Phytotaxa 524 (2): 085–091
https://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.524.2.2

Paracremonium lepidopterorum, a new insect-associated fungus

DA-QIONG MING[1,4], LING-YAN LUO[1,5], XIAO-XIA HE[1,6], MING-SHU WANG[1,7], WEN-XING FANG[1,8], SHU-FENG CHEN[1,9], WAN-HAO CHEN[2,10]*, YAN-FENG HAN[3,11]* & ZONG-QI LIANG[3,12]

[1] *School of Pharmacy, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou 550025, China.*
[2] *Basic Medical School, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou 550025, China.*
[3] *Institute of Fungus Resources, Guizhou University, Guiyang, Guizhou 550025, China.*
[4] *2780370259@qq.com; https://orcid.org/0000-0001-6648-9783*
[5] *1406902981@qq.com; https://orcid.org/0000-0001-6851-6423*
[6] *2646449466@qq.com; https://orcid.org/0000-0002-7678-5188*
[7] *1789711668@qq.com; https://orcid.org/0000-0001-9652-392X*
[8] *3138412616@qq.com; https://orcid.org/0000-0002-8758-8702*
[9] *1834498400@qq.com; https://orcid.org/0000-0002-4217-4485*
[10] *cwhisaria@163.com; https://orcid.org/0000-0001-7240-6841*
[11] *swallow1128@126.com; https://orcid.org/0000-0002-8646-3975*
[12] *zqliang472@126.com; https://orcid.org/0000-0003-2867-2231*
**Corresponding author*

Abstract

During a survey of entomopathogenetic fungi from Southwest China, a new species, *Paracremonium lepidopterorum* was isolated from a pupa. It differs from other *Paracremonium* species by its shorter cylindrical phialides and absence of slimy head of conidia and chlamydospores. Phylogenetic analyses based on the combined datasets of *ITS+LSU+TUB* confirmed that *P. lepidopterorum* is distinct from other species. The new species is formally described and illustrated, and compared to similar species.

Keywords: 1 new species, combination, morphology, phylogeny, pupa

Introduction

The genus *Paracremonium* was established with the type species *P. inflatum* L. Lombard & Crous and *P. contagium* L. Lombard & Crous (Lombard *et al*. 2015). The typical characteristics of *Paracremonium* were reported to be the formation of sterile coils from which conidiophores radiate, and inconspicuously swollen septa in the hyphae. However, Lynch *et al*. (2016) reported a new insect-associated species *P. pembeum* S.C. Lynch & Eskalen, which lacked the sterile coils and swollen septa. An additional five species have been described, *P. binnewijzendii* Houbraken, van der Kleij & L. Lombard, *P. variiforme* Z.F. Zhang, F. Liu & L. Cai, *P. moubasheri* Al-Bedak & M.A. Ismail, *P. apiculatum* Z.F. Zhang & L. Cai and *P. ellipsoideum* Z.F. Zhang & L. Cai (Crous *et al*. 2017, Zhang *et al*. 2017, 2020, Al-Bedak *et al*. 2019).

Paracremonium species are widely distributed and have diverse habitats. *Paracremonium inflatum* and *P. contagium* were associated with human infections (Lombard *et al*. 2015). *Paracremonium pembeum* was isolated from the ambrosia beetle *Euwallacea* sp. as a fungal symbiont (Lynch *et al*. 2016). *Paracremonium apiculatum*, *P. binnewijzendii* and *P. moubasheri* were isolated from the soil of Sanjiao Cave, the soil of a stream embankment and an alkaline mud sample, respectively (Zhang *et al*. 2020, Crous *et al*. 2017, Al-Bedak *et al*. 2019). *Paracremonium ellipsoideum* and *P. variiforme* were isolated from sewage within Sanjiao Cave and from water in a karst cave, respectively (Zhang *et al*. 2017, 2020).

During a survey of entomopathogenetic fungi from Southwest China, a new *Paracremonium* species was found. It is described and illustrated as *P. lepidopterorum*.

Accepted by Eric McKenzie: 24 Oct. 2021; published: 3 Nov. 2021

Materials and methods

Specimen collection and identification

The fungus-infected insect specimens were collected from Duyun County (26°21′27.96″N, 107°22′48.22″E), Guizhou Province, on October 1, 2019. Isolation of the fungi was done as described by Chen *et al*. (2019). The surface of the specimens was rinsed with sterile water, followed by surface sterilization with 75% ethanol for 3–5 s. A part of the insect body was cut off and inoculated with haemocoel on potato dextrose agar (PDA) and on PDA to which 1% w/v peptone (PDAP) had been added. Fungal colonies emerging from specimens were isolated and cultured at 22 °C for 14 d under 12 h light/12 h dark conditions following protocols described by Zou *et al*. (2010). Accordingly, strains DY10351 and DY10352 were obtained. The specimens and the isolated strains were deposited in the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code, GZAC), Guiyang City, Guizhou, China.

Macroscopic and microscopic morphological characteristics of the fungi were examined and growth rates determined from PDA cultures incubated at 25 °C for 14 d. Hyphae and conidiogenous structures were mounted in lactophenol cotton blue or 20% lactate solution and observed with an optical microscope (OM, DM4 B, Leica, Germany).

DNA extraction, PCR amplification and nucleotide sequencing

DNA extraction was carried out in accordance with Liang *et al*. (2011). The extracted DNA was stored at −20 °C. The β-tubulin gene (*TUB*) was amplified using Bt2a/Bt2b primers according to Glass & Donaldson (1995). The internal transcribed spacer (*ITS*) region and large subunit ribosomal RNA (*LSU*) genes were amplified by PCR using ITS4/ ITS5 and LROR/ LR5, which was described by White *et al*. (1990). PCR products were purified using the UNIQ-10 column PCR products purification kit (no. SK1141; Sangon Biotech (Shanghai) Co., Shanghai, China) in accordance with the manufacturer's protocol and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

Sequence alignment and phylogenetic analyses

The DNA sequences generated in this study were assembled and edited using Lasergene software (version 6.0, DNASTAR). Generated *ITS*, *LSU* and *TUB* sequences were aligned with those published by Lombard *et al*. (2015), Lynch *et al*. (2016), Crous *et al*. (2017), Zhang *et al*. (2017), Al-Bedak *et al*. (2019), Zhang *et al*. (2020) and others selected on the basis of BLAST algorithm-based searches in GenBank. *Nalanthamala vermoesenii* (Biourge) Schroers (isolates CBS 110893 and CBS 230.48) was chosen as outgroup taxon. Multiple datasets of *ITS*, *LSU* and *TUB* were aligned using MAFFT v7.037b (Katoh & Standley 2013) and alignments were edited with MEGA6 (Tamura *et al*. 2013). Sequences were concatenated with SequenceMatrix v.1.7.8 (Vaidya *et al*. 2011). Partition homogeneity test in PAUP4.0b10 (Swofford 2002) was done by using the command 'hompart'.

Maximum likelihood (ML) analyses were constructed with RAxMLGUI (Silvestro & Michalak 2012). The GTRGAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites. For Bayesian inference (BI), a Markov chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v.3.2 (Ronquist *et al*. 2012) for the combined sequence datasets. The selection of the best-fit nucleotide substitution model for each locus was calculated by the Akaike Information Criterion (AIC) with jModelTest 2 (Darriba *et al*. 2012). The GTR+I+G model was selected for the concatenated *ITS+LSU+TUB* sequences. The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4,000 trees, representing the burn-in phase of the analyses were discarded, while the remaining 16,001 trees were used for calculating posterior probabilities in the majority rule consensus tree. After the analysis was finished, each run was examined using the program Tracer v1.5 (Drummond & Rambaut 2007) to determine burn-in and confirm that both runs had converged. The final alignment is available from TreeBASE under submission ID: 26945 (http://www.treebase.org).

Results

Phylogenetic analyses

The phylogenetic tree was generated from the ML and BI analysis on the *ITS+LSU +TUB* datasets. Statistical support (≥ 50%/0.5) is shown at the nodes for ML bootstrap support/BI posterior probabilities (Fig. 1). The strain numbers are noted after the name of each species. The concatenated sequences included 12 taxa, and consisted of 1,376 characters (*ITS*: 502, *LSU*: 672 and *TUB*: 202) with gaps.

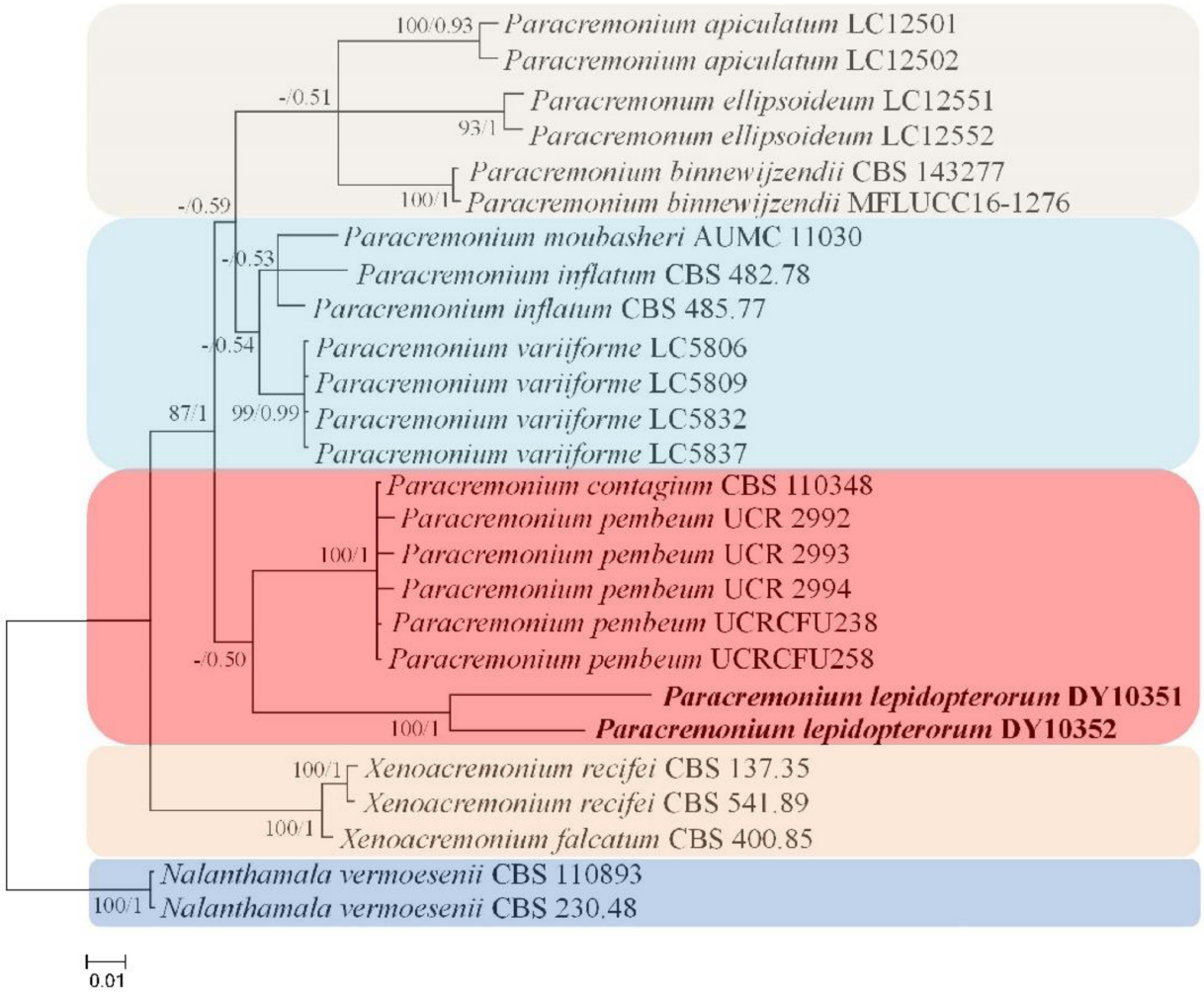

FIGURE 1. Phylogenetic analysis of *Paracremonium lepidopterorum* and related species based on combined partial *ITS+LSU+TUB* sequences. Statistical support values (≥50%) are shown at nodes, and presented as bootstrap values/Bayesian posterior probabilities.

The RAxML analysis of the combined dataset (*ITS+LSU+TUB*) yielded the highest scoring tree (Fig. 1) with a final ML optimization likelihood value of –5,087.254791. Parameters for the GTR model of the concatenated data set was as follows: estimated base frequencies; A = 0.224525, C = 0.271212, G = 0.277793, T = 0.226470; substitution rates AC = 0.845458, AG = 1.562294, AT = 1.081924, CG = 0.806079, CT = 3.892737, GT = 1.000000; gamma distribution shape parameter α = 0.334304. In the phylogenetic tree (Fig. 1), *P. lepidopterorum* clustered with *P. contagium* and *P. pembeum* in a subclade and formed an independent branch.

Paracremonium lepidopterorum D.Q. Ming, W.H. Chen, Y.F. Han & Z.Q. Liang *sp. nov.* (Fig. 2) Mycobank No.: MB 837492

Type:—CHINA. Guizhou Province: Duyun City, Doupeng Mountain (26°21′27.96″N, 107°22′48.22″E), on a pupa, 1 October 2019, Wanhao Chen, holotype GZAC DY1035; ex-type culture GZAC DY10351. Sequences from the strain DY10351 have been deposited in GenBank with accession numbers: *ITS*=MW000352, *LSU*=MW000470, *TUB*=MW015086.

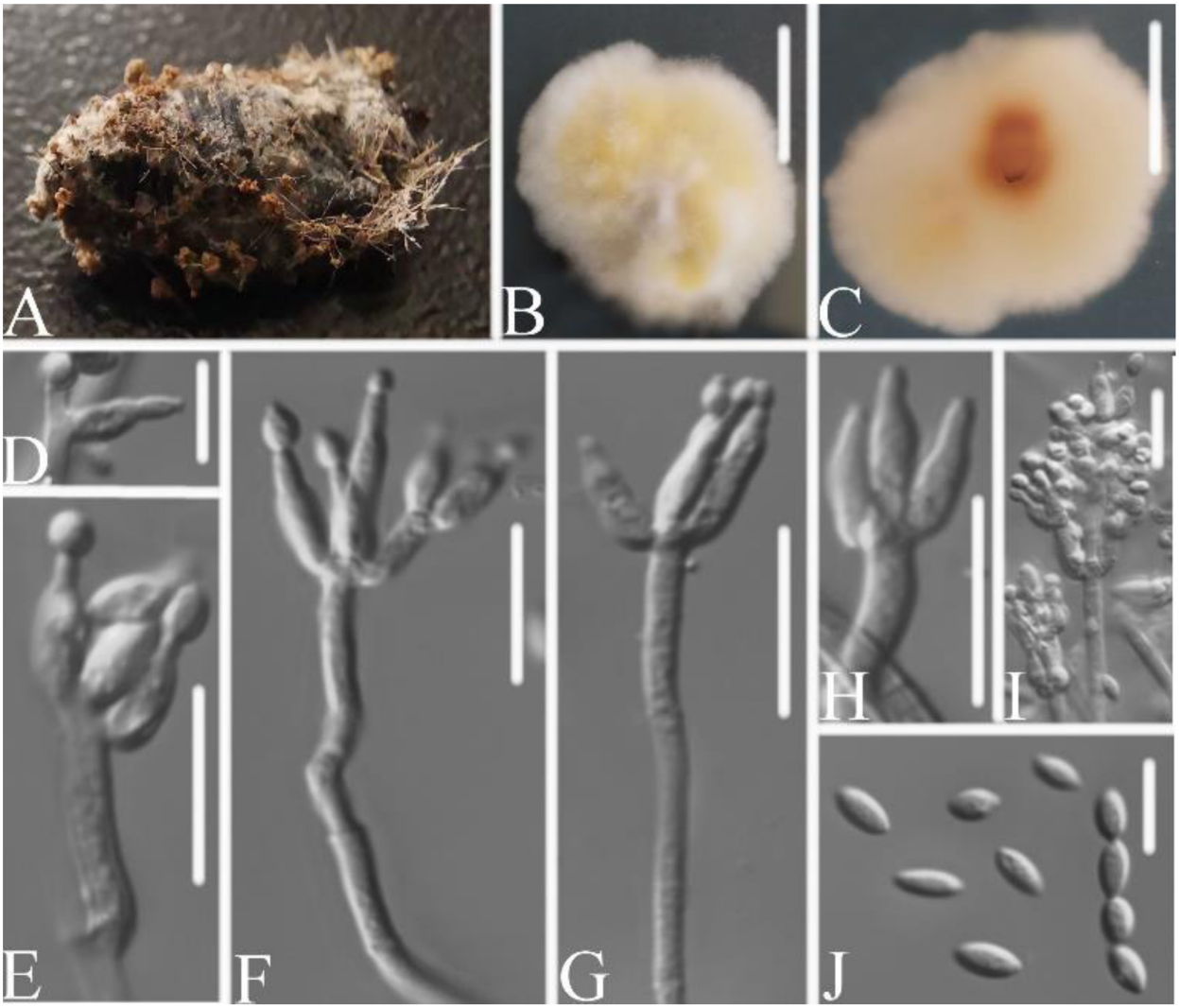

FIGURE 2. *Paracremonium lepidopterorum*. A. Infected pupa. B–C. Culture on PDA, showing the top (B) and the underside (C). D–I. Phialides and conidia formed on PDA. J. Conidia formed on PDA. Scale bars: B, C = 10 mm, D–J = 10 μm.

Colonies on PDA, attaining a diameter of 23–25 mm after 14 days at 25 °C, white to yellowish, thin, reverse yellowish. *Hyphae* septate, hyaline, smooth-walled, 1.4–1.5 μm wide. *Conidiophores* mononematous, hyaline, smooth-walled, with whorls of 2–6 phialides, or penicillium-like from hyphae directly. *Phialides* 5.4–8.5 × 2.8–3.3 μm, consisting of a cylindrical, somewhat inflated base, tapering to a thin neck. *Conidia* hyaline, smooth-walled, mostly fusiform, 4.4–5.9 × 2.6–3.0 μm, forming divergent and basipetal chains. Chlamydospores not observed. In culture both phialides and conidia are of similar general shape and size to those found on the pupa.

Etymology:—referring to the fungus colonizing pupa in the order Lepidoptera.

Additional materials examined:—CHINA. Guizhou Province: Qiannan Buyi and Miao Autonomous Prefecture, Duyun City, Doupeng mountain (26°21′27.96″N, 107°22′48.22″E), on a pupa, 1 October 2019, Wanhao Chen (DY10352), Sequences from this strain have been deposited in GenBank with accession numbers: *ITS*=MW000462, *LSU*=MW000667, *TUB*=MW015087.

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

Discussion

Phylogenetic analyses based on the combined dataset of *ITS*+*LSU*+*TUB* suggests that strains DY10351 and DY10352 belong to the genus *Paracremonium*. When compared with the typical characteristics of *Paracremonium* species (Table 1), strain DY10351 was similar to *P. apiculatum* and *P. variiforme* in that all three species lack chlamydospores

and a slimy head of conidia. Strain DY10351 can be distinguished from *P. apiculatum* and *P. variiforme* by having cylindrical phialides and fusiform conidia. Thus, strain DY10351 is described here as a new species, *Paracremonium lepidopterorum*.

Phylogenetic analyses of *Paracremonium* have been previously based on *acl1*, *act*, *cmdA*, *his3*, *ITS*, *LSU*, *RPB1*, *RPB2*, *TEF* and *TUB* genes (Lombard *et al*. 2015). Some of these sites were applied in the taxonomy of *Paracremonium* (Lynch *et al*. 2016, Crous *et al*. 2017, Zhang *et al*. 2017, 2020, Al-Bedak *et al*. 2019). The loci *ITS*, *LSU* and *TUB* were applied in the present study. Concatenated analyses of (*ITS*+*LSU*+*TUB*) produced ML and Bayesian trees that were largely congruent. The majority of branches were strongly supported in both analyses. The two strains of *P. lepidopterorum* clustered together, and have a close relationship with *P. contagium* and *P. pembeum*. However, *P. lepidopterorum* can be distinguished from these two species by having shorter cylindrical phialides and absence of a slimy head of conidia. Therefore, molecular phylogenetic results supported the morphologically base d conclusion that *P. lepidopterorum* is a new species.

TABLE 1. Morphological comparison of all *Paracremonium* species.

Species	Morphological characteristics				References
	Conidiogenous cells/phialides (μm)	Conidia heads	Conidia (μm)	Chlamydospores	
Paracremonium apiculatum	Acicular, 14–24 × 1.5–3.0	Absent	Subglobose to globose, 3.5–5.0 wide	Absent	Zhang *et al*. 2020
P. binnewijzendii	Subcylindrical, (17.5–)30–55(–65) × 1.5–3	Present	Ellipsoidal to fusoid, occasionally curved (6–)7–11(–13) × (1.5–)2.5–3.5(–4.5)	Absent	Crous *et al*. 2017
P. contagium	Elongate ampulliform, 25–50 × 2–3	Present	Ellipsoidal to fusiform, slightly to strongly curved, 4–6(–7) × 2–3	Absent	Lombard *et al*. 2015
P. ellipsoideum	Acicular, 22–38 × 2.5–3.5	Present	Ellipsoidal, 5.5–8.0 × 3.5–5.0	Absent	Zhang *et al*. 2020
P. inflatum	Elongate ampulliform, 20–85 × 2–4	Present	Ellipsoidal to fusiform, slightly to strongly curved, 5–6 × 1–2	Absent	Lombard *et al*. 2015
P. lepidopterorum	Cylindrical, 5.4–8.5 × 2.8–3.3	Absent	Fusiform, 4.4–5.9 × 2.6–3.0	Absent	This study
P. moubasheri	Elongate-ampulliform, 1–1.5 wide	Present	Fusiform, (7–)10–12(–16) × 2–3	Present	Al-Bedak *et al*. 2019
P. pembeum	(14.0–)31.0–41.5 long	Present	Globose to ellipsoidal, (3.0–)4.5–7.0 × (0.5–)1.0–2.5	Present	Lynch *et al*. 2016
P. variiforme	Elongate-ampulliform, 18–41 × 2–3.5	Absent	Clavate, ovoid or elliptical, 9–14.5 × 4–6	Absent	Zhang *et al*. 2017

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 31860002), High-level Innovative Talents Training Object in Guizhou Province (No. Qiankehepingtairencai [2020]6005), Science and Technology Foundation of Guizhou Province (No. Qiankehejichu [2020]1Y060) and Engineering Research Center of General Higher Education in Guizhou Province (Qianjiaohe(2015)337).

References

Al-Bedak, O.A., Ismail, M.A. & Mohamed, R.A. (2019) *Paracremonium moubasheri*, a new species from an alkaline sediment of Lake Hamra in Wadi-El-Natron, Egypt with a key to the accepted species. *Studies in Fungi* 4 (1): 216–222. https://doi.org/10.5943/sif/4/1/23

Chen, W.H., Liu, C., Han, Y.F., Liang, J.D., Tian, W.Y. & Liang, Z.Q. (2019) Three novel insect-associated species of *Simplicillium* (Cordycipitaceae, Hypocreales) from Southwest China. *MycoKeys* 58: 83. https://doi.org/10.3897/mycokeys.58.37176

Crous, P.W., Wingfield, M.J., Burgess, T.I., Carnegie, A.J., Hardy, G.S.J., Smith, D., Summerell, B.A., Cano-Lira, J.F., Guarro, J., Houbraken, J., Lombard, L., Martín, M.P., Sandoval-Denis, M., Alexandrova, A.V., Barnes, C.W., Baseia, I.G., Bezerra, J.D.P., Guarnaccia, V., May, T.W., Hernández-Restrepo, M., Stchigel, A.M., Miller, A.N., Ordoñez, M.E., Abreu, V.P., Accioly, T., Agnello, C., Agustin Colmán, A., Albuquerque, C.C., Alfredo, D.S., Alvarado, P., Araújo-Magalhães, G.R., Arauzo, S., Atkinson, T., Barili, A., Barreto, R.W., Bezerra, J.L., Cabral, T.S., Rodríguez, F.C., Cruz, R.H.S.F., Daniëls, P.P., da Silva, B.D.B., de Almeida, D.A.C., de Carvalho Júnior, A.A., Decock, C.A., Delgat, L., Denman, S., Dimitrov, R.A., Edwards, J., Fedosova, A.G., Ferreira, R.J., Firmino, A.L., Flores, J.A., García, D., Gené, J., Giraldo, A., Góis, J.S., Gomes, A.A.M., Gonçalves, C.M., Gouliamova, D.E., Groenewald, M., Guéorguiev, B.V., Guevara-Suarez, M., Gusmão, L.F.P., Hosaka, K., Hubka, V., Huhndorf, S.M., Jadan, M., Jurjević, Ž., Kraak, B., Kučera, V., Kumar, T.K.A., Kušan, I., Lacerda, S.R., Lamlertthon, S., Lisboa, W.S., Loizides, M., Luangsa-ard, J.J., Lysková, P., Mac Cormack, W.P., Macedo, D.M., Machado, A.R., Malysheva, E.F., Marinho, P., Matočec, N., Meijer, M., Mešić, A., Mongkolsamrit, S., Moreira, K.A., Morozova, O.V., Nair, K.U., Nakamura, N., Noisripoom, W., Olariaga, I., Oliveira, R.J.V., Paiva, L.M., Pawar, P., Pereira, O.L., Peterson, S.W., Prieto, M., Rodríguez-Andrade, E., Rojo De Blas, C., Roy, M., Santos, E.S., Sharma, R., Silva, G.A., Souza-Motta, C.M., Takeuchi-Kaneko, Y., Tanaka, C., Thakur, A., Smith, M.T., Tkalčec, Z., Valenzuela-Lopez, N., van der Kleij, P., Verbeken, A., Viana, M.G., Wang, X.W., Groenewald, J.Z. & Lombard, L. (2017) Fungal Planet description sheets: 625–715. *Persoonia* 39: 270. https://doi.org/10.3767/persoonia.2017.39.11

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

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

Glass, N.L. & Donaldson, G.C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61 (4): 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

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

Liang, J.D., Han, Y.F., Zhang, J.W., Du, W., Liang, Z.Q. & Li, Z.Z. (2011) Optimal culture conditions for keratinase production by a novel thermophilic *Myceliophthora thermophila* strain GZUIFR-H49-1. *Journal of Applied Microbiology* 110: 871–880. https://doi.org/10.1111/j.1365-2672.2011.04949.x

Lombard, L., Van der Merwe, N.A., Groenewald, J.Z. & Crous, P.W. (2015) Generic concepts in Nectriaceae. *Studies in Mycology* 80: 189–245. https://doi.org/10.1016/j.simyco.2014.12.002

Lynch, S.C., Twizeyimana, M., Mayorquin, J.S., Wang, D.H., Na, F., Kayim, M., Kasson, M.T., Thu, P.Q., Bateman, C., Rugman-Jones, P., Hulcr, J., Stouthamer, R. & Hulcr, J. (2016) Identification, pathogenicity and abundance of *Paracremonium pembeum sp. nov.* and *Graphium euwallaceae sp. nov.*—two newly discovered mycangial associates of the polyphagous shot hole borer (*Euwallacea* sp.) in California. *Mycologia* 108 (2): 313–329. https://doi.org/10.3852/15-063

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

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

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

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

Vaidya, G., Lohman, D.J. & Meier, R. (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27 (2): 171–180.
https://doi.org/10.1093/molbev/mst197

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, New York. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang, Z.F., Liu, F., Zhou, X., Liu, X.Z., Liu, S.J. & Cai, L. (2017) Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. *Persoonia* 39: 1–31.
https://doi.org/10.3767/persoonia.2017.39.01

Zhang, Z.F., Zhou, S.Y., Eurwilaichitr, L., Ingsriswang, S., Raza, M., Chen, Q., Zhao, P., Liu, F. & Cai, L. (2020) Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. *Fungal Diversity* 106: 29–136.
https://doi.org/10.1007/s13225-020-00453-7

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