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FULL PAPER

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The teleomorph of *Leptographium yunnanense*, discovered in crosses among isolates from Thailand, China, and Japan

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Abstract A teleomorph was discovered in crosses among isolates of *Leptographium yunnanense* isolated from *Pinus* spp. originating in Thailand, China, and Japan. The ascocarps are black, globose to subglobose, and lacking necks. Ascospores are hyaline, 1-celled, surrounded by hyaline sheaths, and appear cucullate in side view, quadrangular in face view, and triangular in end view. Three species were known to have teleomorphs morphologically similar to the present fungus. However, their anamorphs were distinguishable from *L. yunnanense*. Thus, this teleomorph is described as *Grosmannia yunnanensis*.

Key words *Grosmannia* · *Leptographium yunnanense* · Mating test · New species · *Pinus* · Teleomorph

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Introduction

Leptographium yunnanense X.D. Zhou, K. Jacobs, M.J. Wingf. & M. Morelet was described by Zhou et al. (2000) from the bark beetle *Tomicus piniperda* L. infesting *Pinus yunnanensis* Franchet in Yunnan, southwestern China, although the bark beetle is now recognized as a distinct species, *Tomicus* sp. (Duan et al. 2004). In Japan, the fungus was isolated from *T. piniperda* infesting Japanese red pine, *P. densiflora* Sieb. & Zucc., and reported as *Graphium* sp. (Masuya et al. 1998). Later, it was identified as *L. yunnanense* based on morphological comparison with Chinese isolates of *L. yunnanense* (unpublished data). During a survey of ophiostomatoid fungi in northern Thailand in 2003 and 2004, *L. yunnanense* was isolated from logs of *P. khasya* Royle (= *P. kesiya* Royle ex Gordon) infested by the bark beetles *Polygraphus major* Stebbing and *Cryphalus kesiyae* Browne (Yamaoka et al. 2007). The fungus appears to adapt to an association with bark beetles infesting *Pinus* species belonging to subsection *Sylvestres* of the section *Pinus*, distributed in tropical and temperate monsoon areas of Asia (Yamaoka et al. 2007).

Leptographium Lagerb. & Melin is one of the anamorph genera associated with *Ophiostoma* Syd. & P. Syd. sensu lato (Harrington 1987; Jacobs and Wingfield 2001). The teleomorphs were transferred to *Grosmannia* Goid., based on phylogenetic analyses (Zipfel et al. 2006). Although numerous *Leptographium* species have been connected to their *Grosmannia* teleomorphs, there are many species, including *L. yunnanense*, lacking a known teleomorph (Jacobs and Wingfield 2001).

Some isolates of *L. yunnanense* obtained from northern Thailand produced protoperithecium-like structures on agar or on bark of Japanese red pine placed on agar (Yamaoka, unpublished data). This finding raised the question whether the fungus might be heterothallic and whether a mature teleomorph might result from crosses between sexually compatible isolates. In this study, we undertook mating experiments with isolates from China, Thailand, and Japan and successfully induced the teleomorph of

L. yunnanense. The teleomorph is described as a new species.

Materials and methods

Isolates used for mating test

Isolates of *L. yunnanense* acquired from China, Thailand, and Japan (Table 1) were used for mating tests. Eight isolates [ThP2-1, ThP2-2 (= BCC 20302), ThP7-1, ThP7-2 (= BCC 20304), ThP2-11 (= BCC 20301), ThP2-12, ThP7-11 (= BCC 20303), and ThP7-12] were collected from galleries of the bark beetle, *Polygraphus* (*Pol.) major*, collected in northern Thailand in 2003 and 2004. These insects had infested small trees or twigs (about 3 cm in diameter) of *Pinus khasya* and were associated with stained sapwood (Yamaoka et al. 2007).

Four Japanese isolates [MCC-95 (= JCM 14938), MCC-96 (= JCM 11873, MAFF410952), MCC-97 (= JCM 14939), and MCC-100 (= JCM 11874, MAFF410953)] made from adult beetles and a gallery of *T. piniperda* in *P. densiflora* collected in Tsukuba, Ibaraki, Central Honshu, Japan, also were used in the study. Two Chinese isolates, CMW5152 and CMW5304, from *T. piniperda* infesting *P. yunnanensis* Franchet in Yunnan, southwestern China, were also used. The isolate CMW 5304 is the ex-type strain of the holotype of the fungus (PREM 56579).

Single ascospore isolates were established from ascocarps resulted from a mating between ThP2-1 and ThP7-2, ThP2-2 and ThP7-2, and MCC-95 and MCC-96. A mass of ascospores taken from an ascocarp was suspended in 1 ml 10% dimethylsulfoxide (DMSO) in a plastic Petri dish. The ascospore suspension was diluted with sterilized distilled water and spread on the surface of 1.5% water agar plates. After incubation at 17°C in the dark for 2 to 3 days, the plates were inspected under a light microscope (150 × magnification) to locate single germinating ascospores sufficiently removed from others to be transferred easily. Ten single ascospore isolates (A-1 to A-10) were established from an ascocarp produced between ThP2-1 and ThP7-2. Seven single ascospore isolates (B-1 to B-7) were also established from those produced between ThP2-2 and ThP7-2. Ten single ascospore isolates (M-1 to M-10) were established from an ascocarp produced between MCC-95 and MCC-96.

In the specimens of *P. khasya* collected form northern Thailand in 2004, ascocarps were found embedded in phloem tissue associated with galleries of *Pol. major*. Isolations (ThP2-11, ThP2-12, ThP7-11, and ThP7-12) were

Table 1. Isolates used in the mating tests

Isolate	Origin	Locality of collection	Date of collection
ThP2-1	Sapwood of *Pinus (P.) khasya* colonized by *Polygraphus (Pol.) major*	Parking area of Sirithan Water Fall, Northern Thailand	24 Sept., 2003
ThP2-2 (= BCC 20302)	Sapwood of *P. khasya* colonized by *Pol. major*	Parking area of Sirithan Water Fall, Northern Thailand	24 Sept., 2003
ThP2-11 (= BCC 20301)	Sapwood of *P. khasya* colonized by *Pol. major*	Doi Suthep, Northern Thailand	21 Nov., 2004
ThP2-12	Sapwood of *P. khasya* colonized by *Pol. major* and *Cryphalus kesiyae*	Doi Suthep, Northern Thailand	21 Nov., 2004
ThP7-1	Gallery of *P. major* in *P. khasya*	Parking area of Sirithan Water Fall, Northern Thailand	24 Sept., 2003
ThP7-2 (= BCC 20304)	Gallery of *P. major* in *P. khasya*	Parking area of Sirithan Water Fall, Northern Thailand	24 Sept., 2003
ThP7-11 (= BCC 20303)	Gallery of *P. major* in *P. khasya*	Doi Suthep, Northern Thailand	21 Nov., 2004
ThP7-12	Sapwood of *P. khasya* invaded by *P. major*	Doi Suthep, Northern Thailand	21 Nov., 2004
A-1–A-10	Single ascospore isolate from an ascocarp resulted from mating between ThP2-1 and ThP7-2		
B-1–B-7	Single ascospore isolate from an ascocarp resulted from mating between ThP2-2 and ThP7-2		
C-1–C-10	Single ascospore isolate from an ascocarp produced on phloem of *P. khasya* near galleries of *P. major* collected in Doi Suthep, Northern Thailand on 21 Nov., 2004		
MCC-95 (= JCM 14938)	Adult beetle of *Tomicus piniperda* in *P. densiflora*	Tsukuba, Ibaraki, Japan	4 June, 1995
MCC-97 (= JCM 14939)	Adult beetle of *T. piniperda* in *P. densiflora*	Tsukuba, Ibaraki, Japan	18 May, 1995
MCC-96 (= JCM 11873, MAFF 410952)	Adult beetle of *T. piniperda* in *P. densiflora*	Tsukuba, Ibaraki, Japan	2 June, 1995
MCC-100 (= JCM 11874, MAFF 410953)	Gallery of *T. piniperda* in *P. densiflora*	Tsukuba, Ibaraki, Japan	7 June, 1995
M-1–M-10 [M-1 (= JCM 14940), M-2 (= JCM 14941)]	Single ascospore isolate from an ascocarp resulted from mating between MCC-95 and MCC-96		
CMW5152	*T. piniperda* infesting *P. yunnanensis*	YiNiang, Yunnan, China	Aug., 1995
CMW5304	*T. piniperda* infesting *P. yunnanensis*	Vimen, Yunnan, China	Mar., 1997

made from these collections, and single ascospore isolates (C-1 to C-10) were also established from a single ascocarp produced in phloem tissues, using the same method described above.

Some of the cultures used in the present study were deposited in the culture collection in the National Institute of Agrobiological Sciences (NIAS) Genebank, the Ministry of Agriculture, Forestry and Fishery (MAFF), Tsukuba, Japan, in the Japan Collection of Microorganisms (JCM), Wako, Japan and in Biotec Culture Collection (BCC), Pathumthani, Thailand.

Mating tests

Small agar blocks (about 5 × 5 mm) from the colonies of two isolates grown on 2% malt extract Ebios agar [2% MEBA; 20 g Difco malt extract, 1 g Ebios (Brewer's yeast preparation; Tanabe, Osaka), 15 g agar/1000 ml distilled water] were placed approximately 1 cm apart on fresh 2% MEBA in 9-cm plates. After the plates were incubated at 17°C in the dark for 10 days, a few small pieces (about 20 mm × 5 mm × 3 mm) of autoclaved Japanese red pine bark were placed perpendicular to the zone where the two colonies touched, to smear and mix spores of the two cultures. The plates were incubated for additional 3 weeks to induce ascocarp production.

Morphological observations

Ascocarps and ascospores resulted from the mating of isolates were used for morphological characterization. In addition, ascocarps produced in phloem tissues of *P. khasya* from northern Thailand were used to describe structures as they occur naturally. Ascocarps were mounted on glass slides in Polyvinyl alcohol or 1% lacto-fuchsin and studied under an Olympus BHS-N differential interference contrast microscope. Anamorphs of the isolates were also observed by the same method.

Ascospores of the present fungus had three distinct views. To describe shapes of the ascospores, we followed the method used by Griffin (1968). The three different views of the ascospores were referred to as end, side, and face views. The end view is parallel to the long axis of the spore and the other two views are at right angles to the long axis. The side view is asymmetrical compared with the face view and at right angles to the face view.

Dried specimens of cultures including teleomorph structures were deposited with the Herbarium of the Life and Environmental Sciences, University of Tsukuba (TSH), the National Science Museum (TNS), Tsukuba, Japan, Department of Plant Pathology, Chiang Mai University, Chiang Mai, Thailand, Institute of Forest Ecology, Environment and Protection (CXY), Chinese Academy of Forestry, Beijing, China, and the Forestry and Agricultural Biotechnology Institute (PREM), University of Pretoria, Pretoria, South Africa.

Results

Mating tests

Results of mating tests among 25 isolates obtained from Thailand are shown in Table 2. A total of 161 crosses were performed, and 47 crosses resulted in production of ascocarps on small pieces of autoclaved bark of *P. densiflora* placed on 2% MEBA and on the surface of the medium near the pieces of bark. Two mating types were found among the isolates (Table 2), which were tentatively designated as "A" and "a". When the opposite mating type strains were paired, ascocarps were produced after 3 to 4 weeks of incubation at 17°C. The mating type of isolate B-4 was regarded as "a", although it did not mate with most of the isolates examined. It was not possible to determine the mating type of isolate B-5 because it did not mate with any isolates examined (Table 2).

Japanese isolates MCC-95, MCC-96, MCC-97, and MCC-100 and ten single ascospore isolates were also assigned mating types based on the mating tests (Table 3). Single ascospore isolates established from an ascocarp produced in phloem tissues of *P. khasya* also mated with the Japanese isolates (Table 4). The two Chinese isolates (CMW 5152 and CMW 5304) were considered as mating type "A" and "a", respectively, even though a mating test using these two isolates was unsuccessful (Table 5).

Morphological observations

Ascocarps resulting from the mating tests were black and globose to subglobose without necks. Ascospores were one-celled and surrounded by hyaline sheaths, appearing cucullate in side view. Dimensions of ascocarps and ascospores are shown in Table 6. There were no observable differences in morphology of teleomorphs produced on artificial media and those on collected on natural substrates in the field.

Taxonomy

Grosmannia yunnanensis Yamaoka, Masuya & M.J. Wingf., sp. nov. Figs. 1–4

Anamorph: *Leptographium yunnanense* X.D. Zhou, K. Jacobs, M.J. Wingf. & M. Morelet, Mycoscience 41:576 (2000).

Ascocarpia in medio agari vel in cortice in superficie medii posito, superficialia vel immersa atra, globosa vel subglobosa, 199–365 × 206–413 µm diametro; collum absens. Asci evanescentes. Ascosporae hyalinae, unicellulares, cucullatae a latere visae, quadrangulares a facie visae, triangulares ab apice visae (4.5–)5.5–7.5(–8.0) × 3.0–4.5 µm.

Holotypus: TNS-F-12520, dried dual cultures MCC-95 (= JCM14938) × CMW5304 grown on 2% malt extract Ebios agar with pieces of autoclaved bark of *Pinus densiflora* (2% MEBAB) at 17°C.

Table 2. Results of mating tests using isolates from Thailand

Isolate	ThP 2-1	ThP 2-2	ThP 2-11	ThP 2-12	ThP 7-1	ThP 7-2	ThP 7-11	ThP 7-12	A-1	A-2	A-4	A-5	A-6	A-8	A-9	A-10	A-3	A-7	B-3	B-6	B-1	B-7	B-2	B-4	B-5	Mating types
ThP 2-1	–	–			+	+				–	–						+	+	–	–				–		A
ThP 2-2		–	–	–	+	+	+	+		–	–						+	+	–	–				–		A
ThP 2-11			–			+																				A
ThP 2-12				–		+																				A
ThP 7-1					–	–				+	+						–	–	+	+				–		a
ThP 7-2						–	–	–		+	+						–	–	+	+				–		a
ThP 7-11							–																			a
ThP 7-12								–																		a
A-1									–	–	–	–	–	–	–	–	+	+								A
A-2										–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	A
A-4											–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	A
A-5												–	–	–	–	–	+	+								A
A-6													–	–	–	–	+	+								A
A-8														–	–	–	–	+								A
A-9															–	–	+	+								A
A-10																–	+	+								A
A-3																	–	–	+	+	+	+	+	–	–	a
A-7																		–	+	+	+	+	+	–	–	a
B-3																			–	–	–	–	–	+	–	A
B-6																				–	–	–	–	+	–	A
B-1																					–	–	–	–	–	A
B-7																						–	–	–	–	A
B-2																							–	–	–	A
B-4																								–	–	a?
B-5																									–	?

+, production of ascocarps; –, no production of ascocarps

Table 3. Results of mating tests using isolates from Japan and tester isolates from Thailand

Japanese isolates	Japanese isolates											Tester isolates from Thailand								Mating types
	MCC-95	MCC-97	MCC-96	MCC-100	M-1	M-3	M-5	M-6	M-7	M-2	M-4	M-8	M-9	M-10	Mating type A			Mating type a		
															ThP 2-2	A-4	B-3	B-6	ThP 7-2	A-3	A-7	B-4	
MCC-95	–	–	+	+	–		–		–	+			+	+	–	–	–	–	+	+	+	+	A
MCC-97		–	+	+																			A
MCC-96			–	–	+		+		–	–			–	–	+	+	+	+	–	–	–	–	a
MCC-100				–					+														a
M-1					–	–	–		–	+	+	+	+	+	–	–	–	–	+	+	+	+	A
M-3						–	–		–	+	+	+	+	+									A
M-5							–		–	+	+	+	+	+	–	–	–	–	+	+	+	+	A
M-6								–	–	+	+	+	+	+									A
M-7									–	+	+	+	+	+	–	–	–	–	+	+	+	+	A
M-2										–	–	–	–	–	+	+	+	+	–	–	–	–	a
M-4												–	–	–									a
M-8													–	–									a
M-9														–	+	+	+	+	–	–	–	–	a
M-10															+	+	+	+	–	–	–	–	a

Table 4. Results of mating tests using single ascospore isolates from Thailand and tester isolates from Japan

Thailand isolates	Tester isolates from Japan		Mating types
	Mating type A	Mating type a	
	M1	M2	
C-1	–	+	A
C-6	–	+	A
C-9	–	+	A
C-2	+	–	a
C-3	+	–	a
C-4	+	–	a
C-5	+	–	a
C-7	+	–	a
C-8	+	–	a
C-10	+	–	a

Etymology: *yunnanensis* from specific epithet of the anamorph species name.

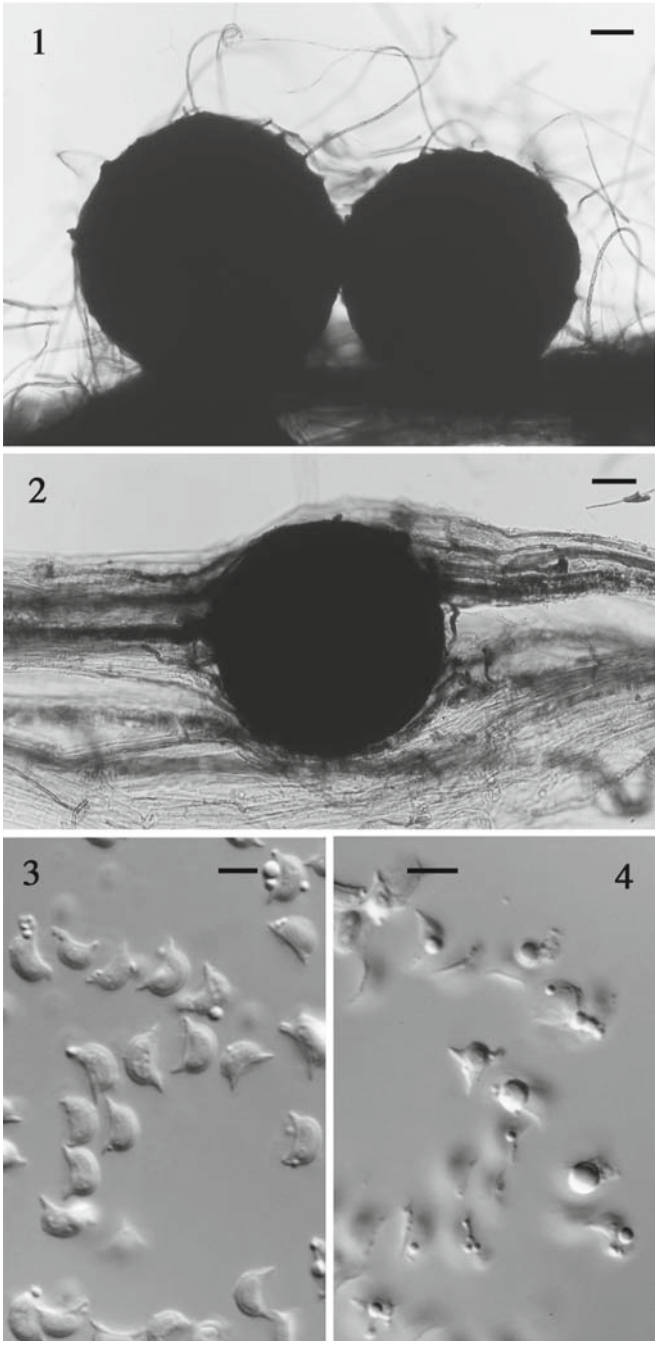
Ascocarps (Figs. 4, 5) superficial or embedded in agar medium and on bark placed on the surface of the agar, black, globose to ellipsoidal, 199–365 × 206–413 µm in diameter, lacking a neck, wall of the ascocarp composed of thick-walled polygonal or irregularly shaped cells, 12–27 × 10–17 µm. Asci evanescent, not seen. Ascospores (Figs. 6, 7) hyaline (white in mass), one-celled surrounded by hyaline sheaths, appearing cucullate in side view, quadrangular in face view, triangular in end view, (4.5–)5.5–7.5(–8.0) × 3.0–4.5 µm including sheath about 0.5 µm in thickness.

Anamorph: *Leptographium yunnanense*.

Dried specimens deposited: TNS-F-12520 (holotype) and TSH-C454 (= CXY 1200, PREM 59428), dried dual cultures MCC-95 (= JCM14938) × CMW5304 grown on 2% malt extract Ebios agar with pieces of autoclaved bark of *P. densiflora* (2% MEBAB) at 17°C; TSH-C444, dried dual cultures MCC-97 (= JCM14939) × MCC100 (= JCM 11874) grown on 2% MEBAB at 17°C; TNS-F-12519 and TSH-C445, dried dual cultures MCC-95 × MCC96 (= JCM 11873) grown on 2% MEBAB at 17°C; TSH-C446, dried dual cultures MCC-97 × B-4 grown on 2% MEBAB at 17°C; TSH-C447, dried dual cultures B-6 × MCC-96 grown on 2% MEBAB at 17°C; TSH-C442, dried dual cultures ThP2-1 × ThP7-2 (= BCC 20304) grown on 2% MEBAB at 17°C; TNS-F-12518 and TSH-C443, dried dual cultures ThP2-2 (= BCC 20302) × ThP7-2 grown on 2% MEBAB at 17°C; TSH-C457, dried dual cultures ThP2-2 × MCC-96 grown on 2% MEBAB at 17°C; TSH-C456 (= CXY 1202. PREM 59430), dried dual cultures ThP2-2 × CMW5304 grown on 2% MEBAB at 17°C; TSH-C455 (= CXY 1201, PREM 59429), dried dual cultures CMW5152 × MCC-96 grown on 2% MEBAB at 17°C; TSH-C460, dried dual cultures M-1 (= JCM 14940) × M-2 (= JCM 14941) grown on 2% MEBAB at 17°C; TSH-C462, dried bark of *Pinus khasya* near a gallery of *Polygraphus major,* Doi Suthep, Northern Thailand, 21 Nov. 2004, by Y. Yamaoka.

Table 5. Results of mating tests using isolates from Thailand, Japan, and China

Chinese isolates	Chinese isolates		Tester isolates from Thailand and Japan												Mating types
			Mating type A							Mating type a					
	CMW 5152	CMW 5304	ThP 2-2	A-4	B-3	B-6	MCC-95	M-1	M-5	M-7	ThP 7-2	A-3	A-7	B-4	MCC-96	M-2	M-9	M-10	
CMW 5152	–	–	–	–	–	–	–	–	–	–	+	–	+	–	+	+	+	+	A
CMW 5304		–	+	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	a



Figs. 1–4. *Grosmannia yunnanensis*. **1** Ascocarps produced on the surface of the pine bark placed on 2% MEBA. **2** Ascocarp produced in pine bark placed on 2% MEBA. **3**, **4** Ascospores. *Bars* **1**, **2** 50 µm; **3**, **4** 5 µm

Discussion

In this study, we describe the teleomorph of *Leptographium yunnanense* as *Grosmannia yunnanensis*. This discovery followed a chance observation of ascomata in cultures from areas other than China, where the anamorph of the fungus was originally collected. The appearance of ascomata allowed us to produce single ascospore cultures and to dem-

Table 6. Morphological comparison of *Grosmannia yunnanensis* with morphologically similar *Grosmannia* and *Ophiostoma* species

Characteristics	*Grosmannia yunnanensis*	*Grosmannia aurea* (Jacobs and Wingfield 2001)	*G. robusta* (Jacobs and Wingfield 2001)	*O. trinacriforme* (Jacobs and Wingfield 2001)	*Leptographium yunnanense* (Zhou et al. 2000)
Perithecium					
Diameter of base (µm)	199–365 × 206–413	300–400	200–400	(225–)260(–345)	
Ascospore					
Form	Hat-shaped, cucullate	Hat-shaped, cucullate*	Reniform, cucullate*	Hat-shaped	
Size (µm) including sheath	(4.5–)5.5–7.5(–8.0) × 3.0–4.5	3–6 × 2–4 4.2–5.8 × 2.6–3.7*	3–5 × 2–3 3.2–5.3 × 1.6–3.2*	(3.6–)4.8(–5.4) × (1.8–)2.2(–3.1)**	
Conidiophore					
Length (µm)	80–240	(100–)369–770(–1350)	31–112(–116)	(125–)207–377(–662)	74–227(–233)
Stipe					
Length (µm)	8–80	(35–)150–490(–785) Up to 442*	9–36(–39) 14–46*	(70–)145–297(–587)	11–66(–112)
No. of septa	0–3	3–19	0–2, 0–1*	3–10	0–4
Conidiogenous apparatus					
Length (µm)	50–210	(35–)135–350(–900) Up to 600*	20–67(–70) 29–70*	(35–)58–72(–95)	(40–)83–88(–127)
Conidia					
Form	Oblong to obovoid with truncate bases and rounded apices	Oblong with truncate bases and rounded apices	Oblong with truncate bases and rounded apices	Oblong to obovoid with truncate bases and rounded apices	Oblong to obovoid
		Elongate, slightly falcate*[a], cylindrical to obclavate*[b]	Cylindrical*[c], globose-ovoid*[d]	Cylindrical to ellipsoid-ovoid**	
Size (µm)	3–13 × 2–6	(5–)7–9(–12) × 2–4 Up to 5.0 × 29*[a] 2.5–7.0 × 1.8–2.5*[b]	3–7 × 2–6 5.3–11.0 × 3.2–4.3*[c] 8.0 × 5.5*[d]	4–6(–7) × 1–2 4–7.4 × 1.6–3.8**	(4–)7–8(–11) × 2–6
Conidial droplet					
Color	Hyaline at first, becoming cream-colored	Hyaline at first, becoming amber-yellow	Hyaline at first, becoming cream-colored	Hyaline to cream-colored at first, becoming amber	Hyaline at first, becoming cream-colored

Sources: * Robinson-Jeffrey and Davidson (1968); ** Parker (1957)
Conidia form and size with the same alphabet (a, b, c, d) were correspond each other

onstrate that the fungus is heterothallic and that two mating types are required for sexual outcrossing.

Classification of the teleomorph of *L. yunnanense* in the genus *Grosmannia* is consistent with the recent phylogenetic treatment, showing that species of *Leptographium* (or with *Leptographium* anamorphs) form a monophyletic lineage distinct from *Ophiostoma* (Zipfel et al. 2006). *Grosmannia* was resurrected to include those species of *Ophiostoma* sensu lato with *Leptographium* anamorphs. *Grosmannia yunnanensis* can be classified in this genus because of its *Leptographium* anamorph. Its ascospores with cucullate sheaths also represent a morphological feature restricted to species of *Grosmannia*.

Morphological characteristics of the teleomorph of *L. yunnanense* as well as the other species of *Grosmannia* and *Ophiostoma* with ascocarps lacking necks and having cucullate ascospores are shown in Table 2. Ascocarp dimensions and ascospore shapes are similar for all four species. However, ascospores of the teleomorph of *L. yunnanense* are larger than those of the other species. The anamorph of *L. yunnanense* is morphologically most similar to that of *G. robusta* (R.C. Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., but conidiophores and conidia of this fungus are larger than those of *G. robusta*. Conidiophores of *L. yunnanense* differ morphologically from those of *G. aurea* (R.C. Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. and of *O. trinacriforme* (A.K. Parker) T.C. Harr.

Kim et al. (2005) conducted molecular phylogenetic analyses of some species of *Leptographium*, mainly those associated with *T. piniperda*. Comparison of DNA sequences of rDNA, β-tubulin, and actin showed that *L. yunnanense* was clearly distinguishable from *G. aurea* and *G. robusta*. *Ophiostoma trinacriforme* was not included in their analyses, and its relatedness to *G. yunnanensis* is unclear.

Based on mating tests, all the Japanese isolates of *G. yunnanensis* were clearly separated into two mating types. All Japanese isolates mated with the isolates of the opposite mating type. Most isolates from Thailand and China also grouped into two mating types. Thus, we suggest that the mating type of *G. yunnanensis* is bipolar. However, some isolates from Thailand and China (isolates A-3, A-4, B-4, CMW 5152, and CMW 5304) did not mate with all isolates considered to be a different mating type, although they were able to mate with Japanese isolates. This finding could reflect a lack of fertility in some isolates.

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