persoonia. 2009;23:41–7.
109. Hodges CS, May LC. A root disease of pine, *Araucaria*, and *Eucalyptus* in Brazil caused by a new species of *Cylindrocladium*. Phytopathology. 1972;62:898–901.
110. Kim M-S, Klopfenstein NB, Hanna JW, Cannon P, Medel R, López A. First report of Armillaria root disease caused by *Armillaria tabescens* on *Araucaria araucana* in Veracruz. Mexico Plant Dis. 2010;94:784.
111. Brown BN, Wylie FR. Diseases and pests of Australian forest nurseries: past and present. In: Sutherland JR, Glover SG, editors. Proceedings of the first meeting of IUFRO Working Party S. 2.07–09 (Diseases and Insects in Forest Nurseries). Victoria, British Columbia, Canada: Forestry Canada; 1991. pp. 3–15.
112. Bolland L. *Phellinus noxius*: cause of a significant root-rot in Queensland hoop pine plantations. Aust For. 1984;47:2–10.
113. Bolland L. Variation in *Phellinus noxius* (Corner) GH Cunn. In: Abstracts of Doctoral Thesis. Australian Forestry 52:135–93; 1980. pp. 141. https://doi.org/10.1080/00049158.1989.10674548.
114. Chang T, Yang W. *Phellinus noxius* in Taiwan: distribution, host plants and the pH and texture of the rhizosphere soils of infected hosts. Mycol Res. 1998;102:1085–8.
115. Ann P-J, Chang T-T, Ko W-H. *Phellinus noxius* brown root rot of fruit and ornamental trees in Taiwan. Plant Dis. 2002;86:820–6.
116. Tsai J-N, Ann P-J, Liou R-F, Hsieh W-H, Ko W-H. *Phellinus noxius*: molecular diversity among isolates from Taiwan and its phylogenetic relationship with other species of *Phellinus* based on sequences of the ITS region. Bot Stud. 2017;58:9.
117. Oudemans P, Coffey MD. A revised systematics of twelve papillate *Phytophthora* species based on isozyme analysis. Mycol Res. 1991;95:1025–46.
118. Dos Santos ÁF, Tessmann DJ, Alves TC, Vida JB, Harakava R. Root and crown rot of Brazilian pine (*Araucaria angustifolia*) caused by *Phytophthora cinnamomi*. J Phytopathol. 2011;159:194–6.
119. Von Broembsen S. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. Phytophylactica. 1984;16:221–6.
120. González G, Opazo A. Enfermedades fungosas y otras. In: Baldini A, Pancel L, editors. Agentes de daño en el bosque nativo. Santiago de Chile: Editorial Universitaria; 2002. pp. 89-199.
121. Robertson GI. Susceptibility of exotic and indigenous trees and shrubs to *Phytophthora cinnamomi* Rands. N Z J Agric Res. 1970;13:297–307.
122. Arentz F, Simpson JA. Distribution of *Phytophthora cinnamomi* in Papua New Guinea and notes on its origin. Trans Br Mycol Soc. 1986;87:289–95.
123. Lee SB, Taylor JW. Phylogeny of five fungus-like protoctistan *Phytophthora* species, inferred from the internal transcribed spacers of ribosomal DNA. Mol Biol Evol. 1992;9:636–53.
124. Raabe RD, Connors IL, Martinez AP, Nelson SC. Checklist of plant diseases in Hawaii: including records of microorganisms, principally fungi, found in the State. Honolulu: University of Hawaii; 2009.
125. Spaulding P. Foreign diseases of forest trees of the world: an annotated list. Vol. 197. Washington, DC: US Government Printing Office; 1961. Available from: https://books.google.co.za/books?hl=en&lr=&id=4jPnUVheGdAC&oi=fnd&pg=PA1&dq=araucaria+araucana+mauritius&ots=8Wjf6EEFEF&sig=c4MgaoIBzHn5T774qMzHG2-06XM&redir_esc=y#v=onepage&q=araucaria%20araucana%20mauritius&f=false.
126. Galvez E, Larach A, Riquelme N, Celis JL, Guajardo J, Besoain XA. *Araucaria araucana* root rot caused by *Phytophthora multivora* and *P. citrophthora.* Phytopathology. 2018;108:S1.186.
127. Jung T, Orlikowski L, Henricot B, Abad-Campos P, Aday AG, Aguín Casal O, et al. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of Phytophthora diseases. Forest Pathol. 2016;46:134–63.
128. Erwin DC, Ribeiro OK. Phytophthora diseases worldwide. St. Paul: APS Press; 1996.
129. dos Santos AF, Thomazi H, Duarte HSS, Machado EB, Silva CN, Tessmann DJ. First report of root rot caused by *Rosellinia bunodes* on a poplar species (*Populus deltoides*) in Brazil. Plant Dis. 2017;101:632–3.
130. Cunningham GH. The Polyporaceae of New Zealand. Trans Proc R Soc N Z. 1928;58:202–50.

131. Buchanan PK, Hood IA. New species and new records of Aphyllophorales (Basidiomycetes) from New Zealand. NZ J Bot. 1992;30:95–112.
132. Cockle KL, Martin K, Robledo G. Linking fungi, trees, and hole-using birds in a neotropical tree-cavity network: pathways of cavity production and implications for conservation. For Ecol Manage. 2012;264:210–9.
133. Rajchenberg M, Robledo G. Pathogenic polypores in Argentina. Forest Pathol. 2013;43:171–84.
134. Torres-Torres MG, Guzmán-Dávalos L, de Mello GA. *Ganoderma* in Brazil: known species and new records. Mycotaxon. 2013;121:93–132.
135. Chase TE, Ullrich RC, Korhonen K. Homothallic isolates of *Heterobasidion annosum*. Mycologia. 1985;77:975–7.
136. Zhao C-L, Saba M, Khalid AN, Song J, Pfister DH. *Heterobasidion amyloideopsis* sp. nov. (Basidiomycota, Russulales) evidenced by morphological characteristics and phylogenetic analysis. Phytotaxa. 2017;317:199–210.
137. Simpson JA. *Heterobasidion annosum* on *Araucaria cunninghamii* in Papua New Guinea. Plant Dis Rep. 1978;62:404–5.
138. Maijala P, Harrington TC, Raudaskoski M. A peroxidase gene family and gene trees in *Heterobasidion* and related genera. Mycologia. 2003;95:209–21.
139. De Beer ZW, Duong TA, Barnes I, Wingfield BD, Wingfield MJ. Redefining *Ceratocystis* and allied genera. Stud Mycol. 2014;79:187–219.
140. Goos RD. A new genus of the Hyphomycetes from Hawaii. Mycologia. 1970;62:171–5.
141. Zipfel RD, De Beer ZW, Jacobs K, Wingfield BD, Wingfield MJ. Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Stud Mycol. 2006;55:75–97.
142. Butin H. A new species of *Ceratocystis* causing blue-stain in *Araucaria araucana*. Can J Bot. 1968;46:61–3.
143. De Hoog GS, Scheffer RJ. *Ceratocystis* versus *Ophiostoma*: a reappraisal. Mycologia. 1984;76:292–9.
144. Butin H. A new species of *Ophiostoma* causing blue-stain in *Araucaria angustifolia* (Bertol.) O. Kuntze. J Phytopathol. 1978;91:230–4.
145. Hausner G, Reid J. Notes on *Ceratocystis brunnea* and some other *Ophiostoma* species based on partial ribosomal DNA sequence analysis. Can J Bot. 2003;81:865–76.
146. Silveira RMBd, Guerrero RT. Os gêneros Rigidoporus, Murr. e Flaviporus Murr. (Basiciomycetes) no Parque Nacional de Aparados da Serra, RS. Acta Botanica Brasilica. 1989;3:29–45. https://doi.org/10.1590/S0102-33061989000300004.
147. Hood IA, Dick M. *Junghuhnia vincta* (Berkeley) comb. nov., root pathogen of *Pinus radiata.* N Z J Bot. 1988;26:113–6.
148. Bolland L. *Chaetoporus radulus* on hoop pine in Queensland. Australas Plant Pathol. 1974;3:60.
149. Dick MA. Junghuhnia root disease. Scion: Forest Pathology in New Zealand No. 18 (2nd Edition). 2009;1–6.
150. Ivory MH. Poria root disease of exotic forest trees in east Africa. East Afr Agric For J. 1973;39:180–8.
151. Huang C-L, Wang Y-Z. New records of endophytic fungi associated with the Araucariaceae in Taiwan. Collect Res. 2011;24:87–95.
152. Zapata M, Schafer M. *Diplodia africana* causing twig death on *Araucaria araucana*, a new host and first record for Chile. New Disease Rep. 2019;40:2.
153. Besoain X, Guajardo J, Camps R. First report of *Diplodia mutila* causing gummy canker in *Araucaria araucana* in Chile. Plant Dis. 2017;101:1328.
154. Corrêa PRR, Auer CG, Santos ÁFd. Patogenicidade de *Sphaeropsis sapinea* em árvores jovens de *Araucaria angustifolia.* Summa Phytopathologica. 2012;38:84–6.
155. Besoain X, Guajardo J, Larach A, Riquelme N, Galvez E, Tapia L, et al. First report of *Diplodia seriata* causing gummy canker in *Araucaria araucana* wild populations in south-central Chile. Plant Dis. 2019;103:2684.
156. Tan YP, Shivas RG, Marney TS, Edwards J, Dearnaley J, Jami F, et al. Australian cultures of Botryosphaeriaceae held in Queensland and Victoria plant pathology herbaria revisited. Australas Plant Pathol. 2019;48:25–34.
157. Vegni G. Segnalazione della «Necrosi del colletto» della *Araucaria imbricata*. Riv Patol Vegetale. 1964;4:142–5.
158. Voglino P. Trunk rot of *Araucaria imbricata*. Difesa delle Piante. 1932;9:17–20.
159. Kliejunas JT. Bleeding canker of Norfolk Island pine in Hawaii. Plant Dis Rep. 1976;60:84–7.
160. Darge WA. First report of *Lasiodiplodia theobromae* causing needle blight and stem canker diseases on *Araucaria heterophylla* in Ethiopia. J Hortic Res. 2017;25:15–8.
161. Li GQ, Liu FF, Li JQ, Liu QL, Chen SF. Botryosphaeriaceae from *Eucalyptus* plantations and adjacent plants in China. Persoonia. 2018;40:63–95.
162. Pérez S, Guerrero J, Galdames R. First report of *Neofusicoccum nonquaesitum* in Chile causing branch dieback and decline in *Araucaria araucana*. Plant Dis. 2018;102:1460.
163. Slippers B, Summerel BA, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ. Preliminary studies on *Botryosphaeria* species from Southern Hemisphere conifers in Australasia and South Africa. Australas Plant Pathol. 2005;34:213–20.
164. Sakalidis ML, Hardy GESJ, Burgess TI. Use of the genealogical sorting index (GSI) to delineate species boundaries in the *Neofusicoccum parvum–Neofusicoccum ribis* species complex. Mol Phylogenet Evol. 2011;60:333–44.
165. Dobbie K. Dieback of *Araucaria heterophylla*. Forest Health News No. 173 [Internet]. SCION: Pests and diseases of forestry in New Zealand; 2007 [cited 13 August 2020]. Available from: https://www.nzffa.org.nz/farm-forestry-model/the-essentials/forest-health-pests-and-diseases/Pests-diseases-by-tree-species/araucaria-heterophylla/Norfolk-pine-dieback/.
166. Golzar H, Burgess TI. *Neofusicoccum parvum*, a causal agent associated with cankers and decline of Norfolk Island pine in Australia. Australas Plant Pathol. 2011;40:484–9.
167. ArborCarbon (Pty Lt). Investigation into the cause(s) of premature decline of Norfolk Island Pine. Perth: Town of Cottesloe; 2020. Report No.: J20490. Available from: https://www.cottesloe.wa.gov.au/council-meetings/ordinary-council-meeting/24-november-2020-ordinary-council-meeting/274/documents/10110-arbor-carbon-final-report-on-norfolk-island-pine-decline-nov-2020.pdf.
168. Butin H. Studien zur Morphologie und Biologie von *Mikronegeria fagi* Diet. et Neg. J Phytopathol. 1969;64:242–57.
169. Riess K, Schön ME, Lutz M, Butin H, Oberwinkler F, Garnica S. On the evolutionary history of *Uleiella chilensis*, a smut fungus parasite of *Araucaria araucana* in South America: Uleiellales ord nov. in Ustilaginomycetes. PloS one. 2016;11:e0147107.
170. Barth OM. Os esporos de *Uleiella paradoxa* Schroet (Uredinales). Sellowia. 1964;16:179–89.
171. Thirumalachar MJ. Critical notes on some plant rusts. Bull Torrey Bot Club. 1949;76:339–42.
172. Butin H, Speer EO. Über einige parasitische Ascomyceten auf Nadeln der Brasilianischen Araukarie. Sydowia. 1978;31:9–26.
173. Crous PW, Wingfield MJ, Burgess TI, Hardy GESJ, Gené J, Guarro J, et al. Fungal planet description sheets: 716–784. Persoonia. 2018;40:239–392.
174. Butin H. Beitrag zur ascomyceten flora von Chile. Sydowia. 1975;27:267–92.
175. Chetverikov PE, Beaulieu F, Beliavskaia AY, Rautian MS, Sukhareva SI. Redescription of an early-derivative mite,

Pentasetacus araucariae (Eriophyoidea, Phytoptidae), and new hypotheses on the eriophyoid reproductive anatomy. Exp Appl Acarol. 2014;63:123–55.
176. Butin H. Zwei neue *Caliciopsis*-arten auf chilenischen koniferen. J Phytopathol. 1970;69:71–7.
177. Crous PW, Kang J-C. Phylogenetic confirmation of *Calonectria spathulata* and *Cylindrocladium leucothoes* based on morphology, and sequence data of the β-tubulin and ITS rRNA genes. Mycoscience. 2001;42:51–7.
178. Wu H-X, Tian Q, Li WJ, Hyde KD. A reappraisal of Microthyriaceaea. Phytotaxa. 2014;176:201–12.
179. Arentz F. Forest nursery diseases in Papua New Guinea. In: Sutherland JR, Glover SG, editors. Proceedings of the first meeting of IUFRO Working Party S. 2.07–09 (Diseases and Insects in Forest Nurseries). Victoria, British Columbia, Canada: Forestry Canada; 1991. pp. 97–9.
180. Voglino P. On a wilt of *Araucaria imbricata.* Difesa delle Piante. 1933;10:37–9.
181. Masser G. Fungi Exotici, II. Bulletin of miscellaneous information, Royal Botanic Gardens, Kew. 1899;153/154:164-85.
182. Saccardo. Fungilli imperfecti novi. J Bot. 1915;15:357–63.
183. Karsten PA, Hariot P. Fungilli imperfecti novi. Journal de Botanique. 1890;15:357–63.
184. Masser G. Bulletin of miscellaneous information. Royal Gardens, Kew. London: Darling and Son; 1899.
185. Crous PW, Wingfield MJ, Schumacher RK, Akulov A, Bulgakov TS, Carnegie AJ, et al. New and interesting fungi. 3. Fungal Syst Evol. 2020;6:157–231.
186. Alfieri Jr SA, Langdon KR, Wehlburg C, Kimbrough JW. Index of plant diseases in Florida, Bulletin 11. Florida Department of Agriculture and Consumer Services, Division of Plant Industry; 1984. Available from: https://palmm.digital.flvc.org/islandora/object/uf%3A104219#page/221/mode/1up.
187. Saccardo PA. Sylloge fungorum (Vol XXV). Sumptibus Coheredum Saccardo. Trotter A, editor. Avellino, Italy: Typis pergola; 1931.
188. Saccardo PA. Fungi ex insula Melita (Malta) lecti a Doct. Caruana-Gatto et Doct. G. Borg annis MCMXIII et MCMIV. Nuovo Giornale Botanico Italiano. 1915;22:24–76.
189. Butin H. Zwei neue arten der gattung *Phaeocryptopus* Naumov. J Phytopathol. 1970;68:269–75.
190. Kobayashi T, Nakashima C, Nishijima T. Notes on some plant-inhabiting fungi collected from the Nansei Islands (1). Mycoscience. 2003;44:473–9.
191. Bissett J, Palm ME. Species of *Phyllosticta* on conifers. Can J Bot. 1989;67:3378–85.
192. Butin H. *Rhizothyrium parasiticum* sp. nov.(Coelomycetes), ein blattparasit auf *Araucaria araucana* (Mol.) C. Koch. Journal of Phytopathology. 1986;115:313–7.
193. Macedo DM, Vitorino MD, Veiga JL, Souza AH, Barreto RW. First report of *Athelia rolfsii* causing damping off in *Araucaria bidwillii* (Bunya Pine) in Brazil. Plant Disease. 2018;102:1667–.
194. Ntima OO. The Araucarias. Fast growing timber trees of the lowland tropics, No.3. Oxford: Commonwealth Forestry Institute, University of Oxford; 1968.
195. Ferreira FA, Muchovej JJ. Diseases of forest nurseries in Brazil. In: Sutherland JR, Glover SG, editors. Proceedings of the first meeting IUFRO Working Party S. 2.07–09 (Diseases and Insects in Forest Nurseries). Victoria, British Columbia, Canada: Forestry Canada; 1991. pp. 3–15.
196. Gadgil PD. Fungi of New Zealand. Vol. 4. Fungi on trees and shrubs in New Zealand. Hong Kong: Fungal Diversity Press; 2005.
197. Schoch CL, Crous PW, Wingfield BD, Wingfield MJ. Phylogeny of *Calonectria* based on comparisons of β-tubulin DNA sequences. Mycol Res. 2001;105:1045–52.
198. Kamara AM, El-Lakany MH, Badran OA, Attia YG. Seed pathology of Araucaria spp. I. A survey of seed-borne fungi associated with four *Araucaria* spp. Aust For Res. 1981;11:269–74.
199. Gerlach W. *Fusarium robustum* spec, nov., der erreger einer Stammfäule an *Araucaria angustifolia* (Bertol.) O. Kuntze in Argentinien? J Phytopatol. 1977;88:29–37.
200. O'Donnell K. Phylogenetic evidence indicates the important mycotoxigenic strains Fn-2, Fn-3, Fn-2B and Fn-M represent a new species of *Fusarium*. Mycotoxins. 1997;1997:1–10.
201. Lindquist JC, Merlo PA. Decay of *Araucaria angustifolia* caused by *Fusarium robustum* Gerlach. (Abstr.). Rev Fac Agron. 1980;56:1–3.
202. El-Lakany MH, Kamara AM, Badran OA, Attia YG. Seed pathology of *Araucaria* spp., 2. Fungal species associated with *Araucaria heterophylla* seed. Australian Forest Research. 1981;11:275–81.
203. Evans-Ruhl G, Latin RX, Pecknold PC, Scott DH, Mitchell B, Harmer L. Compilation of plant diseases and disorders in Indiana. Proc Indiana Acad Sci. 1981;91:120–39.
204. Liew ECY, Offord A, Pinaria A, Pavich C, Summerell BA. Effects of metalaxyl and phosphonate on *Phytophthora* root rot of Wollemi pine. 9th International Congress of Plant Pathology, ICPP 2008. Journal of Plant Pathology. 2008;90 (Supplement 2).S2.413–4.
205. Bullock S, Summerell BA, Gunn LV. Pathogens of the Wollemi pine, *Wollemia nobilis*. Australas Plant Pathol. 2000;29:211–4.
206. Puno V, Laurence M, Guest D, Liew E. Detection of *Phytophthora multivora* in the Wollemi Pine site and pathogenicity to *Wollemia nobilis*. Australas Plant Pathol. 2015;44:205–15.
207. Sivanesan A, Shivas RG. Studies on *Mycosphaerella* species in Queensland. Aust Mycol Res. 2002;106:355–64.
208. Slippers B, Wingfield MJ. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal Biol Rev. 2007;21:90–106.
209. IUCN. The IUCN Red List of Threatened Species, Version 2020–3. [Internet]. 2021 [cited 2 Feb 2021]. Available from: https://www.iucnredlist.org/search/stats?taxonomies=101489&searchType=species.
210. Peakall R, Ebert D, Scott LJ, Meagher PF, Offord CA. Comparative genetic study confirms exceptionally low genetic variation in the ancient and endangered relictual conifer, *Wollemia nobilis* (Araucariaceae). Mol Ecol. 2003;12:2331–43.
211. Offord C, Porter C, Meagher P, Errington G. Sexual reproduction and early plant growth of the Wollemi pine (*Wollemia nobilis*), a rare and threatened Australian conifer. Ann Bot. 1999;84:1–9.
212.•• Bradshaw R, Bellgard S, Black A, Burns B, Gerth M, McDougal R, et al. *Phytophthora agathidicida*: research progress, cultural perspectives and knowledge gaps in the control and management of kauri dieback in New Zealand. Plant Pathology. 2020;69:3–16. **This review summarizes most of the accumulated knowledge about one of the most studied and serious diseases on species of the Araucariaceae, including aspects of its biology, socioeconomic and ecological impacts, and management strategies.**
213. Schmidt H. Dinámica de un bosque virgen de Araucaria-Lenga (Chile). Bosque. 1977;2:3–11.
214. Sanguinetti J, Kitzberger T. Patterns and mechanisms of masting in the large-seeded southern hemisphere conifer *Araucaria araucana*. Austral Ecol. 2008;33:78–87.
215. Paludo GF, Duarte RI, Bernardi AP, Mantovani A, Reis MSd. The size of *Araucaria angustifolia* (Bertol.) Kuntze entering into reproductive stages as a basis for seed management projects. Rev Árvore. 2016;40:695–705.
216. Kang K-S, Cappa EP, Hwang J. Growth characteristics of 100 open-pollinated families in an early-age test of *Agathis*

loranthifolia in West Java, Indonesia. J Korean For Soc. 2010;99:213–9.

217. Picone AP. Habitat, population structure and the conservation status of *Araucaria bidwillii* Hook. in the Australian Wet Tropics. [MSc thesis]. [Townsville, Australia]: James Cook University; 2015.
218. Zimmer HC, Auld TD, Benson J, Baker PJ. Recruitment bottlenecks in the rare Australian conifer *Wollemia nobilis*. Biodivers Conserv. 2014;23:203–15.
219. Ogden J, Wardle GM, Ahmed M. Population dynamics of the emergent conifer *Agathis australis* (D. Don) Lindl.(kauri) in New Zealand II Seedling population sizes and gap-phase regeneration. N Z J Bot. 1987;25:231–42.
220. Clearly M. The tension between conservation and tourism in New Zealand: a case study on Kauri dieback disease. [Internet]. Our Environment; 2020 [cited 10 February 2021]. Available from: https://ourenvironment.ac.nz/2020/04/26/the-tension-between-conservation-and-tourism-in-new-zealand-a-case-study-on-kauri-dieback-disease/.
221. Steward G, Hansen L, Dungey H. Economics of New Zealand planted kauri forestry–a model exercise. N Z J For. 2014;59:31–6.
222. Aagesen DL. Indigenous resource rights and conservation of the monkey-puzzle tree (*Araucaria araucana*, araucariaceae): A case study from Southern Chile. Econ Bot. 1998;52:146–60.
223.●● Burgess TI, Wingfield MJ. Pathogens on the move: a 100-year global experiment with planted eucalypts. Bioscience. 2017;67:14–25. **This review, using *Eucalyptus* as a model genus, describes and exemplifies the scenarios in which the movement of plant material and changes in management can drive disease epidemics in both, plantations and natural forests.**
224. Kelly CL, Pickering CM, Buckley RC. Impacts of tourism on threatened plant taxa and communities in Australia. Ecol Manag Restor. 2003;4:37–44.
225. Desprez-Loustau M-L, Aguayo J, Dutech C, Hayden KJ, Husson C, Jakushkin B, et al. An evolutionary ecology perspective to address forest pathology challenges of today and tomorrow. Ann For Sci. 2016;73:45–67.
226. Slippers B, Stenlid J, Wingfield MJ. Emerging pathogens: fungal host jumps following anthropogenic introduction. Trends Ecol Evol. 2005;20:420–1.
227. Santini A, Liebhold A, Migliorini D, Woodward S. Tracing the role of human civilization in the globalization of plant pathogens. ISME J. 2018;12:647–52.
228. Grulke NE. The nexus of host and pathogen phenology: understanding the disease triangle with climate change. New Phytol. 2011;189:8–11.
229. Desprez-Loustau M-L, Robin C, Reynaud G, Déqué M, Badeau V, Piou D, et al. Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. Can J Plant Path. 2007;29:101–20.
230. Bassett I, Horner I, Hough E, Wolber F, Egeter B, Stanley M, et al. Ingestion of infected roots by feral pigs provides a minor vector pathway for kauri dieback disease *Phytophthora agathidicida*. Forestry. 2017;90:640–8.
231. Tella JL, Lambertucci SA, Speziale KL, Hiraldo F. Large-scale impacts of multiple co-occurring invaders on monkey puzzle forest regeneration, native seed predators and their ecological interactions. Glob Ecol Conserv. 2016;6:1–15.
232. Gallo L, Izquierdo F, Sanguinetti L, Pinna A, Siffredi G, Ayesa J, et al. *Araucaria araucana* forest genetic resources in Argentina. In: Vinceti B, Amaral W, Meilleur B, editors., et al., Challenges in managing forest genetic resources for livelihoods: examples from Argentina and Brazil. Rome: International Plant Genetic Resources Institute; 2004. p. 115–43.
233. Shaw C, MacKenzie M, Toes E, Hood I. Cultural characteristics and pathogenicity to *Pinus radiata* of *Armillaria novae-zelandiae* and *A. limonea*. N Z J For Sci. 1981;11:65–70.
234. Lewis KS, Black A, Condron LM, Waipara NW, Scott P, Williams N, et al. Land-use changes influence the sporulation and survival of *Phytophthora agathidicida*, a lethal pathogen of New Zealand kauri (*Agathis australis*). For Pathol. 2019;49:e12502.
235. Beauchamp A. The detection of *Phytophthora* Taxon "Agathis" in the second round of surveillance sampling—with discussion of the implications for kauri dieback management of all surveillance activity. New Zealand: Shared Services Threats and Transformation, Department of Conservation; 2013.
236. Donoso SR, Peña-Rojas K, Espinoza C, Galdames E, Pacheco C. Producción, permanencia y germinación de semillas de *Araucaria araucana* (Mol.) K. Koch en bosques naturales, aprovechados por comunidades indígenas del sur de Chile. Interciencia. 2014;39:338–43.
237. Stenlid J, Oliva J, Boberg JB, Hopkins AJ. Emerging diseases in European forest ecosystems and responses in society. Forests. 2011;2:486–504.
238. Garreaud RD, Boisier JP, Rondanelli R, Montecinos A, Sepúlveda HH, Veloso-Aguila D. The central Chile mega drought (2010–2018): a climate dynamics perspective. Int J Climatol. 2020;40:421–39.
239. Schumacher V, Justino F, Fernández A, Meseguer-Ruiz O, Sarricolea P, Comin A, et al. Comparison between observations and gridded data sets over complex terrain in the Chilean Andes: precipitation and temperature. Int J Climatol. 2020;40:5266–88.
240. Desprez-Loustau M-L, Marçais B, Nageleisen L-M, Piou D, Vannini A. Interactive effects of drought and pathogens in forest trees. Ann For Sci. 2006;63:597–612.
241. Sturrock R, Frankel S, Brown A, Hennon P, Kliejunas J, Lewis K, et al. Climate change and forest diseases. Plant Pathol. 2011;60:133–49.
242. Bostock RM, Pye MF, Roubtsova TV. Predisposition in plant disease: exploiting the nexus in abiotic and biotic stress perception and response. Annu Rev Phytopathol. 2014;52:517–49.
243. Brodribb TJ, McAdam SA, Jordan GJ, Martins SC. Conifer species adapt to low-rainfall climates by following one of two divergent pathways. Proc Natl Acad Sci. 2014;111:14489–93.
244.● Zimmer HC, Brodribb TJ, Delzon S, Baker PJ. Drought avoidance and vulnerability in the Australian Araucariaceae. Tree Physiol. 2016;36:218–28. **This article studies the mechanisms that some Araucariaceae have to cope with water scarcity and exposes the low tolerance of members in this family compared to other conifers.**
245.● Marchioro CA, Santos KL, Siminski A. Present and future of the critically endangered *Araucaria angustifolia* due to climate change and habitat loss. Forestry. 2020;93:401–10. **This study exposes a good example of severe habitat loss due to anthropogenic disturbances and climate change in an endangered species in the Araucariaceae.**
246. Castro MB, Barbosa ACMC, Pompeu PV, Eisenlohr PV, de Assis PG, Apgaua DMG, et al. Will the emblematic southern conifer *Araucaria angustifolia* survive to climate change in Brazil? Biodivers Conserv. 2020;29:591–607.
247. Saavedra A, Willhite E. Observations and recommendations regarding *Araucaria araucana* branch and foliage mortality (daño foliar de la *Araucaria*) in the national parks of south-central Chile. USDA Forest Service; 2017. Technical report. Available from: https://doi.org/10.13140/rg.2.2.20807.14248.
248. Pauchard A, Kueffer C, Dietz H, Daehler CC, Alexander J, Edwards PJ, et al. Ain't no mountain high enough: plant invasions reaching new elevations. Front Ecol Environ. 2009;7:479–86.

249. Pauchard A, Milbau A, Albihn A, Alexander J, Burgess T, Daehler C, et al. Non-native and native organisms moving into high elevation and high latitude ecosystems in an era of climate change: new challenges for ecology and conservation. Biol Invasions. 2016;18:345–53.

250. Stone JK, Coop LB, Manter DK. Predicting effects of climate change on Swiss needle cast disease severity in Pacific Northwest forests. Can J Plant Path. 2008;30:169–76.

251.•• Simler-Williamson AB, Rizzo DM, Cobb RC. Interacting effects of global change on forest pest and pathogen dynamics. Ann Rev Ecol Evol Syst. 2019;50:381–403. **This review deals with the diverse manners in which changes in environmental conditions may drive emergence or intensification of pests and diseases, including the effects on the biotic stressors, on the hosts, and on the ecology of both.**

252. Burgess TI, Oliva J, Sapsford SJ, Sakalidis ML, Balocchi F, Paap T. Anthropogenic disturbances and the emergence of native diseases; a threat to forest health. Current Forestry Reports. 2022;[This Issue].

253. NSW Department of Environment and Conservation. Wollemi Pine (*Wollemia nobilis*) recovery plan. Hurstville NSW.: NSW Department of Environment and Conservation; 2006. Report No.: DEC 2006/51.

254. ProForest. Conducting forest health research abroad. [Internet]. Proactive forest health and resilience; 2017 [cited 01 Sept 2021]. Available from: http://proforesthealth.org/research/projects/conducting-forest-health-research-abroad.

255. Winkworth RC, Bellgard SE, McLenachan PA, Lockhart PJ. The mitogenome of *Phytophthora agathidicida*: evidence for a not so recent arrival of the “kauri killing” *Phytophthora* in New Zealand. PloS one. 2021;16:e0250422.

256. Hawksworth DL. The oldest sequenced fungal specimen. Lichenologist. 2013;45:131–2.

257.• Oliva J, Ángel R, Stenlid J. Functional ecology of forest disease. Ann Rev Phytopathol. 2020;58:343–61. **This review highlights the difficulties of assessing the impact of diseases on forests under a changing environment, with special consideration to diseases of complex aetiologies, and it proposes a framework to address this from a functional ecology perspective.**

258. Paap T, Wingfield MJ, Burgess TI, Hulbert JM, Santini A. Harmonising the fields of invasion science and forest pathology. NeoBiota. 2020;62:301–32.

259. Paap T, Wingfield MJ, Burgess TI, Wilson JR, Richardson DM, Santini A. Invasion frameworks: a forest pathogen perspective. Curr For Rep. 2022. https://doi.org/10.1007/s40725-021-00157-4.

260. Wondafrash M, Wingfield MJ, Wilson JR, Hurley BP, Slippers B, Paap T. Botanical gardens as key resources and hazards for biosecurity. Biodivers Conserv. 2021;30:1929–46.

261. Paap T, Burgess TI, Wingfield MJ. Urban trees: bridge-heads for forest pest invasions and sentinels for early detection. Biol Invasions. 2017;19:3515–26.

262. Sims LL, Garbelotto M. *Phytophthora* species repeatedly introduced in Northern California through restoration projects can spread into adjacent sites. Biol Invasions. 2021;23:2173–90.

CHAPTER 2

Pewenomyces kutranfy gen. nov. et sp. nov. causal agent of an important canker disease on *Araucaria araucana* in Chile

Felipe Balocchi
Michael J. Wingfield
Rodrigo Ahumada
Irene Barnes

Received: 7 October 2020 | Revised: 6 January 2021 | Accepted: 18 January 2021

DOI: 10.1111/ppa.13353

ORIGINAL ARTICLE

Plant Pathology An International Journal edited by the British Society for Plant Pathology

WILEY

Pewenomyces kutranfy gen. nov. et sp. nov. causal agent of an important canker disease on *Araucaria araucana* in Chile

Felipe Balocchi[1] | **Mike J. Wingfield**[2] | **Rodrigo Ahumada**[3] | **Irene Barnes**[2]

[1]Department of Plant and Soil Science, FABI, University of Pretoria, Pretoria, South Africa

[2]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

[3]Bioforest S.A., Concepcion, Chile

Correspondence
Irene Barnes, Department of Biochemistry, Genetics and Microbiology, FABI, University of Pretoria, Pretoria, 0002, South Africa.
Email: irene.barnes@fabi.up.ac.za

Abstract

Araucaria araucana, (commonly referred to as araucaria, pewen, or monkey puzzle tree) is an ancient conifer endemic to the Chilean and Argentinian mountain ranges where it has a sacred relevance to indigenous communities. During 2015, a serious disease was noticed on trees of all ages in most of the natural distribution of this iconic tree. Four areas were surveyed, and the most important symptoms of the disease were cankers on branches and stems resulting in copious resin exudation. Trees were monitored for a period of two years and isolations were made from the cankers. Field observations showed that the disease typically begins on the leaves or at the leaf bases and progresses downwards to initiate cankers that can girdle branches or stems within a two-year period. Black ascomata, resembling those of *Caliciopsis* species previously described from *A. araucana*, were consistently found developing in the cankers from which isolations were made. Phylogenetic analyses of the ITS, nucSSU, and nucLSU gene regions showed that the fungus resides in the Coryneliaceae but is distinct from other genera in that family. The morphological characteristics and phylogenetic position of the fungus show that it represents a new genus and species, described here as *Pewenomyces kutranfy* gen. nov. et sp. nov. Pathogenicity trials on trees under field conditions confirmed that this newly described fungus is able to cause cankers on *A. araucana* similar to those found under natural conditions.

KEYWORDS

canker pathogen, Coryneliaceae, disease emergence, novel genus, novel taxon, pewen

1 | INTRODUCTION

Araucaria araucana (araucaria, pewen, or pehuén) is an ancient conifer endemic to the Chilean and Argentinian mountain ranges, distributed from ~37°24′ to ~40°03′ S. It is a dioecious, evergreen tree, with an extended lifespan and is adapted to grow at high altitudes and low temperatures, including at least three months of snow (González & Veblen, 2006). The tree is sacred to the Mapuche people who represent one of the largest indigenous cultures in Chile (Herrmann, 2005; Rodriguez et al., 1983). The genus *Araucaria* resides in the Araucariaceae, along with the genera *Agathis* and *Wollemia*, both of which are mostly distributed in the southern Asia–Pacific region. *A. araucana* and *Araucaria angustifolia* (Brazilian pine) are the only two extant species in the genus and family that are native to South America (Kershaw & Wagstaff, 2001).

During 2015, severe crown disease symptoms were observed on *A. araucana* trees in most of its natural range in Chile (CONAF, 2018) and Argentina (Vélez et al., 2020). Trees exhibited a variety of symptoms on their leaves and branches, leading to the hypothesis that the problem could be related to abiotic factors and stress, potentially including climate change. Furthermore, it was suggested that such stress could predispose the trees to damage by native pests and pathogens (Vélez et al., 2018).

Very little is known regarding the diseases of *A. araucana* in its natural environment. Several pathogens have been recorded, including the heteroecious rust *Mikronegeria fagi*, some minor leaf pathogens such as *Rhizothyrium parasiticum* and *Phaeocryptopus araucariae*, and root rot caused by *Armillaria mellea* sensu lato (Butin, 1970, 1975, 1986; Butin & Peredo, 1986; González & Opazo, 2002). The identification of all the fungi associated with these diseases was based only on morphology and came at a time when there was no DNA sequence-based phylogenetic inference able to confirm their taxonomic placement. Additionally, most of these pathogens have been referred to only once in the literature, illustrating the scarcity of the information available on them.

More recent studies on *A. araucana* have led to the description of several fungi on these trees, although little is known regarding their role in causing disease. These include *Diplodia africana*, *Diplodia mutila*, and *Neofusicoccum nonquaesitum* residing in the Botryosphaeriaceae, and two species of *Mortierella* (Mortierellales: Mortierellomycotina), namely *Mortierella alpina* and *Mortierella* sp. (Vélez et al., 2020). *D. africana* was associated with twig die-back on a single *A. araucana* tree within its natural distribution (Zapata & Schafer, 2019), and *D. mutila* and *N. nonquaesitum* were found on ornamental trees outside their natural range (Besoain et al., 2017; Pérez et al., 2018). The two *Mortierella* species were seldom recovered, and each was only recorded from a single location in the Argentinian *Araucaria* population (Vélez et al., 2020). None of these fungi are known to be primary pathogens and they appear to be associated with localized problems rather than linked to a widely distributed disease.

A preliminary inspection of diseased *A. araucana* trees conducted in 2017 by M. J. Wingfield and R. Ahumada (unpublished data) revealed that affected trees consistently had severe cankers on their branches and stems. This led to more intensive surveys where a fungus resembling a *Caliciopsis* sp. (Coryneliaceae) was consistently found sporulating on mature cankers. Two species of *Caliciopsis*, *Caliciopsis brevipes* and *Caliciopsis cochlearis*, have been morphologically described from *A. araucana* in Chile (Butin, 1970). One of these, *C. brevipes*, was associated with leaf deformation (Butin, 1970) but no pathogenicity tests have been conducted with the fungus. The other species, *C. cochlearis*, is known as a saprophyte on dying leaves and decaying bark of several conifers including *A. araucana* (Butin, 1970, 1986).

The objectives of this study were to conduct surveys of the canker disease on *A. araucana* in Chile and to monitor the progression of the disease symptoms over time. In addition, isolations were made from cankers, and specifically of the fungus resembling a species of *Caliciopsis* consistently associated with this symptom, for identification purposes. The fungus was identified based on multigene phylogenetic inferences using DNA sequence data from the nucLSU, nucSSU, and ITS gene regions, as well as morphological characteristics. Its pathogenicity was also considered in inoculation tests.

2 | MATERIALS AND METHODS

2.1 | Monitoring of disease symptoms

Several field surveys were conducted of Chilean National Parks and private land-holdings where *A. araucana* forests occur. These included Ralco National Reserve, Conguillío National Park, and Villarrica National Park in the Andes mountain range and private properties in Trongol Alto in the coastal range (Nahuelbuta; Figure 1). These surveys focused on monitoring stem and branch cankers to determine their distribution and disease progress over time. Trees with cankers at different stages of development were monitored at four time points between December 2017 and December 2019. A total of 27 trees at different locations were selected and marked, and branches on these trees were labelled. All the trees were GPS referenced, and photographic images were captured for them in December 2017, April 2018, February 2019, and December 2019. This made it possible to track the progression of disease symptoms from the first appearance of small sites of discolouration to the eventual death of the branches.

2.2 | Collection of samples and isolation of fungi

Branch canker samples were collected during December 2017 and January 2018 from the survey sites described above (Figure 1). Five collection points were established at each site except for Trongol Alto, which had three collection sites. Five samples from different trees were collected at each sampling point. Samples consisted of 20–60 cm branch segments bearing fresh, resinous cankers. Seven stem cankers were also collected at later stages from Conguillío National Park, three during December 2018, and four during December 2019.

For fungal isolations, all healthy leaves surrounding the cankers were removed and affected leaves were cut to their bases to reveal the discoloured tissue. This material was then briefly surface-disinfested by immersion in 70% ethanol for 10 s and 5% sodium hypochlorite for 1 min, and then rinsed with sterile distilled water. Outer layers of discoloured tissue were removed and small pieces of tissue were dissected from the margins of the discoloured area and plated on Petri dishes containing 50% potato dextrose agar (19.5 g/L PDA, 10 g/L agar; Merck) or 2% malt extract agar (MEA, 20 g/L malt extract, 20 g/L agar; Biolab). Isolations were incubated at 20–25 °C for 14 days and the obtained fungi were transferred to fresh 2% MEA plates. All isolates were then transferred to 2% water-agar (WA, agar 20 g/L; Biolab) and grown for 2–4 days, after which single hyphal tips were isolated and transferred to 2% MEA on Petri dishes.

In addition to isolations from diseased tissues, single spore isolations were made directly from fruiting bodies (ascomata) present in cankers on branches. In these cases, whole fruiting bodies were each placed on the surface of WA in Petri dishes and rolled across the agar surface using a sterile dissecting needle to release the spores. After 24–48 hr incubation, germinating spores were individually transferred to new Petri dishes containing 2% MEA. Isolates were broadly grouped into morphotypes based on colony shape and colour.

2.3 | DNA extraction, PCR, and sequencing reactions

Isolates representing a dominant fungal morphotype obtained from isolations from lesion margins and fruiting bodies were selected for

Map: Subsecretaría de Medio Ambiente, 2018 | Esri, HERE, Garmin, NGA,

Site / Collection point	Ralco National Reserve	Conguillío National Park: Los Paraguas	Villarrica National Park: Puesco	Trongol Alto
Point 1	-37.964705° , -71.327459°	-38.697836° , -71.817216°	-39.570510° , -71.502642°	-37.553434° , -73.188438°
Point 2	-37.962620° , -71.327679°	-38.698653° , -71.816826°	-39.572706° , -71.499235°	-37.571061° , -73.194365°
Point 3	-37.954698° , -71.331507°	-38.697493° , -71.814460°	-39.575582° , -71.493489°	-37.564893° , -73.205764°
Point 4	-37.951548° , -71.333419°	-38.698075° , -71.813826°	-39.581883° , -71.478997°	- -
Point 5	-37.949798° , -71.337132°	-38.697527° , -71.815577°	-39.584783° , -71.470167°	- -

FIGURE 1 Location of the study sites within the natural range of *Araucaria araucana* in the Andes mountain range and Coastal range. GPS coordinates for each sampling point within each site are provided

sequence comparison. Pure cultures of these isolates were grown on 2% MEA for 7–21 days for DNA extractions. Mycelium was collected and placed in 2 ml Eppendorf tubes, freeze-dried overnight and then ground in 2 ml Eppendorf tubes with metal beads using a mixer mill (MM 301, Retsch GmbH) set at 30 oscillations/s for 3 min. A phenol/chloroform protocol, described in Barnes et al. (2001) was used to extract DNA, with the exception of the first centrifuge stage being carried out for 60 min at 4 °C rather than at room temperature. RNase was added after the final suspension and incubated at 65 °C for 60 min. DNA concentrations were measured with a NanoDrop spectrophotometer (Thermo Scientific NanoDrop ND-1000) and standardized to 30 ng/μl working stock for PCRs.

The internal transcribed spacer (ITS) gene region was amplified with the primers ITS1/ITS4 (White et al., 1990) for all the isolates, and a subsequent group of isolates was selected to amplify the large ribosomal subunit (nucLSU) using the primers LROR/LR5 (Rehner & Samuels, 1995; Vilgalys & Hester, 1990) and the small ribosomal subunit (nucSSU) with the primers NS1/NS3/NS4/NS8 (White et al., 1990). PCRs were carried out using MyTaq DNA polymerase (Bioline) in 25 μl reactions containing 5 μl of MyTaq Reaction Buffer 5×, 0.5 μl of 10 μM of each primer, 0.3 μl DNA polymerase, and 17.7 μl of sterile distilled water (SABAX). The thermal cycling conditions included an initial denaturation stage of 95 °C for 3 min; followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 56 °C for 30 s and elongation at 72 °C for 45 s; and a final elongation stage at 72 °C for 4 min. PCR amplicons were stained with GelRed (2 μl per 4 μl of PCR product) and electrophoresis was carried out by running the product on a 1% agarose gel for 12 min at 110 V.

PCR amplicons were cleaned by sodium acetate precipitation (Duong et al., 2013) and eluted in sterile distilled water. The forward and reverse sequences were separately sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Thermo Fisher) following the manufacturer's protocol. The obtained products were cleaned using sodium acetate precipitation. Sequencing of the products was carried out at the Bioinformatics and Computational Biology Unit, University of Pretoria. The forward and reverse sequences obtained for each isolate were visualized and assembled into consensus sequences with CLC Main Workbench v. 8.0.1 (https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-clc-main-workbench/).

2.4 | Phylogenetic analyses

Consensus sequences were aligned to NCBI's GenBank database (NCBI, http://www.ncbi.nlm.nih.gov) using the BLAST utility to obtain a preliminary identification of all isolates. Based on the similarity output results, sequence data sets were generated for the ITS, nucLSU, and nucSSU gene regions available in GenBank for taxa relevant in determining the phylogenetic placement of the fungus (Table 1). This included isolates of most sequenced species residing in the Coryneliaceae, representative species for all subclasses in the Eurotiomycetes, and three species of Lecanoromycetes that served as the outgroup. Selection of isolates was made favouring ex-type cultures and/or cultures that had most of the gene regions sequenced. Additional sequences were produced, following the same methods described above, for two isolates (CBS 140.64 and CBS 142066) obtained from the culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands (CBS) and these were included in the sequence data sets.

The sequences in the databases were aligned using the online version of MAFFT (Katoh et al., 2019) (https://mafft.cbrc.jp/alignment/server/), and visualized and edited with MEGA v. 7.0.26 (Kumar et al., 2016). The sequences for each gene region were first aligned separately for individual phylogenetic analyses and then concatenated using CLC Bio Main Workbench to perform a combined analysis of the three gene regions. Maximum-likelihood trees for the four data sets (the individual sequences and the concatenated sequence) were generated with IQ-Tree Web server (Trifinopoulos et al., 2016) (http://iqtree.cibiv.univie.ac.at/) using the model ranked best according to the Bayesian information criterion (BIC) by ModelFinder (Kalyaanamoorthy et al., 2017). Bayesian inference trees for all four data sets were performed with Mr Bayes v. 3.2.6 by the Markov chain Monte Carlo (MCMC) method with four independent chains. Ten million generations with sampling every 100 steps were performed. Stationarity was corroborated by visualizing the data with Tracer v. 1.6.0 and burn-in was set to the first 10% of generations. Phylogenetic trees were visualized and edited with FigTree v. 1.3.1, iTOL v. 4 (https://itol.embl.de/), and Inkscape v. 0.92.4.

2.5 | Morphology

Ascomata and spermogonia were collected from plant material and cultures photographed and measured using a Zeiss SteREO Discovery.V.12 modular stereomicroscope and the software AxioVision (AxioVs40x64 v. 4.9.1.0). A total of 14 ascomata and five spermogonia were measured from canker samples, and 10 additional spermogonia were measured from 3-month-old cultures on MEA. Ascomata and spermogonia were also used to prepare semipermanent slides in lactic acid sealed with nail polish. Ascomata were cut with a microtome (Leica CM1520). Measurements of 19 asci, 50 ascospores, and 25 spermatia from cankers and 50 spermatia from cultures were made using the Nikon Eclipse Ni-u microscope with a Nikon DS-Ri2 camera and the software NIS-Elements v. 4.30.

Four representative isolates (CMW54230, CMW54240, CMW54244, and CMW54251) obtained from different trees and from three different sites were selected to study culture growth and characteristics. For this purpose, cultures were grown at temperatures ranging from 5 to 35 °C in 5 °C intervals on 2% MEA and oatmeal agar (OMA) in 90 mm Petri dishes in the dark. The area of culture growth was traced on the plates with a marker pen every 4 days for 28 days with three replicates of each isolate at each temperature. The total area of the cultures was measured using image processing with ImageJ software (Guerrero et al., 2012; Wang, 2017). In the case of cultures at temperatures where there was no growth, these were incubated for an additional 14 days at 20 °C to assess the viability of the original inoculum. Colours of cultures were designated according to the colour charts of Rayner (1970).

2.6 | Pathogenicity tests

Two isolates obtained from cankers (CMW54240 and CMW54244) were used for pathogenicity tests conducted on *A. araucana* trees during December 2018 in Villarrica National Park sector Puesco, where they had originally been collected. Isolates were grown on PDA at 20 °C for 14 days prior to inoculation. Trees chosen for inoculation were randomly distributed in two areas of approximately 1 ha, in open areas and under canopy cover. Healthy branches of trees between 1.5 and 3 m in height were inoculated by removing a leaf segment and placing a plug of mycelium-colonized agar onto the wounds, which was subsequently covered with Parafilm to avoid contamination and desiccation. A single branch was inoculated on each of six trees for each of the isolates and the control (sterile PDA plugs).

After 6 weeks, inoculated branch segments were cut from the trees and transferred in brown paper bags to the laboratory for assessment. Leaves were removed from the branches, the bark removed with a sterile scalpel to expose discolouration, and lesion lengths (mm) were measured. The percentage of the branch that had been girdled was measured by cutting the lesions transversely at the point of inoculation, capturing an image of the section, and

TABLE 1 Details of cultures and gene regions used for phylogenetic analyses

			GenBank accession no.		
Taxonomic position	**Species**	**Isolate**	**ITS**	**nucLSU**	**nucSSU**
Eurotiomycetes					
Chaetothyriomycetidae					
Chaetothyriales					
Cyphellophoraceae	*Cyphellophora guyanensis*	MUCL 43737 ^{ET}	NR132880.1	KC455253.1	NG065005.1
	Cyphellophora laciniata	CBS 190.61 ^{ET}	JQ766423.1	FJ358239.1	FJ358307.1
Pyrenulales					
Pyrenulaceae	*Pyrenula nitida*	F-5929	JQ927458.1	DQ329023.1	–
	Pyrgillus javanicus	AFTOL-ID 342	DQ826741.1	DQ823103.1	DQ823110.1
Verrucariales					
Verrucariaceae	*Verrucaria lecideoides*	AFTOL-ID 2295	EU010256.1	EF643798.1	–
	Verrucaria muralis	AFTOL-ID 2265	EU010261.1	EF643803.1	EF689878.1
Coryneliomycetidae					
Coryneliales					
Coryneliaceae	*Caliciopsis beckhausii*	MA 18186 ^{NT}	NR132090.1	NG060418.1	–
	Caliciopsis calicioides	Voucher 211	JX968549.1	–	–
	Caliciopsis eucalypti	CBS 142066 ^{ET}	NR154836.1	NG059013.1	**MT359910**
	Caliciopsis indica	GFCC 4947 ^{ET}	NR119752.1	GQ259980.1	–
	Caliciopsis orientalis	AFTOL-ID 1911/CBS 138.64 ^{ET}	NR145392.1	NG058741.1	DQ471039.1
	Caliciopsis pinea	AFTOL-ID 1869/CBS 139.64	KP881691.1	DQ678097.1	DQ678043.1
	Caliciopsis pseudotsugae	CBS 140.64 ^{ET}	**MT334517**	**MT334518**	**MT359911**
	Caliciopsis valentina	MA 18176/IGB290 ^{T}	NR132091.1	NG060419.1	–
	Corynelia africana	PREM 57242/ARW 247 ^{T}	NR153901.1	NG058910.1	KP881719.1
	Corynelia fructigena	PREM 57240/ARW 250 ^{T}	NR153902.1	NG058911.1	KP881720.1
	Pewenomyces kutranfy	CMW54230 ^{PT}	**MT334514**	**MT334519**	**MT359912**
	P. kutranfy	CMW54240 ^{ET}	**MT334515**	**MT334521**	**MT359913**
	P. kutranfy	CMW54244 ^{PT}	**MT334516**	**MT334520**	**MT359914**
	Hypsotheca eucalyptorum	CBS 145576 ^{T}	MK876393.1	MK876434.1	–
	Hypsotheca maxima	CPC 24674/COAD 1983 ^{eT}	NR160329.1	NG064416.1	–
	Hypsotheca nigra	MA 18191/ IGB305	–	KP144011.1	–
	Hypsotheca pleomorpha	CPC 32144/CBS 144636	MK442588.1	MK442528.1	–
	Lagenulopsis bispora	PREM 57232/ARW 249 ^{eT}	NR154120.1	NG060325.1	NG061200.1
	Tripospora tripos	PREM 61200/ARW 677	KP881712.1	KP881718.1	–
Eurotiomycetidae					
Arachnomycetales					
Arachnomycetaceae	*Arachnomyces minimus*	CBS 324.70 ^{T}	–	NG056963.1	AJ315167.1
	Arachnomyces nodosetosus	CCF 3975	HM205102.1	HM205103.1	HM205104.1
Euriotiales					
Aspergillaceae	*Aspergillus inflatus*	CBS 575.70A	MH859856.1	MH871635.1	–
	Hamigera avellanea	CBS 295.48 ^{T}	NR156333.1	–	NG061105.1
	Hamigera striata	CBS 377.48 ^{T}	MH856405.1	MH867954.1	–
	Leiothecium ellipsoideum	CBS 147.75	MH860904.1	MH872639.1	–
	Penicillium crustosum	DAOM 215345	JN942857.1	JN938953.1	JN939046.1
Thermoascaceae	*Byssochlamys nivea*	CBS 100.11 ^{NT}	NR144910.1	NG058631.1	NG061072.1

(Continues)

TABLE 1 (Continued)

Taxonomic position	Species	Isolate	GenBank accession no.		
			ITS	nucLSU	nucSSU
	Thermoascus crustaceus	CBS 181.67 ^{T}	NR144915.1	NG064060.1	NG062804.1
Trichocomaceae	*Rasamsonia emersonii*	CBS 266.71	MH860109.1	MH871885.1	–
	Sagenomella griseoviridis	CBS 470.78 ^{T}	MH861167.1	MH872930.1	–
	Talaromyces islandicus	CBS 126825	MH864247.1	MH875699.1	–
Onygenales					
Arthrodermataceae	*Arthroderma ciferrii*	CBS 272.66 ^{iT}	NR144888.1	NG057027.1	NG062604.1
	Arthroderma curreyi	CBS 138.26	KT155805.1	AY176726.1	AJ315165.1
	Ctenomyces serratus	CBS 187.61 ^{NT}	NR144890.1	NG058765.1	NG062605.1
Eremascaceae	*Eremascus albus*	CBS 975.69	MH859498.1	MH871279.1	FJ358348.1
Gymnoascaceae	*Amauroascus verrucosus*	NFCCI 2672 ^{T}	NR160558.1	JQ517293.1	JQ517294.1
	Gymnoascus reesii	CBS 410.72	MH860507.1	AY176749.1	GU733366.1
Onygenaceae	*Onygena corvina*	CBS 225.60	MH857958.1	MH869510.1	–
	O. corvina	CBS 281.48	–	FJ358287.1	FJ358352.1
Mycocaliciomycetidae					
Mycocaliciales					
Mycocaliciaceae	*Brunneocarpos banksiae*	CBS 141465 ^{T}	NR147648.1	MH878228.1	–
	Chaenothecopsis diabolica	H Tuovila 06-035 ^{PT}	NR120164.1	JX119118.1	–
	Chaenothecopsis golubkovae	Titov 6707 (UPS)	AY795859.1	AY795996.1	–
	Chaenothecopsis sitchensis	HT22	–	KF157988.1	KF157976.1
	Mycocalicium subtile	Wedin 6889 (UPS)	–	AY853379.1	–
	Phaeocalicium polyporaeum	ZW-Geo60-Clark	AY789363.1	AY789362.1	AY789361.1
Sclerococcomycetidae					
Sclerococcales					
Dactylosporaceae	*Rhopalophora clavispora*	CBS 637.73 ^{T}	NR152542.1	KX537757.1	NG061246.1
	Sclerococcum vrijmoediae	NTOU4002 ^{T}	KJ958534.1	KC692153.1	KC692152.1
Lecanoromycetes					
Acarosporomycetidae					
Acarosporales					
Acarosporaceae	*Sarcogyne plicata*	AFTOL-ID 4830	–	KJ766657.1	KJ766791.1
Lecanoromycetidae					
Caliciales					
Caliciaceae	*Calicium glaucellum*	Tibell 22319 (UPS)	AY450569.1	AY453646.1	–
	Calicium salicinum	CBS 100898	–	KF157982.1	KF157970.1

Note: Sequences generated in this study are in bold. ^{T} Sequence from type material; ^{ET} sequence from ex-type culture; ^{NT} sequence from neotype culture; ^{eT} sequence from epitype; ^{iT} sequence from isotype; ^{PT} sequence from paratype. Designation of cultures and culture collections: AFTOL-ID, Assembling the Fungal Tree of Life (AFTOL) project (www.lutzonilab.net/aftol); ARW, personal number of Alan Wood, ARC-Plant Protection Research Institute, South Africa; CBS, Centraalbureau voor Schimmelcultures, Netherlands; CCF, Culture Collection of Fungi, Department of Botany, Charles University in Prague, Czech Republic; Clark, Clark University, USA; CMW, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; COAD, Coleção Octávio de Almeida Drumond, Universidade Federal de Viçosa, Brazil, CPC, Culture collection of Pedro Crous, housed at CBS, Netherlands; DAOM, Canadian National Mycological Herbarium, Ottawa Research and Development Centre, Canada; F, personal number of Zdenek Palice, Institute of Botany, Academy of Sciences of the Czech Republic; GFCC, Goa University Fungus Culture Collection and Research Unit, India; H Tuovila, HT, personal number of Hanna Tuovila, University of Helsinki, Finland; MA, Real Jardín Botánico, Spain; MUCL, Mycotheque de l'Universite Catholique de Louvain, Belgium; NFCCI, National Fungal Culture Collection of India, India; NTOU, National Museum of Natural Science Herbarium, Taiwan; PREM, National Collection of Fungi, South Africa; UPS, Museum of Evolution, Sweden.

measuring the angle of discoloured tissue with ImageJ software (https://imagej.nih.gov/ij/index.html) using the circumference of the branch pith as the reference centre point. The captured data were analysed statistically using R software by performing analysis of variance (ANOVA) and posterior Tukey's test (HSD) for each kind of measurement individually.

Reisolations were made from lesion margins of all inoculated branches including control treatments. For this purpose, the lesions were surface disinfested with 70% ethanol (10 s), followed by 5% sodium hypochlorite (1 min), and then cleaned with sterile distilled water. Small segments were cut from the leading edges of the lesions and incubated in Petri dishes containing 50% PDA for 7–14 days. The identification of the emerging isolates was confirmed by sequencing of the ITS region.

3 | RESULTS

3.1 | Monitoring of disease symptoms

Decline symptoms were observed on *Araucaria* trees of all ages, including young seedlings, at all four sites assessed. The predominant symptom observed was a progressive death of branches (Figure 2a,b) starting most frequently from the base of the crown and occasionally moving laterally. Whole branches or parts of trees were observed yellowing from mid-November and throughout December and January (spring to summer). Most of these tree parts had died by the middle of February, but branches continued to die up to April. After 1–2 years, the dead leaves had fallen, with the dry medulla remaining. Girdling cankers were consistently associated with dying branches of most diseased trees at the sites on the Andes mountain range, but these were rarely found on trees on the coastal range (Trongol Alto in Nahuelbuta). A low incidence of branches infested by insects and by secondary leaf pathogens were consistently observed in all sites.

Cankers on the Andean *Araucaria* populations were found on trees of all ages, on branches of different sizes and in some cases on the main stems of a large proportion of the trees. In addition, at the same sites, most of the branches that had recently died exhibited a canker near or directly at its base. Monitoring of specific lesions for a 2-year period provided clear evidence that the cankers resulted in branch death. Variability in the rate of spread of the discolouration and the production of secondary symptoms was observed in lesions that were tracked over time. Freshly developing cankers were observed on trees growing at all the sites inspected in the Andes at all three times of inspection (November and December in 2017, 2018, and 2019). This showed that infection could occur between the end of winter (August) and the beginning of spring (September–October).

Initial infections included reddish-brown areas of discolouration, approximately 3 mm long at the bases of individual isolated leaves, which in most cases were chlorotic or dead (Figure 3a). As infection progressed it caused slight swelling of the tissues leading to small cracks that exuded copious amounts of resin. The infection continued to spread at the base of the leaves, both longitudinally and laterally, resulting in the death of attached leaves in a ring-like pattern (Figures 2c,d and 3b,c). Over time, cankers that had developed in these rings of infection became darker and cracks coalesced into larger lesions. After 1–2 years, cankers had girdled the branches and the leaves distal to these cankers became chlorotic and died (Figures 2d and 3c). Cankers were more often found at the bases of branches (Figure 2e) and/or secondary branches and these consistently led to the death of the distal portions of those branches. Cankers were also observed in the middle of secondary branches, which resulted in the death of either the distal portion or entire secondary branch. Similar cankers were observed on the trunks of young trees (Figure 2h), and, in some cases, these resulted in the death of tree parts above those cankers.

3.2 | Collection of samples and isolation of fungi

Fresh and well-developed cankers were easily collected from all three sites visited in the Andes mountain range, and these consistently displayed tissue discolouration and resin exudation. However, cankers found in Trongol Alto (Nahuelbuta, Coast range), which had a drier appearance, were scarce, including some sample collection points where no cankers were found. In such cases, samples of branch segments that contained small areas of brownish red discolouration at the base of one to three leaves were collected.

Many different fungi were obtained in the primary isolations from the margins of lesions of samples from all sites. Most were recovered only once or at low frequency. Based on culture morphology, only a single fungus was consistently recovered from branch and stem cankers. These were also only from samples collected from sites in the Andes mountain range. No dominant fungal morphotype was recovered from samples collected at the Coastal range site (Trongol Alto, Nahuelbuta).

The main morphotype recovered from cankers from the sites in the Andes mountain range was a pale white colony, with radial aerial mycelium that grew slowly on the PDA, reaching 25 mm diameter after 14 days at 20 °C. A total of 49 isolates of this dominant fungal morphotype were obtained from branch cankers and 11 were obtained from cankers on the main stems of trees. Additionally, 14 isolates of an identical morphotype were obtained from the single spore isolations made from 14 different ascocarps collected from four different cankers.

3.3 | Phylogenetic analysis

Isolates representing the dominant morphotype obtained by isolation from lesion margins and from fruiting bodies found on cankers had identical ITS sequences. BLAST searches with these sequences had the highest identity to species of *Caliciopsis*. This was consistent with expectation due to the morphology of the fruiting bodies from which isolations were made. The closest matches were *Caliciopsis calicioides* (acc. no. JX968549.1) with only 86.36% identity, *Caliciopsis indica* (acc. no. NR_119752.1) with 86.01% identity, and *Caliciopsis eucalypti* (acc. no. NR_154836.1) with 85.08% identity.

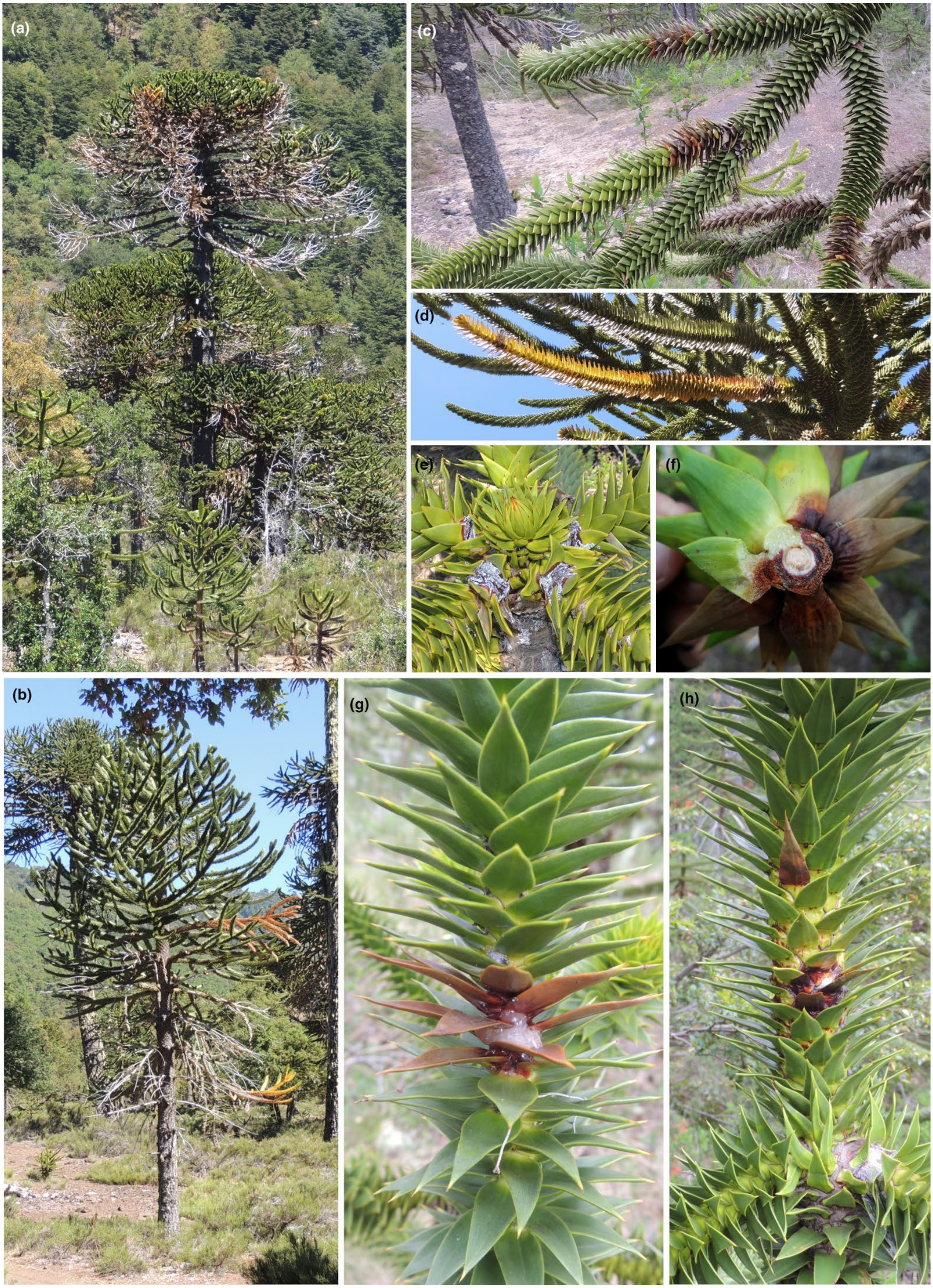


FIGURE 2 Symptoms of canker disease observed on *Araucaria araucana*. (a,b) Adult and young trees showing progressive death of branches; (c,d) ring-shaped cankers on branches; (e) cankers on the bases of branches; (f) transverse cut of young branch canker showing girdling of the phloem; (g) fresh canker with exudation of resin; (h) fresh canker on main trunk of a young tree

Three isolates of the dominant morphotype were included in the data sets with sequences obtained from GenBank for phylogenetic analyses. When aligned, these resulted in a total of 45 taxa for the ITS gene region, 50 for the nucLSU gene region, 28 for the nucSSU gene region, and 56 taxa for the combined data set. Maximum-likelihood and Bayesian-inference analyses using the individual

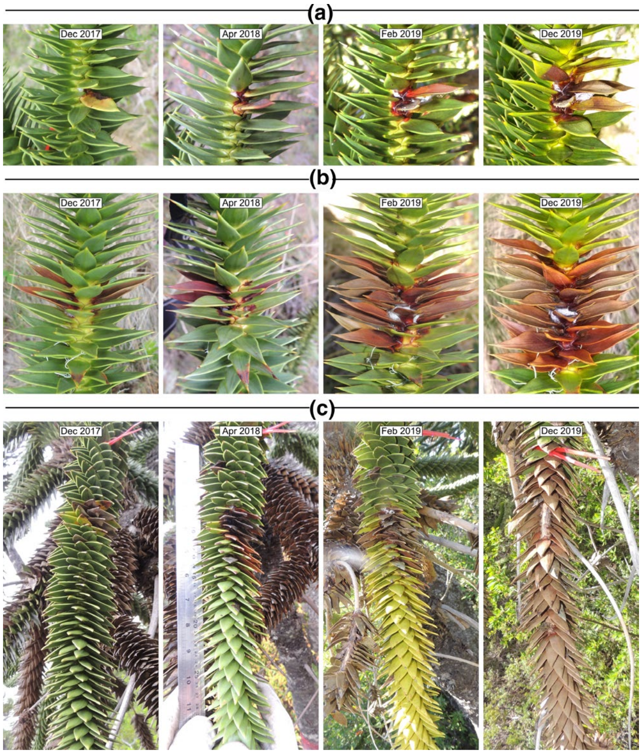


FIGURE 3 Long-term symptom progression of canker disease on three branches of *Araucaria araucana* over a two-year period from December 2017 to December 2019. (a) Initial stage of infection starting from one diseased leaf; (b) intermediate stage of infection including complete girdling; (c) advanced stages of infection with the appearance of secondary symptoms and death of branches

(Figures S1, S2 and S3) as well as the combined gene region (Figure 4) consistently grouped the isolates in an independent clade at the base of the Coryneliaceae family. This supported a conclusion that the fungus represented a novel genus. High branch support (bootstrap above 95%) was obtained for most nodes in the Coryneliaceae clade in phylogenetic trees made for all data sets. Results obtained with the ITS, nucLSU, and the combined data sets consistently separated *Caliciopsis* from *Hypsotheca* and showed that *Caliciopsis* is paraphyletic. However, ITS and nucLSU trees were not consistent in the position of species belonging to other genera in Coryneliaceae (*Corynelia*, *Lagenulopsis*, and *Tripospora*), placing them in a single clade sister to either *Hypsotheca* or *Caliciopsis*, respectively.

3.4 | Morphology

Ascocarps were only observed in cankers and these structures were never found in culture. These were also the most commonly occurring and obvious fruiting structures found on cankers (Figure 5e–h), and were most often found around and/or inside cracks in the bases of the leaves (Figure 5m). Ascocarps were black straight or tilted column-like structures with an ascigerous swelling, most commonly in the middle and tending to an upper position closer to the apex, bearing a reddish-brown mass of spores (mazaedium) at their tips. Asci were long, pedicellate and bearing eight reddish-brown ascospores with verrucose ornamentation (only seen at high amplification; Figure 5i–l). Spermogonia were identified from observing the ascocarps in development (Figure 5d,e); these emerged subepidermally from the base of diseased leaves (Figure 5a–d) in several samples. Spermatia obtained from the spermogonia on cankers were hyaline and obtuse or ovoid (Figure 5c). Ascocarps developed from the same stroma as the emerging spermogonia. Cultures in MEA were white when young, mostly submerged, with little aerial mycelium growing radially and turned darker with time, producing a hazel to umber brown colour. Some cultures produced spermogonia from young mycelium at the edge of the colonies, forming a concentric halo in the centre, while in other cultures they only formed at the margins when older.

3.5 | Taxonomy

Results of the phylogenetic analyses carried out for the most commonly encountered morphotype did not identify this fungus as any species for which sequence data were available. The fungus resided outside of the clade representing the genus *Caliciopsis* and in a clade of its own. Additionally, the results of morphological analyses indicated that the set of characters for the fungus isolated in this study were unique and different from *Caliciopsis* species described on *Araucaria* trees from Chile. Based on its unique morphology and phylogenetic placement, this fungus is described in a novel genus and species, as follows:

3.5.1 | *Pewenomyces* Balocchi, I. Barnes & M. J. Wingf gen. nov.

MycoBank MB834914

Etymology: From "pewen", the name of *Araucaria* in the Mapudungun, language of the native Chilean and Argentinian Mapuche culture.

Type species: *Pewenomyces kutranfy* Balocchi, I. Barnes & M.J. Wingf

Ascomata Individually or gregarious, arising individually or in pairs from a subepidermal stroma that may or may not erupt and become evident. Black in colour, ventricose, sometimes straight but most commonly tilted, ascigerous swelling position variable, mostly subapical to medium but also submedian, rarely collapsed to one side, very rarely branched. A powdery reddish-brown mass of mature ascospores accumulate on tip of mature ascomata. Ascomal wall of *textura porrecta* to *textura prismática*, interior *textura intricata*. *Asci* containing 8 spores, spathulate, ovoid to ellipsoid in spore bearing part, with a long and slender stipe, deliquescent. *Ascospores* reddish brown, thick-walled, mostly globose, but some ovoid to ellipsoid, verrucose. *Spermogonia* gregarious, erupting from plant tissue, black in colour, globose or piriform at maturity, creamy white spore exudation coming from an ostiole when mature. *Spermatia* hyaline, asymmetric, oblong, ovoid or reniform, aseptate, smooth.

Notes. *Pewenomyces* ascomatal morphology and arrangements are more similar to those of *Hypsotheca* and *Caliciopsis* than

FIGURE 4 Maximum-likelihood phylogenetic tree for the concatenated nucSSU, ITS, and nucLSU gene sequences for the subclasses and part of the subsequent orders within Eurotiomycetes. The new taxon (in red) is located independently at the base of the subclass Coryneliomycetidae (monotypic: order Coryneliales, also monotypic: family Coryneliaceae). Numbers on branches correspond to bootstrap values (*n* = 1,000) and posterior probabilities values from Bayesian inference, respectively. *: Sclerococcomycetales

to other genera in the Coryneliaceae. Ascomatal characteristics and dimensions have typically been used to separate genera in Coryneliaceae, and the existence of a stipe under the ascigerous swelling has been seen as the diagnostic morphological feature for *Caliciopsis* (Fitzpatrick, 1942b). More recently, the existence of a stipe has emerged as a common feature of *Caliciopsis*, *Hypsotheca*, and *Pewenomyces*, separating these three genera from the rest of the family. As a consequence, the existence of a stipe in *Pewenomyces* ascocarps serves as evidence that this genus does not reside with the remaining and unsequenced genera of the Coryneliaceae, that is, *Coryneliospora*, *Coryneliopsis*, *Coryneliella*, and *Fitzpatrickella* (Benny et al., 1985a, 1985b; Fitzpatrick, 1942a).

3.5.2 | *Pewenomyces kutranfy* Balocchi, I. Barnes & M. J. Wingf sp. nov. (Figures 5 and 6)

MycoBank MB834915

Etymology: From indigenous Chilean and Argentinian Mapuche language mapudungun; “kutran”, meaning a disease and the “-fy”,

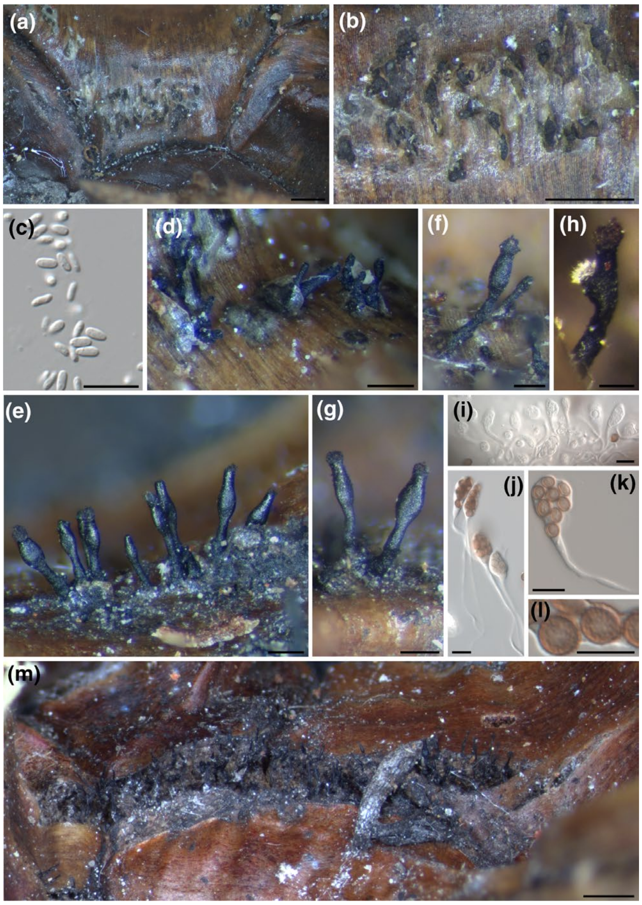


FIGURE 5 Fruiting bodies on branch cankers of *Araucaria araucana*. (a,b) Asexual stage (spermogonia) erupting subepidermally; (c) spermatia; (d,e) ascomata developing from stroma of spermogonia; (f,g) mature ascomata; (h) ascomata bearing a lateral spermogonia; (i,j) immature asci; (k) mature ascus bearing mature ascospores; (l) ascospores showing verrucose ornamentation; (m) ascomata's habit on cracks on base of the leaves. Scale bars: a, b, m = 1 mm; d, f, h = 0.1 mm; e, g = 0.2 mm; c, i–l = 10 μm

suffix from Latin "-ficare", to "make into". Thus, illustrating the fact that the fungus causes disease.

Ascomata Individual or gregarious, emerging from cracks at the base of leaves and stems. Black in colour, elongate, ventricose with a submedian to supramedian ascigerous swelling, (358) 473 ± 91 (600) μm tall, (122) 148 ± 23 (173) μm wide in the broadest part and (83) 103 ± 18.3 (126) μm at the tip. Ascomal wall composed of a few layers of dark *textura porrecta* cells and hyaline cells of *textura prismatica*, centre of *textura intricata*. *Asci* spathulate, long stalked (pedicellate), eight-spored, (13) 15.8 ± 1.7 (22) μm long and (8) 10.2 ± 0.7 (11) μm wide in spore-bearing part. *Ascospores* reddish brown, thick walled, verrucose, mostly globose or subglobose, rarely ellipsoidal, (4.0) 4.7 ± 0.5 (6.4) μm long and (3.4) 4.3 ± 0.5 (5.3) μm wide. *Spermogonia* erupting from plant tissue, at base of affected leaves, spherical to obpyriform, exuding a small white semitranslucent drop of spores. *Spermatia* hyaline, oblong, ovoid, or reniform (2.5) 3.6 ± 0.6 (4.6) long and (1.1) 1.3 ± 0.2 (1.7) wide.

Culture characters: Slow growing cultures variable in morphology. Optimum temperature for growth between 15 and 20 °C reaching 32–35 mm after 28 days in MEA and 24–27 mm on oatmeal agar (OMA; Figure 6). Faster growth observed at 5 and 10 °C than at 25 °C. No growth seen at 30 or 35 °C after 28 days on any tested culture medium. Cultures at 35 °C failed to grow when plates were returned to 20 °C for 14 days, indicating these high temperatures result in their death. Cultures on MEA and incubated at 20 °C were white when young, mostly submerged, with little aerial mycelium, growing radially, with a saffron white to light hazel brown colour underneath. Cultures gradually becoming darker and hazel to umber brown in colour, some with saffron to buff grey aerial mycelium at the centre. The reverse side of plates dark, especially at the edges, with a hazel brown to sepia colour. Fully grown cultures flat, round, with either entire or curled margins. Cultures on OMA white, sunken, with aerial mycelium absent or very minimally present, growing sparsely with curled margins. On reverse, cultures white, becoming creamy coloured with time.

Spermogonia were inconsistently observed, only in MEA, and occurred more frequently at lower temperatures (5–15 °C). Produced from the young mycelium at the edges of the colonies, forming a concentric halo at the centre or edge of the colony depending on when they are developed. Gregarious or in clusters, obpyriform to spherical when solitary, dark brown to black with a milky white to yellowish spore mazaedium in the top when mature. Spermatia are hyaline, aseptate, shape varies from symmetrical oblong to reniform to asymmetrical obovoid with an apparent constriction in the middle, (3.1) 5.6 ± 1.1 (8.8) μm long and (1.4) 2.1 ± 0.5 (3.4) μm wide.

Substrate: Cankers on branches of *Araucaria araucana*

Specimens examined: Chile, Araucanía (IX), Villarrica National Park sector Puesco, 13 December 2017, Felipe Balocchi, HOLOTYPE PREM 63075 (dried culture), ex-type culture CBS 146709 = CMW54240 = AR128 (isolated from lesion margins of cankers on branches of *Araucaria araucana*), GenBank: ITS = MT334515, nucLSU = MT334521, nucSSU = MT359913; PARATYPE PREM 63077 (dried culture), ex-type culture CBS 146711 = CMW54244 = AR161 (isolated from lesion margins of cankers on branches of *Araucaria araucana*), GenBank: ITS = MT334515, nucLSU = MT334521, nucSSU = MT359913. Chile, Araucanía (IX), Conguillío National Park sector Los Paraguas, 11 December 2017, Felipe Balocchi, PARATYPE PREM 63076 (dried culture), ex-type culture CBS 146710 = CMW54230 = AR050 (isolated from lesion margins of cankers on branches of *Araucaria araucana*), GenBank: ITS = MT334514, nucLSU = MT334519, nucSSU = MT359912. Chile, Biobío (VIII), Ralco National Reserve, from cankers in the branches of *Araucaria araucana*, 28 December 2017, Felipe Balocchi, living culture CMW54251 = AR221 (isolated from lesion margins of cankers on branches of *Araucaria araucana*).

Notes. Although *P. kutranfy* described here resides in a novel monotypic genus, it was relevant to compare its morphology with *Caliciopsis* species described previously from *A. araucana* (Butin, 1970). In this regard, ascomata were more similar in size to

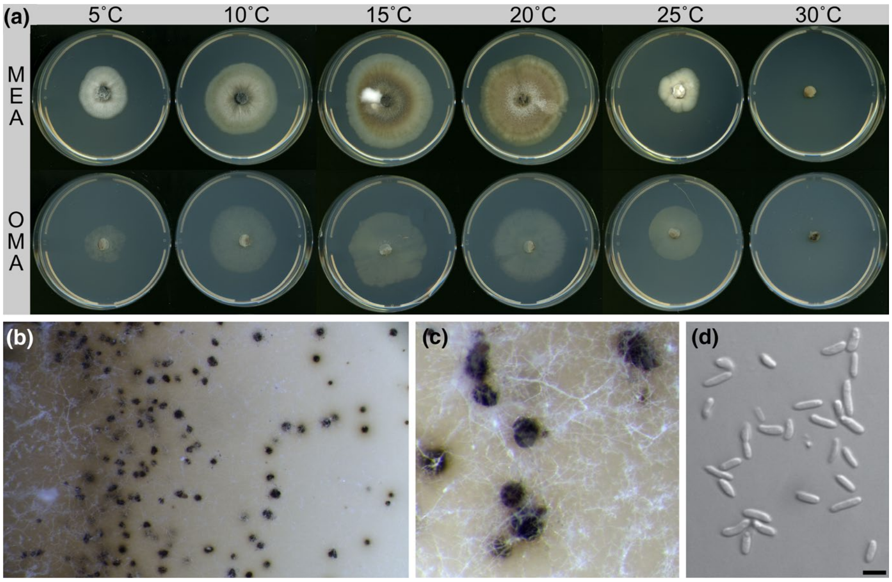


FIGURE 6 Culture characteristics of *Pewenomyces kutranfy* isolate CMW54240. (a) Culture growth after 28 days at six temperatures and two culture media: malt extract agar (MEA) and oatmeal agar (OMA); (b,c) fruiting bodies (spermogonia) on cultures on MEA; (d) spermatia. Scale bar: 5 μm

TABLE 2 Comparison of morphological characteristics of *Caliciopsis* species and *Pewenomyces kutranfy* on *Araucaria araucana*

Structure	Character	*Caliciopsis brevipes*[a,b]	*Caliciopsis cochlearis*[a,b]	*Pewenomyces kutranfy*
Ascomata	Height (μm)	600–1,000	350–700	358–600 (473 ± 91)
	Width (μm)	200–280	80–140	122–173 (148 ± 23)
	Tip width (μm)	100–160	50–75	83–126 (103 ± 18)
Asci	Length (μm)	18–22	16–18	13–22 (15.8 ± 1.7)
	Width (μm)	8–10	10–14	8–11 (10.2 ± 0.6)
Ascospores	Length (μm)	5.6–7.0	4.5–5.5	4.0–6.4 (4.7 ± 0.5)
	Width (μm)	4.5–6.0	4.0–4.6	3.4–5.3 (4.3 ± 0.5)
Spermogonia	Height (μm)	140–250	100–200	NA
	Width (μm)	140–250	NA	100
Spermatia	Length (μm)	4.2–5.5	3.2–4.2	2.5–4.6
	Width (μm)	2.0–3.0	1.0–1.5	1.1–1.7
	Shape	Allantoid/ellipsoidal	Allantoid	Oblong/ovoid

Abbreviation: NA, not available.

[a] Butin (1970).

[b] Benny et al. (1985b).

C. cochlearis than to those of *C. brevipes* (Table 2). Most features were found to be more variable than reported in literature, even for some previously considered diagnostic features such as the position of the ascigerous swelling. Although the ascomata on the cankers most commonly had a subapical ascigerous swelling (similar to those found in *C. cochlearis*), several ascocarps had a median to submedian swelling (similar to *C. brevipes*). The ascocarps were similar to those of *C. cochlearis* in height, but ascocarps on cankers were wider and the subepidermally erupting stromata remained visible at the base of most ascocarps. This was in contrast to those in *C. cochlearis* where ascocarps appear to erupt directly from the plant tissue. Dimensions of asci and ascospores obtained from ascocarps on cankers were also more variable than those described for *C. brevipes* and *C. cochlearis*. Although ascospore dimensions were most similar to those of *C. cochlearis*, those found in *P. kutranfy* were slightly larger. Spermatia obtained from spermogonia on cankers were shorter and wider than any of the two *Caliciopsis* species mentioned above; rather than being allantoid shape, they were mostly ovoid to obtuse.

3.6 | Pathogenicity

Six weeks after inoculation, distinct lesions had developed on all branches inoculated with the two isolates of *P. kutranfy*. All the infected branches had copious resin exudation covering the point of inoculation and a red-brown discolouration was observed spreading to the bases of the attached leaves (Figure 7). Removal of the outer layers of plant tissue exposed the longitudinal and lateral spread of the discolouration, and cutting the lesions transversely exposed the spreading of the discolouration through the phloem, reaching the cambium, and

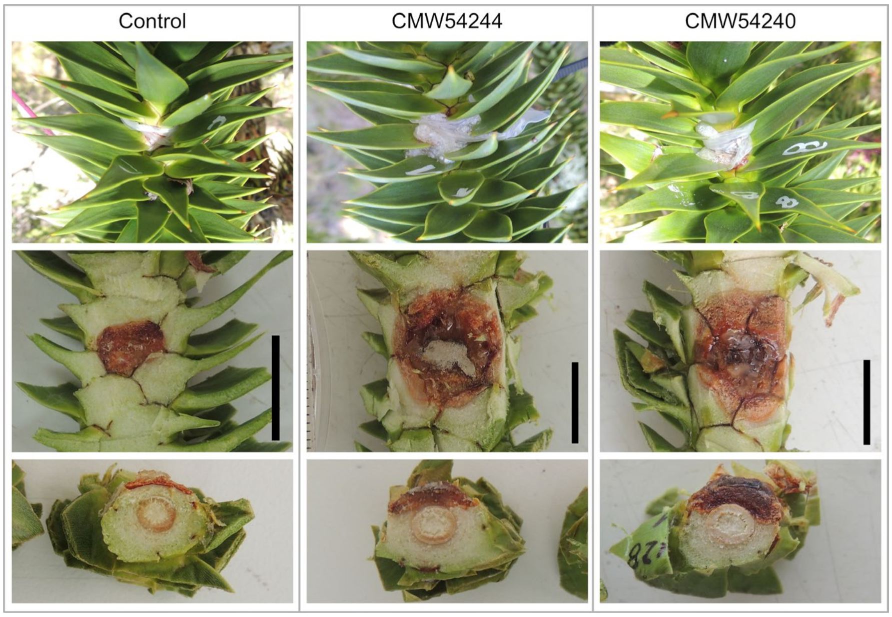


FIGURE 7 Results of inoculations with two isolates of *Pewenomyces kutranfy* and a control treatment on branches of *Araucaria araucana* after 6 weeks, showing differences in resin exudation (top), discolouration (middle), and girdling the branch (below). Scale bars: 10 mm

girdling the branch. Lesions produced by the isolate CMW54240 were 13.2 ± 2.4 mm long and had girdled 44 ± 3.3% of the branch circumference while isolate CMW54244 produced lesions 12.4 ± 2.5 mm long and girdled 41.2 ± 4.8% of the branch circumference. Differences between lesion sizes and girdling percentages produced with the two isolates were not significant according to Tukey's test ($p < .05$) (Figure S4).

In contrast, none of the control branches had resin exudation and were free of discolouration other than at the surface of the wound, which became dry. The size of the wounds remained the same as the initial inoculation wounds, which were 7.3 ± 1.4 mm long and the wound shape was the same as the initial leaf-base. When the outer tissues were removed, no discolouration was found in any of the inoculated branches. Cutting lesions transversally showed no signs of infection in the phloem, only a superficial layer of dry cells, which in circumference covered only 30.9 ± 2.8%. Lesion size and girdling percentage of both isolates of *P. kutranfy* were significantly different with those of the control.

Reisolations from the lesion margins resulted in 100% recovery of the inoculated fungus, while isolations from the control treatments yielded only contaminant fungi.

4 | DISCUSSION

The results of this study showed that an aggressive canker disease is directly related to branch die-back and decline of *A. araucana* trees in the main distribution of these trees in the Chilean Andes. A previously unidentified fungus in the Coryneliaceae was consistently associated with these cankers. Based on phylogenetic inference and supported by morphological characteristics, the fungus is described here in the new genus *Pewenomyces* as *P. kutranfy*. In field pathogenicity tests, *P. kutranfy* was shown to cause cankers similar to those observed under natural conditions.

Pewenomyces is most similar morphologically to *Caliciopsis* and to *Hypsotheca*, two genera that have similar morphological characters. The genus *Hypsotheca* was resurrected by Crous et al. (2019) to relocate three *Caliciopsis* species, based mainly on DNA sequence data. Those authors showed that species of *Caliciopsis* that had not had their identity confirmed by DNA sequence data might not actually belong to the genus, and thus, their morphology may not be representative of the genus. If only the nine species confirmed as *Caliciopsis* by sequence data are taken into account (Table 3), the common morphological traits are ascomata over 1 mm tall (except for *C. indica* and *C. moriondi*), with the ascigerous swelling terminal to subterminal (except *C. calicioides*), ascospores ellipsoidal to subglobose or ovoid (except *C. indica*) with smooth walls (Fitzpatrick, 1942b; Garrido-Benavent & Pérez-Ortega, 2015; Migliorini et al., 2020; Pratibha et al., 2010). Because the genus *Hypsotheca* has only recently been resurrected and one of its species, *Hypsotheca eucalyptorum*, was described only from culture, the description of morphological characteristics for this genus is limited, especially regarding the sexual morph. Observations of the ascomata of three sequenced species appear to show that these species form smaller fruiting bodies with the ascigerous swelling at a lower position (median or submedian, with the exception of *H. maxima*). However, both *Caliciopsis* and *Hypsotheca* include species that do not conform to these characteristics, which limits them as diagnostic features. It is also noted that morphological differences between these genera and *Pewenomyces* described in the present study are scarce; *Hypsotheca* species produce a hyphomycete state in cultures (Crous et al., 2019), which has not been reported for any *Caliciopsis* species and was not found in *Pewenomyces*. Furthermore, *P. kutranfy* produces ornamented (verrucose) ascospores rather than the smooth-walled ascospores in *Caliciopsis*, or the foveolate ornamentation seen in *Hypsotheca*.

The morphology of *P. kutranfy* was unlike that of either of the two *Caliciopsis* species (*C. brevipes* and *C. cochlearis)* described on *A. araucana* by Butin (1970). Even though neither of these species

TABLE 3 Main morphological characters for the species confirmed by sequencing as *Caliciopsis* and *Hypsotheca*

	Ascomatal dimensions				Asci	Ascospores			Spermogonia	Spermatia	
Species	Stroma	Height (μm)	Diameter (μm)	Ascigerous swelling	Dimensions (μm)	Ornamentation	Shape	Dimensions (μm)	Diameter (μm)	Shape	Dimensions (μm)
Caliciopsis beckhausii[g]	Absent	(500) 700–1,100 (1,500)	(75) 90–130 (160)	Median tending to subterminal	15–21 × 7–10	Smooth	Globose to subglobose (ellipsoidal)	(5.4) 6.4–8.4 (11) × (4.8) 5.7–7.5 (10)	65–150 (250)	Subcylindrical to allantoid	(3.5) 3.9–4.5 (4.7) × (1.2) 1.3–1.7
C. calicioides[a,d]	Present	1,400–1,800	200–340	Median or submedian	15–20 × 8–11	Smooth	Subfusiform to ellipsoidal (oval)	(6) 7 (10) × 3.5 (5)	140	—	2.5–3.0
C. eucalypti[b]	—	—	—	—	—	—	—	—	150–200	Bean-shaped obtuse ends	3–4 × 1.5
C. indica[k]	Absent	205–300	35–70	Terminal	18–28 × 4–7	Smooth	Globose	2.5 – 4.5 (Ø)	—	—	—
C. moriondi[i]	Present	(450) 821–869 (1,240)	(81) 137–147 (268)	Terminal	12.0–14.2 × 5.3–7.4	—	Subglobose to ellipsoidal	(3) 4.4 (6.2) × (1.8) 2.5 (3.5)	—	Slightly fusiform	—
C. orientalis[f]	—	2,000	150–200	Subterminal	16–19 × 7–9	—	Ellipsoidal	4.5–6 × 3.5–4.5	100–200	Allantoid	3.5 × 1.0
C. pinea[d,f,h]	Present	500–1,000	175–275	Terminal	12–17 × 5–8	—	Ellipsoidal to ovoidal or globose	3.5–6 × 2–4	100–150	Rod-shaped to allantoid	2.5–3.5 × 1.0
C. pseudotsugae[f]	—	1,000–3,000	250	—	12–19 × 5–9	—	Ellipsoidal to subglobose	(3) 4–5 (6) × 2–4	40–140	Allantoid	3.5–5.0 × 1.0–1.5
C. valentina[g]	Present	(600) 700–1,100 (5,000)	(100) 115–145 (160)	Subterminal	20–35 × 6–10	Smooth	Subglobose to ellipsoidal	(5.3) 6.2–7.8 (9.6) × (4) 5.0–6.4 (8)	120–170	Subcylindrical to allantoid	(4) 4.2–4.8 (5) × (1) 1.2–1.6
Hypsotheca eucalyptorum[c,l]	—	—	—	—	—	—	—	—	180–200	Subcylindrical with obtuse ends	(3) 3.5–4.0 (4.5) × 1.5 (2)
H. maxima[d]	Present	1,000–1,500	125–150	Median tending to subterminal	15–17 × 10	—	Globose, subglobose	5–6 × 4–5	—	Narrow-fusiform	11–24 × 4
H. nigra[a,e]	Present	≤500	170–24	Usually basal, submedian	—	Foveolate	—	4–5 (Ø)	—	—	—
H. pleomorpha[j]	Absent	250–700 (900)	65–130	Submedian to suprabasal	10–13 × 7–8	Smooth	Depressed globose to subellipsoid	3.3–3.7 (Ø)	80–150	Oblong to allantoid	3–5 × 1–2

Note: (Ø) for ascospore dimensions indicates that measurement of only a single dimension is provided.

[a] Benny et al. (1985b).

[b] Crous et al. (2016).

[c] Crous et al. (2019).

[d] Fitzpatrick (1920).

[e] Fitzpatrick (1942b).

[f] Funk (1963).

[g] Garrido-Benavent and Pérez-Ortega (2015).

[h] McCormack (1936).

[i] Migliorini et al. (2020).

[j] Pascoe et al. (2018).

[k] Pratibha et al. (2010).

[l] Described only from colonies in culture medium.

was reported to cause cankers, our initial hypothesis was that *P. kutranfy* might represent one of them based on general morphological characteristics and the niche in which these fungi occur. However, *C. brevipes* and *C. cochlearis* are more similar to each other than to any other *Caliciopsis* species and share some common features not seen in any other *Caliciopsis* species, such as verrucose ornamentation of the ascospores (Benny et al., 1985b). Taking into consideration the fact that DNA sequence data for some originally described *Caliciopsis* species has resulted in placing them in a different genus, it is reasonable to expect that Butin's species most likely reside in *Pewenomyces* rather than in *Caliciopsis*. Resolving this question must await collections from *A. araucana* that fit morphological characteristics of *C. brevipes* and *C. cochlearis* and from which cultures can be made.

Pathogenicity tests in this study showed that *P. kutranfy* is able to cause cankers similar to those found in the field and is thus considered as a new canker pathogen in the Coryneliaceae. This family of fungi is emerging as an important group of tree pathogens. It now includes a group of *Caliciopsis* species known to cause cankers on several conifer species, such as *C. moriondi* (Migliorini et al., 2020) and *Caliciopsis pinea* on *Pinus* spp., *Caliciopsis pseudotsugae* on Douglas-fir (*Pseudotsuga* spp.) and *Caliciopsis orientalis* on *Tsuga* spp. (Funk, 1963). In addition, there are two *Hypsotheca* species, including *Hypsotheca nigra* that causes galls on twigs of different *Juniperus* spp. and *Hypsotheca pleomorpha* causing cankers on different *Eucalyptus* species (Pascoe et al., 2018). Among these, Caliciopsis canker disease on *Pinus strobus* caused by *C. pinea* in the USA (Munck et al., 2015) and the canker disease caused by *H. pleomorpha* on *Eucalyptus* spp. in Australia, have resulted in recent outbreaks of diseases on both planted and natural forests. This would be similar to the canker disease caused by *P. kutranfy* described in the present study that occurs in natural *A. araucana* forests.

Culture growth studies with *P. kutranfy* showed that this fungus has a very evident tolerance to cold temperatures. This is consistent with its area of occurrence and suggests an adaptation to high elevations and extreme cold in winter that are typical of mountain ecosystems such as in the Andes (Diaz et al., 2003; Mellado-Mansilla et al., 2018). Other species in the Coryneliaceae have also been described from Chilean Andes environments (Butin, 1970; Fitzpatrick, 1942b; Léveillé, 1846), indicating that members of the Coryneliaceae may have been present in these environments for long periods of time.

Climate change could be among the drivers of the disease on *A. araucana* considered in this study. Weather conditions are known to have been outside of their usual range at the time when disease symptoms were first recorded on trees in the Chilean Andes. These conditions included an extended period of drought, referred to as the mega-drought of 2010–2018 (Garreaud et al., 2020), and a general increase in temperatures in the Andes mountain range at the same time (Schumacher et al., 2020). This led to the hypothesis that drought and/or extreme temperatures in summer resulted in stress to the trees, predisposing them to pest and pathogen damage. Although this scenario might in part explain the diversity of secondary agents found at all sites considered in this study, the fact that *P. kutranfy* grows poorly at temperatures above 20 °C is in conflict with the hypothesis that increased summer temperatures were responsible. In contrast, a rise in winter or early spring temperatures observed during the same period offer a more plausible explanation of the driver of canker disease. Higher temperatures in winter, especially at high elevations, would have a direct effect on fungal pathogens; for example, it would affect the survival of inoculum during winter as well as fungal biology, including reproduction and infection rates (Simler-Williamson et al., 2019).

The effect of climate change on forest pests and pathogens has become increasingly important in recent years. Changes in climatic conditions can affect the impact or emergence of diseases via diverse mechanisms (Ramsfield et al., 2016). Results of this study suggest that the emergence of the canker disease caused by *P. kutranfy* could be linked to a rise in winter temperatures. Such conditions could allow a native pathogen to cause damage not previously possible or facilitate the invasion process for an introduced pathogen. Although there is more evidence from this study to suggest that *P. kutranfy* is native to the environment in which it is causing disease, this question remains to be resolved.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the funding bodies for this work, namely the Tree Protection Cooperative Programme (TPCP), the Forest and Agricultural Biotechnology Institute (FABI) and the University of Pretoria, South Africa, the Chilean National Forest Corporation (Corporación Nacional Forestal, CONAF), and Bioforest S.A. Arauco, Chile. We would also like to acknowledge the personnel of the phytosanitary division and laboratory technicians at Bioforest S.A. for their assistance in laboratory and field work.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Felipe Balocchi https://orcid.org/0000-0003-4790-6283
Mike J. Wingfield https://orcid.org/0000-0001-9346-2009
Rodrigo Ahumada https://orcid.org/0000-0002-2397-2721
Irene Barnes https://orcid.org/0000-0002-4349-3402

REFERENCES

Barnes, I., Roux, J., Wingfield, M.J., Coetzee, M.P. & Wingfield, B.D. (2001) Characterization of *Seiridium* spp. associated with cypress canker based on β-tubulin and histone sequences. *Plant Disease*, 85, 317–321.

Benny, G.L., Samuelson, D.A. & Kimbrough, J.W. (1985a) Studies on the Coryneliales. I. *Fitzpatrickella*, a monotypic genus on the fruits of *Drimys*. *Botanical Gazette*, 146, 232–237.

Benny, G.L., Samuelson, D.A. & Kimbrough, J.W. (1985b) Studies on the Coryneliales. IV. *Caliciopsis*, *Coryneliopsis*, and *Coryneliospora*. *Botanical Gazette*, 146, 437–448.

Besoain, X., Guajardo, J. & Camps, R. (2017) First report of *Diplodia mutila* causing gummy canker in *Araucaria araucana* in Chile. *Plant Disease*, 101, 1328.

Butin, H. (1970) Zwei neue *Caliciopsis*-arten auf chilenischen koniferen. *Journal of Phytopathology*, 69, 71–77.

Butin, H. (1975) Beitrag zur ascomyceten flora von Chile. *Sydowia*, 27, 267–292.

Butin, H. (1986) *Rhizothyrium paraskicum* sp. nov. (Coelomycetes), ein blattparasit auf *Araucaria araucana* (Mol.) C. *Koch. Journal of Phytopathology*, 115, 313–317.

Butin, H. & Peredo, H.L. (1986) Hongos parásitos en coníferas de América del Sur, con especial referencia a Chile. *Bibliotheca Mycologica*, 101, 1–100.

CONAF. (2018) Consejo de la Sociedad Civil CONAF se interioriza de daño que afecta a araucarias. Available at: https://www.conaf.cl/consejo-de-la-sociedad-civil-conaf-se-interioriza-de-dano-que-afecta-a-araucarias/ [Accessed 1 February 2021]

Crous, P., Schumacher, R.K., Akulov, A., Thangavel, R., Hernández-Restrepo, M., Carnegie, A. et al. (2019) New and interesting fungi. 2. *Fungal Systematics and Evolution*, 3, 57–134.

Crous, P., Wingfield, M., Guarro, J., Cheewangkoon, R., van der Bank, M., Swart, W. et al. (2016) Fungal Planet description sheets: 469–557. *Persoonia*, 37, 218.

Diaz, H.F., Grosjean, M. & Graumlich, L. (2003) Climate variability and change in high elevation regions: past, present and future. *Climatic Change*, 59, 1–4.

Duong, T.A., De Beer, Z.W., Wingfield, B.D. & Wingfield, M.J. (2013) Characterization of the mating-type genes in *Leptographium procerum* and *Leptographium profanum*. *Fungal Biology*, 117, 411–421.

Fitzpatrick, H.M. (1920) Monograph of the Coryneliaceae. *Mycologia*, 12, 206–237.

Fitzpatrick, H.M. (1942a) Revisionary studies in the Coryneliaceae. *Mycologia*, 34, 464–488.

Fitzpatrick, H.M. (1942b) Revisionary studies in the Coryneliaceae. II. The genus *Caliciopsis*. *Mycologia*, 34, 489–514.

Funk, A. (1963) Studies in the genus *Caliciopsis*. *Canadian Journal of Botany*, 41, 503–543.

Garreaud, R.D., Boisier, J.P., Rondanelli, R., Montecinos, A., Sepúlveda, H.H. & Veloso-Aguila, D. (2020) The central Chile mega drought (2010–2018): a climate dynamics perspective. *International Journal of Climatology*, 40, 421–439.

Garrido-Benavent, I. & Pérez-Ortega, S. (2015) Unravelling the diversity of European *Caliciopsis* (Coryneliaceae, Ascomycota): *Caliciopsis valentina* sp. nov. and *C. beckhausii* comb. nov., with a worldwide key to *Caliciopsis*. *Mycological Progress*, 14, 10.

González, G. & Opazo, A. (2002) Enfermedades fungosas y otras. In: Baldini, A. and Pancel, L. (Eds.) *Agentes de Daño en el Bosque Nativo*. Santiago de Chile: Editorial Universitaria, pp. 89–199.

González, M.E. & Veblen, T.T. (2006) Climatic influences on fire in *Araucaria araucana–Nothofagus* forests in the Andean cordillera of south-central Chile. *Écoscience*, 13, 342–350.

Guerrero, N.R., Quintero, M.A.O. & Naranjo, J.C.P. (2012) Determinación del área foliar en fotografías tomadas con una cámara web, un teléfono celular o una cámara semiprofesional. *Revista Facultad Nacional de Agronomía – Medellín*, 65, 6399–6405.

Herrmann, T.M. (2005) Knowledge, values, uses and management of the *Araucaria araucana* forest by the indigenous Mapuche Pewenche people: a basis for collaborative natural resource management in southern Chile. *Natural Resources Forum*, 29, 120–134.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587.

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, 20, 1160–1166.

Kershaw, P. & Wagstaff, B. (2001) The southern conifer family Araucariaceae: history, status, and value for paleoenvironmental reconstruction. *Annual Review of Ecology and Systematics*, 32, 397–414.

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.

Léveillé, J. (1846) Descriptions des champignons de l'herbier du Muséum de Paris. *Annales des Sciences Naturelles (Ser.*, 3) 5, 249–305.

McCormack, H.W. (1936) The morphology and development of *Caliciopsis pinea*. *Mycologia*, 28, 188–196.

Mellado-Mansilla, D., Díaz, I.A., Godoy-Güinao, J., Ortega-Solís, G. & Moreno-Gonzalez, R. (2018) Bosque Pehuén Park's flora: a contribution to the knowledge of the Andean montane forests in the Araucanía Region, Chile. *Natural Areas Journal*, 38, 298–311.

Migliorini, D., Luchi, N., Pepori, A.L., Pecori, F., Aglietti, C., Maccioni, F. et al. (2020) *Caliciopsis moriondi*, a new species for a fungus long confused with the pine pathogen *C. pinea*. *MycoKeys*, 73, 87–108.

Munck, I.A., Livingston, W., Lombard, K., Luther, T., Ostrofsky, W.D., Weimer, J. et al. (2015) Extent and severity of caliciopsis canker in New England, USA: An emerging disease of eastern white pine (*Pinus strobus* L.). *Forests*, 6, 4360–4373.

Pascoe, I., Smith, I., Dinh, S.-Q. & Edwards, J. (2018) *Caliciopsis pleomorpha* sp. nov. (Ascomycota: Coryneliales) causing a severe canker disease of *Eucalyptus cladocaly*x and other eucalypt species in Australia. *Fungal Systematics and Evolution*, 2, 45–56.

Pérez, S., Guerrero, J. & Galdames, R. (2018) First report of *Neofusicoccum nonquaesitum* in Chile causing branch dieback and decline in *Araucaria araucana*. *Plant Disease*, 102, 1460.

Pratibha, J., Amandeep, K., Shenoy, B. & Bhat, D. (2010) *Caliciopsis indica* sp. nov. from India. *Mycosphere*, 1, 65–72.

Ramsfield, T., Bentz, B., Faccoli, M., Jactel, H. & Brockerhoff, E. (2016) Forest health in a changing world: effects of globalization and climate change on forest insect and pathogen impacts. *Forestry*, 89, 245–252.

Rayner, R.W. (1970) *A Mycological Colour Chart*. Kew, UK: Commonwealth Mycological Institute and British Mycological Society.

Rehner, S.A. & Samuels, G.J. (1995) Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. *Canadian Journal of Botany*, 73, 816–823.

Rodriguez, R., Matthei, S. & Quezada, M.M. (1983) *Flora Arbórea de Chile*. Concepción: Editorial de la Universidad de Concepción.

Schumacher, V., Justino, F., Fernández, A., Meseguer-Ruiz, O., Sarricolea, P., Comin, A. et al. (2020) Comparison between observations and gridded data sets over complex terrain in the Chilean Andes: Precipitation and temperature. *International Journal of Climatology*, 40, 5266–5288.

Simler-Williamson, A.B., Rizzo, D.M. & Cobb, R.C. (2019) Interacting effects of global change on forest pest and pathogen dynamics. *Annual Review of Ecology, Evolution, and Systematics*, 50, 381–403.

Trifinopoulos, J., Nguyen, L.-T., von Haeseler, A. & Minh, B.Q. (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, 44, W232–W235.

Vélez, M.L., Marfetán, J.A., Salgado Salomón, M.E. & Taccari, L.E. (2020) *Mortierella* species from declining *Araucaria araucana* trees in Patagonia, Argentina. *Forest Pathology*, 50, e12591.

Vélez, M.L., Salgado Salomón, M.E., Marfetan, A., Tirante, S.I., Mattes, F.H., Avila, M. et al. (2018) *Caracterización Desecación del Dosel y Sanidad de* Araucaria araucana *en Argentina*. Technical report. Available at: https://doi.org/10.13140/RG.2.2.14227.78889 [Accessed 6 August 2020]

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172, 4238–4246.

Wang, F. (2017) SIOX plugin in ImageJ: area measurement made easy. *UV4Plants Bulletin*, 2016, 37–44.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. (Eds.) *PCR Protocols: A Guide to Methods and Applications*. New York: Academic Press, pp. 315–322.

Zapata, M. & Schafer, M. (2019) *Diplodia africana* causing twig death on *Araucaria araucana*, a new host and first record for Chile. *New Disease Reports*, 40, 2.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Balocchi FA, Wingfield MJ, Ahumada R, Barnes I. *Pewenomyces kutranfy* gen. nov. et sp. nov. causal agent of an important canker disease on *araucaria araucana* in chile. *Plant Pathol*. 2021;00:1–17. https://doi.org/10.1111/ppa.13353

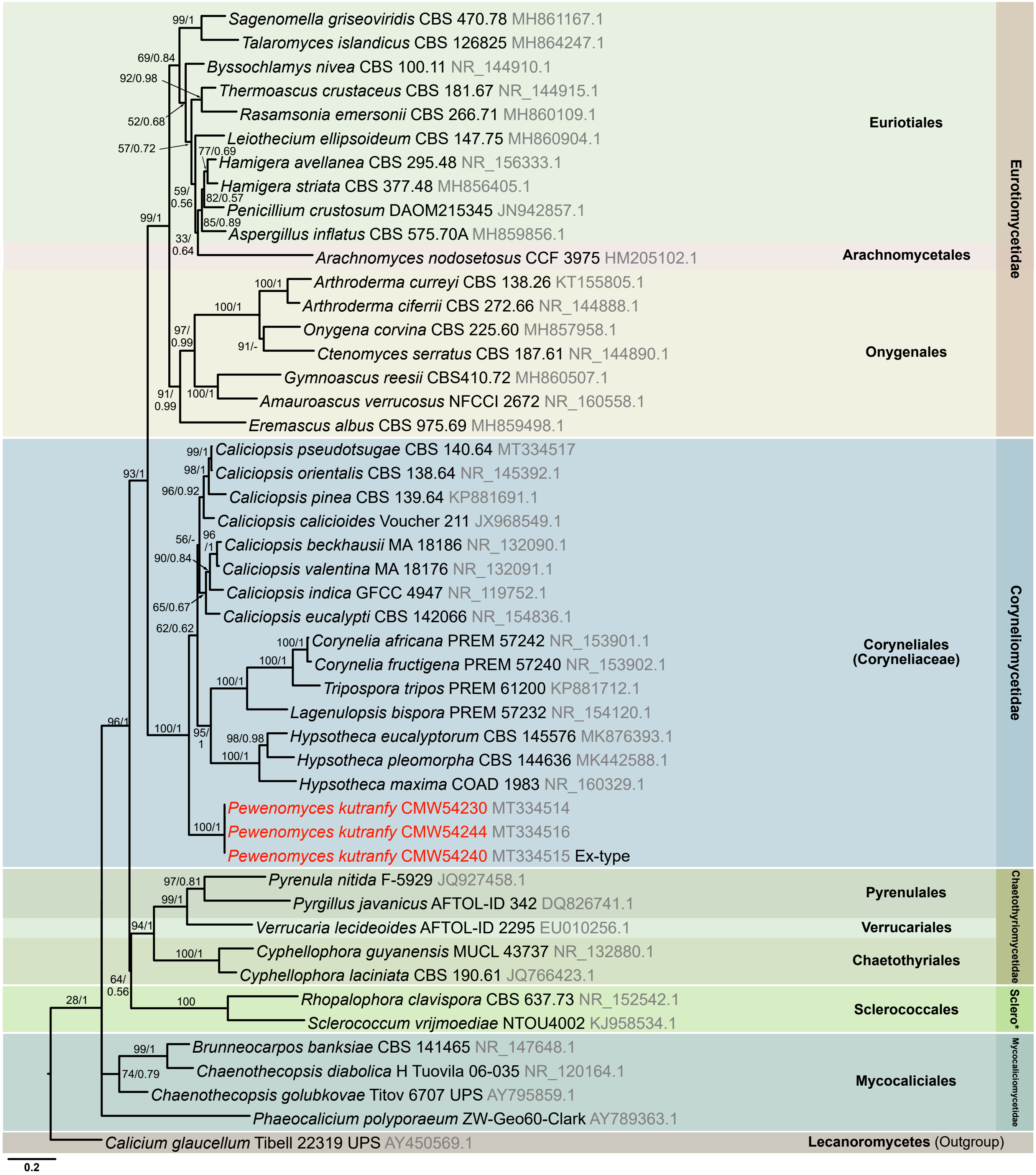


Figure S1. Maximum likelihood phylogenetic tree for the ITS region of the subclasses and part of the subsequent orders within Eurotiomycetes. New taxon (in red) is located basally on its own in the subclass Coryneliomycetidae (monotypic: order Coryneliales, also monotypic: family Coryneliaceae). Numbers on branches correspond to Bootstrap values (n=1000) and posterior probabilities values from Bayesian inference. Tree is rooted on species from the sister class Lecanoromycetes. *: Sclerococcomycetales.

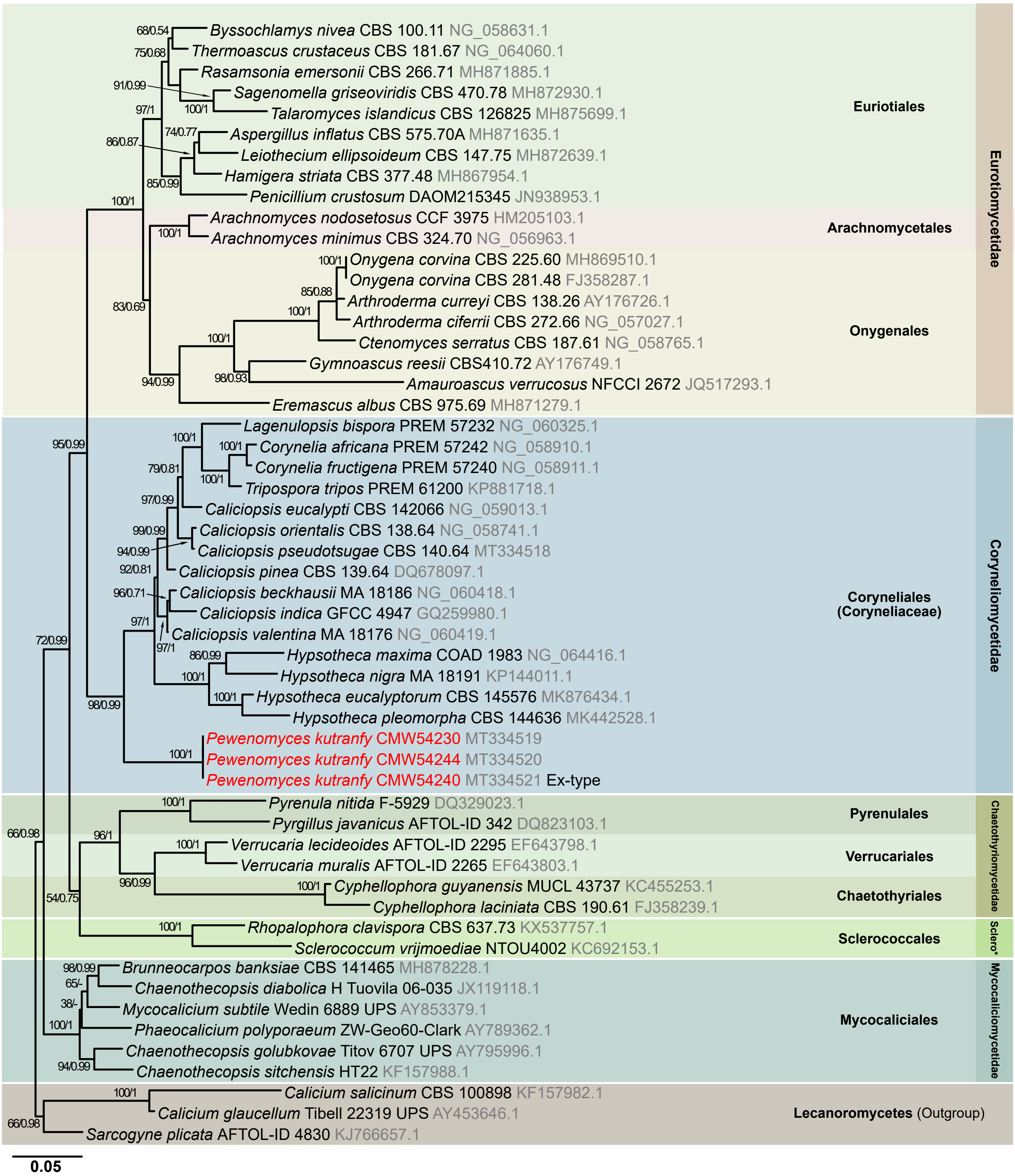


Figure S2. Maximum likelihood phylogenetic tree for the nucLSU (28S) region of the subclasses and part of the subsequent orders within Eurotiomycetes. New taxon (in red) is located basally on its own in the subclass Coryneliomycetidae (monotypic: order Coryneliales, also monotypic: family Coryneliaceae). Numbers on branches correspond to Bootstrap values (n=1000) and posterior probabilities values from Bayesian inference. Tree is rooted on species from the sister class Lecanoromycetes. ***:** Sclerococcomycetales.

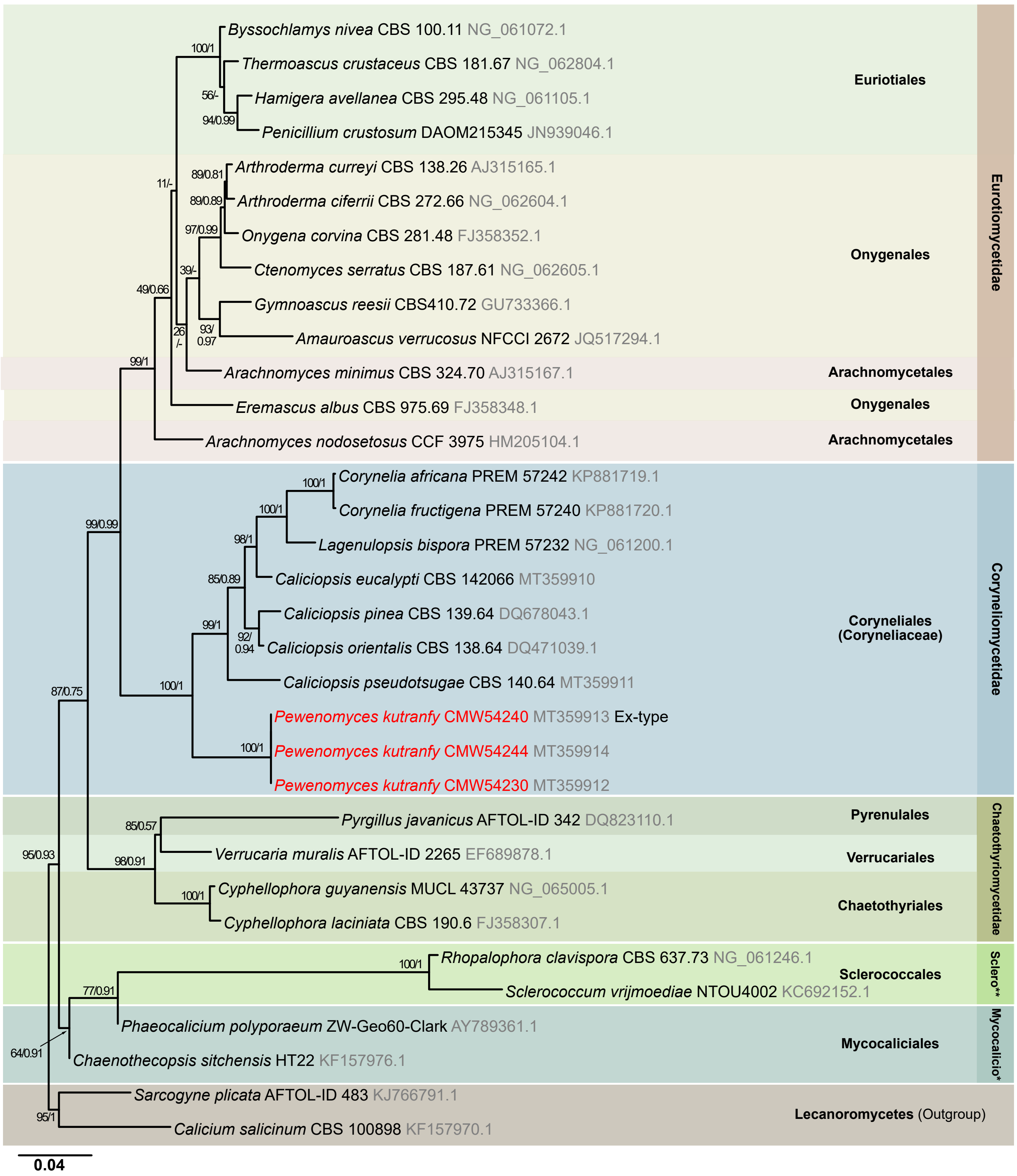
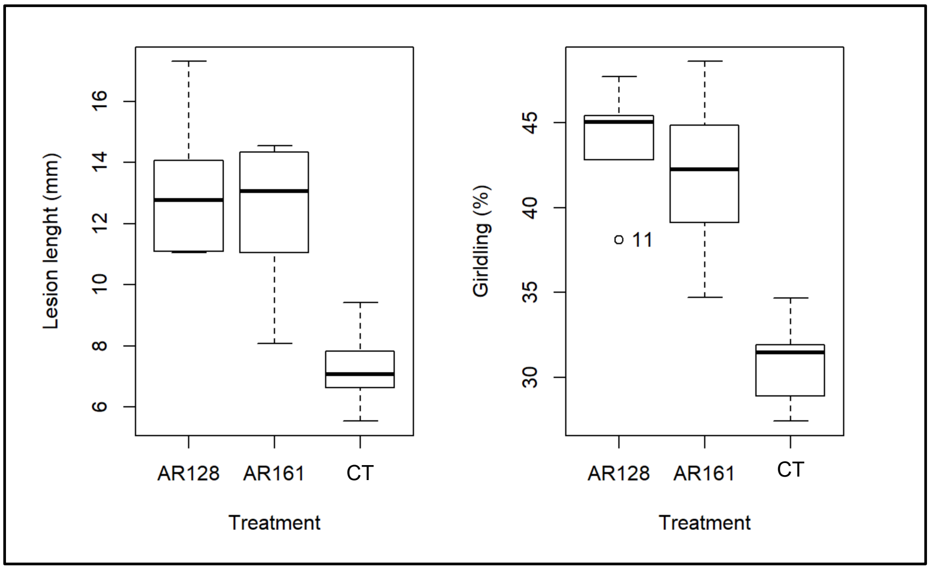


Figure S3. Maximum likelihood phylogenetic tree for the nucSSU (18S) region of the subclasses and part of the subsequent orders within Eurotiomycetes. New taxon (in red) is located basally on its own in the subclass Coryneliomycetidae (monotypic: order Coryneliales, also monotypic: family Coryneliaceae). Numbers on branches correspond to Bootstrap values (n=1000) and posterior probabilities values from Bayesian inference. Tree is rooted on species from the sister class Lecanoromycetes. ***:** Mycocaliciomycetidae; ****:** Sclerococcomycetales.



Lesion lenght

Shapiro-Wilk normality test

```
data:  Lesion_size
W = 0.96346, p-value = 0.6971
```

Bartlett test of homogeneity of variances

```
data:  Lesion_size and Treatment
Bartlett's K-squared = 1.1656, df = 2, p-value = 0.5583
```

Analysis of Variance

```
Response: Lesion_size

                Df      Sum Sq   Mean Sq  F value  Pr(>F)
Treatment       2       107.867  53.933   11.411   0.001149**
Residuals       14       66.172  4.727
```

Tukey multiple comparisons of means

```
95% family-wise confidence level
Fit: aov(formula = analysis_length)

                diff        lwr        upr        p adj
AR161-AR128     -0.8216667  -4.106868  2.463535   0.7928454
CONTROL-AR128   -5.8870000  -9.332548  -2.441452  0.0014305
CONTROL-AR161   -5.0653333  -8.510881  -1.619785  0.0047277
```

Girdling

Shapiro-Wilk normality test

```
data:  Girdling
W = 0.92768, p-value = 0.199
```

Bartlett test of homogeneity of variances

```
data:  Girdling and Treatment
Bartlett's K-squared = 1.3091, df = 2, p-value = 0.5197
```

Analysis of Variance

```
Response: Girdling

                Df      Sum Sq   Mean Sq  F value  Pr(>F)
Treatment       2       530.59   265.296  18.383   0.0001213**
Residuals       14       202.05  14.432
```

Tukey multiple comparisons of means

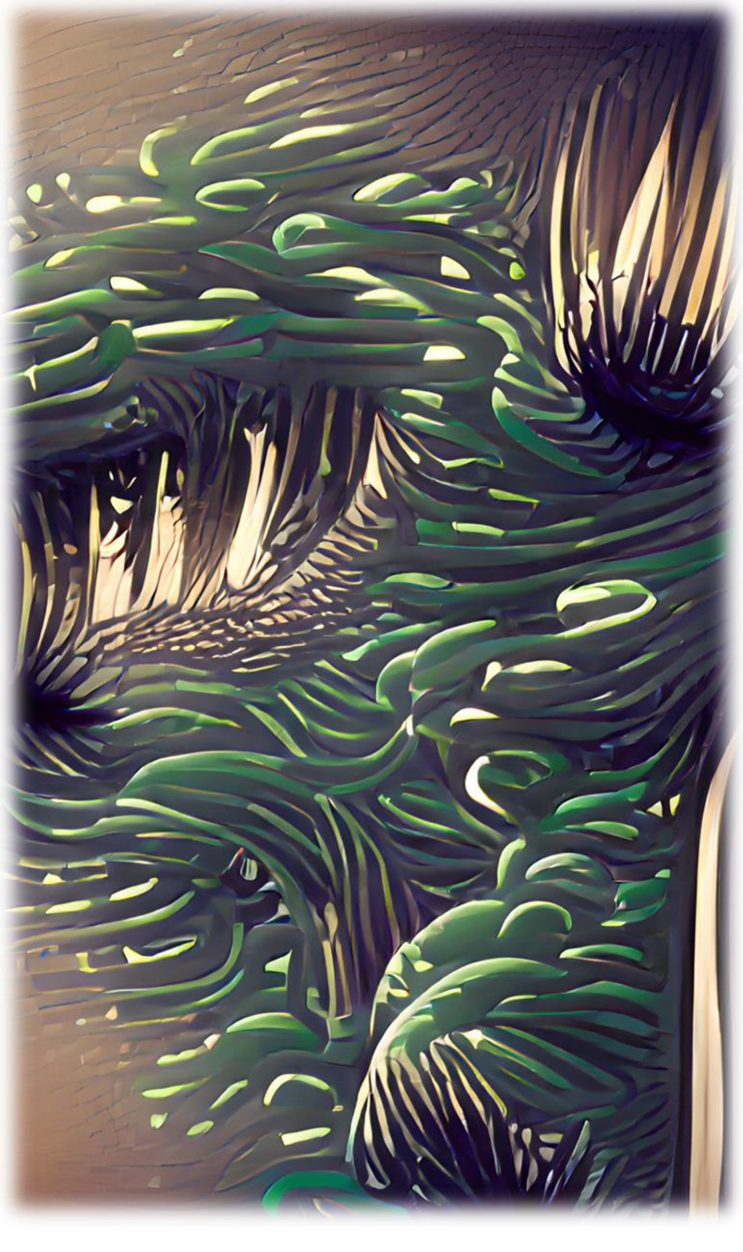
```
95% family-wise confidence level
Fit: aov(formula = analysis_girdling)

                diff        lwr         upr        p adj
AR161-AR128     -2.069815   -7.810354   3.670724   0.6227428
CONTROL-AR128   -13.146583  -19.167311  -7.125855  0.0001484
CONTROL-AR161   -11.076769  -17.097497  -5.056040  0.0007515
```

Figure S4: Results for the statistical analysis performed with data from the pathogenicity test. Boxplot (top) and results of main tests conducted are shown for lesion size (middle) and girldling (bottom) measurements.

CHAPTER 3

Three new species of *Pewenomyces* (*Coryneliaceae*) from *Araucaria araucana* in Chile



Felipe Balocchi
Seonju Marincowitz
Michael J. Wingfield
Rodrigo Ahumada
Irene Barnes

ABSTRACT

The *Coryneliaceae* is a relatively small family of mainly pathogenic fungi occurring on a diversity of hosts with a wide global distribution. Members of the family are recognized by their black, upright and elongated ascomata. Historically, the taxonomy of this group was mainly based on morphological characters, but in more recent years DNA sequence data have resulted in new revisions. The genus *Pewenomyces* was recently described based on *P. kutranfy*, a canker pathogen on *Araucaria araucana* in Chile. Morphologically, this fungus resembles species in *Caliciopsis* and *Hypsotheca*. During the study in which *Pewenomyces* was described, three putative species were identified from the same host, two of which were observed only from cultures obtained by isolating from plant tissues. At the time of describing *P. kutranfy*, there was uncertainty regarding its novelty because two species of *Caliciopsis* (*C. brevipes* and *C. cochlearis*), a closely related genus in the *Coryneliaceae*, had previously also been described from the same host and location, but for which DNA sequence data were not available. In this study, phylogenetic analyses that were carried out for the three putative *Pewenomyces* species using sequences for seven gene regions, confirmed that they were distinct species. Herbarium specimens for the two *Caliciopsis* species were obtained for morphological comparisons and phylogenetic analyses. These data showed that none of the novel *Pewenomyces* corresponded to the previously described *Caliciopsis* species. The three putative species are consequently described here as *Pewenomyces lalenivora* sp. nov., *P. tapulicola* sp. nov. and *P. kalosus* sp. nov.

1. INTRODUCTION

The *Coryneliaceae* is a family of ascomycetes residing in the order *Coryneliales* and in the subclass *Coryneliomycetidae* (Wood et al. 2016). The family accommodates nine genera and approximately sixty species. These fungi are plant pathogens, biotrophs, hyperparasites or saprobes with a wide global distribution and occurring on a diverse assemblage of hosts. Species in the *Coryneliaceae* are characterized by producing distinctive black elongated urceolate ascomata (stipitate in some genera), long-stiped spathulate asci, lacking paraphyses, and pigmentated one-celled ascospores. Delineation of species and genera has largely relied on morphological features of these ascomata (Fitzpatrick 1920, 1942a; Benny et al. 1985a, b, c, d; Garrido-Benavent and Pérez-Ortega 2015; Wood et al. 2016), including for example, their shape and dimensions, the presence or absence of a definite stalk below the ascigerous cavity, ascigerous cavity position, ascomatal proliferation and microscopic characters such as shape and number of ascospores per ascus.

Since the description of the *Coryneliaceae*, which initially included only the genera *Corynelia* and *Tripospora* (Saccardo 1886, 1891), the family has frequently been relocated within the ascomycetes as new genera and species were described and ultimately with its definitive placement in the *Eurotiomycetes* (Fitzpatrick 1920, 1942a; Wood et al. 2016). The genus *Caliciopsis* was described by Peck (1880) and was placed in different families until it was eventually allocated to the *Coryneliaceae* in 1920 (Fitzpatrick 1920, 1942b). *Caliciopsis* was distinguished from the other genera in the family mainly due to species having a definite, and in most cases, elongated stalk below the ascigerous cavity. Additionally, species of *Caliciopsis* were distinguished by non-morphological characters such as being able to grow in artificial media unlike most other members of the family that are obligate parasites, and their ability to infect woody tissues sometimes as conifer pathogens. Various *Caliciopsis* species have been discovered and described from diverse locations and hosts (Fitzpatrick 1942b; Batista 1956; Funk 1963; Huguenin 1969; Butin 1970; Funk and Kuijt 1974; Marmolejo 1999; Rikkinen 2000; Pratibha et al. 2010; Garrido-Benavent and Pérez-Ortega 2015; Crous et al. 2016). Ultimately, *Caliciopsis* has emerged as the most species-rich genus in the *Coryneliaceae*, accommodating approximately half of the species in the family.

Only a small proportion of species in the *Coryneliaceae* have been studied by comparisons of DNA sequences. Recent phylogenetic analyses of species in the family, using various gene regions (Geiser et al. 2006; Wood et al. 2016), have supported the phylogeny proposed by Fitzpatrick (1920) for the genera *Corynelia*, *Lagenulopsis*, *Tripospora* and *Caliciopsis,* with *Caliciopsis* being basal in the family. These studies also revealed a higher species diversity in *Caliciopsis* that had not been evident based on morphological characteristics. Some examples include the descriptions of *Caliciopsis moriondi* (Migliorini et al. 2020), long confused with the severe canker pathogen *Caliciopsis pinea*; and the resurrection of the genus *Hypsotheca* to accommodate three former *Caliciopsis* species, namely, *H. nigra* (=*Caliciopsis nigra*), *H. maxima* (=*Caliciopsis maxima*) and *H. pleomorpha* (=*Caliciopsis pleomorpha*) (Fitzpatrick 1942b; Crous et al. 2018; Pascoe et al. 2018; Crous et al. 2019a).

The novel genus *Pewenomyces* was recently discovered and described in the *Coryneliaceae* (Balocchi et al. 2021). This description was based on the pathogenic species *Pewenomyces kutranfy,* which was shown in the same study to cause cankers on *Araucaria araucana* in Chile. Two species of *Caliciopsis*, namely *Caliciopsis brevipes* and *Caliciopsis cochlearis*, had previously been described on *A. araucana* (Butin 1970) and it was initially suspected that *P. kutranfy* might correspond to one of these species. However, morphological comparisons showed that *P. kutranfy* was distinct from both those *Caliciopsis* spp. and a multi-gene phylogeny showed that it should reside in a distinct genus.

During the course of the study in which *P. kutranfy* was discovered (Balocchi et al. 2021), ascomata belonging to a second *Caliciopsis*-like fungus were found on some of the more mature canker samples, or on associated dead tissues. These fruiting structures appeared slightly different to those defined as *P. kutranfy*. A preliminary phylogenetic analysis based on the ITS region placed this fungus in *Pewenomyces*, but in a clade separate from *P. kutranfy*. This species was also recovered in isolations from dead plant tissues, that yielded isolates of another two possible new species of *Pewenomyces*. Similar to the situation that emerged when *P. kutranfy* was described, it was suspected that some of these three putatively undescribed species could represent one or both of the two *Caliciopsis* species previously described from *A. araucana* in Chile (Butin 1970), but for which no cultures are available.

The aim of this study was to consider the identity of the isolates from *A. araucana* related to *Pewenomyces* and in relation to previously described *Caliciopsis* species occurring on the same host.

2. MATERIALS AND METHODS

2.1 Collection of samples, fungal isolations and herbarium material

Isolates used in this study were obtained from branch cankers on *Araucaria araucana* and isolated on culture medium as described by Balocchi et al. (2021). Samples were collected between December 2017 and January 2018 from three conservation areas on the Andes Mountain range (Ralco Natural Reserve, Conguillio National Park and Villarrica National Park) and one private site on the Coastal range (Trongol Alto) (Balocchi et al. 2021). A collection of 28 isolates was obtained from these samples by isolating from diseased or dead plant tissue, as well as fruiting bodies (ascomata) present on mature cankers.

Additional sampling was done between December of 2019 and January of 2020 from the same sites. These included twigs and small branches of *A. araucana* with galls and fruiting bodies (*Caliciopsis*-like ascomata) similar to those described by Butin (1970) and Butin and Peredo (1986). Single spore isolations from these fruiting bodies were made by picking up mature ascomata from the galls, placing them on 2% water agar (WA, 20 g L^{-1} agar, Difco, Maryland, USA) in Petri dishes and rolling these across the surface of the agar to release the spores.

The isolation plates were incubated for 48h at ~22 °C and the germlings were transferred to new Petri dishes containing 2% malt extract agar (MEA: 20 g L^{-1} malt extract and 20 g L^{-1} agar, Biolab, Midrand, South Africa). Additional cultures used for the phylogenetic analyses included three isolates of *Pewenomyces kutranfy* (CMW 54230, CMW 54240, CMW 54244) preserved in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria; and isolates CBS 138.64 (*Caliciopsis orientalis*) and CBS 139.64 (*Caliciopsis pinea*) obtained from the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, Netherlands.

Herbarium material used by Butin (1970) for the description of two *Caliciopsis* spp. from *Araucaria araucana* was obtained from the Zürich Z+ZT Herbaria (University of Zürich and ETH Zürich). Three specimens were used in this study; the holotype specimen for *Caliciopsis brevipes* (ZT Myc 58038, on *A. araucana*), the holotype for *C. cochlearis* (ZT Myc 58041, on *Pilgerodendron uviferum* (D. Don) Florin) and a paratype for *C. cochlearis* (ZT Myc 58041, on *A. araucana*).

2.2 DNA extractions, PCR and sequencing

The same methods as described in Balocchi et al. (2021) were used to extract DNA from cultures. These included growing the isolates for 7-21 days on 2% MEA, collecting mycelium in 2 mL Eppendorf tubes, freeze drying the mycelium, grinding it with metal beads, and extracting DNA using a phenol/chloroform protocol (Barnes et al. 2001) with modifications (Balocchi et al. 2021). The obtained DNA was measured with a NanoDrop spectrophotometer (Thermo Scientific NanoDrop ND-1000) and a working stock of 30 ng/µL was prepared for each sample.

An attempt was made to extract DNA from the three available herbarium specimens using the kit DNeasy® UltraClean® Microbial Kit (Qiagen, Germany) following the manufacturer’s instructions. Because material was scarce, only two fruiting bodies (ascomata) were used per specimen. The DNA concentrations obtained were extremely low and thus a working stock was not prepared for these samples.

The ITS region was amplified and sequenced (as explained below) for all isolates obtained from cankers using primers ITS1 and ITS4 (White et al. 1990). Based on a preliminary phylogenetic analysis of these sequences (Supplementary Figure 1), isolates were separated into three groups representing putative species. Three representative isolates per group were selected to sequence six additional gene-regions. These included (i) nc LSU rDNA with primers LROR and LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1995), (ii) nc SSU rDNA with primers NS1, NS3, NS4 and NS8 (White et al. 1990), (iii) the RNA polymerase II second largest subunit (*RPB2*) with primers RPB2-5f2 and RPB2-7cR (Liu et al. 1999; Sung et al. 2007), (iv) the β-tubulin-1 gene region (*BT1*) with primers Bt1a and Bt1b (Glass and

Donaldson 1995), (v) the β-tubulin-2 gene region (*BT2*) with primers Bt2a and Bt2b (Glass and Donaldson 1995), and (vi) the translation elongation factor 1 alpha (*TEF1*) large intron with primers EF1-782F and EF-2 (O'Donnell et al. 1998; Carbone and Kohn 1999). PCRs were carried out with MyTaq™ DNA Polymerase (Bioline©) in 25 µL reactions containing 5 µL of MyTaq Reaction Buffer 5X, 0.5 µL of 10 µM of each primer, 0.3 µL of DNA polymerase and 17.7 µL of sterile deionized water. The thermal cycling conditions included an initial denaturation step of 95 ˚C for 3 min, followed by 35 cycles of denaturing at 95 ˚C for 30 s, annealing at 56 ˚C (52 ˚C for *TEF1* and *BT1*) for 30 s and elongation at 72 ˚C for 45 s, and a final elongation step at 72 ˚C for 4 min. PCR amplicons were dyed with GelRed® (2 µL per 4 µL of PCR product) and electrophoresis was carried out by running the product on a 1% agarose gel for 12 min at 110V. PCR and sequencing of the ITS gene region for the isolates obtained from fruiting bodies on galls was done following the same methods described above. No additional gene regions were considered necessary for these isolates.

PCRs with the DNA obtained from the herbarium specimens were attempted with three primer sets (i) primers ITS1 and ITS4 for the full ITS region (ITS-1 + 5.8S rDNA + ITS-2), (ii) primers ITS1 and ITS2 for the ITS-1 region, and (iii) primers ITS3 and ITS4 for the ITS-2 region. PCRs were performed with the same polymerase kit as mentioned above but in 14 µL reactions containing 2.5 µL of MyTaq Reaction Buffer 5X, 0.25 µL of each primer, 0.15 µL of DNA polymerase, 8.9 µL of SABAX water and 2 µL of template DNA. The thermal cycling conditions were extended and included an initial denaturation step of 95 ˚C for 10 min, followed by 10 cycles of denaturing at 95 ˚C for 30 s, annealing at 56 ˚C for 45 s and elongation at 72 ˚C for 1 min, then 30 cycles of denaturing at 95 ˚C for 30 s, annealing at 55 ˚C (+ 0.05 ˚C/cycle), and elongation at 72 ˚C for 1 min; and a final elongation step at 72 ˚C for 4 min. Amplicons were visualized using electrophoresis as outlined above.

PCR products were cleaned with ExoSAP-IT™ PCR Product Cleanup (Applied Biosystems™, Thermo Fisher, Waltham, MA, USA) following the instructions provided by the manufacturer. Amplicons were sequenced in both directions using the BigDye® Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems™, Thermo Fisher, Waltham, MA, USA) following the manufacturer's instructions. The obtained PCR products were cleaned using sodium acetate precipitation (Duong et al. 2013). Sequencing of the products was carried out at the Sequencing Facility at the University of Pretoria. The forward and reverse sequences obtained for each isolate were visualized and assembled into consensus sequences with CLC Main Workbench 21.0.3 (https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-clc-main-workbench/).

2.3 Phylogenetic analyses

The consensus ITS sequences generated for 28 isolates obtained from cankers in the preliminary analyses were aligned to compare their identities and then used to construct a Maximum likelihood tree. Representative isolates of each putative species were aligned, using BLAST, against the NCBI's GenBank database (NCBI; http://www.ncbi.nlm.nih.gov). Based on the similarity output results, a first group of datasets were generated for the ITS, nc LSU rDNA and nc SSU rDNA gene region sequences available in GenBank for relevant taxa in the *Coryneliaceae.* These included the sequences generated for representative isolates used in the present study and two species of *Hamigera* (Eurotiales; Aspergillaceae) that served as the outgroup taxa (Table 1). A second group of datasets were generated for the ITS, *TUB1*, *TUB2*, *TEF1* and *RPB2* gene regions each, including sequences from GenBank and those generated in this study, which included the selected isolates for three putative novel species, isolates of *P. kutranfy*, and two *Caliciopsis* spp.

Sequences for each dataset were aligned using the online MAFFT service (Katoh et al. 2017) (https://mafft.cbrc.jp/alignment/server/) and visualized and edited with MEGA X 10.1.8 (Kumar et al. 2018) and MESQUITE v3.61 (Maddison and Maddison 2019). The sequences for each gene region were first aligned separately for individual phylogenetic analyses and then concatenated using CLC Bio Main Workbench to perform a combined analysis. Combined datasets included (i) ITS + nucLSU + nucSSU for the *Coryneliaceae* including *Hamigera* spp. as outgroups, and (ii) ITS + *TUB1* + *TUB2* + *TEF1* + *RPB2* for *Pewenomyces* spp. including *Caliciopsis* spp. as outgroup taxa. Maximum likelihood trees for the ten datasets (individual and the combined) were generated with IQ-Tree Web server

(Trifinopoulos et al. 2016) (http://iqtree.cibiv.univie.ac.at/) using the model ranked best by BIC in ModelFinder (Kalyaanamoorthy et al. 2017). Bayesian analyses were carried using MrBayes on XSEDE (v3.2.7a) via the CIPRES Science Gateway V3.3 (Miller et al. 2010, 2011). The likelihood model parameters were adjusted for each dataset to fit models obtained previously with ModelFinder as mentioned above. Analyses were carried out using the Markov Chain Monte Carlo (MCMC) method with four independent chains. Ten million generations with sampling every 1,000 steps were performed and burn-in was set to the first 25% generations. Phylogenetic trees were visualized and edited with FigTree v1.3.1 and Affinity Designer 1.8.5.703.

A third phylogenetic analysis was performed that included sequences from cultures obtained from ascomata on freshly collected leaf galls and from one of the herbarium specimens (ITS-1 region). These were aligned to the first ITS dataset mentioned above, including representatives for the *Coryneliaceae*, and *Hamigera* spp. as outgroup. Alignment and phylogenetic analyses, including maximum likelihood and Bayesian inference, were done following same methods described above.

2.4 Morphological studies

2.4.1 Microscopy: canker samples and twigs with galls

Plant samples including segments of branches of *A. araucana* with cankers, twigs of the same tree species with galls, and herbarium specimens mentioned above, were examined for fruiting bodies using a Zeiss SteREO Discovery.V.12 modular dissection microscope. Where present, ascomata and spermogonia were photographed and measured using the same dissection microscope with the software AxioVision (AxioVs40x64 v.4.9.1.0). Semi-permanent slides were made of the fruiting bodies for each sample, using distilled water or lactic acid (85%) as a mountant for fresh samples and 5% KOH for preserved specimens. Slides were visualized using a Nikon Eclipse Ni-u microscope with a Nikon DS-Ri2 camera and the software NIS-Elements v.4.30 was used to make measurements and capture photographic images. In some cases, slides were visualized and photographed using a Zeiss Axioskop 2 plus microscope with an Axiocam 105 colour camera and the software ZEN 2.5 (blue edition). Macroand microscopic structures observed in the samples were named using the terminology provided by Kirk et al. (2008).

2.4.2 Culture and morphological characteristics

Between four and eight isolates of each putative new *Pewenomyces* species were grown in Petri dishes containing 2% MEA by distributing mycelium fragments made from older cultures in two straight lines per plate. These were incubated for 28–35 days at ~22 °C under natural light. Semi-permanent slides using 85% lactic acid as a mountant were made of the fruiting bodies that developed on the cultures by crushing them to release the spores. Sections through fruiting structures were also made using a CM1520 cryostat (Leica). Fruiting bodies and fungal structures were visualized and measured using a Nikon Eclipse N*i* microscope with a Nikon DS-Ri2 camera, and the software NIS Elements (Nikon, Tokyo, Japan) was used to make measurements as well as to capture photographic images. Up to 50 measurements were made for each characteristic morphological structure where these were available. The size ranges are presented as minimum-maximum (avg ± standard deviation).

Culture growth rate was studied for the three putatively new taxa using three representative isolates per taxon that were obtained from different locations. Isolates were grown in 90 mm Petri dishes containing 2% MEA and oatmeal agar (OMA) and incubated for 28 days in darkness at temperatures ranging between 5 °C and 35 °C at 5 °C intervals. Three plates per isolate on each culture medium were used per temperature, and the total area of growth of the colonies was measured every four days. The total area of each colony at each point in time was marked at the bottom of the plate, then captured photographically, and measured using the software ImageJ (Guerrero et al. 2012; Wang 2017). Colours on the cultures were designated based on the colour charts of Rayner (1970).

All relevant isolates used in this study were deposited at the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Additionally, ex-type cultures of species newly described in this study were deposited in the collection of the

Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, the Netherlands, and as dried specimens in the South African National Collection of Fungi (PREM), Roodeplaat, South Africa.

3. RESULTS

3.1 Phylogenetic analyses

Preliminary phylogenetic analyses using the ITS sequences of 28 isolates, including those obtained from living and dead plant tissue and fruiting bodies, grouped them into three distinct clades close to *P. kutranfy* (Supplementary Figure 1). These groups were thus treated as three putatively new *Pewenomyces* species in downstream analyses. Isolates of *Pewenomyces* sp. 1 included a mixture of those obtained from plant tissue and from ascomata found on moribund cankers, while isolates of *Pewenomyces* sp. 2 and sp. 3 were obtained only from plant tissue. Isolates in each of these groups of putative species had identical or near identical ITS sequences, with identities above 99% when compared to each other. When sequences for representative isolates in each of these putative new species were aligned to those in the GenBank database, they consistently matched to *Pewenomyces kutranfy* (acc. No. MT334520) with identities ranging from 94.5 to 96.6%. The second closest matches varied between different *Caliciopsis* species, including *Caliciopsis calicioides* (Acc. No JX968549) and *Caliciopsis indica* (Acc. No. NR_119752) with identities ranging from 85.4 to 86.5%.

Phylogenetic analyses using nine representative isolates for the three putative new *Pewenomyces* species, together with other sequences of species within the *Coryneliaceae*, utilizing sequences for the ITS, nc LSU rDNA, nc SSU rDNA individually (Supplementary Figure 2) and combined (Fig. 1a), consistently placed the isolates in three distinct clades, supported with high bootstrap values close to but distinct from *P. kutranfy*. These analyses included several species of *Caliciopsis* and *Hypsotheca*, which were consistently distinct from *Pewenomyces* with high bootstrap values. Phylogenetic analyses using sequences for a second set of gene regions (ITS, *TUB1*, *TUB2*, *TEF1* and *RPB2*), individually (Supplementary Figure 3) and combined (Fig. 1b), supported the previous analyses where isolates grouped in three strongly supported independent clades, all distinct from *P. kutranfy*.

Phylogenetic analyses for each of the individual gene regions showed strong support for the fact that the three putative new species and *P. kutranfy* were distinct from each other (Supplementary Figure 3). However, the topologies of the trees for the different gene regions varied in the arrangement of these groups in the *Pewenomyces* clade. *Pewenomyces* sp. 1 and *Pewenomyces* sp. 2 consistently grouped together as sister species using all gene regions, however, the position of *Pewenomyces* sp. 3 varied depending on the gene regions used for the analyses. Gene regions such as *RPB2*, *TEF1* and nc SSU rDNA placed this species basal for the genus, while analyses with the ITS, *BT1* and *BT2* sequences resolved it in a sister clade with *P. kutranfy*. Analyses done with the nc LSU rDNA (Supplementary Figure 2) sequences resulted in the most robust topology for the genus based on bootstrap values, placing *P. kutranfy* as basal and *Pewenomyces* sp. 3 as sister clade to another containing the remaining two species.

The ITS phylogenetic analyses placed cultures obtained from ascomata on leaf galls (Fig. 2), believed to possibly be *Caliciopsis brevipes*, and the herbarium specimen ZT Myc 58043 (partial sequence; *Caliciopsis cochlearis* paratype, on *Araucaria araucana*) within the *Pewenomyces* clade, and more specifically, within the clade including the putative new species *Pewenomyces* sp. 1. The sequence from the herbarium specimen was slightly distant from others in the *Pewenomyces* sp. 1 clade, probably due to the fact that only a fragment of a sequence (~300 bp) was available for this material. Phylogenetic analyses with the dataset trimmed to shorter sequences (~330 bp) resulted in exactly the same topology but with a reduced distance between the sequence for the herbarium specimen and other members of the clade (data not shown). Despite this fact, both analyses, using full- and trimmed sequences, resulted in a robust phylogeny for the *Pewenomyces* clade, with robust support placing both sequences for the fresh specimens and paratype specimen for *C. cochlearis*, within *Pewenomyces* and separate from *Caliciopsis*. Full length sequences for isolates from the ascomata on fresh galls samples were identical (100% identity) to those for the other isolates representing *Pewenomyces* sp. 1.

3.2 Morphology of fruiting structures

3.2.1 Canker samples

Samples from well-developed cankers on branches of *A. araucana* had a large number of dead leaves around the diseased tissue and they were covered in dried resin (Fig. 3a). Fruiting structures (spermogonia and ascomata) were abundant on these samples, spanning the area from the leaf bases, where cracks on the plant tissues had developed, to the surfaces of surrounding dead leaves. Fruiting structures of *P. kutranfy*, the causal agent of these cankers (Balocchi et al. 2021), were abundant at the leaf bases (Fig. 3b, c) where cracks on leaf tissues originated. In contrast, fruiting bodies from which *Pewenomyces* sp. 1 was isolated were predominantly distributed on the surrounding dead leaves (Fig. 3d–h), either emerging directly from the surface of dead tissues or from cracks that had expanded from the cankers. These fruiting structures had, in most cases, dimensions similar to those of *P. kutranfy* but were distinguishable from them by qualitative traits of their fruiting structures. These included the shape of the ascomata, position of ascigerous swelling, presence/absence of underlying stroma rupturing tissues and shape of the spermogonia. Microscopic features for the two species also differed in characters such as the range of shapes of the spermatia or ascospore dimensions and pigmentation. Ascospores of *Pewenomyces* sp. 1 that were close to maturity (those inside developing asci, smaller in size and not fully pigmentated) had verrucose ornamented walls (Fig. 3k), but those fully mature (having reached full dimensions, pigmentation and including those already released from the asci in the mazaedium) had smooth walls (Fig. 3m, n). This in contrast with the mature verrucose ornamented ascospores of *P. kutranfy* (Balocchi et al. 2021).

3.2.2 Leaf gall samples

Ascomata found on the *A. araucana* twig samples with leaf galls (Fig. 4), which resided in *Pewenomyces* sp. 1 based on the phylogenetic analyses (Fig. 2), had a morphology identical to those found on dead leaves surrounding cankers. Additionally, fruiting structures on these gall samples were also present only where plant tissues had died, but in this case, spermogonia were also found on mechanically ruptured tissues (Fig. 4h) rather than being only immersed on leaf tissues as was found on the samples from cankers.

3.2.3 Herbarium specimens

Fruiting structures on the paratype specimen of *Caliciopsis cochlearis* (ZT Myc 58043, on *Araucaria araucana*, Fig. 5), for which DNA sequences were obtained and that were placed alongside *Pewenomyces* sp. 1 in the phylogenetic analyses (Fig. 2), overlapped considerably in their morphology with the ones found on fresh galls and on dead leaves on the canker samples. Small differences were observed for the ascomata in the paratype specimen when compared to the two other fresh sample types (e.g. size of the ascigerous swelling, shape of ascomata). However, their microscopic features were almost identical, and ascospores of all the three specimens initially had verrucose ornamentation but were smooth walled when mature.

Both herbarium specimens representing the holotypes of *C. brevipes* (ZT Myc 58038, on leaf galls on *Araucaria araucana*, Fig. 6) and *C. cochlearis* (ZT Myc 58041, on dead leaves of *Pilgerodendron uviferum*, Fig. 7) had ascomata with distinctive morphologies that did not resemble those of any other sample used in this study. None of the spermogonia collected from any of these specimens contained spermatia, and thus, comparisons for these structures were not possible.

Ascomata on the holotype specimen of *C. brevipes* were much larger than any other specimen observed in this study (Table 2) and had a distinctive shape (Fig. 6d–f). These had much thinner stalks and the ascigerous swellings were wider, straighter, and longer with a less pronounced transition to the tips resulting in a robust column-shaped structure rather than one that is urceolate as is found in the other species. In addition, ascospores from these ascocarps were very evidently verrucose ornamented when mature (Fig. 6h–k). The morphology of the ascomata on the holotype specimen for *C. brevipes* did not match with those on freshly collected leaf gall samples. This was initially expected because they were collected from the same host, plant organ, and symptom and from a similar location to those reported in the herbarium specimen.

Ascomata on the holotype specimen of *C. cochlearis* were smaller than any other specimen observed in this study and had also a distinct shape. These structures had subapical ascigerous swellings with longer stalks and wider tips relative to its size (Fig. 7d–h). All ascospores in this specimen, whether mature or immature, were smooth walled. The holotype and paratype specimens of *C. cochlearis* were morphologically different to each other, and in the latter case, much closer to the morphology of the putative new species *Pewenomyces* sp. 1.

4. TAXONOMY

The results of phylogenetic analyses for three groups of isolates considered in this study confirmed that they all reside in *Pewenomyces*, and that they are distinct from *P. kutranfy*. Differences in morphological features of the colonies for these groups, including the type of fruiting structure, colony shape and colour, as well as growth rate at different temperatures (documented below) were consistent with the results obtained in phylogenetic analyses that resolved four species in *Pewenomyces*, including *P. kutranfy*. Additionally, morphological features of ascomata on cankers resolving as putative new species *Pewenomyces* sp. 1 were identical to the ascomata found on fresh galls and closely resembled those on the herbarium specimen that was deposited as a paratype for *Caliciopsis cochlearis*. This was also supported in the phylogenetic analyses with sequences obtained from both the fresh and herbarium samples. The morphological features of these fruiting structures, including those in the paratype specimen, were clearly distinct from two other specimens representing the holotypes of *Caliciopsis brevipes* and *Caliciopsis cochlearis*. Based on these results, we recognize three new species of *Pewenomyces*, including two species represented by isolates obtained from plant tissue and a third obtained from both plant tissue and fruiting structures on similar samples.

4.1 ***Pewenomyces lalenivora*** Balocchi & Marinc., sp. nov (*Pewenomyces* sp. 1)

Figures 3, 4 and 8

Mycobank MB 844552

Etymology: The name originates from indigenous Chilean and Argentinian Mapuche language mapudungun; *lalen*, meaning to be dying, illustrating that this fungus is found on dying and/or dead material.

Type material: CHILE, Araucanía (IX), Conguillío National Park, sector Los Paraguas, –38.697836°, –71.817216°, spermogonia and ascomata on mature cankers on branches of *Araucaria araucana*, 12 February 2019, F. Balocchi, holotype PREM 63252, ex-type culture CMW 56868, CBS #.

Description: Stroma not seen. Spermogonia gregarious, individually or in pairs when in leaf surface, grouped in clumps when inside cracks on leaves, black, spherical to subprolate, most commonly sunken in leaf tissues, occasionally standing loose on ruptured tissues, 47–86 × 50–152 (68 ± 13 × 82 ± 21) µm. Ascomata developing from the same spermogonia, superficial, gregarious, individually or in pairs, emerging from in cracks on leaf surfaces, black, elongated, upright, ventricose, 201–597 (426 ± 88) µm tall, submedian ascigerous swelling 54–197 × 43–112 (129 ± 29 × 77 ± 15) µm, basal stalk 47–192 × 35–66 (97 ± 42 × 48 ± 10) µm, elongated beak 79–309 (208 ± 62) µm long, 24–48 (35 ± 6) µm wide at the middle, 26–70 (44 ± 9) µm wide at the tip, where a brownish red mazaedium of ascospores accumulate. Asci hyaline, spathulate with long pedicel, 13–23 × 7–10 (17.4 ± 2.5 × 8.5 ± 0.8) µm at the spore-bearing part, bearing eight ascospores, deliquescent. Ascospores hyaline when immature, becoming light brown, verrucose ornamented when close to maturity, reddish brown to dark brown, smooth walled when fully mature, spherical to subspherical, 3–5 × 3–5 (4.4 ± 0.4 × 3.8 ± 0.4) µm. Spermatia hyaline, 1-celled, oblong, reniform or fusiform, 2–5 × 1–2 (3.8 ± 0.4 × 1.4 ± 0.3) µm. Conidiomata on 2% MEA, stromatic, embedded in aerial mycelium or immersed, solitary or aggregated loosely, globose to applanate, unilocular or multilocular, simple or convoluted, wall composed of brown, thick-walled cells, textura intricata, 74–504 × 63–766 (197 ± 98 × 201 ± 158) µm. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, hyaline, ovoid, discrete, with limited number of sympodial growth, ampuliform, lageniform or short cylindrical, 2–8 × 1–3 (5.9 ± 1.5 × 2.5 ± 0.5) µm. Conidia hyaline, 1-celled, ellipsoidal to cylindrical, often gradually tapering to base, straight or curved, 4–6 × 1–2 (4.6 ± 0.5 × 1.8 ± 0.2) µm.

Culture characteristics. Colonies slow growing, variable in morphology. Optimum growth temperature 15–20 °C, reaching 44–55 mm diam (20.3 ± 3 cm^2) after 28 days on 2% MEA, 42–51 mm diam (17.8 ± 2.5 cm^2) on OMA. Cultures at 25 °C having more condensed mycelial growth, darker pigmentation. Cultures at 30 and 35 °C showing no growth after 28 days on 2% MEA, OMA. Cultures at 35 °C failed to grow when plates were incubated at 20 °C for additional 14 days. Cultures at 20 °C on 2% MEA white when young, round with smooth edges, mostly submerged, with little aerial mycelium, with a buff colour underneath, fully grown cultures with crenate edges, becoming slightly honey pigmented with centre hazel to dark brick pigmented underneath. Cultures at 20 °C on OMA white, round, sunken with smooth or crenate margins throughout the growth study. Cultures on 2% MEA developed black globose spermogonia at either their centre or edges, slightly sunken in the medium, or hidden under a layer of mycelium, producing a translucent spore exudate becoming milky white with age.

Other specimens examined: CHILE, Araucanía (IX), Conguillío National Park, sector Los Paraguas, –38.697836°, –71.817216°, isolates obtained from diseased plant tissues from cankers on branches of *Araucaria araucana*, 11 December 2017, F. Balocchi, PREM 63253, cultures CMW 54223, CMW 54224, Biobío (VIII), Nahuelbuta mountain range, Trongol Alto, –37.553434°, –73.188438°, spermogonia and ascomata on leaf galls on *Araucaria araucana*, 5 December 2019, F. Balocchi, PREM XXX; Ralco Natural Reserve, –37.962620°, –71.327679°, isolate obtained from diseased plant tissues from cankers on branches of *Araucaria araucana*, 27 December 2017, F. Balocchi, PREM 63254, culture CMW 54250.

Distribution and ecology: This fungus occurred on samples of *A. araucana* from the Chilean Coastal range (Nahuelbuta) and the Andes Mountain range. Unlike the other species treated in this study, it is typically found on decaying tissues or galls on twigs. Based on the conditions of the tissues from which *P. lalenivora* was collected, it is either a saprophyte or a secondary opportunistic pathogen.

Notes: *Pewenomyces lalenivora* could be confused with *P. kutranfy*, when both species are found together on a canker, or with *C. brevipes* that also infects leaf galls on *A. araucana*. Ascomata of *C. brevipes* are, however, much larger than those of these two *Pewenomyces* species and have a distinctive shape. Ascomata of *P. kutranfy* and *P. lalenivora* have similar sizes, but they have different shapes. The latter species has a basal stalk wider and shorter than the beak, different from *P. kutranfy,* in which the stalk is longer and usually thinner than the beak or apex. Ascospores of *P. lalenivora* also differ from those of *P. kutranfy* being smaller (~1 µm in length) and more darkly pigmentated, and from *P. kutranfy* and *C. brevipes* in that they become smooth-walled at maturity.

Some ascomata of *P. lalenivora* occurring on cracks on leaf surfaces had an extension of stromatic tissue beneath the stalk emerging from within the leaf tissues. This resulted in the length of the fruiting structure being up to 1 mm, a feature not observed for *P. kutranfy*. Spermogonia of *P. lalenivora* differed from those of *P. kutranfy* in being gregariously distributed without an evident stroma visible and by being rounder and having smooth surfaces. Spermatia obtained from these spermogonia commonly overlapped in their morphology with those of *P. kutranfy* being mostly oblong. However, these spermatia were variably reniform or fusiform rather than ovoid as found in *P. kutranfy*. Cultures of *P. lalenivora* resemble those of *P. kutranfy*, but they are clearer in colour (white to yellowish tones, not becoming brown or dark), and have a different texture; glabrous with scarce or no aerial mycelium, especially when grown at 20 °C or higher.

4.2 ***Pewenomyces tapulicola*** Balocchi & Marinc., sp. nov (*Pewenomyces* sp. 2)

Figure 9.

Mycobank MB 844553

Etymology: The name is taken from indigenous Chilean and Argentinian Mapuche language mapudungun; *tapül*, meaning the leaves of a tree, indicating the substrate from where the fungus was obtained.

Type material: CHILE, Araucanía (IX), Conguillío National Park sector Los Paraguas, –38.698075°, –71.813826°, isolated from diseased tissue from cankers on branches of *Araucaria araucana*, 11 December 2017, F. Balocchi, holotype PREM 63248, ex-type culture CMW 54234, CBS #.

Description: Sexual morph not observed. Asexual morph: Conidiomata on 2% MEA immersed, stromatic, subglobose to applanate, unilocular or multilocular, simple or convoluted, walls composed of pale brown to brown, thick-walled cells, textura angularis, 82–426 × 64–1 154 (242 ± 90 × 317 ± 235) µm. Conidiophores reduced to conidiogenous cells. Conidiogenous cells borne along peridial wall, hyaline, short-cylindrical to ampuliform, 5–7 × 2–4.5 (5.5 ± 0.7 × 3 ± 0.7) µm. Conidia hyaline, 1-celled, ellipsoidal, 4–7 × 2–3 (4.6 ± 0.6 × 2 ± 0.3) µm. Spermatia hyaline, 1-celled, oblong with round apices, straight or curved 2–4 × 1–1.5 (3.1 ± 0.4 × 1.3 ± 0.1) µm. Sclerotia-like structures present in some cultures, composed of brown, thin-walled cells, textura angularis.

Culture characteristics. Colonies slow growing, variable in morphology. Optimum growth temperature 15–20 °C, reaching 43–55 mm diam. (21.2 ± 3.0 cm^2) after 28 days on 2% MEA, 49.8–58.8 mm diam. (23.6 ± 4.3 cm^2) on OMA. Growth at 25 °C strongly reduced in both media, with increased mycelium density and pigmentation on 2% MEA. Cultures at 30 and 35 °C showing no growth after 28 days. Cultures at 35 °C failed to grow when incubated at 20 °C for an additional 14 days. Colonies on 2% MEA at 15 and 20 °C white, round with smooth edges when young, with little to almost no aerial mycelium, occasionally with a few translucent oil drops in the centre, developing grey olivaceous pigmentation with light brown to white edges with age, edges becoming crenate. Cultures on 2% MEA developing black globose spermogonia in centre or edges, slightly sunken in the medium, producing a translucent spore exudate that became milky and brown.

Other specimens examined: CHILE, Araucanía (IX), Conguillío National Park sector Los Paraguas, –38.697493°, –71.814460°, isolated from leaf tissues on branches of *Araucaria araucana*, 11 December 2017, F. Balocchi, PREM 63249, culture CMW 56866; Villarrica National Park sector Puesco, –39.570510°, –71.502642°, isolated from healthy leaf tissues of branches from *Araucaria araucana*, 13 December 2017, F. Balocchi, PREM 63250, culture CMW 54248; Biobío (VIII), Nahuelbuta mountain range, Trongol Alto, –37.553434°, –73.188438°, isolated from leaf tissues on branches of *Araucaria araucana*, 21 December 2017, F. Balocchi, PREM 63251, cultures CMW 54252.

Distribution and ecology: This species was isolated only from a small number of *A. araucana* leaf samples. These samples were collected from one site on the Coastal range (Nahuelbuta) and several sites on the Andes Mountain range. Most samples were from healthy leaf tissues or those with leaf spots caused by other fungi, suggesting that it is either an endophyte or an opportunistic pathogen.

Notes: This species is represented only by cultures, which are similar in morphology to those of *P. lalenivora* and *P. kutranfy*. In older cultures, mycelium becomes dark brown and spore masses that exude from pycnidia become brown. This is distinct from the white to clear brown mycelium and clear-coloured or translucent exudation of conidia observed in *P. lalenivora* and *P. kutranfy*. Isolates of *P. tapulicola* can be easily confused with those of *P. kutranfy* based on their morphology at optimum conditions. However, *P. tapulicola* produces less (or none) aerial mycelium than *P. kutranfy*, sometimes appearing glabrous in texture, and cultures are less vividly brown. When isolates are grown at temperatures above optimal conditions, their mycelium grows more densely, and becomes intensely dark brown. Although both *P. kutranfy* and *P. lalenivora* also undergo changes in mycelium density under similar conditions, these changes are more subtle and pigmentation in both species tends to be reduced rather than more intense. In contrast to *P. lalenivora* and *P. kutranfy*, the conidiomata of *P. tapulicola* are slightly larger, conidiogenous cells are borne directly on the peridial wall, the conidia are ellipsoid in shape, and sclerotia-like structure shapes sometimes develop at the edges of the colonies.

4.3 ***Pewenomyces kalosus*** Balocchi & Marinc., sp. nov (*Pewenomyces* sp. 3)

Figure 10.

Mycobank MB 844554

Etymology: The name comes from the indigenous Chilean and Argentinian Mapuche language mapudungun; *kal*, meaning hair, indicating the colonies have abundant aerial mycelium.

Type material: CHILE, Araucanía (IX), Conguillío National Park sector Los Paraguas, –38.697493°, –71.814460°, isolated from diseased plant tissue from cankers on branches of *Araucaria araucana*, 11 December 2017, F. Balocchi, holotype PREM 63245, ex-type culture CMW 54228, CBS #.

Description: Sexual morph not observed. Asexual morph: Conidiophores micronematous, semimacronematous, macronematous, simple or branched, often reduced to conidiogenous cells. Conidiogenous cells hyaline, polyphialidic, acropleurogenous, cylindrical, tapering towards apex, with conidial mass in slimy droplet, 2–28 × 1–3 (10.5 ± 6.4 × 2 ± 0.4) µm. Conidia hyaline, 1-celled, ellipsoidal to subglobose, cylindrical with round apex and tapering toward pointed base, 3–9 × 1.5–3 (5.2 ± 1.5 × 2.3 ± 0.4) µm, producing secondary conidia by budding.

Culture characteristics: Colonies fast growing, variable in morphology. Optimum growth temperature 20–25 °C, reaching 47–78 mm diam (46.1 ± 4.3 cm^2) on 2% MEA after 16 days and fully covering the plate after ~20 days, 50.4–69.4 mm diam (44.3 ± 8.7 cm^2) on OMA after 16 days and fully covering the plate after 20–24 days. On both culture media growth at 15 °C slightly slower than at optimal temperature, evidently reduced at 10 °C and 30 °C, no growth observed at 35 °C after 28 days. Cultures at 35 °C failed to growth when plates were incubated at 20 °C for an additional 14 days. Cultures on 2% MEA incubated at 20 and 25 °C white when young, both sunken in the medium, growing aerially, with smooth or laciniate margins, becoming darker with age, with aerial mycelium becoming greenish to grey olivaceous, bottoms of colonies becoming sepia. Colonies on OMA grown at similar temperatures white, mostly sunken with little white aerial mycelium developing from the centre of colony as they aged, edges smooth or in some cultures crenate, bottom white to light buff.

Other specimens examined: CHILE. Araucanía (IX): Villarrica National Park sector Puesco, –39.570510°, –71.502642°, isolated from dead plant tissue from cankers on branches of *Araucaria araucana*, 13 December 2017, F. Balocchi, PREM 63247, culture CMW 56881; Biobío (VIII): Ralco National Reserve, –37.951548°, –71.333419°, cankers on branches on *Araucaria araucana* (isolated from dead plant tissue), 27 December 2017, F. Balocchi, PREM 63246, culture CMW 56867.

Distribution and ecology: This fungus is known only from samples on *A. araucana*. Samples were collected from multiple sites where the tree occurs in the Andes Mountain range. Tissues samples from which it was isolated were dying or dead and were on branches with cankers caused by *P. kutranfy*. It is unclear whether *P. kalosus* is pathogenic or not. However, based on the conditions of the tissues from which it was recovered, it seems likely to be a secondary opportunistic pathogen or saprotroph.

Notes: Colonies of *P. kalosus* can be distinguished from those of other *Pewenomyces* species or *Caliciopsis* species considered in this study based on their growth rate, morphology and the presence of a hyphomycete asexual state. Only two species in the *Coryneliaceae*, both in the genus *Hypsotheca* (*H. pleomorpha* and *H. eucalyptorum*) are known to produce a hyphomycete state in culture. This has also been proposed as a diagnostic feature for the genus. The presence of a hyphomycete in *P. kalosus* means that it can no longer be used as diagnostic for *Hypsotheca*. There are, however, differences between the hyphomycete state produced by *P. kalosus*, and those in *Hypsotheca*. These include the production of macronematous conidiophores and secondary conidia by budding, which have not been reported in any species of *Hypsotheca*. Additionally, conidia of *P. kalosus* are smaller than to those for both *Hypsotheca* species.

5. DISCUSSION

Results of this study have led to the discovery of three new species in the genus *Pewenomyces*. This has added substantial structure to the genus, known previously only for *P. kutranfy*, that causes cankers on branches and stems of *A. araucana* in Chile (Balocchi et al. 2021). The three new species were also obtained from branches and/or leaves of these iconic trees. In contrast to *P. kutranfy*, no evidence emerged to suggest that any of the newly described species in this study are primary pathogens. In this regard, *P. tapulicola* and *P. kalosus* were recovered mostly in isolations from dead plant tissues and only in rare cases from healthy tissues, but not from living yet diseased tissues. *Pewenomyces lalenivora* was found sporulating on recently dead or decaying tissues surrounding cankers caused by *P. kutranfy*, or on leaf galls typically attributed to either mites (Chetverikov et al. 2014), or other *Caliciopsis* species (Butin 1970).

Butin (1970) was the first researcher to consider species in the *Coryneliaceae* on *Araucaria* and he described *C. brevipes* and *C. cochlearis* on these trees in Chile. Neither of these species were found in the present study even though our samples included galls similar to those on which *C. brevipes* was described (Butin 1970). Contrary to expectation, the fruiting structures that we found on galls did not resemble those of the holotype specimen for *C. brevipes*. They rather had a morphology identical to those of *P. lalenivora*, described in the present study, and this observation was verified based on DNA sequence comparisons. The paratype specimen of *C. cochlearis* occurring on *A. araucana* was similar to *P. lalenivora* in both morphology and DNA sequence comparisons. However, the holotype and paratype specimens of *C. cochlearis* considered in this study were distinctly different to each other in morphology and were from two different host genera with distinct areas of geographic occurrence. This suggests that these specimens represent different species. In that case, the paratype specimen for which DNA was obtained would reside in *Pewenomyces* and the holotype for which no DNA could be obtained would, at least for the present, remain in *Caliciopsis*. It is also unlikely that *P. kalosus* and *P. tapulicola*, described only from cultures in this study, represents either of the species described by Butin (1970) given their differences in colony morphology and origins. However, due to a lack of DNA available from some of the herbarium specimens, this question remains to be resolved.

Balocchi et al. (2021) proposed that *Pewenomyces* species could be distinguished from *Caliciopsis* and *Hypsotheca* species by their characteristic verrucose ascospores. The results of the present study have expanded the morphological characteristics for *Pewenomyces* and show that there is no single morphological characteristic that defines the three genera. Ascospores have been observed in only two of the four species of *Pewenomyces*, and in the case of *P. lalenivora* these spores are verrucose only when immature but become smooth at maturity. The hyphomycete state of *Hypsotheca* proposed by Crous et al. (2019a) to define that genus can no longer be considered a diagnostic feature because *P. kalosus* also has a hyphomycete state in culture. However, the hyphomycete states of *Hypsotheca* species and *P. kalosus* differ in morphology such as in the size of conidia and the structure of their conidiophores. Similarly, there are morphological differences between the conidiomata that develop in cultures of *Pewenomyces* species and those of *Hypsotheca* (e.g., conidiogenous cell arrangement, shape and dimensions). This suggests that there may be significant morphological differences in the asexual morphs in the two genera that could serve as diagnostic features, and that have been overlooked because most descriptions of fungi in the *Coryneliaceae* have focused mostly on the sexual morphs.

The taxonomy of the *Coryneliaceae* is deeply dependant of phylogenetic inference based on DNA sequence data. A complication in this regard is that such data are available only for a small number of species in the family. This implies that considerable uncertainty remains regarding the taxonomic placement of species in the currently accepted genera. This is especially true for the genera described solely based on morphology, including *Coryneliospora*, *Coryneliopsis*, *Coryneliella*, and *Fitzpatrickella*, and most species of *Caliciopsis* and *Corynelia*. An effort needs to be made to ensure that these fungi are not overlooked and they can be included in future studies. Strategies such as sequencing of preserved specimens and/or collection of fresh specimens should be applied wherever possible. However, as shown in this study, both of these methods may not be successful and alternative manners for their inclusion need to be explored. This is particularly relevant because some of these fungi are emerging as important tree pathogens, including of the *Araucariaceae* (Balocchi et al. 2022).

This study and that of Balocchi et al. (2021) have added four new species to the *Coryneliaceae* on *A. araucana* in Chile. This excludes the two species of *Caliciopsis* previously described by Butin (1970) on these trees. Butin (1970) also mentioned that *C. cochlearis* occurred in four other Chilean native conifer species. They might represent novel species if they were to be reconsidered based on DNA sequence analyses as has been found in the present study and that of Migliorini et al. (2020). There are also various other species residing in the *Coryneliaceae* in Chile (Fitzpatrick 1942b; Benny et al. 1985a, d; Butin and Peredo 1986) that deserve consideration, suggesting that this area of the world represents a centre of diversity of these fungi.

6. ACKNOWLEDGEMENTS

We acknowledge the Zürich herbaria Z+ZT (University of Zürich and ETH Zürich) for providing the herbarium specimens used in this study. We are also grateful to the Kimeltuwe project for providing support regarding the appropriate use of Mapudungun used in naming new species. We also acknowledge Prof Cobus Visagie who provided valuable advice regarding microscopic work and taxonomy.

7. REFERENCES

Assefa A, Abate D, Stenlid J (2014) Characterization of *Corynelia uberata* Fr., a putative fungal pathogen of *Podocarpus falcatus* in Ethiopian forests. Forest Pathology 44:45–55. https://doi.org/10.1111/efp.12067

Balocchi F, Wingfield MJ, Ahumada R, Barnes I (2021) *Pewenomyces kutranfy* gen nov. et sp. nov. causal agent of an important canker disease on *Araucaria araucana* in Chile. Plant Pathology 70:1243–1259. https://doi.org/10.1111/ppa.13353

Balocchi F, Wingfield MJ, Paap T, Ahumada R, Barnes I (2022) Pathogens of the Araucariaceae: How much do we know? Current Forestry Reports 8:1–24. https://doi.org/10.1007/s40725-022-00164-z

Barnes I, Roux J, Wingfield MJ, Coetzee MP, Wingfield BD (2001) Characterization of *Seiridium* spp. associated with cypress canker based on ß-tubulin and histone sequences. Plant Disease 85:317–321. https://doi.org/10.1094/PDIS.2001.85.3.317

Batista AC (1956) A new species of *Caliciopsis* from India and Sumatra. Anais da Sociedade de Biologia de Pernambuco 14:111–118

Benny GL, Samuelson DA, Kimbrough JW (1985a) Studies on the Coryneliales. I. *Fitzpatrickella*, a monotypic genus on the fruits of *Drimys*. Botanical Gazette 146:232–237. https://doi.org/10.1086/337519

Benny GL, Samuelson DA, Kimbrough JW (1985b) Studies on the Coryneliales. II. Taxa parasitic on Podocarpaceae: *Corynelia*. Botanical Gazette 146:238–251. https://doi.org/10.1086/337520

Benny GL, Samuelson DA, Kimbrough JW (1985c) Studies on the Coryneliales. III. Taxa parasitic on Podocarpaceae: *Lagenulopsis* and *Tripospora*. Botanical Gazette 146:431–436. https://doi.org/10.1086/337543

Benny GL, Samuelson DA, Kimbrough JW (1985d) Studies on the Coryneliales. IV. *Caliciopsis*, *Coryneliopsis*, and *Coryneliospora*. Botanical Gazette 146:437–448. https://doi.org/10.1086/337544

Butin H (1970) Zwei neue *Caliciopsis*-Arten auf chilenischen Koniferen. Journal of Phytopathology 69:71–77. https://doi.org/10.1111/j.1439-0434.1970.tb03903.x

Butin H, Peredo HL (1986) Hongos parásitos en coníferas de América del Sur, con especial referencia a Chile. J. Cramer, Berlin-Stuttgart

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556. https://doi.org/10.1080/00275514.1999.12061051

Chetverikov PE, Beaulieu F, Beliavskaia AY, Rautian MS, Sukhareva SI (2014) Redescription of an early-derivative mite, *Pentasetacus araucariae* (Eriophyoidea, Phytoptidae), and new hypotheses on the eriophyoid reproductive anatomy. Experimental and Applied Acarology 63:123–155. https://doi.org/10.1007/s10493-014-9774-2

Crous P, Schumacher RK, Akulov A, Thangavel R, Hernández-Restrepo M, Carnegie A, Cheewangkoon R, Wingfield MJ, Summerell B, Quaedvlieg W, Coutinho TA, Roux J, Wood A, Giraldo A, Groenewald JZ (2019a) New and interesting fungi. 2. Fungal Systematics and Evolution 3:57–134. https://doi.org/10.3114/fuse.2019.03.06

Crous P, Schumacher RK, Wingfield MJ, Akulov A, Denman S, Roux J, Braun U, Burgess T, Carnegie A, Váczy K, Guatimosim E, Schwartsburd P, Barreto R, Hernández-Restrepo M, Lombard L, Groenewald J (2018) New and Interesting Fungi. 1. Fungal Systematics and Evolution 1:169–215. https://dx.doi.org/10.3114%2Ffuse.2018.01.08

Crous PW, Carnegie AJ, Wingfield MJ, Sharma R, Mughini G, Noordeloos ME, Santini A, Shouche YS, Bezerra JDP, Dima B, Guarnaccia V, Imrefi I, Jurjević Ž, Knapp DG, Kovács GM, Magistà D, Perrone G, Rämä T, Rebriev YA, Shivas RG, Singh SM, Souza-Motta CM, Thangavel R, Adhapure NN, Alexandrova AV, Alfenas AC, Alfenas RF, Alvarado P, Alves AL, Andrade DA, Andrade JP, Barbosa RN, Barili A, Barnes CW, Baseia IG, Bellanger JM, Berlanas C, Bessette AE, Bessette AR, Biketova AY, Bomfim FS, Brandrud TE, Bransgrove K, Brito ACQ, Cano-Lira JF, Cantillo T, Cavalcanti AD, Cheewangkoon R, Chikowski RS, Conforto C, Cordeiro TRL, Craine JD, Cruz R, Damm U, de Oliveira RJV, de Souza JT, de Souza HG, Dearnaley JDW, Dimitrov RA, Dovana F, Erhard A, Esteve-Raventós F, Félix CR, Ferisin G, Fernandes RA, Ferreira RJ, Ferro LO, Figueiredo CN, Frank JL, Freire KTLS, García D, Gené J, Gêsiorska A, Gibertoni TB, Gondra RAG, Gouliamova DE, Gramaje D, Guard F, Gusmão LFP, Haitook S, Hirooka Y, Houbraken J, Hubka V, Inamdar A, Iturriaga T, Iturrieta-González I, Jadan M, Jiang N, Justo A, Kachalkin AV, Kapitonov VI, Karadelev M, Karakehian J, Kasuya T, Kautmanová I, Kruse J, Kušan I, Kuznetsova TA, Landell MF, Larsson KH, Lee HB, Lima DX, Lira CRS, Machado AR, Madrid H, Magalhães OMC, Majerova H, Malysheva EF, Mapperson RR, Marbach PAS, Martín MP, Martín-Sanz A, Matočec N, McTaggart AR, Mello JF, Melo RFR, Mešić A, Michereff SJ, Miller AN, Minoshima A, Molinero-Ruiz L, Morozova OV, Mosoh D, Nabe M, Naik R, Nara K, Nascimento SS, Neves RP, Olariaga I, Oliveira RL, Oliveira TGL, Ono T, Ordoñez ME, Ottoni AdM, Paiva LM, Pancorbo F, Pant B, Pawłowska J, Peterson SW, Raudabaugh DB, Rodríguez-Andrade E, Rubio E, Rusevska K, Santiago ALCMA, Santos ACS, Santos C, Sazanova NA, Shah S, Sharma J, Silva BDB, Siquier JL, Sonawane MS, Stchigel AM, Svetasheva T, Tamakeaw N, Telleria MT, Tiago PV, Tian CM, Tkalčec Z, Tomashevskaya MA, Truong HH, Vecherskii MV, Visagie CM, Vizzini A, Yilmaz N, Zmitrovich IV, Zvyagina EA, Boekhout T, Kehlet T, Læssøe T, Groenewald JZ (2019b) Fungal Planet description sheets: 868–950. Persoonia 42:291–473. https://doi.org/10.3767/persoonia.2019.42.11

Crous PW, Wingfield MJ, Guarro J, Cheewangkoon R, van der Bank M, Swart WJ, Stchigel AM, Cano-Lira JF, Roux J, Madrid H, Damm U, Wood AR, Shuttleworth LA, Hodges CS, Munster M, de Jesus Y-M, M, Zuñiga-Estrada L, Cruywagen, E.M, de Hoog GS, Silvera C, Najafzadeh J, Davison EM, Davison PJN, Barrett MD, Barrett RL, Manamgoda DS, Minnis AM, Kleczewski NM, Flory SL, Castlebury LA, Clay K, Hyde KD, Mausse-Sitoe SND, Chen S, Lechat C, Hairaud M, Lesage-Meessen L, Pawlowska J, Wilk M, Sliwinska-Wyrzychowska A, Metrak M, Wrzosek M, Pavlic-Zupanc D, Maleme HM, Slippers B, Mac Cormack WP, Archuby DI, Grünwald NJ, Telleira MT, Dueñas M, Martin MP, Marincowitz S, de Beer ZW, Perez CA, Gene J, Marin-Felix Y, Groenewald

JZ (2016) Fungal Planet description sheets: 469–557. Persoonia 37:218–403. https://doi.org/10.3767/003158516X694499

Duong TA, De Beer ZW, Wingfield BD, Wingfield MJ (2013) Characterization of the mating-type genes in *Leptographium procerum* and *Leptographium profanum*. Fungal Biology 117:411–421. https://doi.org/10.1016/j.funbio.2013.04.005

Fitzpatrick HM (1920) Monograph of the Coryneliaceae. Mycologia 12:206–237. https://doi.org/10.1080/00275514.1920.12016837

Fitzpatrick HM (1942a) Revisionary studies in the Coryneliaceae. Mycologia 34:464–488. https://doi.org/10.1080/00275514.1942.12020916

Fitzpatrick HM (1942b) Revisionary studies in the Coryneliaceae. II. The genus *Caliciopsis*. Mycologia 34:489–514. https://doi.org/10.1080/00275514.1942.12020918

Funk A (1963) Studies in the genus *Caliciopsis*. Canadian Journal of Botany 41:503–543. https://doi.org/10.1139/b63-044

Funk A, Kuijt J (1974) *Caliciopsis struthanthi* n. sp.(Ascomycetes) on Loranthaceae in Central America. Canadian Journal of Botany 52:1149–1150. https://doi.org/10.1139/b74-147

Garrido-Benavent I, Pérez-Ortega S (2015) Unravelling the diversity of European *Caliciopsis* (Coryneliaceae, Ascomycota): *Caliciopsis valentina* sp. nov. and *C. beckhausii* comb. nov., with a worldwide key to *Caliciopsis*. Mycological Progress 14:1–11. https://doi.org/10.1007/s11557-015-1034-2

Geiser DM, Gueidan C, Miadlikowska J, Lutzoni F, Kauff F, Hofstetter V, Fraker E, Schoch CL, Tibell L, Untereiner WA (2006) Eurotiomycetes: eurotiomycetidae and chaetothyriomycetidae. Mycologia 98:1053–1064. https://doi.org/10.1080/15572536.2006.11832633

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61:1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Guerrero NR, Quintero MAO, Naranjo JCP (2012) Determinación del área foliar en fotografías tomadas con una cámara web, un teléfono celular o una cámara semiprofesional. Revista Facultad Nacional de Agronomía-Medellín 65:6399–6405

Huguenin B (1969) Micromycètes du Pacifique Sud: 8-Ascomycètes de Nouvelle Calédonie. Cahiers du Pacifique 13:295–305

Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14:587. https://doi.org/10.1038/nmeth.4285

Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 1. https://doi.org/10.1093/bib/bbx108

Kirk P, Cannon P, Minter D, Stalpers J (2008) Dictionary of the fungi (10th Edition). CAB International, Wallingford, UK.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16:1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Maddison WP, Maddison DR (2019) Mesquite: a modular system for evolutionary analysis. Version 3.61 http://www.mesquiteproject.org

Marmolejo JG (1999) A new species of *Caliciopsis* on oaks from Mexico. Mycotaxon 72:195–197

Migliorini D, Luchi N, Pepori AL, Pecori F, Aglietti C, Maccioni F, Munck I, Wyka S, Broders K, Wingfield MJ, Santini A (2020) *Caliciopsis moriondi*, a new species for a fungus long confused with the pine pathogen *C. pinea*. MycoKeys 73:87–108. https://doi.org/10.3897/mycokeys.73.53028

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. 2010 Gateway Computing Environments Workshop (GCE) 1–8. https://doi.org/10.1109/GCE.2010.5676129.

Miller MA, Pfeiffer W, Schwartz T (2011) The CIPRES science gateway: a community resource for phylogenetic analyses. Proceedings of the 2011 TeraGrid Conference: extreme digital discovery 41:1–8. https://doi.org/10.1145/2016741.2016785

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences 95:2044–2049. https://doi.org/10.1073/pnas.95.5.2044

Pascoe IG, Smith IW, Dinh S-Q, Edwards J (2018) *Caliciopsis pleomorpha* sp. nov.(Ascomycota: Coryneliales) causing a severe canker disease of *Eucalyptus cladocalyx* and other eucalypt species in Australia. Fungal Systematics and Evolution 2:45–56. https://doi.org/10.3114/fuse.2018.02.04

Peck H (1880) Report of the Botanist. In: New York State Museum (ed) Thirty-third Annual report of the state museum of Natural History. Regents of the University of the State of New York, Albany, pp 11–55

Pettersson OV, Su-lin LL, Lantz H, Rice T, Dijksterhuis J, Houbraken J, Samson RA, Schnürer J (2011) Phylogeny and intraspecific variation of the extreme xerophile, *Xeromyces bisporus*. Fungal Biology 115:1100–1111. https://doi.org/10.1016/j.funbio.2011.06.012

Pratibha J, Amandeep K, Shenoy B, Bhat D (2010) *Caliciopsis indica* sp. nov. from India. Mycosphere 1:65–72

Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, UK

Rehner SA, Samuels GJ (1995) Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. Canadian Journal of Botany 73:816–823. https://doi.org/10.1139/b95-327

Rikkinen J (2000) Two new species of *Caliciopsis* (Coryneliaceae) from Hunan Province, China. Karstenia 40:147–151. http://hdl.handle.net/10138/309680

Saccardo P (1886) Sylloge Fungorum Omnium hucusque Cognitorum: Additamenta ad volumina I-IV. Sapienza University of Rome, Rome

Saccardo P (1891) Sylloge Fungorum Omnium hucusque Cognitorum. (Vol. IX). Sapienza University of Rome, Rome

Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98:1041–1052. https://doi.org/10.1080/15572536.2006.11832632

Sung G-H, Sung J-M, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Molecular phylogenetics and evolution 44:1204–1223. https://doi.org/10.1016/j.ympev.2007.03.011

Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic acids research 44:W232–W235. https://doi.org/10.1093/nar/gkw256

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of bacteriology 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Vu D, Groenewald M, De Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald J, Cardinali G, Houbraken J (2018) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in Mycology 91:23–36. https://doi.org/10.1016/j.simyco.2018.05.001

Wang F (2017) SIOX plugin in ImageJ: area measurement made easy. UV4Plants Bulletin 2016:37–44

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18:315–322

Wood AR, Damm U, van der Linde EJ, Groenewald JZ, Cheewangkoon R, Crous PW (2016) Finding the missing link: Resolving the Coryneliomycetidae within Eurotiomycetes. Persoonia 37:37. https://doi.org/10.3767/003158516X689800

Yanai M, Yoshida S, Ueda S, Udagawa S (2016) Identification of *Hamigera* species isolated from foods as heat-resistant fungi. Japanese Journal of Mycology 57:47–58. https://doi.org/10.18962/jjom.57.1_47

8. TABLES

Table 1. Taxa, isolates, and gene regions used for phylogenetic analyses.

Taxon	Isolate	GenBank accession numbers							References
		ITS	nc LSU	nc SSU	*RPB2*	*TEF1*	*BT1*	*BT2*	
Coryneliomycetidae **Coryneliales** **Coryneliaceae**									
Caliciopsis beckhausii	MA 18186^{NT}	NR_132090	NG_060418	–	–	–	–	–	Garrido-Benavent and Pérez-Ortéga, 2015
C. calicioides	Voucher 211	JX968549	–	–	–	–	–	–	Assefa et al. 2014
~*C. cochlearis* (paratype specimen)	**ZT Myc 58043**^{PT}	**OM982902****	–	–	–	–	–	–	This study
C. eucalypti	CBS 142066^{ET}	NR_154836	NG_059013	MT359910	–	–	–	–	Crous et al. 2016
C. indica	GFCC 4947^{ET}	NR_119752	GQ259980	-	–	–	–	–	Pratibha et al. 2011
C. orientalis	CBS 138.64^{ET}	NR_145392	NG_058741	DQ471039	DQ470939	**OM982872**	**OM982886**	**OM982900**	Wood et al. 2016, Schoch et al. 2006, Geiser et al. 2006, this study
C. pinea	CBS 139.64	KP881691	DQ678097	DQ678043	EF411067	**OM982873**	**OM982887**	**OM982901**	Wood et al. 2016, Schoch et al. 2006, Geiser et al. 2006, this study
C. pseudotsugae	CBS 140.64^{ET}	MT334518	MT334517	MT359911	–	–	–	–	Balocchi et al. 2021
C. valentina	MA 18176/IGB290^{T}	NR_132091	NG_060419	–	–	–	–	–	Garrido-Benavent and Pérez-Ortéga, 2015
Corynelia africana	PREM 57242/AW 247^{T}	NR_153901	NG_058910	KP881719	–	–	–	–	Wood et al. 2016
Co. fructigena	PREM 57240/ARW 250^{T}	NR_153902	NG_058911	KP881720	–	–	–	–	Wood et al. 2016
Co. uberata	PREM 61207/ARW 686^{ET}	NR_153903	–	–	–	–	–	–	Wood et al. 2016
	Specimen voucher D-046	JF811344	–	JQ663846	–	–	–	–	Assefa et al. 2014
Hypsotheca eucalyptorum	CBS 145576^{T}	MK876393	MK876434	–	–	–	–	–	Crous et al. 2019b
H. maxima	CPC 24674/COAD 1983^{eT}	NR_160329	NG_064416	–	–	–	–	–	Crous et al. 2018
H. nigra	MA 18191/IGB305	–	KP144011.1	–	–	–	–	–	Garrido-Benavent and Pérez-Ortéga, 2015
H. pleomorpha	CPC 32144/CBS 144636	MK442588	MK442528	–	–	–	–	–	Crous et al. 2019a
Lagenulopsis bispora	PREM 57232/ARW 249^{eT}	NR_154120	NG_060325	NG_061200	–	–	–	–	Wood et al. 2016
Pewenomyces kutranfy	CMW54230/ CBS 146710^{PT}	MT334519	MT334514	MT359912	**OM937813**	**OM982869**	**OM982883**	**OM982897**	Balocchi et al. 2021, this study
	CMW54240/CBS 146709^{ET}	NR_172182	MT334515	MT359913	**OM937814**	**OM982870**	**OM982884**	**OM982898**	Balocchi et al. 2021, this study
	CMW54244/CBS 146711^{PT}	MT334520	MT334516	MT359914	**OM937815**	**OM982871**	**OM982885**	**OM982899**	Balocchi et al. 2021, this study

P. lalenivora sp. nov (sp. 1)	**CMW56868**^{ET}	**OM937779**	**OM937794**	**OM937803**	**OM937812**	**OM982868**	**OM982882**	**OM982896**	This study
	CMW54223^{PT}	**OM937777**	**OM937792**	**OM937801**	**OM937810**	**OM982866**	**OM982880**	**OM982894**	This study
	CMW54250^{PT}	**OM937778**	**OM937793**	**OM937802**	**OM937811**	**OM982867**	**OM982881**	**OM982895**	This study
P. tapulicola sp. nov (sp. 2)	**CMW54234**^{ET}	**OM937774**	**OM937789**	**OM937798**	**OM937807**	**OM982863**	**OM982877**	**OM982891**	This study
	CMW54248^{PT}	**OM937775**	**OM937790**	**OM937799**	**OM937808**	**OM982864**	**OM982878**	**OM982892**	This study
	CMW54252^{PT}	**OM937776**	**OM937791**	**OM937800**	**OM937809**	**OM982865**	**OM982879**	**OM982893**	This study
P. kalosus sp. nov (sp. 3)	**CMW54228**^{ET}	**OM937772**	**OM937786**	**OM937795**	**OM937804**	**OM982860**	**OM982874**	**OM982888**	This study
	CMW56881^{PT}	**OM937771**	**OM937787**	**OM937796**	**OM937805**	**OM982861**	**OM982875**	**OM982889**	This study
	CMW56867^{PT}	**OM937773**	**OM937788**	**OM937797**	**OM937806**	**OM982862**	**OM982876**	**OM982890**	This study
Isolates from ascomata on leaf galls (~*C. brevipes*)	**CMW56882**	**OM937780**	–	–	–	–	–	–	This study
	CMW56883	**OM937781**	–	–	–	–	–	–	This study
	CMW56884	**OM937782**	–	–	–	–	–	–	This study
	CMW56885	**OM937783**	–	–	–	–	–	–	This study
	CMW56886	**OM937784**	–	–	–	–	–	–	This study
	CMW56887	**OM937785**	–	–	–	–	–	–	This study
Tripospora tripos	PREM 61200/ARW 677	KP881712	KP881718	–	–	–	–	–	Wood et al. 2016
Eurotiomycetidae									
Eurotiales									
Aspergillaceae									
Hamigera avellanea	CBS 295.48^{T}	NR_156333	–	NG_061105	–	–	–	–	Petterson et al. 2011, Yanai et al. 2016
H. striata	CBS 377.48^{T}	MH856405	MH867954	–	–	–	–	–	Vu et al. 2018

Sequences generated in this study are in bold. ^{T} *Sequence from type material;* ^{ET} *sequence from ex-type culture;* ^{NT} *sequence from neotype culture;* ^{eT} *sequence from epitype;* ^{PT} *sequence from paratype. Designation of cultures and culture collections: AFTOL-ID, Assembling the Fungal Tree of Life (AFTOL) project (www.lutzonilab.net/aftol); ARW, personal number of Alan Wood, ARC-Plant Protection Research Institute, South Africa; CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; GFCC, Goa University Fungus Culture Collection and Research Unit, India; CMW, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; COAD, Coleção Octávio de Almeida Drumond, Universidade Federal de Viçosa, Brazil; CPC, Culture collection of Pedro Crous, housed at the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands, Netherlands; MA, Real Jardín Botánico, Spain; PREM, National Collection of Fungi, South Africa; ZT Myc, Zürich Herbaria Z+ZT, ETH Zürich, Switzerland.* ***** *Isolates identified by ascocarp morphology.* ****** Sequence for the internal transcribed spacer 1 (ITS1) gene region (not including 5.8S rDNA and ITS2). ~ Originally believed to belong to this species.

Table 2. Comparison of morphological characters between *Caliciopsis* and *Pewenomyces* species associated to *Araucaria araucana* from literature, samples collected in this study and herbarium material.

Structure	**Character**	***Pewenomyces kutranfy***[b]	***Pewenomyces* sp. on mature cankers** (Fig. 3)	**Fresh galls samples** (Fig. 4)	***Caliciopsis brevipes***		***Caliciopsis cochlearis***		
					Literature[a]	**ZT Myc 58038 Holotype** (Fig. 6)	**Literature**[a]	**ZT Myc 58041 Holotype** (Fig. 7)	**ZT Myc 58043 Paratype** (Fig. 5)
Ascoma	Height (µm)	(358) 381–564 (600)	(341) 390–540 (597)	(201) 290–427 (447)	600–1,000	(636) 642–764 (820)	350–700	353– 390 (398)	(277) 286–489 (607)
	Width (µm)	124–171 (173)	(58) 68–95 (112)	(43) 56–83 (88)	200–280	(97) 128–195 (214)	80–140	(42) 53–86	(74) 92–115
	Tip width (µm)	(83) 85–122 (126)	(30) 38–52 (57)	(26) 31–57 (70)	100–160	(86) 86–127 (152)	50–75	(44) 51–63	34–59
	Ascigerous cavity position	subapical to median	submedian to median	submedian to median	submedian to median	submedian to median	subapical to median	subapical to median	subapical to submedian
	Ascigerous cavity length (µm)	–	(80) 111–160 (197)	(54) 87–153 (163)	–	(277) 306–379 (390)	180–260	(89) 110–146	142–218
Asci	Length (µm)	(13) 13.9–18.2 (22)	(14.6) 16.4–19.8 (20.6)	(13.1) 14.3–19.8 (23.4)	18–22	(13) 14.5–17.9 (20)	16–18	(12.2) 12.9–15.7 (16.5)	(14.6) 15.8–18.3
	Width (µm)	(7.9) 10.2–11.8 (11)	(7.7) 8.0–9.6 (10.2)	(6.6) 7.6–9.1 (9.5)	8–10	(6.7) 8.4–11 (12.4)	10–14	(7.8) 8.4–9.8 (10)	(8.5) 8.9–10.9 (11.4)
Ascospores	Length (µm)	(4.0) 4.2–5.3 (6.4)	(3.4) 4.2–4.9 (5.1)	(3.6) 4.0–4.7 (5.4)	5.6–7	(3.9) 4.1–4.8 (5.3)	4.5 – 5.5	(3.3) 4.1–4.8 (5.1)	(3.5) 3.9–4.6 (5.1)
	Width (µm)	(3.4) 3.8–4.7 (5.3)	(2.9) 3.5–4.2 (4.6)	(2.7) 3.5–4.2 (4.5)	4.5–6	(3.3) 3.7–4.3 (4.7)	4.0 – 4.6	(3.1) 3.7–4.4 (4.7)	(3.1) 3.5–4.2 (4.9)
	Ratio (L:W)	1.1	1.0–1.3 (1.6)	1.0–1.3 (1.5)	–	1.1	–	1.1	1.1
Spermogonia	Height (µm)	–	–	(47) 55–81 (86)	140–250	–	100–200	–	–
	Width (µm)	100	(50) 66–125 (152)	(51) 63–87 (91)	140–250	–	–	–	143–198
Spermatia	Length (µm)	2.5–4.6	(3.1) 3.7–4.3 (4.5)	(2.2) 3.2–4.2 (4.8)	4.2–5.5	–	3.2–4.2	–	–
	Width (µm)	1.1–1.7	(1.1) 1.2–1.5 (1.6)	(0.6) 1.0–1.7 (2.2)	2–3	–	1.0–1.5	–	–
	Shape	oblong/ovoid	oblong to fusiform/ovoid	oblong to fusiform/ovoid	allantoid to ellipsoidal	–	allantoid	–	–

[a]Butin 1970; [b]Balocchi et al. 2021.

9. FIGURES

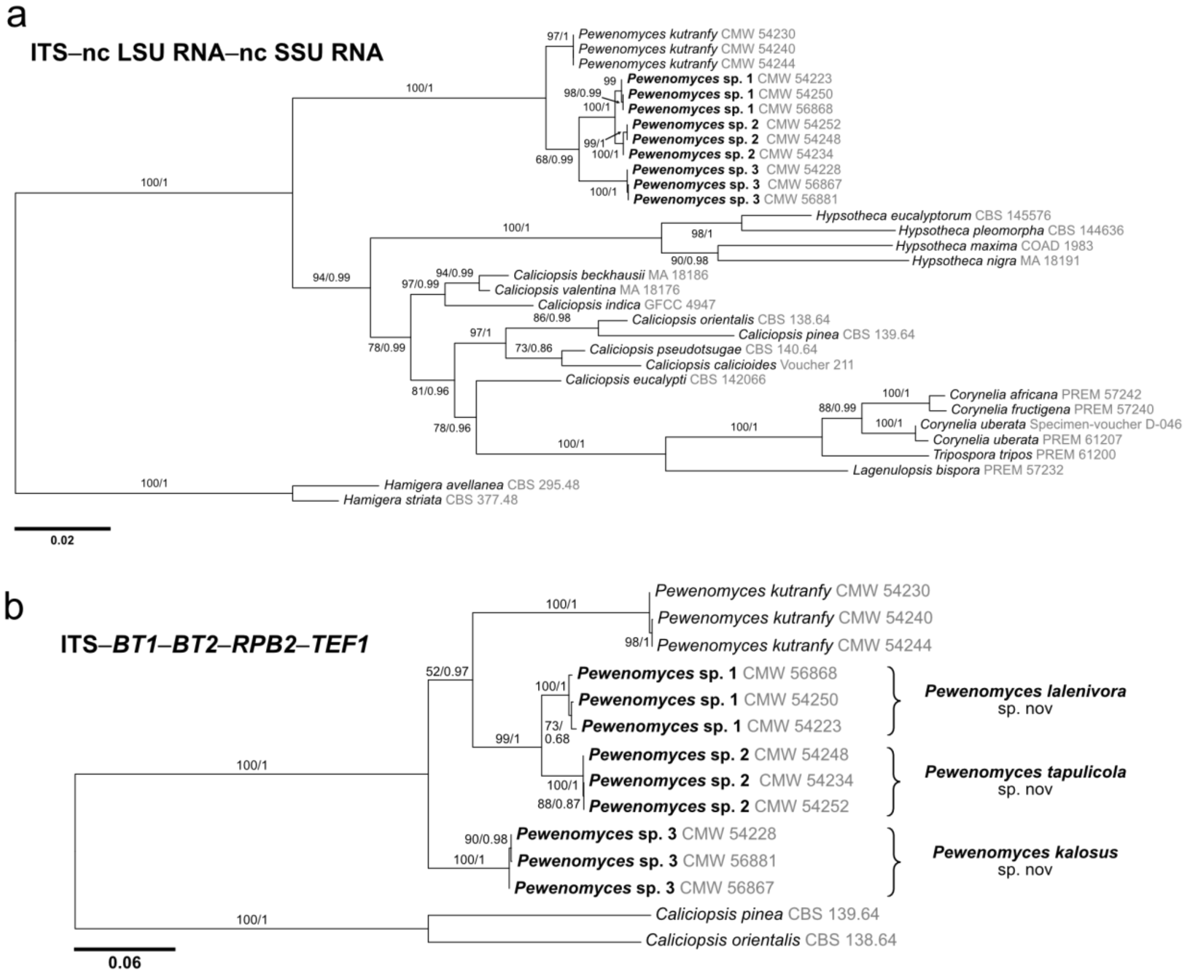


Fig. 1 Multigene phylogenetic trees for the *Coryneliaceae* including sequences from cultures obtained from cankers on *Araucaria araucana*. **a**. Maximum likelihood tree for the concatenated nc SSU rDNA, ITS and nc LSU rDNA for the *Coryneliaceae*, placing novel isolates (in bold) inside *Pewenomyces*. **b**. Maximum likelihood tree for the concatenated ITS, *BT1*, *BT2*, *RPB2* and *TEF1* gene regions for the genus *Pewenomyces*, separating new isolates (in bold) in three novel phylogenetic species. Numbers on branches correspond to bootstrap (*n* = 1000) and posterior probabilities for Bayesian inference, respectively. Scale bars: substitutions per site.

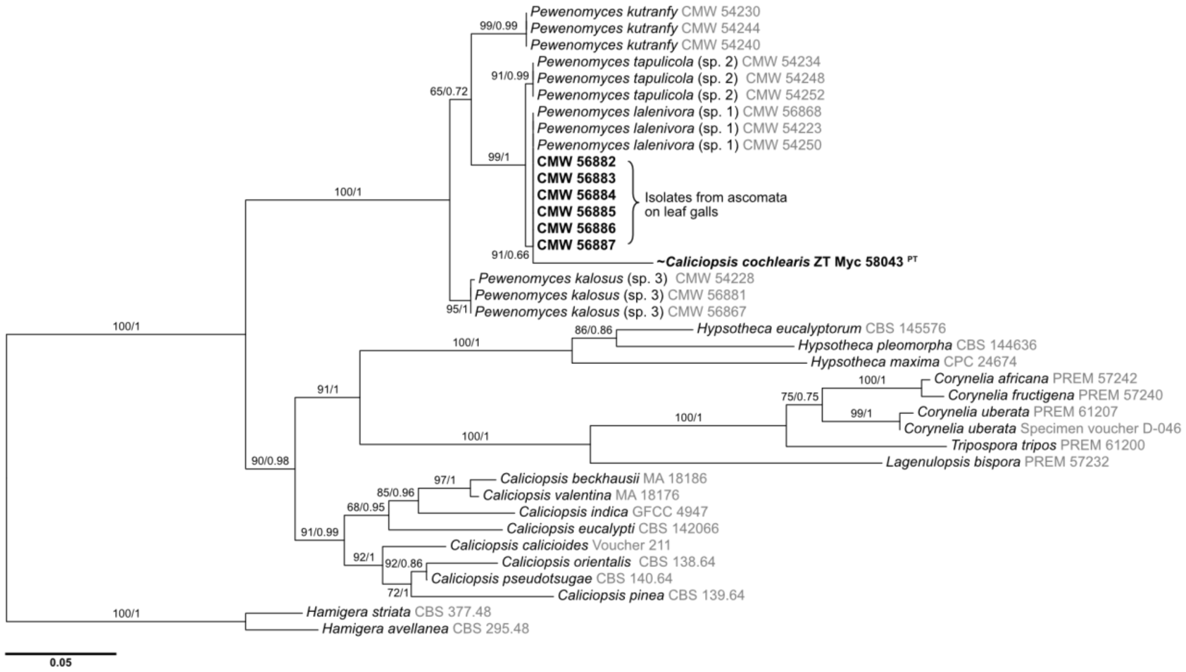


Fig. 2 Maximum likelihood tree for the ITS region for the *Coryneliaceae* including sequences from cultures obtained from ascomata on leaf galls (in bold), and the ITS-1 region sequence for the herbarium specimen ZT Myc 58043 (*Caliciopsis cochlearis* paratype, also in bold). Numbers on branches correspond to bootstrap (n = 1000) and posterior probabilities for Bayesian inference, respectively. Scale bar: substitutions per site.

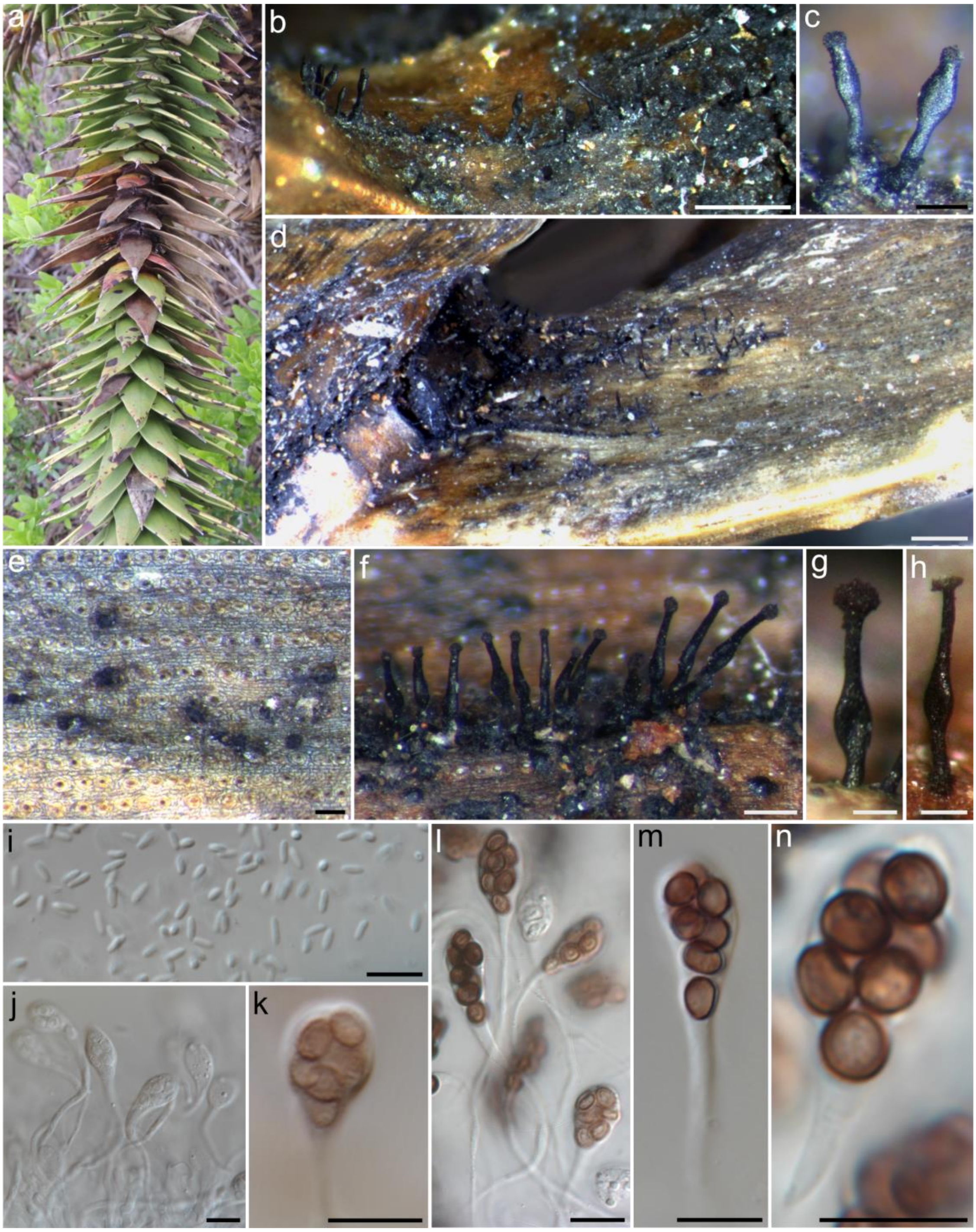


Fig. 3 *Pewenomyces* species sporulating on mature cankers on branches of *Araucaria araucana*. **a**. Mature canker on branch. **b**-**c**. Ascomata of *Pewenomyces kutranfy* at the base of leaves and emerging from the phloem. **d**-**n**. Fruiting structures of a second *Pewenomyces* species (sp. 1) emerging from dead leaf tissues. **d.** Dead leaf tissue surface with fruiting structures. **e**. Spermogonia. **f**-**h**. Ascomata. **i**. Spermatia. **j**. Immature asci. **k**. Asci close to maturity with ascospores showing verrucose ornamentation. **l**. Asci reaching maturity. **m**-**n**. Mature asci bearing smooth-walled ascospores. Scale bars: **b**, **d** = 1 mm; **c**, **e**, **f** = 200 µm; **g**, **h** = 100 µm; **i**–**n** = 10 µm.

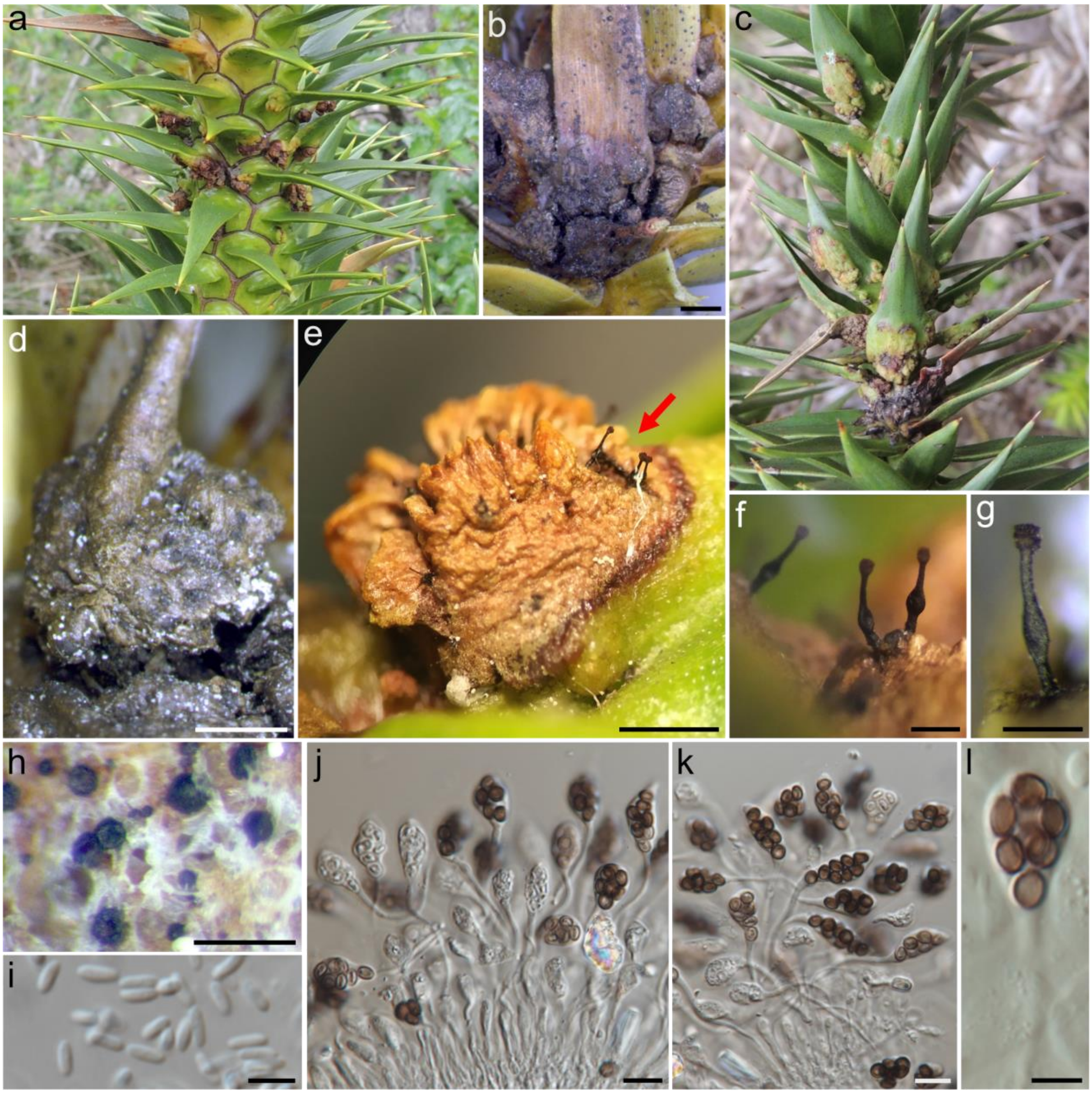
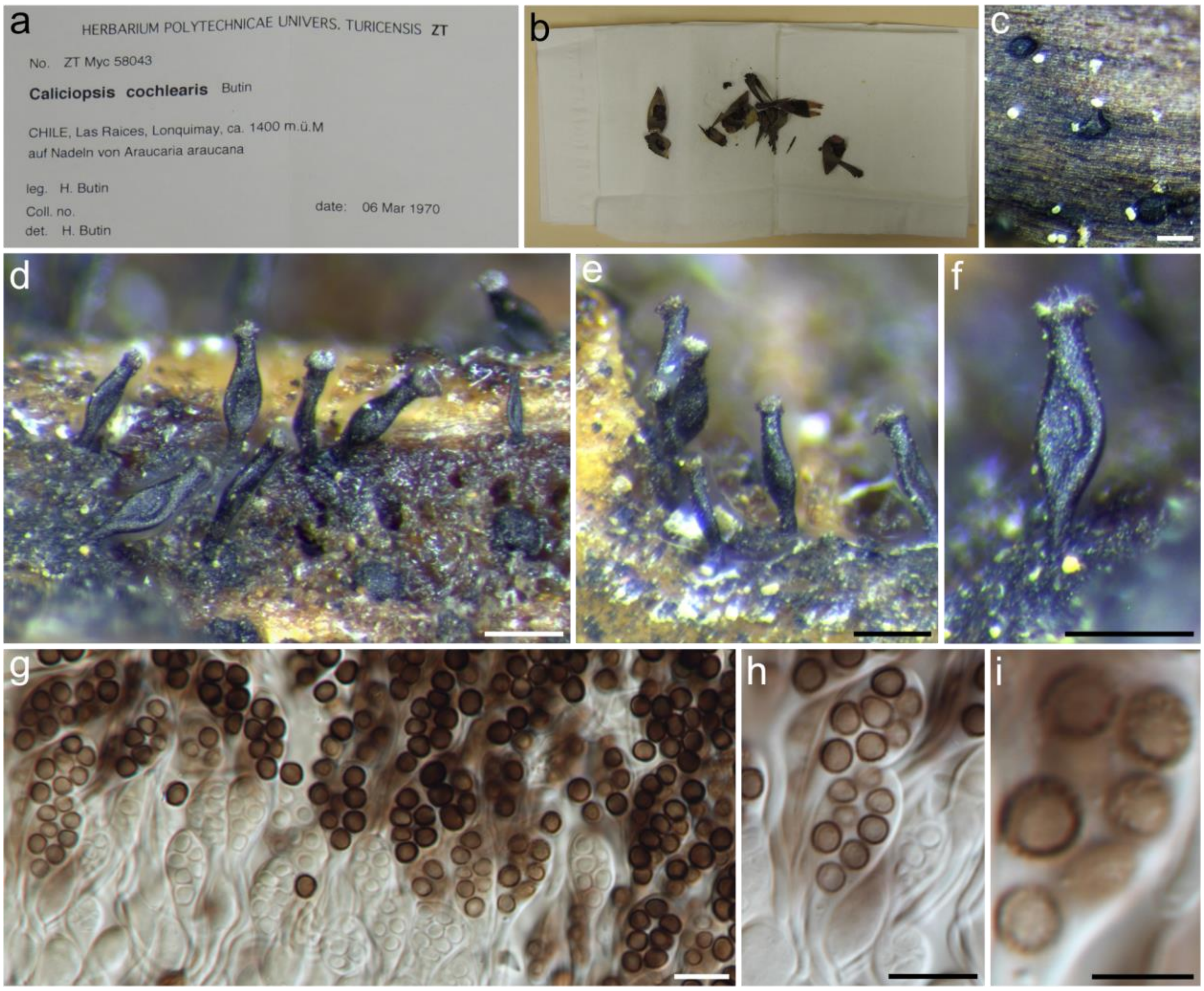


Fig. 4 *Caliciopsis*-like fungus on galls on *Araucaria araucana* collected from sites in the coast range (Nahuelbuta) and on the Andes Mountain range. **a**. Galls on the base of leaves on a young main stem. **b**. Old galls on the base of leaves on a mature branch. **c**. Galls on the base of leaves of young twigs. **d-g**. Spermogonia and ascomata emerging from dead tissues. **h**. Round spermogonia on ruptured tissues. **i**. Spermatia. **j-k**. Immature and mature asci and ascospores. **l**. Smooth-walled mature ascospores. Scale bars: **b** = 2 mm; **d**, **e** = 1 mm; **f–h** = 200 µm; **j** = 10 µm; **l**, **i** = 5 µm.



HERBARIUM POLYTECHNICAE UNIVERS. TURICENSIS ZT

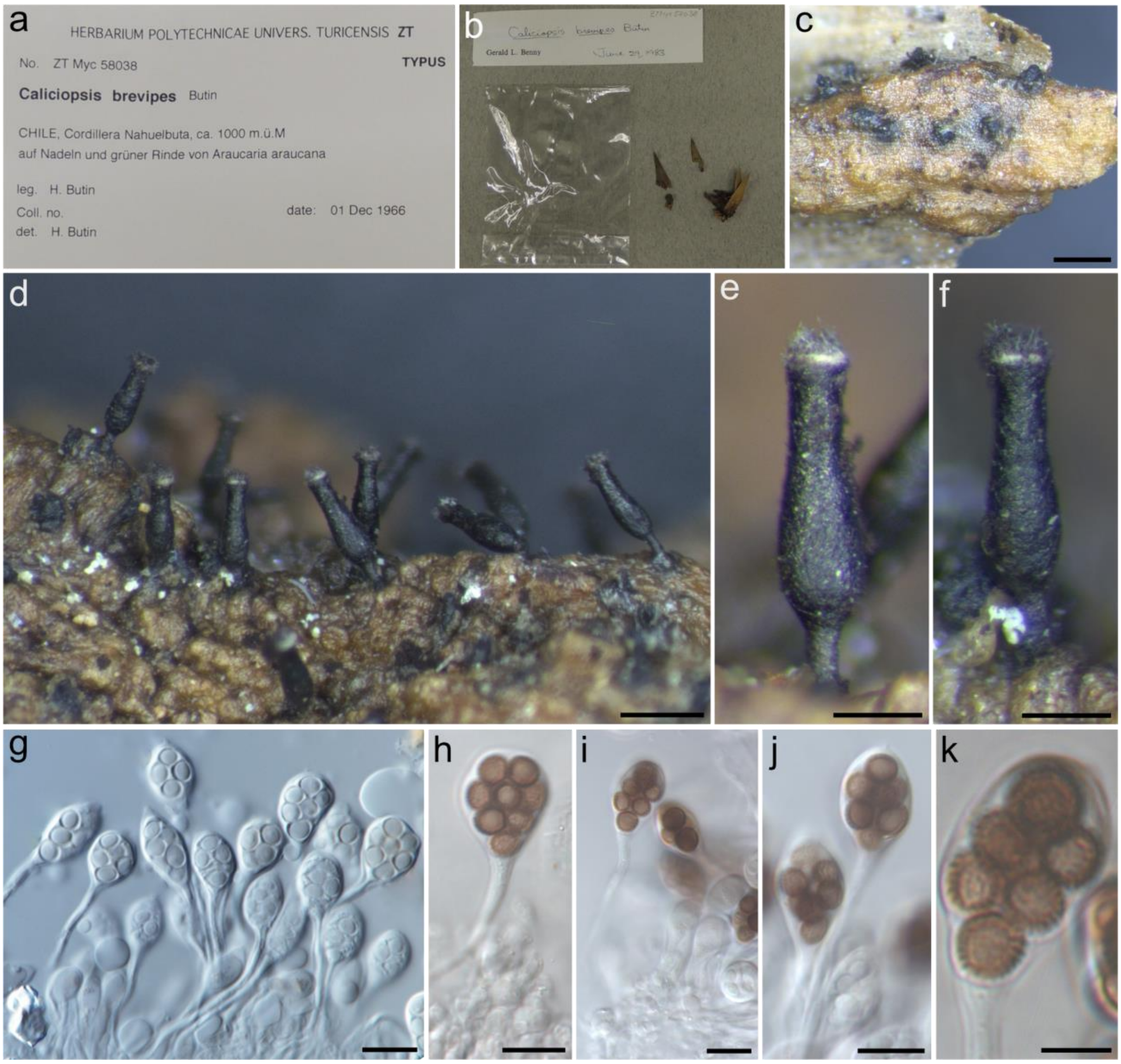
No. ZT Myc 58043

Caliciopsis cochlearis Butin

CHILE, Las Raices, Lonquimay, ca. 1400 m.ü.M
auf Nadeln von Araucaria araucana

leg. H. Butin
Coll. no. date: 06 Mar 1970
det. H. Butin

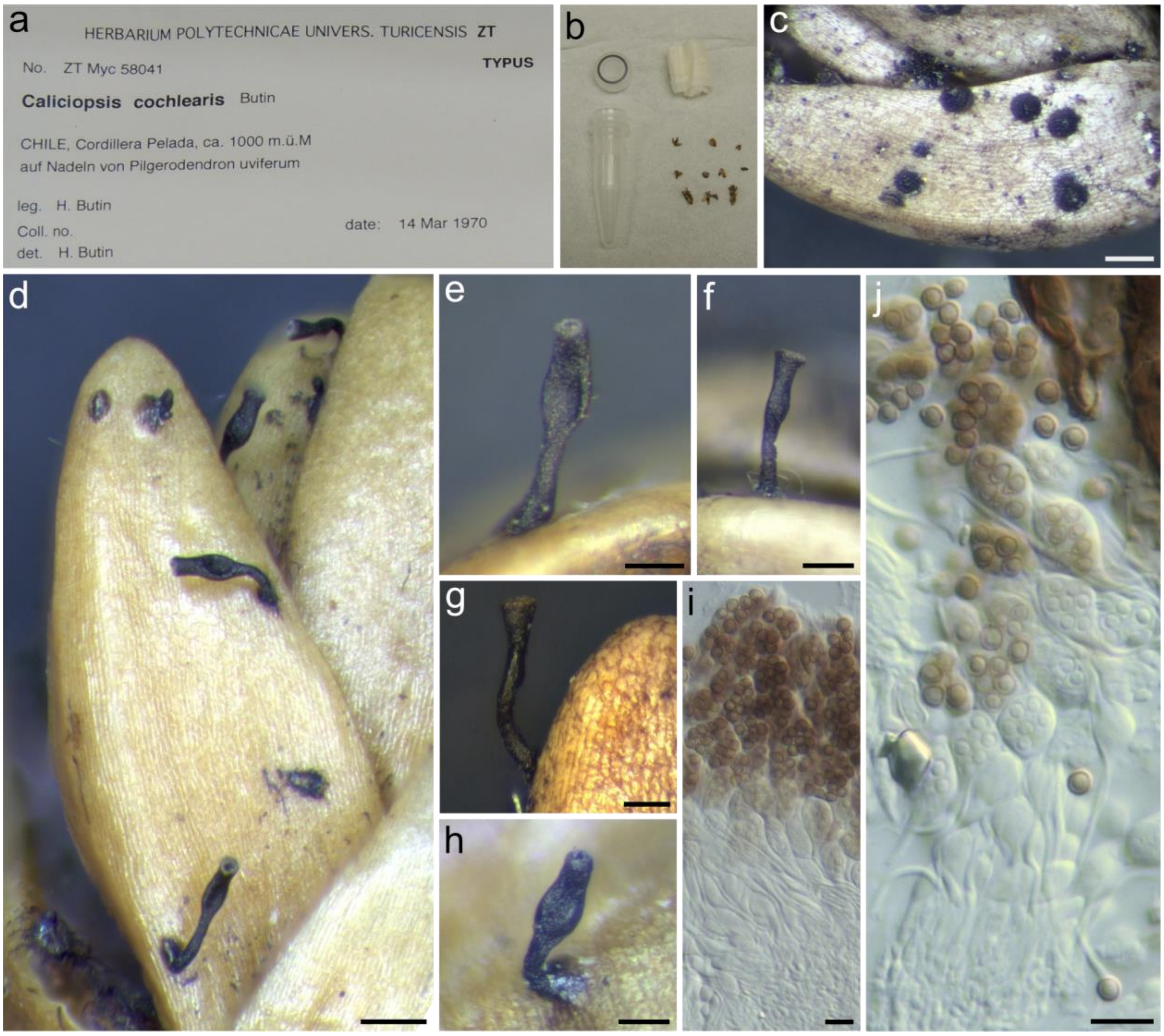
Fig. 5 Micrograph of herbarium specimen ZT Myc 58043 of *Caliciopsis cochlearis* paratype on leaves of *Araucaria araucana*. **a-b**. Labels and specimen identification. **c**. Spermogonia. **d-f**. Ascocarps. **g-h**. Mature and immature asci and ascospores. **i**. Immature ascospores showing verrucose ornamentation. Scale bars: **c–f** = 200 µm; **g–h** = 10 µm; **i** = 5 µm.



HERBARIUM POLYTECHNICAE UNIVERS. TURICENSIS ZT
No. ZT Myc 58038
TYPUS
Caliciopsis brevipes Butin
CHILE, Cordillera Nahuelbuta, ca. 1000 m.ü.M
auf Nadeln und grüner Rinde von Araucaria araucana
leg. H. Butin
Coll. no.
date: 01 Dec 1966
det. H. Butin

ZTMyc 58038
Caliciopsis brevipes Butin
Gerald L. Benny
June 29, 1983

Fig. 6 Micrograph of herbarium specimen ZT Myc 58038 of *Caliciopsis brevipes* holotype on leaf galls on *Araucaria araucana*. **a**-**b**. Labels and specimen identification. **c**. Spermogonia. **d**-**f**. Ascocarps. **g**. Immature asci. **h**-**j**. Mature asci and ascospores. **k**. Mature ascospores showing verrucose ornamentation. Scale bars: **c–d** = 500 µm; **e–f** = 200 µm; **g–j** = 10 µm; **k** = 5 µm.



HERBARIUM POLYTECHNICAE UNIVERS. TURICENSIS ZT

No. ZT Myc 58041 TYPUS

Caliciopsis cochlearis Butin

CHILE, Cordillera Pelada, ca. 1000 m.ü.M
auf Nadeln von Pilgerodendron uviferum

leg. H. Butin
Coll. no. date: 14 Mar 1970
det. H. Butin

Fig. 7 Micrograph of herbarium specimen ZT Myc 58041 of *Caliciopsis cochlearis* holotype on leaves of *Pilgerodendron uviferum*. **a**-**b**. Labels and specimen identification. **c**. Spermogonia. **d**-**h**. Ascocarps. **i**-**j**. Mature and immature asci and ascospores. Scale bars: **c–d** = 200 µm; **e–h** = 100 µm; **i–j** = 10 µm.

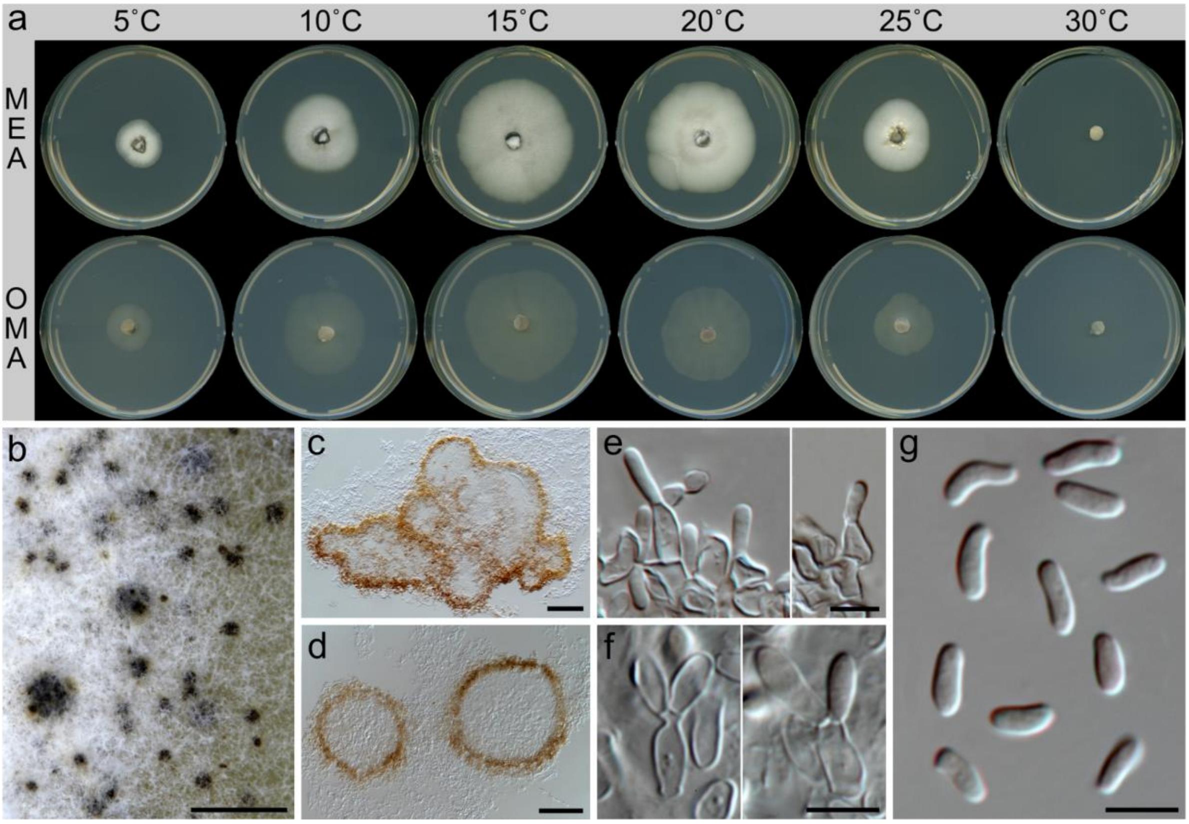


Fig. 8 Micrograph of *Pewenomyces lalenivora* (sp. 1; ex-holotype CMW 56868). **a**. Cultures grown on two different media (MEA, OMA) at 5–30 °C for 28 d in the dark. **b**. Colony showing conidiomata embedded in aerial mycelium. **c-d**. Vertical section of conidiomata. **e-f**. Conidiogenous cells showing simple (**e**) or sympodial growth (**f**). **g**. Conidia. Scale bars: **b** = 1 mm; **c**, **d** = 100 µm; **e–g** = 5 µm.

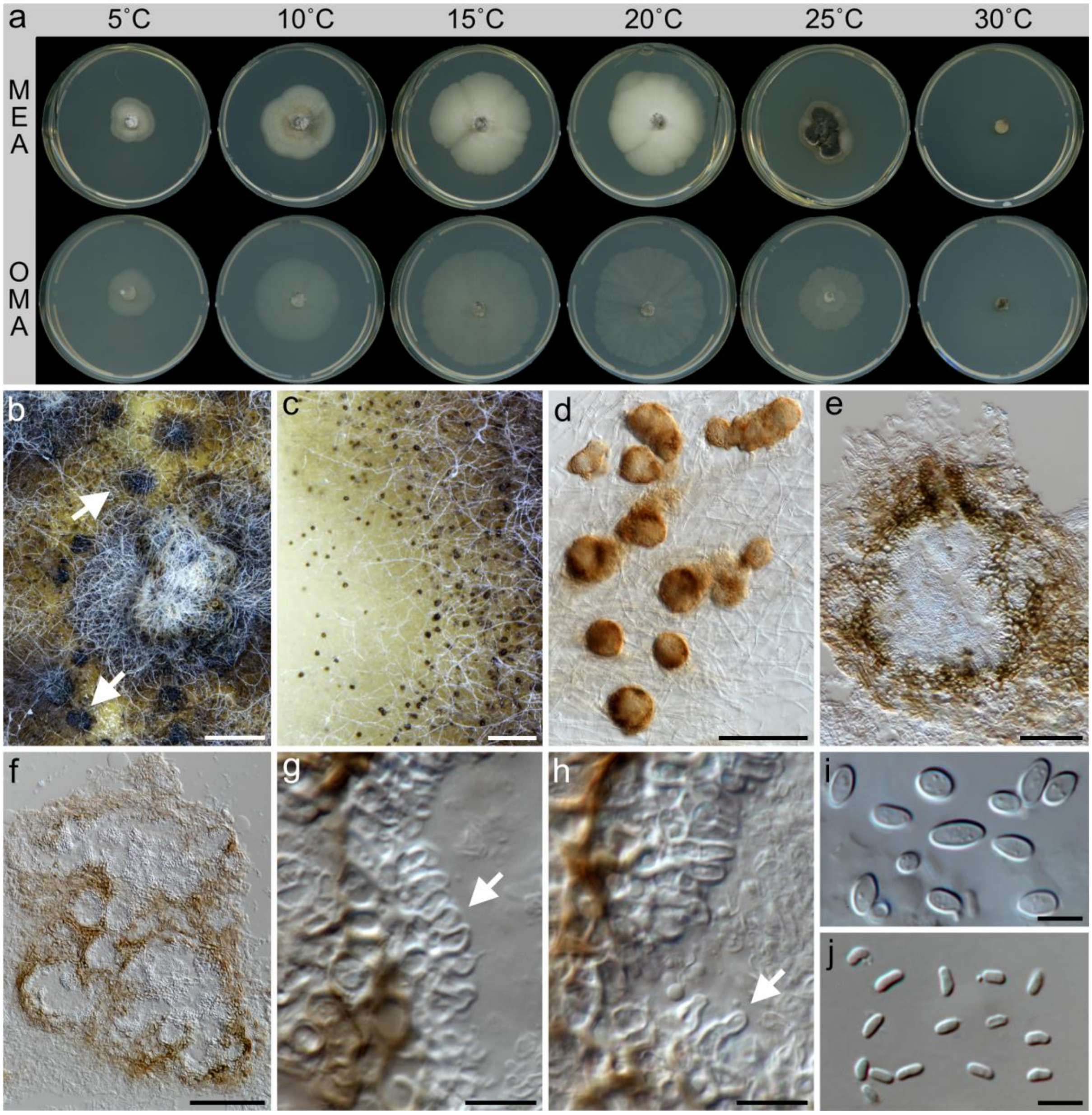


Fig. 9 Micrograph of *Pewenomyces tapulicola* (ex-holotype CMW 54234). **a**. Cultures grown on two different media (MEA, OMA) at 5–30 °C for 28 d in the dark. **b**. Conidiomata (arrows) semi-immersed in 2% MEA. **c-d**. Sclerotia-like structures. **e-f**. Vertical section of conidiomata: unilocular (**e**), multilocular (**f**). **g-h**. Conidiogenous cell borne on peridial wall (arrow). **i**. Conidia. **j**. Spermatia. Scale bars: **b** = 1 mm; **c** = 500 µm; **f** = 100 µm; **d** = 100 µm; **e** = 50 µm; **g**, **h** = 10 µm; **i**, **j** = 5 µm.

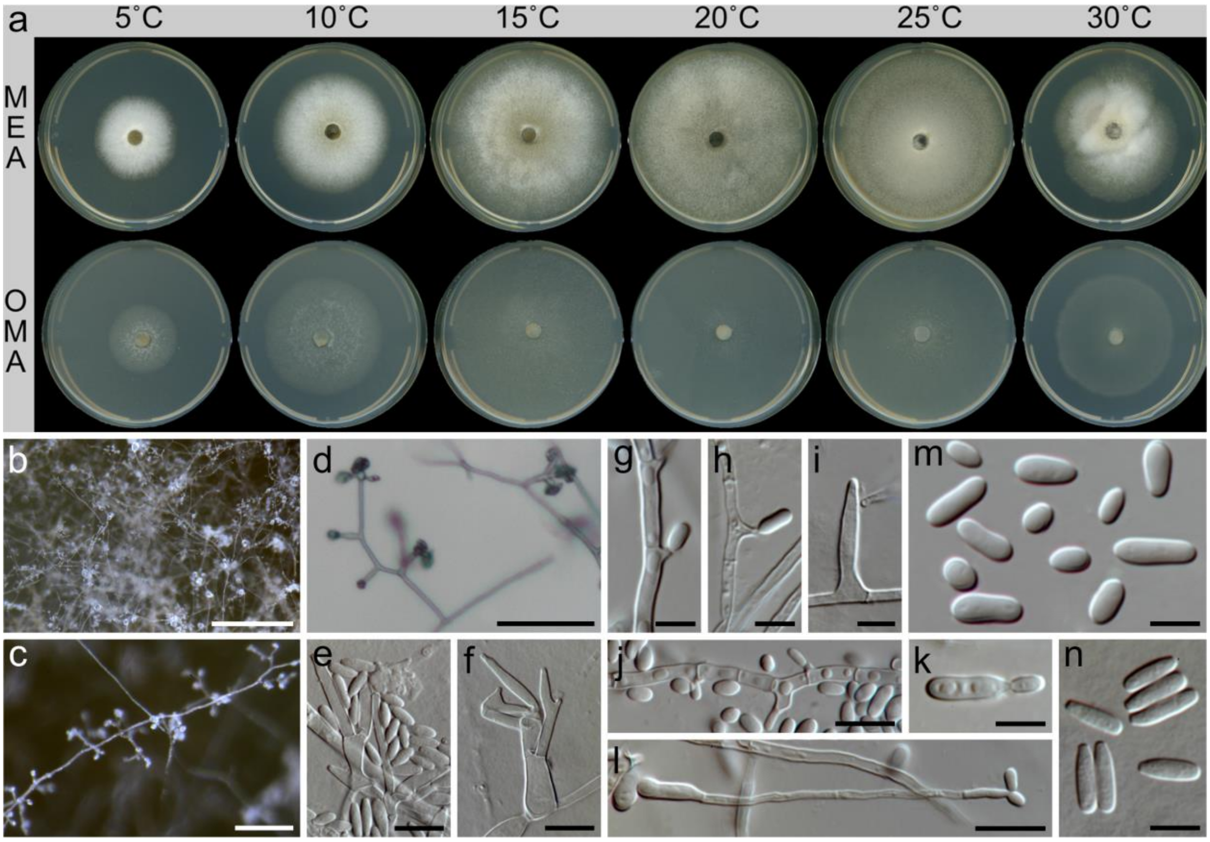
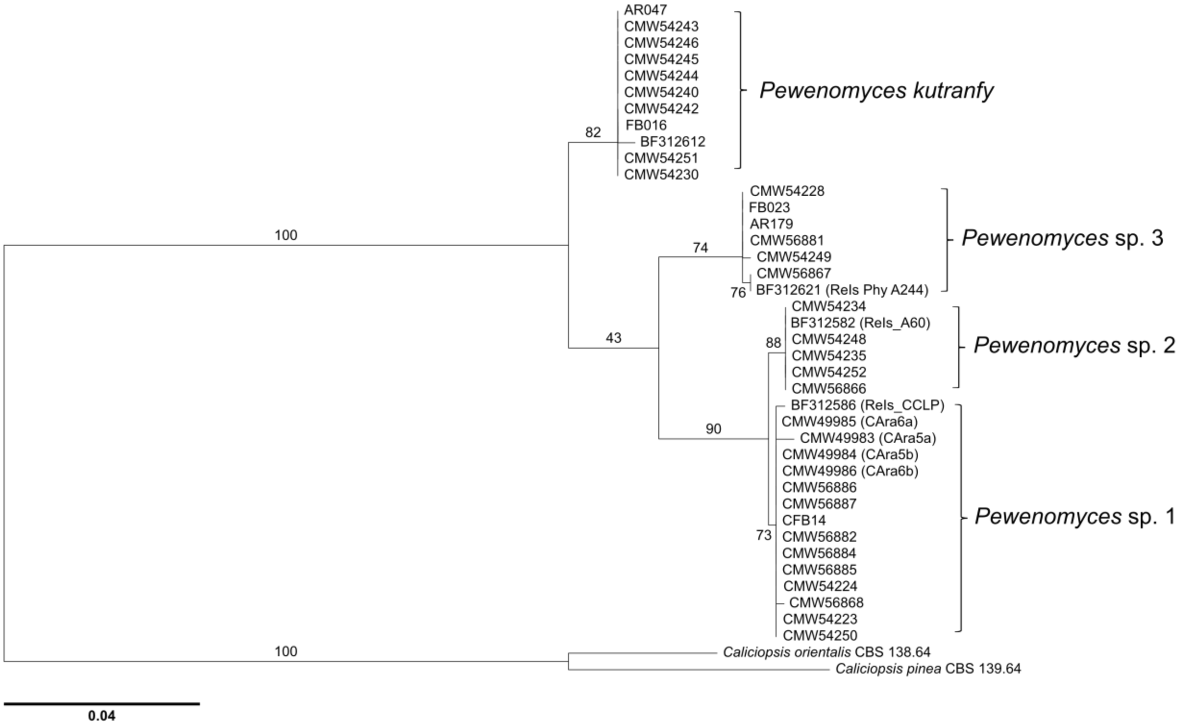
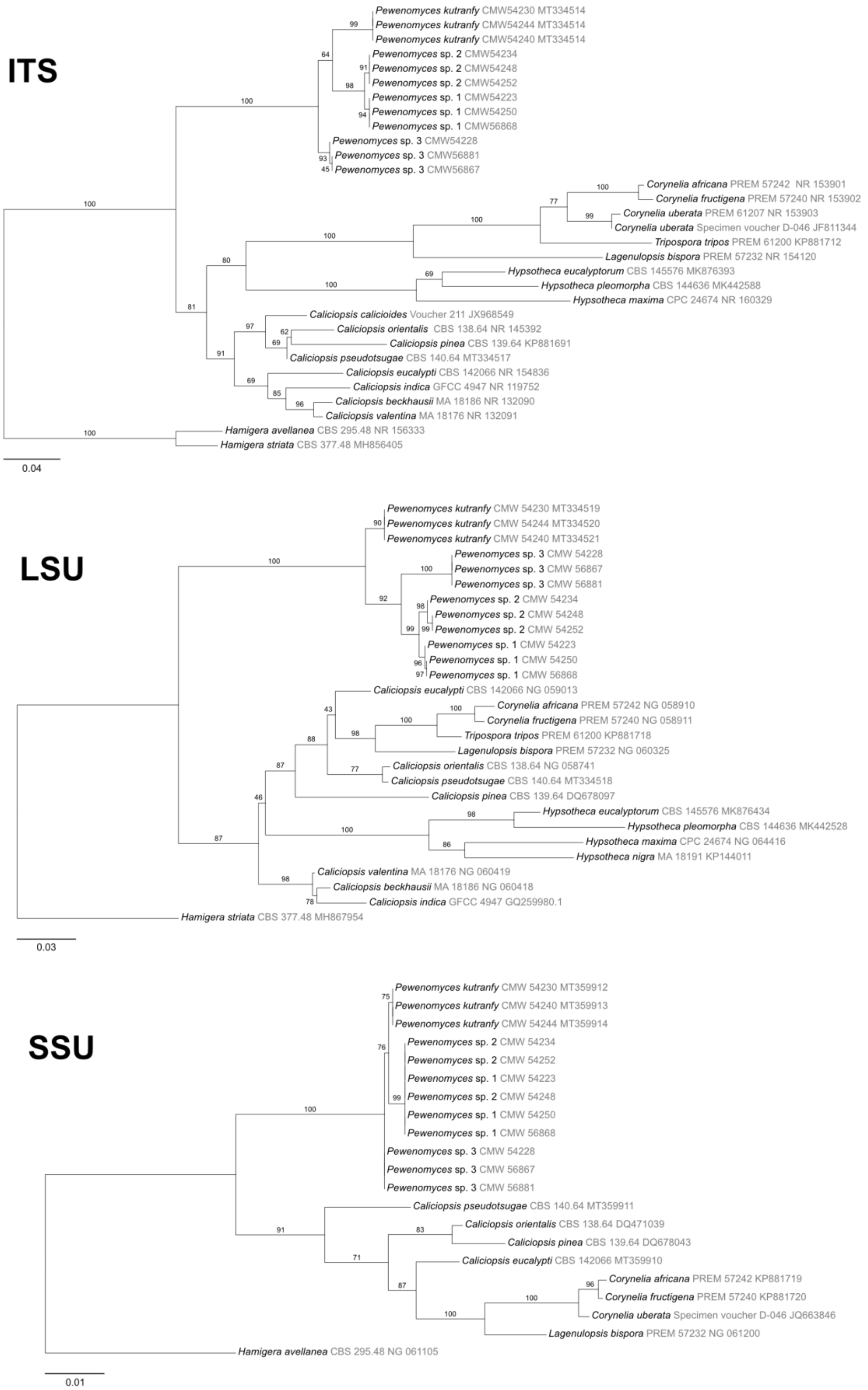


Fig. 10 Micrograph of *Pewenomyces kalosus* (sp. 3; ex-holotype CMW 54228). **a**. Cultures grown on two different media (MEA, OMA) at 5–30 °C for 28 d in the dark. **b-d**. Conidiophores with slimy droplets on aerial hyphae on 2% MEA. **e-j**. Various conidiophores: macronematous (**e**-**f**), micronematous (**g-h**), semimacronematous (**i-j**). **k**. Conidium producing secondary spore. **l**. Germinating conidium producing secondary spores. **m-n**. Conidia. Scale bars: **b** = 500 µm; **c** = 100 µm; **d** = 50 µm; **e, f, j, l** = 10 µm; **g–i, k, m, n** = 5 µm.

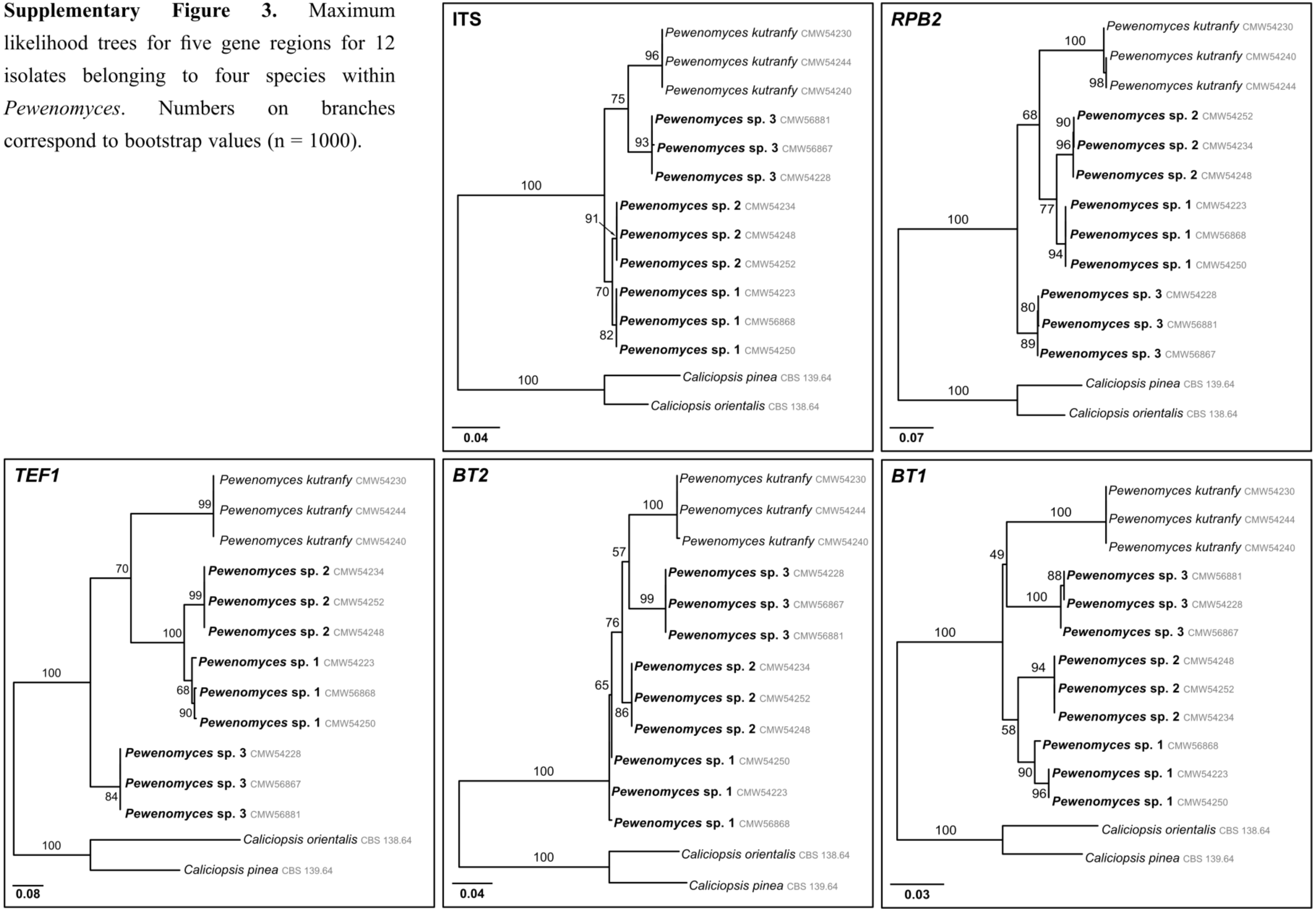
10. SUPPLEMENTARY FIGURES



Supplementary Figure 1. Preliminary phylogeny (maximum likelihood tree for the ITS gene region) for isolates obtained from dead and discouloured tissues and fruiting bodies (ascomata) on samples of *Araucaria araucana* including three putative new species of *Pewenomyces*. Numbers on branches indicate Bootstrap values (n=1000).



Supplementary Figure 2. Maximum likelihood trees for the ITS (top), nc LSU rDNA (middle) and nc SSU rDNA (bottom) for the *Coryneliaceae*, including three new species of *Pewenomyces*.



Supplementary Figure 3. Maximum likelihood trees for five gene regions for 12 isolates belonging to four species within *Pewenomyces*. Numbers on branches correspond to bootstrap values (n = 1000).

CHAPTER 4

Two new *Resinogalea* species from resin-covered branches of *Araucaria araucana* in Chile, and a reclassification of the genus in *Cryptocaliciomycetidae*

Felipe Balocchi
Cobus M. Visagie
Michael J. Wingfield
Rodrigo Ahumada
Irene Barnes

ABSTRACT

Araucaria araucana is an ancient conifer native to the mountain ranges in Chile and Argentina. These trees host a large number of organisms, mainly insects, strongly or even exclusively associated to them. The recent emergence a novel canker disease has emphasised the importance of fungi associated with these iconic trees and has resulted in the discovery of various new species. In this study we considered the identity of an unknown calicioid fungus consistently found on resin on the branches of *A. araucana*. Preliminary phylogenetic analyses placed isolates in the recently described sub-class *Cryptocaliciomycetidae,* close to *Cryptocalicium blascoi*. However, the morphology of the ascomata and its occurrence in a unique niche suggested that the closest relative could be *Resinogalea humboldtensis* (*Bruceomycetaceae*, *incertae sedis*), a fungus with similar fruiting structures on resin of *Araucaria humboldtensis* and found in New Caledonia. There are, however, no living cultures or sequence data available for either *R. humboldtensis* or *Bruceomyces castoris* making a sequence based comparison to these species impossible. Morphological comparisons of the fruiting structures on *A. araucana* confirmed that the anatomical traits of the ascomata of the unknown calicioid fungus and *R. humboldtensis* are almost identical and resemble each other more than *B. castoris* or *C. blascoi*. Phylogenetic analyses using three gene regions resolved the fungus found on *A. araucana* as a new species, forming a well-supported but distinct clade close to *C. blascoi*. Further analyses using three additional gene regions revealed that our isolates included a second novel species obtained solely from plant material impregnated with resin. As a result, we have reclassified the genus *Resinogalea* in the subclass *Cryptocaliciomycetidae* and introduce two new species described here as *Resinogalea araucana* sp. nov and *Resinogalea tapulicola* sp. nov.

1. INTRODUCTION

Araucaria araucana (*Araucariaceae*), commonly known as Araucaria, pewén or the monkey puzzle tree, is an ancient species native to the mountain ranges of Chile and Argentina. These trees are associated with a diverse range of organisms that are in several cases believed to have co-evolved with them and that are scientifically rare (Kuschel 2000). Insects on these trees have been well studied and several species have been described that are strictly associated with *Araucaria* (Kuschel 2000; Carpintero and Dellapé 2006; Beéche 2017). In contrast, very little is known regarding the microbiology of these iconic trees, a situation that also extends to most members of the *Araucariaceae* (Balocchi et al. 2022b).

Recent studies on fungi associated with *A. araucana* led to the discovery of an important canker disease on these trees (Balocchi et al. 2021). The new genus *Pewenomyces* and four new species residing in the *Coryneliaceae* were found associated with this disease, of which *P. kutranfy* [MB 80651] was found to be the causal pathogen (Balocchi et al. 2022a). During studies to resolve the identity of the canker disease on *A. araucana* in Chile, ascomata of a rarely occurring stipitate, capitate and mazaediate (~calicioid) fungus were found emerging from dry resin patches associated with cankers or other sources of damage (e.g., insect feeding, mechanical damage of uncertain origin).

Based on morphological features and niche preference the unusual ascomata were thought to be most similar to the recently described *Resinogalea humboldtensis* [MB 817094] (*Bruceomycetaceae, incertae sedis*; MB 817090, MB 90556 and MB 501288 respectively) (Rikkinen et al. 2016), which produces mazaediate and stipitate open ascomata. *Resinogalea humboldtensis* was described from resin patches due to insect damage on *Araucaria humboldtensis* in New Caledonia. However, the description of *R. humboldtensis* was based solely on morphology and no living cultures or DNA sequences are available for it. The same is true for the only other member and type species of the *Bruceomycetaceae*; *Bruceomyces castoris* (≡ *Brucea castoris*) [MB 803287] (Rikkinen 2003). Consequently, the taxonomic position of both species remains unresolved. The aim of this study was to consider the identity of the fungus found on *A. araucana*, using both morphological characterisation and phylogenetic inference.

2. METHODS

2.1. Sample collections and fungal isolations

Araucaria araucana samples were collected different areas in Chile where these trees occur naturally (Balocchi et al. 2021). These included the Conguillío National Park (sector Los Paraguas), Villarrica National Park (sector Puesco), Ralco Natural Reserve and private areas in the Coastal range (Nahuelbuta, Trongol Alto). Sample collections were carried out between 2017 and 2020 during the Chilean summer periods (December–February). Samples consisted mainly of segments of branches, and a few stems from natural regeneration, that exhibited varied sources of damage including fungal cankers, or damage caused by insect feeding, which had led to the production of resin on them.

Isolations from plant material were made by first surface disinfesting the tissues by immersion in 70% ethanol for 10 s followed by 1 min in 5% sodium hypochlorite. Small pieces of tissue were then dissected and plated on ½ strength potato dextrose agar (½PDA: 19.5 g/L PDA, 10 g/L agar; Merck) or 2% malt extract agar (MEA: 20 g/L malt extract, 20 g/L agar; Biolab) in Petri dishes. Plates were incubated for 14 d at 20–25 °C. Fungal colonies of interest were transferred to new MEA plates in order to produce pure cultures. Isolates were further purified by transferring them to 2% water-agar (WA: agar 20 g/L; Biolab), and allowing these to grow for 2–4 d and transferring single hyphal tips to MEA. Direct isolations from ascomata were made by collecting ascospores with a sterile needle and spreading these onto WA. After 2–4 days, germinating single spores were transferred to MEA.

All relevant isolates used in this study were deposited at the culture collection CMW of the Forestry and Agricultural Biotechnology Institute, University of Pretoria. Reference isolates used for taxonomic purposes were deposited in the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, The Netherlands, and in the fungarium of the South African National Collection of Fungi (PREM) housed at the Agricultural Research Council (ARC; Plant Health and Protection, South Africa). An isolate of *Caliciopsis pinea* (CBS 139.64) obtained from the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), was also used in this study.

2.2. DNA extraction, PCR and Sequencing

DNA extractions were performed using the Prepman® Ultra Sample Preparation Reagent kit (Thermo Fisher Scientifc, Waltham, MA, USA) following the manufacturer's protocols or a phenol-chloroform method. For the latter, isolates were grown on MEA for 21 d, after which mycelium was collected in 2 mL Eppendorf tubes and freeze dried overnight. Mycelia was ground by adding metal beads and shaking this in a mixer mill (MM 301, Retsch GmbH). DNA was extracted following the protocol described by Barnes et al. (2001) and modified by Balocchi *et al.* (2021). The quality and quantity of DNA extractions were measured on a NanoDrop spectrophotometer (Thermo Scientific NanoDrop ND-1000) and working stocks of 30 ng/µL were subsequently prepared for each sample.

The ITS gene region was amplified for all isolates using the primer pair ITS1 & ITS4 (White et al. 1990) and sequenced as described below. A preliminary phylogenetic analysis based on ITS resolved isolates into two clades. Six isolates from each clade were selected and five additional gene regions were sequenced for them. These regions included: (i) the nuclear 28S large ribosomal subunit (LSU) with primers LROR & LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1995); (ii) the nuclear 18S small ribosomal subunit (SSU) with primer pairs NS1 & NS4 and NS3 & NS8 (White et al. 1990); (iii) the RNA polymerase II second largest subunit (*RPB2*) with primers RPB2-5f2 & RPB2-7cR (Liu et al. 1999; Sung et al. 2007), (iv) the mini chromosome maintenance protein complex 7 (*MCM7*) with primers Cer-MCM7F & Cer-MCM7R (De Beer et al. 2014); and (v) the translation elongation factor 1-alpha (*TEF1*) large intron with primers EF1-782F & EF-2 (O'Donnell et al. 1998; Carbone and Kohn 1999). PCRs were prepared in 25 µL reaction volumes and contained 5 µL 5X MyTaq™ Reaction Buffer (Bioline, London, UK), 0.5 µL (1 µL for *MCM7*) of each primer (10 µM), 0.3 µL MyTaq™ DNA Polymerase and 17.7 µL (16.7 µL for *MCM7*) sterile deionized water. The thermal cycling conditions included an initial denaturation stage of 95 ˚C for 3 min, followed by 35 cycles of denaturing at 95 ˚C for 30 s, annealing at 56 ˚C (52 ˚C for *TEF1* and *MCM7*) for 30 s and elongation at 72 ˚C for

45 s, followed by a final elongation step at 72 ˚C for 4 min. Successful amplifications were confirmed by staining PCR amplicons with GelRed® (2 µL per 4 µL of PCR product) and electrophoresing the product on a 1% agarose gel for 12 min at 110V.

PCR amplicons were cleaned from excess primers and unincorporated nucleotides either by sodium acetate precipitation (Duong et al. 2013) or using ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems™, Thermo Fisher, Waltham, MA, USA) following the manufacturer's instructions. Amplicons were sequenced in both directions using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems™, Thermo Fisher, Waltham, MA, USA) following the manufacturer's instructions. Sanger sequencing was carried out at the sequencing facility of the University of Pretoria. The sequences obtained for each isolate were visualized and assembled into consensus sequences with CLC Main Workbench v.21.0.3 (Qiagen, Hilden, Germany). New sequences were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/; Table 1).

2.3. Phylogenetic analyses

For an initial identification of the isolates obtained from the sampling, the ITS sequences were compared for 28 isolates, including those obtained from plant material and from fruiting structures. Sequences were compared against each other as well as to the GenBank database using the Basic Local Alignment Search Tool (BLAST). Based on the results, 12 isolates were selected for further analyses based on the type of tissue from which they were isolated and the location from which they were collected.

Two reference sequence datasets were constructed for phylogenetic analyses (see Table 1). The first dataset included ITS, LSU and SSU sequences for taxa relevant in determining the phylogenetic placement of the fungus within *Eurotiomycetes* and was largely based on Prieto et al. (2021). This included isolates of representative species for the *Eurotiomycetidae*, *Cryptocaliciomycetidae, Coryneliomycetidae*, *Chaetothyriomycetidae*, *Sclerococcomycetidae* and *Mycocaliciomycetidae* and two *Calicium* species (*C. salicinum* and *C. glaucellum*; *Lecanoromycetes*) that served as the outgroup.

Isolate selection was made favouring ex-type cultures and/or cultures that had most of the gene regions sequenced. The second dataset was constructed with the aim of studying the phylogenetic relationship between our isolates using *Cryptocalicium blascoi* and *Caliciopsis pinea* (*Coryneliomycetidae, Coryneliales*) as the outgroup and applying the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept (Taylor et al. 2000). In this case sequences for the ITS, *RPB2*, *MCM7* and *TEF1* were included.

All sequence alignments were done using the MAFFT v.7 online service (Katoh et al. 2019) and visualized and edited, where necessary, using MEGA v.7.0.26 (Kumar et al. 2016). Both single gene (ITS, *RPB2*, *MCM7* and *TEF1*) and multigene (SSU-ITS-LSU) phylogenetic analyses were made. For the multigene analysis, alignments were concatenated using CLC Main Workbench. Maximum likelihood (ML) trees were constructed using the IQ-TREE Web server (Trifinopoulos et al. 2016) selecting the most suited nucleotide substitution model (partitioning the data in case of concatenated trees) according to the Bayesian information criterion (BIC) using ModelFinder (Kalyaanamoorthy et al. 2017) built into IQTREE. Phylogenetic trees were visualized using FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) and edited using Affinity Designer v.1.10.5.1342 (Serif (Europe) Ltd, Nottingham, UK).

2.4. Morphological observations

2.4.1. Fruiting structures on branch samples

Fruiting structures on *A. araucana* branches were visualized, photographed, and measured using a Zeiss AXIO Zoom. V16 dissection microscope with an AxioCaM 512 colour camera and the software Zen Blue v.3.2 software (Carl Zeiss CMP GmbH, Göttingen, Germany). For microscopic observations, fruiting structures were collected from the samples and were either sectioned using a CM1520 cryostat (Leica) or placed directly on glass microscope slides in lactic acid (10%) as the mountant. Structures were visualized, photographed, and measured using a AXIO Imager.A2 compound microscope with the same camera and software used in the case of the dissection microscope. Up to 50 measurements were made for each characteristic morphological structure where available, and boxplot graphs were constructed with measurements of each character to visualize the distribution of the data. Dissection microscope images were processed using Helicon Focus v.7.5.4 (Extended Depth of Field analysis; HeliconSoft, Kharkiv, Ukraine), and all photographic plates were prepared in Affinity Designer v.1.10.5.1342 (Serif (Europe) Ltd, Nottingham, UK).

2.4.2. Colony growth rate and morphology

Six isolates of each of the two putative new species were chosen to represent the geographical range from which they had been isolated. A spore/mycelium suspension was prepared for each isolate by cutting agar blocks from MEA colonies and suspending these in 10% glycerol. Inoculations from suspensions were made onto test media at three equidistant spots (1 µL each) using a micropipette and allowed to dry for 10 min before plates were sealed with Parafilm® and incubated. Three culture media (2% MEA, PDA and WA) and seven temperatures were considered for the study (5–35 °C, in intervals of 5 °C) with a single plate per isolate for each medium at each temperature. All plates were incubated for 21 d and colony diameters were measured twice a week. Colony surface areas were measured from images taken on day 21 using ImageJ v.1.52 (Abràmoff et al. 2004; Guerrero et al. 2012) software with an adjusted scale. Plates incubated at temperatures where there was no growth were incubated at 20 °C for two additional weeks to determine whether the original inoculum had remained viable. For descriptions, colour names and codes were based on Kornerup and Wanscher (1967). Some isolates were incubated for an additional ~3 months periods and observations noted in descriptions.

3. RESULTS

3.1. Isolations and preliminary identification

Isolations from *A. araucana* plant tissue covered in resin resulted in 23 isolates. Isolates originated from all four areas considered. An additional 25 isolates were recovered through direct isolations made from five fruiting structures that had developed on the samples. These samples originated from Conguillío National Park (Andes Mountain range) and Trongol Alto (Coastal range).

DNA extraction and ITS sequencing were performed for all isolates originating from plant tissue (n=23), and one from each fruiting structure (n=5). A preliminary phylogenetic analysis resolved them into two closely related clades. The first clade contained 22 isolates including all the of isolates originating from fruiting structures, and the second clade contained six isolates. When sequences of both clades were compared using a BLAST search against the GenBank database, the closest hits were *Cryptocalicium blascoi* (GenBank MW999967; Identity = 543/611(89%), 39 gaps (6%)), followed by species in *Penicillium* such as *P. scabrosum* (GenBank MT995062; Identity = 507/617(82%), 35 gaps (5%)), *P. hirayamae* (GenBank MH858553; Identity = 502/612(82%), 28 gaps (4%)), *P. copticola* (GenBank MF326615; Identity = 427/509(84%), 16 gaps (3%)) and *P. terrigenum* (GenBank KT336534; Identity = 508/617(82%), 27 gaps (4%)).

3.2. Phylogenetic analyses

A multigene phylogeny of SSU-ITS-LSU was assembled to resolve the phylogenetic position of the 12 isolates obtained from the *A. araucana* samples (six per clade described above). Alignments for SSU (52 taxa, 3911 bp), ITS (57 taxa, 835 bp) and LSU (61 taxa, 1024 bp) were concatenated resulting in a matrix containing 62 taxa that was 5770 bp long. Best likelihood models according to the Bayesian information criterion (BIC) for the ITS, LSU and SSU were GTR+F+I+G4, TN+F+I+G4 and TNe+I+G4 respectively. ML analyses resolved isolates into two clades in the subclass *Cryptocaliciomycetidae* with *Cryptocalicium blascoi* as sister with high bootstrap support (Fig. 1).

Single gene phylogenies were calculated to assess the taxonomy of the isolates considered in this study by applying GCPSR. Substitution models for the aligned ITS (16 taxa, 617 bp), *MCM7* (14 taxa, 618 bp), *RPB2* (14 taxa, 873 bp), *TEF1* (13 taxa, 451 bp), LSU (15 taxa, 847 bp) and SSU (14 taxa, 2013 bp) datasets were, respectively, TNe+G4, K2P+I, TNe+I, TNe+G4, TN+F+I and K2P+I. All gene regions resolved the isolates in two concordantly well-supported clades (Fig. 2), and with the exception of SSU, all regions resolved them in a clade separate from *C. blascoi*.

3.3. Morphology

Sexual fruiting structures (ascomata) were observed only on branch samples, never in culture, and all isolates obtained from them belonged to a single clade based on DNA sequence comparisons. The ascomata on the samples emerged directly from the resin or from plant tissue surrounded by it (Fig. 3), and were very similar to those of *R. humboldtensis* (*Bruceomycetaceae*; Rikkinen et al. 2016). Both species produced ascomata having a similar morphology and dimensions (Fig. 4, Table 2) and shared additional features, such as the cover of mineral pruina on their stipes (Figs. 3, 4f), the production of mycelium that colonizes resin (Fig. 4f), a capitulum covered with resin (resin helmet) (Fig. 3i), erythrocyte-shaped smooth to slightly roughened ascospores (Fig. 4j), and both occurred on resin patches of two different *Araucaria* species (Fig. 3a, b). In contrast, ascomata of *Bruceomyces castoris*, type species for the *Bruceomycetaceae*, do not share any of these features, and have distinctly larger dimensions in most of its structures (e.g., ascomata, ascospores), and have distinctive characters of its own (e,g., shape of the ascomata and ascospore ornamentation) (Rikkinen 2003; Table 2).

Cryptocalicium blascoi (Prieto et al. 2021), which, based on DNA sequences, is the closest described species to the fungus found on *A. araucana*, shares broad morphological and niche features with our fungus and *R. humboldtensis*. These include growing on conifer resin, ascomata developing a definite capitulum, having a coverage with mineral pruina and producing in some cases a columnar protruding mazaedium. However, ascomata of *C. blascoi* has considerable differences with *R. humboldtensis* and the fungus on *A. araucana* with regards to dimensions (e.g., size of ascomata) and colours (mazaedium, mineral pruina; Table 2).

All isolates obtained from *A. araucana* samples had a broadly similar morphology when grown on MEA or ½PDA. However, these differed considerably when compared to those described for *C. blascoi* (Prieto et al. 2021) in terms of morphology, growth rate and microscopic characters (specified below). Further observations of the isolates from *A. araucana* revealed slight but consistent morphological differences between isolates from in the two phylogenetic clades (Figs. 5, 6), and these also had different growth rates and optimal temperatures (Fig. 7).

4. TAXONOMY

Analyses using DNA sequence comparisons and morphological comparisons showed that the fungi obtained from *A. araucana* represent two distinct species, and that they belong in the *Cryptocaliciomycetidae*. They are, however, distinct to the only known species of *Cryptocalicium*, i.e., *C. blascoi*, both in terms of DNA sequence identity, and morphology. Although DNA sequence data and colony morphology are not available for *R. humboldtensis*, which is monotypic, this fungus shares an almost identical morphology, anatomy, and niche with the fungus found in the present study sporulating on resin covering the branches of *A. araucana*. Additionally, the two species are more similar to each other than either of them are to *Bruceomyces castoris* (Table 2), the type species of the *Bruceomycetaceae*, in which *R. humboldtensis* currently resides. Consequently, two new species in *Resinogalea* are described and a proposal is made to relocate the genus to the *Cryptocaliciomycetidae* alongside *Cryptocalicium*.

4.1 ***Resinogalea araucana*** Balocchi & Visagie, sp. nov

Figures 3, 4 and 5

Mycobank MB xxxxx

In: Class *Eurotiomycetes*, subclass *Cryptocaliciomycetidae*, order *Cryptocaliciales*, family *Cryptocaliciaceae*, genus *Resinogalea*

Etymology: Latin, '*araucana*', named for its only known host, *Araucaria araucana*, on which this fungus colonizes resin patches commonly resulting from wounds.

Type specimen: Chile, Araucanía (IX), Conguillío National Park sector Los Paraguas, -38.697836°, -71.817216° branches of *Araucaria araucana* (single spore isolate from ascomata on branch), 02 May 2018, F. Balocchi, PREM = xxx (dried isolate in metabolically inactive state; holotype), ex-type cultures CMW 57581 = CBS xxx = NCFB4

ITS barcode: xxxx. Alternative identification markers: LSU = xxx; MCM7 = xxx; RPB2 = xxx; SSU = xxx; TEF1 = xxx.

Description: In nature — Ascomata emerging from dry resin patches in branches of *Araucaria araucana*, stipitate, capitate, (556–)766–1070(–1540) µm tall (excluding mazaedium); stipe cylindrical, subulate or rarely dumbbell-shaped; bent wholly or at the base, rarely straight, black, rugose, covered with a granular orange to reddish-brown mineral pruina, (257–)364–598(–1069) × (70–)92–214(–275) µm; stipe medulla composed of *textura intricata-porrecta*, brownish pigmentation; stipe outermost layer composed of 15–20 layers of *textura prismatica-porrecta*, long parallel hyphae, cylindrical, septate, slightly thick walled, hyaline inner layers becoming dematiaceous towards outer layers, hyphae 2–3 µm wide; strongly melanized mycelium emerging from the base of the stipe, hyphae darkly pigmentated and thick walled, (3–)6–9(–13.5) × 3–5(–7.5) µm; capitulum spherical when immature, ampulliform or urceolate when mature, (204–)293–394(–467) µm wide, (121–)230–344(–500) µm tall (not including mazaedium), black, rugose, sometimes covered with white to yellowish white resin; margins at the apical opening discrete, irregular, sometimes crenate-like, light brown; mazaedium well-developed in mature ascomata, reddish-brown, irregularly shaped or in some cases columnar, up to 290 µm in length; ectal excipulum poorly differentiated from the medullary excipulum, both combined consisting of 8–14 layers of *textura prismatica–porrecta*, periclinally arranged hyphae, darkly pigmented in the outer layers and becoming hyaline in the inner, (8–)11–17(–32) × 3–6(–9) µm; subhymenium hyaline or with very slight yellowish pigmentation, composed of *textura angularis-intricata*. Asci clavate, pedicellate, bitunicate, evanescent, eight-spored, often with biseriately arranged spores, sporiferous part (15.5–)19–23(–30) × 4–6 µm, pedicel 6–18.5(–32) × 1.5–3 µm. Ascospores non-septate, hyaline when immature, brownish with age, sometimes, smooth to finely roughened, broadly ellipsoidal, some globose or ellipsoidal, erythrocyte-like, 4–5.5(–6.5) × (3.5–)4–5.5 µm (aspect ratio (1–)1.1–1.3(–1.5)). Paraphyses hyaline, filiform, obtuse, non-branched, with 1–5 septa, 64–121(–172) × 2–3 µm. Phialide-like structures sporadically found inside the capitulum, potentially emerging from walls, hyaline, single-celled, subglobose, ellipsoidal or ampulliform, guttulate, (3–)4.5–7.5(–8.5) × 2–3(–4) µm, bearing single conidia-like structures, globose to obovoid, sometimes tilted to one side, hyaline when immature, becoming brownish when older, smooth or finely roughened, 4–6(–7) × 3.5–5.5 µm.

In culture — Colony diameters: (mm after 14 d (after 21 d)): MEA at 5 °C microcolonies (3–5), at 10 °C 6–9 (13–15), at 15 °C 13–21 (17–28), at 20 °C 19–26 (29–35), at 25 °C 18–24 (29–37); PDA at 5 °C microcolonies (3–4), at 10 °C 8–11 (13–17), at 15 °C 15–18 (21–26), at 20 °C 20–24 (28–

35), at 25 °C 19–27 (27–40), at 30 °C 6–15 (8–21), at 35 °C no growth; WA at 5 °C microcolonies (2–5), at 10 °C 5–8 (10–13), at 15 °C 10–14 (15–20), at 20 °C 14–19 (22–27), at 25 °C 14–23 (25–36); OA at 20 °C 18–24 (30–36).

Colony characters: MEA 25 °C 21 d: Colonies velvety, concentric darkening or without pattern, umbonate, margins entire to slightly irregular, subsurface, obverse greyish yellow to dark yellow (4B4–C8), aerial mycelium yellowish white to orange white (4A2–5A2) at margins, pale to light yellow (4A3–5) centrally, reverse beige to blond (4C3–4) orange yellow to dark yellow (4B8–C7) centrally. MEA 20°C 21 d: Colonies slimy, glabrous and sulcate, rarely dry and fluffy, umbonate, with full or slightly crenate margins, obverse greyish yellow to dark yellow (4B3–C8), aerial mycelium yellowish white to orange white (4A2–5A2) at margins, yellowish white to light yellow (4A2–5) centrally, ***reverse*** beige to blond (4C3–4) at margins, orange yellow to dark yellow (4B8–C7) centrally. MEA 20–25 °C 6 wks or older: Colonies slimy, glabrous, sulcate, irregular margins, producing sporodochia as mucilaginous spore masses on the surface, in concentric halos or with no pattern, dark brown to brownish black (6F4–H8). PDA 25 °C 21 d: Colonies velvety, glabrous, some cultures slimy only at centres, concentric or slightly petaloid pattern, umbonate, with full or slightly crenate margins, ***obverse*** yellowish grey to orange white (4B2–5A2), margins sunken in the medium, aerial mycelium greyish yellow to pale orange (4B4–5A3) at margins, becoming slightly darker or sometimes grey (4B1–D1) towards the centre, reverse yellowish white to yellowish grey (4A2–B2) at margins, pale yellow to olive (2D4–4A3) at centre, sometimes olive (2F7).

Microscopic characters: Somatic hyphae at colony periphery hyaline, smooth, thin-walled, branched, transversely septate, 1.5–4.5 µm diam., anastomosing and developing coils, becoming inflated and melanized as cultures age. Conidiomata most commonly absent, sporodochial in old cultures, composed of pseudostromatic thick-walled melanized hyphae, setae absent. Conidiophores reduced, unbranched, rarely single branched or stromatic. Conidiogenous cells monophialidic or undifferentiated, acropleurogenous; *denticles* sometimes present, up to 3 µm in length; *terminal conidiogenous loci* 13.5–25 (–35) µm in length from septa; *phialides* ampulliform, curved or straight and tapering towards the apex, collarette distinct, (5.5–)7–10(–15) × 2.5–3(–4) *µm*. Conidia produced in slimy heads, hyaline, sometimes dematiaceous when colonies age, aseptate, smooth walled, some with indistinct hilum, globose to broadly ellipsoidal, but also some larger ellipsoidal to ovoid conidia in PDA and WA, variable in size, in MEA (3–)3.5–4.5(–6.5) × 3–3.5(–4) *µm* (aspect ratio 1–1.2(–1.9)), in PDA 3.5–6(–8) × (3–)3.5–5 *µm* (aspect ratio 1–1.4(–2.2)).

Habitat and distribution: Resinogalea araucana produces a sexual state on dry resin patches on damaged tissues in branches of *Araucaria araucana*. It was isolated into culture directly from resin or plant tissues covered with it. The type of damage inducing the exudation of the resin in which this fungus was found varied between samples, and included cankers caused by other pathogenic fungi, or insect feeding, and suggests that the presence of this fungus is merely associated with the presence of dry resin. Isolates originate from all collection sites in the Andes and Coastal range suggesting that its distribution overlaps that of *Araucaria araucana*. Its occurrence on other tree species has not been explored and a larger distribution may thus exist.

Additional specimens examined: Chile, Araucanía (IX), Conguillío National Park sector Los Paraguas, -38.697836°, -71.817216° branches of *Araucaria araucana*, 02 May 2018, F. Balocchi, PREM = xxx (dried metabolically inactive specimen, paratype), culture CMW 57580 = CBS xxx = NCFB1; -38.697836°, -71.817216°, branches of *Araucaria araucana* (isolates from plant tissue covered in resin), 11 Dec 2017, F. Balocchi, culture AR023 = CMW 53536. — Villarrica National Park sector Puesco, -39.572706°, -71.499235°, branches of *Araucaria araucana* (isolates from plant tissue covered in resin), 13 Dec 2017, F. Balocchi, culture AR140 = CMW 53539 = CBS xxx. — Biobío (VIII), Ralco Natural Reserve, -37.962620°, -71.327679°, cankers on branches on *Araucaria araucana* (isolates from plant tissue covered in resin), F. Balocchi, culture AR224 = CMW 53543. — Nahuelbuta mountain range, Trongol Alto, -37.564893°, -73.205764°, branches of *Araucaria araucana* (isolates from plant tissue covered in resin), 21 Dec 2017, F. Balocchi, culture AR290 = CMW 53544 = CBS = xxx; -37.553434°, -73.188438°, ascomata on branches of *Araucaria araucana*, 5 December 2019, F. Balocchi, PREM xxx.

4.2 ***Resinogalea tapulicola*** Balocchi & Visagie, sp. nov

Figure 6

Mycobank MB xxxxx

In: Class *Eurotiomycetes*, subclass *Cryptocaliciomycetidae*, order *Cryptocaliciales*, family *Cryptocaliciaceae*, genus *Resinogalea*

Etymology: From indigenous Chilean and Argentinian Mapuche language mapudungun; ‘*tapül*’, meaning the leaves of a tree, and the substrate from which the fungus was obtained.

Type specimen: Chile, Araucanía (IX), Conguillío National Park sector Los Paraguas, -38.697836°, -71.817216°, branches of *Araucaria araucana* (isolates from plant tissue covered in resin), 11 Dec 2017, F. Balocchi, holotype PREM = xxx (dried culture in metabolically inactive state), ex-type culture AR073 = CMW 53537 = CBS xxx.

ITS barcode: xxxx. Alternative identification markers: LSU = xxx; MCM7 = xxx; RPB2 = xxx; SSU = xxx; TEF1 = xxx.

Description: In nature — not observed.

In culture — Colony diameters : (mm after 14 d (after 21 d)): MEA at 5 °C microcolonies (3–4), at 10 °C 9–10 (15–17), at 15 °C 15–18 (24–26), at 20 °C 22–27 (33–39), at 25 °C 23–31 (33–47); PDA at 5 °C microcolonies (3–5), 10 °C 9–12 (16–19), 15 °C 17–20 (25–30), 20 °C 21–29 (32–44), at 25 °C 27–34 (39–49), 30 °C 11–19 (15–27), 35 °C no growth; WA at 5 °C microcolonies (3–5), at 10 °C 7–9 (10–16), at 15 °C 13–16 (17–24), at 20 °C 15–23 (22–34), at 25°C 17–24 (29–36); OA at 20 °C 20–24 (29–37).

Colony characters: MEA 25 °C 21 d: Colonies velvety to tomentose, mycelium growth in a rosaceous and/or concentric pattern, umbonate, margins irregular, obverse dark yellow to greyish yellow (4B4–C8) at margins, aerial mycelium yellowish white to orange grey (4A2–5B2) at margins, pale to light yellow (4A3–5) centrally, reverse greyish yellow to blond (4B3–C4) at margins, becoming dark yellow to brownish yellow (4B8–5C7) centrally. MEA 20–25 °C 6 wks or older: slimy, glabrous, rarely velvety, umbonate, mycelium growing in a rosaceous and/or concentric pattern, margins irregular, soluble pigments golden brown to reddish brown (5D7–8E6), ***obverse*** orange grey to light brown (6B2–D4), aerial mycelium sometimes present, white (3A1) to greyish red (7B4), ***reverse*** brownish orange to brown (5C3–F6), , producing sporodochia as mucilaginous spore masses on the surface, in concentric halos or with no pattern, light brown to dark brown (6D6–F8). PDA 25 °C 21 d: slimy, glabrous to tomentose, mycelium growing in a rosaceous or stellate pattern, margins irregular, ***obverse*** edges sunken in the medium, yellowish grey (2B2–4B2), aerial mycelium white to pale yellow (3A1–3) at margins, becoming olive to dark grey (1F1–3E8) centrally, ***reverse*** pale grey to yellowish grey (3B1–3) at margins, becoming dull yellow to olive (3B4–E5) centrally, sometimes darker (3F7).

Microscopic characters: Somatic hyphae at colony periphery hyaline, smooth, thin-walled, branched, transversely septate, 1.5–4 µm diam, anastomosing, often developing coils, becoming inflated and melanized with age. Conidiomata most commonly absent, sporodochial in old cultures, composed of interwoven thick-walled melanized hyphae, setae absent. Conidiophores reduced, unbranched, single branched, becoming monoverticillate to richly branched. Conidiogenous cells monophialidic, acropleurogenous; *denticles* sometimes present, up to 4.5 µm in length; *terminal conidiogenous loci* (10.5–)19–28(–52) µm in length from septa; *phialides* ampulliform, sometimes constricted at the middle, curved or straight and tapering towards the apex, with distinct collarette, (6–)8–12(–16) × (2–)2.5–3.5(–4) *µm*. Conidia produced in slimy heads, hyaline and thin-walled in young colonies or nutrient poor medium, thick-walled and/or dematiaceous when colonies age, aseptate, smooth walled, some with indistinct hilum, on MEA globose to broadly ellipsoidal, rarely ellipsoidal, 3–6 × 3–5 *µm* (aspect ratio 1.0–1.2(–1.5)), on PDA and WA subglobose to ellipsoidal, rarely globose, (3–)3.5–5(–8) × 3–4(–4.5) *µm*, (aspect ratio 1.0–1.4(–2)).

Habitat and distribution: The sexual state of this fungus has not been observed and was only observed in pure cultures isolated from plant tissues that had resin on them. Isolates originate from collection sites in the Andes and Coastal range suggesting that its distribution overlaps that of *Araucaria araucana*. Its occurrence on other tree species has not been explored and a larger distribution may thus exist.

Additional isolates examined: Chile, Araucanía (IX), Conguillío National Park sector Los Paraguas, -38.697836°, -71.817216°, branches of *Araucaria araucana* (isolates from plant tissue covered in resin), 11 Dec 2017, F. Balocchi, culture AR011 = CMW 53535; culture AR074 = CMW 53538. — Villarrica National Park sector Puesco, -39.575582°, -71.493489°, branches of *Araucaria* *araucana* (isolates from plant tissue covered in resin), 13 Dec 2017, F. Balocchi, culture AR149 = CMW 53540 = CBS xxx; AR155 = 53542. — Biobío (VIII), Nahuelbuta mountain range, Trongol Alto, -37.564893°, -73.205764°, branches of *Araucaria araucana* (isolates from plant tissue covered in resin), 15 Jan 2019, F. Balocchi, culture FB041 = CMW 57582 = CBS xxx.

Distinguishing characters for Resinogalea araucana *and* R. tapulicola: Based on its sexual structures, *Resinogalea araucana* closely resembles *R. humboldtensis* and to a lesser extent, *Cryptocalicium blascoi* in terms of overall morphology. Ascomata for the second *Resinogalea* species found on *A. araucana* were not found, and thus, could not be compared. Ascomata of *R. araucana* are slightly shorter and wider than those of *R. humboldtensis*, the capitulum in *R. araucana* is more robust and only rarely cracking as it is common for *R. humboldtensis*, the colour of the mineral pruina covering the stipe is orange to darker-red, rather than yellowish green, and its ascospores are slightly larger. *Cryptocalicium blascoi* produces much smaller ascomata (smaller dimensions on most of its microscopical features too) and it is characterized by a greenish mazaedium, contrasting to the reddish-brown mazaedium of both *Resinogalea* spp.

Cultures of *R. araucana* and *R. tapulicola* resemble each other and could easily be confused. Cultures of *R. tapulicola* grow faster and at warmer temperatures than *R. araucana*, have a drier texture (not slimy), the mycelium commonly grows producing a petaloid pattern and a soluble pigment is released into the medium that becomes stained a reddish colour. Isolates of *R. tapulicola* produce more abundant coiled structures in its mycelium, have verticillated or richly branched conidiophores, and sporodochia are produced from filamentous melanized hyphae rather than pseudo-stromatic tissues as seen in *R. araucana*. *Resinogalea araucana* and *R. tapulicola* have several common morphological features in culture that serve to distinguish them from cultures of *Cryptocalicium blascoi*. These include their colony morphology (i.e., yellowish coloured filamentous colonies) with production of sporodochial spore masses, and microscopic features such as conidia produced from lateral denticles, well-developed phialides and dematiaceous conidia.

5. DISCUSSION

Results of this study revealed two novel species of *Resinogalea* for which the names *R. araucana* and *R. tapulicola* have been provided. These unusual fungi were isolated from resinous areas on the branches of *Araucaria araucana* in Chile. Ascomata were observed only in the case of *R. araucana* while *R. tapulicola* is known only from culture. Based on broad morphological features of the *R. araucana* ascomata and the very specific niche in which these fungi occur, it is likely that their closest relative is *R. humboldtensis* (Rikkinen et al. 2016).

Resinogalea humboldensis is a calicioid fungus also found on resin on branches of *A. humboldtensis* in New Caledonia. Our results showed that *R. araucana* produces ascomata having a similar shape and dimensions, as well as almost identical microscopical features to those described for *R. humboldtensis* (Rikkinen et al. 2016; Table 2). Additionally, these species share some uncommon features, such as a stipe covered with a mineral layer, erythrocyte-shaped ascospores, and a round capitulum covered with resin. Although all evidence suggests a close relationship between *R. araucana*, *R. tapulicola* and *R. humboldtensis*, the lack of cultures for the latter and thus an inability to compare DNA sequences precludes a conclusive confirmation of this relationship.

The results of our analyses using DNA sequence data for *R. tapulicola* and *R. araucana* showed that they reside in the recently described subclass *Cryptocaliciomycetidae* (Prieto et al. 2021). This subclass, and underlying taxonomic levels, were described based on the single species *C. blascoi*, found on bark and resin droplets on *Cupressaceae* species in the Mediterranean, and represent a discrete lineage of calicioid fungi in the *Eurotiomycetes*. These taxa were not known at the time *R. humboldtensis* was described, and consequently it was assigned to the *Bruceomyetaceae* (*Ascomycota*, *incertae sedis*) alongside *Bruceomyces castoris* (type species; Rikkinen 2003; Rikkinen et al. 2016). Similar to *R. humboldtensis*, there were no DNA sequences or cultures available for *B. castoris*. Based on the results of the present study including DNA sequence data and morphology, we propose to relocate the genus *Resinogalea* to the *Cryptocaliciaceae* family (*Cryptocaliciales*, *Cryptocaliciomycetidae*), alongside *Cryptocalicium*. *Bruceomyces* (*B. castoris*) however, is morphologically distinct from *Resinogalea* spp.

and *C. blascoi*. These taxa will need to be resolved separately and for the present best remain in their current positions.

Prieto et al. (2021) in their study of *Cryptocalicium* showed how broader morphological features such as ascomata type fail to provide sufficiently precise data to distinguish between higher taxonomic groups (e.g., classes or subclasses). In contrast, the use of only DNA based data can lead to the omission of species described solely by morphology that have not yet been sequenced (Balocchi et al. 2022a). The present study represents an attempt to include unresolved taxa described solely based on morphological characteristics (i.e., *Resinogalea humboldtensis*) linked to available DNA data. Species that lack sequences can be easily omitted (e.g., absence on predominantly used databases such as GenBank), and where possible efforts need to be made to obtain sequences for the species that lack them (e.g., sequencing of old herbarium specimens). This situation has shown to be relevant for fungi associated with *A. araucana* (this study; Balocchi et al. 2022a) and the rest of the *Araucariaceae* (Balocchi et al. 2022b).

This study adds two more fungal species only known to be associated with *Araucaria* species. Several fungi have been described on *A. araucana* in Chile (Butin 1968, 1970a, b; Grinbergs and Yarrow 1970; Butin 1975, 1986; Riess et al. 2016; Balocchi et al. 2021; Balocchi et al. 2022a) that have not been found in any other host or location. This is also true for a range of insect species (Giganti and Dapoto 1990; Kuschel 2000; Mecke and Galileo 2004; Beéche 2017) and mites (Chetverikov et al. 2014), showing the diverse assemblage of organisms strictly associated with these trees. Although limited, studies on closely related tree species such as *A. angustifolia* (Butin and Speer 1978; da Silva et al. 2015a, b) or *A. humboldtensis* (Beimforde et al. 2017) have also found rare fungal species associated with them. This includes *R. humboldtensis,* discussed in this study (Rikkinen et al. 2016). *Araucaria* forests, and to a larger extent, *Araucariaceae* forests, have remained poorly studied in terms of microbial biodiversity (Balocchi et al. 2022b). Studies such as the present one allow a glimpse into the yet to be discovered diversity harboured by these ancient and iconic trees.

6. ACKNOWLEDGMENTS

We would like to acknowledge the personnel of the forest health division and laboratory technicians at Bioforest S.A. for their assistance in laboratory and field work. We would also like to acknowledge Dr Seonju Marincowitz and Dr Tuan Duong for their assistance at different stages of the study.

7. REFERENCES

Abràmoff MD, Magalhães PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics international 11:36-42.

Balocchi F, Marincowitz S, Wingfield MJ, Ahumada R, Barnes I (2022a) Three new species of *Pewenomyces* (Coryneliaceae) from *Araucaria araucana* in Chile. Mycological Progress

Balocchi F, Wingfield MJ, Ahumada R, Barnes I (2021) *Pewenomyces kutranfy* gen nov. et sp. nov. causal agent of an important canker disease on *Araucaria araucana* in Chile. Plant Pathology 70:1243–1259. https://doi.org/10.1111/ppa.13353

Balocchi F, Wingfield MJ, Paap T, Ahumada R, Barnes I (2022b) Pathogens of the Araucariaceae: How much do we know? Current Forestry Reports 8:1–24. https://doi.org/10.1007/s40725-022-00164-z

Barnes I, Roux J, Wingfield MJ, Coetzee MP, Wingfield BD (2001) Characterization of *Seiridium* spp. associated with cypress canker based on ß-tubulin and histone sequences. Plant Disease 85:317–321. https://doi.org/10.1094/PDIS.2001.85.3.317

Beéche MA (2017) *Yanara*, new genus of Oecophoridae (Lepidoptera) associated with *Araucaria araucana* (Araucariaceae) from southern Chile. Boletín Museo Nacional de Historia Natural 66:21–28. http://boletinmnhn.cl/index.php/ojs/article/view/69

Beimforde C, Seyfullah LJ, Perrichot V, Schmidt K, Rikkinen J, Schmidt AR (2017) Resin exudation and resinicolous communities on *Araucaria humboldtensis* in New Caledonia. Arthropod-Plant Interactions 11:495–505. https://doi.org/10.1007/s11829-016-9475-3

Butin H (1968) A new species of *Ceratocystis* causing blue-stain in *Araucaria araucana*. Canadian Journal of Botany 46:61–63. https://doi.org/10.1139/b68-012

Butin H (1970a) Zwei neue Arten der Gattung *Phaeocryptopus* Naumov. Journal of Phytopathology 68:269–275. https://doi.org/10.1111/j.1439-0434.1970.tb02510.x

Butin H (1970b) Zwei neue *Caliciopsis*-Arten auf chilenischen Koniferen. Journal of Phytopathology 69:71–77. https://doi.org/10.1111/j.1439-0434.1970.tb03903.x

Butin H (1975) Beitrag zur Ascomyceten flora von Chile. Sydowia 27:267–292.

Butin H (1986) *Rhizothyrium parasiticum* sp. nov. (Coelomycetes), ein Blattparasit auf *Araucaria araucana* (Mol.) C. Koch. Journal of Phytopathology 115:313–317. https://doi.org/10.1111/j.1439-0434.1986.tb04343.x

Butin H, Speer EO (1978) Über einige parasitische Ascomyceten auf Nadeln der Brasilianischen Araukarie. Sydowia 31:9–26.

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556. https://doi.org/10.1080/00275514.1999.12061051

Carpintero DL, Dellapé PM (2006) *Pehuencoris*, a new genus of Cardiastethini (Heteroptera: Anthocoridae) from southern Argentina and Chile (Patagonia). Zoological Science 23:1039–1042. https://doi.org/10.2108/zsj.23.1039

Chetverikov PE, Beaulieu F, Beliavskaia AY, Rautian MS, Sukhareva SI (2014) Redescription of an early-derivative mite, *Pentasetacus araucariae* (Eriophyoidea, Phytoptidae), and new hypotheses on the eriophyoid reproductive anatomy. Experimental and Applied Acarology 63:123–155. https://doi.org/10.1007/s10493-014-9774-2

da Silva SS, Gusmão LFP, Castañeda-Ruiz RF (2015a) Conidial fungi on *Araucaria angustifolia: Trichoconis foliicola* sp. nov. and two new records from Brazil. Mycotaxon 130:1051–1059. https://doi.org/10.5248/130.1051

da Silva SS, Gusmão LFP, Castañeda-Ruiz RF (2015b) *Cryptocoryneum parvulum*, a new species on *Araucaria angustifolia* (Brazilian pine). Mycotaxon 130:465–469. https://doi.org/10.5248/130.465

De Beer ZW, Duong T, Barnes I, Wingfield BD, Wingfield MJ (2014) Redefining *Ceratocystis* and allied genera. Studies in Mycology 79:187–219. https://doi.org/10.1016/j.simyco.2014.10.001

Duong TA, De Beer ZW, Wingfield BD, Wingfield MJ (2013) Characterization of the mating-type genes in *Leptographium procerum* and *Leptographium profanum*. Fungal Biology 117:411–421. https://doi.org/10.1016/j.funbio.2013.04.005

Giganti H, Dapoto G (1990) Coleópteros de los bosques nativos del Departamento Aluminé (Neuquén-Argentina). Bosque 11:37–44. https://doi.org/10.4206/bosque.1990.v11n2-04

Grinbergs J, Yarrow D (1970) *Rhodotorula araucariae* sp. n. Antonie van Leeuwenhoek 36:455–457. https://doi.org/10.1007/BF02069046

Guerrero NR, Quintero MAO, Naranjo JCP (2012) Determinación del área foliar en fotografías tomadas con una cámara web, un teléfono celular o una cámara semiprofesional. Revista Facultad Nacional de Agronomía-Medellín 65:6399–6405. http://ref.scielo.org/h6c5r7

Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature methods 14:587. https://doi.org/10.1038/nmeth.4285

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in bioinformatics 20:1160–1166. https://doi.org/10.1093/bib/bbx108

Kornerup A, Wanscher JH (1967) Methuen handbook of colour. 2nd edn. Methuen & Co Ltd, London, England

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Kuschel G (2000) Curculionid (Coleoptera: Curculionoidea) fauna of *Araucaria araucana*. Revista Chilena de Entomologia 27:41–51.

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular biology and evolution 16:1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Mecke R, Galileo MHM (2004) A review of the weevil fauna (Coleoptera, Curculionoidea) of *Araucaria angustifolia* (Bert.) O. Kuntze (Araucariaceae) in South Brazil. Rev Bras Zool 21:505–513. https://doi.org/10.1590/S0101-81752004000300013

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences 95:2044–2049. https://doi.org/10.1073/pnas.95.5.2044

Prieto M, Etayo J, Olariaga I (2021) A new lineage of mazaediate fungi in the Eurotiomycetes: Cryptocaliciomycetidae subclass. nov., based on the new species *Cryptocalicium blascoi* and the revision of the ascoma evolution. Mycological Progress 20:889–904. https://doi.org/10.1007/s11557-021-01710-y

Rehner SA, Samuels GJ (1995) Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. Canadian Journal of Botany 73:816–823. https://doi.org/10.1139/b95-327

Riess K, Schön ME, Lutz M, Butin H, Oberwinkler F, Garnica S (2016) On the evolutionary history of *Uleiella chilensis*, a smut fungus parasite of *Araucaria araucana* in South America: Uleiellales ord. nov. in Ustilaginomycetes. PloS one 11:e0147107. https://doi.org/10.1371/journal.pone.0147107

Rikkinen J (2003) New resinicolous ascomycetes from beaver scars in western North America. Annales Botanici Fennici 40:443–450. https://www.jstor.org/stable/23726801

Rikkinen J, Beimforde C, Seyfullah LJ, Perrichot V, Schmidt K, Schmidt AR (2016) *Resinogalea humboldtensis* gen. et sp. nov., a new resinicolous fungus from New Caledonia, placed in Bruceomycetaceae fam. nova (Ascomycota). Annales Botanici Fennici 53:205–215. https://doi.org/10.5735/085.053.0408

Sung G-H, Sung J-M, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Molecular phylogenetics and evolution 44:1204–1223. https://doi.org/10.1016/j.ympev.2007.03.011

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal genetics and biology 31:21–32.

Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic acids research 44:W232–W235. https://doi.org/10.1093/nar/gkw256

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications, vol 18. vol 1. Academic Press, Inc, New York, pp 315–322

8. TABLES

Table 1. Taxa used for phylogenetic analyses, including representatives of the *Eurotiomycetes* and subsequent ranks, and *Lecanoromycetes* used as outgroup.

Taxonomic position	**Isolate**	**ITS**	**LSU**	**SSU**	***RPB2***	***MCM7***	***TEF***
EUROTIOMYCETES							
Chaetothyriomycetidae							
Chaetothyriales							
Cyphellophora guyanensis	MUCL 43737	NR_132880	KC455253	NG_065005	—	—	—
Exophiala eucalyptorum	CBS 121638	KC455245	KC455258	KC455302	—	—	—
Knufia perforans	CBS 885.95	NR_121502	NG_042586	NG_062148	—	—	—
Vonarxia vagans	CBS 123533	FJ839636	FJ839672	KC455310	—	—	—
Pyrenulales						—	—
Pyrenula nitida	F-5929	JQ927458	DQ329023	—	—	—	—
Pyrgillus javanicus	AFTOL-ID 342	DQ826741	DQ823103	DQ823110	—	—	—
Rhynchostoma proteae	CBS 112051	NR_132824	NG_073789	—	—	—	—
Verrucariales						—	—
Catapyrenium cinereum	AFTOL-ID 2230	—	EF643747	EF689829	—	—	—
Verrucaria lecideoides	AFTOL-ID 2295	EU010256	EF643798	—	—	—	—
Verrucaria muralis	AFTOL-ID 2265	EU010261	EF643803	EF689878	—	—	—
Coryneliomycetidae						—	—
Coryneliaceae							
Caliciopsis pinea	CBS 139.64	KP881691	DQ678097	DQ678043	EF411067	**xxx**	OM982873
Caliciopsis pseudotsugae	CBS 140.64	MT334518	MT334517	MT359911	—	—	—
Corynelia africana	PREM 57242	NR_153901	NG_058910	KP881719	—	—	—
Corynelia fructigena	PREM 57240	NR_153902	NG_058911	KP881720	—	—	—
Lagenulopsis bispora	PREM 57232	NR_154120	NG_060325	NG_061200	—	—	—
Pewenomyces kutranfy	CMW 54240	NR_172182	MT334515	NG_074914	—	—	—
Cryptocaliciomycetidae						—	—
Cryptocaliciales							
Cryptocalicium blascoi	ARAN-Fungi 14723	MW999967	MW999967	—	MZ020967	MZ020966	—
	Etayo 31798	MW999969	MW999951	MW999950	—	—	—
Resinogalea araucana sp. nov (sp. 1)	**CMW 53536**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 53539	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 53543	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 53544	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 57580	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 57581	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
Resinogalea tapulicola sp. nov (sp. 2)	**CMW 53535**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 53537	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 53538	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 53540	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 53542	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
	CMW 57582	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**	**xxx**
Eurotiomycetidae						—	—
Arachnomycetales							
Arachnomyces minimus	CBS 324.70	—	NG_056963	AJ315167	—	—	—
Arachnomyces nodosetosus	CCF 3975	HM205102	HM205103	HM205104	—	—	—
Arachnomyces peruvianus	CBS 112.54	NR_160079	NG_057623	—	—	—	—
Euriotiales						—	—
Aspergillus cremeus	NRRL 5081	NR_137455	NG_057314	NG_063231	—	—	—
Aspergillus herbariorum	DAOM 221134	JN942870	JN938918	JN938995	—	—	—
Aspergillus penicillioides	NRRL 4548	NR_131285	NG_057322	NG_063229	—	—	—
Byssochlamys nivea	CBS 100.11	NR_144910	NG_058631	NG_061072	—	—	—
Penicillium expansum	DAOM 215345	JN942855	JN938952	JN938958	—	—	—
Penicillium taxi	CBS 206.57	NR_145184	NG_057624	NG_062613	—	—	—
Sagenomella diversispora	CBS 354.36	NR_164428	NG_063981	NG_060979	—	—	—
Sagenomella verticillata	CBS 414.78	NR_160158	NG_064113	NG_062610	—	—	—
Thermoascus crustaceus	CBS 181.67	NR_144915	NG_064060	NG_062804	—	—	—
Thermoascus verrucosus	CBS 605.74	NR_103601	NG_075362	NG_074947	—	—	—
Trichocoma paradoxa	CBS 788.83	JN899398	FJ358290	FJ358354	—	—	—
Onygenales						—	—
Amauroascus verrucosus	NFCCI 2672	NR_160558	JQ517293	JQ517294	—	—	—

Aphanoascus verrucosus	NBRC 32381	NR_131309	NG_057011	—	—	—	—
Apinisia graminicola	CBS 721.68	—	NG_056945	NG_060969	—	—	—
Arthroderma curreyi	CBS 138.26	KT155805	AY176726	AJ315165	—	—	—
Ctenomyces serratus	CBS 187.61	NR_144890	NG_058765	NG_062605	—	—	—
Eremascus albus	CBS 975.69	MH859498	MH871279	FJ358348	—	—	—
Gymnascella littoralis	CBS 454.73	NR_155105	NG_057810	NG_062769	—	—	—
Nannizziopsis vriesii	CBS 407.71	NR_111874	NG_057976	NG_061166	—	—	—
Paranannizziopsis australasiensis	UAMH 10439	KF477218	—	KF466866	—	—	—
Mycocaliciomycetidae						—	—
Mycocaliciales							
Brunneocarpos banksiae	CBS 141465	NR_147648	MH878228	—	—	—	—
Chaenothecopsis golubkovae	Titov 6707 UPS	AY795859	AY795996	—	—	—	—
Chaenothecopsis sitchensis	HT22	—	KF157988	KF157976	—	—	—
Phaeocalicium polyporaeum	ZW-Geo60-Clark	AY789363	AY789362	AY789361	—	—	—
Sclerococcomycetidae						—	—
Sclerococcales							
Rhopalophora clavispora	CBS 637.73	NR_152542	KX537757	NG_061246	—	—	—
Sclerococcum ricasoliae	A.F.Fla6b	MT153964	MT153993	—	—	—	—
Sclerococcum vrijmoediae	NTOU 4002	KJ958534	KC692153	KC692152	—	—	—
LECANOROMYCETES							—
Lecanoromycetidae						—	—
Caliciales							
Calicium glaucellum	Tibell 22319 UPS	AY450569	AY453646	—	—	—	—
Calicium salicinum	CBS 100898	—	KF157982	KF157970	—	—	—

Note: A.F: Adam Flaku´s personal collection, Department of Lichenology, W. Szafer Institute of Botany, Poland; AFTOL-ID: Assembling the Fungal Tree of Life (AFTOL) project (www.lutzonilab.net/aftol); ARAN-Fungi: ARAN-Fungi Fungarium, the Basque Country, Spain; CBS: Centraalbureau voor Schimmelcultures, Netherlands; CCF: Culture Collection of Fungi, Department of Botany, Charles University in Prague, Czech Republic; Clark: Clark University, USA; CMW: Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DAOM: Canadian National Mycological Herbarium, Ottawa Research and Development Centre, Canada; Etayo: Javier Etayo´s personal collection, Spain; F: Personal number of Zdenek Palice, Institute of Botany, Academy of Sciences of the Czech Republic, HT: personal number of Hanna Tuovila, University of Helsinki, Finland; MUCL: Mycotheque de l'Universite Catholique de Louvain, Belgium; NBRC: Biological Resource Center, NITE, Japan; NFCCI: National Fungal Culture Collection of India, India, NRRL: Agricultural Research Service (ARS) Culture collection, Illinois, USA; NTOU: National Taiwan Ocean University, Taiwan; PREM: National Collection of Fungi, South Africa; UPS: Museum of Evolution, Sweden; UAMH 10439: Centre for Global Microfungal Biodiversity, University of Toronto, Canada.

Table 2. Morphological comparison of ascomata belonging to the novel *Resinogalea* found on *Araucaria araucana*, *Cryptocalicium blascoi*, *Resinogalea humboldtensis* and *Bruceomyces castoris*.

Character	**Dimension**	***Resinogalea araucana* sp. nov**	***Resinogalea humboldtensis***[1]	***Cryptocalicium blascoi***[2]	***Bruceomyces castoris***[3]
Habitat	Host	*Araucaria araucana*	*Araucaria humboldtensis*	*Juniperus* spp.	*Pseudotsuga*, *Abies*, *Acer*, *Alnus*
	Location	Chile	New Caledonia	Spain	USA
Ascomata	Height (µm)	(556) 766–1070 (1540)	650–1500	150–360	800–2500
Stipe	Length (µm)	(257) 364–598 (1069)	–	100–260	–
	Width (µm)	(70) 92–214 (275)	80–140	20–40	(75) 85–135 (200)
Hyphae on stipe	Width (µm)	2–3	(2.5) 3.0–4.5 (5.4)	(0.2–0.4) 1.2–1.6	–
Capitulum	Height (µm)	(121) 230–344 (500)	–	50–100	–
	Width (µm)	(204) 293–394 (467)	–	100–150	(170) 200–310 (430)
Mazaedium	Colour	reddish brown	reddish brown	light ochre to greyish green	pale greyish to reddish-brown
Ectal Excipulum	Cell width (µm)	3–6 (9)	–	3.5–5.5	–
Hymenium	Colour	hyaline or slightly yellowish	hyaline	pale brown	pale to medium brown, oil droplets
	Texture	angularis–intricata	–	angularis–globulosa	
Asci	Qualitative	clavate, pedicellate, bitunicate, evanescent, 8–spored, often with biseriately arranged spores	clavate, 8–spored, often with biseriately arranged spores	clavate, bitunicate, initially thick–walled (wall 1 µm), then thin–walled, evanescent, 8–spored	clavate, 8-spored, with relatively thick wall, not differentiated at the apex
	Length (total)	21–38 (57)	14–28	20–27	15–20
	Width (total)	4–6	6–9	5–7	7–9
	Sporiferous (L)	(15.5–)19–23(–30)	–	10–16	–
	Pedicel (L)	6–18.5(–32)	22–43	–	20–25
	Pedicel (W)	1.5–3	–	1 (diam.)	–
Ascospores	Shape	broadly ellipsoidal varying to globose or ellipsoidal	globose to broadly ellipsoidal, erythrocyte–like	globose to subglobose, rarely ellipsoid	ellipsoidal
	Colour	pale brown	pale brown	pale brown	pale brown
	Ornamentation	smooth or slightly verrucose	smooth, with very slight ornamentation visible in SEM	smooth when viewed in a light microscope	surface with longitudinal wrinkles visible under the light microscope
	Length (µm)	4–5.5 (6.5)	(3.0) 3.6 – 4.7 (5.8)	(3)3.3–4(4.7)	(6.0) 7.0–8.3 (9.8)
	Width (µm)	(3.5) 4–5.5	(2.7) 3.3 – 4.3 (4.6)	2.8–3.5 (4.0)	(4.0) 4.4–5.1 (5.8)
Paraphyses	Shape	filiform, obtuse, non–branched	filiform and non–branched	cylindrical, obtuse	–
	Colour	hyaline	hyaline	hyaline to very pale brown	–
	Septa (n)	(1) 2–4 (5)	–	2–4 septa	–
	Length (µm)	64–121(–172)	–	32–40	–
	Width (µm)	2–3	2.0–4.2	1.5–2	2.5–3.5

[1]Rikkinen *et al.* 2016; [2]Prieto *et al.* 2020; [3]Rikkinen 2003.

9. FIGURES

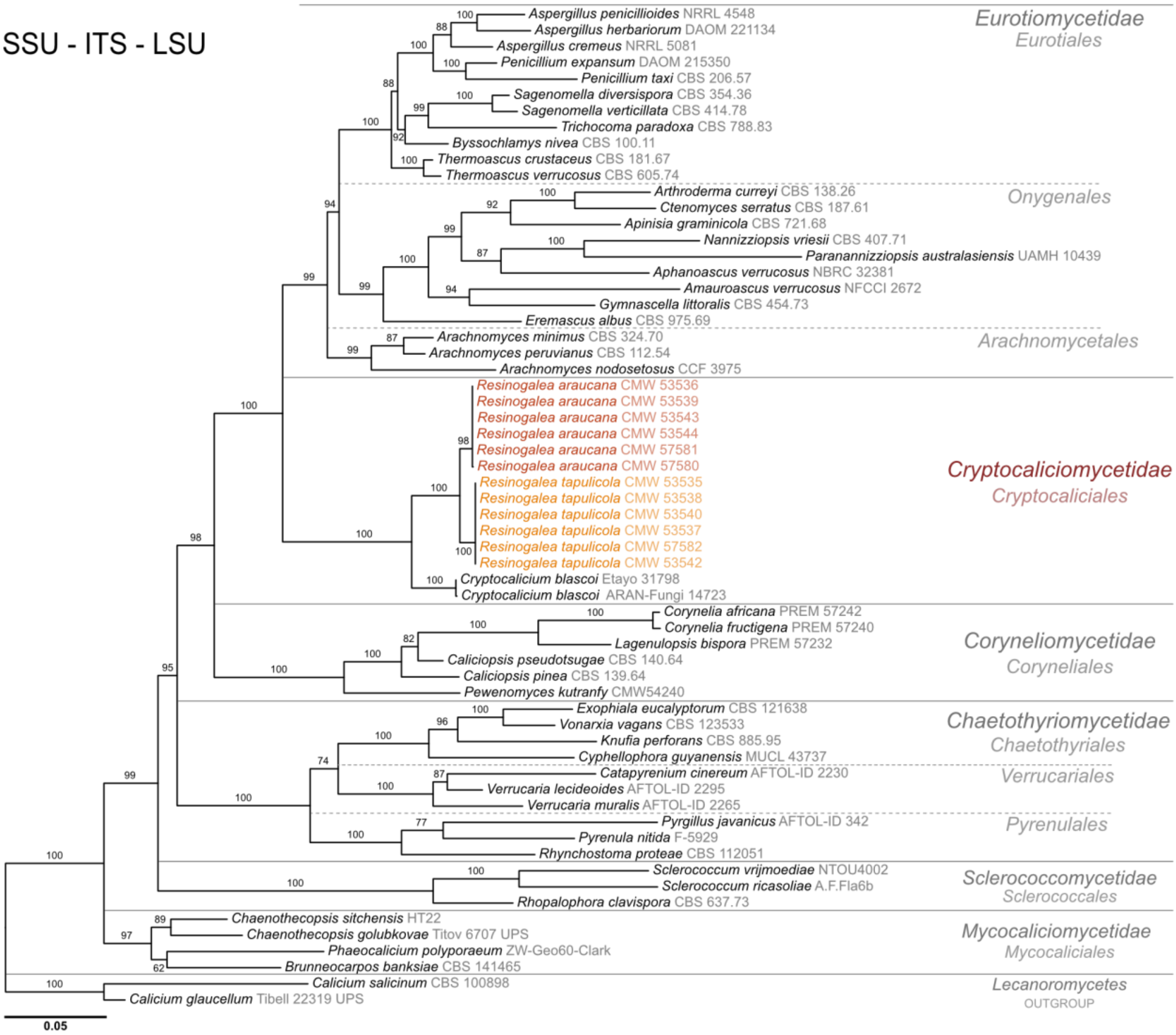


Fig. 1 Maximum-likelihood phylogenetic tree for the concatenated nuc SSU, ITS, and nuc LSU gene sequences for the subclasses and part of the subsequent orders within *Eurotiomycetes*. Two species of *Calicium* (*Lecanoromycetes*) were chosen as the outgroups. Isolates included in this study representing new species are shown in colour. Numbers on branches correspond to bootstrap values (n = 1,000). Scale bar: substitutions per site.

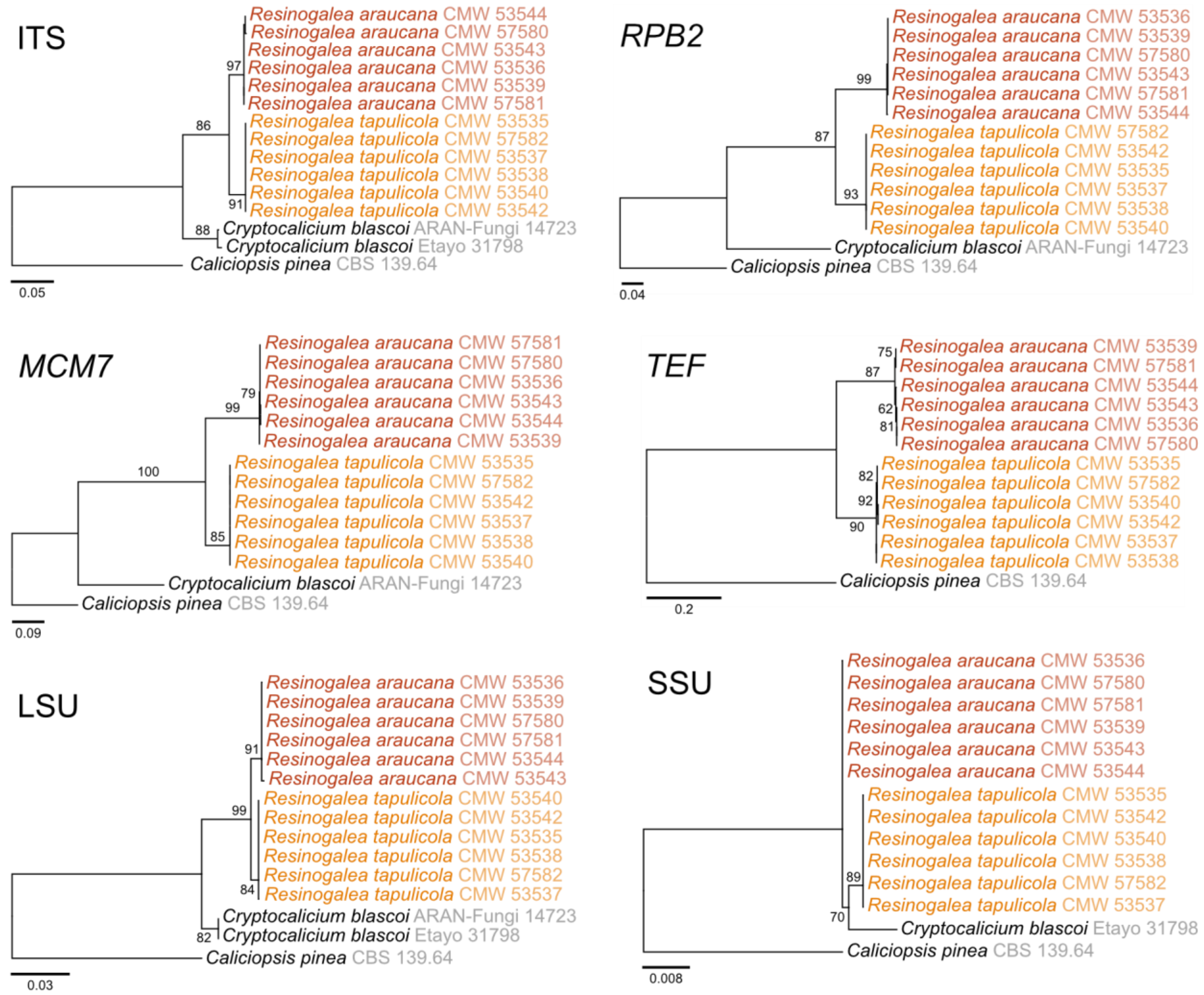


Fig. 2 Maximum likelihood phylogenetic trees for the ITS, *RPB2*, *MCM7*, *TEF*, LSU and SSU gene sequences. *Caliciopsis pinea* (*Coryneliaceae*, *Coryneliales*) was chosen as the outgroup. Isolates included in this study representing new species are shown in colour. Numbers on branches correspond to bootstrap values (n = 1,000). Scale bars: substitutions per site.

Fig. 3 Ascomata from *Resinogalea araucana*. (a–b) Habit, dry resin patches on wounded branches of *Araucaria araucana*; (c–e) Ascomata emerging from and around resin patches; (f–g) Developing ascomata; (h) Mature ascomata with protruding columnar mazaedium; (i) Ascoma with capitulum covered with resin. Scale bars: (a–b) = 2000 µm; (c) = 500 µm; (d–e, h–i) = 200 µm; (f–g) = 100 µm.

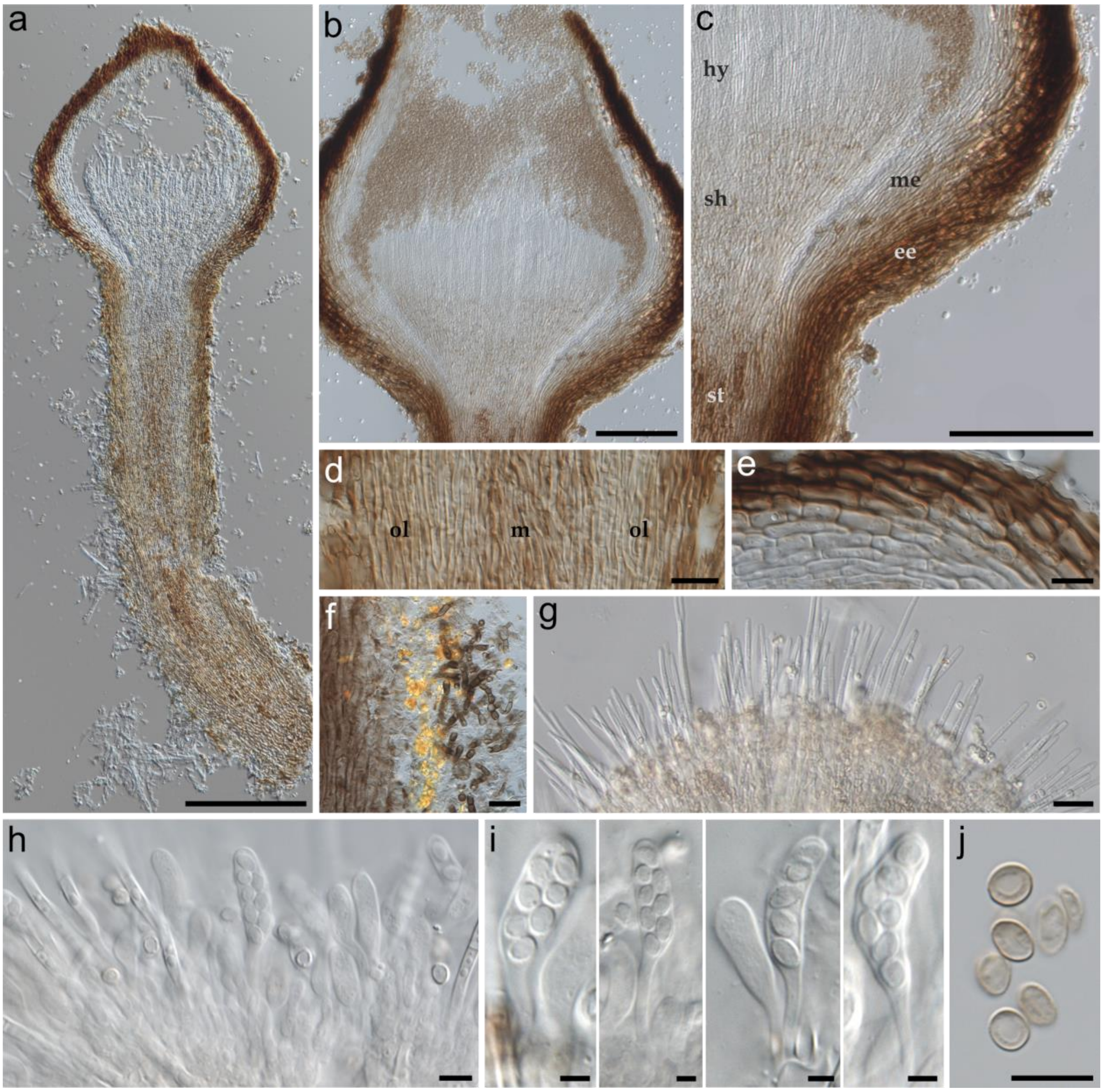


Fig. 4 Micrograph of the ascomata of *Resinogalea araucana*. (a–c) Cross sections of ascomata; hy: hymeniun, sh: subhymenium, me: medullary excipulum, ee: ectal excipulum, st: stipe; (d–e) Textures, d: stipe (m: medulla, ol: outer layers), e: Walls of the capitulum; (f) Mineral pruina and melanized dematiaceous hyphae at the outer layers of the base of the stipe; (g–h) Cross section of the hymenium containing paraphyses and developing asci; (i) Immature asci and ascospores; (j) Mature ascospores. Scale bars: (a) = 200 µm; (b–c) = 100 µm; (d–e) = 50 µm; (f–h) = 20 µm; (i–j) = 10 µm.

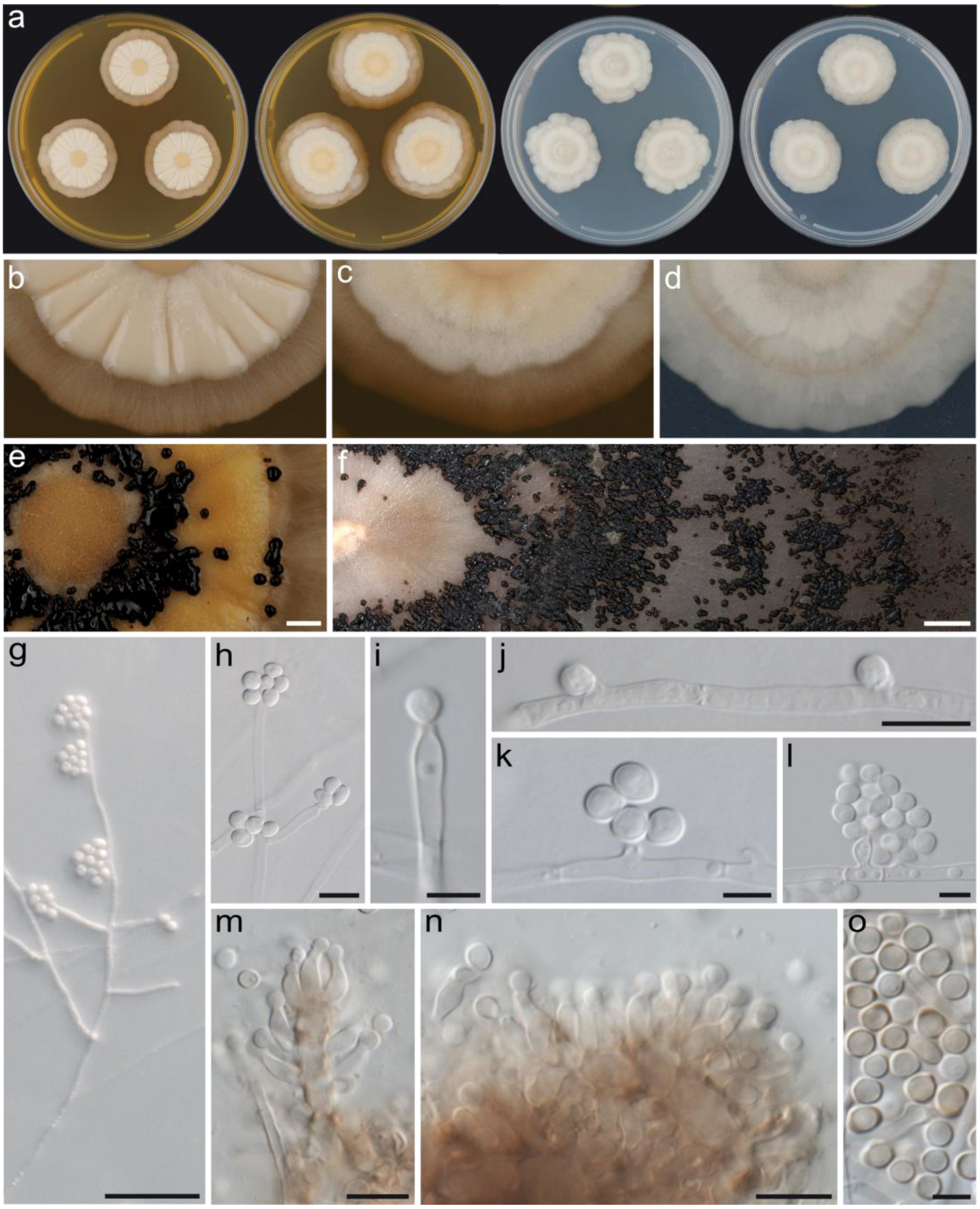


Fig. 5 Culture morphology of *Resinogalea araucana*, isolate CMW 57580. (a) Colonies after 28 d in darkness, from left to right, MEA (20 °C), MEA (25 °C), PDA (20 °C), PDA (25 °C); (b–d) Colony textures, d: MEA at 20 °C, c: MEA at 25 °C, d: PDA at 25 °C; (e–f) Sporodochia in 8-week or older cultures on MEA; (g) Conidiophores on mycelium; (h–l) Conidiogenous cells: terminal (h–i), lateral denticles (j–k), definite phialides (l); (m) Phialides on swollen and melanized hyphae; (n) Phialides emerging from pseudostromatic hyphae on sporodochia; (o) Conidia. Scale bars: (e–f) = 2 mm; (g) = 50 µm; (h, j, m–n) = 10 µm; (i, k–l, o) = 5 µm.

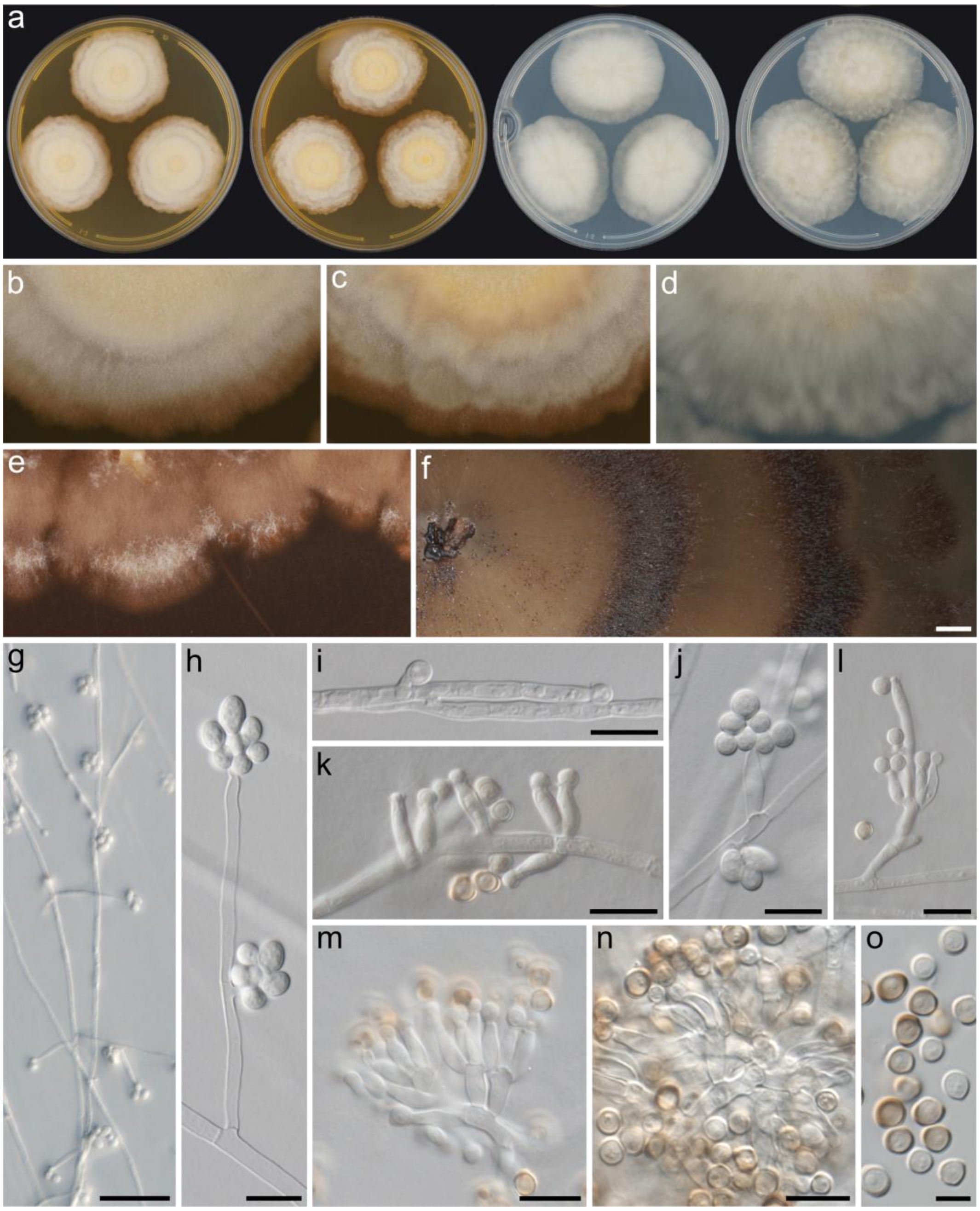


Fig. 6 Culture morphology of *Resinogalea tapulicola*, isolate CMW 53537. (a) Colonies after 28 d in darkness, from left to right, MEA (20 °C), MEA (25 °C), PDA (20 °C), PDA (25 °C); (b–d) Colony textures, b: MEA at 20 °C, c: MEA at 25 °C, d: PDA at 25 °C; (e–f) Morphological features on 8-week or older cultures on MEA: (e) staining of culture medium, (f) production of sporodochia; (g–h) Conidiophores on mycelium; (i–k) Conidiogenous cells: (i) terminal and lateral denticles, (j–k) definite phialides; (l–m) verticillate and richly branched conidiophores; (n) Phialides emerging from inflated and/or melanized hyphae on sporodochia; (o) Conidia. Scale bars: (f) = 2 mm; (g) = 50 µm; (h–n) = 10 µm; (o) = 5 µm.

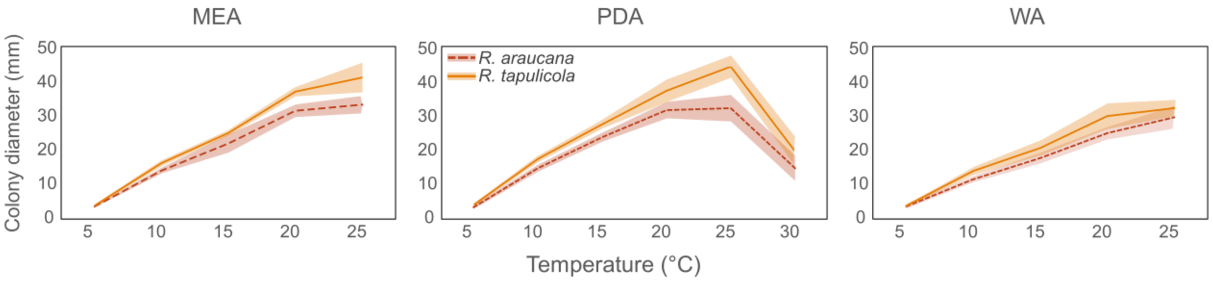


Fig 7 Culture growth after 28 days in darkness for two species of *Resinogalea* (six isolates each) on three culture media (MEA, PDA and WA) and at different temperatures. Solid lines represent average and shaded areas represent standard deviation.

SUMMARY

Araucaria (*Araucaria araucana*) is an ancient conifer endemic to the mountain ranges in Chile and Argentina. These trees are widely recognized by characteristic features such as their uncommon crown structure and their occurrence in enviroments with several months of snow cover. Additionally, they have a sacred value for the indigenous Mapuche-Pewenche comunities that co-inhabit the areas, and is recognized as the national tree of Chile.

A severe dieback disease emerged in natural *A. araucana* forests between 2015 and 2016, which was prevalent across most of the distribution of these trees. Symptoms of the disease included the death of branches, large portions of the crown, and in some rare cases entire trees. Extensinve field surveys revealed the existence of cankers on the branches and ocassionally stems of the diseased trees. Fruiting structures of a fungus in the *Coryneliaceae* were consistently found on diseased tissues, however, there were no reports in the literature referring to fungi in this family causing cankers on *A. araucana*. This unprecedented canker disease on *A. araucana* became the focus of the research of this thesis.

The first chapter of this thesis provides a review of the literature available on the diseases of the *Araucariaceae*, in which *A. araucana* resides. This iconic group of trees contains mostly species having limited geographical distributions, with several species categorized at some level of risk. A few species have had recent reports of diseases emerging, and this consistently illustrated the limited knowledge on the matter. For this review, multiple and diverse searches of the scientific literature were carried out to capture most of the available information. A total of 227 disease reports were found for the whole *Araucariaceae*, however, 85% of these reports represented only eight out of the 38 species in the family. The number of reports per tree species were consistent with their level of economical importance, and resulted in most of the tree species having extremely scarce to no disease reports. Furthermore, 75% of the pathogen-host combinations found in literature had no records of pathogenicity tests beinga carried out, and 78% of them had no records of the identity of the pathogen being confirmed with DNA sequence data. This lack in knowledge sets a threatening scenario for the preservation of these species, especially in a scenario where globalization and climate change are driving the emergence of forest diseases in an increasing trend.

The second chapter of this thesis focused on the canker disease discovered on *A. araucana* in its natural distribution. The aim of this research was to study the symptomatology associated with the canker disease, and to identify the causal agent. Through monitoring diseased trees and specific branches over a two year period, it was demonstrated that these cankers girdled the branches ultimately resulting in their death. Through the collection of samples, microscopic examination and isolations on culture media, a fungus in the *Coryneliaceae* was found predominantly associated with the symptoms. Morphological and phylogenetic analyses using three gene regions demonstrated that this fungus

represented a novel genus and species close to *Caliciopsis*, described herein as *Pewenomyces kutranfy*. Pathogenicity tests on naturally regenerated *A. araucana* trees in the field resulted in isolates of this species causing similar lesions as those originally observed. The results of this chapter confirmed that the cankers on branches resulted in their death, and that these were caused by a fungus representing a new genus and species.

The third chapter focused on a group of isolates obtained from *A. araucana* branches that were preliminary identified as *Pewenomyces* species in Chapter 2, but that remained unresolved. These isolates included a group obtained from fruiting structures on dead leaves around cankers and others obtained through isolations from plant tissues. Preliminary phylogenetic analyses separated these isolates into three distinct clades. The aim of this chapter was to consider the identities of these isolates, in relation to the previously described *P. kutranfy*, and two species of *Caliciopsis* (*C. brevipes* and *C. cochlearis*) previously described from *A. araucana* based on morphology. No DNA sequences were available for the *Caliciopsis* species, and therefore, fresh collections and herbarium specimens of these were included in the study. Morphological and phylogenetic analyses with seven gene regions showed that the studied isolates represent in three distinct species, distinct from *P. kutranfy* and from both *Caliciopsis* species. These were described as *P. lalenivora*, *P. tapulicola* and *P. kalosus*, with the latter two only known from cultures. The results of this chapter added three more species to *Pewenomyces* from *A. araucana*, and three more species to the *Coryneliaceae* from Chile. These findings support the hypothesis of *Pewenomyces* being native to this country.

The fourth and last chapter of this thesis considered the identity of a rare calicioid fungus found emerging from resin on the branches of *A. araucana*. Fruiting structures on the samples had an unusual morphology, resembling closely those of *Resinogalea humboldtensis*, a fungus found on resin on branches of *A. humboldtensis* in New Caledonia. *Resinogalea humboldtensis* was placed in the *Bruceomycetaceae* along with *Bruceomyces castoris* based solely on morphology, with no DNA sequence or living cultures available for either species. The aim of this study was to consider the identity of the calicioid fungus on *A. araucana*. Isolates in culture media were obtained from the calicioid fungus on *A. araucana* by isolating from ascospores on the fruiting structures and from plant tissues covered in resin. Phylogenetic analyses using six gene regions resolved the isolates in two clades in the newly described subclass *Cryptocaliciomycetidae*, along its only other member, *Cryptocalicium blascoi*. However, the fungus from *A. araucana* more closely resembled *R. humboldtensis* than *C. blascoi*, and furthermore, *R. humboldtensis* more closely resembled the fungus on *A. araucana* than *B. castoris*. Therefore, the study in this chapter proposes the relocation of the genus *Resinogalea* to the *Cryptocaliciomycetidae*, close to *Cryptocalicium*, and two new species, *Resinogalea araucana* and *Resinogalea tapulicola* were described. These new species, add to a number of organisms exclusively known from *Araucaria araucana*, including those described in the previous chapters, and suggests that there is a large microbial diversity harboured by these ancient and iconic trees yet to be discovered.

The studies carried out in this thesis, focused on a recently emerged canker disease on *A. araucana* with no known causal agent, and resulted in a review of the literature on diseases of these trees and its relatives, the identification and description of the causal agent of the aforementionded disease, and the description of several novel species exclusively known from these trees. All the fungi described along the research chapters had some degree of relationship with the cankers on branches of *A. araucana*, and were part of the complex and diverse assembly of unknown organisms originally obscuring the identity of its cause. The study describing *Pewenomyces kutranfy* resolved the causal agent for the canker disease and the description of three other *Pewenomyces* demonstrated the diversity of fungi in this group on *A. araucana* and Chilean forests. Additionally, the results of this thesis provide substantial structure to the *Coryneliaceae*, and to the newly described subclass *Cryptocaliciomycetidae* incorporating species neglected due to the lack of DNA sequence data. All these results support the hypothesis that the species newly described in *Pewenomyces* and *Resinogalea* in this thesis are native to the environment where they were found. They also appear to have intimate relationship with *A. araucana* and most likely coevolved with this iconic tree.