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# Morphological characteristics and phylogenetic analyses revealed four new wood inhabiting fungi (Agaricomycetes, Basidiomycota) in Xizang Autonomous Region, China

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#### Abstract

Four new fungi from Xizang in southwest China, *Calocera ramaria, Ceraceomyces rhizomorphus, Leptosporomyces linzhiensis,* and *Ramaria xizangensis* are described and illustrated based on the morphological and molecular evidence. *Calocera ramaria* is characterized by the ramal and bright orange basidiomata, a monomitic hyphal system with simple septa generative hyphae, usually 4-septate basidiospores; *Ceraceomyces rhizomorphus* is characterized by the cream to yellowish basidiomata with rhizomorphs, cylindrical basidiospores; *Leptosporomyces linzhiensis* is characterized by white with pink basidiomata, cylindrical to oblong ellipsoid basidiospores; *Ramaria xizangensis* is characterized by flesh pink basidiomata, branched dichotomously in 4–5 ranks, a monomitic hyphal system with clamped generative hyphae, ellipsoid to cylindrical and densely warted basidiospores.

Key words: Molecular systematic, phylogenetic analysis, taxonomy, wood-decaying fungi

## Introduction

The fruiting bodies of Basidiomycota exhibit complex forms, such as gilled, poroid, toothed, coralloid basidiomata. Numerous taxonomists have endeavored to construct a stable classification system based on these characters (Gäumann 1953). Recently, the analysis of DNA sequences has emerged as a common method for deducing fungal phylogenies and enhancing higher classification frameworks through the integration of genetic traits (Cui et al. 2019; Wijayawardene et al. 2020; Liu et al. 2023).

The abundance of biodiversity in *Abies* forests can be attributed to the plentiful presence of humus and mycorrhizal fungi, which foster an optimal environment for the proliferation of the macrofungal species. Information regarding the fungal diversity in *Abies* communities is scattered over a range of publications

(Ryvarden and Gilbertson 1993; Dai 2022). Ceraceomyces Jülich, a small genus characterized by yellow rhizomorphic basidiomata, was established by Jülich based on the taxon C. tessulatus (Cooke) Jülich (Jülich 1972). This genus, originally from North America, features annual, resupinate, pellicular basidiomata with a smooth or merulioid hymenial surface, a monomitic hyphal system, narrowly clavate basidia, and subglobose, narrowly ovate, ellipsoid to cylindrical basidiospores (Chikowski et al. 2016). Phylogenetic studies revealed that Ceraceomyces was polyphyletic, comprising three distinct groups. The section of Corticium tessulatum Cooke belonged to Polyporales, and Ceraceomyces serpens (Tode) Ginns and C. eludens K.H. Larss. were part of phlebioid clade (Larsson et al. 2004). A recent study indicated that the type species, Corticium tessulatum is classified under the order Amylocorticiales (Binder et al. 2010; Chikowski et al. 2016), as well as species, C. yunnanensis Qi Yuan & C.L. Zhao and C. borealis (Romell) J. Erikss. & Ryvarden (Yuan et al. 2023). Currently, eleven species are recognized in the genus Ceraceomyces, including C. cystidiatus (J. Erikss. & Hjortstam) Hjortstam, C. eludens, C. microsporus K.H. Larss. and C. sublaevis (Bres.) Jülich were accepted in the genus. A genus, Crystallicutis El-Gharabawy, Leal-Dutra & G.W. Griff. was derived from Ceraceomyces based on the crystals in the hymenium and subiculum of the basidiomata, which includes the brown-rot species C. serpens (El-Gharabawy et al. 2021). Both species C. sulphurinus and C. violascens (Fr.) Jscens were recorded in Ceraceomyces, are considered congeneric with Rhizochaete Gresl., Nakasone & Rajchenb. due to the characteristics like the rhizomorphic margin and the purple reaction in KOH.

*Calocera* (Fr.) Fr. is known for its distinctive characteristics, stipitate, fasciculate or scattered, gelatinous basidiomata, dendroid or staghorn-like, subclavate to clavate basidia and probasidia, as well as cylindrical to reniform, septate or non-septate basidiospores (Fisher 1931; Lowy 1971; Peng et al. 1992; Wu et al. 2011; Shirouzu et al. 2017). Recent phylogenetic analyses of the class Dacrymycetes demonstrated that *Calocera* was polyphyletic and species in the genus are scattered throughout the family Dacrymycetaceae together with most of the species of *Dacrymyces* Nees (1817: 89) as well as a few species from other genera such as *Dacryopinax* G.W. Martin (1948: 116) and *Femsjonia* Fr. (Shirouzu et al. 2007; Zamora and Ekman 2020).

The genus *Leptosporomyces* Jülich is characterized by the resupinate basidiomata, white yellow and smooth hymenial surface, a monomitic hyphal system with clamped connections, and thin-wall, smooth, acyanophilous basidiospores. Recent research has indicated that *Leptosporomyces* was polyphyletic, with two taxa, *L. galzinii* (Bourdot) Jülich and *L. raunkiaeri* (M.P. Christ.) Jülich, grouped in the order Atheliales, while *L. septentrionalis* (J. Erikss.) Krieglst. was placed in the order Amylocorticiales (Larsson 2007; Hodkinson et al. 2014; Sulistyo et al. 2021). The generic delimitation of *Fibulomyces* Jülich and *Leptosporomyces* remains controversial, with both being indistinguishable in phylogenetic and morphological analyses, leading to the former being considered as a synonym of the latter (Bernicchia and Gorjón 2010).

*Ramaria* Fr. ex Bonord. is a widely distributed non-gilled Basidiomycete genus (Marr and Stuntz 1973; Petersen 1981; Humpert et al. 2001). The genus is recognized by branched basidiomata, mono- to dimitic hyphal systems with clamped or simple-septate generative hyphae, and smooth to echinulate,

verrucose-reticulate or striate ornamentation basidiospores (Corner 1950; Marr and Stuntz 1973; Petersen 1981; Humpert et al. 2001). The genus has been classified into four subgenera, namely *R*. subg. *Ramaria*, *R*. subg. *Laeticolora* Marr & D.E. Stuntz, *R*. subg. *Lentoramaria* Corner, and *R*. subg. *Echinoramaria* Corner (Marr and Stuntz 1973; Humpert et al. 2001; Exeter et al. 2006; Knudson 2012; Hanif et al. 2019). Initially, *Ramaria* was treated as a subgenus within *Clavaria* (Coker 1923; Doty 1944) until Corner (1970) elevated it to genus rank. Studies based on the morphological and molecular data agree on the paraphyletic state of *Ramaria* (Humpert et al. 2001; Hosaka et al. 2006; Giachini et al. 2010).

In the present paper, species from four genera are collected from Xizang under forest of *Abies*, and the phylogenetic relationships of four taxa are still unclear. Thus, to explore the diversity and taxonomic status with different characters for those taxa will be significant for macrofungi in Xizang, and the taxonomy and phylogeny analysis show that they are new to science.

## Material and method

The specimens were collected from Xizang which were deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. Samples were photographed when fresh in the field, and their habitats were recorded. Microscopic structures were discussed by Zhao et al. (2023). Special color terms were set by Anonymous (1969) and Petersen (1996). A Nikon Digital Sight DS-L3 or Leica ICC50 HD camera (magnification ×1,000) was used to exam hand-cut sections of basidiomata, which were first treated with 5% KOH for a few minutes and then with 1% phloxine B ( $C_{20}H_4Br_4Cl_2K_2O_5$ ). At least 30 basidiospores of each species were examined. The values were expressed as a mean with 5% of the measurements excluded from each end of the range, given in parentheses. Stalks were excluded for basidia measurement, and the hilar appendages were excluded for basidiospore measurement.

#### DNA extraction, amplification, and sequencing

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing) was used to obtain DNA from dried specimens and PCR was performed according to the manufacturer's instructions with some modifications (Yang et al. 2023). ITS were amplified using the primer pairs ITS5/ITS4 (White et al. 1990). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 54 °C for 45 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. The PCR procedure for LSU was as follows: initial denaturation at 94 °C for 3 o s, 50 °C for 1 min, and 72 °C for 1.5 min; and a final extension at 72 °C for 1.5 min; and a final extension at 72 °C for 1.5 min; and a final extension at 72 °C for 1.5 min; and a final extension at 72 °C for 10 min. All newly generated sequences were submitted to GenBank and are listed in Table 1.

Sequences generated for this study were aligned, with additional sequences downloaded from GenBank. Sequences were aligned using MAFFT v.7 (https://mafft.cbrc.jp/alignment/server/), adjusting the direction of nucleotide sequences according to the first sequence (accurate enough for most cases), and selecting the G-INS-i iterative refinement method (Katoh et al. 2019). Alignments were

| Table 1. | Таха | information | and the s | equences | used in | this study. | *Newly | generated | sequences | for this s | study. |
|----------|------|-------------|-----------|----------|---------|-------------|--------|-----------|-----------|------------|--------|
|          |      |             |           |          |         |             |        | 0         |           |            |        |

| Species                      | Locality       | Voucher        | ITS       | LSU        |
|------------------------------|----------------|----------------|-----------|------------|
| Amyloathelia crassiuscula    | Sweden         | GB/K169-796    | DQ144610  | _          |
| Amylocorticium cebennense    | USA            | HHB-2808       | GU187505  | GU187561   |
| Amylocorticium subincarnatum | Sweden         | GB/AS-95       | AY463377  | AY586628   |
| Amylocorticium subsulphureum | USA            | HHB-13817      | GU187506  | GU187562   |
| Anomoporia bombycina         | USA            | CFMR L-6240    | GU187508  | GU187611   |
| Anomoporia vesiculosa        | China          | Dai 22795      | ON413718  | ON413720   |
| Athelia abscondita           | USA            | Govette 633    | OP877120  | 0P902328   |
| Athelopsis subinconspicua    | Sweden         | GB0058732      | LR694197  | LR694174   |
| Bondarzewia occidentalis     | Canada         | AFTOL-ID 452   | D0200923  | D0234539   |
| Byssocorticium caeruleum     | Canada         | RS 09400 (H)   | NR_121454 | -          |
| Calocera bambusicola         | China          | Wu 9910-12     | FJ195751  | _          |
| Calocera cornea              | Sweden         | UPS F 940775   | MN595627  | MN595627   |
| Calocera cornea              | Unknown        | AFTOL ID 438   | AY789083  | AY701526   |
| Calocera cornea              | Sweden         | UPS F 940774   | MN595626  | MN595626   |
| Calocera cornea              | Canada         | CBS 124 84     | AB712437  | AB472738   |
| Calocera quepinioides        | New Zealand    | PDD 107969     | LC131411  | LC131370   |
| Calocera quepinioides        | New Zealand    | PDD 107981     | L C131412 | LC131371   |
| Calocera guepinioides        | New Zealand    | PDD 105005     | LC131407  | LC131366   |
| Calocera quepinioides        | New Zealand    | PDD 107874     | LC131409  | LC131368   |
| Calocera quepinioides        | New Zealand    | PDD 105033     | LC131408  | LC131367   |
| Calocera quepinioides        | New Zealand    | PDD 107929     | LC131410  | LC131369   |
| Calocera lutea               | New Zealand    | PDD 107841     | 1 C131413 | LC131372   |
| Calocera lutea               | New Zealand    | PDD 107842     | LC131414  | LC131373   |
| Calocera nalmata             | New Zealand    | PDD 107830     | 10131415  | LC131374   |
| Calocera palmata             | New Zealand    | PDD 107925     | LC131416  | LC131375   |
| Calocera palmata             | lanan          | CBS 127 51     | MH856777  | MH868295   |
| Calocera ramaria             | China          | CL Zhao 31166  | PP399147  | PP862915   |
| Calocera sinensis            | China          | MHHNU30743     | MK167408  | -          |
| Calocera sinensis            | China          | Wu 0505-3      | E 1105753 |            |
| Calocera sinensis            | China          | Wu 0703-6      | F I195754 |            |
| Calocera sinensis            | China          | ICH 070726     | F 1105755 |            |
| Calocera tihetica            | China          | Dai 20171      | MW549777  | MW750403   |
| Calocera tibetica            | China          | Dai 20171      | MW549778  | MW750404   |
| Calocera viscosa s lat       | Sweden         | LIPS F-940773  | MN595628  | MN595628   |
| Calocera viscosa s lat       | Germany        | ETOL ID1679    | DO520102  | DO520102   |
|                              |                | FD-102188      | KP135/00  | KP135277   |
| Ceraceomyces atlanticus      | Brazil         | LIPM 85888     | NR 153026 | NG 060427  |
| Ceraceomyces atlanticus      | China          | M67            | OP766067  | KY68587/   |
| Ceraceomyces horealis        | Sweden         | KHI 8/32       | EU118610  | -          |
| Ceraceomyces buleans         | Sweden         | 19 27109       | AE000870  |            |
| Ceraceomyces eludens         | Sweden         | 15 22780       | ΔΕ090877  |            |
| Ceraceomyces eludens         | United Kingdom | KM 194563      | OR907143  |            |
|                              |                | LIC 2023077    | KD81//18  |            |
|                              | Sweden         | IS 27153       | AE000873  |            |
| Ceraceomyces microsports     | China          | CI 7bao 21154  | PD200151  |            |
|                              | China          | CLZIIdO 31134  | DD200140  |            |
|                              | China          | CLZ1100 31101  | PP399140  | DD962017   |
|                              | China          | Cl 7hac 21107  | DD200150  | DD862016   |
|                              |                | ED-101245-00   | KD125020  | CI1197607  |
| Caraceomyces tessulatus      |                | MDN 152005020  | 00690647  |            |
| Caracomycos tessulatus       | Swadan         | VIEN 152003030 | VIIE100E1 |            |
|                              | Sweden         | CL Zhoc 19002  | 00100510  | -          |
|                              |                |                | 00132319  | N//704440  |
| Carianhara marmarata         | Dalai          |                |           | IVIN/04448 |
|                              | Beigium        | IVIUUL: 3100/  | GU18/515  | GU18/5/1   |
|                              |                | PDD 10/990     | LU131425  | LU131380   |
| Daciymyces pacnysporus       | New ∠ealand    | PDD 105004     | LC131429  | LC131392   |

| Species                              | Locality    | Voucher           | ITS       | LSU       |
|--------------------------------------|-------------|-------------------|-----------|-----------|
| Dacrymyces parastenosporus           | New Zealand | PDD104960         | LC131431  | LC131394  |
| Dacrymyces stillatus                 | Sweden      | UPS F-939814      | MN595676  | MN595676  |
| Dacrymyces subalpinus                | Japan       | TUFC12834         | AB712465  | AB299060  |
| Dacryonaema macnabbii                | Sweden      | UPS F-940949      | MN595650  | MN595650  |
| Dacryonaema macnabbii                | Sweden      | UPS F-940951      | MN595651  | MN595651  |
| Dacryonaema macrosporum              | Norway      | 0 160045          | MN595659  | MN595659  |
| Dacryonaema macrosporum              | Finland     | UPS F-940998      | MN595660  | MN595660  |
| Dacryonaema rufum                    | Sweden      | UPS F-941003      | MN595645  | MN595645  |
| Dacryonaema rufum                    | Sweden      | UPS F-941005      | MN595646  | MN595646  |
| Dendrdacrys brasiliense              | Brazil      | INPA:241458       | AB744230  | AB723514  |
| Dendrdacrys dendrocalami             | Japan       | TUFC 13914        | AB712453  | AB712428  |
| Fibulomyces mutabilis                | Germany     | HG-B 5753 (GB)    | GQ162817  | -         |
| Ganoderma resinaceum                 | Unknown     | C45               | KX371982  | KX372027  |
| Gautieria parksiana                  | USA         | SNF 236 USA       | AF377059  | -         |
| Gloeocantharellus neoechinosporus    | China       | GDGM 75321        | MK358820  | MK358815  |
| Go. Iudovicianus                     | USA         | TFB 14476         | KJ655570  | KJ655580  |
| Gomphus clavatus                     | Spain       | MA-Fungi 48085    | AJ292292  | -         |
| Hypochniciellum subillaqueatum       | Sweden      | KHL 8493          | AY463431  | AY586679  |
| Hypochniciellum subillaqueatum       | UK          | KM165142          | MZ159402  | -         |
| Kavinia himantia                     | USA         | CFMR: DLL2011-079 | KJ140598  | -         |
| Kavinia alboviridis                  | USA         | CFMR: DLL2011-131 | KJ140634  | -         |
| Lentaria micheneri                   | USA         | RRD6 (TENN)       | MF773634  | -         |
| Lactarius sp.                        | New Zealand | PDD:113066        | MW683864  | MW683691  |
| Lentaria byssiseda                   | USA         | TENN 61159        | FJ596788  | -         |
| Leptosporomyces fuscostratus         | USA         | UC 2022884        | KP814350  | -         |
| Leptosporomyces fuscostratus         | Unknown     | DK 16_251         | OL436970  | -         |
| Leptosporomyces galzinii             | Sweden      | GB 0107211        | LR694202  | LR694180  |
| Leptosporomyces galzinii             | USA         | UC 2023126        | KP814291  | -         |
| Leptosporomyces linzhiensis          | China       | CLZhao 31174      | PP399152  | PP862922  |
| Leptosporomyces linzhiensis          | China       | CLZhao 31183      | PP399153  | PP862918  |
| Leptosporomyces linzhiensis          | China       | CLZhao 31187      | PP399154  | -         |
| Leptosporomyces linzhiensis          | China       | CLZhao 31190      | PP399155  | -         |
| Leptosporomyces raunkiaeri           | USA         | UC 2023053        | KP814293  | -         |
| Leptosporomyces raunkiaeri           | USA         | CFMR: HHB-7628    | GU187528  | GU187588  |
| Leptosporomyces septentrionalis      | USA         | UC 2023047        | KP814348  | -         |
| Leptosporomyces septentrionalis      | Sweden      | GB 0090937        | LR694203  | LR694181  |
| Leptosporomyces septentrionalis      | Norway      | JS 16122          | GU187497  | -         |
| Lobulicium occultum                  | Sweden      | KHL13496b         | MT340827  | -         |
| Mythicomyces corneipes               | Unknown     | AFTOL-972         | DQ404393  | AY745707  |
| Phaeoclavulina flaccida              | Italy       | AMB n. 17671      | MK796107  | MK796156  |
| Phlebiella christiansenii            | Finland     | KHL 11689         | EU118659  | -         |
| Phlebiella vaga                      | Sweden      | KHL 11065         | EU118660  | EU118661  |
| Piloderma fallax                     | Finland     | CFMR: S-12        | GU187535  | -         |
| Plicaturopsis crispa                 | China       | LWZ 20201017-11   | ON897938  | ON885398  |
| Plicaturopsis crispa                 | Brazil      | URM 85888         | NR_153926 | NG_060427 |
| Ramaria abietina                     | USA         | u066              | KY510818  | -         |
| Ramaria acrisiccescens               | USA         | OSC 112057        | KY354738  | KY354711  |
| Ramaria admiratia                    | USA         | TENN: 69114       | NR_137862 | NG_059504 |
| Ramaria amyloidea                    | USA         | OSC 69891         | EU837196  | KP637036  |
| Ramaria apiculata var. brunnea       | USA         | CBS:149.74        | MH860840  | MH872577  |
| Ramaria araiospora                   | Germany     | OSC 108707        | EU846298  | -         |
| Ramaria aurantiisiccescens           | USA         | OSC 104868        | EU837197  | -         |
| Ramaria aurea                        | Italy       | AMB 18352         | MN637783  | MN637796  |
| Ramaria botrytis                     | Italy       | AMB n. 18201      | NR_189799 | NG_241889 |
| Ramaria botrytis                     | Argentina   | GM 19044          | OP177707  | OP177871  |
| Ramaria botrytis                     | USA         | snf213            | AF377055  | -         |
| Ramaria botrytis f. musicolor        | Italy       | ZT Myc 57160      | KY626144  | -         |
| Ramaria botrytis var. aurantiiramosa | USA         | OSC 140667        | JX310410  | -         |

| Species                                 | Locality   | Voucher        | ITS       | LSU      |
|---|------------|----------------|-----------|----------|
| Ramaria botrytis var. aurantiiramosa    | USA        | WTU-F-043053   | KX574471  | _        |
| Ramaria celerivirescens                 | USA        | OSC 140471     | JX310392  | JX269125 |
| Ramaria claviramulata                   | USA        | WTU-F-043055   | KX574472  | KX671009 |
| Ramaria conjunctipes                    | USA        | OSC: 110613    | KC346861  | -        |
| Ramaria coulterae                       | USA        | OSC 69929      | EU669320  | EU669320 |
| Ramaria dendrophora                     | Argentina  | GM 20020       | OP177716  | OP177880 |
| Ramaria dendrophora                     | Argentina  | GM 19094       | OP177715  | OP177879 |
| Ramaria fennica                         | Italy      | AMB n. 17522   | MK682678  | -        |
| Ramaria flavescens                      | Italy      | AMB 17404      | KY354743  | -        |
| Ramaria flavescens                      | Italy      | AMB 17404      | MK493036  | -        |
| Ramaria flava                           | Italy      | AMB 17393      | MK493035  | -        |
| Ramaria flavinedulis                    | Argentina  | GM 19056       | OP177717  | OP177881 |
| Ramaria flavinedulis                    | Argentina  | GM 19035       | 0P177720  | 0P177884 |
| Ramaria flavobrunnescens var. aromatica | USA        | AGK 059        | JQ408240  | _        |
| Ramaria foetida                         | USA        | AGK 058        | JQ408239  | JQ408239 |
| Ramaria formosa                         | USA        | OSC1064203     | EU525994  | _        |
| Ramaria fumosiavellanea                 | USA        | WTU-F-063048   | MK169345  | _        |
| Ramaria gelatiniaurantia                | USA        | OSC 65737      | KP658144  | -        |
| Ramaria inedulis                        | Chile      | 12648          | 0P177723  | 0P177887 |
| Ramaria inedulis                        | Argentina  | GM 19047       | 0P177722  | 0P177886 |
| Ramaria largentii                       | USA        | OSC 67012      | KP658130  | KP637058 |
| Ramaria luteovernalis                   | Italv      | MCVE 28637     | NR 155720 | KT357477 |
| Ramaria maculatipes                     | USA        | OSC 112051     | KY354749  | KY354721 |
| Ramaria magnipes                        | USA        | WTU-F-063057   | MK169351  | MK493050 |
| Ramaria mvceliosa                       | USA        | AGK 035        | J0408230  | _        |
| Ramaria obtusissima                     | USA        | TFB 14473      | KJ655554  | KJ655575 |
| Ramaria patagonica                      | Argentina  | 403            | 0P177710  | 0P177874 |
| Ramaria patagonica                      | Argentina  | GM 19106       | 0P177713  | 0P177877 |
| Ramaria pseudoflava                     | Italv      | AMB 17392      | MK493046  | _        |
| Ramaria rasilisporoides                 | Pakistan   | MH-2013        | MG760613  |          |
| Ramaria rasilisporoides                 | USA        | WTU-F-043029   | MK169346  | _        |
| Ramaria rubella                         | USA        | OSC 115946     | EU669317  | EU669343 |
| Ramaria rubella f. rubella              | USA        | AGK 049        | J0408236  | _        |
| Ramaria rubribrunnescens                | USA        | OSC 119676     | EU652352  | EU652387 |
| Ramaria rubribrunnescens                | USA        | OSC 66051      | KY354750  | KY354722 |
| Ramaria sandaracina var. sandaracina    | Canada     | UBC F28386     | KP454028  | _        |
| Ramaria sp.                             | India      | KD-14-006      | KT824242  | _        |
| Ramaria stricta                         | Germany    | CBS 165.48     | MH856299  | _        |
| Ramaria stricta var. concolor           | USA        | AGK 011        | JQ408221  | _        |
| Ramaria stuntzii                        | USA        | OSC 73315      | KP658122  | KP637048 |
| Ramaria subbotrytis                     | Spain      | MA-Fungi 48088 | AJ408361  | _        |
| Ramaria subtilis                        | Spain      | MA-Fungi 48055 | AF442098  | _        |
| Ramaria suecica                         | USA        | OSC 115933     | KP658148  | KP637079 |
| Ramaria testaceoflava                   | USA        | OSC 107885     | KP658128  | AY586708 |
| Ramaria verlotensis                     | USA        | WTU-F-063047   | KX574480  | KX671016 |
| Ramaria xizangensis                     | China      | CLZhao 31169   | PP399156  | PP862919 |
| Ramaria xizangensis                     | China      | CLZhao 31180   | PP399157  | PP862920 |
| Ramaria xizangensis                     | China      | CLZhao 31204   | PP399158  | PP862921 |
| Ramaria formosa                         | Italy      | AMB 18529      | MT055910  | MT053203 |
| Ramaricium polyporoideum                | USA        | TENN: 065654   | MF992160  | MF992160 |
| Stereopsis vitellina                    | Sweden     | F 703241       | LR694211  | LR694189 |
| Turbinellus floccosus                   | USA        | MO 285170      | MN319564  | MN319563 |
| Unilacryma unispora                     | Sweden     | UPS F 941268   | MN595672  | MN595672 |
| Unilacryma unispora                     | Sweden     | UPS F 941277   | MN595665  | MN593500 |
| Xenasmatella ardosiaca                  | Costa Rica | KHL 12928      | EU118658  | _        |
| Xenasmatella ardosiaca                  | USA        | CBS 126045     | MH864060  | MH875515 |

manually adjusted to maximize alignment and minimize gaps with BioEdit v.7.0.9 (Hall 1999). A dataset of concatenated ITS and LSU sequences was used to determine the phylogenetic position of the new species. Maximum likelihood (ML) analysis was performed using the CIPRES Science Gateway (Miller et al. 2010) based on the dataset using the RA × ML-HPC BlackBox tool, with setting RA × ML halt bootstrapping automatically and 0.25 for maximum hours and obtaining the best tree using ML search. Other parameters in ML analysis used default settings, and statistical support values were obtained using nonparametric bootstrapping with 1,000 replicates. Bayesian inference (BI) analysis based on the dataset was performed using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2012). The best substitution model for the dataset was selected by ModelFinder (Kalyaanamoorthy et al. 2017) using a Bayesian information criterion, and the model was used for Bayesian analysis. Four Markov chains were run from random starting trees. Trees were sampled every 1,000<sup>th</sup> generation. The first 25% of sampled trees were discarded as burn-in, whereas other trees were used to construct a 50% majority consensus tree and for calculating Bayesian posterior probabilities (BPPs). The aligned sequences were deposited in TreeBase (https://www.treebase.org/ treebase-web/home.html; submission ID 31437).

Branches of the consensus tree that received bootstrap support for ML were greater than or equal to 75%, Bayesian posterior probabilities more than 0.9, respectively.

## Result

#### The Phylogeny of Calocera

BI analysis yielded a similar topology to MP and ML analysis. Only the MP tree is provided here (Fig. 1). Branches that received bootstrap support for ML (ML-BS), and BI (BPP) greater than or equal to 75% (MP-BS and ML-BS) and 0.90 (BPP) were considered as significantly supported, respectively. The ITS and LSU dataset contains sequences from 26 fungal specimens representing twelve *Calocera* taxa. The average SD of split frequencies in BI analyses is 0.005504 (BI). The phylogenetic tree (Fig. 1) reveals the new species has close relationship with *C. tibatica*, sister to *C. viscosa* and *C. cornea*.

#### The Phylogeny of Ceraceomyces

The dataset included ITS and LSU from 29 samples representing 22 taxa. The best model for the concatenated ITS+LSU dataset estimated and applied for BI analysis was "GTR+I+G4", datatype = DNA, nucmodel = 4by4, lset nst = 6, rates = invgamma; state frequencies had a Dirichlet prior (1,1,1,1), and the distribution was approximated using four categories. BI analysis yielded a similar topology to ML analysis, with an average standard deviation of split frequencies of 0.006593. The ML tree was provided (Fig. 2). Branches that received bootstrap support for ML and BI  $\geq$  70%, and 0.75 were considered significantly supported, respectively.

The analysis reveals four clades (Fig. 2), in which three European species *C. eludens*, *C. microsporus*, *C. sublaevis* clustered together and *Rhizochaete americanus* (Nakasone, C.R. Bergman & Burds.) Gresl., Nakasone & Rajchenb. The core clade formed by *C. tessulatus* and *C. atlanticus*, along with



Figure 1. Phylogeny of species in *Calocera* generated by maximum likelihood based on ITS+LSU sequence data. Branches are labeled with maximum likelihood bootstrap  $\geq$  75% and Bayesian posterior probabilities  $\geq$  0.90, respectively. New species are in bold.

*Hypochniciellum subillaqueatum* (Litsch.) Hjortstam. Four specimens from China formed two lineages, namely *Ceraceomyces rhizomorphus* with *C. yunnanensis*, and were sister to *C. borealis*.

#### The Phylogeny of Leptosporomyces

BI analysis yielded a similar topology to MP and ML analysis, with an average standard deviation of split frequencies = 0.008841. Only the MP tree is provided here (Fig. 3). Branches that received bootstrap support for ML (ML-BS), and BI (BPP) greater than or equal to 75% (MP-BS and ML-BS) and 0.90 (BPP) were considered as significantly supported, respectively. Four previously accepted species, *L. galzinii*, *L. fuscostratus* (Jülich) Krieglst., *L. raunkiaeri*, and *L. mundus* (H.S. Jacks. & Dearden) Jülich received strong support in three lineages. The new species *L. linzhiensis* had a close relationship with *L. septentrionalis* with full support.







Figure 3. Phylogeny of species in *Leptosporomyces* generated by maximum likelihood based on ITS+LSU sequence data. Branches are labeled with maximum likelihood bootstrap  $\geq$  75% and Bayesian posterior probabilities  $\geq$  0.90, respectively. New species are in bold.

#### The Phylogeny of Ramaria

BI analysis yielded a similar topology to MP and ML analysis. Only the MP tree is provided here (Fig. 4). Branches that received bootstrap support for ML (ML-BS), and BI (BPP) greater than or equal to 75% (MP-BS and ML-BS) and 0.90 (BPP) were considered as significantly supported, respectively. Four clades were obtained from our phylogenetic analysis, *Ramaria* sub. *Laeticolora*, *Ramaria* Sub. *Ramaria*, *Ramaria* Sub. *Echinormaria* and *Ramaria* sub. *Laeticolora*, *Ramaria*. The species *Ramaria xizangensis* was grouped in *Ramaria* sub. *Laeticolora* along with *R. amyloidea* Marr & D.E. Stuntz, *R. celerivescens* Marr & D.E. Stuntz, and *R. claviramulata* Marr & D.E. Stuntz.



Figure 4. Phylogeny of species in the *Ramaria* generated by maximum likelihood based on ITS+LSU sequence data. Branches are labeled with maximum likelihood bootstrap  $\geq$  75% and Bayesian posterior probabilities  $\geq$  0.90, respectively. New species are in bold.

#### Taxonomy

Calocera ramaria C.L. Zhao & H.M. Zhou, sp. nov. MycoBank No: 852565

Figs 5, 6

**Holotype.** CHINA, Xizang, Linzhi, Sejila Mountain National Forest Park, 29°64'N, 94°71'E, elev. 3852 m, gregarious on humus under *Abies*, 2 August 2023, CLZ-hao 31166 (SWFC).

**Etymology.** *Ramaria* (Lat.): refers to the ramal basidiomata of the specimens. **Diagnosis.** Differed from other species in having ramal basidiomata, septate

hyphae, usually 4-septate basidiospores ( $9.2-11 \times 3.9-4.4 \mu m$ ).

**Fruiting body.** Basidiomata stipitate, gregarious, bright orange when fresh, orange brown when dry, gelatinous when soaked, corneous when dry, ramal, repeatedly branched, apically blunt, up to 6.2 cm high; stipe 0.7–1 mm in diam, become orange to reddish brown corneous when dry.

**Internal features.** Marginal hyphae hyaline, smooth, thin-walled, septate, simple or branched, without clamp connections,  $4-5.5 \mu m$  in diam; internal hyphae hyaline, smooth or scabrous, thin- to slightly thick-walled, interwoven, with nodose-septa, without clamp connections,  $2-3 \mu m$  in diam; hyphidia hyaline, smooth, thin-walled, with a simple septum at base, occasionally terminally branched; basidia hyaline, thin-walled, subclavate to clavate, without basal clamp connection,  $23-31 \times 2-4 \mu m$ ; basidiospores hyaline, smooth, thin-walled, oblong-ellipsoid to navicular, straight or curved, apiculate, usually 4-septate when mature, occasionally 5-septate,  $(9.1-)9.2-11(-11.6) \times (3.5-)3.9-4.4(-4.7) \mu m$ , L = 10.18 µm, W = 4.19 µm, Q = 2.43 (n = 30/1).

#### Ceraceomyces rhizomorphus C.L. Zhao & H.M. Zhou, sp. nov.

MycoBank No: 852584 Figs 7, 8

**Holotype.** CHINA, Xizang, Linzhi, Sejilashan National Forest Park, 29°64'N, 94°71'E, elev. 3848 m, on the fallen branch of *Abies*, 2 August 2023, CLZhao 31188 (SWFC).

**Etymology.** *Rhizomorphus* (Lat.): refers to the basidiomata with rhizomorphs.

**Diagnosis.** Differed from other species in having merulioid, cream to yellowish basidiomata, generative hyphae with clamp connections, cylindrical basidiospores ( $4.7-6.2 \times 1.8-2.3 \mu m$ ).

**Fruiting body.** Basidiomata resupinate, adnate, smooth to tuberculate when fresh, merulioid upon drying, without odor or taste when fresh, up to 6 cm long, 2 cm wide,  $100-200 \,\mu$ m thick. Hymenial surface merulioid, cream to yellowish when fresh, turn to orange yellow upon drying. Margin sterile, white, with rhizomorphs.

**Hyphal structure.** Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin- to slightly thick-walled, branched, interwoven,  $3.5-7 \mu m$  in diameter, IKI-, CB-; tissues turn black in KOH.

**Hymenium.** Cystidia and cystidioles absent; basidia narrowly clavate to clavate, in a dense palisade, with 4 sterigmata and a basal clamp connection,



Figure 5. Basidiomata and microscopic structures of *Calocera ramaria* (holotype, CLZhao 31166, holotype) **A**, **B** basidiomata **C** a section of hymenium **D** basidiospores **E** marginal hyphae **F** internal hyphae. Scale bars: 1 cm (**A**, **B**); 10 μm (**C**–**F**).

 $16-19 \times 3.5-4 \mu m$ ; basidioles dominant, similar to basidia in shape, but slightly smaller.

**Spores.** Basidiospores cylindrical, with suprahilar depression, colorless, smooth, thin-walled, IKI-, CB-, (4.2-)4.7-6.2(-6.4) × (1.5-)1.8-2.3(-2.4)  $\mu$ m, L = 5.49  $\mu$ m, W = 2.05  $\mu$ m, Q = 2.66-2.68 (n = 60/2).



Figure 6. Microscopic structures of *Calocera ramaria* (holotype, CLZhao 31166) **a** basidiospores **b** basidia with basidiospores. Scale bars:  $5 \mu m (a)$ ;  $10 \mu m (b)$ .

Additional specimens examined (*paratypes*). CHINA. Xizang, Linzhi, Sejila Mountain National Forest Park, 29°64'N, 94°71'E, elev. 3848 m, on the trunk of *Abies*, 2 August 2023, CLZhao 31153 (SWFC); CLZhao 31154 (SWFC); CLZhao 31161 (SWFC); CLZhao 31202 (SWFC); on the fallen branch of *Abies*, 2 August 2023, CLZhao 31184 (SWFC); CLZhao 31185 (SWFC); CLZhao 31197 (SWFC).



Figure 7. Basidiomata of Ceraceomyces rhizomorphus A, C CLZhao 31188 (holotype) B, D CLZhao 31185.



Figure 8. Microscopic structures of *Ceraceomyces rhizomorphus* (holotype, CLZhao 31216) **a** basidiospores **b** basidia **c** basidioles **d** a section of hymenium. Scale bars:  $5 \mu m$  (**a**);  $10 \mu m$  (**b**-**d**).

#### Leptosporomyces linzhiensis C.L. Zhao & H.M. Zhou, sp. nov.

MycoBank No: 852585 Figs 9, 10

**Holotype.** CHINA, Xizang, Linzhi, Sjilashan Forest Park, 29°64'N, 94°71'E, elev. 3848 m, on fallen trunk of *Abies*, 2 August 2023, CLZhao 31183 (SWFC).

**Etymology.** *Linzhiensis* (Lat.): refers to the locality (Xizang) of the type specimens.

**Diagnosis.** Differed from other species in having white basidiomata, monomitic hyphal system, cylindrical to oblong ellipsoid basidiospores  $(3.8-4. \times 1.7-2 \mu m)$ .

**Fruiting body.** Basidiomata resupinate, athelioid, membranous upon drying, without odor or taste when fresh, up to 10 cm long, 4 cm wide, 200  $\mu$ m thick. Hymenial surface smooth to cracked, white with pink tint when fresh, turning to yellowish cream upon drying. Margin sterile, white, fimbriate.

**Hyphal structure.** Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin- to slightly thick-walled, branched, interwoven,  $2-5 \mu m$  in diameter, IKI-, CB-; tissues turn black in KOH.

**Hymenium.** Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin-walled, branched, interwoven, 2–3.5  $\mu$ m in diameter, IKI–, CB–. Basidia clavate, with 4 sterigmata and a basal clamp connection, 11.5–13.5 × 3.2–3.8  $\mu$ m.

**Spores.** Basidiospores cylindrical to oblong ellipsoid, colorless, smooth, thinwalled, IKI-, CB-,  $(3.5-)3.8-4.3(-4.7) \times (1.7-)1.7-2(-2.3) \mu$ m, L = 4.02  $\mu$ m, W = 1.88  $\mu$ m, Q = 1.95-2.18 (n = 90/3).

Additional specimens examined (*paratypes*). CHINA, Xizang, Linzhi, Sjilashan Forest Park, 22°57'N, 103°42'E, elev. 2100 m, on fallen trunk of *Abies*, 2 August 2023, CLZhao 31174 (SWFC); on fallen trunk of *Abies*, 2 August 2023, CLZhao 31187 (SWFC); on fallen trunk of *Abies*, 2 August 2023, CLZhao 31190 (SWFC).



Figure 9. Basidiomata of Leptosporomyces linzhiensis (holotype, CLZhao 31183). Scale bars: 1 cm (A); 1 mm (B).





## Ramaria xizangensis C.L. Zhao & H.M. Zhou, sp. nov.

MycoBank No: 852586 Figs 11, 12

**Holotype.** CHINA, Xizang, Linzhi, Sejila Mountain National Forest Park, 29°64'N, 94°71'E, elev. 3850 m, gregarious on the humus under *Abies*, 2 August 2023, CLZhao 31169 (SWFC).

**Etymology.** *Xizangensis* (Lat.): refers to the locality (Xizang) of the type specimens.

**Diagnosis.** Differed from other species in having flesh pink basidiomata, monomitic hyphal system, generative hyphae with clamp connections, ellipsoid to cylindrical, densely warted basidiospores  $(9.7-11.8 \times 3.9-4.9 \ \mu m)$ .

**Fruiting body.** Basidiomata solitary to gregarious, with 8 cm high × 6 cm wide at the widest point, repeat branched dichotomously in 4–5 ranks, flesh pink when fresh, become clay buff with dry; apices obtuse, orange yellow when fresh, becoming fuscous when dry. Stipe  $\geq$  3 cm high, compound to fasciculate in groups of 5, emerging from a common base, concolorous with the branches.

**Hyphal structure.** Hyphal system monomitic, generative hyphae with clamp connections, branched, walls smooth and hyaline; basal stem with tramal hyphae 4–7  $\mu$ m wide and inflated ones up to 10  $\mu$ m, occasionally branched, thin-walled, parallel arranged, hyaline; tramal hyphae of branches 3–4  $\mu$ m wide.

**Hymenium.** Hymenium all along the basidiomata. Basidia clavate, in a dense palisade, with 4 sterigmata and a basal clamp connection. Basidioles elongated clavate, smooth, hyaline, contents homogeneous,  $23.5-34 \times 6-7 \mu m$ .

**Spores.** Basidiospores ellipsoid to cylindrical, densely warted, with 1–2 several guttulae, IKI–, CB–,  $9.7-11.8(-12.5) \times (3.8-)3.9-4.9(-5.1) \mu$ m, L = 10.69  $\mu$ m, W = 4.29  $\mu$ m, Q = 2.49 (n = 30/1).

Additional specimens examined (*paratypes*). CHINA, Xizang, Linzhi, Sejila Mountain National Forest Park, 29°67'N, 94°74'E, elev. 3850 m, gregarious on the humus under *Abies*, 2 August 2023, CLZhao 31180 (SWFC); on ground in forest of *Abies*, 2 August 2023, CLZhao 31204 (SWFC).



Figure 11. Basidiomata of Ramaria xizangensis (holotype, CLZhao 31169). Scale bars: 1 cm (A, B).





## Discussion

Wood decay fungi encompasses the vast group of aphyllophoroid fungi with corticioid, prioid or jelly form of basidiomata (Herter 1910). This classification has historically been used to define the different families of Basidiomycetes. However, molecular studies have revealed that many of these fungi are distributed across various orders within the Basidiomycetes, including the likes

of Amylocorticiales, Atheliales, Dacrymycetales, and Gomphales (Kirk et al. 2018; Wei et al. 2022). As a result, further research is needed to elucidate the relationships and morphological variability of these taxa through phylogenetic analysis.

The Xizang Autonomous Region, situated in the southwest of China, is renowned as one of the most bio-diverse regions in the country. This is attributed to its complex topography and diverse ecosystems, making it a focal point for fungal biodiversity in China. Recently, studies focusing on fungal diversity and the ecology of Basidiomycota in Xizang were carried out (Ke 2016; Pubu et al. 2016; Wang et al. 2023). According to the study (Pubu et al. 2016), 1733 species were collected in Xizang. The fungal research indicated that Sejila Mountain National Forest Park is predominantly composed of spruce and fir trees, which provide an ideal habitat for a rich diversity of macrofungi species to flourish (Zhao and Li 1987). In our study, four species were found from Xizang, *Calocera ramaria, Ceraceomyces rhizomorphus, Leptosporomyces linzhiensis*, and *Ramaria xizangensis*.

*Calocera* is characterized by its yellow, gelatinous basidiomata, resembling *Dacrymyces*. However, *Dacrymyces* displays a broader range of basidiomata forms, including pulvinate, discoid, turbinate, spathulate, flabellate, and cylindrical shapes (Shirouzu et al. 2009; Fan et al. 2021), whereas *Calocera* exhibits branched, dendroid basidiomata. Our results have further confirmed that our newly discovered species features ramal basidiomata and clusters phylogenetically with *Calocera* species, placing it within the genus *Calocera*. In Xizang, two species have been identified, *C. ramaria* and *C. tibetica*, but the latter has wider basidiospores (5–6 µm vs. 3.9–4.4 µm, Fan et al. 2021). In our phylogenies, *C. viscosa* and *C. cornea* were related to *C. ramaria* (Fig. 1); however, *C. viscosa* has 1-septate mature basidiospores, and *C. cornea* differs from *C. ramaria* by its distinctly larger basidiospores (7–10 × 3–4.5 µm vs. 9.2–11 × 3.9–4.4 µm) with one septum (McNabb 1965; Shirouzu et al. 2009).

Previous research has highlighted the polyphyly of *Ceraceomyces* (Chikowski 2016; Yuan et al. 2023), and seven species are retained in *Ceraceomyces*. However, it is worth noting that authentic specimens and DNA data are lacking for *Ceraceomyces* species. Phylogenetically, *C. rhizomorphus* formed a sister group with *C. yunnanensis* and *C. borealis*, but *C. yunnanensis* has smaller basidiospores ( $3-4 \times 1-1.5 \mu m vs. 4.7-6.2 \times 1.8-2.3 \mu m$ , Yuan et al. 2023) and *C. borealis* has larger basidiospores ( $6-8 \times 1.8-2 \mu m vs. 4.7-6.2 \times 1.8-2.3 \mu m$ , Bernicchia and Gorjón 2010).

Ceraceomyces rhizomorphus and C. tessulatus had similar yellowish basidiomata with rhizomorphs when fresh, while C. tessulatus has ellipsoid and larger basidiospores (6–8 × 3.5–4.5  $\mu$ m vs. 4.7–6.2 × 1.8–2.3  $\mu$ m, Bernicchia and Gorjón 2010). Three known species, C. bizonatus, C. reidii, and C. simulans also distributed in Asia. However, C. bizonatus has shorter basidiospores (2.5–3.3  $\mu$ m vs. 4.7–6.2  $\mu$ m, Bernicchia and Gorjón 2010); C. reidii has larger basidiospores (11.5–15 × 4.5–6  $\mu$ m vs. 4.7–6.2 × 1.8–2.3  $\mu$ m, Bernicchia and Gorjón 2010); C. simulans has longer basidiospores (6–7  $\mu$ m vs. 4.7–6.2  $\mu$ m, Bernicchia and Gorjón 2010).

Leptosporomyces linzhiensis is similar to *L. thindii* in having white basidiomata and being distributed in Asia, but the latter has wider basidiospores (Prasher 2015). Leptosporomyces linzhiensis sisters to *L. septentrionalis* by its white basidiomata, and cylindrical basidiospores, but the latter has slightly shorter basidiospores (3–4  $\mu$ m vs. 3.8–4.3  $\mu$ m), and 2–4 basidia (Prasher 2015). *Leptosporomyces linzhiensis* is easily confused with *L. roseus* in, but the latter has shorter basidiospores (2–2.5  $\mu$ m vs. 3.8–4.3  $\mu$ m, Prasher 2015). *Leptosporomyces fuscostratus* has a broad distributional range in the northern hemisphere, but it has wider basidiospores (2–2.8  $\mu$ m vs. 1.7–2  $\mu$ m, Yurchenko and Wołkowycki 2022).

In our phylogeny, *Ramaria* is paraphyletic, which included four clades, *R*. sub. *Laeticolora* and *R*. sub. *Lentoramaria*, *R*. sub. *Ramaria* and *R*. sub. *Echinormaria*. *Ramaria xizangensis* was clustered in *Ramaria* sub. *Laeticolora* with *Ramaria amyloidea*, *R. celerivirescens* and *R. claviramulata*. However, *R. celerivirescens* has slightly wider basidiospores (4–6  $\mu$ m vs. 3.9–4.9  $\mu$ m, Marr and Stuntz 1973). *R. claviramulata* has cream to brownish white basidiomata. *Ramaria xizangensis* is similar to *R. indoyunnaniana* in having pink basidiomata and being distributed in Yunnan, but the latter has shorter basidiospores (7.2–8.3  $\mu$ m vs. 9.7–11.8  $\mu$ m, Petersen and Zang 1986).

According to our field inventory, the four Chinese new species were found in alpine zone near the Sejila Mountain, and the coniferous forest dominant by *Abies* at high altitude with cold and humid environments. Previously, numerous new species have been found in Southwest China (Dai 2022; Zhao et al. 2023), and the present paper confirms the fungal diversity is very rich in the montane forests of Southeast Xizang.

## **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

## **Ethical statement**

No ethical statement was reported.

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#### Author contributions

Data curation: ZHM, ZCL,WF. Formal analysis: ZXC. Methodology: ZHM, ZXC, LJT. Software: ZXC, LJT. Writing - original draft: ZHM, ZCL. Writing - review and editing: ZCL, WF.

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#### **Data availability**

All of the data that support the findings of this study are available in the main text.

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