

FULL PAPER

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## A new species of *Brasiliomyces* (Erysiphaceae) on *Dalbergia cultrata* var. *cultrata* from Thailand

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**Abstract** A powdery mildew fungus on leaves of *Dalbergia cultrata* var. *cultrata* (Fabaceae) collected at the Queen Sirikit Botanical Garden in northern Thailand is proven to be a new species of the genus *Brasiliomyces* and is described as *B. Chiangmaiensis* sp. nov. with light and SEM micrographs. Differences in known *Brasiliomyces* species are discussed, and a key to species of this genus is provided.

**Key words** *Brasiliomyces Chiangmaiensis* · *Dalbergia cultrata* · Powdery mildew · Taxonomy

### Introduction

The genus *Brasiliomyces* Viégas was first established because of its absence of ascomatal appendages (Viégas 1944). Zheng (1984) reexamined type collections of species of this genus and found scarce, rudimentary appendages, at least in some ascomata. Braun (1987) also examined type collections of *Brasiliomyces* species (excluding *B. setosus*) to confirm that rudimentary ascomatal appendages were present. He distinguished *Brasiliomyces* from *Erysiphe* DC. by its thin-walled (one-layered) peridium, and provided the following circumscription:

“Mycelium superficial, conidial state unknown. Erysiphaceous cleistothecia globoid to depressed-globose, usually fairly small, about 45–80 µm in diam, peridium very thin, composed of only one conspicuous layer, wall yellowish to light brown, semitransparent, appendages very sparsely developed, mycelioid, few, often nearly absent or even lacking, asci more than 1, 2–8-spored.”

Only six species of the *Brasiliomyces*, viz., *B. trina* (Harkn.) R.Y. Zheng (North America), *B. entadae* Marasas & Rabie (South Africa), *B. malachrae* (Seaver) Boesew. (South America), *B. setosus* Hodges (Hawaii), *B. cyclobalanopsidis* K.C. Kuo, W.H. Hsieh & L.S. Liu (Taiwan), and *B. kumaonensis* N. Ahmad, A.K. Sarbhoy & Kamal (Northern India), have so far been described (Braun 1987; Hodges 1985; Kou et al. 1992; Ahmad et al. 1998).

In 1999, a species of *Brasiliomyces* was found on leaves of *Dalbergia cultrata* Graham ex Benth var. *cultrata*. This fungus agrees well with the general characteristics of the genus *Brasiliomyces* and is easily distinguishable from the other six species of *Brasiliomyces* by having smaller ascomata and very few asci. *Brasiliomyces Chiangmaiensis* C. To-anun & S. Takamatsu sp. nov. is proposed to accommodate this fungus. This is the first record of *Brasiliomyces* species from Southeast Asia. Molecular phylogenetic studies based on rDNA sequence data of this fungus will be reported elsewhere.

### Materials and methods

#### Sample sources

The leaves of *Dalbergia cultrata* var. *cultrata* with *Brasiliomyces* sp. were collected at the Queen Sirikit Botanical Garden in northern Thailand and examined as fresh material. For comparison, herbarium samples of *B. trina*, *B. malachrae*, and *B. entadae* (IMI 124287) were also examined.

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## Light microscopy

Hyphae, ascomata, appendages, asci, and ascospores of the fungal collections were stripped off from the leaf surfaces with a clean needle, mounted on a microscope slide, and examined in water using a light microscope with phase contrast 20 $\times$ , 40 $\times$ , and 100 $\times$  oil immersion objectives. The following information was noted during the examination of the fresh specimens: size and shape of ascomata, presence or absence of appendages, structure and size of peridial cells, number of asci per ascus, number of ascospores per asci, size and shape of asci and ascospores, and shape and position of hyphal appressoria. Thirty ascomata were measured per sample.

Observation of ascospore germ tubes was conducted by the method described by Hirata (1942). The inner surface cell layer of onion scales was cut with a razor to a size of 1 cm<sup>2</sup> and stripped off by a clean forceps. The cell layer was kept in 80% ethanol for more than 2 weeks and rinsed with tap water for 30 min before use. The cell layer was put on a microscope slide, followed by removing the excess water with a filter paper, and inoculated with ascospores. The inoculated cell layer was floated on distilled water in a petri dish and incubated at 20 $^{\circ}$ –25 $^{\circ}$ C for 24 h until microscopic observation.

## Scanning electron microscopy

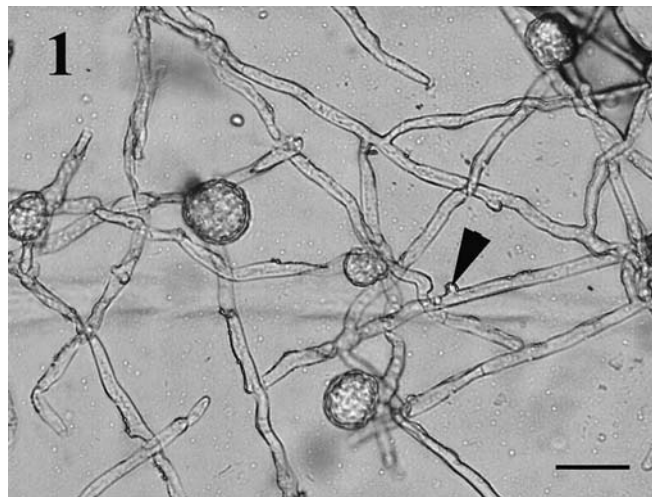
Small pieces of leaf specimens were placed on a glass slide. The leaves were fixed with OsO<sub>4</sub> vaporized from 1% solution in a watch glass at the bottom of a glass box until the specimens were darkened. The glass box was tightly sealed with Parafilm during fixation. The lid of the box was slightly opened to allow the vapor to be released and also the specimens to be dried in a chemical hood. The specimens were attached to an aluminium stub with silver paste and coated with gold using an ion-sputter (model E-1010; Hitachi, Tokyo, Japan). They were observed with a scanning electron microscope (model S-4000; Hitachi) at accelerating voltage 15 kV.

## Results and discussion

### Taxonomy

*Brasiliomyces chiangmaiensis* C. To-anun & S. Takamatsu, sp. nov.

Mycelium amphigenum, plerumque epiphyllum, persistens, maculis irregularibus, albidis formans; hyphae 3–4  $\mu$ m latae; appressoria papillata vel oblongato-clavata; conidiophora et conidia incognita; ascomata sparsa vel gregaria, globosa, (35–)38–45(–48)  $\mu$ m diam., tenuitunicata, peridio flavido vel pallide brunneo, subluvido, ex cellulis daedaleoidibus (10–)15–20  $\mu$ m latis composito; appendices 2–6, simplices, mycelioides, saepe ramosae, tenuitunicatae, non septatae, hyalinae, ad basim brunneae, 5–30  $\times$  3–5  $\mu$ m; asci 2, claviformes, 33–40(–45)  $\times$  27–35  $\mu$ m; ascospores 6, ellipso-



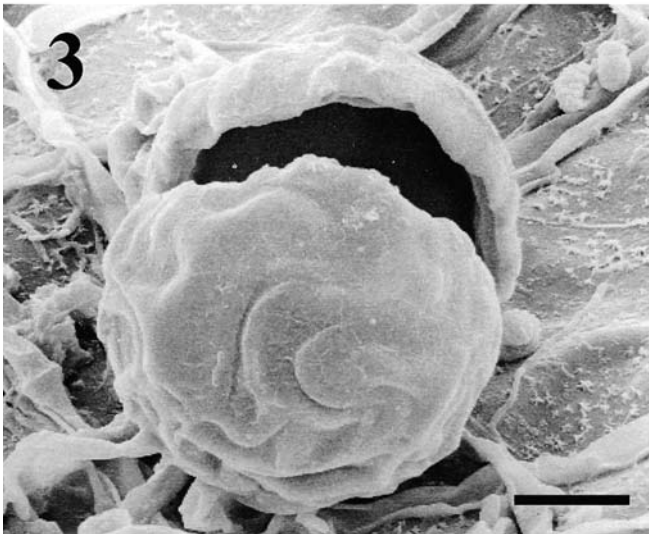
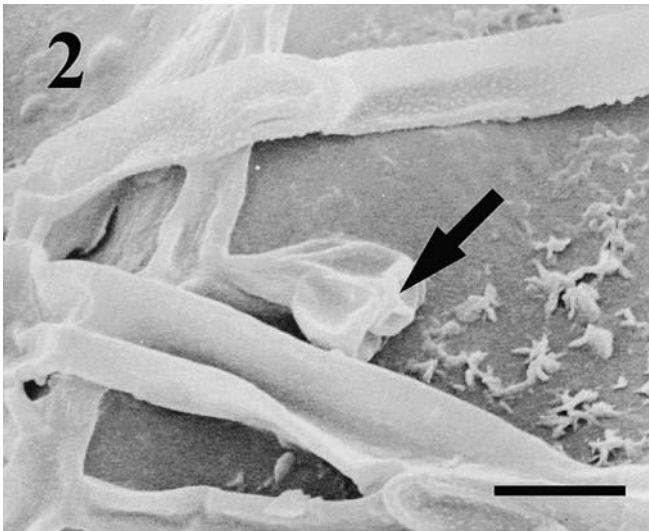
**Fig. 1.** Mycelia, appressoria, and young ascomata of *Brasiliomyces chiangmaiensis*. Bar 20  $\mu$ m

ideae vel ovoideae, olivaceae vel pallide viridulae, guttulate, laeves, 15.5–18.2  $\times$  8.5–10.5  $\mu$ m.

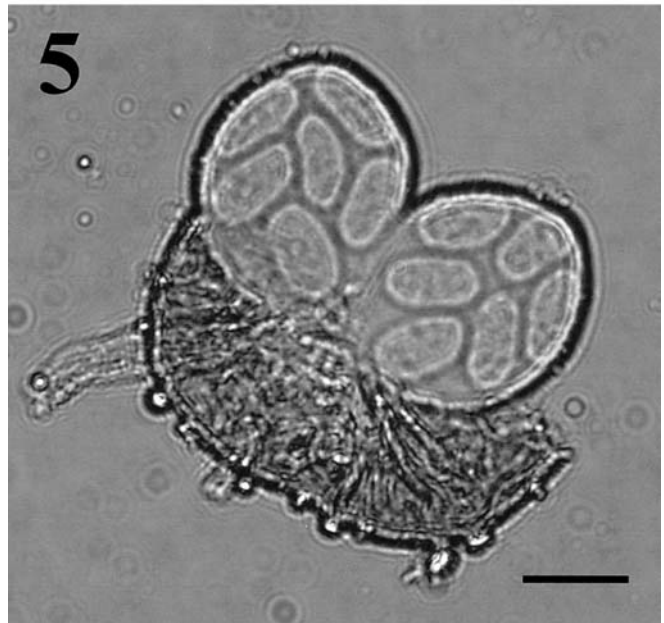
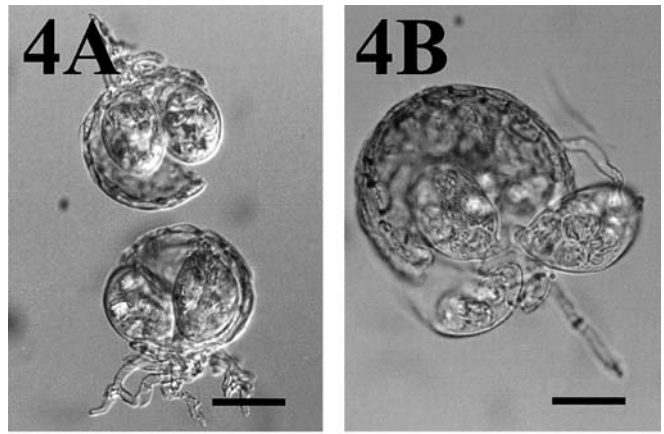
Holotype: on *Dalbergia cultrata* Graham ex Bentham var. *cultrata* (Fabaceae), northern Thailand, Queen Sirikit Botanical Garden, 1999, C. To-anun (HAL1741). Isotype: MUMH 1487.

Mycelium amphigenous, mainly epiphyllous, persistent, forming irregular white patches. Hyphae hyaline, superficial, almost straight to somewhat undulate, 3–4  $\mu$ m wide, branching at right or narrow angles. Appressoria well-developed, nipple- (Fig. 1) to oblong club-shaped (Fig. 2), single or occasionally opposite in pairs. Conidiophores and conidia unknown. Ascomata scattered to gregarious, (35–)38–45(–48)  $\mu$ m diameter (average, 41.4  $\mu$ m); cells of the wall daedaleoid when mature, (10–)15–20  $\mu$ m diameter; peridium thin, one-layered, yellowish to light brown, semi-transparent, with few basal appendages (2–6, sometimes lacking), mycelioid, thin-walled, hyaline, sometimes pale brown near the base, aseptate, often branched, coral-like, almost uniform in width, shorter than the ascomatal diameter, 5–30  $\times$  3–5  $\mu$ m. Asci two, sessile or short-stalked, thin-walled, 33–40(–45)  $\times$  27–35  $\mu$ m (average, 38.1  $\times$  31.5  $\mu$ m), 6-spored. Ascospores ellipsoid-ovoid, olivaceous to pale greenish due to oil drops, 15.5–18.2  $\times$  8.5–10.5  $\mu$ m (average, 17.5  $\times$  9.3  $\mu$ m).

The colonies of *B. chiangmaiensis* can be found on both sides of leaves of *D. cultrata*, but they are more abundant on the upper leaf surface, which is similar to the other species of *Brasiliomyces* (Braun 1987) except *B. setosus* (Hodges 1985). They are at first whitish, and later turn brownish during the formation of ascomata, which often cover the entire surface of the leaves. Lacking the conidial state is very characteristic for *Brasiliomyces*. At present, there are only two genera of powdery mildews without any conidial states, *Brasiliomyces* and *Typhulochaeta*. *Brasiliomyces* is unique among the genera of powdery mildews in producing abundant ascomata without any conidial state in tropical climates (Hanlin and Tortolero 1984), whereas most other



**Fig. 2.** Scanning electron micrograph of an appressorium, oblong club-shaped (arrow), of *Brasiliomyces chiangmaiensis*. Bar 5  $\mu$ m  
**Fig. 3.** Scanning electron micrograph of an ascoma of *Brasiliomyces chiangmaiensis*. Bar 10  $\mu$ m



**Fig. 4.** Ascomata and asci of *Brasiliomyces chiangmaiensis* (A) and *B. entadae* (B). Bars 20  $\mu$ m  
**Fig. 5.** Ascus and ascospores of *Brasiliomyces chiangmaiensis*. Bar 10  $\mu$ m

powdery mildews in this area form only conidia without ascomata (Boesewinkel 1980).

The ascomata (Figs. 3, 4A) of *B. chiangmaiensis* are globose and very small, 38–45  $\mu$ m (48  $\mu$ m, maximum) diameter. These very small ascomata are unique compared with the hitherto known species of the genus *Brasiliomyces*, in which the size of ascomata ranges from 50 to 70  $\mu$ m (Braun 1987; Hodges 1985). Furthermore, *B. chiangmaiensis* differs from the other species of *Brasiliomyces* in having only two asci containing six ascospores per ascoma (Fig. 5).

Characteristics of the present fungus indicate that this fungus belongs in the genus *Brasiliomyces*, and it distinctly differs from the six *Brasiliomyces* species in its size of ascomata and number of asci. *Brasiliomyces chiangmaiensis* is close to *B. entadae* Marasas & Rabie (IMI 124287), which

is also reported to occur on a legume (Fabaceae). Therefore, the differences between the two fungi have been examined in detail; the results are shown in Table 1.

At present, *Brasiliomyces* comprises seven species, i.e., *B. setosus*, *B. trina*, *B. malachrae*, *B. entadae*, *B. cyclobalanopsidis*, *B. kumaonensis*, and *B. chiangmaiensis*, which can be distinguished as follows.

#### Key to the species of *Brasiliomyces*

1. Mycelium hypophyllous, with a large number of setae produced along the upper sides of hyphae, usually three asci per ascoma, asci (4–)5–6-spored, parasitic on Sapindaceae (*Sapindus oahuensis*), Hawaii . . . . . *B. setosus*
- 1'. Mycelium mainly epiphyllous, without setae on hyphae . . . . . 2



**Fig. 6.** Peridium cells of ascoma of *Brasiliomyces chiangmaiensis*. Bar 20  $\mu$ m

**Fig. 7.** Germination of ascospores of *Brasiliomyces chiangmaiensis* on onion epidermal cells. Bar 20  $\mu$ m

**Table 1.** Characteristics of *Brasiliomyces entadae* (IMI 124287) and *B. chiangmaiensis*

Main characteristics	<i>B. entadae</i>	<i>B. chiangmaiensis</i>
Diameter of ascomata	(45–) 50–75 (–80) $\mu$ m	(35–) 38–45 (–48) $\mu$ m
Number of asci	(3–) 4–5	2
Size of asci	45–55 (–65) $\times$ 30–40 $\mu$ m	33–40 (–45) $\times$ 27–35 $\mu$ m
Host family/subfamily and species	Fabaceae/Mimosoideae ( <i>Entada spicata</i> )	Fabaceae/Faboideae ( <i>Dalbergia cultrata</i> )
Distribution	South Africa	Southeast Asia (Thailand)

2. Ascomata small, 26–45(–48)  $\mu$ m diameter, containing only 2 asci ..... 3
- 2'. Ascomata 45–95  $\mu$ m diameter, containing (2–)3–5 asci ..... 4
3. Appendages none, perithecial wall composed of flattened cells with short protuberances on the surface of ascoma, asci 8-spored, parasitic on Fagaceae (*Cyclobalanopsis glauca*), Taiwan ..... *B. cyclobalanopsidis*
- 3'. Appendages 2–6, cell of perithecial wall daedaleoid, asci 6-spored, parasitic on Faboideae (*Dalbergia cultrata* var. *cultrata*), Southeast Asia (North Thailand) ..... *B. chiangmaiensis*
4. Parasitic on Fagaceae ..... 5
- 4'. Parasitic on host families other than Fagaceae ..... 6
5. Ascomata comparatively large, 80–90(–95)  $\mu$ m diameter, asci 4–6-spored, parasitic on *Quercus* sp., North India ..... *B. kumaonensis*
- 5'. Ascomata smaller, 50–70  $\mu$ m diameter, asci 2-spored, ascospores large, up to 40  $\mu$ m in length, parasitic on *Quercus agrifolia*, North America ..... *B. trina*
6. Asci (2–)4–5-spored, cells of ascomatal wall irregularly polygonal, parasitic on Malvaceae, South America ..... *B. malachrae*
- 6'. Asci 5–8-spored, ascomatal wall daedaleoid, parasitic on Mimosoideae (*Entada spicata*), South Africa ..... *B. entadae*

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