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Four new filamentous fungal species from newly-collected and hive-stored bee pollen

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Abstract

Hive-stored pollen, as one of the major nutrition sources for honeybees, is the mixture of fresh bee pollen with honey, plant resins and wax. In this study, four new species (i.e. *Arthrinium locuta-pollinis*, *Chrysosporium alvearium*, *Nigrograna locuta-pollinis* and *Trichoderma pollinicola*) were identified, when we explored the culturable fungi in newly-collected bee pollen and hive-stored pollen produced by Italian honey bees (*Apis mellifera ligustica*) in the flowering season of rape (*Brassica campestris*). The four new species were described on the basis of morphological comparisons and multi-locus phylogenetic analyses, and their relationships with morphologically similar and phylogenetically closely related taxa are discussed.

Key words – *Arthrinium* – *Chrysosporium* – Morphology – *Nigrograna* – Phylogenetic analyses – *Trichoderma*

Introduction

Bee pollen, one of the products obtained from the hive due to the activity of honeybees, is abundant in organic molecules, with demonstrated antifungal, antibacterial, antiviral, anti-inflammatory, immune stimulating and analgesic activities (Kroyer & Hegedus 2001, Almaraz-Abarca et al. 2004). Newly-collected bee pollen is not always served as the direct food source for honey bees. Bee pollen is sometimes stored in comb cells of hives for a few days, mixing with honey, plant resins and wax, and then consumed by honey bees (Brovarskyi et al. 2017, Kieliszek et al. 2018). During this process, bacteria and yeasts have been considered playing an important role on lactic acid fermentation (Foote 1957, Haydak 1958, Gilliam 1979). However, filamentous fungi thus far received rare attention in apicultural research even they are widely known for their ability to degrade and synthesize numerous compounds (Gilliam et al. 1989).

Up to now, only a few fungal taxa have been reported from newly-collected bee pollen and hive-stored pollen based on morphological identification, such as *Alternaria alternata*, *Aspergillus* spp., *Aureobasidium pullulans*, *Bettsia alvei*, *Cladosporium oxysporum*, *Epicoccum purpurascens*, *Eremascus fertilis*, *Fusarium oxysporum*, *Gymnoascus setosus*, *Monilia* spp., *Mucor erectus*, *Oospora favorum* and *Penicillium* spp. (Egorova 1971, Sainger et al. 1978, Gilliam et al. 1989), and fungi associated with these two types of pollen remained poorly explored.

In our fungal exploration from newly-collected bee pollen and hive-stored pollen in the flowering season of *Brassica campestris*, four new filamentous species are discovered. They are

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described and illustrated based on the morphological comparisons and phylogenetic analyses in the present study. While the fungal community composition and distinction between these two kinds of bee pollen will be published elsewhere.

Materials & Methods

Sample collection and fungal isolation

Newly-collected pollen and hive-stored pollen samples were collected from Italian honey bee (*Apis mellifera ligustica*) colonies located in Yicheng, Hubei province in China, where the blooming rape (*Brassica campestris*) covered more than 500 hectares in March 2016 (Fig. 1). Three colonies with consistent population were used to trap bee pollen and produce stored pollen. Each colony was comprised of 10 frames of adult bees (about 8000–10 000 adult bees). Before we established the bee colonies, no pollen was stored in beehives, but which contained some bee larvae and honey. New frames were placed in the colonies to provide space for the worker bees to store pollen.

Figure 1 – Sampling site and samples. a Sampling site beside fields of *Brassica campestris*. b–c Worker bees (*Apis mellifera ligustica*) collecting pollen of rape flowers. d Newly-collected pollen obtained by pollen traps. e Red arrow indicating hive-stored pollen under fermentation in the comb cells. f–g Hive-stored pollen.

In the flowering season of rape, standard pollen traps were used to collect bee pollen samples (Fig. 1) (Giesecke et al. 2010). Fresh bee pollen was immediately collected from pollen baskets (corbicula) of incoming honey bees. Fourteen days after the flowering season of rape, hive-stored pollen samples were dug by sterile medicine spoons (Kieliszek et al. 2018). One hundred grams of newly-collected pollen and hive-stored pollen samples were collected from each colony, which

made 3 samples for each kind of pollen. All samples were put in sterile 10 mL centrifuge tubes and took back to laboratory immediately and preserved at 4 °C.

Fungi were isolated following a modified dilution plate method from newly-collected and hive-stored pollen samples (Zhang et al. 2015). One gram of each sample was suspended in 10 mL sterile water in a 15 mL sterile centrifuge tube. The tubes were shaken by Vortex vibration meter thoroughly. The suspension was then diluted to a series of concentrations, i.e. 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Two hundred microliters suspensions from each dilution were spread onto potato-dextrose agar (PDA, Aobox, ABX-00279) and yeast-peptone-dextrose agar (YPD) containing ampicillin (50 μg/ mL) and streptomycin (50 μg/ mL), onto De Man, Rogosa and Sharpe agar (MRS, Aobox, 02-293) containing amphotericin (8 μg/ mL), by three replicates.

All the plates were incubated at room temperature (ca. 25–28 °C) for 1–4 weeks, and from which the single colonies were picked up and inoculated onto new PDA plates every 2 d. All fungal strains were stored at 4 °C in the LC Culture Collection (personal culture collection held in lab of Dr. Lei Cai) for further studies. The dry cultures of novel species were deposited in the Herbarium of Microbiology, Academia Sinica (HMAS), while living cultures were deposited in the China General Microbiological Culture Collection Center (CGMCC).

Morphology

Morphological characterization was made for isolates cultivated on potato dextrose agar (PDA; Difco), and alternatively on synthetic nutrient-poor agar (SNA, 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 0.6 ml NaOH (1 M) and 13.2 g agar/liter distilled water) or amended with double-autoclaved pine needles placed onto the agar surface (Smith et al. 1996), malt extract agar (MEA, malt extract (Oxoid CM0059) 50 g, $ZnSO_4 \cdot 7H_2O$ 0.01g, $CuSO_4 \cdot 5H_2O$ 0.005 g/liter distilled water), and cornmeal agar (CMA, cornmeal 20 g, peptone 20 g, glucose 20 g, agar 15 g/liter distilled water) according to the genus level placement of the novel species. Cultures were incubated at 25 °C and another five temperatures for *Trichoderma* (15 °C, 20 °C, 30 °C, 37 °C, 40 °C) according to Jaklitsch & Voglmayr (2015). Cultures were examined periodically for the development of reproductive structures. Microscopic preparations were made mounted on lactic acid, and at least 30 measurements per structure were documented and examined under a Nikon Eclipse 80i microscope using differential interference contrast (DIC). Taxonomic descriptions and nomenclature were deposited in MycoBank.

Molecular analyses

Total genomic DNA was extracted from fungal mycelia using a modified CTAB protocol (Guo et al. 2000). Multi genes were amplified and sequenced for each new species, and the primer sets were listed in Table 1. ITS, *TEF1-a* and *TUB2* sequences were obtained for *Arthrinium*, LSU, ITS, *CAL* and *RPB2* sequences obtained for *Chrysosporium*, LSU, ITS, *RPB2* and *TEF1-a* obtained for *Nigrograna*, and ITS, *CAL*, *RPB2* and *TEF1-a* obtained for *Trichoderma*. However, single ITS phylogenetic analysis was finally performed for *Chrysosporium* due to the lack of reference sequences of other loci. PCR amplification protocols were performed as described by Gao et al. (2017), but the denaturing temperatures were adjusted to 59 °C for *RPB2*, and 55 °C for the other loci. Purification and sequencing of PCR amplifications were carried out by the Omegagenetics Company, Beijing, China. MEGA 6.06 was used to obtain consensus sequences from DNA sequences generated from forward and reverse primers. All reference and novel sequences obtained in this study were deposited in NCBI's GenBank database (See supplementary file: Table S1). The alignments were deposited in TreeBASE (www.treebase.org, S22986).

Sequences alignment was performed with MAFFT 7, and was manually improved with MEGA 6.06. Bayesian inference (BI) and maximum likelihood (ML) methods were implemented in this study. Bayesian analyses were performed using MRBAYES 3.2.2 (Ronquist et al. 2012) as outlined by Liu et al. (2014). ML analyses were performed using RAXML 7.0.3 with 1000 replicates under the GTR-GAMMA model (Stamatakis 2006).

Table 1 Primers used in this study

Locus	Primer	Primer sequences 5' to 3'	Orientation	Reference
large subunit ribosomal DNA (LSU)	LR0R	ACCCGCTGAACTTAAGC	Forward	Rehner & Samuels (1994)
	LR5	ATCCTGAGGGAAACTTC	Reverse	Rehner & Samuels (1994)
ITS	ITS1	TCCGTAGGTGAACCTGCGG	Forward	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC	Reverse	White et al. (1990)
beta-tubulin (*TUB2*)	T1	AACATGCGTGAGATTGTAAGT	Forward	O'Donnell & Cigelnik (1997)
	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	Forward	Glass & Donaldson (1995)
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	Reverse	Glass & Donaldson (1995)
translation elongation factor 1-alpha (*TEF1-a*)	EF 1-728F	CAT CGA GAA GTT CGA GAA GG	Forward	Carbone & Kohn (1999)
	EF 1-1567R	ACHGTRCCRATACCACCRATCTT	Reverse	Rehner (2001)
	EF 1-2218R	ATGACACCRACRGCRACRGTYTG	Reverse	Rehner (2001)
calmodulin (*CAL*)	CAL-228F	GAGTTCAAGGAGGCCTTCTCCC	Forward	Carbone & Kohn (1999)
	CAL-737R	CATCTTTCTGGCCATCATGG	Reverse	Carbone & Kohn (1999)
RNA polymerase II second largest subunit (*RPB2*)	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	Forward	Liu et al. (1999)
	fRPB2-7cR	CCCATRGCTTGYTTRCCCAT	Reverse	Liu et al. (1999)

Results

Phylogenetic analyses

Arthrinium. Phylogenetic analysis of *Arthrinium* was performed on the concatenated dataset of ITS, *TUB2* and *TEF1-a*, with *Nigrospora gorlenkoana* (CBS 480.73) as outgroup (Fig. 2). The concatenated dataset contained 720 characters with alignment gaps for ITS, 1025 for *TUB2*, and 602 for *TEF1-a*. The maximum likelihood (ML) tree confirmed the tree topology of the Bayesian consensus (BS) tree. Strains from the newly-collected bee pollen and hive-stored pollen separated into two well-supported clades in the phylogenetic tree of *Arthrinium* (Fig. 2), which represented one known species, *A. rasikravindrii*, and one novelty described in this study.

Chrysosporium. Phylogenetic analysis of *Chrysosporium* and related species was performed based on ITS sequences (Fig. 3). Fifty-six sequences from *Chrysosporium* and *Aphanoascus* were included in the ITS dataset, with *Corynascus sepedonium* and *Thermothelomyces thermophila* as outgroups. The dataset contained 791 characters with alignment gaps. The ML tree confirmed the

tree topology of BS tree. Strains from the hive-stored pollen formed a distinct clade which closely related to *C. submersum* and *C. hubeiense* (Fig. 3).

Nigrograna. Phylogenetic analysis of *Nigrograna* was performed on the concatenated dataset of LSU, ITS, *RPB2* and *TEF1-a*, with *Occultibambusa fusispora* (MFLUCC 11-0127) as outgroup (Fig. 4). The concatenated dataset contained 748 characters with alignment gaps for LSU, 439 for ITS, 807 for *RPB2*, and 691 for *TEF1-a*. The maximum likelihood (ML) tree confirmed the tree topology of the Bayesian consensus (BS) tree. Strains from the hive-stored pollen formed a distinct and well-supported clade in the 4-locus tree and showed phylogenetic distance from all other species in *Nigrograna* (Fig. 4).

Figure 2 – Phylogenetic tree of *Arthrinium* calculated with maximum likelihood analysis on a combined dataset of three-locus sequences (ITS, *TUB2*, *TEF1-a*) by running RAxML v.7.0.3. The RAxML bootstrap support values (> 50%) are displayed at the nodes. Thickened branches indicate branches also present in the Bayesian tree with > 0.95 posterior probabilities. Strains in bold indicate ex-type cultures. Strains obtained in this study are in red colour.

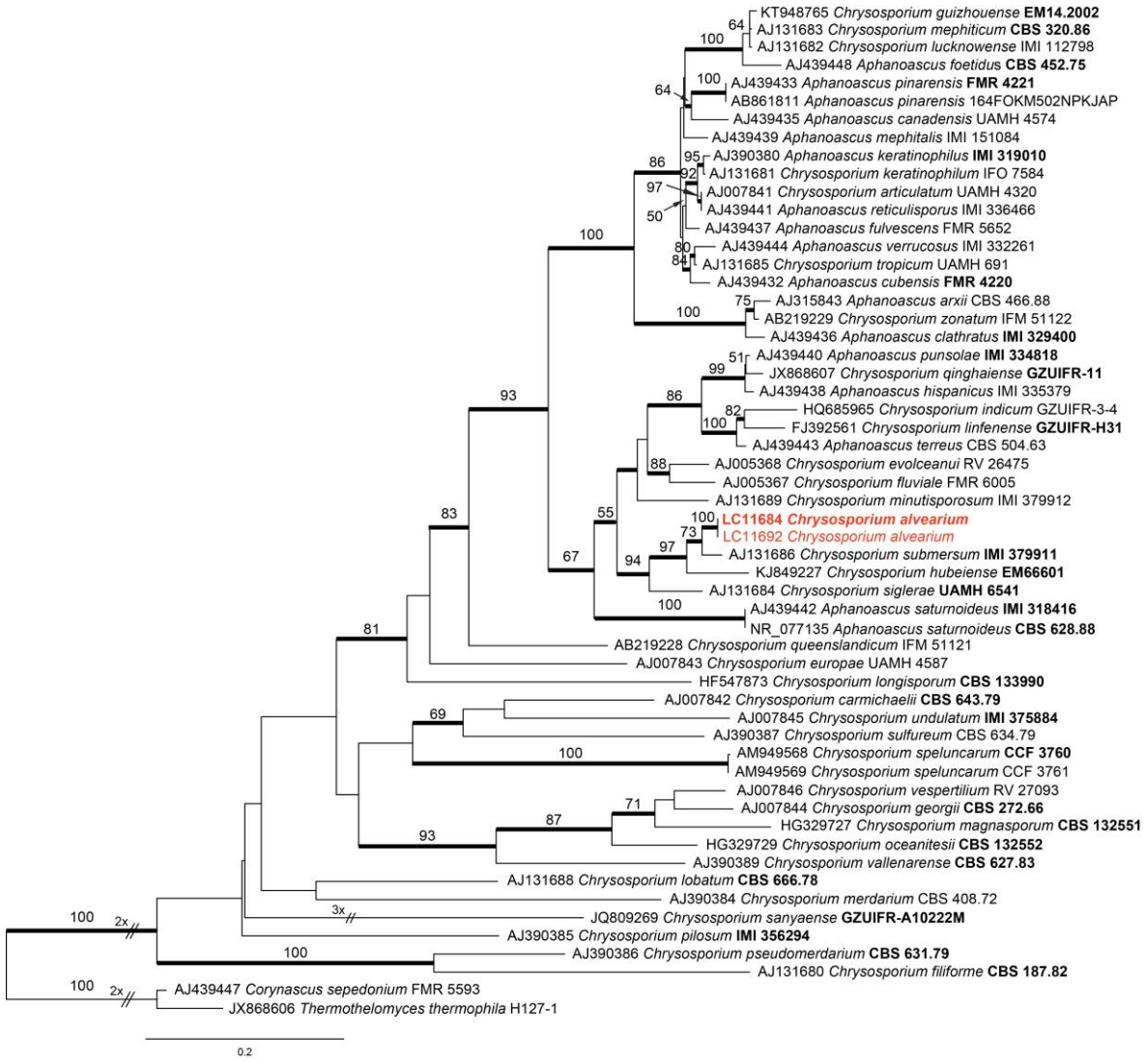

Figure 3 – Phylogenetic tree of *Chrysosporium* and related taxa calculated with maximum likelihood analysis on ITS sequences by running RAxML v.7.0.3. The RAxML bootstrap support values (> 50%) are displayed at the nodes. Thickened branches indicate branches also present in the Bayesian tree with > 0.95 posterior probabilities. Strains in bold indicate ex-type cultures. Strains obtained in this study are in red colour.

Trichoderma. Trichoderma isolates obtained in this study were confirmed belonging to the *T. harzianum* species complex based on the BLASTn searches of the NCBI GenBank nucleotide database and preliminary *TEF1-a* gene tree (results not shown). Multi-locus phylogenetic analysis of *T. harzianum* species complex was then performed on the concatenated dataset of ITS, *RPB2*, *ACT*, *CAL* and *TEF1-a*, with *T. aggressivum* (CBS 100525) as outgroup (Fig. 5). The concatenated dataset contained 535 characters with alignment gaps for ITS, 836 for *RPB2*, 299 for *ACT*, 431 for *CAL* and 597 for *TEF1-a*. The maximum likelihood (ML) tree confirmed the tree topology of the Bayesian consensus (BS) tree. Strains from the newly-collected pollen formed a distinct and well-supported clade in the 5-locus tree and showed phylogenetic distance from all other species in *T. harzianum* species complex (Fig. 5).

Taxonomy

Arthrinium locuta-pollinis F. Liu & L. Cai, sp. nov. Fig. 6

MycoBank: MB824505; Facesoffungi number: FoF05221

Etymology – *locuta-pollinis*, named after the origin of this species, stored pollen.

Colonies on PDA flat, surface initially white and becoming yellowish in the center, with abundant aerial mycelia, reaching 9 cm in 7 d at 25 C. On MEA, colonies umbonate, entire edge, surface initially white and becoming yellowish in the center, with abundant aerial mycelia. Hyphae hyaline, or pale brown, branched, septate, 2–5 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells pale brown, smooth, subglobose to ampulliform to doliiform, 3–7.5 × 3–6 µm (av. ± SD = 4.9 ± 1.13 × 3.8 ± 0.77 µm). Conidia pale brown to brown with hyaline equatorial rim, smooth, globose to subglobose, 5.5–9 × 4.5–8 µm (av. ± SD = 7.1 ± 0.55 × 6.4 ± 0.66 µm), or ellipsoidal, 8–15× 5–9.5 µm (av. ± SD = 10.7 ± 1.47 × 7.1 ± 0.85 µm). Elongated cells (sterile cells) formed on solitary loci on hyphae, pale brown or brown, smooth, ellipsoidal to clavate, 11.5–21 × 3.5–8 µm (av. ± SD = 15.7 ± 2.22 × 5.7 ± 1.08 µm).

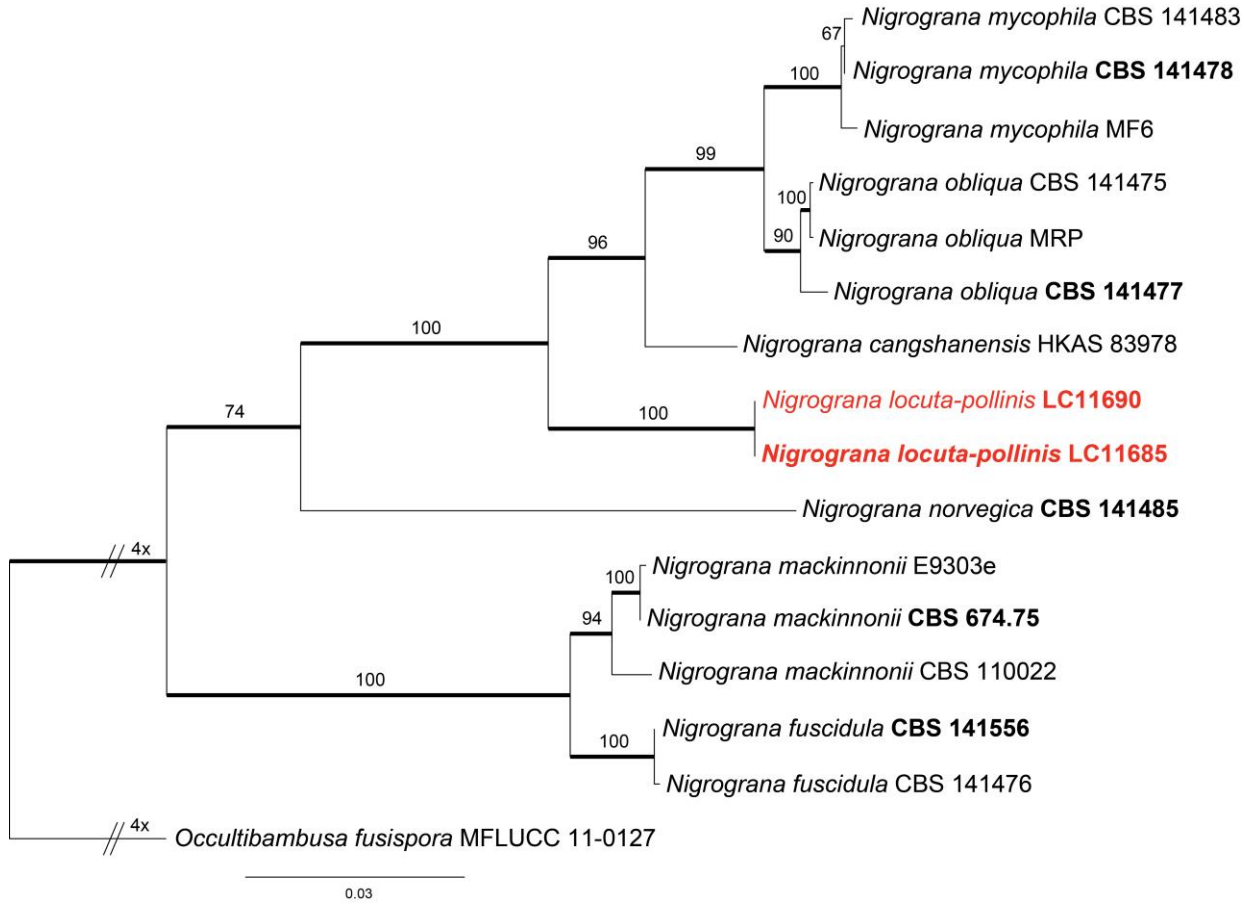

Figure 4 – Phylogenetic tree of *Nigrograna* calculated with maximum likelihood analysis on a combined dataset of four-locus sequences (ITS, LSU, *RPB2*, *TEF1-a*) by running RAxML v.7.0.3. The RAxML bootstrap support values (> 50%) are displayed at the nodes. Thickened branches indicate branches also present in the Bayesian tree with > 0.95 posterior probabilities. Strains in bold indicate ex-type cultures. Strains obtained in this study are in red colour.

Materials examined – CHINA, Hubei Province, from hive-stored pollen collected in the Italian honey bee colonies in the flowering season of *Brassica campestris*, 31 Mar 2016, Y.Z. Zhao (holotype HMAS 247779) – ex-holotype living culture CGMCC 3.18782 = LC 11683 = LF1844; *ibid.* living cultures LC 11688 = LF2064, LC 11689 = LF2065.

Notes – *Arthrinium locuta-pollinis* is phylogenetically closely related to *A. mediterranei*, *A. marii* and *A. hispanicum*, but differs in distinct morphological characters and nucleotide differences. *Arthrinium locuta-pollinis* produces globose, subglobose or ellipsoidal conidia, while which are globose or subglobose in *A. hispanicum*, *A. marii* and *A. mediterranei*. Furthermore, *A. locuta-pollinis* differs from *A. mediterranei* in producing smaller globose or subglobose conidia (5.5–9 × 4.5–8 µm vs. 9–9.5 × 7.5–9 µm) and longer sterile cells (11.5–21 × 3.5–8 µm vs. 7–7.5 ×

6.5–7 µm) (Larrondo & Calvo 1992), and differs from *A. hispanicum* in producing obviously longer sterile cells (11.5–21 µm vs. < 7.5–8.5 µm) (Larrondo & Calvo 1992).

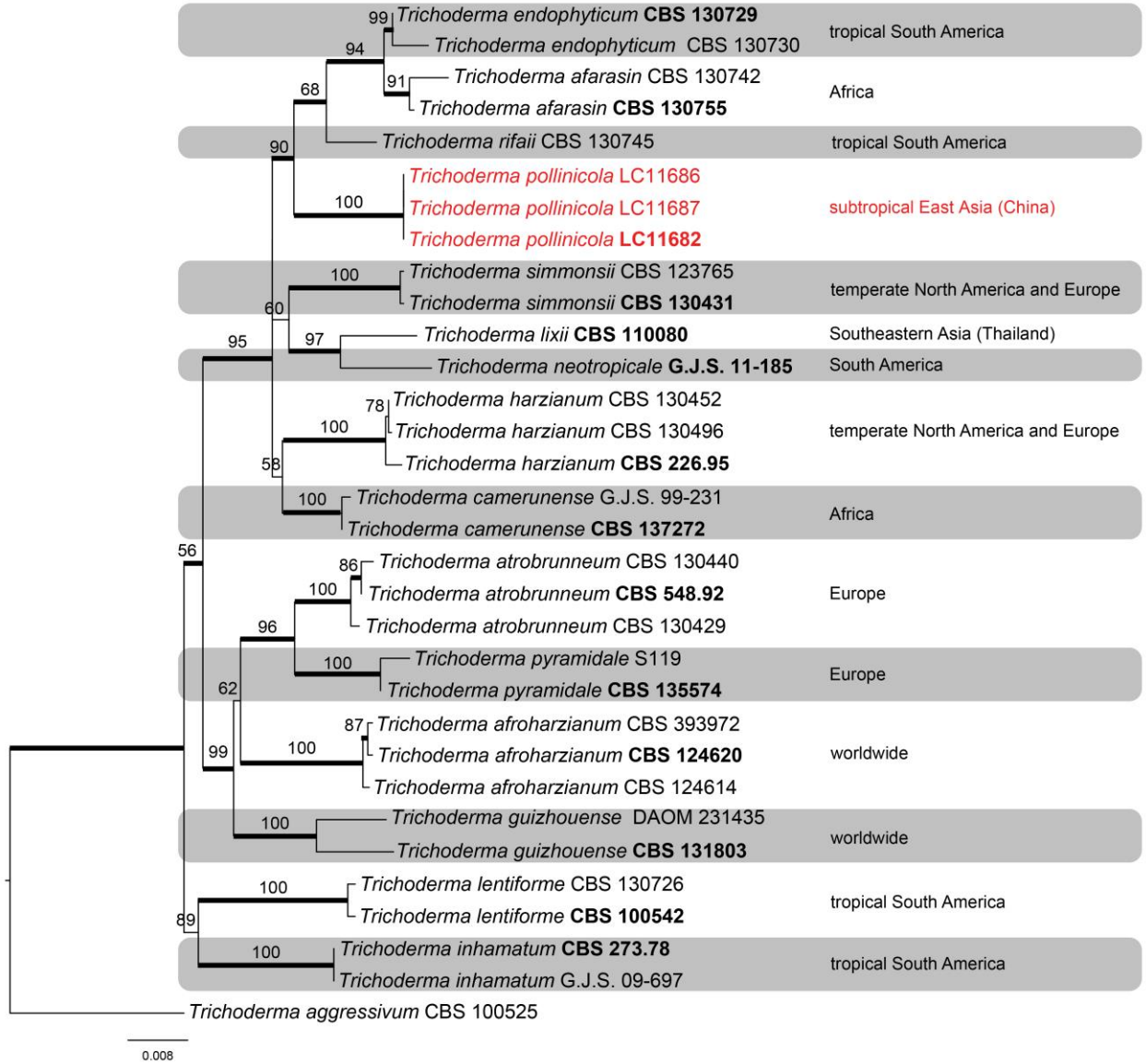

Figure 5 – Phylogenetic tree of *Trichoderma harzianum* species complex calculated with maximum likelihood analysis on a combined dataset of five-locus sequences (ITS, *ACT*, *CAL*, *RPB2*, *TEF1-a*) by running RAxML v.7.0.3. The RAxML bootstrap support values (> 50%) are displayed at the nodes. Thickened branches indicate branches also present in the Bayesian tree with > 0.95 posterior probabilities. Strains in bold indicate ex-type cultures. Strains obtained in this study are in red colour.

Chrysosporium alvearium F. Liu & L. Cai, sp. nov. Fig. 7

MycoBank: MB824506; Facesoffungi number: FoF05222

Etymology – *alvearium*, referring to the place where the fungus was collected, hive.

Description – Colonies on PDA flat or with slightly elevated center, with a daily growth rate of 2–2.5 mm in the dark at 25 C, white coloured, powdery, irregular at the margin; reverse white. Hyphae hyaline, branched, smooth, septate, 1.5–2.5 µm diam. Terminal and lateral conidia sessile or on short or long right-angled side protrusions, solitary or in chains of up to 10 conidia, hyaline, smooth, globose, pyriform, clavate, or obovoid, rarely cylindrical, 1-celled, 4–9 × 2–7.5 µm (av. ± SD = 5.6 ± 0.8 × 4.4 ± 1.1 µm). Intercalary conidia abundant, solitary or in chains, smooth,

globose, barrel-shaped, ellipsoid to obvoid, 4–11 × 3.5–9.5 μm (av. ± SD = 7 ± 1.1 × 5.9 ± 1.0 μm). Racquet hyphae and chlamydospores present. Sexual morph not observed.

Materials examined – CHINA, Hubei Province, from hive-stored pollen collected in the Italian honey bee colonies in the flowering season of *Brassica campestris*, 31 Mar 2016, Y.Z. Zhao (holotype HMAS 247780) – ex-holotype living culture CGMCC 3.18783 = LC 11684 = LF1882; *ibid.* living cultures LC 11692 = LF2097, LC 11693 = LF2098.

Notes – Traditionally morphologically defined *Chrysosporium* is polyphyletic with affiliations to at least three orders of the Ascomycota (Vidal et al. 2000, Pitt et al. 2013). The genus awaits taxonomic revision if the type species *C. merdarium* could be epitypified (Pitt et al. 2013). Morphologically our species is characterized by white colonies and aleurioconidia on undifferentiated hyphae, in agreement to the current morphological circumscription of *Chrysosporium* (Carmichael 1962). *Chrysosporium alvearium* is phylogenetically closely related to *C. submersum* and *C. hubeiense* (Fig. 3), but their ITS sequences only shows 97% and 94% similarities respectively. Morphologically, *C. alvearium* differs from *C. submersum* by lower growth rate on PDA (28–35 mm/14d vs. 50–60 mm/14d) and the absence of 1–3-septate conidia. In addition, the conidia of *C. alvearium* are commonly in longer chains than that of *C. submersum* (rarely, up to four conidia) (Vidal et al. 2002). In contrast to *C. hubeiense*, *C. alvearium* produces longer terminal and lateral conidia (4–9 × 2–7.5 μm vs. 2.2–4.3 × 1.6–2.3 μm) (Zhang et al. 2016).

Nigrograna locuta-pollinis F. Liu & L. Cai, sp. nov.

MycoBank: MB824507; Facesoffungi number: FoF05223

Etymology – *locuta-pollinis*, named after the origin of this species, stored pollen.

Diagnosis – Growth on PDA after 14 d at room temperature (ca. 25 C) flat, entire edge, olivaceous grey, aerial mycelia fluffy, 49 mm diam.; on MEA flat, entire edge, olivaceous grey, aerial mycelia sparse, 18 mm diam.; on CMA flat, entire edge, olivaceous to olivaceous black, aerial mycelia sparse, 49 mm diam. Cultures sterile.

Reference phylogeny – Fig. 4, present study.

Molecular and phylogenetic notes – *Nigrograna locuta-pollinis* forms a distinct and strongly supported monophyletic clade (ML bootstrap 100 %, Bayesian posterior probabilities = 1.00) within genus *Nigrograna* (Fig. 4). It differs from its closest phylogenetic neighbor, *N. cangshanensis*, by unique fixed alleles in three loci based on alignments of the separate loci demonstrated in the supplementary file (See supplementary file: Fig. S1).

Materials examined – CHINA, Hubei Province, from hive-stored pollen collected in the Italian honey bee colonies in the flowering season of *Brassica campestris*, 31 Mar 2016, Y.Z. Zhao (holotype HMAS 247781) – ex-holotype living culture CGMCC 3.18784 = LC 11685 = LF1889; *ibid.* living cultures LC 11690 = LF2070, LC 11691 = LF2071.

Notes – *Nigrograna* was erected by Gruyter et al. (2012), and currently includes five species (Jaklitsch & Voglmayr 2016). These species were reported from bark of moderately decayed twigs of shrubs and trees and sometimes as human pathogens. They usually produce sexual morph in their life cycle. *Nigrograna locuta-pollinis* was isolated from hive-stored pollen, expanding the known habitat of this genus, but proved sterile when cultivated on several different media (PDA, SNA ± pine needle, CMA, MEA, etc.).

Trichoderma pollinicola F. Liu & L. Cai, sp. nov. Fig. 8

MycoBank: MB824508; Facesoffungi number: FoF05224

Etymology – *pollinicola*, referring to the substrate where the fungus was first discovered, pollen.

Description – Characteristics in culture: Colony radius after 72 h at 15 C on PDA 8–11 mm, on SNA 9–15 mm, on CMA 17–18 mm; 20 C on PDA 28–30 mm, on SNA 32–34 mm, on CMA 30–31 mm; 25 C on PDA 53–54 mm, on SNA 49–50 mm, on CMA 44–52 mm; at 30 C on PDA 48–49 mm, on SNA 46–49 mm, on CMA 60–65 mm; at 37 C on PDA 12–16 mm, on SNA 7–8 mm, on CMA 8–10 mm. Not growing at 40 C.

On PDA after 96 h at 25 C aerial hyphae abundant, cottony, radial; conidia appearing within 48–72 h, typically abundant and disposed in three concentric rings around the inoculum. On CMA after 96 h at 25 C aerial hyphae abundant, cottony, radial; conidia appearing within 48 h, forming abundantly and disposed around the inoculum in the range of 2.5 cm radius. On SNA after 96 h at 25 C, aerial hyphae sparse, appearing in several concentric rings. Conidia noted within 72–96 h, forming abundantly on aerial hyphae, conidial pustules spreading in concentric rings, 0.5–1.5 mm in diam. Conidiophores pyramidal, comprising a distant main axis, side branches paired or unpaired, often at acute angles with the main axis, re-branching up to 3 levels. Phialides solitary or in whorls of 2–4, lageniform to ampulliform, 4.5–9.5 × 2.5–4 µm (av. ± SD = 6.7 ± 1.2 × 3.0 ± 0.2 µm), length/width ratio = 2.2, base 1.5–2.5 µm wide (av. = 2 µm), supporting cells 2–4 µm wide (av. = 3.1 µm). Conidia globose or subglobose, rarely ovoid, 2.5–3.5 × 2–3 µm (av. ± SD = 2.8 ± 0.1 × 2.6 ± 0.1 µm), length/ width ratio = 1.1, smooth, green. Chlamydospores not observed.

Materials examined – CHINA, Hubei Province, from newly-collected pollen collected in the Italian honey bee colonies in the flowering season of *Brassica campestris*, 31 Mar 2016, Y.Z. Zhao (holotype HMAS 247782) – ex-holotype living culture CGMCC 3.18781 = LC 11682 = LF1542; *ibid.* living cultures LC 11686 = LF2050, LC 11687 = LF2051.

Notes – Strains of *Trichoderma pollinicola* formed a well-supported and distinct clade in the *T. harzianum* species complex based on multi-locus (ITS, *ACT*, *CAL*, *TEF1-a*, *RPB2*) phylogenetic analysis (Fig. 5). It differs from the phylogenetically related species *T. rifaii* in lower growth rate (e.g. colony radius after 72 h at 25 °C on PDA 53–54 mm vs. 62–67 mm, on SNA 49–50 mm vs. 55–65mm) (Chaverri et al. 2015). *Trichoderma pollinicola* is similar to *T. rifaii* in microstructure, but they can be distinguished from each other either by *RPB2* (96% similarity, 32 bp differences) or *TEF1-a* (96% similarity, 21 bp differences) sequence data.

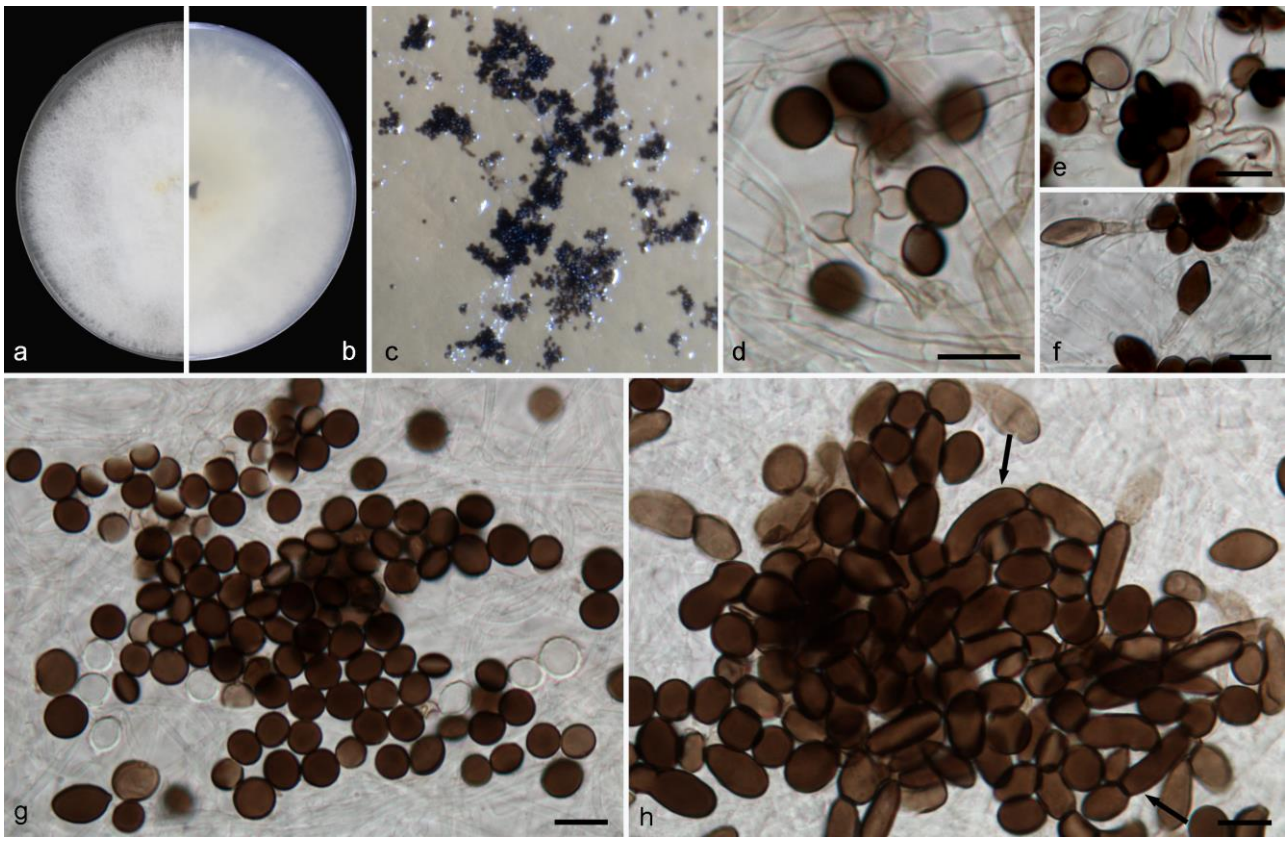

Figure 6 – *Arthrinium locuta-pollinis* (ex-holotype CGMCC 3.18782 = LC 11683). a–b Colonies after 7 d on PDA. c. Colony on MEA producing conidia mass. d–f Conidiogenous cells giving rise to conidia. g Conidia. h Conidia and elongated conidia (might be sterile cells, indicated by arrows). Bar = 10 µm.

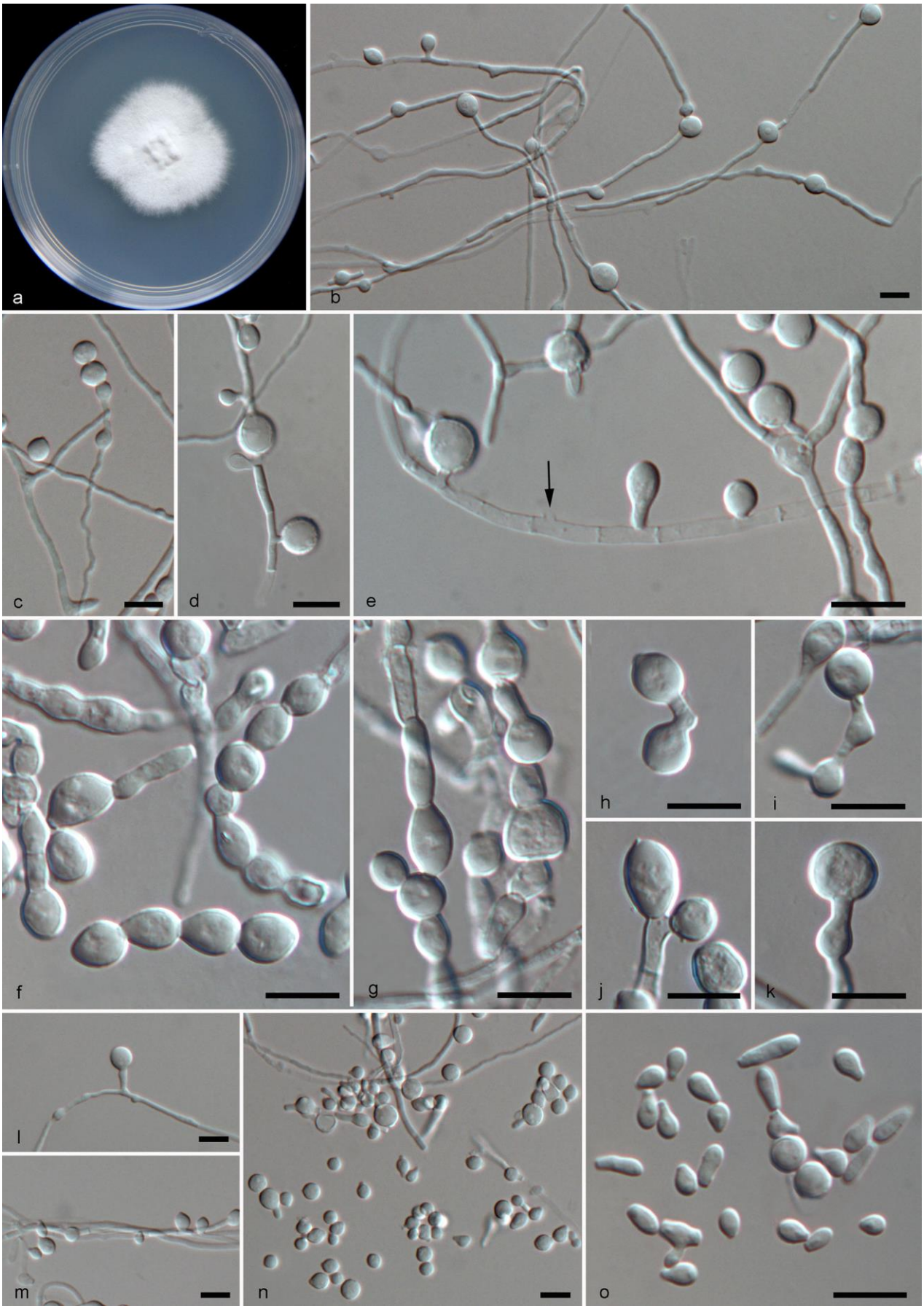

Figure 7 – *Chrysosporium alvearium* (ex-holotype CGMCC 3.18783 = LC 11684). a Colony after 10 d on PDA. b–e, h–m Conidiogenous structures. f–g Conidia in chains. n–o Conidia. Scale Bar = 10 μm.

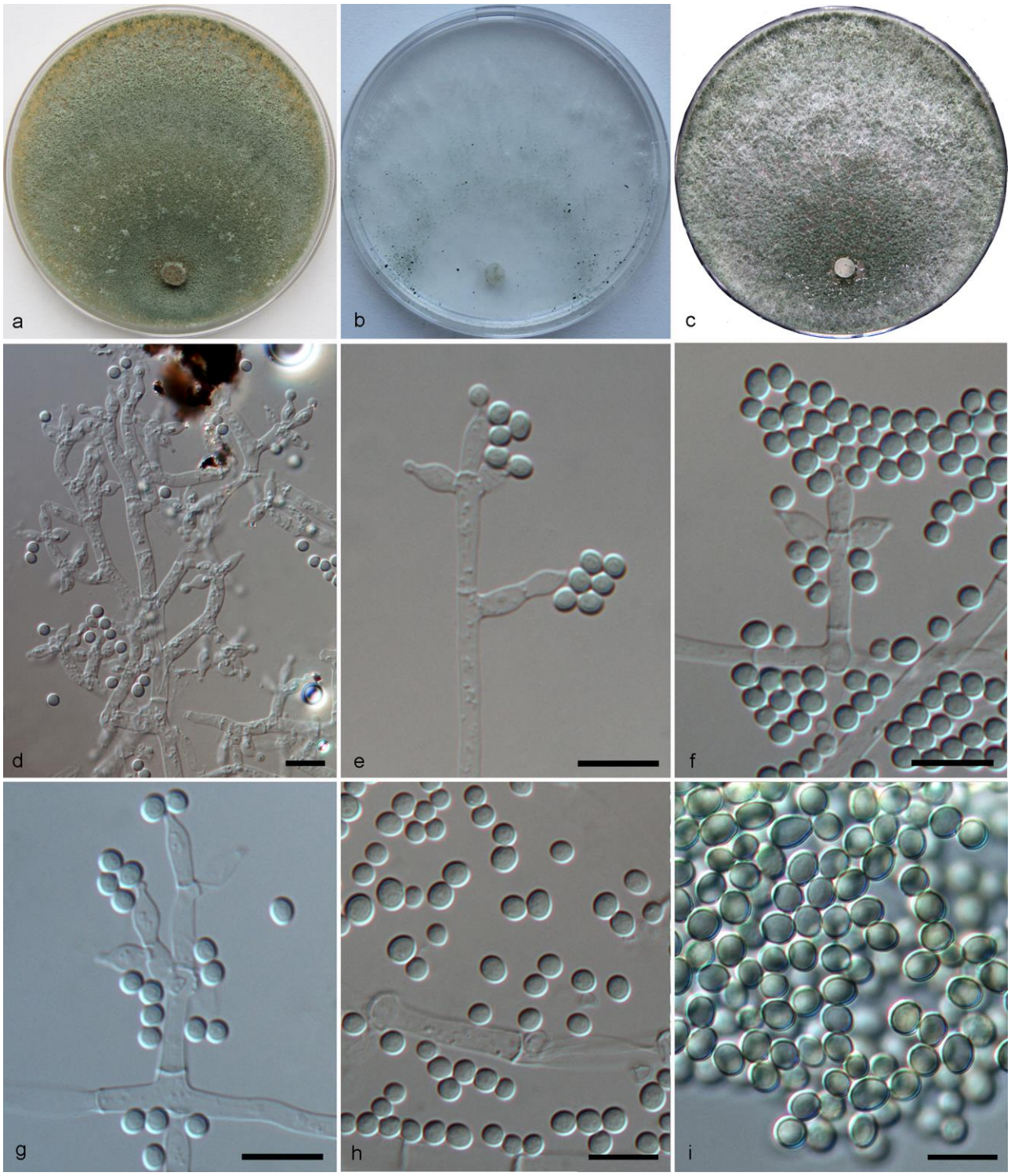

Figure 8 – *Trichoderma pollinicola* (ex-holotype CGMCC 3.18781 = LC 11682). a–c Colonies after 7 d at 25 °C (a. PDA. b. SNA. c. CMA). d Conidiophores. e–g Phialides. h. Hyaline to light green conidia when young. i Green conidia with age. Scale Bar = 10 μm.

Discussion

During our survey of culturable fungi from newly-collected and hive-stored pollen, four new species were identified, i.e. *Arthrinium locuta-pollinis*, *Chrysosporium alvearium*, *Nigrograna locuta-pollinis* and *Trichoderma pollinicola*. As far as we know, nine previously described *Chrysosporium* species were recorded from mason bees (*Osmia* spp.), beehives, bee pollen and honey, i.e. *C. botryoides*, *C. farinicola*, *C. globiferum* and its two varieties, *C. hispanicum*, *C. holmii*, *C. medium*, *C. merdarium* and its variety, *C. minor* and *C. pyriforme*. Most of above species

representing extreme xerophiles have already been combined into *Xerochrysium* or *Bettsia* except *C. merdarium* (Pitt et al. 2013). Although *C. alvearium* was isolated from hive-stored pollen from beehives and the genus *Chrysosporium* is polyphyletic, it shows very low similarity to *Xerochrysium* or *Bettsia*.

Species in the *T. harzianum* complex were recently indicated having a tendency of specialization for habitat (Chaverri et al. 2015). Some were demonstrated to be endophytic, while others were only isolated from soil. In the present study, *T. pollinicola* was isolated from the newly-collected pollen of *Brassica campestris*, however we could not determine whether *T. pollinicola* was the endophyte of *B. campestris* or environment related fungus. It is worth noting that, we did not obtain any *Trichoderma* strain when performing fungal isolation from the flowers of *B. campestris* at the same time (results not shown). As far as we know, only one species in *Trichoderma*, *T. pseudokoningii*, was previously recorded from *B. campestris* from Canada (Rifai 1969), while which belongs to Longibrachiatum clade (Jaklitsch & Voglmayr 2015).

Arthrinium species are geographically distributed in various substrates, e.g. host plants, air and soil (Wang et al. 2018). *Arthrinium camelliae-sinensis* has recently been reported from pollen product of *Brassica campestris* ('Huaxing' brand) which made from fresh-collected pollen (Wang et al. 2018). Whereas in the present study, *A. locuta-pollinis* is isolated from hive-stored pollen of *Brassica campestris*, but not from newly-collected pollen. It is probably that *A. locuta-pollinis* was introduced through bees' activity or from air. According to the multi-locus phylogeny (Fig. 2), *A. mediterranei*, *A. marii* and *A. hispanicum* showed phylogenetic identity. Further comparison revealed that ITS and *TUB2* sequences of type strains of above three species are identical, and the *TEF* sequences of *A. mediterranei* and *A. hispanicum* are absent in any public database for comparison. Further researches are therefore required to determine their conspecific or heterogeneity.

Culture media such as YPD and MRS are commonly used for bacteria and yeast isolation. Surprisingly we obtained some exclusive species comparing to the most commonly used PDA medium (results not shown), for example, the two new species *A. locuta-pollinis* and *C. alvearium* were only isolated from YPD, and *T. pollinicola* was only from MRS. While *N. locuta-pollinis* was only isolated on PDA. The exclusive fungi obtained from YPD and MRS may be more in favor of the yeast/bacterial dominated environment. Another possibility is that, when the diluted concentration of pollen is low enough, the growth space of *A. locuta-pollinis*, *C. alvearium* and *T. pollinicola* were not be occupied and restrained by favorable yeast or bacteria on YPD and MRS. Above results demonstrated that the application of different culture media and dilution concentration of materials could help to reveal more microbial biodiversity.

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Supplementary files:

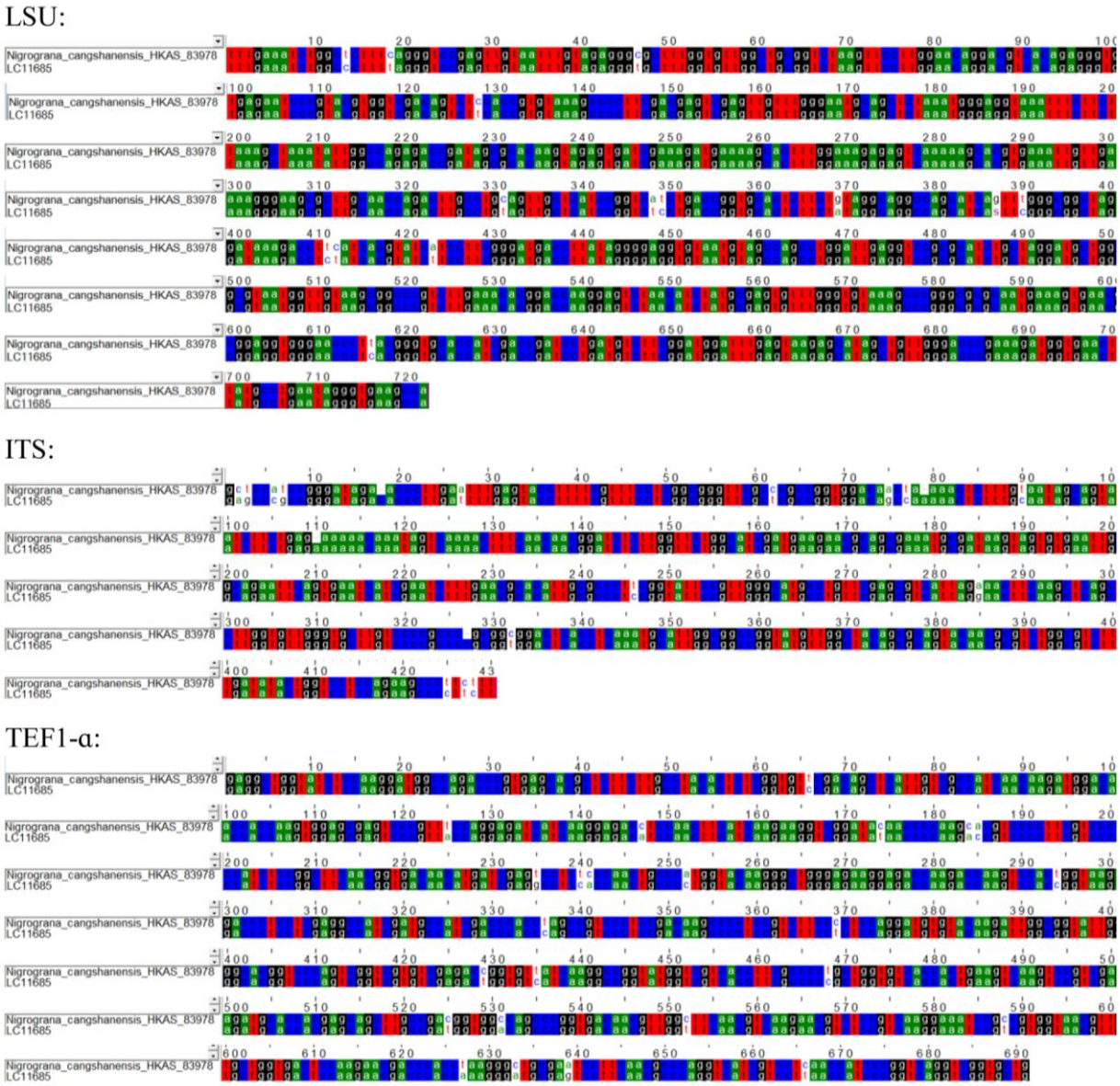

Figure S1 – Sequence alignments of *Nigrograna locuta-pollinis* and *N. cangshanensis*

Table S1 Strains used in this study.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
Fig. 2	*Arthrinium arundinis*	CBS 114316	Leaf of *Hordeum vulgare*	KF144884	KF144974	KF145016				
		CBS 124788	Living leaves of *Fagus sylvatica*	KF144885	KF144975	KF145017				
	A. aureum	CBS 244.83*	Air	AB220251	KF144981	KF145023				
	A. bambusae	LC7106 = WM340	Leaf of bamboo	KY494718	KY705186	KY806204				
		LC7113 = WM347	Leaf of bamboo	KY494720	KY705188	KY806205				
		LC7128 = WM362	Leaf of bamboo	KY494730	KY705198	KY705126				
	A. camelliae-sinensis	LC5007	*Camellia sinensis*	KY494704	KY705173	KY705103				
		LC8181 = LF1498	*Brassica capestris*	KY494761	KY705229	KY705157				
	A. dichotomanthi	LC4950	*Dichotomanthus tristaniaecarpa*	KY494697	KY705167	KY705096				
		LC8175	*Dichotomanthus tristaniaecarpa*	KY494755	KY705223	KY705151				
		LC8176	*Dichotomanthus tristaniaecarpa*	KY494756	KY705224	KY705152				
	A. euphorbiae	IMI 285638b	Unknown	AB220241	AB220288	-				
	A. guizhouense	LC5318	Air	KY494708	KY705177	KY705107				
		LC5322	Air	KY494709	KY705178	KY705108				
	A. gutiae	CBS 135835	Gut of a grasshopper	KR011352	KR011350	KR011351				
	A. hispanicum	IMI 326877*	Maritime sand	AB220242	AB220289	-				

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
	A. hydei	CBS 114990*	Culms of *Bambusa tuldoides*	KF144890	KF144982	KF145024				
		LC7103 = WM337	Leaf of bamboo	KY494715	KY705183	KY705114				
		LC7105 = WM339	Leaf of bamboo	KY494717	KY705185	KY705116				
	A. hyphopodii	MFLUCC 15-0003*	Culms of *Bambusa tuldoides*	KR069110	-	-				
	A. japonicum	IFO 30500	Unknown	AB220262	AB220309	-				
		IFO 31098	Unknown	AB220264	AB220311	-				
	A. jatrophae	MMI00052*	Healthy petiole of *Jatropha podagrica*	JQ246355	-	-				
	A. jiangxiense	LC2831	Leaf of bamboo	KY494686	KY806201	KY705085				
		LC4494	*Phyllostachys sp.*	KY494690	KY705160	KY705089				
		LC4577	*Maesa sp.*	KY494693	KY705163	KY705092				
		LC5015	*Imperata cylindrica*	KY494705	KY705174	KY705104				
	A. kogelbergense	CBS 113333*	Dead culms of Restionaceae	KF144892	KF144984	KF145026				
	A. locuta-pollinis	**LC11683 = LF1844***	**Hive-stored pollen of *Brassica campestris***	**MF939595**	**MF939622**	**MF939616**				
		LC11688 = LF2064	**Hive-stored pollen of *B. campestris***	**MF939597**	**MF939623**	**MF939618**				
		LC11689 = LF2065	**Hive-stored pollen of *B. campestris***	**MF939596**	**MF939624**	**MF939617**				
	A. longistromum	MFLUCC 11-0481*	Decaying bamboo culms	KU940141	-	-				

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
		MFLUCC 11-0479	Decaying bamboo culms	KU940142	-	-				
	A. malaysianum	CBS 102053*	*Macaranga hullettii* stem colonised by ants	KF144896	KF144988	KF145030				
	A. marii	CBS 497.90*	Air	AB220252	KF144993	KF145035				
	A. mediterranei	IMI 326875*	Air	AB220243	AB220290	-				
	A. mytilomorphum	DAOM 214595	Dead blades of *Andropogon*	KY494685	-	-				
	A. obovatum	LC4940	*Lithocarpus* sp.	KY494696	KY705166	KY705095				
		LC8178	*Lithocarpus* sp.	KY494758	KY705226	KY705154				
		LC8177	*Lithocarpus* sp.	KY494757	KY705225	KY705153				
	A. ovatum	CBS 115042*	*Arundinaria hindsii*	KF144903	KF144995	KF145037				
	A. phaeospermum	CBS 114314	Leaf of *Hordeum vulgare*	KF144904	KF144996	KF145038				
		CBS 114315	Leaf of *Hordeum vulgare*	KF144905	KF144997	KF145039				
		CBS 114317	Leaf of *Hordeum vulgare*	KF144906	KF144998	KF145040				
		CBS 114318	Leaf of *Hordeum vulgare*	KF144907	KF144999	KF145041				
	A. phragmites	CPC 18900*	Culms of *Phragmites australis*	KF144909	KF145001	KF145043				
	A. pseudosinense	CPC 21546*	Leaf of bamboo	KF144910	-	KF145044				
	A. pseudoparenchymaticum	LC7234 = WM468	Leaf of bamboo	KY494743	KY705211	KY705139				

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
		LC8174	Leaf of bamboo	KY494754	KY705222	KY705150				
		LC8173	Leaf of bamboo	KY494753	KY705221	KY705149				
	A. pseudospegazzinii	CBS 102052*	*Macaranga hullettii* stem colonised by ants	KF144911	KF145002	KF145045				
	A. pterospermum	CPC 20193*	Leaf lesion of *Machaerina sinclairii*	KF144913	KF145004	KF145046				
	A. puccinioides	CBS 549.86	Leaf of *Lepidosperma gladiatum*	AB220253	AB220300	-				
	A. rasikravindrii	CBS 337.61	Cissus	KF144914	-	-				
		LC8180 = LF1684	Bee bread	KY494760	KY705228	KY705156				
		LC8179 = LF1737	Bee bread	KY494759	KY705227	KY705155				
		LC7119 = WM353	Leaf of bamboo	KY494724	KY705192	KY705121				
		LC7142 = WM376	Leaf of bamboo	KY494735	KY705203	KY705131				
		NFCCI 2144*	Soil	JF326454.2	-	-				
	A. sacchari	CBS 212.30	*Phragmites australis*	KF144916	KF145005	KF145047				
		CBS 301.49	Bamboo	KF144917	KF145006	KF145048				
	A. saccharicola	CBS 191.73	Air	KF144920	KF145009	KF145051				
		CBS 334.86	Dead culms of *Phragmites australis*	AB220257	KF145010	KF145052				
		CBS 463.83	Dead culms of *Phragmites australis*	KF144921	KF145011	KF145053				

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
	A. serenense	IMI 326869*		AB220250	AB220297	-				
	A. subglobosum	MFLUCC 11-0397*	Dead bamboo culms	KR069112	-	-				
	A. subroseum	LC7215 = WM449	Leaf of bamboo	KY494740	KY705208	KY705136				
		LC7291 = WM525	Leaf of bamboo	KY494751	KY705219	KY705147				
		LC7292 = WM526	Leaf of bamboo	KY494752	KY705220	KY705148				
	A. thailandicum	MFLUCC 15-0202*	Dead bamboo culms	KU940145	-	-				
		LC5630	Rotten wood	KY494714	KY806200	KY705113				
	A. urticae	IMI 326344	Unknown	AB220245	AB220292	-				
	A. xenocordella	CBS 478.86*	Soil from roadwa	KF144925	KF145013	KF145055				
		LC3486	*Camellia sinensis*	KY494687	KY705158	KY705086				
	A. yunnanum	MFLUCC 15-0002*	Decaying bamboo culms	KU940147	-	-				
	Nigrospora gorlenkoana	CBS 480.73	*Vitis vinifera*	KX986109	KY019456	KY019420				
Fig. 3	*Aphanoascus arxii*	CBS 466.88	Soil	AJ315843						
	A. canadensis	UAMH 4574		AJ439435						
	A. clathratus	IMI 329400*	Soil	AJ439436						
	A. cubensis	IMI 356789, FMR 4220*	Soil	AJ439432						

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
	A. foetidus	CBS 452.75*	*Myomys daltoni*	AJ4394 48						
	A. fulvescens	FMR 5652		AJ4394 37						
	A. hispanicus	IMI 335379		AJ4394 38						
	A. keratinophilus	IMI 319010*	Soil	AJ3903 80						
	A. mephitalis	IMI 151084	Dung	AJ4394 39						
	A. pinarensis	164FOKM502N PKJAP	*Gallus gallus*	AB8618 11						
		IMI 360509, FMR 4221*	Soil	AJ4394 33						
	A. punsolae	IMI 334818*	Soil	AJ4394 40						
	A. reticulisporus	IMI 336466	Soil	AJ4394 41						
	A. saturnoideus	CBS 628.88*	Soil	NR_077135						
		IMI 318416*	Soil	AJ4394 42						
	A. terreus	CBS 504.63	Soil	AJ4394 43						
	A. verrucosus	IMI 332261	Soil	AJ4394 44						
	Chrysosporium alvearium	**LC11692 = LF2097**	**Hive-stored pollen of *B. campestris***	**MF939599**	**MF939626**		**MF939608**	**MF939581**		**MF939591**
		LC11693 = LF2098	**Hive-stored pollen of *B. campestris***	**MF939600**	**MF939627**		**MF939609**	**MF939582**		**MF939590**
		LC11684 = LF1882*	**Hive-stored pollen of *B. campestris***	**MF939598**	**MF939625**		**MF939607**	**MF939580**		**MF939589**
	C. articulatum	UAMH 4320		AJ0078 41						

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
	C. carmichaelii	CBS 643.79*		AJ007842						
	C. europae	UAMH 4587		AJ007843						
	C. evolceanui	RV 26475		AJ005368						
	C. filiforme	CBS 187.82*	*Pinus contorta* var. *latifolia*	AJ131680						
	C. fluviale	FMR 6005		AJ005367						
	C. georgii	CBS 272.66*	Soil	AJ007844						
	C. guizhouense	EM14.2002*	Soil	KT948765						
	C. hubeiense	EM66601*	Soil	KJ849227						
	C. indicum	GZUIFR-3-4		HQ685965						
	C. keratinophilum	IFO 7584		AJ131681						
	C. linfenense	GZUIFR-H31*	Soil	FJ392561						
	C. lobatum	CBS 666.78*	*Apodemus*	AJ131688						
	C. longisporum	CBS 133990*	dermic lesion of *Erpeton tentaculatum*	HF547873						
	C. lucknowense	IMI 112798*	Soil	AJ131682						
	C. magnasporum	CBS 132551*	Catharacta skua Brunnich pellet	HG329727						
	C. mephiticum	CBS 320.86*	Soil	AJ131683						

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
	C. merdarium	CBS 408.72*	Dung	AJ390384						
	C. minutisporosum	IMI 379912		AJ131689						
	C. oceanitesii	CBS 132552*	Dead junvenile of *Oceanites oceanicus*	HG329729						
	C. pilosum	IMI 356294*	River sediment	AJ390385						
	C. pseudomerdarium	CBS 631.79*	Beach sand	AJ390386						
	C. qinghaiense	GZUIFR-11*	Soil	JX868607						
	C. queenslandicum	IFM 51121	Soil	AB219228						
	C. sanyaense	GZUIFR-A10222M*	Rhizosphere Soil of palm	JQ809269						
	C. siglerae	UAMH 6541*	Soil	AJ131684						
	C. speluncarum	CCF 3761	Bat guano	AM949569						
		CCF 3760*	Guano of *Rhinolophus euryale*	AM949568						
	C. submersum	IMI 379911*	River sediment	AJ131686						
	C. sulfureum	CBS 634.79	Cheese rind	AJ390387						
	C. tropicum	UAMH 691		AJ131685						
	C. undulatum	IMI 375884*	Soil	AJ007845						
	C. vallenarense	CBS 627.83, ATCC 64421*	Keratinous substrate	AJ390389						

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
Fig. 4	*C. vespertilium*	RV 27093		AJ007846						
	C. zonatum	IFM 51122	Soil	AB219229						
	Corynascus sepedonium	FMR 5593		AJ439447						
	Thermothelomyces thermophila	H127-1	Soil	JX868606						
	Nigrograna cangshanensis	HKAS 83978*	Submerged wood	KY511063		KY511066	-	KY511064		
	N. fuscidula	CBS 141476	Dead branches of *Sambucus nigra*	KX650547		KX650522	KX650576	KX650547		
		CBS 141556*	Dead branches and twigs of *Sambucus nigra*	KX650550		KX650525	-	KX650550		
	N. locuta-pollinis	**LC11685 = LF1889***	**Hive-stored pollen of *B. campestris***	**MF939601**		**MF939613**	**MF939610**	**MF939583**		
		LC11690 = LF2070	**Hive-stored pollen of *B. campestris***	**MF939602**		**MF939614**	**MF939611**	**MF939584**		
		LC11691 = LF2071	**Hive-stored pollen of *B. campestris***	**MF939603**		**MF939615**	**MF939612**	**MF939585**		
	N. mackinnonii	CBS 110022	Man, mycetoma of patient	KF015653		KF407985	KF015704	GQ387614		
		CBS 674.75*	Man, black grain mycetoma	NR_132037		KF407986	KF015703	GQ387613		
		E9303e	*Malvaviscus concinnus*	JN545759		LN626673	LN626666	LN626681		
	N. mycophila	CBS 141478*	Twigs of *Acer campestre*	KX650553		KX650526	-	KX650553		
		MF6	*Corylus avellana*	KX650554		KX650527	-	KX650554		
		CBS 141483	Twigs of *Acer pseudoplatanus*	KX650555		KX650528	KX650577	KX650555		

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	TUB	TEF	RPB2	28S	ACT	CAL
	N. norvegica	CBS 141485*	Twig of *Tilia platyphyllos*	KX650556		-	KX650578	KX650556		
	N. obliqua	CBS 141475	*Sambucus racemosa*	KX650558		KX650530	KX650579	KX650558		
		CBS 141477*	Twigs of *Salix caprea*	KX650560		KX650531	KX650580	KX650560		
		MRP	*Ribes uva-crispa*	KX650561		KX650532	KX650581	KX650561		
	Occultibambusa fusispora	MFLUCC 11-0127	Bamboo	KU940125		KU940195	KU940172	KU863114		
Fig. 5	*Trichoderma afarasin*	CBS 130742	Cola altissima trunk endophyte	FJ442259		FJ463400	FJ442778		FJ442468	FJ442312
		CBS 130755*	Soil	AY027784		AF348093	-		FJ442536	FJ442388
	T. afroharzianum	CBS 124620*	On basidioma of Moniliophthora roreri on fruit of Theobroma	FJ442265		FJ463301	FJ442691		-	FJ442370
		CBS 124614	On basidioma of Moniliophthora roreri on fruit of Theobroma	FJ442233		FJ463298	FJ442709		-	FJ442372
		IMI 393972	Soil	AY027781		AF348106	-		FJ442535	AF442882
	T. aggressivum	CBS 100525	Mushroom compost	AF057600		AF348095	AF545541		FJ442433	AF442859
	T. atrobrunneum	CBS 130440	Soil	FJ442273		FJ463360	FJ442724		FJ442492	FJ442329
		CBS 130429	Decaying Pinus sylvestris	AF443926		AF443943	FJ442735		FJ442525	AF442886
		CBS 548.92*	Decorticated wood of Fagus sp.	AF443924		AF443942	-		FJ442528	AF442883
	T. camerunense	CBS 137272*	Soil	AY027780		AF348107	-		FJ442537	AF442875

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	**TUB**	**TEF**	**RPB2**	**28S**	**ACT**	**CAL**
		G.J.S. 99-231	Soil	AY027783		AF348108	-		FJ442538	AF442874
	T. endophyticum	CBS 130729*	Theobroma gileri trunk endophyte	FJ442243		FJ463319	-		FJ442445	FJ442292
		CBS 130730	Theobroma gileri trunk endophyte	FJ442242		FJ463314	FJ442721		FJ442446	FJ442293
	T. guizhouense	DAOM 231435	Soil	EF191296		EF191321	-		-	FJ577721
		CBS 131803*	Soil	JN191311		JN215484	JQ901400		-	-
	T. harzianum	CBS 226.95*	Soil	AJ222720		AF348101	AF545549		FJ442567	AF442864
		CBS 1304452 = G.J.S. 04-71	Castanea sativa twig endophyte	FJ442673		FJ463396	FJ442779		FJ442494	FJ442369
		CBS 130496 = G.J.S. 05-107	*Ricinus communis* stem endophyte	FJ442679		FJ463329	FJ442708		FJ442569	FJ442333
	T. inhamatum	CBS 273.78*	Soil	FJ442680		AF348099	FJ442725		FJ442561	AF442891
		G.J.S. 09-697	Soil	-		KP115272	-		-	-
	T. lentiforme	CBS 130726	*Theobroma cacao* trunk endophyte	FJ442681		FJ851872	FJ442786		FJ442440	FJ442287
		CBS 100542*	Decorticated wood	AF469189		AF469195	-		AF469193	AF469191
	T. lixii	CBS 110080*	Decayed *Ganoderma basidiocarp*	AF443920		AF443938	-		FJ442533	AF442872
	T. neotropicale	G.J.S. 11-185*	*Hevea guianensis* trunk endophyte	HQ022407		HQ022771	-		KP115268	KP115279
	T. pollinicola	**LC11682 = LF1542***	**Newly-collected pollen of *B. campestris***	**MF939592**		**MF939619**	**MF939604**		-	**MF939586**
		LC11686 = LF2050	**Newly-collected pollen of *B. campestris***	**MF939593**		**MF939620**	**MF939605**		-	**MF939587**

Table S1 Continued.

Figs	Species	Strain number[1]	Host	GenBank accessions[2]						
				ITS	**TUB**	**TEF**	**RPB2**	**28S**	**ACT**	**CAL**
		LC11687 = LF2051	**Newly-collected pollen of *B. campestris***	**MF939594**		**MF939621**	**MF939606**		-	**MF939588**
	T. pyramidale	CBS 135574*	Olea europaea	-		KJ665699	KJ665334		-	-
		S119	*Quercus pubescens*	-		KJ665696	-		-	-
	T. rifaii	CBS 130745	*Theobroma cacao* trunk endophyte	FJ442621		FJ463321	FJ442720		FJ442471	FJ442315
	T. simmonsii	CBS 123765	Decaying bark	AF443918		AF443936	FJ442798		FJ442524	AF442870
		CBS 130431*	Decaying bark	AF443917		AF443935	FJ442757		FJ442526	AF442869

[1] ATCC: American Type Culture Collection, Manassas, VA, USA; CBS: Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; FMR: Facultat de Medicina i Ciències de la Salut, Reus, Spain; IFO: Institute for Fermentation, Osaka, Japan; IMI: Culture collection of CABI Europe UK Center, Egham, UK; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai Thailand; LC: working collection of Lei Cai, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; UAMH: Centre for Global Microfungal Biodiversity, University of Toronto, Toronto, Canada;
[2] Sequences newly generated in this study are indicated in bold.