Morphology and Phylogeny Reveal Five Novel Species in the Genus Cordyceps (Cordycipitaceae, Hypocreales) From Yunnan, China

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INTRODUCTION

Cordyceps Fr. is a well-known genus of arthropod-pathogenic fungi. It was shown that many species of Cordyceps played a significant role in the cycling of matter in an ecological system, had a high ecological and economic value for biocontrol and bioactive compounds, and served as a model system for research on fungal insect pathology (Zha et al., 2018; Chen W. H. et al., 2019). Complexes of some cordycipitoid fungal species and their natural host, such as C. militaris Fr., C. chanhua Z. Z. Li, F. G. Luan, Hywel-Jones, C. R. Li and S. L. Zhang, and C. kyusyuensis Kawam, have received significant attention in traditional medicine industry due to the detection of...
bioactive compounds with anti-aging, anti-tumor, antioxidant, anti-inflammatory, and immuno-modulatory effects (Castillo et al., 2018; Zhao et al., 2018; Lou et al., 2019; Li et al., 2021; Zhang et al., 2021). Cordyceps tenuipes (Peck) Kepler, B. Shrestha and Spatafora has been applied in a variety of functional foods in Japan and South Korea, possessing nutritional, immune-regulatory, antitumor, analgesic, antibacterial, and anti-malaria effects (Wang Y. et al., 2020). Cordyceps species reproduce via sexual (ascospores) or asexual (conidia) spores, or both (Mora et al., 2017). The host range of Cordyceps embraces 7 orders of Arthropoda, namely, Araneae, Coleopteran, Dermaptera, Hemiptera, Hymenoptera, Lepidoptera, and Orthoptera, where Coleoptera and Lepidoptera are the two significant orders to host beyond the estimated 200 Cordyceps spp. as recorded (Shrestha et al., 2016; Kepler et al., 2017; Mongkolsamrit et al., 2020; Wang Y. B. et al., 2020).

Fries (1818) was credited with coining Cordyceps as a genus in Pyrenomycetes from a hybrid of the Greek word cordyle and the Latin word caput, meaning a club and a head, respectively. However, this genus was then treated as a tribe level of Sphaeria and described as stroma erect, stipe simple or branching, a sterile stalk supporting the perithecia at the periphery, and projecting openings at the apex (Shrestha et al., 2014). Nearly 20 different genera have been accounted as synonyms of Cordyceps in various sources (Shrestha et al., 2014). Shrestha et al. (2014) provided a comprehensive review regarding the taxonomic history of Cordyceps and concluded that the genus is the oldest valid genus in Cordycipitaceae (Hypocreales, Ascomycota) and is typified by a sexual morph. Owing to the cylindrical shape of the stroma, C. militaris, the type species of Cordyceps, was already described in the 17th- and early 18th-century literature (Shrestha et al., 2014).

Species of Cordyceps species produce three types of ascospore, namely disarticulating ascospores (e.g., C. militaris), intact ascospores (e.g., Blackwollomyces cardialis (G. H. Sung and Spatafora) Spatafora and Luangsa-ard and Blackwollomyces pseudomilitaris Hywel-Jones and Sivichai), and bola-ascospores (e.g., C. bifusispora O. E. Eriksson), with superficial to partially immersed perithecia on fleshy stromata that are pallid and long segments, followed by surface sterilization with 30% H2O2.

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Materials and Methods

Sampling
Cordyceps samples were newly collected from Kunming, Weishan, Jinghong, and Pu’er of Yunnan, southwestern China. Voucher specimens were deposited in the YHH (Yunnan Herbal Herbarium) of Yunnan University. The isolated strains were deposited in YFCF (Yunnan Fungal Culture Collection) of Yunnan University.

Fungal materials, including the hosts, were photographed and recorded. Isolation of the fungi was achieved as per Wang Y. B. et al. (2020). The stromata or synnemata were cut into 5-mm-long segments, followed by surface sterilization with 30% H2O2.
for 30 s to 1 min, and then rinsed with sterile water five times, dried with sterilized filter paper. Then, a part of the insect body was cut off, and the resulting segments and insect body were inoculated onto potato dextrose agar (PDA: potato 200 g/L, dextrose 20 g/L, and agar 20 g/L) plates containing 0.1 g/L streptomycin and 0.05 g/L tetracycline.

Morphological Studies
Given the field notes, color images of the materials, and complementary literature data, macro-morphological characteristics, such as the host, fungi location, color, and shape of the stromata, and perithecial orientation (superficial, immersed, semi-immersed; ordinal or oblique) were examined under a dissecting microscope (Olympus SZ61), where the insect hosts were recognized with the support from professional entomologists.

The sexual characteristics, such as perithecia, ascii, and ascospores, were firstly mounted on glass slides with lactophenol cotton blue solution after removing from the stroma, whereas the asexual characteristics, such as phialides and conidia, were firstly inoculated on glass slides with a thin layer of PDA medium for a couple of days, and the colonies were photographed and measured every week.

Molecular Studies
DNA Extraction and PCR Amplification
Total DNA was extracted from the fungal mycelia on PDA plates or herbarium materials using the modified CTAB procedure (Doyle and Doyle, 1987). For DNA amplification, the primer pairs nrSSU-CoF and nrSSU-CoR (Wang Y. B. et al., 2015) and LR5 and LR0R (Vilgalys and Hester, 1990; Rehner and Samuels, 1994) were used for the nrSSU and nrLSU, EF1α-EF and EF1α-ER (Bischoff et al., 2006; Sung et al., 2007) were used to amplify the translation elongation factor 1α (tef-1α), and primers RBP1-5’F and RBP1-5’R, and RBPB2-5’F and RBPB2-5’R (Bischoff et al., 2006; Sung et al., 2007) were used to amplify the largest and second-largest subunits of RNA polymerase II (rpb1 and rpb2), respectively.

The polymerase chain reaction (PCR) matrix was composed of 2.5 μl of PCR 10 × Buffer (2 mmol/L Mg2+ ) (Transgen Biotech, Beijing, China), 0.25 μl of Taq DNA polymerase (Transgen Biotech, Beijing, China), 2 μl of dNTP (2.5 mmol/L), 1 μl of DNA template (500 ng/μl), 1 μl of forward primers (10 μmol/L), 1 μl of reverse primers (10 μmol/L), and 17.25 μl of sterile ddH2O. Amplification reactions were performed in a BIO-RAD T100TM thermal cycler (BIO-RAD Laboratories, Hercules, CA, United States). The PCR program was performed as described by Sung et al. (2007) and Wang Y. B. et al. (2020). Products of PCR were purified with the Gel Extraction and PCR Purification Combo Kit Beijing Genomics Institute (Shenzhen, China) and then sequenced on an automatic sequence analyzer (BGI Co., Ltd, Shenzhen, China) using the same primers as those used in amplification.

DNA Sequence Alignments
The samples’ nrSSU, nrLSU, tef-1α, rpb1, and rpb2 nucleotide sequences were compared with those deposited in the GenBank database. To understand the relationship of our sample with those in the GenBank, nrSSU, nrLSU, tef-1α, rpb1, and rpb2 sequences of the representative Cordyceps s. s. species available in GenBank were retrieved and combined with our sequences (Table 1). Five datasets, the nrSSU, nrLSU, tef-1α, rpb1, and rpb2 sequences, were aligned and manually checked on Bioedit v7.0.9 (Hall, 1999). In order to examine phylogenetic conflicts among these datasets, the partition homogeneity (PH) test was performed with 1,000 randomized replicates, using heuristic searches with simple addition of sequences in PAUP* 4.0b10 (Swofford, 2002); the phylogenetic signals in the five gene markers showed no conflict.

Phylogenetic Analyses
Phylogenetic trees were visualized with FigTree v1.4.0 (Rambaut, 2006) and edited in Microsoft PowerPoint, then saved as .PDF format and finally converted to .JPG format using Adobe Illustrator CS6 (Adobe Systems Inc., United States). The finalized alignments and trees were submitted in TreeBASE (Submission ID: 29339).

Molecular Analyses of the concatenated five-gene datasets were conducted using ML and BI methods. The GTR + I + G were chosen as the best models for nrSSU-nrLSU-tef-1α-rpb1-rpb2, using the Akaike Information Criterion (AIC) implemented in MrModeltest v 2.3 (Nylander, 2004), and then the partitioned analyses were separately conducted. For ML analyses, raxml v 8.2.7 was employed. All parameters were kept as default with an exception that the model was chosen as GTRGAMMAI. The statistic supports were calculated using 1,000 replicates of non-parametric bootstrapping. BI analysis was carried out with MrBayes v 3.2.6 using the selected models for 5 million generations with the value of stopval set to 0.01 via the stopval command. At the same time, other parameters were kept as default and trees were summarized. Statistic supports were obtained using sumt command complemented in MrBayes by discarding the first 25% generations as burn-ins. The Bayesian trees were sampled every 100 generations. The first 25% trees were discarded as burn-ins, and the remaining trees were employed to create a consensus tree using sumt command.

RESULTS
Sequence Alignment
The combined 101-taxon 5-gene dataset consisted of 4,627 base pairs of sequence data (nrSSU 1060 bp, nrLSU 877 bp, tef-1α 999 bp, rpb1 719 bp, and rpb2 972 bp). A total of 1,039 were parsimony-informative (nrSSU 45 bp, nrLSU 77 bp, tef-1α...
### TABLE 1 | Names, voucher information, host, and corresponding GenBank accession numbers of the taxa used in this study.

| Taxon                        | Voucher information | Host                     | GenBank Accession Number | References                                      |
|-----------------------------|---------------------|--------------------------|--------------------------|-------------------------------------------------|
|                            |                     |                          | nrSSU | nrLSU  | tef-1α  | rpb1  | rpb2  |                         |
| **Cordyceps albocitrina**   | spat 07-174         | Coleoptera               | MF416575                  | MF416467 | MF416629 | Kepler et al., 2017    |
| **Cordyceps amoene-rosea**  | CBS 107.73          | Coleoptera; Nitidulidae  | AY526464                  | MF416550 | MF416494 | Luangsar-ard et al., 2005 |
| **Cordyceps amoene-rosea**  | CBS 729.73          | Arachnida; Araneae       | MF416604                  | MF416551 | MF416495 | Luangsar-ard et al., 2005 |
| **Cordyceps araneae**       | BCC 85065           | Arachnida; Araneae       | MT003037                  | MT017850 | MT017810 | Mongkolsamrit et al., 2020 |
| **Cordyceps araneae**       | BCC 85066           | Arachnida; Araneae       | MT003038                  | MT017851 | MT017811 | Mongkolsamrit et al., 2020 |
| **Cordyceps araneae**       | BCC 88291           | Arachnida; Araneae       | MT003039                  | MT017852 | MT017812 | Mongkolsamrit et al., 2020 |
| **Cordyceps bifusispora**   | spat 08-129         |                            | MF416576                  | MF416523 | MF416468 | Kepler et al., 2017    |
| **Cordyceps bifusispora**   | spat 08-133.1       |                            | MF416577                  | MF416524 | MF416469 | Kepler et al., 2017    |
| **Cordyceps blackwelliae**  | TBRC 7255           | Coleoptera (larva)       | MF140703                  | MF140823 | MF140772 | Mongkolsamrit et al., 2018 |
| **Cordyceps blackwelliae**  | TBRC 7256           | Coleoptera (larva)       | MF140702                  | MF140822 | MF140771 | Mongkolsamrit et al., 2018 |
| **Cordyceps brevispora**    | BCC 78209           | Lepidoptera (larva)      | MT003044                  | MT017855 | MT017817 | Mongkolsamrit et al., 2020 |
| **Cordyceps brevispora**    | BCC 79252           | Lepidoptera (larva)      | MT003045                  | MT017856 | MT017836 | Mongkolsamrit et al., 2020 |
| **Cordyceps bullispora**    | YFCC 8400           | Lepidoptera (pupa)       | OL468555                  | OL468575 | OL473523 | This study             |
| **Cordyceps bullispora**    | YFCC 8401           | Lepidoptera (pupa)       | OL468556                  | OL468576 | OL473524 | This study             |
| **Cordyceps caloceroides**  | MCA 2249            | Araneae                  | MF416578                  | MF416525 | MF416470 | Kepler et al., 2017    |
| **Cordyceps cateniannulata**| CBS 152.83          | Coleoptera (adult)       | AY526465                  | MG665226 | JQ425687 | Luangsar-ard et al., 2004; Mongkolsamrit et al., 2018 |
| **Cordyceps cateniobliqua** | YFCC 3367           | Coleopteran adult        | MN576765                  | MN576821 | MN576991 | Wang Y. B. et al., 2020 |
| **Cordyceps cateniobliqua** | YFCC 5935           |                            | MN576766                  | MN576822 | MN576992 | Wang Y. B. et al., 2020 |
| **Cordyceps cateniobliqua** | YFCC 153.83         | Adoxophytes privatana    | AS26466                   | JQ425688 | MG665236 | Luangsar-ard et al., 2004; Mongkolsamrit et al., 2018 |
| **Cordyceps cf. ochraceostromata** | ARSEF 5691 |                      | EF468964                  | EF468819 | EF468759 | Kepler et al., 2012    |
| **Cordyceps cf. pruinosa**  | spat 08-115         |                            | MF416586                  | MF416532 | MF416476 | Kepler et al., 2017    |
| **Cordyceps cf. pruinosa**  | spat 09-021         |                            | MF416587                  | MF416533 | MF416477 | Kepler et al., 2017    |
| **Cordyceps cf. pruinosa**  | NHJ 10627           | Limacodid pupa (Lepidoptera) | EF468967                  | EF468822 | EF468763 | Sung et al., 2007     |
| **Cordyceps cf. pruinosa**  | NHJ 10684           | Limacodid pupa (Lepidoptera) | EF468968                  | EF468823 | EF468761 | Sung et al., 2007     |
| **Cordyceps cf. pruinosa**  | EFCC 5693           |                            | EF468966                  | EF468821 | EF468762 | Sung et al., 2007     |
| **Cordyceps cf. pruinosa**  | EFCC 5197           |                            | EF468965                  | EF468820 | EF468760 | Sung et al., 2007     |
| **Cordyceps cf. takaoantana** | NHJ 12623         | Lepidoptera               | EF468984                  | EF468838 | EF468778 | Sung et al., 2007     |
| **Cordyceps chaetoclavata** | YHH 15101          |                            | MN576722                  | MN576778 | MN576948 | Wang Y. B. et al., 2020 |
| **Cordyceps chiangdaensis** | BCC 68469           | Coleoptera               | MF140732                  | KT261403 |                   | Tasanathai et al., 2016; Mongkolsamrit et al., 2018 |
| **Cordyceps chiangdaensis** | YFCC 857            | Coleoptera; Bateridaceae  | MW181781                  | MW173993 | MW168234 | Zha et al., 2019      |
| **Cordyceps cicadae**       | GACP 07071701       | Hemiptera                | MK761207                  | MK761212 | MK770631 |                         |

(Continued)
| Taxon | Voucher information | Host | GenBank Accession Number | References |
|-------|---------------------|------|--------------------------|------------|
|       |                     |      | nrSSU | nrLSU | tef-1α | rpb1 | rpb2 |
| Cordyceps cicadae | RCEF HP090724-31 | Hemiptera: Cicadidae | MF416005 | MF416552 | MF416496 | MF416653 | MF416653 | Kepler et al., 2017 |
| Cordyceps cocoonihabita | YFCC 3415 | | MNS576723 | MNS576779 | MNS576949 | MNS576939 | MNS576895 | Wang Y. B. et al., 2020 |
| Cordyceps cocoonihabita | YFCC 3416 | | MNS576724 | MNS576780 | MNS576950 | MNS576840 | MNS576896 | Wang Y. B. et al., 2020 |
| Cordyceps coleopterorum | CBS 110.73 | Coleoptera (larva) | JF415985 | JF415988 | JF416028 | JN049903 | JF416006 | Kepler et al., 2012 |
| Cordyceps exasperata | MCA 2288 | Lepidoptera (larva) | MF416962 | MF416538 | MF416482 | MF416639 | | Kepler et al., 2017 |
| Cordyceps farinosa | CBS 111113 | | AYS26474 | AYS216554 | AYS216499 | AYS216656 | AYS216566 | Luangsa-ard et al., 2004; Kepler et al., 2017 |
| Cordyceps fumosorosea | YFCC 4561 | Lepidoptera | MNS576761 | MNS576817 | MNS576877 | MNS576877 | MNS576931 | Wang Y. B. et al., 2020 |
| Cordyceps fumosorosea | CBS 244.31 | Butter | MF416609 | MF416557 | MF416503 | MF416660 | MF416454 | Kepler et al., 2017 |
| Cordyceps fumosorosea | CBS 375.70 | Food | MF416450 | MF416658 | | | MF416452 | Kepler et al., 2017 |
| Cordyceps fumosorosea | CBS 107.10 | | MGS65227 | HMG161735 | | | | Luangsa-ard et al., 2005 |
| Cordyceps grylli | MFLU 17-1023 | | MK963048 | MK963055 | MK963093 | | | Unpublished |
| Cordyceps grylli | MFLU 17-1024 | | MK963049 | MK963056 | MK963194 | | | Unpublished |
| Cordyceps inthanonensis | BCC 7928 | Lepidoptera (pupa) | MT017854 | MT017853 | – | MT017814 | MT017831 | Mongkolsamrit et al., 2020 |
| Cordyceps inthanonensis | BCC 56302 | Lepidoptera (pupa) | MT030040 | MT017853 | – | MT017814 | MT017831 | Mongkolsamrit et al., 2020 |
| Cordyceps inthanonensis | BCC 55812 | Lepidoptera (larva) | MT030041 | – | – | MT017815 | MT017832 | Mongkolsamrit et al., 2020 |
| Cordyceps jakajanicola | BCC 79816 | Hemiptera | MNS275696 | MNS338479 | MNS338484 | MNS338484 | MNS338490 | Crous et al., 2019 |
| Cordyceps jakajanicola | BCC 79817 | Hemiptera | MNS275697 | MNS338480 | MNS338485 | MNS338485 | MNS338490 | Crous et al., 2019 |
| Cordyceps lepidopterorum | TBRC 7263 | Lepidoptera (larva) | MF140711 | MF408031 | MF407801 | | | Mongkolsamrit et al., 2018 |
| Cordyceps lepidopterorum | TBRC 7264 | Lepidoptera (larva) | MF140700 | MF408032 | MF407801 | | | Mongkolsamrit et al., 2018 |
| Cordyceps longiphialis | YFCC 8402 | Rotten wood | OL468557 | OL468577 | OL473525 | OL739571 | OL473536 | This study |
| Cordyceps longiphialis | YFCC 8403 | Rotten wood | OL468558 | OL468578 | OL473526 | OL739572 | OL473537 | This study |
| Cordyceps militaris | YFCC 8657 | Lepidoptera (pupa) | MNS76762 | MNS76818 | MNS76898 | MNS76878 | MNS76932 | Wang Y. B. et al., 2020 |
| Cordyceps militaris | YFCC 8640 | Lepidoptera (pupa) | MNS76763 | MNS76819 | MNS76899 | MNS76879 | MNS76933 | Wang Y. B. et al., 2020 |
| Cordyceps morakotii | BCC 55820 | Hymenoptera (ant pupa) | MF140730 | KT261399 | | | | Tasanathai et al., 2016 |
| Cordyceps morakotii | BCC 68938 | Hymenoptera (ant pupa) | MF140731 | KT261398 | | | | Tasanathai et al., 2016 |
| Cordyceps nabanheensis | YFCC 8409 | Lepidoptera | OL468564 | OL468584 | OL473532 | OL739578 | OL473543 | This study |
| Cordyceps nabanheensis | YFCC 8410 | Lepidoptera | OL468565 | OL468585 | OL473533 | OL739579 | OL473544 | This study |
| Cordyceps neopruinosa | BCC 91361 | Lepidoptera (pupa) | MT030047 | MT017858 | | | | Mongkolsamrit et al., 2020 |
| Cordyceps neopruinosa | BCC 91362 | Lepidoptera (pupa) | MT030048 | MT017859 | | | | Mongkolsamrit et al., 2020 |
| Cordyceps nudus | HUA 186125 | Araneae (Mygalomorphae) | KC810778 | KC810752 | KC810722 | | | Chirix et al., 2017 |

(Continued)
TABLE 1 (Continued)

| Taxon                        | Voucher information | Host                                | GenBank Accession Number | References            |
|------------------------------|---------------------|-------------------------------------|--------------------------|-----------------------|
|                              |                     |                                     | nrSSU                    |                       |
| *Cordyceps nidus*            | HUA 186186          | Araneae (Mygalomorphae)             | KY360301                 | Chirivi et al., 2017  |
| *Cordyceps ninchukispora*    | EGS 38.165          | Plant (Beilschmiedia erythrophloia) | EF468991                 | Sung et al., 2007     |
| *Cordyceps ninchukispora*    | EGS 38.166          | Plant (Beilschmiedia erythrophloia) | EF468992                 | Sung et al., 2007     |
| *Cordyceps ningxiaensis*     | HMJAU 25074         |                                     | KF309671                 | Yan and Bau, 2015     |
| *Cordyceps ningxiaensis*     | HMJAU 25076         |                                     | KF309673                 | Yan and Bau, 2015     |
| *Cordyceps pseudotenuipes*   | YFCC 8404           | Lepidoptera                         | OL468559                 | This study            |
| *Cordyceps pseudotenuipes*   | YFCC 8405           | Lepidoptera                         | OL468560                 | This study            |
| *Cordyceps oncoporae*        | ARSEF 4358          | Lepidoptera; Oncoperaaintricate     | AF339581                 | Sung et al., 2007     |
| *Cordyceps polyarthra*       | MCA 996             | Lepidoptera                         | MF416597                 | Kepler et al., 2017   |
| *Cordyceps polyarthra*       | MCA 1009            | Lepidoptera                         | MF416598                 | Kepler et al., 2017   |
| *Cordyceps prunosa*          | ARSEF 5413          | Lepidoptera; Limacodidae            | AY184979                 | Spatafora et al., 2007|
| *Cordyceps qingchengensis*   | MFLU 17-1022        | Lepidoptera; Bombycidae             | MK761206                 | Zha et al., 2019      |
| *Cordyceps rosea*            | spat 09-053         | Lepidoptera larva                   | MF416590                 | Sung et al., 2001     |
| *Cordyceps roseostromata*    | ARSEF 4871          |                                     | AF339573                 |                      |
| *Cordyceps shuifuluensis*    | YFCC 5230           |                                     | MNS576721                | Wang Y. B. et al., 2020|
| *Cordyceps simaoensis*       | YFCC 8406           | Lepidoptera                         | OL468551                 | This study            |
| *Cordyceps simaoensis*       | YFCC 8407           | Lepidoptera                         | OL468552                 | This study            |
| *Cordyceps simaoensis*       | YFCC 8408           | Lepidoptera                         | OL468553                 | This study            |
| *Cordyceps sp.*              | CBS 102184          | Spider (Arachnida)                  | AF339613                 | Kepler et al., 2012   |
| *Cordyceps sp.*              | EFCC 2535           |                                     | EF468980                 | Sung et al., 2007     |
| *Cordyceps sp.*              | YFCC 5833           |                                     | MN576764                 | Torres et al., 2005   |
| *Cordyceps spegzazzinii*     | ARSF 7850           |                                     | DQ196435                 |                      |
| *Cordyceps subtenuipes*      | YFCC 6051           |                                     | MN576719                 | Wang Y. B. et al., 2020|
| *Cordyceps subtenuipes*      | YFCC 6064           |                                     | MN576720                 | Wang Y. B. et al., 2020|
| *Cordyceps succavus*         | MFLU 18-1890        |                                     | MK086058                 | Unpublished           |
| *Cordyceps teniueps*         | TBRC 7265           | Lepidopteran (pupa)                 | MF140707                 | Mongkolsumrit et al., 2018|
| *Cordyceps teniueps*         | TBRC 7266           | Lepidopteran (pupa)                 | MF140708                 | Mongkolsumrit et al., 2018|
| *Cordyceps teniueps*         | ARSEF 5135          | Lepidopteran (pupa)                 | MF416612                 | Kepler et al., 2012, 2017|
| *Cordyceps teniueps*         | YFCC 4266           |                                     | MN576774                 | Wang Y. B. et al., 2020|
| *Cordyceps yinlangensis*     | YJ 06221            | Ant                                 | MT577003                 | Li et al., 2020       |
| *Liangia sinensis*           | YFCC 3103           |                                     | MN576726                 | Wang Y. B. et al., 2020|
| *Liangia sinensis*           | YFCC 3104           |                                     | MN576727                 | Wang Y. B. et al., 2020|

Boldface: data generated in this study.

References:

Chirivi et al., 2017
Sung et al., 2007
Yan and Bau, 2015
Kepler et al., 2017
Sung et al., 2007
Spatafora et al., 2007
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Wang Y. B. et al., 2020
Kepler et al., 2012
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Wang Y. B. et al., 2020
Mongkolsumrit et al., 2018
Mongkolsumrit et al., 2018
Kepler et al., 2012, 2017
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Wang Y. B. et al., 2020
Wang Y. B. et al., 2020

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370 bp, rpb1 234 bp, and rpb2 313 bp). A total of 101 taxa were complete for all five genes, and the number of taxa for each gene was as follows: nrSSU 71 taxa, nrLSU 97 taxa, tef-1a 95 taxa, rpb1 82 taxa, and rpb2 72 taxa (Table 1).

Molecular Phylogeny
In this study, we generated nrSSU, nrLSU, tef-1a, rpb1, and rpb2 sequences by ten living cultures and one wild material, and their accession numbers are shown in Table 1. Sequences of Liangia sinensis YFCC 3103 and YFCC 3104 in the Cordycipitaceae were chosen as outgroups in the phylogenetic analyses.

Five major (I–IV) clades and five new species could be recognized in Cordyceps s. s. (Figure 1); collections from southwestern China were grouped into five separate species (in boldface, see below) (C. bullispora, C. longipillalis, and C. nabanheensis in clade I, and C. pseudotenuipes and C. simaenois in clade III). Clade I included C. pruinose and 19 other species, with 98% bootstrap support and 1 Bayesian PP support (Figure 1). In clade II, C. militaris and eight other species were grouped together (BS = 70%, PP = 1) (Figure 1). Clade III harbored C. tenuipes and 18 other taxa (BS = 97%, PP = 1) (Figure 1). Clade IV included C. cf. takaomontana NHJ 12623, C. javanica, C. amoenerosea, and C. catenobioula (BS = 91%, PP = 1). Clade V included only two exemplars of the species, C. grylli (Figure 1).

TAXONOMY
The key morphological characteristics that distinguish current Cordyceps s. s. species are summarized in the literature (Tables 2, 3). Including the five new species, there are 66 species of Cordyceps s. s. involved in the current study, among which we have compared 51 species of the sexual morphs in Cordyceps s. s. in Table 2 and 38 species of the asexual morphs in Cordyceps s. s. in Table 3.

Cordyceps bullispora H. Yu, Q. Y. Dong and Z. Y. Zhao, sp. nov. (Figure 2)
MycoBank: MB 842328

Etymology: Referring to button-like structures on the spores.

Type: The Taiji Mountains Nature Reserve, Mizhi Town, Midu County, Yunnan, China. September 20, 2019, H. Yu (YH 20011, holotype; YFCC 8400, ex-holotype living culture).

Teleomorph: Stromata solitary, 10–20 mm long, unbranched, orange-yellow, cylindrical to enlarging apically. The host is covered by a white mycelial surface. Rhizoids flexuous, arising from the head region of host larva buried in soil, 7–10 mm deep under the ground. Stipes cylindrical, white to reddish-orange, 0.1–1.2 mm wide. Fertile parts clavate, orange to reddish-orange, 3.5–7.4 × 0.5–1.5 mm.

Anamorph: Two types of conidial arrangement. Acremonium-like conidia aggregated in heads at the apex of phialides; Mariannaea-like, conidia in imbricate chains, connected laterally.

Colonies on PDA are moderately fast-growing, attaining a diameter of 31–34 mm in 21 days at 25°C, pulvinate, with high mycelial density, Whitish to orange-yellow, reverse deep yellow. Hyphae smooth-walled, branched, septate, hyaline, 1.1–2.7 µm wide. Cultures readily produced phialides and conidia after 2 weeks on potato dextrose agar at room temperature. Phialides arising from aerial hyphae, solitary, 5.6–20.7 × 1.8–3.3 µm, cylindrical, tapering gradually or abruptly toward the apex. Conidia hyaline, one-celled, cylindrical or slightly allantoid, oblong-elliptical to ellipsoidal, 4.9–11.1 × 1.9–4.5 µm.

Habitat and known distribution: On larva of Lepidoptera buried below ground at elevation 2000 m in northwestern Yunnan, China.

Additional specimens examined: The Taiji Mountains Nature Reserve, Mizhi Town, Midu County, Yunnan, China. On pupae of Lepidoptera, September 20, 2019. YH 200112, YFCC 8401.

Comments: Cordyceps bullispora was characterized by unbranched stromata, with cylindrical or orange to reddish-orange fertile parts, Rhizoids flexuous, and the host was the lepidopteran larva. For timing reasons, the fertile part of the specimen was not yet mature at the time of collection in the field. The asexual morph of PDA culture produces cylindrical phialides, which are monothetic, oblong-elliptical to ellipsoidal conidia with a button-like shape.

Based on nrLSU, nrSSU, tef-1a, rpb1, and rpb2 multigene analyses, C. cocoonihabita was revealed to have a close relationship with C. pruinosa and C. ninchukispora (Wang Y. B. et al., 2020). Multigene analyses of ITS, nrLSU, rpb1, rpb2, and tef-1a revealed that C. neopruinosa had a close relationship with C. pruinosa and C. ninchukispora (Mongkolsamrit et al., 2020). C. cocoonihabita, C. neopruinosa, C. pruinosa, and C. ninchukispora all had close relationships, where they shared many similar morphological characteristics, such as they were all characterized by orange- to red-colored stromata and superficial perithecia. C. bullispora shared such features, and our phylogenetic analysis indeed indicated that C. bullispora was closely relevant to a previously undescribed taxon C. cf. pruinosa (spat 08-115, spat 09-221) and was separated from C. cocoonihabita, C. neopruinosa. C. pruinosa, and C. ninchukispora in this clade. However, the perithecia in C. neopruinosa were more prolonged and broader than those reported in Cordyceps pruinosa and C. ninchukispora (330–450 × 150–240 µm vs. 400 × 100 µm vs. 95–145 × 50–60 µm, respectively). C. cocoonihabita had a longer stroma. The micromorphological arrangement of conidia was Isaria-like characteristics and was significantly different from C. pruinosa and C. ninchukispora, which had respective morphology of Mariannaea G. Arnaud and Acremonium Link. C. bullispora had rhizoid stromata, superficial perithecia, wider phialides, and longer conidia 4.9–11.1 × 1.9–4.5 µm. The insect host of C. bullispora and C. neopruinosa all occurred on lepidopteran pupae, and the host of C. ninchukispora was the seed of Beilschmiedia Nees (Liang, 1983, 1991; Su and Wang, 1986; Mongkolsamrit et al., 2020).

Cordyceps yinjiangensis Li et al. (2020) was recently described from Guizhou. Morphologically, it differed from C. bullispora by cylindrical and orange to reddish-orange fertile parts, rhizoids several, flexuous, and the host was the lepidopteran larva. The asexual morph from PDA culture produced cylindrical phialides, which were monothetic and oblong-elliptical
FIGURE 1 | Both ML and BI analyses generate a phylogenetic tree from the concatenated nrSSU, nrLSU, tef-1α, rpb1, and rpb2 datasets. There were no discrepancies between the topology resulting from Bayesian and ML analysis for supported nodes. Bootstrap values ($\geq 60\%$) derived from ML analyses and posterior probabilities from Bayesian inference ($\geq 0.50$) are shown either above or beneath the branches of the nodes. Isolates in bold type are those analyzed in this study.

Dong et al. Five Novel Cordyceps Species
### TABLE 2 | Comparison between the sexual morphs in *Cordyceps*.

| Species                     | Stromata (mm)          | Fertile part (mm)        | Perithecia (µm)       | Asci (µm)                        | Ascospores (µm) | Part-spores (µm) | References                     |
|-----------------------------|------------------------|--------------------------|-----------------------|----------------------------------|-----------------|------------------|--------------------------------|
| *Cordyceps araneae*         | Solitary or gregarious, 4–8 × 0.5 | Clavate, elliptical to fusiform, 2.5–5 × 1–2 | Semi-immersed, 450–500 × 150–200 | Cylindrical, 8-spored, (60)145–220(250) × 2–2.5 | Bola-shaped, (250)280–340(400), central part filiform, 0.3 µm broad, 3 or 4-septate | Mongkolsamrit et al., 2020 |
| *Cordyceps belizensis*      | 100                    |                           |                        | Immersed, 480–570 × 260–300      | Cylindrical, 260–300 × 6 | Filiform         | 4–8 × 1.5                   | Mains, 1940                  |
| *Cordyceps bifusispora*     | Simple, 13 × 0.5–0.7    | Cylindrical, 6 × 1.3     |                        | Immersed, 300 × 150–170          | Cylindrical, 8-spored, 200–300 × 3–4.5 | Bifusiform, 3-septate, 145–220 × 0.4–1.6 | Eriksson, 1982               |
| *Cordyceps blackwelliae*    | Gregarious, cylindrical to clavate, 8–10 × 1–1.5 | 4–6 × 1.5–2              | Superficial, (300–302–332 (350) × (150–155–189 (200) | Cylindrical, 8-spored, 300 × 2.5–3 | Cylindrical, 8-spored, 300 × 1.5–3 | Bola-shaped, 3- or 4-septa, (250–259–283.5 (290) × 1 | Mongkolsamrit et al., 2018 |
| *Cordyceps brasiliensis*    | Sub-solitary or branch, 60–73 × 1.5–2 | 15–20 × 3–5              | Immersed, 350–700 × 175–315 | Worm-form, 70–333 × 3.5–5        | Filiform         | 3.5–7 × 0.7       | Fang et al., 1995            |
| *Cordyceps brevistroma*     | Solitary or gregarious, 2.5–10 × 0.5 | Clavate, subglobose, 2–5 × 1–1.5 | Semi-immersed, ovoid, 250–330(350) × 130–185(200) | Cylindrical, 8-spored, (110)140–220(250) × 2–5 | Whole, bola-shaped, 150–200 × 0.5 | This study                   | Mongkolsamrit et al., 2020 |
| *Cordyceps bullispora*      | Solitary, 10–20, rhizoids flexuous, 3.5–7.4 × 0.5–1.5 | Clavate, 5.6 × 0.7–1.1   | Superficial, 402–610 × 280–427 | Cylindrical, 8-spored, 274–385 × 3.7–4.8 | Filiform         | 127–260 × 0.9–1.2  | Wang Y. B. et al., 2020      |
| *Cordyceps chaetoclavata*   | Solitary, 23 × 0.8     | Clavate, 5.6 × 0.7–1.1   | Superficial, 402–610 × 280–427 | Cylindrical, 8-spored, 274–385 × 3.7–4.8 | Filiform         | 127–260 × 0.9–1.2  | Wang Y. B. et al., 2020      |
| *Cordyceps chanhua*         | Solitary, simple       |                           |                        | Partly immersed, 475–602 × 222–319 | Cylindrical, 8-spored, 235–280 × 2.1–3 | Filiform         | 246–360 × 1.5–1.8  | Li et al., 2021              |
| *Cordyceps chiangdaciensis* | Solitary to gregarious, 2–7 × 1–1.5 |                        | Superficial, 200–450 × 70–170 | Cylindrical, 175–315 × 2–3 | Filiform         | 200–300 × 1       | Tasanathai et al., 2016      |
| *Cordyceps chichibuënsis*   | Solitary, 13–15 × 1–1.5 | Semi-immersed, 400 × 230–260 | 5.5 µm wide            | Clavate, 165 × 6 |                        |                          | Kobayasi and Shimizu, 1980   |
| *Cordyceps chihiuenis*      | Solitary or 2–3, 10–20 × 1–2 | Elliptical, 5–6 × 3–4 | Superficial, 340–440 × 200–240 | Immersed, 350 × 100 |                        |                          | Liang et al., 2002; Zha et al., 2021 |
| *Cordyceps cocccina*        | Gregarious, 4–35 × 0.3–0.5 | Cylindrical or clavate, 3–4 × 1.25–1.75 | Cylindrical, 8-spored, 205–330 × 2.1–3.3 | Filiform, 140–269 × 1.4–2.1 |                        |                          | Petch, 1924; Shrestha, 2011 |
| *Cordyceps cocoonhabita*    | Two or gregarious, 15.2–57.8 | Clavate, 3.5–7.4 × 0.5–1.5 | Superficial, 346–435 × 125–199 | Cylindrical, 8-spored, 205–330 × 2.1–3.3 | Filiform, 3–septate, 340–375–414 × 1.5–2 |                        | Wang Y. B. et al., 2020      |
| *Cordyceps cuncuncinae*     | Solitary, rarely two, 59–92–105 × 7–8–10 | Ovals to subglobose, 15–18–21 × 12–15–18 | Immersed, 772–793–820 × 257–279–314 | Cylindrical, porentous, 8-spored, 364–391–422 × 6–7–8 | Filiform, 3–septate, 340–375–414 × 1.5–2 |                        | Palfner et al., 2012         |
| *Cordyceps cylindrica*      | Single, 36 × 2–2.6     | Cylindrical, clavate with obtuse top, 13 × 3.7–4 | Immersed, fusiform to elliptical or flask-shaped, 850–1000 × 200–225 | 4.5–5.5 µm wide |                        |                          | Kobayasi and Shimizu, 1977   |
| *Cordyceps dermapteoigena*  | Solitary, simple, 15 × 1 | Cylindrical, 7 × 1.5, sterile apex 5 mm long | Embedded, 405–450 × 180–230 | 6–7.2 µm wide | Filiform, multi-septate, (4.8–9–15 × 2–3 |                        | Kobayasi and Shimizu, 1977   |
| *Cordyceps doiana*          | Solitary, simple, 30 × 0.3–0.4 | Cylindrical, 9 × 0.7–0.8 | Semi-superficial, 2/3 embedded, 250 × 170 | 125–135–6 × 7 | 4–5 × 1              |                        | Liang et al., 2003           |

(Continued)
| Species                        | Stromata (mm)                        | Fertile part (mm) | Perithecia (µm)                           | Asci (µm)         | Ascospores (µm)     | Part-spores (µm) | References                  |
|-------------------------------|--------------------------------------|-------------------|-------------------------------------------|-------------------|--------------------|-------------------|----------------------------|
| **Cordyceps formosana**       | Cylindrical or branched, 8.7–21.6     | 5.3–8.0 x 1.5–4.0 | Semi-immersed, 360–520 x 230–330          | Linear, 230–335 x 6.0–7.2 | 5.0–9.8 x 1.4–2.0 | Cylindrical, 3–4 x 1–1.5 | Olatunji et al., 2018      |
| **Cordyceps inthanonensis**   | Multiple, 6–25 mm long                | Cylindrical to clavate, half of stroma, 3–5 mm wide | Semi-immersed, ovoid, 600–720 x 220–420 | Cylindrical, 450–600 x 4–6 | Cylindrical, 3–4 x 1–1.5 | Mongkolsumrith et al., 2020 |
| **Cordyceps ishikariensis**   | Gregarious, 45–65 x 1.5–2             | Fertile part is slightly wider than stipe | Semi-immersed, 500–670 x 240–300          | 250–360 x 4       | Shrestha, 2011     |
| **Cordyceps jakajanicola**    | Gregarious, simple, 32–45             | On the terminal end c. 1/3 of the stroma | Semi-immersed, 400–600 x 300–400          | Cylindrical, 265–360 x 4–5 | Bola-shaped, 250–310 x 1 | Crous et al., 2019        |
| **Cordyceps kuburiensis**     | Solitary, 8 x 1–1.5, rhizoids flexuous | Cavate to subglobose, 1.5–5 x 1–2.5 | Pseudo-immersed, obpyriform, (350–370–460–550) x (120–140–190–240) | Cylindrical, 280 x 3–5 | Filiform, 250 x 1 | Crous et al., 2019        |
| **Cordyceps kyusyuensis**     | Multiple, 15–20 mm long               | Cylindrical, 10–12 mm long | Semi-superficial, ovoid, 410–580 x 210–330 | 4 µm wide         | 4–5 x 1            | Kobayasi, 1981          |
| **Cordyceps longiphialis**    | Two, 13–25                           | Clavate, 4–15 x 2.0–2.5 | Superficial, 380–612 x 167.5–268.3 | Fusiform to cylindrical, 113–200 x 1.1–2.7 | Bola-shaped, 110–184 x 0.8–1.3 | This study                  |
| **Cordyceps militaris**       | Solitary or gregarious, 8–70 mm long  | Clavate, half of stroma | Immersed to semi-immersed, 500–720 x 300–480 | Cylindrical, 300–510 x 3.5–5 | Filiform, multi-septate | 2–4.5 x 1–1.5 | Mains, 1958; Liang et al., 2007 |
| **Cordyceps morakotii**       | Simple or compound, 3–7 x 0.5–1       | Cylindrical to ovoid | Superficial, 200–300 x 70–120            | Cylindrical, 150–200 x 3–5 | Bola-shaped, 200–250 x 1, 3-septate | Tasanathai et al., 2016     |
| **Cordyceps nabanheensis**    | Solitary or gregarious, 14–23, rhizoids flexuous, 2.0–6.5 x 0.7–1.5 | Clavate | Superficial, 224–322.6 x 71.2–317.3 | Filiform, 100–120 x 1.0 | (4–6)–10 x 1 | Chirivi et al., 2017      |
| **Cordyceps nidus**           | Gregarious, simple, 10–42 x 0.5–2     | Subcylindrical, 2.5–18 x 0.5–3 | Pseudodissomes, 300–500 x (630) x 110–190 x (205) | Cylindrical, (145–) 190–360 x 2–4 | Filiform, multi-septate | 3–6.8–7 x 1.0–1.4 | Su and Wang, 1986         |
| **Cordyceps ninchukispora**   | 1–6 branches, 13.8–22.4 x 0.3–0.9    | 5.7–14.2 x 0.8–0.9 | Superficial, 95–145 x 50–60               | Long cylindrical, 75–105 x 2.1–3.1 | Ninchukiform, 90–110 x 1.2, 3–4 septate | Wright, 1993               |
| **Cordyceps ningxiaensis**    | 1–2 branches, 5–15 x 0.3–1.2          | Spherical to ovoid, 1.2–3 x 1.2–2.8 | Immersed, 288–400 x 103–240              | Cylindrical, 8-spored, 168–205 x (3.7–) x (1.5–) x (6.6) | Filiform, multi-septate | 3.6–7.8–7 x 1.0–1.4 | Yan and Bau, 2015          |
| **Cordyceps oncoperae**       | 1–4 branches, 35 mm long, Acute apex, 4–10 x 2–3 | Ovoid, (350–410 x 180–230–380) | Cylindrical, 8-spored, (188–200–224 x (256) x (5–6)–6.5 | Filiform, 104–139 x 1.5–2 | Cylindrical, 400–500 x 5–6 | 8–10 x 1 | Mains, 1959                 |
| **Cordyceps parvula**         | Solitary, 5 mm long                   | Subglobose, clavate, 4–10 x 2–3 | Superficial, ovoid, (320/330–390/410) x (175/180–270/350) | Filiform, 150–175–260/300 x 2–2.5 | Bola-shaped, (180/210–225/250) x 0.5 | Mongkolsumrith et al., 2020 |
| **Cordyceps parvistroma**     | Solitary, 5 mm long                   | Subglobose, clavate, 4–10 x 2–3 | Superficial, ovoid, (320/330–390/410) x (175/180–270/350) | Filiform, 150–175–260/300 x 2–2.5 | Bola-shaped, (180/210–225/250) x 0.5 | Mongkolsumrith et al., 2020 |

(Continued)
| Species                      | Stromata (mm)                          | Fertile part (mm) | Perithecia ($\mu$m)            | Asci ($\mu$m) | Ascospores ($\mu$m) | Part-spores ($\mu$m) | References                      |
|------------------------------|----------------------------------------|-------------------|--------------------------------|---------------|---------------------|----------------------|-------------------------------|
| **Cordyceps polyarthra**     | Gregarious, 30–44                       | Cylindrical to narrowly clavate, 12–14 | Semi-immersed, 220–300 x 180–200 | Cylindrical, 167–217.5 x 3.5–4.5 | Filiform, multi-septate | Cylindrical, 4.5–11 x 0.5–1.0 | Catania et al., 2018           |
| **Cordyceps polystromata**   | Gregarious, 11–37 x 3.0–9.8              | Cylindrical to clavate, 5–17 x 2.3–8.6 | Superficial, 522.3–663.4 x 296.4–576.7 (583.5 x 142.2) | Cylindrical, 34.2–172.8 x 4.1–6.5 (68.4 x 1.7) | Linear, 34.2–172.8 x 0.9–2.6 (2.1 x 1.6) | Cylindrical, 1.3–3.0 x 1.1–2.2 | Duan, 2019                     |
| **Cordyceps pruinosa**       | Solitary, 15–47.5                       | Narrow-clavate or subcylindrical, 7 x 1.5 | Superficial, narrow-oval or ovoid-cylindrical, 400 x 100 | Cylindrical, 1.3–4 x 2.3–8.6 | Filiform and filiform, 4–6 x 0.5–1.0 | Cylindrical, 6 x 1 | Petch, 1924; Liang, 1983; Liang et al., 2007 |
| **Cordyceps pseudomilitaris**| 1-3, simple, 12–25 x 1–2                | 2.5–9(–10) x 1.5–2.5 | Semi-immersed, 320–500 x 225–350 | Cylindrical, 210–395 x 5–6 | Filiform, multi-septate, 200–380 x 1 | Cylindrical, 1.3–2.5 x 1.2–2.2 | Catania et al., 2018; Olariu et al., 2018 |
| **Cordyceps qingchengensis** | 1-3 branches, 25 mm long                | 7–9 x 2.0–2.5     | Partially immersed, sharply pointed, 335–490 x 145–240 | Cylindrical, 8–spored, 180–200 x 2.4–4 | Filiform, partly bifiform, 180–220 x 0.45–0.65 | Cylindrical, 6 x 1 | Zha et al., 2019                |
| **Cordyceps rosea**          | Solitary, 11 mm long                    | Cavate             | Immersed, ovoid, 330–380 x 160–230 | Cylindrical, 10 x 3–4 | Whole, multi-septate | Cylindrical, (3.6–) 4.8–6 (7.2) x (1.2–)1.5–2 | Kobayasi and Shimizu, 1982    |
| **Cordyceps rostrata**       | Solitary, simple, 35 mm long            | Cylindrical, 10 x 2 | Superficial, subglobose, 420–525 x 255–375 | Cylindrical, 6 x 0.5–1.0 | Filiform, multi-septate, 100–250 x 0.5–1 | Cylindrical, (3.6–) 4.8–6 (7.2) x (1.2–)1.5–2 | Liang, 2003                    |
| **Cordyceps simaoensis**     | Solitary or gregarious, 7–25.1          | Elliptical to fusiform, 1–4 x 1.5–3 | Immersed, 638.4–757.6 x 371–531.1 | Clavate to nearly cylindrical, 8–spored, 66.9–126.1 x 1.9–2.7 | Filiform, multi-septate, 3.0–4.0 x 1.0 | This study                     |
| **Cordyceps singeri**        | 1–2, simple, 10–20 x 0.5–1.5            | Cylindrical to clavate, 1 x 3–4 | Embedded, 325–520 x 220–475 | Cylindrical, (187–)425–475 x 3–4(–4.5) | Filiform, multi-septate, 3.0–4.0 x 1.0 | Cylindrical, (3.6–) 4.8–6 (7.2) x (1.2–)1.5–2 | Catania et al., 2018           |
| **Cordyceps spegazzinii**    | Solitary, simple, 7–9 mm long           | Cylindrical to clavate, 1 x 3–4 | Superficial to partially immersed, 400–460 x 200–240 | Cylindrical, 8–spored, 200–250 x 2.5–3 | Filiform, multi-septate, 100–250 x 0.5–1 | Cylindrical, (3.6–) 4.8–6 (7.2) x (1.2–)1.5–2 | Torres et al., 2005            |
| **Cordyceps submilitaris**   | 20–30 x 1–1.5                           | 10–25 x 2         | Embedded, 450–620 x 300–430 | Cylindrical, 300–420 x 3–4 | Filiform | Cylindrical, 3.6–4.5 µm long | Mains, 1940                   |
| **Cordyceps subulifera**     | Solitary, 25 mm long                    | Clavate, 4 x 1.5   | Pseudomissed, 250–650 x 300–430 | Cylindrical, 275–510 x 3.5–5.2 | Filiform, multi-septate, 180–410 x 1.2–1.7 | Cylindrical, 2.8–6.5 µm long | Wang Y. B. et al., 2020        |
| **Cordyceps succavus**       | Solitary, 40–50 x 3–6                    | Cylindrical, 15–20 x 4–5 | Semi-immersed, 534–655 x 179–278 | Cylindrical, 8–spored, 486–600 x 3.6–4.9 | Filiform, 466–594 x 0.9–1.2 | Cylindrical, (8–)19–12 x 1.8–2.0 | Hyde et al., 2019              |
| **Cordyceps suoniensis**     | Gregarious (2–4), 15 x 2–3              | Cylindrical, 7 x 3 | Pseudomissed, 400–500 x 280–300 | Cylindrical, 8–spored, 3.6–4.8 µm wide | Filiform | Cylindrical, 6–8 x 0.5–0.8 | Liang et al., 2003, 2007       |
| **Cordyceps takaomontana**   | Solitary or gregarious, 8–10 mm         | Cylindrical, 8–10 x 1.5–2 | Superficial, 375–450 x 145–195 | Filiform, 1200 x 2.4–3 | Filiform | Cylindrical, 6 x 1 | Zha et al., 2019                |
| **Cordyceps translucens**    | 10 x 1                                  | Globose or ovoid, 2.5 x 2 | Superficial, 0.5 x 0.3 | Cylindrical, 8–spored | Cylindrical, 6 x 1 | Petch, 1924                   |

**Boldface:** data generated in this study.
### TABLE 3 | Comparison between the asexual morphs in Cordyceps.

| Species                  | Conidiophores (µm) | Phialides | Phialides size (µm) | Conidia (µm) | Other key characteristics                                                                 | References                      |
|--------------------------|--------------------|-----------|---------------------|--------------|-------------------------------------------------------------------------------------------|--------------------------------|
| Cordyceps albocitrinus   | 3.8–10.4 × 1.1–1.4 |           | 3.3–11.3 × 0.9–1.2  | 3.7 × 1.1    | In chains 1–5, ellipsoidal or cylindrical, 0.7–3.5 × 0.6–1.8 Synnemata up to 8–10 × 0.3–0.8 mm | Duan, 2019                     |
| Cordyceps amoene-rosea   | 90–150 × 2.0–2.5   | Verteclate| 4.0–7.5 × 1.5–3.0   |              | Subglobose to ellipsoidal, irregularly cylindrical, 2.9–3.5 × 1.7–2.2                       | Samson, 1974                   |
| Cordyceps araneae        |                    | Solitary  | 5–8 × 1.5–2, basal  |              | Fusoid to ovoid, 3–6 × 1–2                                                                   | Mongkolsamrit et al., 2020     |
| Cordyceps bifusispora    |                    | Solitary  | 9–50 × 1.5–2, flask  |              | Globose, ovoid or cylindrical, 1- to 8-celled, 2.5–3.5 × 2.4–4.5                            | Liang et al., 2007             |
| Cordyceps blackwelliae   |                    | Verticile | (6–)6.5–8(–9) ×     |              | Cylindrical to ellipsoidal or reniform, (3–)5–7(8) × 2–3.5 Synnemata numerous, up to       | Mongkolsamrit et al., 2018     |
| Cordyceps brevistroma    |                    | Solitary  | 6–9(12) × 1.5–2, basal |              | Ovoid to fusiform, 3–4 × 1–2                                                                | Mongkolsamrit et al., 2020     |
| Cordycepsbullispora      | Solitary           |           | 5.6–20.7 × 1.8–3.3  |              | Cylindrical or slightly allantoid, oblong-ellipsoidal to ellipsoidal, 4.9–11.1 × 1.9–4.5 | This study                     |
| Cordyceps cateniannulata |                    | Verteclate| 3–8 × 1.5–3, basal  |              | In chain, ovate to ellipsoidal, 2.3–5 × 1–1.5                                              | Liang, 1981                    |
| Cordyceps cateniobliqua  | 90–150 × 1–1.5     | Verteclate| 8.5–12 × 1–1.5 and 5–8 × 2–2.5, basal portion ellipsoidal |              | Synnemata unbranched, red                                                                    | Liang, 1981                    |
| Cordyceps chanhua        |                    | Verteclate| 4.2–7(–13.5) × 2.3–3.5–(5.2) |         | Chlamydospores 13–26.5 × 3–12 µm                                                             | Li et al., 2021                |
| Cordyceps chiangdaoensis |                    |           | 5–22.5 × 1–2        |              | Ovoid to cylindrical, 4–10 × 1.5–2                                                            | Tasanathtai et al., 2016       |
| Cordyceps cocoonhabita   | 5.9–9.3 × 1.4–2.0  | Solitary, | 4.0–16.7 µm long, basophil portion 1.5–2,7, neck 0.5–1.2 |              | In chains or solitary, ovate to fusiform, 1.6–3.0 × 0.7–1.5                                 | Wang Y. B. et al., 2020         |
| Cordyceps farinosa       | 60–150(300) × 1–1.5| Verteclate| 5.7–8 × 1–2         |              | In chains, fusiform, 3–4 × 1–2                                                                | Liang et al., 2007             |
| Cordyceps formosana      | 6–22.5 × 1.5–2.6   | Solitary  | 4.0–16.7 µm long, basophil portion 1.5–2,7, neck 0.5–1.2 |              | In chains or solitary, ovate to fusiform, 1.6–3.0 × 0.7–1.5                                 | Liang et al., 2007             |
| Cordyceps ghanensis      | 100 × 1.5–2        | Verteclate| 5.7–8 × 1–2         |              | In chains, fusiform, 3–4 × 1–2                                                                | Liang et al., 2018             |
| Cordyceps inthanonensis  | 90–180 × 2.5–3.5   | Verteclate| 5.5–8 × 2–3.5, basal portion ellipsoidal, neck 0.5–0.75 |              | Synnemata branched, up to 30 × 0.4 mm, powdery                                              | Mongkolsamrit et al., 2020     |
| Cordyceps jakajanicola   | Solitary           | Verteclate| (12)14–18.5(20) × 1.5–3, basal portion cylindrical |              | Synnemata branched, powdery and floccose                                                    | Crous et al., 2019             |
| Cordyceps javanica       | Verteclate         | 4–5.3(–6) × 2–3.5(–4), basal portion globose, oval or occasionally conical, neck 0.5 | |              | Synnemata branched, powdery and floccose                                                    | Mongkolsamrit et al., 2018     |
| Cordyceps kuiburiensis   | Verteclate         | 6–19(–10) × 2–2.5, basal portion cylindrical, neck 1–3 × 3–4 × 0.5 | |              | Synnemata branched, powdery and floccose                                                    | Crous et al., 2018             |
| Cordyceps lacustrini      | Verteclate         | 3–4 × 1.5–2, basal portion ellipsoidal, necks 1–3 × 1 | |              | Synnemata branched, powdery and floccose                                                    | Crous et al., 2018             |

(Continued)
| Species                        | Conidiophores (µm) | Phialides               | Phialides size (µm)                                                                 | Conidia (µm)                                                                 | Other key characteristics                                                                 | References                   |
|-------------------------------|--------------------|------------------------|------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-------------------------------|
| *Cordyceps lepidopterorum*    | Solitary, cylindrical, 4.6–11.5 x 1.6–3.2 | Solitary or verticillate with whorls of 2 to 3 | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps longijohialis*     | Solitary           | Verticillate with whorls of 2–3 | 5.5–8 x 4–5, basal portion globose to flask shaped, neck 2–3 x 1                  | In chains, ellipsoidal or elliptical, 6(8–9.5)x3–4                                  | Apical conidia more prominent than other conidia, 4.6–10.0 x 1.4–2.3 µm               | This study                   |
| *Cordyceps militaris*         | Solitary or verticillate | Paeonlomyces-type: cylindrical, (0.5)x0.8–1.5 x 6.15(–20), Verticillium-type: 0.8–1.2 x (8–14)x20(–25) | 5.0–6.5 x 2.0–3.0                                                                | Cylindrical, 2.1–6.0 x 0.8–2.5                                                        |                                                                              | Mongkolsamrit et al., 2018    |
| *Cordyceps nabanheensis*      | Solitary, cylindrical, 4.6–11.5 x 1.6–3.2 | Solitary or verticillate with whorls of 2–3 | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps neopruinosa*       | Solitary or verticillate with whorls of 2–4 | Solitary or verticillate | 5.0–6.5 x 2.0–3.0                                                                | Cylindrical, 2.1–6.0 x 0.8–2.5                                                        |                                                                              | Liang et al., 2007             |
| *Cordyceps ninchukispora*     | Solitary or verticillate | Solitary or verticillate | 5.0–6.5 x 2.0–3.0                                                                | Cylindrical, 2.1–6.0 x 0.8–2.5                                                        |                                                                              | Mongkolsamrit et al., 2020    |
| *Cordyceps parvistroma*       | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps polystromata*      | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps poprawskii*        | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps pruinosa*          | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps pseudotenuipes*    | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps shuifuensis*       | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps simaoensis*        | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps subtenuipes*       | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps tenuipes*          | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps yinjiangensis*     | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |
| *Cordyceps zhujiangensis*     | Solitary or verticillate | Solitary or verticillate | 6.0–19.1 x 1.3–3.5                                                                | Elliptical to oblong, 2.1–4.3 x 1.1–2.7                                                | Synnemata awl-shaped, 2–10 x 1 mm                                                     | Mongkolsamrit et al., 2020    |

Boldface: data generated in this study.
ellipsoidal conidia with a button-like character. Tasanathai et al. (2016) reported an anti-pathogenic species, *C. morakotii*. *C. yinjiangensis* had a close relationship with *C. morakotii* with ant host and conidia formed in an imbricate chain. However, *C. yinjiangensis* was distinct from *C. morakotii*, which had longer phialides (16–20 × 2–3 µm) and bigger aseptate conidia (4–12 × 1–2 µm) (Li et al., 2020).

**Cordyceps longiphialis** H. Yu, Q. Y. Dong and D. X Tang, sp. nov. (Figure 3)

*MycoBank:* MB 842329

**Etymology:** Referring to its longer phialide than close relationship species in this genus.

**Type:** Xinfang Reservoir, Simao District, Pu’er City, Yunnan, China. September 28, 2020, H. Yu (YHH 20013, holotype; YFCC 8402, ex-holotype living culture).

**Teleomorph:** Stromata (Figures 3A,B) arising from rotten wood, two, unbranched, 13–25 mm long. Stipe cylindrical, 4–10 mm long, 1.5–2 mm in diameter, orange-red to crimson, fleshy, glabrous, smooth. Fertile part single, clavate, covered by a spinous surface, 4–15 mm long, 2.0–2.5 mm in diameter, reddish-orange. Perithecia (Figure 3D) crowded or sparse, crimson-yellowish, superficial, vase-form to oblong, 380–612 × 167.5–268.3 µm. Asci (Figure 3E) 8-spored, fusiform to cylindrical, 113–200 × 1.1–2.7 µm when mature; Ascus caps hemispherical, 1.6–3.6 µm in height, 1.7–3.3 µm in width. Ascospores hyaline, bola-shaped, septate, 110–184 × 0.8–1.3 µm, central region filiform, terminal region narrowly fusiform, do not disarticulate into part-spores.

**Anamorph:** Conidial arrangement *Mariannaea*-like. Colonies on PDA are fast-growing, attaining a diameter of 35–37 mm in 14 days at 25°C, white to pale yellow, cottony, with high mycelial density at the centrum, reverse white to pale yellow. Hyphae smooth-walled, septate, hyaline, 0.8–2.5 µm wide. Cultures readily produced phialides and conidia after 2 weeks on potato dextrose agar at room temperature. Phialides usually solitary on hyphae, basal portion cylindrical to clavate, tapering gradually toward the apex; 7.0–7.08 µm long, 0.6–2.1 µm wide at the base, 0.9–2.1 µm at the middle, and 0.6–1.8 µm wide at the

**FIGURE 2** | Cordyceps bullispora. (A) Fungus on the pupa of Lepidoptera. (B,C) Colony obverse (B) and reverse (C) on PDA at 21 days. (D–G,I–L) Phialides. (H) Conidia. Scale bars: (A) = 10 mm; (B,C) = 10 mm; (D) = 25 µm; (E) = 50 µm; (F,G) = 15 µm; (H) = 3 µm; (I) = 5 µm; (J) = 15 µm; (K,L) = 10 µm.
apex. Conidia one-celled, smooth-walled, hyaline, cylindrical, 2.1–6.0 × 0.8–2.5 µm, often formed in an imbricate chain, the size of apical conidia significantly more prominent than other conidia in the chain, 4.6–10.0 × 1.4–2.3 µm.

Habitat and known distribution: Buried in rotten logs below ground, in northwestern Yunnan, China.

Additional specimens examined: Xinfang Reservoir, Simao District, Pu’er City, Yunnan, China, isolated from stromata of rotten wood at elevation 1,350 m. September 28, 2020, H. Yu (YFCC 8403, ex-holotype living culture).

Comments: Phylogenetic analyses showed that *C. longiphialis* was closely related to *C. bullispora*; however, the independent phylogenetic position and different physiological characteristics could distinguish *C. longiphialis* from its sister species, *C. bullispora* (as mentioned above). The distinctive characteristics of *Cordyceps longiphialis* were the cylindrical stromata with a spinous surface, superficial perithecia, much shorter ascospores (110–184 × 0.8–1.3 µm), and much longer phialides (7.0–70.8 µm long). Ascospores of *C. longiphialis* were shorter than *C. neopruinosa* (135–275 × 0.5 µm) and *C. pruinose*
Cordyceps nabanheensis H. Yu and Q. Y. Dong, sp. nov. (Figure 4)

MycoBank: MB 842341

Etymology: The location in Nabanhe National Nature Reserve where the species was collected.

Type: Manlv village, Nabanhe National Nature Reserve, Jinghong City, Yunnan, China. August 16, 2020, H. Yu (YHH 20019, holotype; YFCC 8409, ex-holotype living culture).

Teleomorph: Stromata arising from the pupa of Lepidoptera buried in soil, the host covered by a white mycelial surface, solitary or gregarious, cylindrical to enlarging apically, reddish-orange to crimson, 1.4–2.3 cm long. Rhizoids flexuous, arising from the head region of host larva buried in soil, 7–12 mm deep under the ground. Stipes cylindrical, 12.0–20.9 × 0.6–1.3 mm, fertile parts clavate, 2.0–6.5 × 0.7–1.5 mm. Perithecia superficial, oblong-ovate, 224–322.6 × 71.2–317.3 µm. Ascii and ascospores were not observed.

Anamorph: Conidial arrangement Evlachovaea-like. Colonies on PDA moderately fast-growing, 48–51 mm diameter in 14 days at 25°C, floccose, with high mycelium density; white to orange pinkish, reverse orange-brown. Hyphae smooth-walled, branched, septate, hyaline, septate, 3.5–9.3 µm wide. Cultures readily produced phialides and conidia after 1 week on potato dextrose agar at room temperature showing a powdery appearance due to profuse conidiation. Conidiophores smooth-walled, solitary, cylindrical, 4.6–11.5 × 1.6–3.2 µm. Phialides arising from aerial hyphae, cylindrical or clavate, solitary or in whorls of two to three, tapering abruptly into a narrow neck, 5.6–19.1 × 1.3–3.5 µm. Conidia one-celled, smooth-walled, hyaline, elliptical to oblong, 2.1–4.3 × 1.1–2.7 µm, often formed in an imbricate chain.

Habitat and known distribution: On larvae of Lepidoptera buried below ground at elevation 600 m in northeastern Yunnan, China.

Additional specimens examined: Manlv village, Nabanhe National Nature Reserve, Jinghong City, Yunnan, China, on larvae of Lepidoptera. August 16, 2020 (YHH 20020, paratype; YFCC 8410 ex-paratype living culture).

Comments: Cordyceps araneae was firstly reported from Khon Kaen Province, northeastern Thailand by Mongkolsamrit et al. (2020); C. araneae was a spider cocoon pathogenic fungus producing pale orangestromata, perithecia semi-immersed, narrowly ovoid, 450–500 × 150–200 µm with whole bola-shaped ascospores breaking into part-spores 30–65 × 0.5 µm, and developed the Evlachovaea-like anamorph, phialides solitary or in whorls of two to three, 5–8 × 1.5–2 µm, conidia fusoid to ovoid, 3–5 × 1–2 µm (Mongkolsamrit et al., 2020).

Based on the ITS, ribosomal large subunit, rpbl, rpb2, and tef-1α genes, multigene analyses revealed that C. araneae had a close relationship with C. kuiburiensis, C. brevistroma, and C. nidus, and they were all characterized by orange to reddish-orange, cylindrical to enlarging apically stromata and a conidial arrangement Evlachovaea-like (Chirivi et al., 2017; Crous et al., 2019; Mongkolsamrit et al., 2020). Interestingly, C. nabanheensis shared such features, and our phylogenetic analysis indeed indicated that C. nabanheensis had a close relationship to C. araneae and C. brevistroma. However, C. nabanheensis and C. brevistroma differed from C. araneae and C. kuiburiensis regarding their hosts. Both C. nabanheensis and C. brevistroma occur on Lepidoptera larvae, whereas both C. araneae and C. kuiburiensis occurred on spiders. C. brevistroma had bola-shaped whole ascospores, which was the same shape but shorter than C. araneae reported (150–200 µm vs. 250–400 µm). In the natural specimen, C. kuiburiensis developed the anamorph.

Cordyceps pseudotenuipes H. Yu, Q. Y. Dong, and Y. Wang, sp. nov. (Figure 5)

MycoBank: MB 842330

Etymology: Referring to morphological resemblance of Cordyceps tenuipes and Cordyceps subtenuipes but phylogenetically distinct, “not” C. tenuipes.

Type: Wild Duck Lake Forest Park, Shuanglong Town, Panlong District, Kunming City, Yunnan, China. September 10, 2019, H. Yu and Y. Wang (YHH 20014, holotype; YFCC 8404, ex-holotype living culture).

Teleomorph: Undetermined.

Anamorph: Conidial arrangement Isaria-like. Synnemata arising from the pupae of Lepidoptera buried in soil, synnemata erect, solitary, flexuous, white, up to 0.5 cm long. Stipes cylindrical, 0.5 mm wide, producing a mass of conidia at the branches of synnemata, powdery.

Colonies on PDA attaining a diameter of 53–55 mm in 14 days at 25°C, white to cream-colored, soft cottony aerial mycelium, reverse pale yellow. Hyphae smooth-walled, branched, septate, hyaline. Synnemata arising from the entire body of larvae were irregularly branched, 0.2–1.0 cm long, 0.1–0.3 mm wide; cylindrical or clavate stipes with powdery white heads. Cultures readily produced phialides and conidia after 2 weeks on potato dextrose agar at room temperature showing a granular appearance due to profuse conidiation. Conidiophores cylindrical, hyaline, smooth-walled, 17.9–25.9 × 1.7–2.1 µm. Phialides from aerial mycelium straight to slightly flexuose, solitary or in whorls of two to five on each branch, cylindrical, usually with a slightly swollen basal part, tapering into the apex, 6.8–31.8 × 1.2–3.3 µm. Conidia hyaline, ovoid to ellipsoidal, smooth, one-celled, 3.4–6.5 × 2.2–4.0 µm. Chlamydospores present, one-celled, solitary, eggplant shape or oval to pyriform, 9.2–18.5 × 3.4–7.5 µm, hyaline becoming brown, thick and smooth-walled.

Habitat and known distribution: On the pupa of Lepidoptera buried in the soil. Kunming City, China.

Additional specimens examined: Wild Duck Lake Forest Park, Shuanglong Town, Panlong District, Kunming City, Yunnan, China. September 10, 2019, H. Yu (YHH 20015, paratype; YFCC 8405, ex-paratype living culture).

Comments: Phylogenetically, C. pseudotenuipes formed a separate subclade from the other species of Cordyceps with high credible support (100%). C. pseudotenuipes was similar to C.
FIGURE 4 | Cordyceps nabanheensis. (A–C) Fungus on the pupa of Lepidoptera. (D) Fertile part. (E) Perithecia. (F,G) Colony obverse (F) and reverse (G) on PDA at 14 days. (H) Solitary phialide on hyphae. (I,J–N) Opposite conidiophore and verticillate phialides. (O) Conidia. Scale bars: (A–C) = 10 mm; (D) = 1 mm; (E) = 100 µm; (F,G) = 10 mm; (H,J,K) = 20 µm; (I) = 10 µm; (L–O) = 5 µm.
Cordyceps pseudotenuipes (Peck) Kepler et al. (2017) based on its conspicuous synnemata and Isaria-like asexual conidiogenous structure forming phialides with a swollen basal portion. It differed from C. tenuipes by its unbranched synnemata, white color, phialides with a globose basal part, and smaller ovoid to ellipsoidal wider conidia measuring 3.4–6.5 × 2.2–4.0 μm. C. tenuipes had multiple synnemata, more giant cylindrical to botuliform conidia with a size of 2.0–7.0 × 1.2–2.5 μm (Samson, 1974). The sexual morph of C. tenuipes was proposed as C. takaomontana Yakush and Kumaz, with yellowish stromata and being often concurrent with its asexual morph (Liang et al., 2007). However, the sexual morph of C. pseudotenuipes was not found in the field.
FIGURE 6 | *Cordyceps simaoensis*. (A–C) Fungus on the host of Lepidoptera. (D) Fertile part. (E,F) Perithecia. (G–J) Asci. (K,L) Colony obverse (K) and reverse (L) on PDA at 21 days. (M–S) Conidiophore and phialide. Scale bars: (A,B) = 10 mm; (C) = 5 mm; (D) = 2 mm; (E) = 400 µm; (F) = 100 µm; (G–J) = 10 µm; (K,L) = 10 mm; (M–S) = 20 µm.
Cordyceps simaoensis H. Yu, Q. Y. Dong and Z. Q. Wang, sp. nov. (Figure 6)

MycoBank: MB 842331

Etymology: The location in Simao District where the species was collected.

Type: Xinfang Reservoir, Simao District, Pu’er City, Yunnan, China. September 28, 2020. H. Yu (YHH 20016, holotype; YFCC 8406, ex-holotype living culture).

Teleomorph: Stroma arising from the host’s head, solitary or gregarious, mace-shaped, unbranched, 7–25.1 mm in height. Stipe cylindrical, 22 mm long, 1.1–1.2 mm in diameter, bright yellow, fleshy, glabrous, smooth, enlarging abruptly at fertile portion. Fertile portion single, elliptical to fusiform, 1–3 mm long, 1.5–3 mm in diameter, bright yellow, fleshy, glabrous, smooth, enlarging abruptly at fertile portion. Perithecia crowded, nearly fully immersed, vase-form, oval to oblong, 638.4–757.6 × 371–531.1 μm, ostioles protruding. Ascii (Figures 6G–J) 8-spored, narrowly clavate to nearly cylindrical, 66.9–126.1 × 1.9–2.7 μm; cap 1.2–2.3 μm in height, 2.1–3.5 μm in width. Ascospores not observed.

Anamorph: Conidial arrangement Isaria-like. Colonies on PDA are fast-growing, attaining a diameter of 40–43 mm at 25°C in 21 days, white to bright yellow, cottony, with high mycelial density at the center, forming concentric rings around the inoculum, reverse pale yellow to deep yellow. Hyphae smooth-walled, branched, septate, hyaline, 2.1–2.9 μm wide. Cultures produced phialides and conidia after 45 days on potato dextrose agar at room temperature. Conidiophores are smooth-walled, cylindrical, solitary, or verticillate, 17.1–25.2 × 1.4–1.6 μm. Phialides solitary or verticillate, in whorls of two to three, usually solitary on hyphae, basal portion cylindrical to narrow lageniform, gradually or abruptly tapering toward the apex; 11.7–50.2 μm long, 1.5–3.1 μm wide at the base, 3.4–4.0 μm at the middle, and 0.9–2.0 μm wide at the apex. Conidia is one-celled, hyaline, smooth-walled, fusiform or oval, 2.0–4.9 × 2.0–3.3 μm, often in chains.

Habitat and known distribution: On the pupa of Lepidoptera buried in soil.

Additional specimens examined: Xinfang Reservoir, Simao District, Pu’er City, Yunnan, China. October 6, 2019, H. Yu (YHH 20017 paratype, YFCC 8407 ex-paratype living culture; YHH 20018 paratype, YFCC 8408 ex-paratype living culture).

Comments: Our phylogenetic results demonstrated that the isolated position of this collection was within the Cordyceps species except for their host (see Cordyceps cicadae in Figure 1). The rest of the species could easily disarticulate into part-spores. It was found that all the members in clade III had Isaria-like anamorphs and all four species in clade IV were described as conidiophores verticillate with phialides in whors of 2 to 4 or 5, conidia in chains (Table 3). Clade V consisted solely of Cordyceps grylli, characterized as pathogenic on Gryllidae adults and relatively larger perithecia (up to 650–810 × 270–370 μm) (Teng, 1963; Liang et al., 2007).

Phylogenetic classifications of cordycepitoid fungi showed that most diagnostic characteristics used in current classifications of Cordyceps species (e.g., host, arrangement of perithecia, ascospore fragmentation, conidigenous structures, conidial shape and size) were not phylogenetically informative (Sung et al., 2007; Kepler et al., 2017; Mongkolsamrit et al., 2018; Wang Y. B. et al., 2020). Cordyceps lepidopterorum Mongkolsamrit, Noisiproom, Thanakitipipattana, Spataporn, and Luangsaaard was firstly reported from Chiang Mai Province Thailand by Mongkolsamrit et al. (2018). Cordyceps chanhua, which had long been mistaken as Isaria (Paecilomyces) cicadae of a Brazilian specimen, was recently reported as a new species in the genus Cordyceps s. s., for the discovery of its teleomorph in Mt. Jinggang, Jiangxi, China and analyses of both morphological and phylogenetical evidence (Li et al., 2021). Our phylogenetic trees suggested that C. chanhua (see Cordyceps cicadae in Figure 1) could not be distinguished from C. lepidopterorum (Figure 1). Regarding the morphology, there were no significant differences in the morphological characteristics of anamorph between the two species except for their host (Table 3). Because C. lepidopterorum

DISCUSSION

Considerable changes to the taxonomy of Cordyceps have occurred since the research on entomogenous fungi entered the molecular era. At present, multi-locus phylogenetic analyses have gained importance in delimiting Cordyceps species (Tasanathai et al., 2016; Zha et al., 2019; Li et al., 2020, 2021; Wang Y. B. et al., 2020). In this study, most species of the newly circumscribed genus Cordyceps were analyzed based on phylogenetic inferences of five nuclear molecular markers (nrSSU, nrLSU, tef-1a, rpb1, and rpb2). Both ML and BI analyses produced trees with similar topologies that resolved most Cordyceps lineages in separate terminal branches. Cordyceps s. s. was recognized by five statistically well-supported clades, designated as clade I, clade II, clade III, clade IV, and clade V (Figure 1). There were 20 species in clade I. Morphologically, the 20 species shared relatively complicated host, such as spider, Coleoptera, Lepidoptera, Limacodidae, ant, and even plants. They were also complex and varied in shape (ascosporas bolas-shaped, filiform, ninchukiform, or bifusiform). Clade II was made up of C. militaris and other closely related species. With the exception of C. kyusyuensis and C. rosea, all other species had filamentous ascospores; in addition, unlike C. ancomerae and C. rosea, the rest of the species could easily disarticulate into part-spores. It was found that all the members in clade III had Isaria-like anamorphs and all four species in clade IV were described as conidiophores verticillate with phialides in whors of 2 to 4 or 5, conidia in chains (Table 3). Clade V consisted solely of Cordyceps grylli, characterized as pathogenic on Gryllidae adults and relatively larger perithecia (up to 650–810 × 270–370 μm) (Teng, 1963; Liang et al., 2007).

Phylogenetic classifications of cordycepitoid fungi showed that most diagnostic characteristics used in current classifications of Cordyceps species (e.g., host, arrangement of perithecia, ascospore fragmentation, conidigenous structures, conidial shape and size) were not phylogenetically informative (Sung et al., 2007; Kepler et al., 2017; Mongkolsamrit et al., 2018; Wang Y. B. et al., 2020). Cordyceps lepidopterorum Mongkolsamrit, Noisiproom, Thanakitipipattana, Spataporn, and Luangsaaard was firstly reported from Chiang Mai Province Thailand by Mongkolsamrit et al. (2018). Cordyceps chanhua, which had long been mistaken as Isaria (Paecilomyces) cicadae of a Brazilian specimen, was recently reported as a new species in the genus Cordyceps s. s., for the discovery of its teleomorph in Mt. Jinggang, Jiangxi, China and analyses of both morphological and phylogenetical evidence (Li et al., 2021). Our phylogenetic trees suggested that C. chanhua (see Cordyceps cicadae in Figure 1) could not be distinguished from C. lepidopterorum (Figure 1). Regarding the morphology, there were no significant differences in the morphological characteristics of anamorph between the two species except for their host (Table 3). Because C. lepidopterorum

record also indicated that the C. tenuipes was widely distributed and significantly ecologically diverse in China, with small bright yellow fleshy stromata as teleomorph and Isaria-like anamorph.
was described earlier than *C. chanhua*, *C. lepidopterorum* should be recommended as the scientific name for this species.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

**AUTHOR CONTRIBUTIONS**

Q-YD and HY: conceptualization. Q-YD: methodology, writing—original draft preparation, and formal analysis. Q-YD and YW: software and resources. Z-QW and H-JW: validation. Q-YD, YW, Z-QW, D-XT, Z-YZ, H-JW, and HY: investigation.

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