Detection of *Ophiocordyceps sinensis* and Its Common Adulterates Using Species-Specific Primers

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**INTRODUCTION**

*Ophiocordyceps sinensis* (syn. *Cordyceps sinensis*) is an extremely rare and precious traditional Chinese medicine (TCM) with multiple medicinal values (Wang and Yao, 2011; Quan et al., 2014). This medicinal material is mainly collected in alpine regions over 4,000 m around mountain snowlines on the Tibetan Plateau, the neighboring provinces of the Tibetan autonomous prefectures and the high Himalayas (Yi et al., 2011). As reported in the *New Compilation of Materia Medica*, *O. sinensis* is beneficial to the kidney. Treatment with *O. sinensis* has also been claimed to have curative effects on several conditions, including erectile dysfunction, bronchial diseases, diabetes, cough and cold, jaundice (Ashok Kumar and Kailash Chandra, 2011; Sirisidthi et al., 2015). The main bioactive components found in *O. sinensis* are adenosine, cordycepin, cordymin, cordysinin, ergosterol, guanosine, myriocin, melanin, lovastatin, and sitosterol (Hui-Chen Lo et al., 2013). Due to strict environmental requirements, *O. sinensis* collected in the field is considered...
much more pharmacologically valuable than which obtained through culture; however, at present, most of the natural materials is collected by local farmers who do not have the expertise to differentiate between *O. sinensis* and related species. According to one recent study, even some of the *O. sinensis* materials used for study purposes may contain mycelium from other related species (Dong and Yao, 2010). The increasing rate of adulterated *O. sinensis* preparations not only harms consumers and the reputation of Traditional Chinese Medicine (Qin et al., 2011) but also hampers scientific research on this product.

The present identification of *O. sinensis* relies mostly on morphological characteristics, even though this approach has long been controversial. The genus *Ophiocordyceps* was officially defined by Sung et al. (2007) and Chen et al. (2013) and includes *O. sinensis* and similar species distributed within the alpine regions such as *O. gansuensis*, *O. crassispora*, *O. kanglingensis*, *O. multiaxialis*, *O. nepalensis*, and others. It is difficult to distinguish these species morphologically (Shrestha et al., 2010) and it is even difficult to differentiate *O. sinensis* from the closely related adulterants, such as *Cordyceps gunnii*, *Cordyceps cicadae*, *Cordyceps militaris*, *Cordyceps liangshansensis* and *Ophiocordyceps nutans*. Chemical methods have also been applied to authenticate *O. sinensis* (Hu et al., 2015; Zhang et al., 2015); however, these method required relative large amounts of sample material. Genetic methods such as analysis of internal transcribed spacer sequences (ITS) and random amplified polymorphic DNA (RAPD)-derived molecular markers have also been used to identify *O. sinensis* (Lam et al., 2015). These methods have focused on detection of *O. sinensis* in untreated fungal material rather than processed materials where DNA degradation or fragmentation can occur (Meissner et al., 2007; Shadi et al., 2011). Therefore, in the present study, a method with specific-species primers was developed in order to increase the probability of detection of *O. sinensis* and common fungal adulterants even in processed samples.

**MATERIALS AND METHODS**

**Collection of Samples and Its Sequences**

A total of 89 samples of *O. sinensis* and its adulterants (*C. gunnii*, *C. cicadae*, *C. militaris*, *C. liangshansensis*, *O. nutans*) were collected from Qinghai Province, Tibet and Sichuan Province. The details of these samples are listed in **Table 1**. A total of 131 confirmed ITS sequences of *O. sinensis* were available from previous studies (Chen et al., 2001; Liu et al., 2002; Zhang et al., 2009; Xiang et al., 2014). Additionally, all the known ITS sequences of the genera, *Ophiocordyceps* and *Cordyceps*, were downloaded, and published sequences (sequences in published articles) were selected for further study.

**DNA Extraction, Amplification, and Sequencing**

A total of 20–30 mg stromata of specimens were rinsed with 75% ethanol and milled using a ball-milling machine (Restch, Germany). Genomic DNA was extracted from the resulting powders using a Tiangen Plant DNA Kit (Tiangen Biotech, China). The ITS regions were amplified

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**Table 1** | Information for the samples of *O. sinensis* and its counterfeits used in this study.

| Latin name | Voucher no. | Locality        |
|-----------|------------|-----------------|
| *Ophiocordyceps sinensis* | CSC-1 | Qamdo, Tibet China |
|            | CSC-2 | Qamdo, Tibet China |
|            | CSC-3 | Qamdo, Tibet China |
|            | CSC-4 | Qamdo, Tibet China |
|            | CSC-5 | Qamdo, Tibet China |
|            | CSC-6 | Qamdo, Tibet China |
|            | CSC-7 | Qamdo, Tibet China |
|            | CSC-8 | Qamdo, Tibet China |
|            | CSC-9 | Qamdo, Tibet China |
|            | CSC-10 | Qamdo, Tibet China |
|            | CSC-11 | Qamdo, Tibet China |
|            | CSC-12 | Qamdo, Tibet China |
|            | CSC-13 | Qamdo, Tibet China |
|            | CSC-14 | Qamdo, Tibet China |
|            | CSC-15 | Qamdo, Tibet China |
|            | CSC-16 | Qamdo, Tibet China |
|            | CSC-17 | Qamdo, Tibet China |
|            | CSC-18 | Qamdo, Tibet China |
|            | CSC-19 | Qamdo, Tibet China |
|            | CSC-20 | Qamdo, Tibet China |
|            | CSC-21 | Qamdo, Tibet China |
|            | CSC-22 | Qamdo, Tibet China |
|            | CSC-23 | Qamdo, Tibet China |
|            | CSC-24 | Qamdo, Tibet China |
|            | CSC-25 | Qamdo, Tibet China |
|            | CSC-26 | Qamdo, Tibet China |
|            | CSC-27 | Qamdo, Tibet China |
|            | CSC-28 | Qamdo, Tibet China |
|            | CSC-29 | Qamdo, Tibet China |
|            | CSC-30 | Qamdo, Tibet China |
|            | CSN-1  | Yushu, Qinghai China |
|            | CSN-2  | Yushu, Qinghai China |
|            | CSN-3  | Golog Qinghai China |
|            | CSN-4  | Golog Qinghai China |
|            | CSN-5  | Golog Qinghai China |
|            | CSN-6  | Yushu, Qinghai China |
|            | CSN-7  | Yushu, Qinghai China |
|            | CSN-8  | Qamdo Tibet China |
|            | CSN-9  | Qamdo Tibet China |
|            | CSN-10 | Nakchu, Tibet, China |
|            | CSN-11 | Nakchu, Tibet, China |
|            | CSN-12 | Nakchu, Tibet, China |
|            | CSN-13 | Nakchu, Tibet, China |
|            | CSN-14 | Nakchu, Tibet, China |
|            | CSN-15 | Dege Sichuan China |
|            | CSN-16 | Kangling Sichuan China |
|            | CSN-17 | Kangling Sichuan China |
|            | CSN-18 | Litang Sichuan China |
|            | CSN-19 | Litang Sichuan China |
|            | CSN-20 | Litang Sichuan China |

(Continued)
Continued

| TABLE 1 | Voucher no. | Locality |
|----------|-------------|----------|
| CSN-21   | Dege Sichuan China |
| NQ-1     | Naichu, Tibet China |
| NQ-2     | Naichu, Tibet China |
| NQ-3     | Naichu, Tibet China |
| REG-1    | Ruoergai Sichuan China |
| REG-2    | Ruoergai Sichuan China |
| REG-4    | Ruoergai Sichuan China |
| YS-1     | Yushu, Qinghai China |
| YS-2     | Yushu, Qinghai China |
| YS-3     | Yushu, Qinghai China |
| WZ-1     | Unknown, China (market) |
| WZ-2     | Unknown, China (market) |
| WZ-3     | Unknown, China (market) |
| WZ-4     | Unknown, China (market) |
| WZ-5     | Unknown, China (market) |
| WZ-6     | Unknown, China (market) |
| WZ-7     | Unknown, China (market) |
| WZ-8     | Unknown, China (market) |
| WZ-9     | Unknown, China (market) |
| WZ-10    | Unknown, China (market) |
| WZ-11    | Unknown, China (market) |
| WZ-12    | Unknown, China (market) |
| WZ-13    | Unknown, China (market) |
| WZ-14    | Unknown, China (market) |
| WZ-15    | Unknown, China (market) |
| WZ-16    | Unknown, China (market) |
| WZ-17    | Unknown, China (market) |
| WZ-18    | Unknown, China (market) |
| XC-1     | Changbai Mountain Nature Reserve Jilin China |
| XC-2     | Changbai Mountain Nature Reserve Jilin China |
| GN-1     | Chengtu, Sichuan China (market) |
| GN-2     | Xizang China (market) |
| GN-3     | Hebei China (market) |
| Y-1      | Hebei China (market) |
| CH-1     | Hengduan Mountains Sichuan China |
| CH-2     | Bozhou Anhui China (market) |
| CH-3     | Mopan Jiangsu China (market) |
| LS-1     | Sichuan Chian (market) |
| LS-2     | Sichuan Chian (market) |

using an LA Taq polymerase chain reaction (PCR) kit (Takara Biotech Inc.) with the universal primer pairs 5′-AGGTAACAGTCTGGAAG-3′/4R (5′-GTCTTCGCTTATTGATATGC-3′; Li et al., 2013). The PCR mixture contained 0.1 μL of LA Taq (5 U μL⁻¹), 2.5 μL of 10 × LA Taq PCR buffer II (Mg²⁺ Plus), 1 μL of dNTP mixture (2.5 mM each), 0.6 μL of each primer (10 μM), and 1 μL (~120 ng) of genomic DNA in a total volume of 25 μL. The samples were amplified using a GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA) under the following conditions: initial denaturation at 97°C for 1 min, followed by 30 cycles of denaturation at 97°C for 1 min, annealing at 48°C for 1 min, extension at 72°C for 3 min, and a final elongation step at 72°C for 7 min (Liu et al., 2001).

**Sequence Analysis and Primer Pairs Design**

The sequences were edited and assembled manually using CodonCode Aligner 5.1.4 (CodonCode Co., USA). Analysis of the ITS sequences database was conducted using CodonCode Aligner software to search species-specific motifs. Potential primers were designed and analyzed using Primer 6.0 software (Glantz, 2005) according to the species-specific motifs. All of the *O. sinensis* ITS sequences were aligned with MEGA (Lewis et al., 2013) software to verify the specificity of the primers for DNA from *O. sinensis* and its adulterants (*C. gunnii, C. cicadae, C. militaris, C. liangshanensis, O. nutans*).

**Preparation of *O. sinensis* Decoction and DNA Extraction**

Each sample was rinsed with 75% ethanol and was then milled using a ball-milling machine (Retsch, Germany); 40 mg of each milled sample was used for the genomic DNA extraction with the Tiangen Plant DNA Kit (Tiangen Biotech, China). Sterilized *O. sinensis* raw materials (stroma) were boiled in 500 mL double-distilled water for 60 and 90 min. The decoction was then dried on a stove by boiling, and 40 mg of the dried decoction was used for DNA extraction with the Tiangen Plant DNA Kit (Tiangen Biotech, China).

**DNA Amplification to Verify the Primer Specificity and Utility**

PCR was performed on DNA extracted from *O. sinensis* decoctions and its adulterants. The reaction was carried out in 25 μL volumes comprised of 2 μL dNTP mixture (2.5 mmol/L), 1.0 μL primers DCF4 /DCR4 (2.5 μmol/L), 4 μL template DNA (~30 ng), 2.5 μL 10 × PCR Buffer (Tiangen Biotech, China), 8 μL Taq DNA polymerase and 6.5 μL sterilized water subject to the following conditions: initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 30 s, extension at 72°C for 50 s, and a final elongation step at 72°C for 5 min. DNA from boiled materials was also amplified with ITS universal primer pairs to determine if the DNA was still suitable for amplification of larger sequences. PCR with DNA from adulterants was carried out is in 25–μL volumes comprised of 2 μL dNTP mixture (2.5 mmol/L), 2.0 μL primers (2.5 μmol/L), 2 μL template DNA (~30 ng), including pure DNA of adulterants and DNA mixture of *O. sinensis* and each target DNA for the specific primer (at a ratio of 1:1), 2.5 μL 10 × PCR Buffer (Tiangen Biotech, China), 8 μL Taq DNA polymerase and 6.5 μL sterilized water subject to the same conditions.
Amplification and Concentration Measurement of Diluted DNA
Pure *O. sinensis* DNA was two-fold serially diluted to different multiple to determine the minimum amount of DNA needed for production of amplicons that could be visualized by ethidium bromide staining of agarose gels.

RESULTS
Development of Unique Primers for *O. sinensis*
A total of 314 identified ITS sequences of *O. sinensis*, including 131 sequences generated in the previous study, were obtained with a length of ∼500 bp after alignment; 112 published ITS sequences of 41 different species in *Ophiocordyceps* and 250 published ITS sequences of 26 species in *Cordyceps* were downloaded from the GenBank database. The search for primers specific to the fungal species of interest yielded the primer pairs listed in Table 2. The specificity of the primers DCF4/DCR4 for *O. sinensis* is illustrated in Figures 1A,B. There are at least 3 mismatches between the primers and the corresponding sequences from non-*O. sinensis* species.

Amplification with the Species-Specific Primers DCF4/DCR4 and Universal Primers
The DNA in decoctions boiled for 60 or 90 min was amplified with the universal primer pair 5F/4R, as shown in Figure 2, it appears that the DNA extracted from the *O. sinensis* decoctions was possibly too fractured or otherwise degraded by boiling for 60 or 90 min to serve as template for amplification of the ITS sequence with the universal ITS primers. In contrast, PCR with the *O. sinensis*-specific primer pair yielded DNA that could be visualized after gel electrophoresis. As shown in Figure 3, primers DCF4/DCR4 were also used to amplify five common adulterants (*C. gunnii*, *C. cicadae*, *C. militaris*, *C. liangshanensis*, *O. nutans*), and no amplification product was seen with DNA obtained from them.

Amplification with Specific Primers for Five Common Adulterants
In order to judge whether the sample were adulterated, five specific primer pairs (CCF/CCR, CGF/CGR, CMF/CMR, ONF/ONR, and CLF/CLR) for common adulterants were designed respectively, according to the conserved motifs obtained by aligning the ITS sequences of the targeted species. As shown in Figure 4, lanes 3, 7, 11, 15, and 19, none of the primers amplified DNA from *O. sinensis*, but they generated PCR products isolated from their respective target organism and in mixtures of *O. sinensis* and target organism DNA. We artificially mixed the DNA of *O. sinensis* and its adulterants, visual PCR products were obtained with each primer pair for each targeted species, as shown in Figure 4. The results showed that the method is suitable for the identification of the mixture of *O. sinensis* and its adulterants.

Sensitivity of the PCR Method
To determine suitable concentration of DNA, the pure *O. sinensis* DNA was two-fold serially diluted to different ratio, from two to six times. Within the scope of dilution times from two to four, the amplification result of diluted DNA showed no obvious difference, as shown in Figure 5, ∼8 ng of purified *O. sinensis* DNA in a 25-µL reaction volume were necessary for a band of the PCR products from primers DCF4/DCR4 to be visible in an ethidium bromide-stained gel.

DISCUSSION
*O. sinensis* has a long history of use as a traditional medicine in China. Due to over exploitation in the past decades, *O. sinensis* has been listed as an endangered species. Naturally produced *O. sinensis* is worth more than gold and because of this high value, adulterants have emerged frequently in recent years, which leads to market instability and a decline in consumer confidence.

DNA-based identification has become important for the identification of medical plants (Ali et al., 2014) as this technique is convenient, generally accurate and usable by people without taxonomic knowledge. The ITS sequence has been recently selected as the official marker for fugal genetic identification by the Consortium for the Barcode of Life (Das and Deb, 2015). ITS sequences amplification was used to identify fungi from soils or water as an environmental DNA barcode (Bellemain et al., 2010). Dentinger et al. compared the suitability of cytochrome oxidase subunit I (CO1) gene and ITS sequences for mushrooms and fern allies identification and determined that ITS-based identification is superior (Dentinger et al., 2011). Our previous study focused on the identification of raw *O. sinensis* materials based on ITS sequences (Xiang et al., 2014); however, the suitability for use with processed TCMs was not determined. The current study demonstrated that it is possible to apply PCR-based methodology to determine the presence of *O. sinensis* DNA in TCMs. Therefore, in another previous study, we proposed a mini-barcoding technique using short barcodes with a relatively high identification specificity for TCM (Liu et al., 2016), demonstrating immediate relevance to both science,

**TABLE 2 | Primers of *O. sinensis* and its counterfeits used for PCR amplification.**

| Primer name | Species name | Direction | Primer Sequences (5′–3′) | Amplicon size |
|-------------|--------------|-----------|--------------------------|---------------|
| DCF4        | *O. sinensis* | Forward   | AGTTAACCACCTCCAAACC      | 146           |
| DCR4        | *O. sinensis* | Reverse   | TGCTTTGCTTCTGTAGTA       | 146           |
| CCF         | *C. cicadae*  | Forward   | TACACCTCACCACCTCTCC      | 209           |
| CCR         | *C. cicadae*  | Reverse   | GTGCGACAGAACCAAGAGAT     | 209           |
| CGF         | *C. gunnii*   | Forward   | TACCTACTGTGGCTTCGAG      | 203           |
| CGR         | *C. gunnii*   | Reverse   | GTGCGACAGAACCAAGAGAT     | 203           |
| CMF         | *C. militaris*| Forward   | TGAATACCTCTATGCTGCT      | 167           |
| CMR         | *C. militaris*| Reverse   | ATGCCGACAGCAAAGAGAT      | 167           |
| ONF         | *O. nutans*   | Forward   | AACCTCTCAATCTCTGAGT      | 205           |
| ONR         | *O. nutans*   | Reverse   | GCAAATCAGATTACCTATCG     | 205           |
| CLF         | *C. liangshanensis* | Forward | CAGCGCAAGGAGCATCAC     | 219           |
| CLR         | *C. liangshanensis* | Reverse | GATGCCAGAACCAAGAGA      | 219           |
industry and consumers. Further studies on mini-barcoding for the identification of TCM are necessary and beneficial. Although ITS sequences are commonly used to identify fungi, the requirement for relatively intact DNA to obtain complete ITS amplicons can make this approach difficult when DNA is extracted from processed samples whose DNA might have been degraded. We hypothesized that amplification of shorter regions of ITS might be possible with DNA from processed samples since Lo et al. were able to amplify a 88-bp fragment from TCM material after it had been boiled for 120 min (Lo et al., 2015). The present study also showed that a 146-bp fragment could be amplified from DNA extracted from processed *O. sinensis* samples, whereas amplification of the entire ITS region was not possible. For the first time, a specific primer pair is proposed and
is proved to be a very efficient tool for the identification of *O. sinensis* and its adulterants.

The specificity of this primer pair, allows authentication of *O. sinensis* materials by PCR and amplicon detection along without the need for sequencing. Therefore, analysis times and costs are reduced. The assays can potentially be further simplified and expedited by utilizing isothermal recombinase polymerase amplification and an amplicon detection method that does not involve gel electrophoresis (Del Río et al., 2014). The application of the *O. sinensis*-specific primer pair along with the five primer pairs targeting DNA from common adulterants should allow determining if a sample said to only contain *O. sinensis* actually also, or exclusively, contains adulterants added inadvertently or deliberately.

**CONCLUSION**

In this study, a species-specific primer pair that amplifies a 146-bp sequence unique to the ITS region of *O. sinensis* was established. Besides that, five specific primer pairs for common adulterated species were also established. The method developed in this study provides users with an easy authentication method and may make a major contribution to the detection of counterfeit products of *O. sinensis* in the markets. In conclusion, this method can greatly expand the molecular identification of DNA-degraded materials and result in the rapid authentication of *O. sinensis* and its common adulterants among all its congeners with high accuracy, specificity and low cost.

**AUTHOR CONTRIBUTIONS**

JH designed this study. JH and LX provided experimental data. YL analyzed the raw data and drafted the manuscript. All authors helped to finish the manuscript and approved the final manuscript.

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**REFERENCES**

Ali, M. A., Gyulai, G., Hidvégi, N., Kerti, B., Al Hemaid, F. M., Pandey, A. K., et al. (2014). The changing epitome of species identification - DNA barcoding. *Saudi J. Biol. Sci.* 21, 204–231. doi: 10.1016/j.sjbs.2014.03.003

Ashok Kumar, P., and Kailash Chandra, S. (2011). Traditional uses and medicinal potential of *Cordyceps sinensis* of Sikkim. *J. Ayurveda Integr. Med.* 2, 9–13. doi: 10.4103/0975-9476.78183

Bellemain, E., Carlisen, T., Brochmann, C., Coissac, E., Taberlet, P., and Kausberud, H. (2010). ITS as an environmental DNA barcode for fungi: an *in silico* approach reveals potential PCR biases. *BMC Microbiol.* 10:189. doi: 10.1186/1471-2180-10-189

Chen, Y. Q., Ning, W., Qu, L. H., Li, T. H., and Zhang, W. M. (2001). Determination of the anamorph of *Cordyceps sinensis* inferred from the analysis of the ribosomal DNA internal transcribed spacers and 5.8S rDNA. *Biochem. Syst. Ecol.* 29, 597–607. doi: 10.1016/S0305-1978(00)00100-9

Chen, Z. H., Dai, Y. D., Yu, H., Yang, K., Yang, Z. L., Yuan, F., et al. (2013). Systematic analyses of *Ophiocordyceps lanpingensis* sp. nov., a new species of Ophiocordyceps in China. *Microbiol. Res.* 168, 525–532. doi: 10.1016/j.micres.2013.02.010

Das, S., and Deb, B. (2015). DNA barcoding of fungi using Ribosomal ITS Marker for genetic diversity analysis: a review. *Int. J. Pure Appl. Biosci.* 3, 160–167. Available online at: http://www.ijpab.com/form/2015%20Volume%202015%20issue%203/IJPAB-2015-3-3-160-167.pdf

Del Río, J. S., Yehia, A. N., Acero-Sánchez, J. L., Henry, O. Y., and O’Sullivan, C. K. (2014). Electrochemical detection of *Francisella tularensis* genomic DNA using solid-phase recombinase polymerase amplification. *Biosens. Bioelectron.* 54, 674–678. doi: 10.1016/j.bios.2013.11.035

Dentinger, B. T., Didukh, M. Y., and Moncalvo, J. M. (2011). Comparing COI, and ITS as DNA barcode markers for mushrooms and allies (Agaricomycotina). *PLoS ONE* 6:e25081. doi: 10.1371/journal.pone.0025081

Francisella tularensis

Del Río, J. S., Yehia, A. N., Acero-Sánchez, J. L., Henry, O. Y., and O’Sullivan, C. K. (2014). Electrochemical detection of *Francisella tularensis* genomic DNA using solid-phase recombinase polymerase amplification. *Biosens. Bioelectron.* 54, 674–678. doi: 10.1016/j.bios.2013.11.035

Dentinger, B. T., Didukh, M. Y., and Moncalvo, J. M. (2011). Comparing COI, and ITS as DNA barcode markers for mushrooms and allies (Agaricomycotina). *PLoS ONE* 6:e25081. doi: 10.1371/journal.pone.0025081
Dong, C. H., and Yao, Y. J. (2010). On the reliability of fungal materials used in studies on Ophiocordyceps sinensis. J. Ind. Microbiol. 38, 1027–1035. doi: 10.1007/s12275-010-0187-4

Glantz, S. A. (2005). Primer of Biostatistics: Statistical Software Program Version 6.0. New York, NY: McGraw-Hill Medical.

Hu, H., Xiao, L., Zheng, B., Wei, X., Ellis, A., and Liu, Y. M. (2015). Identification of chemical markers in Cordyceps sinensis by HPLC-MS/MS. Anal. Bioanal. Chem. 407, 8059–8066. doi: 10.1007/s00216-015-8978-6

Hui-Chen Lo, C. H., Lin, F. -Y., and Hsu, T. –H. (2013). A systematic review of the mysterious caterpillar fungus Ophiocordyceps sinensis in Dong-ChongXiaCao (冬蟲夏草 Dong Chóng Xià Cáo) and related bioactive ingredients. J. Trad. Complement. Med. 3, 16–32. doi: 10.1016/S2225-4110(16)30164-X

Lam, K. Y., Chan, G. K., Xin, G. Z., Xu, H., Cui, F. F., Chen, J. P., et al. (2015). Authentication of Cordyceps sinensis by DNA analyses: comparison of ITS sequence analysis and RAPD-derived molecular markers. Molecules 20, 22454–22462. doi: 10.3390/molecules201219861

Lewis, P. O., Kumar, S., Tamura, K., Nei, M., and Lewis, P. O. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. doi: 10.1093/molbev/mst197

Li, X., Song, J., Xin, T., Zhu, Y., Shi, L., Xu, X., et al. (2013). DNA barcoding the commercial Chinese caterpillar fungus. FEMS Microbiol. Lett. 347, 156–162. doi: 10.1111/1574-6968.12233

Li, Y., Wang, L., Wang, X., Chen, X., Han, J., and Pang, X. (2016). A nucleotide signature for the identification of American ginseng and its products. Front. Plant Sci. 7:519. doi: 10.3389/fpls.2016.00519

Li, Z. Y., Liang, Z. Q., Liu, A. Y., Yao, Y. J., Hyde, K. D., and Yu, Z. N. (2002). Molecular evidence for teleomorph-anamorph connections in Cordyceps based on ITS-5.8S rDNA sequences. Mycol. Res. 106, 1100–1108. doi: 10.1017/S0953756202006378

Liu, Z. Y., Yao, Y. J., Liang, Z. Q., Liu, A. Y., Pegler, D. N., and Chase, M. W. (2001). Molecular evidence for the anamorph—teleomorph connection in Cordyceps based on ITS-5.8S rDNA sequences. Mycol. Res. 105, 827–832. doi: 10.1017/S095375620100377X

Lo, Y. T., Ming, L., and Shaw, P. C. (2015). Identification of constituent herbs in ginseng decoctions by DNA markers. Chin. Med. 10, 1–8. doi: 10.1186/s13102-015-0029-x

Meissner, C., Bruse, P., Mueller, E., and Oehmichen, M. (2007). A new sensitive short pentaplex (ShOp) PCR for typing of degraded DNA. Forensic Sci. Int. 166, 121–127. doi: 10.1016/j.forsciint.2006.04.014

Qin, S., Zhao, J., Liu, X., Hua, C., Shu, Y., and Yue, G. (2011). Current market state investigation and strategy of Cordyceps sinensis (Berk.) Sacc. Lishizhen Med. Mater. Med. Res. 22, 1236–1237.

Quan, Q. M., Wang, Q. X., Zhou, X. L., Li, S., Yang, X. L., Zhu, Y. G., et al. (2014). Comparative phylogenetic relationships and genetic structure of the caterpillar fungus Ophiocordyceps sinensis and its host insects inferred from multiple gene sequences. J. Microbiol. 52, 99–105. doi: 10.1007/s12275-014-3391-y

Shadi, S., Xin, Z., Janzen, D. H., Winnie, H., Jean-François, L., Jacobus, L. M., et al. (2011). Pyrosequencing for mini-barcoding of fresh and old museum specimens. PLoS ONE 6:e21252. doi: 10.1371/journal.pone.0021252

Shrestha, B., Zhang, W., Zhang, Y., and Liu, X. (2010). What is the Chinese caterpillar fungus Ophiocordyceps sinensis? (Ophiocordycipitaceae)? Mycol. Int. J. Fungal Biol. 1, 228–236. doi: 10.1080/21501203.2010.536791

Sirisidthi, K., Kosai, P., and Jiraungkoorskul, W. (2015). Anti-hyperglycemic activity of Ophiocordyceps sinensis: a review. Indian J. Agric. Res. 49, 400–406. doi: 10.11885/ijjare.v49i5.5801

Sung, G.-H., Hywel-Jones, N. L., Sung, J.-M., Luangs-ard, J. J., Shrestha, B., and Spatafora, J. W. (2007). Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. Stud. Mycol. 57, 5–59. doi: 10.3141/sim.2007.57.01

Wang, X. L., and Yao, Y. J. (2011). Host insect species of Ophiocordyceps sinensis: a review. Zookeys 127, 43–59. doi: 10.3897/zookeys.127.802

Xiang, L., Son, J., Xin, T., Zhu, Y., Shi, L., Xu, X., et al. (2014). “DNA barcoding the commercial chinese Caterpillar Fungus,” in The 14th National Conference on Traditional Chinese Medicine and Nature Medicine Paper Abstract (Beijing), 156–162.

Yi, L., Wang, X. L., Lei, J., Yi, J., Hui, L., Jiang, S. P., et al. (2011). A survey of the geographic distribution of Ophiocordyceps sinensis. J. Microbiol. 49, 913–919. doi: 10.1007/s12275-011-1193-z

Zhang, J., Wang, P., Wei, X., Li, L., Cheng, H., Wu, Y., et al. (2015). A metabolomics approach for authentication of Ophiocordyceps sinensis by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Food Res. Int. 76, 489–497. doi: 10.1016/j.foodres.2015.07.025

Zhang, Y., Xu, L., Shu, Z., Liu, X., An, Z., Mu, W., et al. (2009). Genetic diversity of Ophiocordyceps sinensis, a medicinal fungus endemic to the Tibetan Plateau: Implications for its evolution and conservation. BMC Evol. Biol. 9:290. doi: 10.1186/1471-2148-9-290

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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