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

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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. Although the holotypes for the two *Caliciopsis* species did not yield adequate DNA for a phylogenetic analysis, a detailed morphological study established that these species were clearly different from any of the *Pewenomyces* taxa. The three putative species are consequently described here as *Pewenomyces lalenivora* sp. nov., *P. tapulicola* sp. nov. and *P. kalosus* sp. nov.

Keywords: Caliciopsis . Coryneliales . Coryneliomycetidae . Hypsotheca . Monkey puzzle tree . Taxonomy

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.

MATERIALS AND METHODS

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*).

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/).

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, *BT1*, *BT2*, *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 + *BT1* + *BT2* + *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.

Morphological studies

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). Macro- and microscopic structures observed in the samples were named using the terminology provided by Kirk et al. (2008).

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.

RESULTS

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, *BT1*, *BT2*, *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.

Morphology of fruiting structures

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).

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.

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.

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.

Pewenomyces sp. 1

Pewenomyces lalenivora Balocchi & Marinc., sp. nov

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 149332.

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 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 accumulates. 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 (CBS 149330), 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 63336; 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, CBS 149331.

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* differ from those of *P. kutranfy* being slightly 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.

Pewenomyces sp. 2

Pewenomyces tapulicola Balocchi & Marinc., sp. nov

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 149333.

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 area in both media, with increased mycelial 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 becomes 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, CBS 149334; 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, CBS 149335.

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.

Pewenomyces sp. 3

Pewenomyces kalosus Balocchi & Marinc., sp. nov

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 149329.

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 grow 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, CBS 149328.

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.

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 dependent on 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 those of Wood et al. (2016) and 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.

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Competing interests

The authors declare no competing interests.

Author contributions

IB, MJW and RA guided and supervised this research. The samples used in this study were collected by FB. Morphological investigations and illustrations were done by FB and SM. Molecular data and phylogenetic analyses were performed by FB. FB wrote the first draft and all authors contributed to its development and completion.

Data Availability

All sequence data generated in this study is available on GenBank (see Table 1). The alignment files and original tree outputs are available in the Open Science Framework (OSF) repository, accessible at https://osf.io/83y9s/?view_only=c24d40c35f94477ca0e1f4aeb06249b1.

REFERENCES

Assefa A, Abate D, Stenlid J (2014) Characterization of *Corynelia uberata* Fr., a putative fungal pathogen of *Podocarpus falcatus* in Ethiopian forests. Forest Pathol 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 Pathol 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? Curr For Rep 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 Dis 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. An Soc de Biol Pernamb 14:111–118

Benny GL, Samuelson DA, Kimbrough JW (1985a) Studies on the *Coryneliales*. I. *Fitzpatrickella*, a monotypic genus on the fruits of *Drimys*. Bot Gaz 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*. Bot Gaz 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*. Bot Gaz 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*. Bot Gaz 146:437–448. https://doi.org/10.1086/337544

Butin H (1970) Zwei neue *Caliciopsis*-arten auf chilenischen koniferen. J Phytopathol 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. Exp Appl Acarol 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 System Evol 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 System Evol 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 Descr 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 Descr 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 Biol 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*. Can J Bot 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. Can J Bot 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*. Mycol Prog 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. Appl Environ Microbiol 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. Revist Fac Nac Agron Medellín 65:6399–6405

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

Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat 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. Brief Bioinform 1. https://doi.org/10.1093/bib/bbx108

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

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 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. Mol Biol Evol 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. Proc 2011 TeraGrid Conf: 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. Proc Natl Acad Sci 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 Syst Evol 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 Biol 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. Can J Bot 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. Mol Phylogenet Evol 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 Res 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. J Bacteriol 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. Stud Mycol 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 Bull 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. Jpn J Mycol 57:47–58. https://doi.org/10.18962/jjom.57.1_47

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)	2.47–4.62	(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)	1.09–1.69	(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.

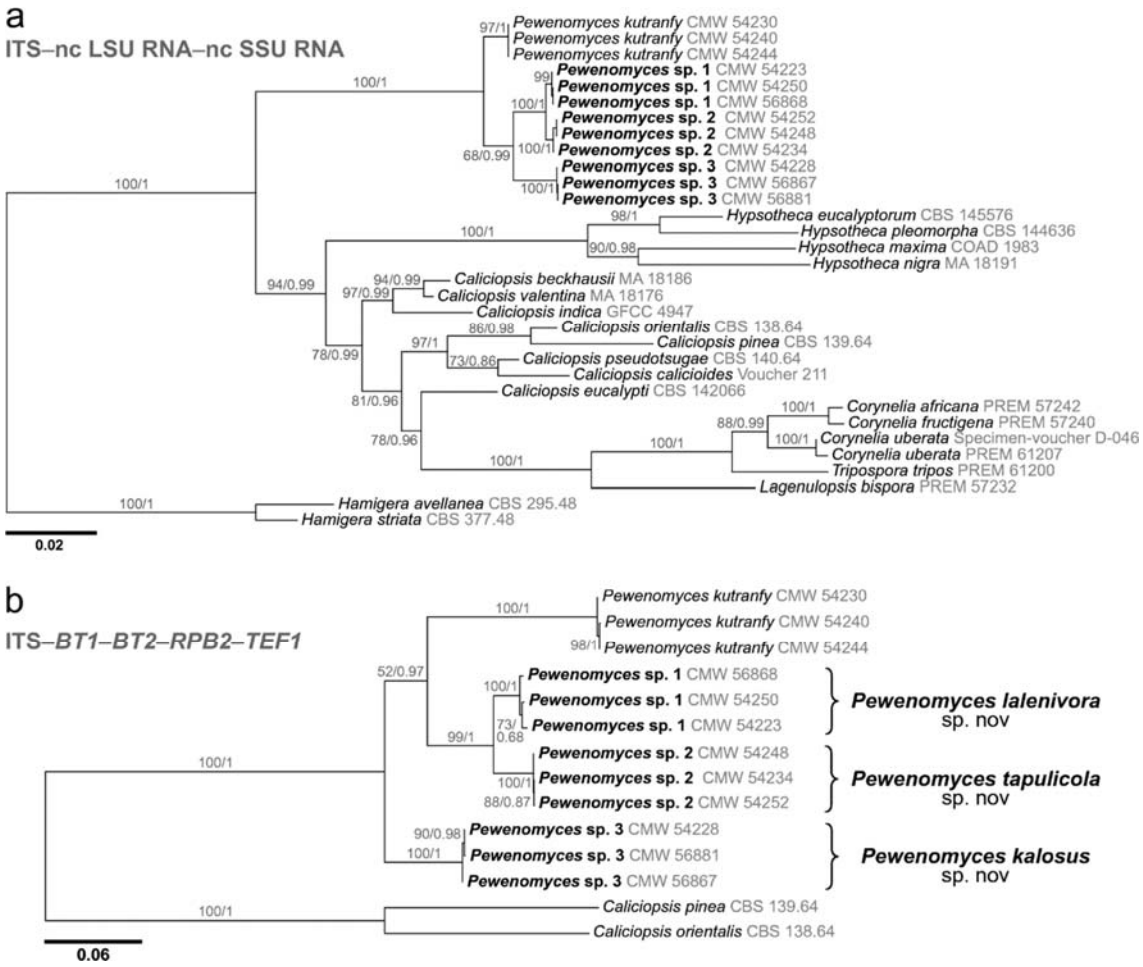

Fig. 1 Multigene phylogenetic trees for the *Coryneliaceae* including sequences from cultures obtained from cankers on *Araucaria araucana*. **a**. Maximum likelihood tree for the concatenated nc SSU rDNA, ITS and nc LSU rDNA for the *Coryneliaceae*, placing novel isolates (in bold) inside *Pewenomyces*. **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.

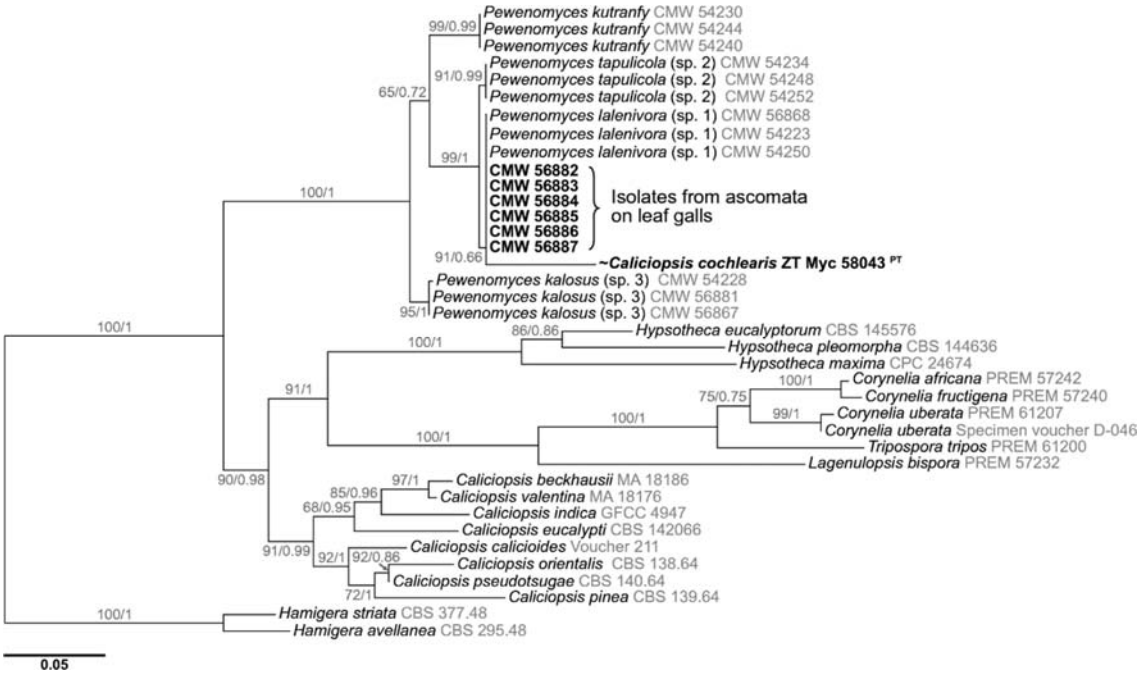

Fig. 2 Maximum likelihood tree for the ITS region for the *Coryneliaceae* including sequences from cultures obtained from ascomata on leaf galls (in bold), and the ITS-1 region sequence for the herbarium specimen ZT Myc 58043 (*Caliciopsis cochlearis* paratype, also in bold). Numbers on branches correspond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

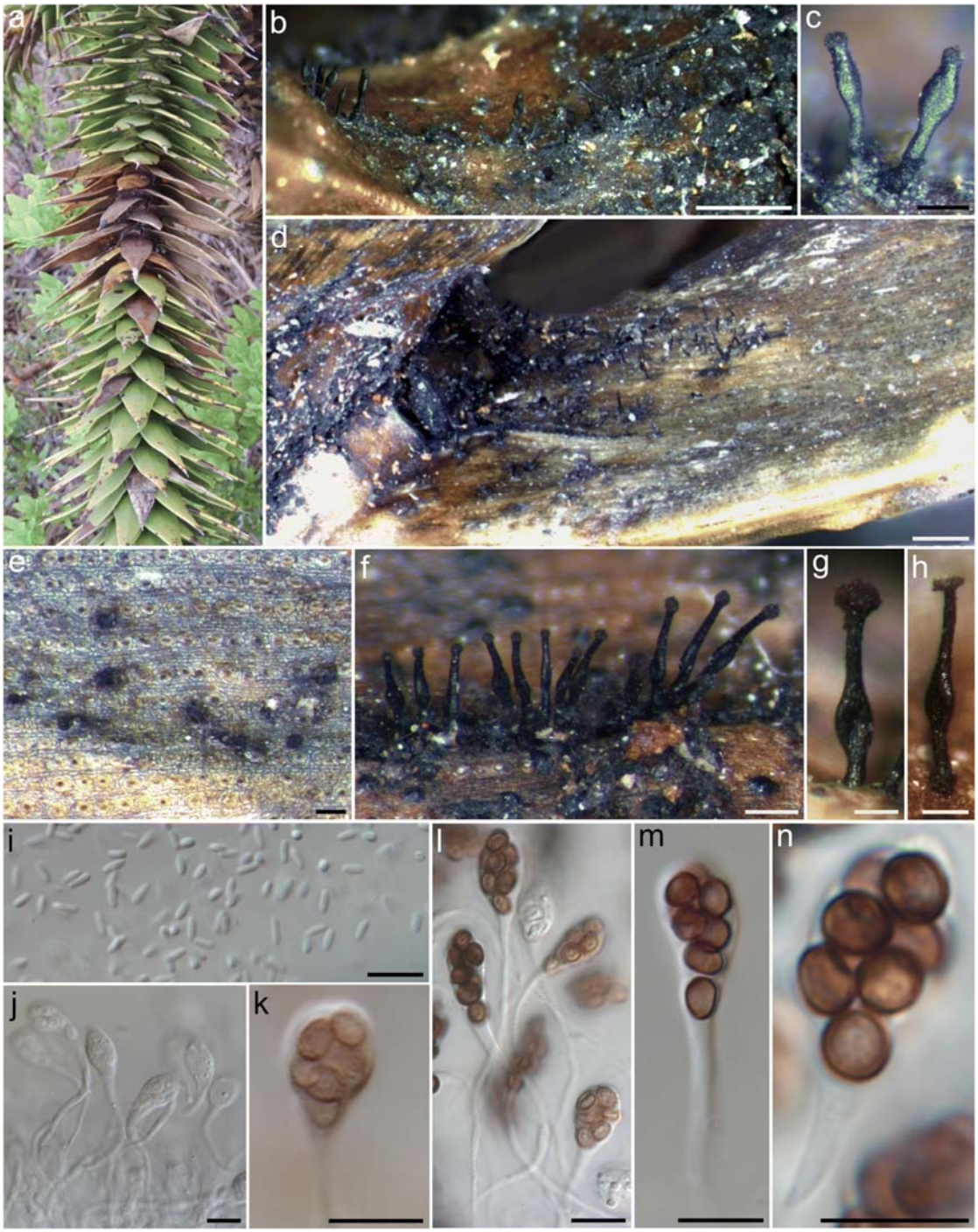

Fig. 3 *Pewenomyces* species sporulating on mature cankers on branches of *Araucaria araucana*. **a**. Mature canker on branch. **b-c**. Ascomata of *Pewenomyces kutranfy* at the base of leaves and emerging from the phloem. **d-h**. Spermogonia and ascomata of a second *Pewenomyces* species (sp. 1) emerging from dead leaf tissues. **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.

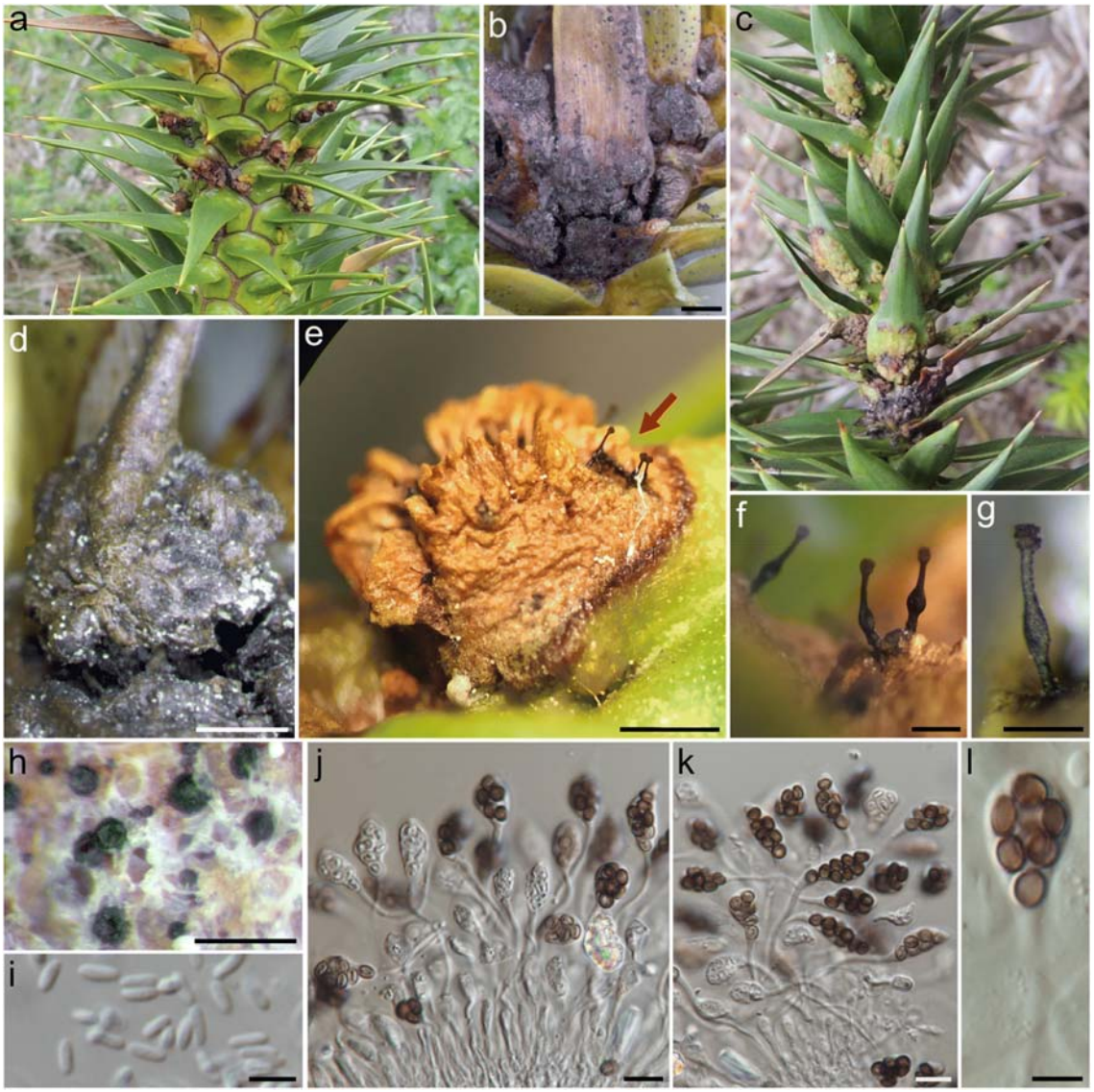

Fig. 4 *Caliciopsis*-like fungus on galls on *Araucaria araucana* collected from sites in the coast range (Nahuelbuta) and on the Andes Mountain range. **a**. Galls on the base of leaves on a young main stem. **b**. Old galls on the base of leaves on a mature branch. **c**. Galls on the base of leaves 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.

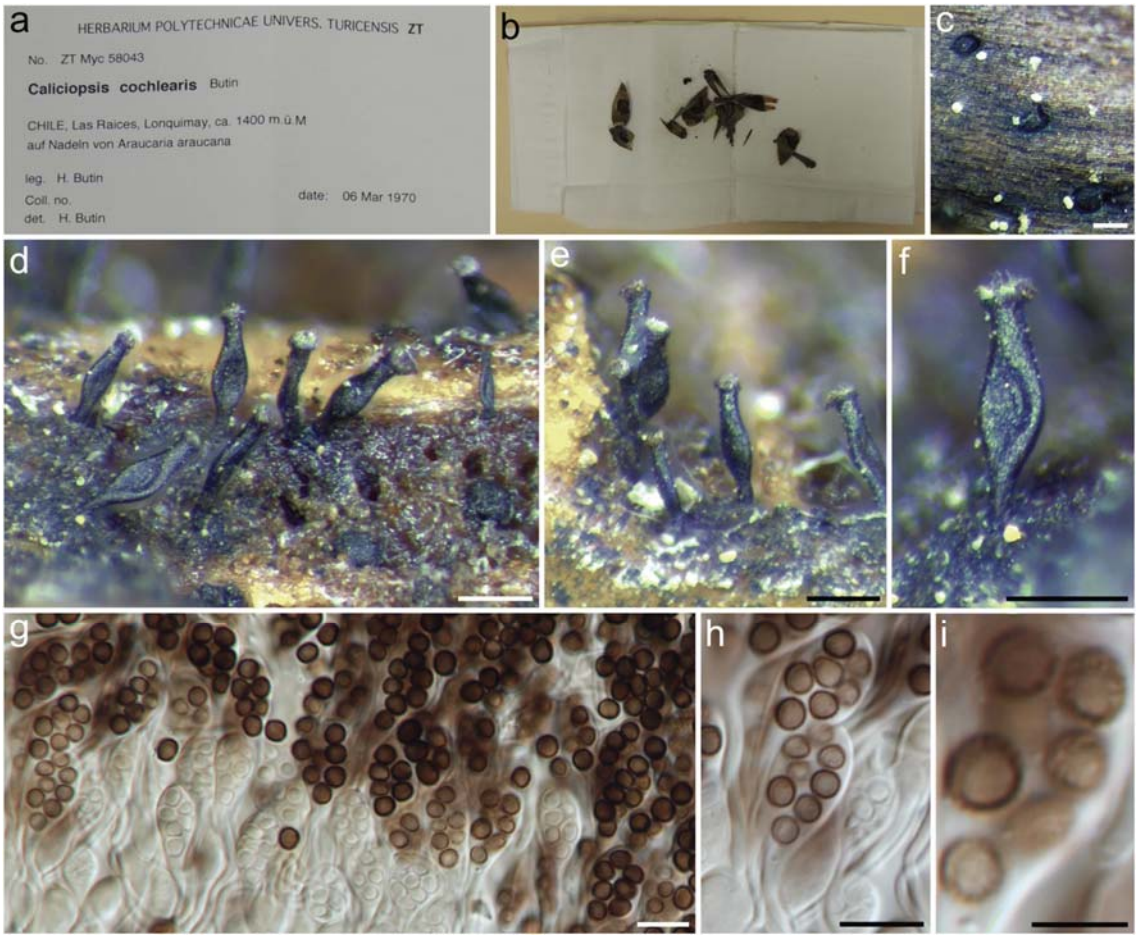

Fig. 5 Micrograph of herbarium specimen ZT Myc 58043 of *Caliciopsis cochlearis* paratype on leaves of *Araucaria araucana*. **a-b**. Labels and specimen identification. **c**. Spermogonia. **d-f**. Ascocarps. **g-h**. Mature and immature asci and ascospores. **i**. Immature ascospores showing verrucose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

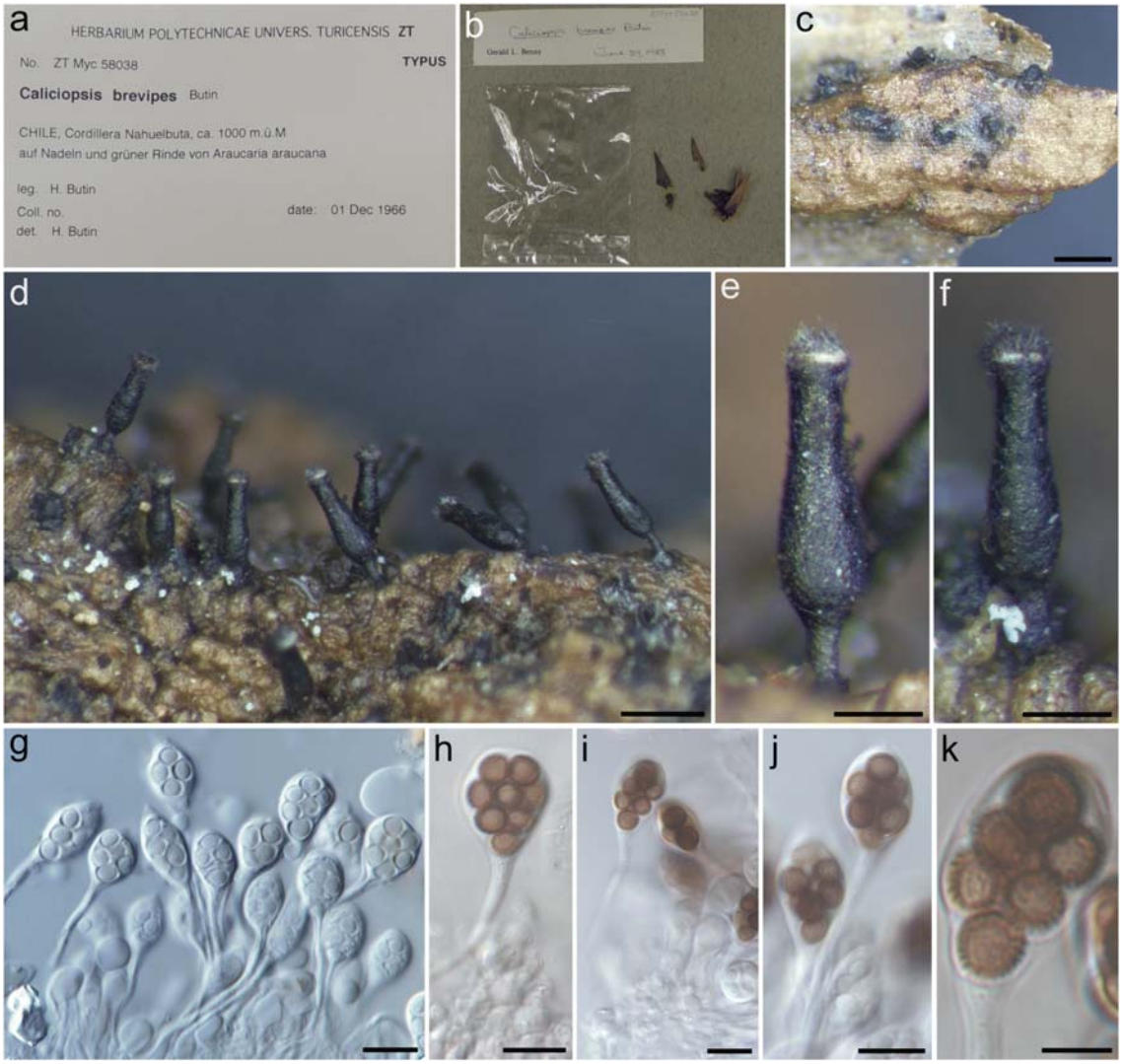

Fig. 6 Micrograph of herbarium specimen ZT Myc 58038 of *Caliciopsis brevipes* holotype on leaf galls on *Araucaria araucana*. **a-b**. Labels and specimen identification. **c**. Spermogonia. **d-f**. Ascocarps. **g**. Immature asci. **h-j**. Mature asci and ascospores. **k**. Mature ascospores showing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

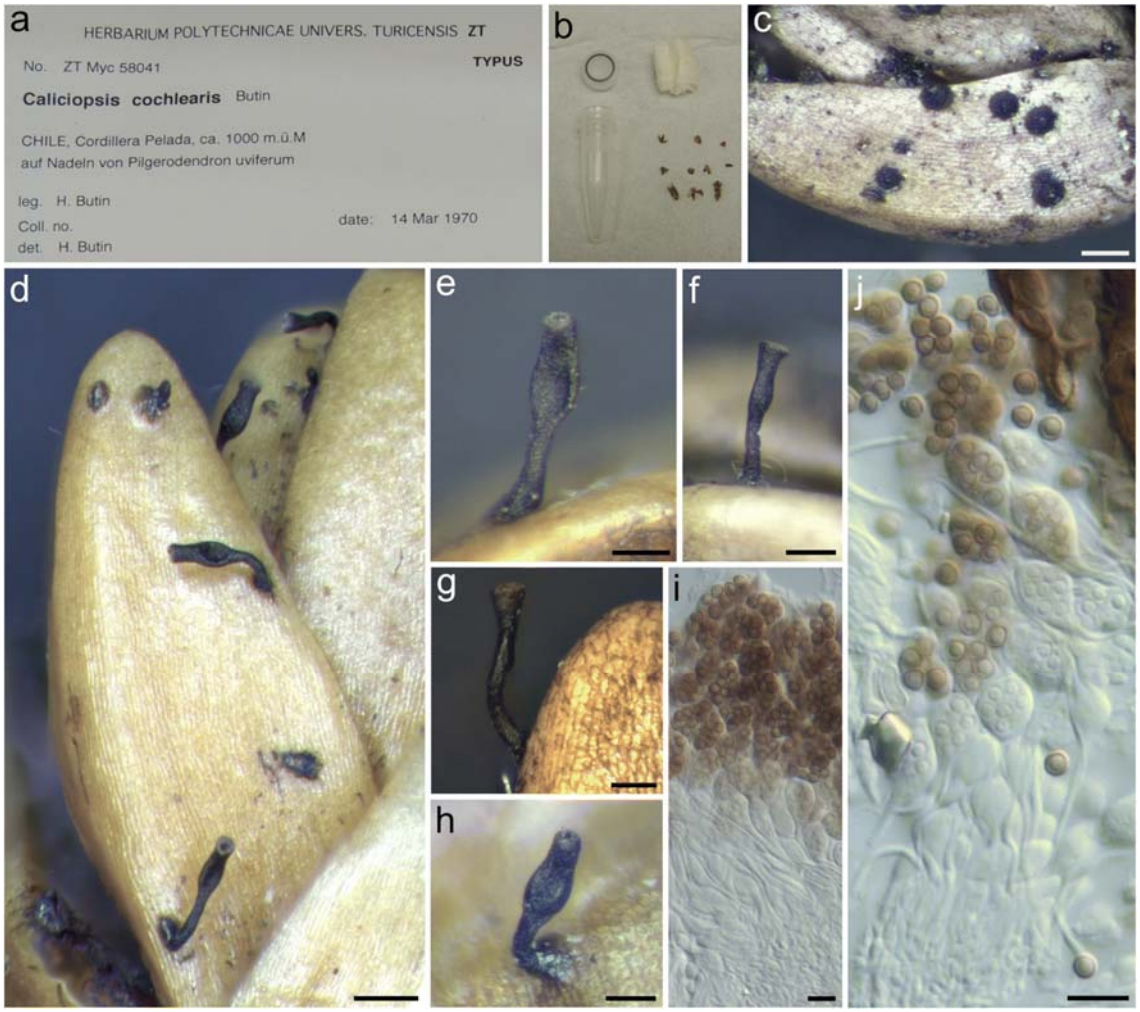

Fig. 7 Micrograph of herbarium specimen ZT Myc 58041 of *Caliciopsis cochlearis* holotype on leaves of *Pilgerodendron uviferum*. **a**-**b**. Labels and specimen identification. **c**. Spermogonia. **d**-**h**. Ascocarps. **i**-**j**. Mature and immature asci and ascospores. Scale bars: **c–d** = 200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

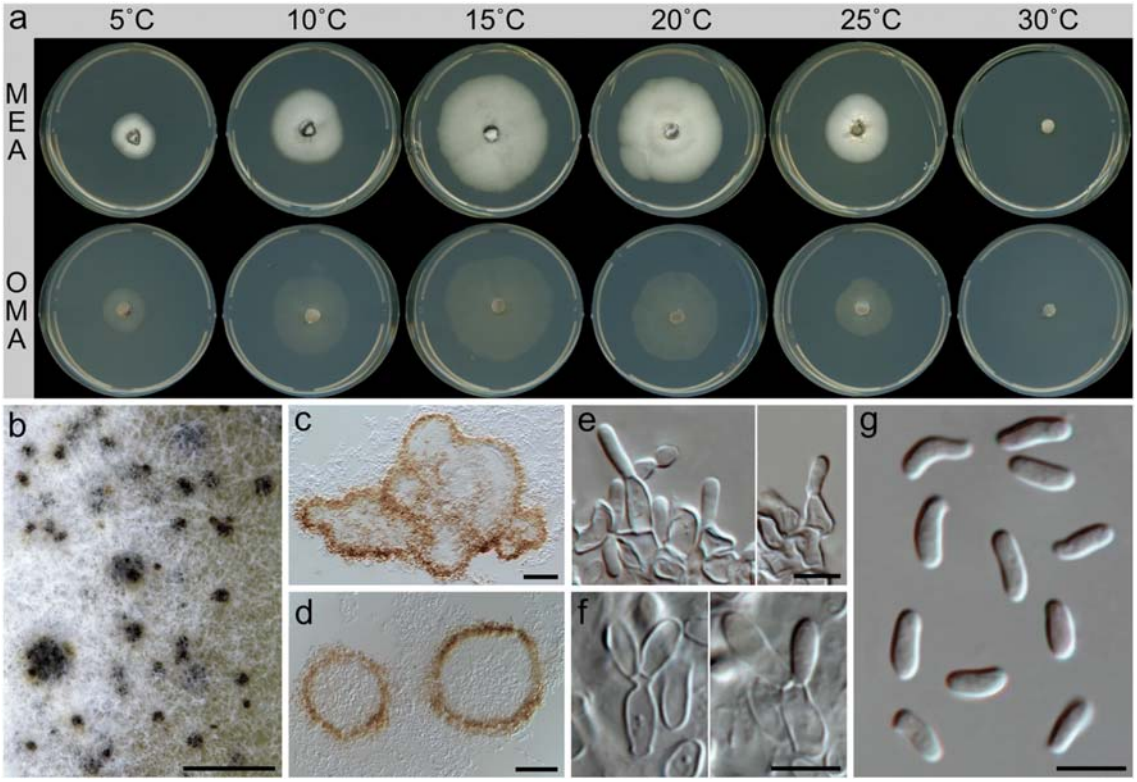

Fig. 8 Micrograph of *Pewenomyces lalenivora* (sp. 1; ex-holotype CMW 56868). **a**. Cultures grown on two different media (MEA, OMA) at 5–30 °C for 28 d in the dark. **b**. Colony showing conidiomata embedded in aerial mycelium. **c-d**. Vertical section of conidiomata. **e-f**. Conidiogenous cells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

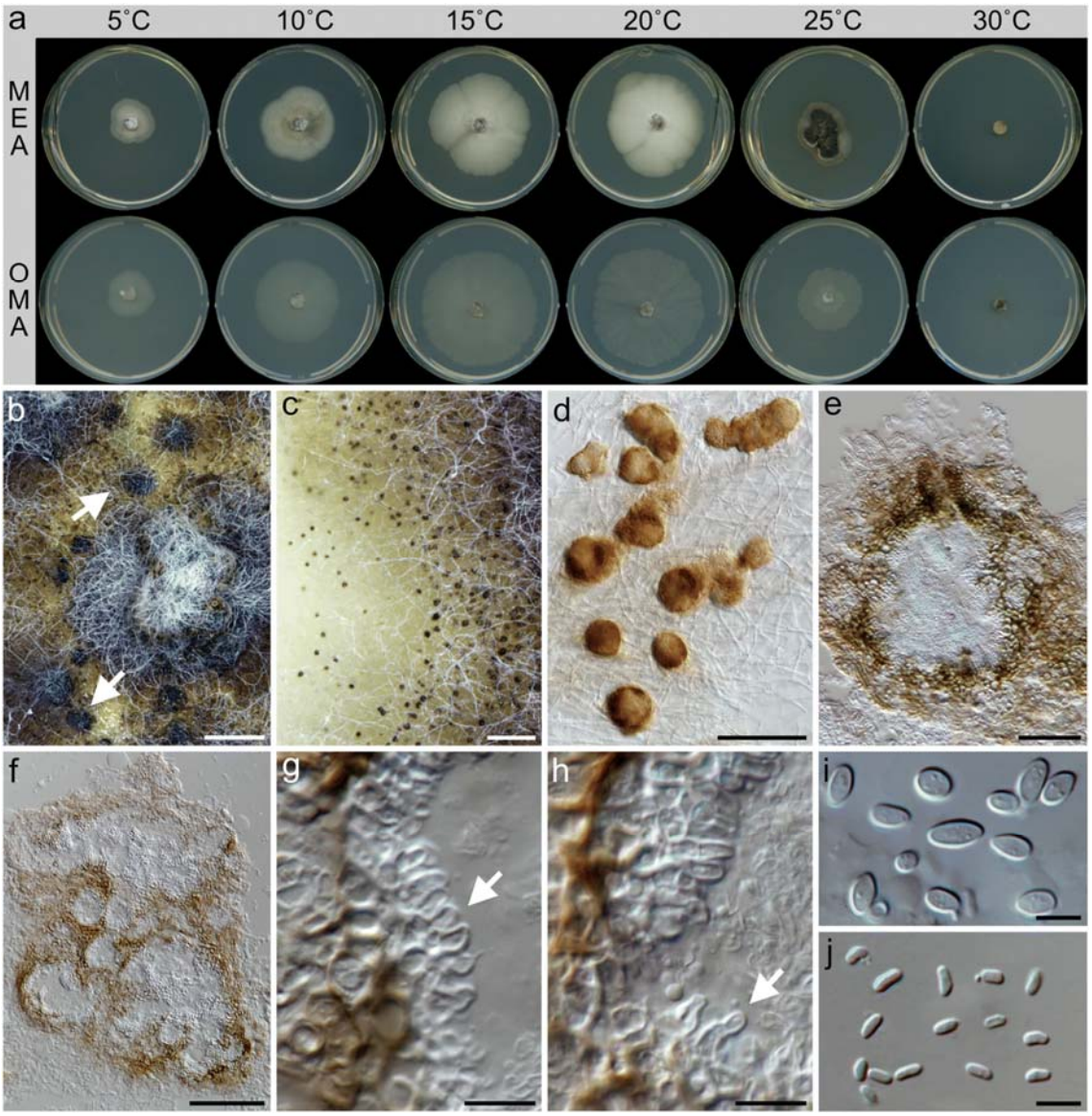

Fig. 9 Micrograph of *Pewenomyces tapulicola* (ex-holotype CMW 54234). **a**. Cultures grown on two different media (MEA, OMA) at 5–30 °C for 28 d in the dark. **b**. Conidiomata (arrows) semi-immersed in 2% MEA. **c-d**. Sclerotia-like structures. **e-f**. Vertical section of conidiomata: unilocular (**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.

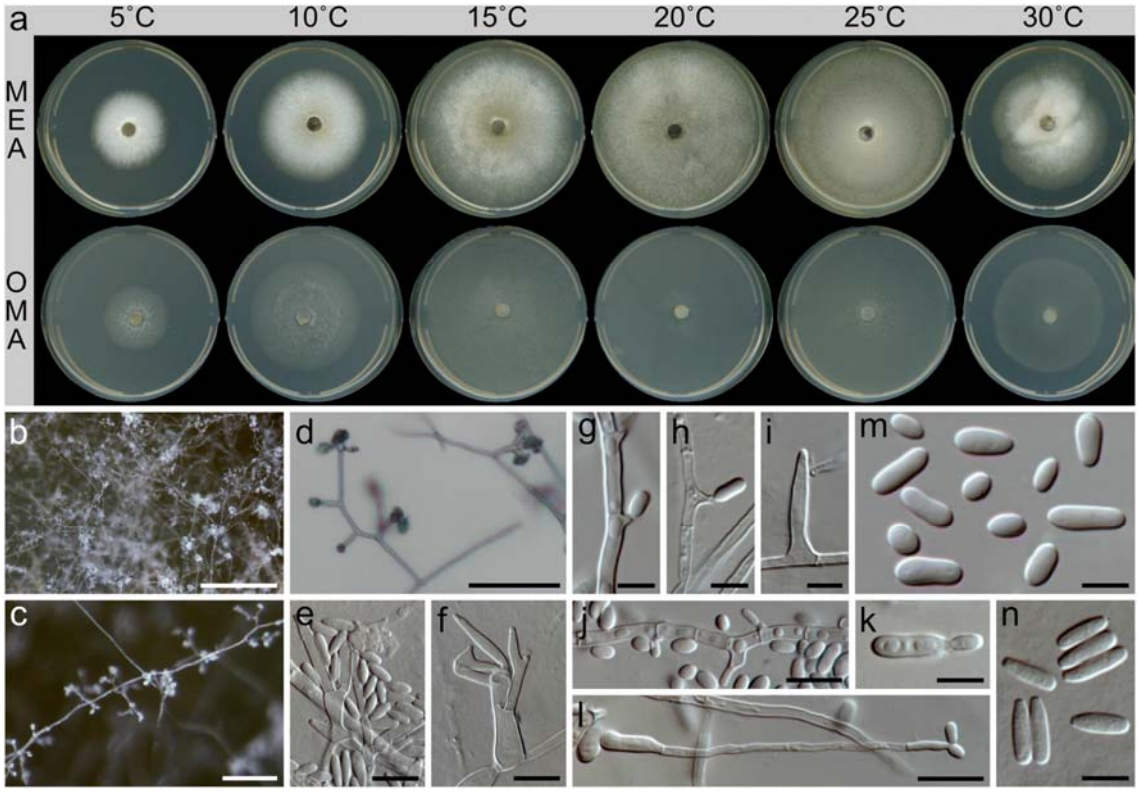

Fig. 10 Micrograph of *Pewenomyces kalosus* (sp. 3; ex-holotype CMW 54228). **a**. Cultures grown on two different media (MEA, OMA) at 5–30 °C for 28 d in the dark. **b-d**. Conidiophores with slimy droplets on aerial hyphae on 2% MEA. **e-j**. Various conidiophores: macronematous (**e-f**), micronematous (**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.