Cryptic Diversity of Isaria-like Species in Guizhou, China

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Abstract: Many Isaria-like species have recently been moved into more appropriate genera. However, more robust molecular phylogenetic analyses are still required for Isaria-like fungi to ensure accurate taxonomic identification. We analyzed these Isaria-like strains using multi-gene phylogenetics. Cryptic diversity was discovered in several Isaria farinosa strains, and two new species, Samsoniella pseudogunnii and S. pupicola, are proposed. Our results reveal that more attention needs to be paid to cryptic intraspecific diversity across different isolates and genotypes of the Isaria-like species, some of which will need to be transferred to Samsoniella. Interestingly, S. hepiali, with a very broad host distribution, has been widely used as a medicinal and edible cordycipitoid fungus.

Keywords: cryptic diversity; intraspecific; Isaria-like; multi-gene analysis

1. Introduction

The genus Isaria was originally establish based on the species Isaria terrestris Fr. [1]. Brown and Smith [2] transferred some species described in Isaria Pers. and Spicaria Harting into Paecilomyces, which possess a conidiogenous structure similar to that of Paecilomyces variotii Bainier. de Hoog [3] redescribed the genus Isaria and chose Isaria felina (DC.) Fr. as the lectotype. Typical characteristics include denticulate conidiogenous cells without elongation that arise in clusters from subtending cells or are solitarily from undifferentiated hyphae; mostly present synnemata; and globose, ellipsoidal, or subcylindrical conidia, mostly with a rounded base [3]. Samson [4] divided the genus Paecilomyces into two sections and all entomogenous species were placed in the section Isarioidea. Hodge et al. [5] reintroduced the genus Isaria with the type species Isaria farniosa (Holm.) Fr. and most entomopathogenic mesophilic Paecilomyces species were transferred to Isaria (Hypocreales, Clavicipitaceae) [6–8].

Kepler et al. [9] proposed the rejection of Isaria in favor of Cordyceps and transferred Isaria species into Cordyceps. Mongkolsamrit et al. [10] introduced some Isaria-like species and the new genus Samsoniella Mongkols., Noisrip., Thanakitp., Spatafora, and Luangsarad. Chen et al. [11,12] reported four Isaria-like species: Akanthomyces araneogenus Z.Q. Liang, W.H. Chen, and Y.F. Han; Samsoniella coleopterorum W.H. Chen, Y.F. Han, and Z.Q. Liang; Samsoniella hymenopterorum W.H. Chen, Y.F. Han, and Z.Q. Liang; and Samsoniella lepidopterorum W.H. Chen, Y.F. Han, and Z.Q. Liang. Currently, many species previously placed in the genus Isaria have been transferred to more appropriate genera. However, robust molecular phylogenetic analyses are still needed for Isaria-like fungi to ensure accurate taxonomic identification with comparable results across different isolates and genotypes [10].

We previously collected many Isaria-like morphs of invertebrate-pathogenic fungi from Guizhou Province, China. Some demonstrated close phylogenetic relationships with Isaria farinosa (Holm.) Fr. based on the analysis of associated ITS sequences. In the
present study, we applied multi-gene (ITS, LSU, RPB1, RPB2, TEF) phylogenetic analysis to reevaluate the taxonomic position of these strains, as well as the cryptic diversity among the different isolates of *I. farinosa*, and to describe new taxa to accommodate the cryptic diversity of *Isaria*-like fungi.

2. Materials and Methods

2.1. Fungal Materials and Identification

The strains used in this study were isolated from infected insect and spider specimens collected in different areas of Guizhou Province, China, including Dali Forest in Rongjiang County, Yaorensan National Forest Park in Sandu County, Mount Fanjing in Yinjiang County, Tongmuling in Guiyang City, and Doupengshan in Duyun City. Isolation of strains was conducted as described by Chen et al. [13]. Fungal colonies emerging from specimens were isolated and cultured at 25 °C for 14 days under 12 h light/12 h dark conditions following protocols described by Zou et al. [14]. Accordingly, the living isolates were obtained. The specimens and the isolated strains were deposited in the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code, GZAC), Guiyang City, Guizhou, China.

Macroscopic and microscopic morphological characteristics of the fungal isolates were examined, especially for the arrangement, shape, and measurement of phialides and conidia, and also the growth rates of cultures incubated at 25 °C for 14 days were determined in Potato Dextrose Agar (PDA) (Potato powder 6%, Agar 20%, Glucose 20%, Beijing Solarbio Technology Co., Ltd., China). Hyphae and conidiogenous structures were mounted in lactophenol cotton blue or 20% lactate solution and observed with an optical microscope (OM, DM4 B, Leica, Germany).

2.2. DNA Extraction, Polymerase Chain Reaction Amplification and Nucleotide Sequencing

DNA extraction was carried out with a fungal genomic DNA extraction kit (DP2033, BioTeke Corporation) in accordance with Liang et al. [15]. The extracted DNA was stored at −20 °C. The amplification of the internal transcribed spacer (ITS) region, the large subunit ribosomal RNA (LSU) gene, the RNA polymerase II largest subunit 1 (RPB1), the RNA polymerase II largest subunit 2 (RPB2), and the translation elongation factor 1 alpha (TEF) by PCR was described by White et al. [16], Rakotonirainy et al. [17], Castlebury et al. [18], and van den Brink et al. [19], respectively. PCR reactions for five loci of all strains were performed in a total volume of 25 µL containing 12.5 µL 2× PowerTaq PCR Master Mix (Tiangen Biotech (Beijing) Co., LTD, China), 1 µL of each primer (10 µM), 1 µL of genomic DNA (20–100 ng), and 9.5 µL of sterile water. Primer sequence information is shown in Table 1. PCR products were purified and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank (the accession number is shown in Table 2).

| Table 1. Primers information for 5-locus DNA sequences. |
|---------------------------------------------------------|
| Name | Length | Direction | Sequence 5'-3' | Optimised PCR Protocols | References |
|------|--------|-----------|---------------|------------------------|------------|
| ITS  | 22     | forward   | GGAAGTGAAAAGTGTGTAACAGG | (95 °C: 30 s, 51 °C: 50 s, 72 °C: 45 s) × 33 cycles | [16]       |
| ITS4 | 20     | reverse   | TCCCTCCGCATTGATATGC |                        |            |
| LSU  | 17     | forward   | ACCCGCTGAACTTAAGC | (94 °C: 30 s, 51 °C: 1 min, 72 °C: 2 min) × 33 cycles | [17]       |
| LR5  | 17     | reverse   | GCCCGCTGACCTTAAACGC |                        |            |
| RPB1 | 20     | forward   | CAYCCCGTGGTTCGTTAAGG | (94 °C: 30 s, 55 °C: 30 s, 72 °C: 1 min) × 33 cycles | [18]       |
| RPB1Cr| 23     | reverse   | CCNGCDATNTCRTTRTCCATRTA |                        |            |
| RPB2 | 20     | forward   | GACGACCGTGATCGTTTGG | (94 °C: 30 s, 54 °C: 40 s, 72 °C: 1 min 20 s) × 33 cycles | [19]       |
| TEF  | 23     | reverse   | GCCCATCCTGTTGTTCCCAT |                        |            |
| 983F | 20     | reverse   | GCCYCCCTGAGCCCATCGTTTGG | (94 °C: 30 s, 58 °C: 1 min 20 s, 72 °C: 1 min) × 33 cycles | [19]       |
| 2218R| 20     | reverse   | ATGACACCRACRGACRCRGTG |                        |            |
Table 2. List of strains and GenBank accession numbers of sequences used in this study.

| Species | Strain No. | Host/Substratum | GenBank Accession No. |
|---------|------------|-----------------|----------------------|
| Akanthomyces aculeatus | HUA 772 | Lepidoptera; Sphingidae | - | KC519370 - - - KC519366 |
| A. attenuates | CBS 402.78 | Leaf litter (Acer saccharum) | - | AF339565 EF468888 EF468935 EF468782 |
| A. coccidioperitheciatus | NHJ 6709 | Araneae (Spider) | AY624180 | EU369042 EU369067 - EU369025 |
| A. farinosa | CBS 541.81 | Lepidoptera (Adult moth) | MF416553 MF416655 MF416449 | JQ425686 |
| A. tuberculosis | BCC 16819 | Araneae (Spider) | GQ249987 - - - GQ250037 |
| A. tuberculosis | OSC 111002 | Plant | - | DQ518767 DQ522384 - DQ522338 |
| Ascopolyporus polychrous | P. C. 546 | Plant | - | DQ118237 DQ127236 - DQ118245 |
| B. brongniartii | ARSEF 617 | Lepidoptera; Arctiidae | - | HX880833 HX880905 HX880974 |
| B. brongniartii | ARSEF 1671 | Coleoptera; Scarabaeidae | - | ABO27381 HX880854 HX880926 HX880991 |
| B. coccidioperitheciatus | BOE 16855 | Lepidoptera; Araneidae | JN049867 JF415967 JN049885 | JF415991 JF416009 |
| B. coccidioperitheciatus | BOE 16856 | Soil | - | AF339520 HX880889 HX880961 | EF469057 |
| B. coccidioperitheciatus | BOE 16857 | Plant | - | DQ862027 - DQ862013 DQ862029 |
| Blackwallomyces cardinalis | OSC 93609 | Lepidoptera; Tineidae (Larva) | - | AY184963 EF469088 - EF469059 |
| B. cardinalis | ARSEF 2567 | Soil | - | AF339501 HX880895 - HX880933 |
| B. coccidioperitheciatus | ARSEF 101409 | Lepidoptera (Larva) | - | JN941393 JN992482 - JN992481 |
| B. pseudomilitaris | ARSEF 101410 | Lepidoptera (Larva) | - | JN941394 JN992482 - JN992481 |
| Calcarisporium arbuscula | CBS 221.73 | Hymenomycetes (Agarics sp.) | - | AY271809 - - - |
| C. arbuscula | CBS 900.68 | Lepidoptera; Coleotera; Aranea (Anomalia cuprea) | - | - - - |
| C. cordycipitale | CGMCC 3.17904 | Cordycipitaceae | - | JT945001 JT944260 - JT944260 |
| C. coccidioperitheciatus | CGMCC 3.17905 | Cordycipitaceae | - | JT945001 JT944260 - JT944260 |
| C. xylariolica | HMAS 276836 | Xylariales (Xylaria) | - | JT424603 JT424601 - JT424606 |
| Calonectria ilicicola | CBS 190.50 | Uredinales (Hemileia vastatrix) | - | AYJ29404 AF339548 - EF468847 |
| Cephalosporium curtipes | CBS 154.61 | Coleoptera (Adult) | - | - - - |
| C. cateniobliqua | CBS 153.83 | Lepidoptera | - | - - - JQ425688 |
| C. cf. farinosa | OSNO11004 | Lepidoptera; Coleoptera (Pupa) | - | - - - |
| C. coleopterorum | CBS 111.73 | Coleoptera (Larva) | AY624177 JF415988 JN049903 | JF416006 JF416028 |
| C. farinosa | CBS 111.111 | Coleoptera (Larva) | - | - - - |
| C. farinosa | CBS 107.10 | Coleoptera (Pupa) | - | MF416556 MF416556 MF416556 MF416556 |
| C. fusicaprea | EFCC 5690 | Lepidoptera (Pupa) | - | EF468806 EF468854 EF468909 EF468746 |
| C. cateniobliqua | CBS 152.83 | Coleoptera (Adult) | - | - - - |
| C. fusicaprea | EMSO11004 | Lepidoptera; Coleoptera (Pupa) | - | - - - |
| C. militaris | OSC 93623 | Lepidoptera; Coleoptera (Pupa) | - | - - - |
| Dactylonectria acalcerascens | CBS 125907 | Plant (Vitis vinifera) | JF735333 KM231629 - JF735819 |
| Elaphocordycipae | NBRCC106332 | - | - | - - - |
| E. paradoxa | NBRC 106958 | Larva | - | - - - |
| Engyodontium araneum | CBS 309.85 | Araneae (Spider) | - | - - - |
| Epichloe tephina | ATCC 56429 | Poaceae (Festuca rubra) | JN049832 U17396 - - |
| Flammochaelia aceris | CPC 24422 | Plant (Acer platanoides) | - | KR611883 KR611901 - |
| Flavocillium bifurcatum | YFC 6101 | Noctuidae (Larva) | - | MN576781 MN576841 MN576897 MN576951 |
| Fusarium circinatum | CBS 405.97 | - | - | JF735819 KM231943 |
| Fusarium solani | CBS 189.34 | Soil | - | U61677 - - |
| Gelasinospora tetrasperma | ATTOF-ID-1125 | - | - | DQ470980 - DQ471103 |
| Gibellula longispora | NHJ 12014 | Araneae (Spider) | - | - - - |
| G. pulchra | NHJ 10808 | Araneae (Spider) | - | EU369035 EU369056 - EU369075 |
| G. racketatauda | ARSEF 1915 | Araneae (Spider) | - | DQ518777 DQ522408 - DQ522360 |
| Haplocladium sinense | CBS 567.95 | Nematode | AJ294217 | AF339545 - - |
| Haplocladium sinense | ARSEF 5472 | - | - | NG_060621 - - |
| H. arachniphile | NHJ 10469 | Araneae (Spider) | - | EU369031 EU369047 - EU369008 |
| H. cinera | NHJ 3510 | Araneae (Spider) | - | EU369048 EU369048 - EU369008 |
| H. nhembobide | BCC 41864 | Araneae (Spider) | JN201871 - - |
| H. novoguineensis | NHJ 11923 | Araneae (Spider) | - | EU369032 EU369052 - EU369013 |
Table 2. Cont.

| Species                          | Strain No. | Host/ Substratum                  | GenBank Accession No. | ITS | LSU | RPB1 | RPB2 | TEF |
|----------------------------------|------------|-----------------------------------|-----------------------|-----|-----|------|------|-----|
| Hyperdermium pulvinatum          | P.C. 602   | Hemiptera (Scale insect)          | -                     | DQ118738 | DQ127237 | -    | DQ118746 |
| Hydropisphaera erubescens        | ATCC 36093 | -                                 | -                     | AF193230 | AY545731 | DQ518174 | - |
| H. peziza                        | GJS 92-101 | Plant (Bark)                      | DQ491488              | AY469730 | -     | AY489625 | DQ471043 |
| Hypocrea americana               | ATTOLOID 52| On decorticated conifer wood      | -                     | AY544649 | -     | -     | DQ471043 |
| H. lutea                         | ATCC 208838| -                                 | -                     | AY543791 | -     | DQ522446 | AY543781 |
| H. rufa                          | DAOM JBT1003| -                                 | DQ491488              | -    | -    | JN938665 | - |
| H. discoidea                     | BCC 8237   | -                                 | -                     | DQ518397 | -     | DQ522446 | DQ518477 |
| Hypomyces polyergusidis          | ATCC 76479 | -                                 | -                     | AF193579 | -     | AY457843 | - |
| I. farinosa                      | CEP 004    | Soil                              | JN998783              | -    | -    | -     | JN998764 |
| I. farinosa                      | CEP 005    | Soil                              | JN998784              | -    | -    | -     | JN998764 |
| I. farinosa                      | CEP 029    | Trialeurodes                      | JN998785              | -    | -    | -     | JN998764 |
| Lecanicillium antillanum         | CBS 350.85 | Hemiptera (Coccus viridis)        | KM283800              | KM283859 | -     | -     | KM283823 |
| L. attenuatum                    | CBS 402.78 | Leaf litter of Acer saccharum     | -                     | AF39565  | EF468888 | AF468935 | EF468782 |
| L. araneatum                     | CBS 726.73a| Arachnida (Spider)                | -                     | AF39537  | EF468877 | AF468934 | EF468781 |
| L. fassiporum                    | CBS 164.70 | Hymenomyces (Coltricia perennis)  | -                     | AF39549  | EF468889 | -     | EF468783 |
| L. psalliotae                    | CBS 367.86 | Puccinia graminis                 | -                     | KM283800 | -     | -     | KM283823 |
| L. lecanii                       | CBS101247  | Hemiptera (Coccus viridis)        | JN09836               | KM283859 | -     | -     | KM283823 |
| Leptobacillium chinesense        | LC 1345    | submerged wood                    | -                     | JQ10122  | -     | -     | - |
| L. coelum                       | CDA 734    | Plant (Coffea arabica)            | -                     | MF966032 | -     | -     | - |
| L. fassiporum                    | YFC 3103   | Fungi (Beauveria yunnanensis)     | -                     | MN57678  | MN576842 | MN576898 | MN576952 |
| L. psalliotae                    | YFC 3104   | Fungi (Beauveria yunnanensis)     | -                     | MN57678  | MN576843 | MN576899 | MN576953 |
| Metapochonia goniodes            | CBS 891.72 | Fungi                             | AJ292409              | AF39535  | DQ522450 | DQ522350 | DQ522354 |
| Myrotheciomyces corymbae         | CPC 33206  | Plant (Corbymia variegata)        | NR_160351             | NG_06542 | -     | -     | - |
| Myrotheciomyces corymbae         | CPC 33206  | Plant (Corbymia variegata)        | -                     | -     | -     | -     | - |
| Myrotheciomyces corymbae         | CPC 33206  | Plant (Corbymia variegata)        | -                     | -     | -     | -     | - |
| M. moravica                      | IMI 156855 | Hymenomyces (Russula nigricans)   | -                     | AY489731 | -     | -     | AY489626 |
| M. roridum                       | ATCC 16297 | Soil                              | -                     | AY489708 | -     | -     | AY489603 |
| M. verrucaria                    | ATCC 9095  | Plant (Goosnium sp.)              | -                     | AY489713 | -     | -     | AY489686 |
| Nectria cinnabarina             | CBS 125165 | Plant (Arسicus sp.)              | -                     | HM484548 | HM484562 | KM283420 | HM484527 |
| N. nigrescens                    | CBS 125148 | Plant (Dicytledonous tree)        | -                     | HM484707 | HM484720 | KM283420 | HM484672 |
| Nectriopsis violacea             | CBS 424.64 | Fungi (Fuligo sp.)                | -                     | AY489719 | -     | -     | - |
| N. ramulariae                    | CBS 182.36 | Fungi (Bertia moriformis)         | -                     | -     | -     | -     | - |
| N. rufa                          | CBS 217.67 | Plant (Malus sylvestris)          | -                     | HM484548 | HM484562 | KM283420 | HM484527 |
| N. neomacrispora                 | CBS 217.67 | Plant (Malus sylvestris)          | -                     | HM484548 | HM484562 | KM283420 | HM484527 |
| Neurospora crassa                | JCM (636)  | Fungi (Bertia moriformis)         | -                     | AY681193 | AY681158 | -     | - |
| Neurospora crassa                | CBS 560.74 | Fungi (Bertia moriformis)         | -                     | AY489720 | -     | -     | AY489614 |
| Ophiocordyceps heteropoda        | EFCC 10125 | Cicadidae (Tibicen slotatus)      | JN049852              | EF468812 | EF468914 | EF468752 | - |
| O. sinensis                      | EFCC 7287  | Lepidoptera (Ghoshmoth)            | JN049854              | EF468827 | EF468924 | EF468767 | - |
| O. stylophorus                   | OSC 111000 | Plant (Theobroma cacao)           | JN049828              | DQ518766 | DQ522433 | DQ522337 | - |
| Peltastambora spirostriata       | CBS 110115 | Plant (Theobroma cacao)           | -                     | AY39724  | EF462516 | AY489619 | - |
| Purpureocillium lilacinum        | CBS 284.36 | Soil                              | -                     | AY624227 | EF468989 | EF468941 | EF468792 |
| P. lilacinum                     | CBS 431.87 | Nematode (Meloidogyne sp.)        | HQ482812              | EF468844 | EF468987 | EF468940 | EF468791 |
| Rosapheria moravica              | LMM        | -                                 | -                     | -     | -     | -     | JF440897 |
| Roumeguerella rufula             | GJS 91-64  | -                                 | JF440895              | -     | -     | -     | JF440897 |
| R. rufula                        | CBS 346.85 | Lepidoptera (Pupa)                | -                     | JF415979 | JN049895 | JF145999 | JF146109 |
| Samsoniella albousamantium       | CBS 240.32 | Lepidoptera (Pupa)                | -                     | JF415979 | JN049895 | JF145999 | JF146109 |
| S. alpina                        | YFCC 5818  | Hepialidae (Hepialus baiamensis)   | -                     | MN576809 | MN576869 | MN576923 | MN576979 |
| S. alpina                        | YFCC 5831  | Hepialidae (Hepialus baiamensis)   | -                     | MN576810 | MN576870 | MN576924 | MN576980 |
| S. antleroides                   | YFCC 6016  | Noctuidae (Larvae)                | -                     | MN576803 | MN576863 | MN576919 | MN576973 |
### Table 2. Cont.

| Species                  | Strain No. | Host/ Substratum                                      | GenBank Accession No.          |
|--------------------------|------------|------------------------------------------------------|-------------------------------|
| S. antleroides           | YFCC 6113  | Noctuidae (Larvae)                                    | ITS - MN576804, LSU - MN576864, RPB1 - MN576918, RPB2 - MN576974 |
| S. aurantia              | TBRC 7271  | Lepidoptera                                          | MF140728, MF140791, MF140818, MF140846 |
| S. aurantia              | TBRC 7272  | Lepidoptera                                          | MF140727, MF140792, MF140817, MF140845 |
| S. aurantia              | DY10951    | Lepidoptera (Pupa)                                   | MZ827666, MZ827667, MZ855229 |
| S. cardinalis            | YFCC 5830  | Limacodidae (Pupa)                                   | MN576788, MN576848, MN576902, MN576958 |
| S. cardinalis            | YFCC 6144  | Limacodidae (Pupa)                                   | MN576786, MN576846, MN576900, MN576956 |
| S. coleopterorum         | A19501     | Curculionidae (Snout beetle)                          | MT626376, MT642600, MT610585, MT610586 |
| S. coleopterorum         | A19502     | Curculionidae (Snout beetle)                          | MT626265, MT642603, MT610587, MT610588 |
| S. cristata              | YFCC 6021  | Satuniidae (Pupa)                                    | MN576791, MN576851, MN576905, MN576961 |
| S. cristata              | YFCC 6023  | Satuniidae (Pupa)                                    | MN576792, MN576852, MN576906, MN576962 |
| S. hepiali               | ICMM 82-2  | Fungi (Ophiocordyceps sinensis)                       | MN576789, MN576854, MN576908, MN576964 |
| S..hepiali               | ICMM Cs-4  | Fungi (Ophiocordyceps sinensis)                       | - MN576799, MN576859, MN576913, MN576969 |
| S. hymenopterorum        | A19521     | Vespidae (Bee)                                       | MN128224, MT642601, MT610589, MT610590 |
| S. hymenopterorum        | A19522     | Vespidae (Bee)                                       | MN128081, MN101589, MN101590, MN101591 |
| S. inthanonensis         | TBRC 7915  | Lepidoptera (Pupa)                                   | MN576794, MN576854, MN576908, MN576961 |
| S. inthanonensis         | TBRC 7916  | Lepidoptera (Pupa)                                   | MN576795, MN576855, MN576909, MN576965 |
| S. lanmaoa               | YFCC 6148  | Lepidoptera                                          | - MN576791, MN576851, MN576905, MN576961 |
| S. lanmaoa               | YFCC 6193  | Lepidoptera                                          | - MN576792, MN576852, MN576906, MN576962 |
| S. lepidopterorum        | DL10071    | Lepidoptera (Pupa)                                   | MN128076, MN101592, MN101593, MN101594 |
| S. lepidopterorum        | DL10072    | Lepidoptera (Pupa)                                   | MN128074, MT642605, MT610585, MT610596 |
| S. pseudoguini           | CY407201   | Lepidoptera (Larvae)                                 | MZ827470, MZ827010, MZ855232, MZ855233 |
| S. pseudoguini           | CY407202   | Lepidoptera (Larvae)                                 | MZ831863, MZ831865, MZ855234, MZ855235 |
| S. pseudoguini           | CY101681   | Lepidoptera (Pupa)                                   | MZ827085, MZ827009, MZ855237, MZ855238 |
| S. ramose                | YFCC 6020  | Limacodidae (Pupa)                                   | MN576780, MN576852, MN576916, MN576972 |
| S. tortricidae           | YFCC 6013  | Limacodidae (Pupa)                                   | MN576780, MN576852, MN576921, MN576977 |
| S. tortricidae           | YFCC 6014  | Limacodidae (Pupa)                                   | MN576780, MN576852, MN576920, MN576976 |
| S. yunnanensis           | YFCC 1824  | Limacodidae (Pupa)                                   | MN576782, MN576872, MN576926, MN576982 |
| S. yunnanensis           | YFCC 1527  | Limacodidae (Pupa)                                   | MN576783, MN576873, MN576927, MN576983 |

**Sarocladium bacillisporum**

| Species                  | Strain No. | Host/ Substratum                                      | GenBank Accession No.          |
|--------------------------|------------|------------------------------------------------------|-------------------------------|
| S. dejongiae             | CBS 14929  | Soil                                                 | NR_145039, MH870718, - - - -  |
| S. implicatum            | CBS 9572   | Soil                                                 | HG965023, MH878470, - - - -  |
| S. subulatum             | CBS 217.35 | Soil                                                 | MH855652, NG_070566, - - - -  |
| S. terricola             | CBS 243.59 | Soil                                                 | MH857853, MH863989, - - - -  |

**Shimizuomyces paradoxus**

| Species                  | Strain No. | Host/ Substratum                                      | GenBank Accession No.          |
|--------------------------|------------|------------------------------------------------------|-------------------------------|
| S. lanosoniceum          | CBS 101267 | Soil                                                 | - AJ292395, DQ522643, DQ522356 |
| S. lanosoniceum          | CBS 704.86 | Soil                                                 | AJ292396, AF339553, DQ522643, DQ522358 |
| Sordaria fimicola        | AFTOL-ID 216| Soil                                                 | AQ018178, - - - - - - - - - - |
| Sphaerostilbella aureolens| GS74-87   | Soil                                                 | HG44263, HM446683, FJ42763, - |
| S. berkeleyana           | GS82-274  | Soil                                                 | HG859197, - - - - - - - - - - |
| Stachybotrys             | CBS 243.99 | Soil                                                 | - MG00171, - - - - - - - - - - |
| S. euclyndrospora        | ATCC 18851 | Plant (Bark)                                         | - JN942887, JN938870, - - - - - |
| Stephanonectria keithii  | GJS89-123  | Soil                                                 | - JN942887, JN938869, JN938869, JN938869, JN938870 |

**Tilachlidium brachiatum**

| Species                  | Strain No. | Host/ Substratum                                      | GenBank Accession No.          |
|--------------------------|------------|------------------------------------------------------|-------------------------------|
| T. brachiatum            | CBS 363.97 | Soil                                                 | KM231838, KM231719, KM232414, KM231975 |
| Tolypocladium inflatum   | SCALT1007-002| Soil                                                 | KM231839, HQ232177, KM232415, KM231975 |
| Trichoderma aggregans    | CBS 100525 | Soil                                                 | JN938987, JQ014130, JQ014130, JQ014130 |
| Trichotheceum roseum     | GJS 96-174 | Soil                                                 | JX458661, JQ014130, JQ014130, JQ014130 |
| Valetoniellopsis laxa    | GJS 96-174 | Soil                                                 | JX458660, JQ014130, JQ014130, JQ014130 |

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2.3. Sequence Alignment and Phylogenetic Analyses

Lasergene software (version 6.0, DNASTAR) was applied for the assembling and editing of DNA sequence in this study. The ITS, LSU, RPB1, RPB2, and TEF sequences were downloaded from GenBank, based on Kepler et al. [9], Mongkolsamrit et al. [10,19], Chen et al. [12], Wang et al. [20], and others selected on the basis of BLAST algorithm-based searches in GenBank (Table 2). A single gene data set was aligned and edited by MAFFT v7.037b [21] and MEGA6 [22]. Combined sequences of ITS, LSU, RPB1, RPB2, and TEF were performed by SequenceMatrix v.1.7.8 [23]. The combined datasets (ITS+LSU+RPB2+TEF) and (ITS+LSU+RPB1+RPB2+TEF) were used to determine the family placement of those strains in Hypocreales and the taxonomic position of strains and the cryptic diversity among the different isolates of I. farinosa in Cordycipitaceae.

The combined genes were both analyzed using the Bayesian inference (BI) and maximum likelihood (ML) methods. For BI, the model was selected for Bayesian analysis by ModelFinder [24] in the software PhyloSuite [25]. A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v.3.2 [26] for the combined sequence datasets. The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 16,001 trees were used for calculating posterior probabilities in the majority rule consensus tree. After the analysis was finished, each run was examined using the program Tracer v1.5 [27] to determine burn-in, confirming that both runs had converged. ML analyses were constructed with RAxMLGUI [28]. The GTR+GAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites.

3. Results
3.1. Phylogenetic Analyses

*Gelasinospora tetrasperma* Dowding, *Neurospora crassa* Shear and B.O. Dodge, and *Sordaria fimicola* (Roberge ex Desm.) Ces. and De Not. were used as the outgroup in analysis 1 (Figure 1) (to determine the family placement of those strains in Hypocreales). *Purpureocillium lilacinum* (Thom) Luangsaa-ard, Houbraken, Hywel-Jones, and Samson was used as the outgroup in analysis 2 (Figure 2) (to determine the taxonomic position of strains and the cryptic diversity among the different isolates of *I. farinosa* in Cordycipitaceae).

The concatenated sequences of analysis 1 and 2 included 77 and 62 taxa, respectively, and consisted of 2396 (ITS, 620; LSU, 712; RPB2, 510; and TEF, 554) and 3309 (ITS, 554; LSU, 677; RPB1, 533; RPB2, 671; and TEF, 874) characters with gaps, respectively.

Analysis 1: The selected models for BI analysis were GTR+F+I+G4 parameters for partition ITS and LSU+RPB2, and GTR+F+G4 parameters for partition TEF. The final value of the highest scoring tree was –37,321.078127, which was obtained from an ML analysis of the dataset (ITS+LSU+RPB2+TEF). The parameters of the general time reversible (GTR) model used to analyze the dataset were estimated using the following frequencies: A = 0.230263, C = 0.272892, G = 0.280445, and T = 0.216401; substitution rates AC = 1.451341, AG = 2.441940, AT = 1.532513, CG = 1.182477, CT = 5.701598, and GT = 1.000000; as well as the gamma distribution shape parameter \(\alpha\) = 0.381402. In the phylogenetic tree (Figure 1), both analyses of ML and BI trees were largely congruent, and strongly supported in most branches. DY10951, DY10952, DY101681, DY101682, GY407201, GY407202, YJ06171, and YJ06172 strains had a close relationship with *Cordyceps* Fr., *Akanthomyces* Lebert, and *Simplicillium* W. Gams and Zare, and clustered into Cordycipitaceae.
Cordycipitaceae). The concatenated sequences of analysis 1 and 2 included 77 and 62 taxa, respectively, and consisted of 2396 (ITS, 620; LSU, 712; RPB2, 510; and TEF, 554) and 3309 (ITS, 554; LSU, 677; RPB1, 533; RPB2, 671; and TEF, 874) characters with gaps, respectively.

Figure 1. Phylogenetic placement of the new Isaria-like strains in the order of Hypocreales based on multigene dataset (ITS, LSU, RPB2m and TEF). Statistical support values (≥50%/0.5) are shown at the nodes for ML bootstrap support/BI posterior probabilities.
Figure 2. Phylogenetic placement of the new strains in Cordycipitaceae, based on multigene dataset (ITS, LSU, RPB1, RPB2, and TEF). Statistical support values (≥50%/0.5) are shown at the nodes for ML bootstrap support/BI posterior probabilities.

Analysis 2: The selected models for BI analysis were GTR+F+I+G4 parameters for partition ITS+LSU+RPB2+TEF and GTR+F+G4 parameters for partition RPB1. The final value of the highest scoring tree was –31,206.916701, which was obtained from an ML analysis of the dataset (ITS+LSU+RPB1+RPB2+TEF). The parameters of the general time reversible (GTR) model used to analyze the dataset were estimated using the following frequencies: A = 0.238319, C = 0.279080, G = 0.271674, and T = 0.210926; substitution rates AC = 1.120096, AG = 2.745044, AT = 0.784066, CG = 0.934312, CT = 6.322628, and GT = 1.000000; as well as the gamma distribution shape parameter α = 0.308970. In the phylogenetic tree (Figure 2),
both analyses of ML and BI trees were largely congruent, and strongly supported in most branches. The new strains were all clustered within the genus *Samsoniella*. GY407201 and GY407202 strains clustered with *Samsoniella coleopterorum* W.H. Chen, Y.F. Han, and Z.Q. Liang in a subclade. DY10951 and DY10952 strains clustered with *Samsoniella aurantia* Mongkols., Noirsrip., Thanakitp., Spathafora, and Luangsa-ard in a subclade. PY101681 and DY101682 strains had a close relationship with *Samsoniella aboaurentia* (G. Sm.) Mongkols., Noirsrip., Thanakitp., Spathafora, and Luangsa-ard; *Samsoniella alpina* H. Yu, Y.B. Wang, Y. Wang, and Zhu L. Yang; and *Samsoniella cardinalis* H. Yu, Y.B. Wang, Y. Wang, Q. Fan, and Zhu L. Yang. YJ06171 and YJ06172 strains clustered with *Isaria farinosa* (Holmsk.) Fr. in a subclade and had close relationship with *Samsoniella hepiali* (Q.T. Chen and R.Q. Dai ex R.Q. Dai, X.M. Li, A.J. Shao, Shu F. Lin, J.L. Lan, Wei H. Chen, and C.Y. Shen) H. Yu, R.Q. Dai, Y.B. Wang, Y. Wang, and Zhu L. Yang.

3.2. Taxonomy

3.2.1. *Samsoniella pseudogunnii* W.H. Chen, Y.F. Han, J.D. Liang, and Z.Q. Liang, sp. nov.

MycoBank No.: MB840999

Etymology: referring to similar morphology with *Keithomyces neogunnii*.

Holotype: CHINA, Guizhou, Qiannan Buyi and Miao Autonomous Prefecture, W.H. Chen, Y.F. Han, J.D. Liang, and Z.Q. Liang, sp. nov. (holotype), ex Wanhao Chen, GZAC DY10168 (holotype), ex Duyun City (26°21' 3" N, 107°22' 58.70"

Description: Colonies on PDA, 4.1–4.3 cm diam. after 14 d at 25°C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.0–1.3 µm diam. Erect conidiophores usually arises from aerial hyphae. Phialides are solitary or in whorls of two to nine. Phialides 6.8–11.0 × 2.2–2.4 µm, with a cylindrical basal portion, tapering into a short distinct neck. Conidia in chains, hyaline, fusiform, one-celled, 2.8–3.2 × 1.7–2.1 µm. Chlamydospores and sexual state were not observed. Sizes and shapes of phialides and conidia are similar in culture and on natural substratum.

Known distribution: Tongmuling, Guiyang, Guizhou Province, China.

Notes: *Samsoniella pseudogunnii* was identified as belonging to *Samsoniella* based on the phylogenetic analyses (Figure 2) and has a close relationship with *S. coleopterorum*. However, *Samsoniella pseudogunnii* (Figure 3) has longer phialide, larger conidia, and its larva host belongs to the order Lepidoptera.

![Figure 3. Samsoniella pseudogunnii (A) infected larva (Lepidoptera) (B,C) culture plate, showing the front (B) and the reverse (C) of the colony, cultured on PDA medium (D–I) phialides solitary, conidia adhering ellipsoidal slimy head and conidia (J) conidia. Scale bars: 10 mm (B,C), 10 µm (D–J).](image-url)
3.2.2. Samsoniella Pupicola W.H. Chen, Y.F. Han, J.D. Liang, and Z.Q. Liang, sp. nov.

MycoBank No.: MB841003

Etymology: referring to its pupa-inhabitor.

Holotype: CHINA, Guizhou, Qiannan Buyi and Miao Autonomous Prefecture, Duyun City (26°21'24.71" N, 107°22'48.22" E). On a pupa (Lepidoptera), 1 October 2019, Wanhao Chen, GZAC DY10168 (holotype), ex-type living cultures, DY101681, DY101682.

Description: Colonies on PDA, 2.3–2.4 cm diam. after 14 d at 25°C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.2–2.2 μm diam. Erect conidiophores usually arise from aerial hyphae. Phialides are solitary or in whorls of two to nine. Phialides 7.0–9.2 × 2.5–3.3 μm, with a cylindrical basal portion, tapering into a short distinct neck. Conidia in chains, hyaline, fusiform, one-celled, 2.5–3.3 × 2.2–2.6 μm. Chlamydospores and sexual state were not observed. Sizes and shapes of phialides and conidia are similar in culture and on natural substratum.

Known distribution: Duyun City, Qiannan Buyi and Miao Autonomous Prefecture, Guizhou Province, China.

Additional specimens examined: CHINA, Guizhou, Qiandongnan Miao and Dong Autonomous Prefecture, Rongjiang County (26°01'58.70" N, 108°24'48.06" E), on a lepidopteran pupa, 1 October 2018, W.H. Chen, GZAC DL1014.

Notes: Samsoniella pupicola was identified as belonging to Samsoniella, based on the phylogenetic analyses (Figure 2) and has a close relationship with S. alboaurantium, S. alpina, and S. cardinalis. However, S. pupicola (Figure 4) is distinguished from S. alboaurantium by having larger fusiform conidia, distinguished from S. alpina by having white colony and fusiform conidia, and distinguished from S. cardinalis by having shorter phialides.

Figure 4. Samsoniella pupicola (A) infected pupa (Lepidoptera) (B,C) culture plate, showing the front (B) and the reverse (C) of the colony, cultured on PDA medium (D–J) phialides solitary, conidia adhering ellipsoidal slimy head and conidia (K) conidia. Scale bars: 10 mm (B,C), 10 μm (D–K).

3.2.3. Samsoniella aurantia Mongkols., Noisrip., Thanakitp., Spatafora, and Luangsa-ard, Mycologia 110(1): 249

Description: Colonies on PDA, 3.7–4.2 cm diam. after 14 d at 25°C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, pale green and pale pink in the middle of the colony, reverse yellowish and pale brown in the middle. Prostrate hyphae smooth, septate, hyaline, 1.3–2.6 μm diam. Erect conidiophores usually arise from aerial hyphae. Phialides are solitary or in whorls of two to ten. Phialides 3.6–7.7 × 1.3–1.6 μm, with a cylindrical basal portion, tapering into a short distinct neck. Conidia in chains, hyaline,
fusiform, occasionally cylindrical, one-celled, 2.6–3.9 × 1.7–2.2 μm. Chlamydospores and sexual state were not observed. Sizes and shapes of phialides and conidia are similar in culture and on natural substratum.

Specimens examined: CHINA, Guizhou, Qiannan Buyi and Miao Autonomous Prefecture, Duyun City (26°21′24.71″ N, 107°22′48.22″ E). On a pupa (Lepidoptera), 1 October 2019, Wanhao Chen, GZAC YJ0617, DY1044, living cultures, DY10951, DY10952.

Note: DY10951 and DY10952 strains were identified as belonging to Samsoniella, based on the phylogenetic analyses (Figure 2), and clustered with Samsoniella aurantia in a clade. The characteristics of DY10951 and DY10952 (Figure 5) strains are similar to that of S. aurantia, which had fusiform conidia (2–4 × 1–2 μm) and larger phialide (5–13 × 2–3 μm). Besides, the pairwise dissimilarities of ITS sequences show no difference within 554 bp between DY10951 and S. aurantia. Thus, molecular phylogenetic results and morphologically based conclusions support the idea that DY10951 and DY10952 strains were S. aurantia.

![Figure 5. Samsoniella aurantia (A) infected pupa (Lepidoptera) (B,C) culture plate, showing the front (B) and the reverse (C) of the colony, cultured on PDA medium (D–J) phialides solitary, conidia adhering ellipsoidal slimy head and conidia (K) conidia. Scale bars: 10 mm (B,C), 10 μm (D–K).]

3.2.4. Samsoniella hepiali (Q.T. Chen, and R.Q. Dai ex R.Q. Dai, X.M. Li, A.J. Shao, Shu F. Lin, J.L. Lan, Wei H. Chen, and C.Y. Shen) H. Yu, R.Q. Dai, Y.B. Wang, Y. Wang, and Zhu L. Yang, Fungal Diversity 103: 31

Description: Colonies on PDA, 5.8–5.9 cm diam. after 14 d at 25°C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.1–1.8 μm diam. Erect conidiophores usually arise from aerial hyphae. Phialides are solitary or in whorls of two to eight. Phialides 6.0–7.8 × 1.5–1.8 μm, with a cylindrical basal portion, tapering into a short distinct neck. Conidia in chains, hyaline, fusiform, one-celled, 2.1–2.5 × 0.9–1.6 μm. Chlamydospores and sexual state were not observed. Sizes and shapes of phialides and conidia are similar in culture and on natural substratum.

Specimens examined: CHINA, Guizhou, Tongren City, Yinjiang (N 27°55′17.1″, E 108°41′25.2″), on an ant, 1 October 2019, Wanhao Chen, GZAC YJ0617, DY1044, living cultures, YJ06171, YJ06172.

Note: YJ06171 and YJ06172 strains were identified as belonging to Samsoniella, based on the phylogenetic analyses (Figure 2), and clustered with Samsoniella hepiali in a clade. The characteristics of YJ06171 and YJ06172 (Figure 6) strains were very closely linked with S. hepiali, which had fusiform or oval conidia (1.8–3.3 × 1.4–2.2 μm) and larger phialide (3.5–13.6 × 1.3–2.1 μm). Besides, the pairwise dissimilarities of LSU sequences show no
difference within 677 bp between YJ06171 and S. hepiali. Thus, molecular phylogenetic results and morphologically based conclusions supported the idea that YJ06171 and YJ06172 strains were S. hepiali.

Figure 6. *Samsoniella hepiali* (A) infected ant (Formicidae) (B,C) culture plate, showing the front (B) and the reverse (C) of the colony, cultured on PDA medium (D–J) phialides solitary, conidia adhering ellipsoidal slimy head and conidia (N) conidia. Scale bars: 10 mm (B,C), 10 μm (D–N).

4. Discussion

The taxonomic delimitation of *Isaria* was originally based on morphological characteristics. However, *Isaria* shares many morphological characters with other genera in Hypocreales, which has resulted in a turbulent taxonomic history [10,29]. D’Alessandro et al. [30] noted that the morphological characteristics used to classify the genus *Isaria* frequently do not resolve new isolates into clearly defined species and need additional molecular markers in phylogenetic analyses. In the present study, *Isaria*-like strains collected from Guizhou Province, China, and previously identified by morphological characteristics, were reanalyzed using multi-gene (ITS, LSU, RPB1, RPB2, TEF) phylogenetic methodology. We proposed two new species of *Samsoniella* in this study.

The species *Isaria farinosa* is a well-known entomopathogenic fungi with worldwide distribution and a wide host range [31]. Kepler et al. [9] transferred *Isaria farinosa* to the genus *Cordyceps* as *C. farinosa* (Holmsk.) Kepler, B. Shrestha, and Spatafora based on a phylogenetic analysis of the CBS 111113 strain. We analyzed several strains of *Isaria farinosa* in the present study. Some properly belonged in the genus *Samsoniella*. CEP 004, CEP 005, CEP 029, YJ06171, and YJ06172 strains were identified as *S. hepiali*. Strains DY10951 and DY10952 were identified as *S. aurantia*. OSC 111005 and OSC 111006 strains were identified as new species but are absent in delineating morphological characteristics. Our results reveal cryptic diversity present in *Isaria farinosa* (now treated as *Cordyceps farinosa*) and illustrated that more attention should be paid on cryptic intraspecific diversity across different fungi isolates and genotypes.

The genus *Samsoniella* was established for the typical species *S. inthanonensis* Mongkol-samrit, Noisripoom, Thanakitpipattana, Spatafora, and Luangs-ard, and two other species (*S. alboaurantia* (G. Sm.) Mongkols., Noisrip., Thanak., Spatafora, and Luangs-ard and *S. aurantia* Mongkols., Noisrip., Thanak., Spatafora, and Luangs-ard) [10]. *Samsoniella* species all have *Isaria*-like morphological characteristics, and cluster in an independent clade with close relationship to the genus *Akanthomyces*. The species *S. alboaurantia* was established based on two strains, CBS 240.32 and CBS 262.58, which previously belonged to *Isaria farinosa* (originally designated *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. and G. Sm.) [10]. Lin et al. [32] revised the taxonomy of some *Isaria*-like strains originally identified as *Isaria farinosa* by morphological characteristics using multi-gene phylogenetic analysis. All the strains were identified as *Samsoniella hepiali* (Q.T. Chen and R.Q. Dai ex R.Q. Dai,
X.M. Li, A.J. Shao, Shu F. Lin, J.L. Lan, Wei H. Chen, and C.Y. Shen) H. Yu, R.Q. Dai, Y.B. Wang, Y. Wang, and Zhu L. Yang. In the present study, YJ06171 and YJ06172 strains were also identified as *Samsoniella hepiali*. Our results revealed that more isolates and genotypes, originally designated as *Isaria*, will need to be transferred to *Samsoniella*.

*Samsoniella hepiali* (otherwise known as *Paecilomyces hepiali*) is isolated from a field collection of natural *Ophiocordyceps sinensis* insect–fungi complex [33], and is widely used as a medicinal and edible cordycepitoid fungus, creating a great economic value [20]. Lin et al. [32] reported six isolates of *Samsoniella hepiali* from Anhui Province, China, which were isolated from leafhopper, larva, and cicada. CEP 004, CEP 005, CEP 029 strains from Buenos Aires, Argentina, were isolated from whitefly and soil [30]. YJ06171 and YJ06172 strains from Guizhou Province, China were isolated from ant. It is interesting that *Samsoniella hepiali* and its hosts are widely distributed in China and Argentina. This result will help us to assess the extent and distribution of genetic diversity of *Samsoniella hepiali* on a large scale, understand its biology and demographic history, and guide biodiversity conservation programs.

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