



# *Phaeotremella camelliae* sp. nov. (Phaeotremellaceae, Tremellales), A Novel Yeasts Isolated from Tea-Oil Fruits in Jiangxi Province, China

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## Abstract

Sixty-two isolates among the 65 yeast strains isolated from Jiangxi province, China, were identified into 15 known species based on the sequence analysis of the D1/D2 domains of the LSU rRNA and ITS region. The other three strains, GaoanZ14, GaoanC57, and GaoanC191, isolated from tea-oil fruits, were identified as two undescribed species of *Phaeotremella* based on the multiple gene sequence analysis, physiological, and biochemical comparisons. Strains GaoanC57 and GaoanC191 had one substitution difference both in the D1/D2 domains of the LSU rRNA and ITS region. They formed a separate branch from the other *Phaeotremella* species in the D1/D2 and multiple genes trees, and differed from the known species by at least 10 nucleotide substitutions in the D1/D2 domains and more than 6% mismatches in the ITS region. The phylogenetic analysis indicated that those two strains represent a novel species of *Phaeotremella*, for which the names *Phaeotremella camelliae* sp. nov. (Holotype CGMCC 2.6141, Mycobank MB832699) is proposed. Only one strain, GaoanZ14, represents the other undescribed species of *Phaeotremella*, so it will be described in latter when more strains are found.

## Introduction

The order Tremellales is the largest known group in the class Tremellomycetes, which is a heterogeneous group showing a high diversity in both morphology and life cycles [1, 2]. It comprises teleomorphic species, for example *Tremella* spp., and anamorphic species, such as yeast species of *Bullera*, *Cryptococcus* and *Fellomyces* [1–5]. The family Phaeotremellaceae, with the type genus *Phaeotremella*, was proposed in the *Tremellales* by Liu et al. based on the

multiple gene phylogenetic analyses [6]. According to the phylogenetic analyses, five *Tremella* species (*T. foliacea*, *T. mycetophiloides*, *T. mycophaga*, *T. neofoliacea* and *T. simplex*) and two *Cryptococcus* species (*C. fagi* and *C. skinneri*) were transferred to the genus *Phaeotremella* [6]. Spirin et al. revised the taxonomy of the *Phaeotremella foliacea* complex based on morphological, ecological, geographic, and multiple gene sequences analyses, and consequently proposed four new taxonomic combinations and one new species in the *Phaeotremella*, which were *P. fimbriata* comb. nov., *P. frondosa* comb. nov., *P. fuscossuccinea* comb. nov., *P. roseotincta* comb. nov. and *P. eugeniae* sp. nov. [7]. Recently three new *Phaeotremella* species, namely *P. lacus*, *P. lactea* and *P. ovata*, were describing by Li et al. [8, 9].

In this study, more than 60 yeast strains were isolated from seven samples of tea-oil (*Camellia oleifera*) and *Gardenia jasminoides* in Gaoan county, Jiangxi province, China. Among them two yeast strains, GaoanC57 and GaoanC191, isolated from two samples of dried tea-oil fruits were identified as a novel species of *Phaeotremella*, namely *Phaeotremella camelliae* sp. nov., based on the multiple gene sequence analysis and phenotypic comparison.

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## Material and Methods

### Yeast Isolation and Phenotypic Properties

Seven samples of the fruits of tea-oil (*Camellia oleifera*) and *Gardenia jasminoides* that dried but still attached to the plant were collected from different adjacent trees, respectively, nearly in the same area of Gaoan county (28° 27' 55.43" N, 115° 31' 37.70" E, 71 m asl.) in Jiangxi province, southwest China, in February 2019. Those fruits were cut into pieces and placed in 15 ml YM liquid medium (1.0% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, pH 6.0 with chloramphenicol 100 µg/ml) and incubated at 25 °C for 24 h in a shaking bed (150 rpm). All culture broth was firstly filtered through a 10 µm pore size filter (wanda, China) to remove filamentous fungi and debris, then the filtered broth was filtered through 3 µm pore size filter (wanda, China), finally the 3 µm filter membrane was removed and soaked in the Erlenmeyer flask with 10 ml sterile water at 25 °C for 10 min in a shaking bed (250 rpm). The soaked liquid was gradiently diluted and spread on YM plates (1.0% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast, 2.0% agar, pH 6.0 with chloramphenicol 100 µg/ml) and incubated at 25 °C for 3–7 days. All yeast colonies were purified by repeated streaking on YM agar plates.

Morphological, physiological and biochemical characteristics were examined according to standard methods Kurtzman et al. [10]. All physiological tests were conducted in liquid media. The potential sexual cycles of new species were investigated using YM, PDA, V8 (10% V8 juice, 2% agar) and corn meal agar (CM, 5% infusion corn meal, 1.5% agar, Difco) at 10 °C and 25 °C for one month.

### Phylogenetic Analysis

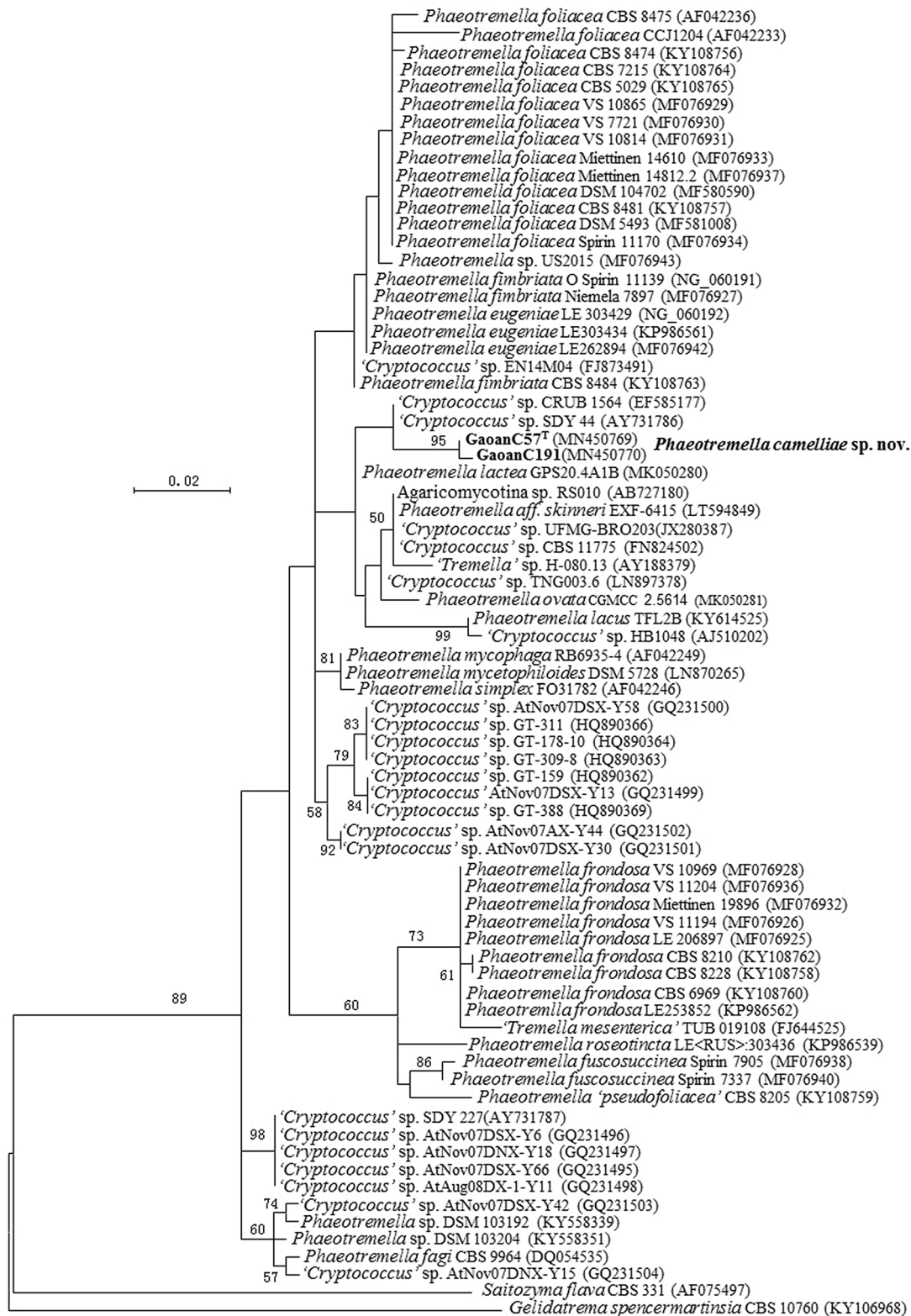
Genomic DNA was extracted from yeast cells that were actively growing on YPD medium, using the DNA extraction kit (Aidlab BioDev., Beijing, China). The ITS region (including the 5.8S rRNA gene) and the D1/D2 domains of the LSU rRNA gene were amplified using the protocols described previously [11]. The SSU rRNA gene, the second subunits of the RNA polymerase II gene (RPB2) and the translation elongation factor 1-a (TEF1) gene were amplified and sequenced according to Wang et al. [12]. The GenBank accession numbers for the sequences of the ITS region, LSU rRNA D1/D2 domains, SSU rRNA, RPB2 and TEF1 were MN450766, MN450768, MN450769, MN450770, MN450795, MN450796, MN450800 and MN450801, respectively. Those new sequences were assembled and inspected using the DNAMAN software

(Lynnon Biosoft), and were subsequently aligned with Muscle program [13]. Positions that were ambiguous to align were excluded manually. Then, multiple sequence-alignments for ITS, D1/D2 and TEF1 were concatenated as a combined file with MEGA 7.0 [14]. The evolution model of the two datasets, the D1/D2 dataset and the combined three genes dataset, were determined using the model selection in MEGA 7.0 [14]. The Maximum-likelihood phylogenetic trees based on the two datasets were constructed to show the phylogenetic relationship of the isolates with their closest phylogenetic neighbors using the best-fit model GTR + I + G. Confidence values were estimated from bootstrap analyses of 1000 replicates [15].

## Results and Discussion

A total of 65 strains were isolated from seven samples of the fruits of tea-oil and *Gardenia jasminoides* collected from Gaoan county, Jiangxi province, China. Among them 62 strains belong to 15 known species identified with the “less than 1%” thresholds in either the ITS or D1/D2 regions recommended by Fell et al. [16], Scorzetti et al. [17] and Kurtzman and Fell [18], which are *Apiotrichum porosum* (2), *Apiotrichum xylopi* (2), *Bullera alba* (5), *Candida melibiosica* (5), *Candida sanyiensis* (2), *Candida solani* (1), *Curvibasidium cygneicollum* (3), *Cystofilobasidium capitatum* (2), *Cystofilobasidium infirmominium* (1), *Tausonia pullulans* (14), *Hannaella pagnoccae* (1), *Occultifur externus* (3), *Occultifur kilbournensis* (2), *Papiliotrema flavescens* (17) and *Saitozyma podzolica* (2). Among those 65 strains, three ones, GaoanZ14, GaoanC57 and GaoanC191, represent two undescribed species of *Phaeotremella*. GaoanC57 and GaoanC191 differed from each other by one substitution in the D1/D2 domains of the LSU rRNA and two substitutions in the ITS region, respectively, which indicated that they are conspecific. Only one strain, GaoanZ14, represent the other undescribed species, so we did not formally described it in this study.

A blast search based on D1/D2 sequences against GenBank database showed that GaoanC57 and GaoanC191 had the highest match with *Phaeotremella ovata*, *Phaeotremella lactea*, *Phaeotremella fimbriata* (NG\_060191) and *Phaeotremella eugeniae* (NG\_060192) with about 98% similarity (10–14 nucleotides differences). The ITS blast result showed that those two strains also closely related to the *Phaeotremella* species, such as *Phaeotremella foliaceae* (MF076902), *P. lactea* and *Phaeotremella lacus*, and differed from those strains by more than 34 nucleotides (~6%) mismatches. The phylogenetic tree constructed from the sequences of the D1/D2 domains indicated that those two strains formed a clade with ‘*Cryptococcus*’ sp. CRUB 1564 (EF585177), ‘*Cryptococcus*’ sp. SDY 44 (AY731786), *P. lactea*, *P. ovata*, *P. lacus*



**Fig. 1** Phylogenetic tree drawn from the Maximum-Likelihood analysis of sequences of the D1/D2 domains of LSU, depicting the relationship of *Phaeotremella camelliae* with closely related taxa. Bar: 0.02. Bootstrap percentages over 50% from 1000 replicates are shown

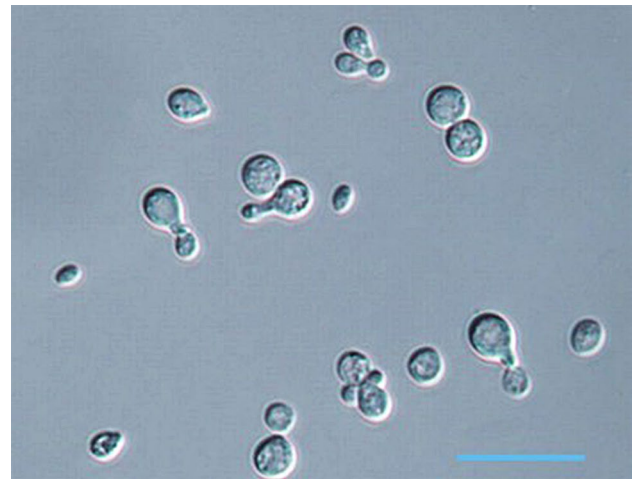
and so on (Fig. 1). The three genes tree also supported that they had a close relationship with *P. ovata*, *P. lactea* and *P. lacus* (Fig. 2). The above results suggested that those two strains represent a novel *Phaeotremella* species, for which *Phaeotremella camelliae* sp. nov. was proposed to accommodate them.

**Description of *Phaeotremella camelliae* Sun, Jiang, Li and Wang sp. nov. MycoBank MB832699**

*Etymology*: the specific epithet *camelliae* refers to *Camellia*, the plant genus from which the type strain was isolated.

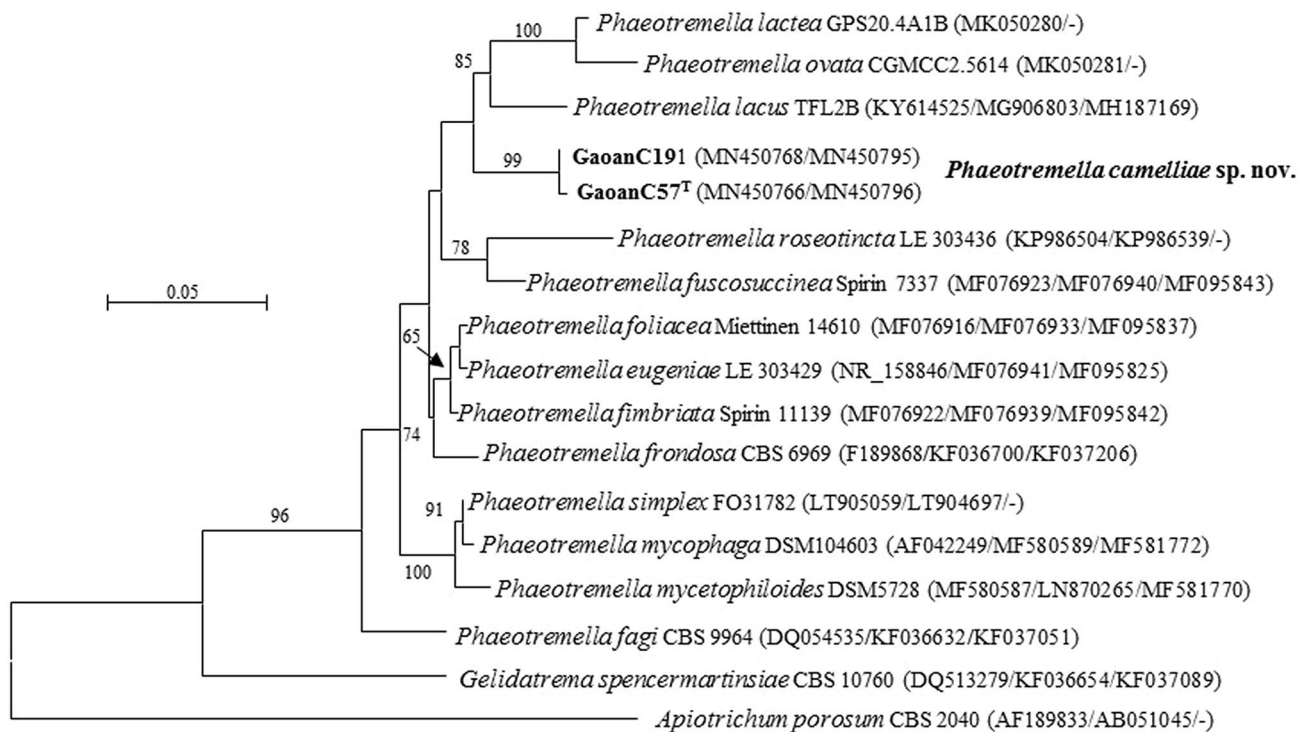
In YM broth, after 7 days at 25 °C, cells are ovoid, ellipsoidal and subglobose, 3.3–8.6 × 5.8–10.0 μm and single or pairs, budding is monopolar (Fig. 3), a sediment is present. On YM agar, after 1 month at 25 °C, the streak culture is cream to yellow, butyrous, smooth and glossy. The margin is entire. In Dalmau plate culture on corn meal agar at 25 °C for up to 3 weeks, pseudohyphae and hyphae are not formed. Ballistoconidia are not produced at 25 °C after 1 month. Sexual cycles were not observed in the culture of single or pair strains on YMA, PDA, V8 and CM agar at 25 °C after 1 month.

Glucose fermentation is negative. Glucose, galactose, sucrose, maltose, cellobiose, trehalose, melibiose, soluble starch, D-xylose, L-arabinose, D-ribose, L-rhamnose,



**Fig. 3** Micrographs of budding cells of *Phaeotremella camelliae* grown in YM broth at 25 °C for 7 days. Bar: 20 μm

D-glucosamine (weak), D-mannitol, DL-lactate, succinate and myo-inositol are assimilated. L-sorbose, lactose, melezitose, raffinose, inulin, D-arabinose, N-Acetyl-D-glucosamine, methanol, ethanol, glycerol, erythritol, ribitol, galactitol, Methyl-α-D-glucoside, D-glucitol, salicin, citrate and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, L-lysine



**Fig. 2** Maximum-Likelihood phylogenetic trees depicting the phylogenetic position of *Phaeotremella camelliae*, based on concatenated sequences of the D1/D2 domains of LSU, ITS (including 5.8S rRNA)

region and TEF1. Bar: 0.05. Bootstrap percentages over 50% from 1000 replicates are shown

and cadaverine dihydrochloride (weak) are assimilated, but ethylamine hydrochloride is not. Maximum growth temperature is 30 °C. Growth in vitamin-free medium is positive (weak). Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. The holotype, CGMCC 2.6141, isolated from the fruit of *Camellia oleifera* collected in Gaoan county in Jiangxi province, southwest China, in February 2019. It has been deposited in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. The ex-type strain has been deposited in Agricultural Culture Collection (ACCC), Beijing, China, as ACCC 21432 (= GaoanC57) and Vietnam Collection of Industrial Microorganisms (VCIM), Hanoi, Vietnam, as VCIM 4236.

Physiologically, *P. camelliae* differs from its closely related species, *P. lacus*, *P. lactea* and *P. ovata* (Fig. 2) in its inability to assimilate lactose, melibiose, galactitol, Methyl- $\alpha$ -D-glucoside and salicin and its ability to assimilate DL-Lactate (Table 1).

## Ecology of *Phaeotremella*

Twelve species of *Phaeotremella* including nine teleomorphic and three anamorphic species have been accepted at present [6–9]. Among teleomorphic species, seven ones have been found to be mycoparasitic [7]. *P. fimbriata*, *P. frondosa*, *P. fuscusuccinea* and *P. foliacea* share similar ecology, which are mostly associated with *Stereum* spp., and *P. mycetophiloides*, *P. mycophaga* and *P. simplex* are parasitic on *Aleurodiscus* spp. [7]. *P. roseotincta* and *P. eugeniae* grow on deciduous trees, but the fungal host species of them are still unknown [7]. The anamorphic yeast species of the genus *Phaeotremella*, including three known species and a bunch of yet undescribed species (Fig. 1), inhabit water, soils and plant material. Specifically, *P. lacus* was isolated from freshwater of crater lake in China. *P. lactea* and *P. ovata* were isolated from leaves of unidentified plant in Tibet and Heilongjiang province, respectively, China. ‘*Cryptococcus*’ sp. CRUB 1564 [20], ‘*Cryptococcus*’ sp. SDY 44 and ‘*Cryptococcus*’ sp. SDY 227 [21] were isolated from the acidic water in Argentina and Iberian Pyrite Belt,

**Table 1** Physiological and biochemical characteristics of *P. camelliae* and its closely related species

Characteristic	<i>P. camelliae</i>	<i>P. ovata</i> <sup>a</sup>	<i>P. lactea</i> <sup>a</sup>	<i>P. lacus</i> <sup>b</sup>	<i>P. frondosa</i> <sup>c</sup>	<i>P. foliacea</i> <sup>c</sup>	<i>P. fagi</i> <sup>d</sup>
Galactose	+	+	+	–	v	v	+
Sucrose	+	+	+	+	–	–	+
Maltose	+	+	+	–	+	–	+
Cellobiose	+	+	+	+	+	+	–
Lactose	–	+	+	+	–	–	+
Melibiose	–	+	+	+	–	v	+
Raffinose	–	–	+	–	–	–	+
Melezitose	+	+	+	–	–	–	+
Inulin	–	+	+	–	–	–	–
Soluble starch	+	+	–	+	–	v	–
D-Arabinose	–	+	+	n/a	+	+	l
L-Rhamnose	+	+	+	–	+	+	+
Ribitol	–	+	+	–	+	+	–
Galactitol	–	+	–	–	v	v	+
D-Mannitol	+	+	+	–	+	+	+
D-Glucitol	–	+	+	–	+	+	+
Methyl- $\alpha$ -D-glucoside	–	+	–	+	–	v	+
Salicin	–	+	+	+	–	v	–
DL-Lactate	+	–	–	–	v	v	lw
Citrate	–	–	–	–	+	+	v
Nitrate	+	+	w	+	–	–	–

+ positive, – negative, l latent, w weak, lw latent and weak, v variable, n/a not available

<sup>a</sup>Results obtained from Li et al. [8]

<sup>b</sup>Results obtained from Li et al. [9]

<sup>c</sup>Results obtained from Bandoni and Boekhout [1], the data of *P. frondosa* here were that of originally named as *P. foliacea* [1]; the data of *P. foliacea* here were that of originally named as *P. neofoliacea* and *P. skinneri* [1]

<sup>d</sup>Results obtained from Middelhoven [19]

respectively. ‘*Cryptococcus*’ sp. UFMG-BRO203 was isolated from bromeliad water tanks in Brasil. ‘*Cryptococcus*’ sp. HB1048 [22] and Agaricomycotina sp. RS010 [23] were isolated from soil in Austria and Japan, respectively. *Phaeotremella* aff. *skinneri* EXF-6415 was isolated from the high CO<sub>2</sub> meadow mofette soil in Slovenia. ‘*Cryptococcus*’ sp. TNG003.6 was isolated from volcano soil in Ecuador. *Phaeotremella* sp. DSM 103,204 beech soil in Czech Republic. *P. fagi* was isolated from rotten beech wood (*Fagus sylvatica*) in Netherlands, *P. lactea*, *P. ovata* and five strains of ‘*Cryptococcus*’ sp. (GT-311, GT-178-10, GT-309-8, GT-159 and GT-388) were isolated from plant leaves in China. ‘*Cryptococcus*’ sp. CBS 11775 was from roots of *Carex limosa* in Russia. *Phaeotremella* sp. DSM 103192 was isolated from spruce litter in Czech Republic. The yeast strain of *P. foliaceae* (*Cryptococcus skinneri*) CBS 5029 is known from insect frass under bark of *Tsuga heterophylla* in Oregon, USA [24]. ‘*Tremella*’ sp. H-080.13 was isolated from nectar in USA. The newly described species *P. camelliae* was isolated from the fruit of *Camellia oleifera* in Jiangxi province, China. ‘*Cryptococcus*’ sp. EN14M04 was isolated from mushroom in Taiwan, China, which indicated that this strain is associated with fungal species, but the sexual stage was not reported. The above data indicated that the yeast stages of *Phaeotremella* occur widely in the natural environments.

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## Compliance with Ethical Standards

**Conflicts of interest** The authors declare that they have no conflicts of interest.

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