



Ophiocordyceps highlandensis, a new entomopathogenic fungus from Yunnan, China

ZHU L. YANG^{1*}, JIAO QIN^{1,4}, CHENGFENG XIA², QUN HU³ & QING-QING LI³

¹ Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China

² State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China

³ Yunnan Haoruo Institute of Biotechnology, Kunming 650201, China

⁴ University of Chinese Academy of Sciences, Beijing 100049, China

*e-mail: fungi@mail.kib.ac.cn

Abstract

A new species of Ophiocordycipitaceae, *Ophiocordyceps highlandensis*, from southwestern China is described using morphological and molecular evidence. It is morphologically characterized by the combination of the following characters: dark-brown to blackish stromata on larvae of Scarabaeidae, fully immersed perithecia with non-protruding ostioles, 3-septate filiform ascospores breaking easily into four part-spores (20) $33\text{--}55 \times 1.5\text{--}2 \mu\text{m}$ and a hymeniform cortex layer of stipe. Molecular phylogenetic analyses using DNA nucleotide sequences of the nuclear ribosomal small subunit, and the genes encoding the largest subunit of RNA polymerase II and the second-largest subunit of RNA polymerase II indicated that *O. highlandensis* was related to *O. konnoana*, *O. barnesii*, *O. nigrella*, *O. ravenelii* and *O. superficialis*. *Ophiocordyceps highlandensis* and its related species were all characterized by dark-brown stromata and an affinity for melolonthid larval hosts. A description, line drawings, phylogenetic placement and comparison with allied taxa are presented.

Keywords: Ascomycetes·new taxon·taxonomy

Introduction

The genus *Ophiocordyceps* Petch (1931: 73) was proposed to accommodate the species of *Cordyceps* Fr. (1818: 316) that produce non-disarticulating ascospores. However, this genus was then treated as a subgenus of *Cordyceps* (Kobayasi 1941) or dispersed in multiple subgenera of *Cordyceps* (Mains 1958) in the last century. Sung *et al.* (2007a) resurrected this genus with an emendation based on molecular and morphological evidence. Presently, *Ophiocordyceps* harbors about 150 entomopathogenic fungi (Sung *et al.* 2007a; Kirk *et al.* 2008), a few of them, such as *O. sinensis* (Berk. 1843: 207) G.H. Sung *et al.* (2007a: 46) and *O. sobolifera* (Hill ex Watson 1763: 271) G.H. Sung *et al.* (2007a: 46), are renowned for their values in traditional Chinese medicine.

This genus is widely distributed in China, and has been received much attention in China in the past (Teng 1963; Zhang *et al.* 1989; Zang & Kinjo 1998; Liang *et al.* 2001, 2002, 2003; Sung *et al.* 2007a; Chen *et al.* 2011; Chen *et al.* 2013; Wen *et al.* 2013, 2014). During our study of the higher fungi in southwestern China, we have found a taxon of this genus, stromata of which were observed emerging above leaf litter with the host buried ca 20–40 mm below ground. A preliminary morphological comparison indicated that our collections of the fungus are close to *O. barnesii* (Thwaites 1875: 110) G.H. Sung *et al.* (2007a: 40) and *O. nigrella* (Kobayasi & Shimizu 1983b: 145) G.H. Sung *et al.* (2007a: 45) in the Chinese literature (Teng 1963; Tai 1979; Bi *et al.* 1994; Zang & Kinjo 1998; Chen & Chen 2007; Liang *et al.* 2007, 2009; Chen *et al.* 2010; Wu *et al.* 2013). Further detailed morphological study indicated that our collections differ from *O. barnesii* and *O. nigrella* significantly (Massee 1895; Petch 1924; Kobayasi & Shimizu 1983a, b; Luangsa-Ard *et al.* 2010).

In this study, we used morphological data together with DNA nucleotide sequence analysis of the nuclear small subunit ribosomal DNA (nrSSU), and the genes encoding the largest subunit of RNA polymerase II (*rpb1*) and the second-largest subunit of RNA polymerase II (*rpb2*) to assess the phylogenetic position of the fungus, because both

Recently, a new species, *Cordyceps neosuperficialis* T.H. Li *et al.* (2008: 366), was described from southern China. Morphologically, it differs from *O. highlandensis* by its filiform stromata with superficial perithecia, much narrower multiseptate ascospores (140–180 × 0.8–1.1 μm) breaking up into 4.5–7.5 μm long part-spores (Li *et al.* 2008). Growing also on larvae of Scarabaeidae, *Cordyceps obliquiordinata* Kobayasi & Shimizu (1982: 114) is somewhat similar to *O. highlandensis*. However, the latter species has oblique perithecia and significantly shorter and narrower part-spores 7–8 × 1 μm (Kobayasi & Shimizu 1982; Shimizu 1994).

Acknowledgements

The authors are very grateful to Prof. Fu-Rong Gui (Yunnan Agricultural University, Kunming) and Dr. Cheng-Ye Wang (Institute of Resource Insects of the Chinese Academy of Forestry, Kunming) for the identification of the host of the fungus. Special thanks are due to the anonymous reviewers for constructive comments and suggestions. This study was supported by the National Basic Research Program of China (973 Program, No. 2014CB138305), and Yunnan High-End Technology Professionals Introduction Program (2010CI117).

References

- Bi, Z.S., Zheng, G.Y. & Li, T.H. (1994) *Macrofungus Flora of Guangdong Province*. Guangdong Science and Technology Press, Guangzhou, 879 pp. [in Chinese]
- Castlebury, L.A., Rossman, A.Y., Sung, G.-H., Hyten, A.S. & Spatafora, J.W. (2004) Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycological Research* 108 (8): 864–872.
- Chang, H. & Zhang, P. (2003) Comparison and analysis of the cordycepin in *Cordyceps barnesii* Thwaites and *Cordyceps sinensis*. *Journal of Shanxi Agricultural University* 23 (4): 345–347.
- Chen, J.Y., Cao, Y.Q., Yang, D.R. & Li, M.H. (2011) A new species of *Ophiocordyceps* (Clavicipitales, Ascomycota) from southwestern China. *Mycotaxon* 115 (1): 1–4.
- Chen, Y.J. & Chen, M.H. (2007) Research on the artificial culture of *Cordyceps nigrella* sporophore. *Edible Fungi of China* 26 (1): 21–23. [in Chinese]
- Chen, Z.H., Dai, Y.D., Yu, H., Yang, K., Yang, Z.L., Yuan, F. & Zeng, W.B. (2013) Systematic analyses of *Ophiocordyceps lanpingensis* sp. nov., a new species of *Ophiocordyceps* in China. *Microbiology Research* 168 (8): 525–532.
- Chen, Z.H., Yu, H., Zeng, W.B., Yang, J.Y., Yuan, J. & Chen, Y.J. (2010) Solid fermentation technique for cordycepin production by *Cordyceps nigrella* strain enig-56. *Acta Edulis Fungi* 17 (1): 84–86.
- Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19: 11–15.
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids symposium series* 41: 95–98.
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth & Bisby's dictionary of the fungi*, 10th edn. CABI International, Wallingford. 771 pp.
- Kobayasi, Y. (1941) The genus *Cordyceps* and its allies. *Science Reports of the Tokyo Bunrika Daigaku* (Section B, no. 84) 5: 53–260.
- Kobayasi, Y. & Shimizu, D. (1980) *Cordyceps* species from Japan 2. *Bulletin of the National Science Museum. Series B Botany (Journal)* 6 (3): 77–96.
- Kobayasi, Y. & Shimizu, D. (1982) *Cordyceps* species from Japan 5. *Bulletin of the National Science Museum. Series B Botany (Journal)* 8 (4): 111–123.
- Kobayasi, Y. & Shimizu, D. (1983a) *Cordyceps* species from Japan 6. *Bulletin of the National Science Museum. Series B Botany (Journal)* 9 (1): 1–21.
- Kobayasi, Y. & Shimizu, D. (1983b) *Iconography of vegetable wasps and plant worms*. Hoikusha Publishing Co Ltd, Osaka, 280 pp. [in Japanese]
- Li, T.H., Deng, C.Y. & Song, B. (2008) A distinct species of *Cordyceps* on coleopterous larvae hidden in twigs. *Mycotaxon* 103: 365–369.
- Liang, Z.Q., Liu, A.Y. & Huang, J.Z. (2002) Some entomogenous fungi from Wuyishan and Zhangjiajie Natural Reserves. *Cordyceps spp. Mycosystema* 21 (2): 162–166.

- Liang, Z.Q., Liu, A.Y. & Jiang, Y.C. (2001) Two new species of *Cordyceps* from Jinggang Mountains. *Mycosystema* 20 (3): 306–309.
- Liang, Z.Q., Liu, A.Y. & Liu, Z.Y. (2007) *Cordyceps*. In: *Flora Fungorum Sinicorum*, vol. 32. Science Press, Beijing, 190 pp. [in Chinese]
- Liang, Z.Q., Liu, Z.Y., Han, Y.F. & Liu, A.Y. (2009) Atlas of *Cordyceps* in China. Guizhou Science and Technology Press, Guiyang, 124 pp. [in Chinese]
- Liang, Z.Q., Wang, B. & Kang, J.C. (2003) Several rare entopathogenic fungi from Western Sichuan mountains. *Fungal Diversity* 12: 129–134.
- Luangsa-ard, J.J., Ridkaew, R., Mongkolsamrit, S., Tسانathai, K. & Hywel-Jones, N.L. (2010) *Ophiocordyceps barnesii* and its relationship to other melolonthid pathogens with dark stromata. *Fungal Biology* 114 (9): 739–745.
- Mains, E.B. (1940) Species of *Cordyceps*. *Mycologia* 32: 310–320.
- Mains, E.B. (1941) *Cordyceps stylophora* and *Cordyceps ravenelii*. *Mycologia* 33: 611–617.
- Mains, E.B. (1958) North American entomogenous species of *Cordyceps*. *Mycologia* 50: 169–222.
- Massee, G. (1895) A revision of the genus *Cordyceps*. *Annals of Botany* 9: 1–44.
- Nylander, J. (2004) MrModeltest v2.3. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.
- Petch, T. (1924) Studies in entomogenous fungi IV. Some Ceylon *Cordyceps*. *Transactions of the British Mycological Society* 10: 28–45.
- Petch, T. (1931) Notes on entomogenous fungi. *Transactions of the British Mycological Society* 16: 55–75.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (12): 1572–1574.
- Shimizu, D. (1994) *Color iconography of vegetable wasps and plant worms*. Seibundo Shinkosha, Tokyo, 380 pp.
- Smith, S.A. & Dunn, C.W. (2008) Phyutility: a phyloinformatics tool for trees, alignments and molecular data. *Bioinformatics* 24 (5): 715–716.
- Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22 (21): 2688–2690.
- Sung, G.-H., Hywel-Jones, N.L., Sung, J.-M., Luangsa-ard, J.J., Shrestha, B. & Spatafora, J.W. (2007a) Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology* 57: 5–66.
- Sung, G.-H., Sung, J.-M., Hywel-Jones, N.L. & Spatafora, J.W. (2007b) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* 44 (3): 1204–1223.
- Swofford, D.L. (2002) *PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0b10*. Sinauer Associates, Sunderland.
- Tai, F.L. (1979) *Sylloge fungorum sinicorum*. Science Press, Beijing, 1527 pp. [in Chinese]
- Teng, S.C. (1963) *Fungi of China*. Science Press, Beijing, 586 pp. [in Chinese]
- Wen, T.C., Xiao, Y.P., Li, W.J., Kang, J.C. & Hyde, K.D. (2014) Systematic analyses of *Ophiocordyceps ramosissimum* sp. nov., a new species from a larvae of Hepialidae in China. *Phytotaxa* 161 (3): 227–234.
<http://dx.doi.org/10.11646/phytotaxa.161.3.6>
- Wen, T.C., Zhu, R.C., Kang, J.C., Huang, M.H., Tan, D.B., Ariyawansa, H., Hyde, K.D. & Liu, H. (2013) *Ophiocordyceps xuefengensis* sp. nov. from larvae of *Phassus nodus* (Hepialidae) in Hunan Province, southern China. *Phytotaxa* 123 (1): 41–50.
<http://dx.doi.org/10.11646/phytotaxa.123.1.2>
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols, a guide to methods and applications*. Academic Press, San Diego, 315–322 pp.
- Wu, X.L., Mao, X.L., Tolgor, B., Song, B., Li, T.H., Zhao, Y.X., Chen, S.L., Zeng, N.K., Huang, S.Z., Wen, T.C. & Deng, C.Y. (2013) *Medicinal Fungi of China*. Science Press, Beijing, 925 pp. [in Chinese]
- Yang, Z.L. (2011) Molecular techniques revolutionize knowledge of basidiomycete evolution. *Fungal Diversity* 50: 47–58.
- Zang, M. & Kinjo, N. (1998) Notes on the alpine *Cordyceps* of China and nearby nations. *Mycotaxon* 66: 215–229.
- Zhang, K.Y., Wang, C.J. & Yan, M.S. (1989) A new species of *Cordyceps* from Gansu, China. *Transaction Mycology Society Japan* 30: 295–299.