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FULL PAPER

Yuichi Yamaoka · Hayato Masuya · Wen-Hsin Chung
Hideaki Goto · Chaiwat To-Anun · Seiji Tokumasu

Two species of *Leptographium* isolated from blue-stained sapwood of *Pinus khasya* and bark beetles in Thailand

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Abstract Two species of *Leptographium* were isolated from blue-stained sapwood of *Pinus khasya* and bark beetle galleries in pine trees near Chiang Mai, Thailand. Based on morphological observations, these two species were identified as *L. pini-densiflorae* and *L. yunnanense*. This is the first record of these fungi in Thailand. *Leptographium yunnanense* appeared to be associated with *Polygraphus major*.

Key words *Leptographium pini-densiflorae* · *Leptographium yunnanense* · New record · *Pinus khasya* · *Polygraphus major*

Introduction

The genus *Leptographium* includes important pathogens that kill trees and seedlings and cause blue-stain of sapwood (Jacobs and Wingfield 2001). *Leptographium* species have been recorded in America, Africa, Asia, Australia, and Europe, but information about *Leptographium* species in Southeastern Asia is limited. Only three species are reported from this area. *Leptographium elegans* M. J. Wingf., Crous & Tzean, *L. hugesii* K. Jacobs, M. J. Wingf. & T. C. Harr., and *L. pineti* K. Jacobs & M. J. Wingf. have been reported from Taiwan (Wingfield et al. 1994), Vietnam (Jacobs et al. 1998), and Indonesia (Jacobs et al. 2000), respectively. In 2003 and 2004, we conducted a survey of ophiostomatoid fungi in Northern Thailand as part of a cooperative research project on fungal diversity in the tropical monsoon areas of Asia. In the present article, we report two species of *Leptographium* new to Thailand that were isolated from *Pinus khasya* Royle (= *P. kesiya* Royle ex Gordon) invaded by bark beetles.

Y. Yamaoka (✉) · W.-H. Chung[1]
Graduate School of Life & Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan
Tel. +81-29-853-6708; Fax +81-29-853-6708
e-mail: yyamaoka@sakura.cc.tsukuba.ac.jp

H. Masuya
Forestry and Forest Products Research Institute, Ibaraki, Japan

H. Goto
Forestry and Forest Products Research Institute, Kyushu Research Center, Kumamoto, Japan

C. To-Anun
Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand

S. Tokumasu
Sugadaira Montane Research Center, University of Tsukuba, Nagano, Japan

Present address:
[1]Department of Plant Pathology, National Chung Hsing University, Taiwan, ROC
This is contribution No. 206, Laboratory of Plant Parasitic Mycology, Graduate School of Life & Environmental Sciences, University of Tsukuba

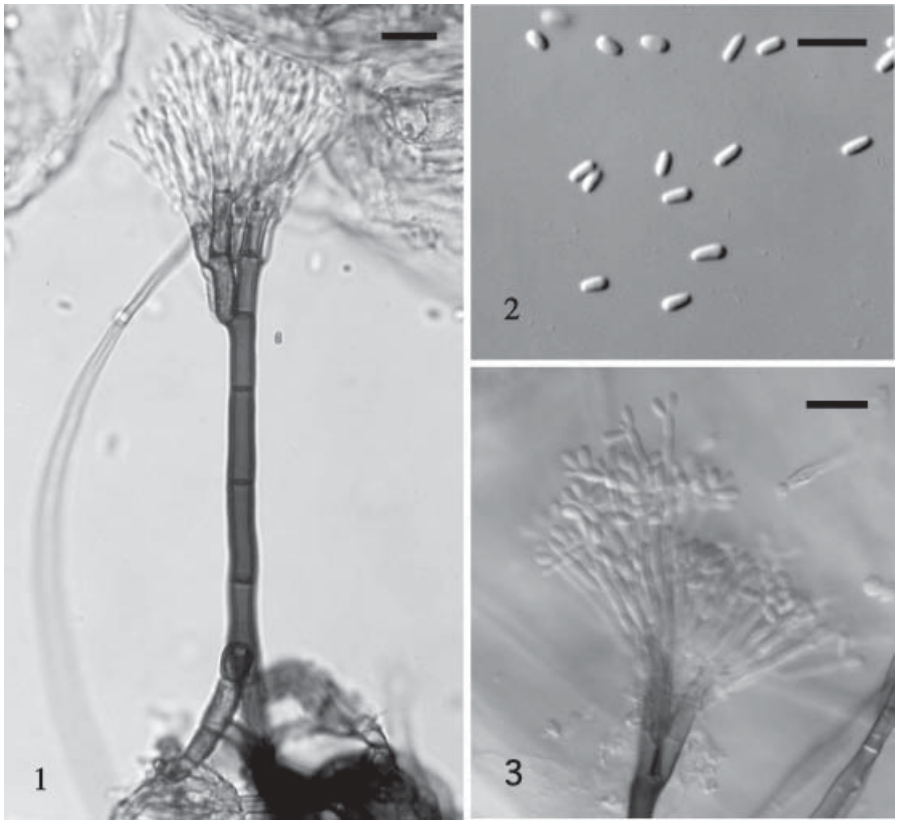
Materials and methods

Four samples were collected from dying or dead *P. khasya* invaded by bark beetles near Chiang Mai, Northern Thailand (Table 1). Sample numbers 1 and 3 were a small dying tree and a fresh fallen twig. They were mainly invaded by *Polygraphus major* Stebbing and also invaded by *Coccotrypes longior* (Eggers) and *Cryphalus kesiyae* Browne, respectively. Sample number 2 was a large dead standing tree. Weevils, *Stenoscelis* sp. (Curculionidae), were collected from under the bark of the tree. Sample number 4 was a dead fallen tree of *P. khasya* invaded by bark beetles. Unfortunately, adult beetles were not collected from this sample, so the invading beetles could not be identified.

Ophiostomatoid fungi were isolated from adult beetles in egg galleries, egg gallery walls, and stained sapwood under galleries. Methods for isolation were the same as previously described by Yamaoka et al. (2004). Cultures used for identification were grown on 2% malt extract agar (2% MA; 20 g malt extract, 15 g agar/1000 ml distilled water) and 2% malt extract Ebios agar [2% MEBA; 20 g malt extract, 1 g Ebios (Brewer's yeast preparation; Tanabe), 15 g agar/1000 ml distilled water]. Small (about 1 cm × 5 mm × 3 mm) pieces of autoclaved bark from Japanese red pine (*Pinus*

Table 1. Samples used for isolation studies

Sample no.	Samples	Associated beetles	Locality of collection	Date of collection
1	Small tree of *Pinus khasya* invaded with bark beetles	*Polygraphus major, Coccotrypes longior*	Parking area of Sirithan Water Fall	24 Sept. 2003
2	Dead standing large tree of *P. khasya*	*Stenoscelis* sp.	Queen Sirikit Botanic Garden	27 Sept. 2003
3	Fresh-fallen twig of *P. khasya* invaded by bark beetles	*Polygraphus major, Cryphalus kesiyae*	Doi Suthep	21 Nov. 2004
4	Dead fallen tree of *P. khasya* invaded by bark beetles	Unknown	Queen Sirikit Botanic Garden	17 Nov. 2004



Figs. 1–3. *Leptographium pini-densiflorae* (BCC 20300). **1** Conidiophore. **2** Conidia. **3** Conidiogenous apparatus. *Bars* 10 µm

densiflorae Siebold & Zucc.) were later added to the plates.

Frequency of occurrence of each species of fungi from beetles in the galleries, egg galleries, and stained sapwood was computed using the following formula:

$$F = (NF/NT) \times 100$$

where *F* represents the frequency of occurrence (%) of the fungus from each niche; *NT* represents the total number of substrate units from which isolations were made, and *NF* represents the number of substrate units from which a particular fungus was isolated. In the frequencies of occurrence of each species from sapwood under the galleries, *NT* represents the total number of radii from which isolations were made and *NF* represents the number of radii from which a particular fungus was isolated.

Representative cultures of the two *Leptographium* species isolated in the present study have been deposited in the Biotech Culture Collection (BCC), Biotec Central Research Unit, Pathumthani, Thailand.

Results and discussion

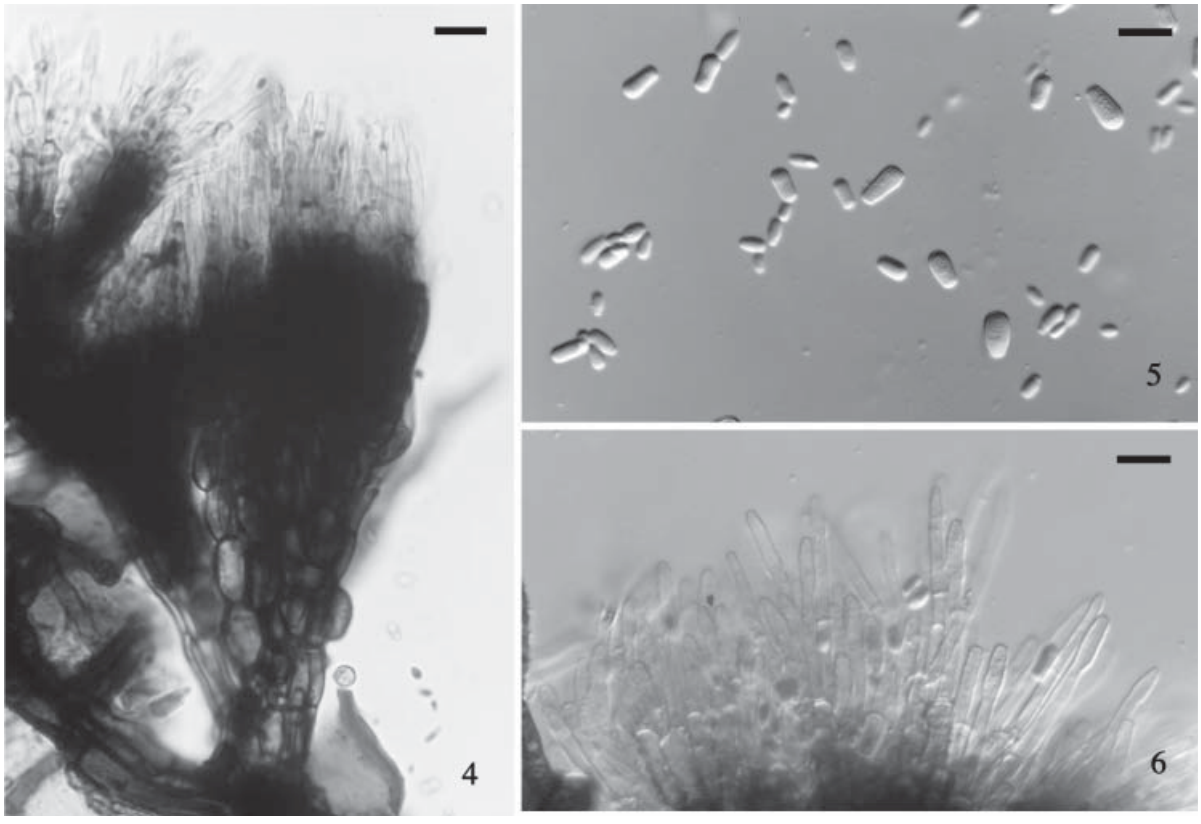
Based on morphological observations of the isolated ophiostomatoid fungi, two *Leptographium* species were identified. One was *L. pini-densiflorae* Masuya & M. J. Wingf. The conidiogenous apparatus of this species has 3 primary branches and 3 branch series and rhizoidal hyphae at the base of the stipe (Fig. 1). Conidiogenesis is annelidic or sympodial (Fig. 3) and conidia oblong to ellipsoidal (Fig. 2). Morphological characteristics of this fungus collected in Thailand fit well the description of *L. pini-densiflorae* by Masuya et al. (2000). Optimum growth temperature of *L. pini-densiflorae* is near 30°C. This fungus is able to grow at 35°C, one important characteristic of *L. pini-densiflorae* (Masuya et al. 2000).

Leptographium pini-densiflorae was isolated from *Stenoscelis* sp. (Table 2). Unfortunately, however, the sample numbers were very small, so we could not make a conclusion about the association of the fungi and the beetles. This

Table 2. Frequencies of occurrence (%) of *Leptographiium* species isolated from beetles, galleries of beetles, and sapwood

Species	Sample no. 1			Sample no. 2		Sample no. 3						Sample no. 4	
	Polygraphus major			*Stenoscelis* sp.		*Polygraphus major*			*Cryphalus kesiyae*			Unknown	
	AB	EG	Sap	AB	Sap	AB	EG	Sap	AB	EG	Sap	EG	Sap
Leptographium pini-densiflorae	0	0	0	33.3	0	0	0	0	0	0	0	40.0	18.8
L. yunnanense	0	38.5	61.5	0	0	100	100	100	76.9	90.0	83.3	0	0
No. of substrates	3	13	13	6	5	15	9	8	13	10	6	10	16

AB, adult beetles in egg galleries; EG, walls of egg galleries; Sap, sapwood



Figs. 4–6. *Leptographium yunnanense* (BCC 20302). **4** Conidiophore. **5** Conidia. **6** Conidiogenous apparatus. *Bars* 10 µm

fungus was also isolated from sample number 4. Beetles invading this sample could not be identified. So far, this species has only been reported from *Pinus densiflora* in Japan. This is the first record of the fungus outside Japan. Masuya et al. (2002) mentioned that the fungus is associated with the bark beetles *Tomicus piniperda* L., *T. minor* (Hartig), *Cryphalus fulvus* Niisima, and *Orthotomicus angulatus* (Eichhoff) and an unidentified beetle.

The second species identified was *L. yunnanense* X.D. Zhou, K. Jacobs, M.J. Wingf. & M. Morelet. This fungus produces synnema-like structures, but branching starts from a short stipe and occurs repeatedly to form a synnema-like structure (Fig. 4). Conidia are oblong to obovoid with a truncated end (Fig. 5), and annelation of the conidiogenous cells is very clear (Fig. 6). Morphological characteristics of this fungus collected from Thailand agree with the description of *L. yunnanense* by Zhou et al. (2000), except that the conidiogenous cells of this fungus [(20.0–)28.0–56.0(–60.0) µm] are longer than the description [(18.0–)23.0–26.0(–32.0) µm]. Optimum growth temperature of *L. yunnanense* is near 25°C. The fungus is not able to grow at 35°C. These characteristics also agree with the description of *L. yunnanense* by Zhou et al. (2000).

Leptographium yunnanense was first reported by Zhou et al. (2000) from *Pinus yunnanensis* Franchet in Yunnan, South-western China. Later, Masuya et al. (2002) reported the fungus was isolated from *Pinus densiflora* in Japan. This is the third record of this fungus in the world. Zhou et al. (2000) also reported *P. khasya* and *P. densata* Masters as substrates. *Pinus densata* is considered as a natural hybrid of *P. yunnanensis* and *P. tabulaeformis* Carr. These pine species reported as host plants of *L. yunnanense* are classi-

fied into the same subsection *Sylvestres*, section *Pinus* (Critchfield and Little 1966; Farjon 1984).

Both Zhou et al. (2000) and Masuya et al. (2002) reported a strong association between the fungus and the bark beetle *T. piniperda*. However, *L. yunnanense* was isolated from adult beetles of *P. major* and *C. kesiyae* in their egg galleries, their gallery walls, and stained sapwood near the galleries with high frequencies of occurrence in Thailand (Table 2). *Polygraphus major* attacks young healthy conifer trees and small branches, and *C. kesiyae* is often found in the same branches as *P. major* (Beaver and Browne 1975). *Tomicus piniperda* is also known to kill healthy trees of *P. yunnanensis* by mass attack (Zhou et al. 2000). *Leptographium yunnanense* may prefer to associate with bark beetles possessing the ability to attack healthy pine trees.

Our results indicated that both *L. pini-densiflorae* and *L. yunnanense* are associated not only with *T. piniperda* but also other bark beetles and use *Pinus* species belonging to subsection *Sylvestres*, section *Pinus*, distributed in the tropical monsoon areas and temperate monsoon areas of Asia as host plants or substrates. It is understandable that common species exists in Southwestern China and Northern Thailand, because these areas are geographically close and the distribution areas of host pines overlap (Critchfield and Little 1966). However, there is no direct geographical connection between this area and Japan, so that the distribution patterns of the fungi appear to be isolated from each other. More information is needed about these fungi from Eastern Asia to clarify the ecology of the fungi.

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References

Beaver RA, Browne FG (1975) The Scolytidae and Platypodidae (Coleoptera) of Thailand. A checklist with biological and zoogeographical notes. Orient Insects 9:283–311

Critchfield WB, Little EL Jr (1966) Geographic distribution of the pines of the world. Miscellaneous publication 991. USDA, Washington, DC

Farjon A (1984) Pines. Brill, Leiden

Jacobs K, Wingfield MJ (2001) *Leptographium* species. Tree pathogens, insect associates, and agents of blue-stain. The American Phytopathological Society Press, St. Paul, MN

Jacobs K, Wingfield MJ, Crous PW, Harrington TC (1998) *Leptographium engelmannii*, a synonym of *Leptographium abietinum*, and description of *Leptographium hughesii* sp. nov. Can J Bot 76:1660–1667

Jacobs K, Wingfield MJ, Bergdahl DR (2000) New *Leptographium* species from Indonesia and Eastern North America. Mycoscience 41:595–606

Masuya H, Wingfield MJ, Kaneko S, Yamaoka Y (2000) *Leptographium pini-densiflorae* sp. nov. from Japanese red pine. Mycoscience 41:425–430

Masuya H, Yamaoka Y, Kaneko S (2002) *Leptographium* species isolated from bark beetles infesting Japanese red pine in Japan. Abstracts, The 7th international mycological congress, Oslo, Norway, 11–17 August, 2002, p 219

Wingfield MJ, Crous PW, Tzean SS (1994) *Leptographium elegans*: a new species from Taiwan. Mycol Res 98:781–785

Yamaoka Y, Masuya H, Ohtaka N, Kaneko S, Abe JP (2004) Three new *Ophiostoma* species with *Pesotum* anamorphs associated with bark beetles infesting *Abies* species in Nikko, Japan. Mycoscience 45:277–286

Zhou XD, Jacobs K, Morelet M, Ye H, Lieutier F, Wingfield MJ (2000) A new *Leptographium* species associated with *Tomicus piniperda* in south-western China. Mycoscience 41:573–578