Morphological and phylogenetic characterisations reveal three new species of *Samsoniella* (Cordycipitaceae, Hypocreales) from Guizhou, China

Wan-Hao Chen¹, Yan-Feng Han², Jian-Dong Liang¹, Wei-Yi Tian¹, Zong-Qi Liang²

¹ Basic Medical School, Guizhou University of Traditional Chinese Medicine, Guiyang 550025, Guizhou, China ² Institute of Fungus Resources, Department of Ecology, College of Life Sciences, Guizhou University, Guiyang 550025, Guizhou, China

Corresponding author: Yan-Feng Han (swallow1128@126.com)

Academic editor: T. Lumbsch  |  Received 17 July 2020  |  Accepted 23 September 2020  |  Published 19 October 2020

Citation: Chen W-H, Han Y-F, Liang J-D, Tian W-Y, Liang Z-Q (2020) Morphological and phylogenetic characterisations reveal three new species of *Samsoniella* (Cordycipitaceae, Hypocreales) from Guizhou, China. MycoKeys 74: 1–15. https://doi.org/10.3897/mycokeys.74.56655

Abstract

*Samsoniella* species have been found on lepidopteran larvae or pupae buried in soil or leaf litter. Three new species, *Samsoniella hymenopterorum*, *S. coleopterorum* and *S. lepidopterorum*, parasitic on hymenopteran larvae, coleopteran larvae and lepidopteran pupae, respectively, are reported. Morphological comparisons with extant species and DNA-based phylogenies from analysis of a multigene (ITS, *RPB1*, *RPB2* and *TEF*) dataset supported the establishment of the new species. Unusually, all three new species have mononematous conidiophores. The new species are clearly distinct from other species in *Samsoniella* occurring in separate subclades.

Keywords

Isaria-like, morphology, nutritional preference, phylogeny

Introduction

The genus *Isaria* Pers. was introduced for entomogenous fungi with mononematous or symnematous conidiophores, usually consisting of several verticillate branches, each bearing a dense whorl of phialides characters. The phialides consist of a cylindrical or swollen basal portion, terminating in a thin, often long neck and
produce divergent conidial chains (Samson 1974). However, entomogenous species, morphologically similar to Isaria, can be found distributed throughout the Hypocreales (Luangsa-ard et al. 2004).

Kepler et al. (2017) proposed the rejection of Isaria in favour of Cordyceps, owing to the confusion surrounding the application of Isaria and combined 11 species into Cordyceps. Mangkolsamrit et al. (2018) described some Isaria-like species and proposed the new genus Samsoniella Mangkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard. The typical characteristics of Samsoniella are oval to fusiform conidia and bright red-orange teleomorphic stromata and anamorphic synnemata. Samsoniella species inhabit lepidopteran larvae and pupae in leaf litter or soil. Currently, Samsoniella consists of three species, S. alboaurantia (G. Sm.) Mangkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, S. aurantia Mangkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard and S. inthanonensis Mangkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard.

Three infected insect specimens were collected during a survey of entomogenous fungi in south-western China. Morphological and molecular phylogenetic analyses suggested that these isolates represented three new species, which are described here as Samsoniella hymenopterorum sp. nov., S. coleopterorum sp. nov. and S. lepidopterorum sp. nov.

**Materials and methods**

**Specimen collection and identification**

Three fungus-infected insect specimens were collected from Xishui County (28°29’56.70”N, 106°24’31.04”E) (A1950 and A1952) and Dali, Rongjiang County (26°01’58.70”N, 108°24’48.06”E) (DL1007), Guizhou Province, on 20 July and 1 October 2018, respectively. Isolation of the fungi was done as described by Chen et al. (2019). The surface of the specimens was rinsed with sterile water, followed by surface sterilisation with 75% ethanol for 3–5 sec. A part of the insect body was cut off and inoculated with haemocoel on potato dextrose agar (PDA) and PDA, to which 1% w/v peptone (PDAP) had been added. Fungal colonies emerging from specimens were isolated and cultured at 22 °C for 14 d under 12 h light/12 h dark conditions following protocols described by Zou et al. (2010). Accordingly, strains A19501, A19502, A19521, A19522, DL10071 and DL10072 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 fungi were examined and growth rates determined from PDA cultures incubated at 25 °C for 14 d. 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).
DNA extraction, PCR amplification and nucleotide sequencing

DNA extraction was carried out in accordance with Liang et al. (2011). The extracted DNA was stored at −20 °C. Translation elongation factor 1 alpha (TEF) and RNA polymerase II largest subunit 2 (RPB2) genes were amplified using 983F/2218R and RPB2-5F/RPB2-7Cr primers, according to van den Brink et al. (2012). The RNA polymerase II largest subunit 1 (RPB1) gene was amplified with the primer pair CRPB1 and RPB1-Cr (Castlebury et al. 2004). The internal transcribed spacer (ITS) region was amplified by PCR using ITS4/ITS5, which was described by White et al. (1990). PCR products were purified using the UNIQ-10 column PCR products purification kit (no. SK1141; Sangon Biotech (Shanghai) Co., Shanghai, China) in accordance with the manufacturer’s protocol and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

Sequence alignment and phylogenetic analyses

The DNA sequences, generated in this study, were assembled and edited using Lasergene software (version 6.0 DNASTAR). Generated ITS, RPB1, RPB2 and TEF sequences were aligned with those published by Mongkolsamrit et al. (2018) and others selected on the basis of BLAST algorithm-based searches in GenBank (Table 1). Purpureocillium lilacinum (Thom) Luangsarad, Houbraken, Hywel-Jones & Samson (isolates CBS 284.36 and CBS 431.87) and Beauveria bassiana (Bals.-Criv.) Vuill. (ARSEF 1564) were chosen as outgroup taxa for the analysis of Samsoniella in Cordycipitaceae and Samsoniella species and closely-related species, respectively. Multiple datasets of ITS, RPB1, RPB2 and TEF were aligned using MAFFT v7.037b (Katoh and Standley 2013) and alignments were edited with MEGA6 (Tamura et al. 2013). Sequences were concatenated with SequenceMatrix v.1.7.8 (Vaidya et al. 2011). The partition homogeneity test in PAUP4.0b10 (Swofford 2002) was undertaken by using the command ‘hompart’.

Maximum Likelihood (ML) analyses were constructed with RAxMLGUI (Silvestro and Michalak 2012). The GTRGAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites. For Bayesian Inference (BI), a Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v.3.2 (Ronquist et al. 2012) for the combined sequence datasets. The selection of the best-fit nucleotide substitution model for each locus was calculated by the Akaike Information Criterion (AIC) with jModelTest 2 (Darriba et al. 2012). The TIM+I+G model was selected for the concatenated ITS+RPB1+RPB2+TEF sequences. The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4,001 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 programme Tracer v1.5 (Drummond and Rambaut 2007) to determine burn-in and confirm that both runs had converged. The final alignment is available from TreeBASE under submission ID: 24710 (http://www.treebase.org).
Table 1. Taxa included in the phylogenetic analyses.

| Species                          | Strain No. | GenBank Accession No. | ITS  | RPB1  | RPB2  | TEF  |
|----------------------------------|------------|-----------------------|------|-------|-------|------|
| A. aculeatus                     | HUA 772    | KC519371              |      |       |       |      |
| A. attenuatus                    | CBS 402.78 | AJ292434              | EF468888 | EF468935 | EF468782 |      |
| A. coccidioperitheciatus         | NHJ 6709   | JN049865              | EU369067 | EU369086 |         |      |
| A. farinosa                      | CBS 541.81 | AY624180              |       |       |       |      |
| A. kanyawimiae                   | TBRC 7242  | MF140751              | MF140784 | MF140808 | MF140838 |      |
| A. lecanii                       | TBRC 7243  | MF140750              | MF140783 | MF140807 | MF140837 |      |
| A. sulphureus                    | TBRC 7244  | MF140752              | MF140836 |           |         |      |
| A. attenuatus                    | CBS 101247 | JN049836              | DQ522407 | DQ522466 | DQ522359 |      |
| A. sulphureus                    | TBRC 7247  | MF140756              | MF140785 | MF140811 | MF140841 |      |
| A. sulphureus                    | TBRC 7248  | MF140758              | MF140787 | MF140812 | MF140843 |      |
| A. sulphureus                    | TBRC 7249  | MF140757              | MF140786 | MF140734 | MF140842 |      |
| A. tuberculatus                  | TBRC 7250  | MF140749              | MF140809 | MF140839 |           |      |
| A. tuberculatus                  | TBRC 7251  | MF140747              | MF140805 | MF140833 |           |      |
| A. tuberculatus                  | TBRC 7252  | MF140748              | MF140806 |           |         |      |
| Acylopogon sulphureus            | RC. 546    | DQ127236              |       |       |       |      |
| Beauveria acridophila            | HUA 179219 | JX003857              | JX003841 | JQ958613 |         |      |
| B. acridophila                   | QCNE 186726| JQ958605              | JX003855 | JQ958618 |         |      |
| B. bassiana                      | ARSEF 1564 | HQ880871              | HQ880833 | HQ880905 | HQ880974 |      |
| B. brongniartii                  | ARSEF 617  | HQ880872              | HQ880854 | HQ880926 | HQ880991 |      |
| B. brongniartii                  | BCC 16585  | JN049867              | JN049885 | JF415991 | JF416009 |      |
| B. calderonica                   | ARSEF 2567 | HQ880817              |       |       |       |      |
| B. diapheromerophila             | MCA 1557   | JQ958608              | JX003851 | JQ958612 |         |      |
| B. diapheromerophila             | QCNE 186722| JQ958599              | JX003848 | JQ958610 |         |      |
| B. diapheromerophila             | QCNE 186714| JQ958603              | JX003850 | JQ958611 |         |      |
| Blackwellomyces cardinalis       | HUA 179217 | JQ958609              | JX003847 |         |         |      |
| B. cardinalis                    | HUA 179218 | JQ958606              | JX003846 | JX003845 | JQ958619 |      |
| B. malaviensis                   | ARSEF 7760 | HQ880897              | HQ880897 | DQ376246 |         |      |
| B. pseudobassiana                | ARSEF 3405 | AY532022              | HQ880864 | HQ880936 | AY531931 |      |
| B. sarahaeidicola                | ARSEF 5689 | JN049827              | DQ522380 | DQ522431 | DQ522335 |      |
| B. staphylinidicola              | ARSEF 5718 | EF468881              | EF468776 |         |         |      |
| Blackwellomyces cardinalis       | OSC 93609  | DQ522370              | DQ522422 | DQ522325 |         |      |
| B. cardinuliss                    | OSC 93610  | EF469088              | EF469059 |         |         |      |
| B. pseudomilitaris               | NBRC 101409| JN943305              | JN992482 |         |         |      |
| Cordyceps amoene-rosea           | CBS 729.73 | MG665235              | HM161732 |         |         |      |
| C. amoene-rosea                  | CBS 107.73 | AY624168              |         |         |         |      |
| C. bifusispora                   | EPCC 5690  | EF468854              | EF468909 |         |         |      |
| C. blackwelliae                  | TBRC 7253  | MF140739              | MF140794 | MF140825 |         |      |
| C. catenamnulatus                | CBS 152.83 | AY624172              | JQ425687 |         |         |      |
| C. catenrhiziformis              | TBRC 7258  | MF140753              | MF140767 |         | MF140850 |      |
| C. chaeformis                    | CBS 153.83 | AY624173              | MG665236 | JQ425688 |         |      |
| C. cl. farinosa                  | OSC 111004 | EF468886              | EF468780 |         |         |      |
| C. cl. ochraceostromata          | ARSEF 5691 | EF468867              | EF468921 | EF468759 |         |      |
| C. cl. takaomontana              | NHJ 12623  | EF468884              | EF468932 | EF468778 |         |      |
| C. chingdaenensis                | TBRC 7274  | KT261393              |         |         |         |      |
Three new species of *Samsoniella*

| Species                  | Strain No. | GenBank Accession No.     |
|--------------------------|------------|---------------------------|
|                          |            | ITS | RPB1 | RPB2 | TEF |
| *C. coleopterorum*        | CBS 110.73 | AY624177 | JN049903 | JF416006 | JF416028 |
| *C. farinosa*             | CBS 111113 | AY624181 | GU979973 | GQ250022 |
| *C. fumosorosea*          | CBS 107.10 | AY624184 | MG665237 | HM161735 |
|                          | CBS 244.31 | AY624182 | MG665238 | HM161736 |
|                          | CBS 375.70 | AY624183 | MG665233 |
|                          | CBS 337.52 | EF411219 |            |
| *C. javanica*             | CBS 134.22 | AY624186 |            |
|                          | TBRC 7259  | MF140745 | MF140780 | MF140804 | MF140831 |
|                          | TBRC 7260  | MF140744 | MF140779 | MF140803 | MF140830 |
|                          | TBRC 7261  | MF140743 | MF140778 | MF140802 | MF140829 |
|                          | TBRC 7262  | MF140746 |            |
| *C. kintrischica*         | ARSEF 7218 | EU553278 |            |
|                          | ARSEF 8058 | GU734764 |            |
| *C. kyuguenensis*         | EFCC 5886  | EF468863 | EF468917 |
| *C. lepidopterorum*       | TBRC 7263  | MF140765 | MF140768 | MF140792 | MF140819 |
|                          | TBRC 7264  | MF140766 | MF140769 | MF140793 | MF140820 |
| *C. militaris*            | OSC 93623  |            |
| *C. morakotii*            | TBRC 7275  | KT261388 |          |
|                          | TBRC 7276  | KT261390 |          |
| *C. nischukiptora*        | EFCC 5197  | EF468868 | EF468760 |
|                          | EFCC 5693  | EF468869 | EF468762 |
|                          | EGS 38.165 | EF468900 | EF468795 |
|                          | EGS 38.166 | EF468901 | EF468794 |
|                          | NHJ 10627  | EF468870 | EF468763 |
|                          | NHJ 10684  | EF468871 | EF468761 |
| *C. oncoperae*            | ARSEF 4358 |            |
| *C. pipers*               | CBS 116719 | DQ27240  | EU369083 | DQ118749 |
| *C. pruinosa*             | ARSEF 5413 | DQ522397 | DQ522451 | DQ522351 |
| *Cordyceps* sp.           | CBS 102184 | EF468907 | EF468948 | EF468803 |
| *C. takaomontana*         | BCC 28612  | FJ765285 |
| *C. tenuipes*             | ARSEF 5135 | AY624196 | JN049896 | JF416000 | JF416020 |
|                          | OSC 111007 | DQ522395 | DQ522449 | DQ522349 |
|                          | TBRC 7265  | MF140741 | MF140776 | MF140827 |
|                          | TBRC 7266  | MF140742 | MF140777 | MF140801 | MF140828 |
|                          | TBRC 7267  | MF140740 | MF140775 | MF140799 | MF140826 |
| *Engyodontium aranearum*  | CBS 309.85 | DQ522387 | DQ522439 | DQ522341 |
| *Gibellula longispora*    | NHJ 12014  | EU369055 | EU369075 | EU369017 |
| *G. ratticaudata*         | ARSEF 1915 | DQ522408 | DQ522467 | DQ522360 |
| *Gibellula* sp.           | NHJ 10788  | EU369058 | EU369078 | EU369019 |
|                          | NHJ 13158  | EU369057 | EU369077 | EU369020 |
|                          | NHJ 10808  | EU369056 | EU369076 | EU369018 |
|                          | NHJ 5401   | EU369059 | EU369079 |
|                          | NHJ 7859   | EU369064 | EU369085 |
| *Hevansia cinerea*        | NHJ 3510   | EU369048 | EU369070 | EU369009 |
| *H. nelamboides*          | BCC 41864  | JN201871 |
| *H. novoguineensis*       | NHJ 4314   | EU369051 | EU369071 | EU369012 |
|                          | NHJ 10469  | EU369047 | EU369008 |
|                          | NHJ 11923  | EU369052 | EU369072 | EU369013 |
|                          | NHJ 13117  | EU369049 | EU369073 | EU369010 |
|                          | NHJ 13161  | EU369050 | EU369011 |
| *Hyperdermium pulvinatum* | PC. 602    | DQ127237 | DQ118746 |
| *Lecanocillium araneorum* | CBS 350.85 | DQ522396 | DQ522450 | DQ522350 |
| *L. araneorum*            | CBS 726.73a| EF468887 | EF468934 | EF468781 |
| *L. fusiporum*            | CBS 164.70 | EF468889 | EF468783 |
| *L. paullitae*            | CBS 101270 | EF469095 | EF469113 | EF469066 |
|                          | CBS 363.86 | EF468890 | EF468784 |
|                          | CBS 532.81 | EF469096 | EF469112 | EF469067 |
Results

Phylogenetic analyses

The phylogenetic tree of *Samsoniella* in Cordycipitaceae (Fig. 1) and *Samsoniella* species and closely related species (Fig. 2) were generated from the ML and BI analysis, based on a combined data set of ITS, *RPB1*, *RPB2* and *TEF* sequence data. Statistical support (≥ 50%/0.5) is shown at the nodes for ML bootstrap support/BI posterior probabilities (Figs 1, 2). The strain numbers are noted after each species’ name. The concatenated sequences of analysis 1 and analysis 2 included 67 and 17 taxa, and consisted of 2,152 (ITS: 528, *RPB1*: 488, *RPB2*: 442 and *TEF*: 694) and 2,194 (ITS: 477, *RPB1*: 565, *RPB2*: 473 and *TEF*: 679) characters with gaps, respectively.

Analysis 1: *Samsoniella* in Cordycipitaceae. The RAxML analysis of the combined dataset (ITS+*RPB1*+*RPB2*+*TEF*) yielded a best scoring tree (Fig. 1) with a final ML optimisation likelihood value of \(-28,809.222105\). Parameters for the GTR model of the concatenated dataset was as follows: estimated base frequencies; A = 0.234094, C = 0.301291, G = 0.260521, T = 0.204093; substitution rates AC = 1.111784, AG = 3.130020, AT = 0.930972, CG = 0.886915, CT = 6.300092, GT = 1.000000; gamma distribution shape parameter \(\alpha\) = 0.390179. In the phylogenetic tree (Fig. 1), *Samsoniella* species were clustered in a clade and resolved into two obvious clades. *Samsoniella* species have a close relationship with *Akanthomyces* species.

Analysis 2: *Samsoniella* species and closely-related species. The RAxML analysis of the combined dataset (ITS+*RPB1*+*RPB2*+*TEF*) yielded a best scoring tree (Fig. 2) with a final ML optimisation likelihood value of \(-9,722.503130\). Parameters for the GTR model of the concatenated data set were as follows: estimated base frequencies;
Three new species of Samsoniella

Figure 1. Phylogenetic relationships of the genus Samsoniella in Cordycipitaceae, based on multigene dataset (ITS, RPB1, RPB2 and TEF). Statistical support values (≥ 50%/0.5) are shown at the nodes for ML bootstrap support/BI posterior probabilities.
A = 0.233473, C = 0.298686, G = 0.261629, T = 0.206212; substitution rates AC = 1.250081, AG = 2.534760, AT = 0.891128, CG = 0.827805, CT = 5.916085, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.674468$. In the phylogenetic tree (Fig. 2), *Samsoniella* species were clustered in a clade and easily distinguished with *Akanthomyces* species. *S. coleopterorum* and *S. lepidopterorum* clustered in a clade (Fig. 2) and formed two independent branches. *S. hymenopterorum* was phylogenetically close to *S. inthanonensis* and *S. aurantia*.

**Figure 2.** Phylogenetic relationships between the genus *Samsoniella* and closely-related species, based on multigene dataset (ITS, RPB1, RPB2 and TEF). Statistical support values ($\geq 50\%/0.5$) are shown at the nodes for ML bootstrap support/BI posterior probabilities.
Three new species of *Samsoniella*

**Samsoniella coleopterorum** W.H. Chen, Y.F. Han & Z.Q. Liang, sp. nov.
MycoBank No: 831735
Fig. 3

**Diagnosis.** Differs from *Samsoniella aurantia* by having smaller conidia and snout beetle host in the family Curculionidae. Differs from *S. lepidoperorum* by having cylindrical to ellipsoidal phialides, smaller fusiform to ellipsoidal conidia and a different host.

**Type.** China, Guizhou Province, Xishui County (28°29'56.70"N, 106°24'31.04"E), July 2018, Jiandong Liang, holotype GZAC A1950, ex-type culture GZAC A19501. Sequences from isolated strain A19501 have been deposited in GenBank with accession numbers: ITS = MT626376, *RPB1* = MT642600, *RPB2* = MN101585 and *TEF* = MN101586.

**Description.** Colonies on PDA, 3.6–4.0 cm diam. in 14 d at 25 °C, white, consisting of a basal felt and cottony, flocose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.1–1.8 μm diam. Erect conidiophores usually arising from aerial hyphae, Isaria-like with phialides in whorls of two to four. Phialides 5.4–9.7 × 1.2–1.8 μm, with a cylindrical to ellipsoidal basal portion, tapering into a short distinct neck. Conidia in chains, hyaline, fusiform, ellipsoidal or subglobose, one-celled, 1.7–2.5 × 1.2–1.8 μm. Chlamydospores and synnemata not observed. Size and shape of phialides and conidia similar in culture and on natural substratum. Sexual state not observed.

**Host.** Snout beetle, family Curculionidae.

**Distribution.** Xishui County, Guizhou Province, China.
Etymology. Referring to its insect host, order Coleoptera.

Remarks. *Samsoniella coleopterorum* was easily identified as belonging to *Samsoniella* based on the phylogenetic analyses (Fig. 1). Comparing with the typical characteristics of three species (Table 2), *S. coleopterorum* has a close relationship with *S. aurantia* by having cylindrical to ellipsoidal phialides and similar in size. However, it differs from *S. aurantia* by having shorter conidia and snout beetle host in the family Curculionidae. Based on the combined dataset of ITS, *RPB1*, *RPB2* and *TEF* sequences, *S. coleopterorum* has a close relationship with *S. lepidopterorum* (Fig. 2). However, *S. coleopterorum* has cylindrical to ellipsoidal phialides, smaller fusiform to ellipsoidal conidia and a different host.

*Samsoniella hymenopterorum* W.H. Chen, Y.F. Han & Z.Q. Liang, sp. nov.
MycoBank No: 831736
Fig. 4

Diagnosis. Differs from *Samsoniella inthanonensis* and *S. aurantia* by having smaller, fusiform to ovoid conidia and a host in the family Vespidae.

Type. China, Guizhou Province, Xishui County, at 28°29′56.70″N, 106°24′31.04″E, July 2018, Jiandong Liang, holotype GZAC A1952, ex-type culture GZAC A19522. Sequences from isolated strain A19522 have been deposited in GenBank with accession numbers: ITS = MN128224, *RPB1* = MT642603, *RPB2* = MT642604 and *TEF* = MN101588.
Three new species of *Samsoniella*

### Description
Colonies on PDA, 6.2–6.4 cm diam. in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.1–1.6 μm diam. Erect conidiophores usually arising from aerial hyphae, Isaria-like with phialides in whorls of three to four. Phialides 6.5–10.6 × 1.2–2.0 μm, with a cylindrical basal portion, tapering to a distinct neck. Conidia in chains, hyaline, fusiform to ovoid, 1-celled, 1.9–2.5 × 1.5–2.1 μm. Chlamydospores and synnemata not observed. Size and shape of phialides and conidia similar in culture and on natural substratum. Sexual state not observed.

**Host.** Bee, family Vespidae.

**Distribution.** Xishui County, Guizhou Province, China.

**Etymology.** Referring to its insect host, order Hymenoptera.

**Remarks.** *Samsoniella hymenopterorum* was identified as belonging to *Samsoniella*, based on the phylogenetic analyses (Fig. 1). Comparing with the typical characteristics of the three species (Table 2), *S. hymenopterorum* has a close relationship with *S. inthanonensis* by having cylindrical basal in phialide and similar in size. However, it is distinguished from *S. inthanonensis* by having smaller, fusiform to ovoid conidia and a host in the family Vespidae. Based on combined dataset of ITS, *RPB1*, *RPB2* and *TEF* sequences, *S. hymenopterorum* is phylogenetically close to *S. aurantia* and *S. inthanonensis* (Fig. 2). However, *S. hymenopterorum* has smaller fusiform to ovoid conidia and a different host.

### Table 2. Morphological comparison of three new species with other *Samsoniella* species.

| Species                  | Morphological characteristics | Hosts/substrates          | Reference                  |
|--------------------------|-------------------------------|---------------------------|---------------------------|
|                          | Phialide (μm)                | Conidia (μm)              |
| *Samsoniella alboaurantium* | 5–8 × 2                      | ovate to lemon-shaped      | soil, lepidopterous pupa  |
| *S. aurantia*            | (5–)3.5–8.5–(13) × 2–3        | fusiform                   | lepidopterous larvae      |
| *S. inthanonensis*       | (4–)6.5–10(–12) × (1–)1.5–2(–3) | short fusiform             | lepidopterous larvae      |
| *S. coleopterorum*       | 5.4–9.7 × 1.2–1.8             | fusiform, ellipsoidal or subglobose | snout beetle            |
| *S. hymenopterorum*      | 6.5–10.6 × 1.2–2.0            | fusiform to ovoid          | bee                       |
| *S. lepidopterorum*      | ellipsoidal                   | fusiform to subglobose     | lepidopterous pupa        |

### Samsoniella lepidopterorum W.H. Chen, Y.F. Han & Z.Q. Liang, sp. nov.
Mycobank No: 831737

**Fig. 5**

**Diagnosis.** Differs from *Samsoniella coleopterorum* by having larger, ellipsoidal phialide conidia and a host in the order Lepidoptera.

**Type.** CHINA, Guizhou Province, Rongjiang County (26°01’56.13”N, 108°24’48.06”E), October 2018, Wanhao Chen, holotype GZAC DL1007 = RJ1807,
Wan-Hao Chen et al. / MycoKeys 74: 1–15 (2020)

Description. Colonies on PDA, 3.7–3.8 cm diam. in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.1–2.2 μm diam. Erect conidiophores usually arising from aerial hyphae, Isaria-like with phialides in whorls of two to four. Phialides 5.2–8.5 (–13.1) × 1.1–1.7 μm, with an ellipsoidal basal portion, tapering into a distinct neck. Conidia in chains, hyaline, fusiform to subglobose, 1-celled, 2.0–2.5 × 1.2–2.0 μm. Chlamydospores and synnemata not observed. Size and shape of phialides and conidia similar in culture and on natural substratum. Sexual state not observed.

Host. Pupa, order Lepidoptera

Distribution. Rongjiang County, Guizhou Province, China

Etymology. Referring to its insect host, order Lepidoptera

Remarks. Samsoniella lepidopterorum was easily identified as belonging to Samsoniella, based on the phylogenetic analyses (Fig. 1). Based on the combined dataset of ITS, RPB1, RPB2 and TEF sequences (Fig. 2) and the typical characteristics of Samsoniella species (Table 2), S. lepidopterorum has a close relationship with S. coleopterorum. However, S. lepidopterorum has larger, ellipsoidal phialide conidia and its pupa host is in the order Lepidoptera.

Discussion

Phylogenetic analyses, based on the combined datasets of (ITS+RPB1+RPB2+TEF), suggest that the three new species are members of the Cordycipitaceae and belong to the genus Samsoniella (Fig. 1). Mongkolsamrit et al. (2018) noted that the typical
Three new species of *Samsoniella* were oval to fusiform conidia, bright red-orange stromata of the sexual morphs and synnemata of the asexual morphs. The phialides in this genus range from cylindrical to possessing a swollen basal portion. *S. coleopterorum*, *S. hymenopterorum* and *S. lepidopterorum* all have cylindrical phialides and fusiform conidia. However, the three new species had mononematous conidiophores rather than synnemata. Synnematous entomopathogenic fungi (such as *Gibellula* spp.) can be found on abaxial leaf surfaces of shrubbery, forest floors and shallow soil layers (Hywel-Jones 1996). As air flow under the forest canopy is slow and humid, the dispersal of conidia through airflow diffusion may be difficult. Therefore, these entomopathogenic fungi may employ a particular strategy, such as producing synnemata and sticky conidia, to accommodate various arthropod activities and facilitate conidium spread (Abbott 2002). The three new species were located in the more open portion of the forest and this may favour the dispersal of dry conidia. Thus, we could speculate that the mononematous conidiophores of the three new species may be the result of a convergent evolution to adapt to the ecological environment.

The evolutionary dynamics of fungi and their hosts are usually described either by co-evolution or by host shifts. Shifts often occur to new hosts that are evolutionarily distant, but which occupy a common ecological niche (Vega et al. 2009). Nutrient requirements often determine whether host shifts occur (Vega et al. 2009). Relationships between insects and fungi have been described as biotrophy, necrotrophy and hemibiotrophy, *inter alia*. The common ancestor of Hypocreaceae and Clavicipitaceae corresponds to a departure from plant-based nutrition to one that specialises on animals and fungi (Spatafora et al. 2007). Prediction of the characteristics and evolutionary placement of any given member should be based on the correlation between molecular-phylogenetic genealogy and nutritional preferences (Spatafora et al. 2007; Vega et al. 2009). Species of *Samsoniella* were originally found on lepidopteran larvae or pupae buried in soil or leaf litter (Mongkolsamrit et al. 2018). Mongkolsamrit et al. (2018) also reported that the true range of host affiliations of *Samsoniella* in nature may not be currently represented. Here, we report *Samsoniella* spp. from coleopteran, hymenopteran larvae and lepidopteran pupae. The presence of different hosts indicates that the nutrient requirements of *Samsoniella* spp. can change with the environment (Spatafora et al. 2007).

In the present study, a four loci phylogenetic analysis showed that *S. coleopterorum*, *S. lepidopterorum* and *S. hymenopterorum* clustered in separate subclades from other *Samsoniella* species. They represent new taxa, based on morphological characteristics, nutritional preferences and phylogenetic analyses.

**Acknowledgements**

This work was supported by the National Natural Science Foundation of China (Grant No. 31860002), High-level Innovative Talents Training Object in Guizhou Province (No. Qiankehepingtairencai [2020]6005), Science and Technology Foundation of
Guizhou Province (No. Qiankehejichu [2020]1Y060), National Survey of Traditional Chinese Medicine Resources (No. Caishe [2017]66, 216) and Engineering Research Center of General Higher Education in Guizhou Province (Qianjiaohe (2015) 337). We also thank Lesley Benyon, PhD, from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

References

Abbott SP (2002) Insects and other arthropods as agents of vector-dispersal in fungi. http://www.thermapure.com/pdf/AbbottInsectdispersal-2.pdf

Castlebury LA, Rossman AY, Sung GH, Hyten AS, Spatafora JW (2004) Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. Mycological Research 108: 864–872. https://doi.org/10.1017/S0953756204000607

Chen WH, Liu C, Han YF, Liang JD, Tian WY, Liang ZQ (2019) Three novel insect-associated species of *Simplicillium* (Cordycipitaceae, Hypocreales) from Southwest China. MycoKeys 58: 83–102. https://doi.org/10.3897/mycokeys.58.37176

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772–772. https://doi.org/10.1038/nmeth.2109

Drummond A, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: e214. https://doi.org/10.1186/1471-2148-7-214

Hywel-Jones N (1996) *Akanthomyces* on spiders in Thailand. Mycological Research 9: 1065–1070. https://doi.org/10.1016/S0953-7562(96)80214-0

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kepler RM, Luangsa-ard JJ, Hywel-Jones NL, Quandt CA, Sung GH, Rehner SA, Aime MC, Henkel TW, Sanjuan T, Zare R, Chen M, Li Z, Rossman AY, Spatafora JW, Shrestha B (2017) A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). IMA Fungus 8: 335–353. https://doi.org/10.5598/imafungus.2017.08.02.08

Liang JD, Han YF, Zhang JW, Du W, Liang ZQ, Li ZZ (2011) Optimal culture conditions for keratinase production by a novel thermophilic *Myceliophthora thermophila* strain GZUIFR-H49-1. Journal of Applied Microbiology 110: 871–880. https://doi.org/10.1111/j.1365-2672.2011.04949.x

Luangsa-ard JJ, Hywel-Jones NL, Samson RA (2004) The order level polyphyletic nature of *Paecilomyces* sensu lato as revealed through 18S-generated rRNA phylogeny. Mycologia 96: 773–780. https://doi.org/10.1080/15572536.2005.11832925

Mongkolsamrit S, Noisripoom W, Thanakitpipattana D, Wutikhun T, Spatafora JW, Luangsa-ard J (2018) Disentangling cryptic species with Isaria-like morphs in Cordycipitaceae. Mycologia 110: 230–257. https://doi.org/10.1080/00275514.2018.1446651

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic
Three new species of *Samsoniella*

inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Samson RA (1974) *Paecilomyces* and some allied hyphomycetes. Studies in Mycology 6: 1–119.

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12(4): 335–337. https://doi.org/10.1007/s13127-011-0056-0

Smith, G (1957) Some new and interesting species of micro-fungi. Transactions of the British Mycological Society 40(4): 481–488. https://doi.org/10.1016/S0007-1536(57)80054-0

Spatafora JW, Sung GH, Sung JM, Hywel-Jones NL, White JF (2007) Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. Molecular Ecology 16: 1701–1711. https://doi.org/10.1111/j.1365-294X.2007.03225.x

Swofford DL (2002) PAUP*: 4.0b10: phylogenetic analysis using parsimony (*and other methods). Sunderland, MA, Sinauer.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27(2): 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

van den Brink J, Samson RA, Hagen F, Boekhout T, de Vries RP (2012) Phylogeny of the industrial relevant, thermophilic genera *Myceliophthora* and *Corynascus*. Fungal Diversity 52: 197–207. https://doi.org/10.1007/s13225-011-0107-z

Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Maniania KN, Monzón A, Ownley BH, Pell JK, Rangel DEN, Roy HE (2009) Fungal entomopathogens: new insights on their ecology. Fungal Ecology 2: 149–159. https://doi.org/10.1016/j.fu neco.2009.05.001

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds.) PCR protocols: a guide to methods and applications. Academic Press, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zou X, Liu AY, Liang ZQ, Han YF, Yang M (2010) *Hirsutella liboensis*, a new entomopathogenic species affecting Cossidae (Lepidoptera) in China. Mycotaxon 111(1): 39–44. https://doi.org/10.5248/111.39