

## *Braunomyces dictyosporus* gen. sp. nov. from Vietnam

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**Abstract:** The generic name *Braunomyces* (ascomycetes, asexual morph), with *B. dictyosporus* as type species, is described, illustrated and discussed, based on material collected in Vietnam on leaf debris of an unidentified broadleaved tree. The new genus is well characterised and quite distinct from other synnematosus and non-synnematosus dematiaceous hyphomycete genera by its unique combination of traits, viz. determinate synnematosus conidiomata, integrated, terminal and intercalary conidiogenous cells with one to several conidiogenous vesicles becoming cupulate with age, tretic conidiogenesis, and mostly cruciately septate, solitary dictyoconidia.

**Key words:**

*Ascomycota*  
asexual morph  
dematiaceous hyphomycetes  
south-east Asia  
synnemata

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### INTRODUCTION

Fungal diversity, especially that of tropical countries, is far from being well explored. Vietnam in south-east Asia is characteristic. Attempts to improve the knowledge on the diversity of Vietnamese fungi have been made within the scope of a Research Program of the Vietnam-Russian Tropical Research and Technological Centre. Numerous specimens of fungi were collected, including various hyphomycetes (asexual fungal morphs, mostly of ascomycetes). Results of examinations and identifications of these fungi were published in a series of papers (Alexandrova *et al.* 2013, Mel'nik 2011, 2012a, b, Mel'nik *et al.* 2012, 2013, Mel'nik & Braun 2013), including numerous new records and some new species. A synnematosus hyphomycete on leaf debris of an unidentified broadleaved tree, superficially similar to species of the genus *Paathramaya* (Subramanian 1956, Bhat 1985, Seifert *et al.* 2011), proved to be a novel species that must be assigned to a new genus. The new genus is discussed and compared with morphologically similar synnematosus and non-synnematosus hyphomycete genera. Attempts to cultivate this fungus proved to be unsuccessful. Conidia failed to germinate, and all fungi cultivated from synnemata belonged to other genera, leading us to conclude that the fungus probably does not grow in culture, or was too old when cultivation was attempted.

### MATERIAL AND METHODS

The freshly collected sample was dried at room temperature (22 °C), and later examined in distilled water and photographed using a Zeiss microscope, Stemi 2000CS, and Axio Imager A1 equipped with Nomarski differential interference contrast optics. The conclusion that it represented an unnamed genus

was accomplished through comparison of descriptions and illustrations in current relevant literature, including Seifert *et al.* (2011) and Seifert & Gams (2011). Type material is deposited at CBS, HAL, and LE.

### TAXONOMY

***Braunomyces* Melnik & Crous, gen. nov.**  
Mycobank MB807593

**Etymology:** In honour of the German mycologist Uwe Braun, on the occasion of his 60<sup>th</sup> birthday in 2013, recognising his outstanding contributions to mycology.

**Diagnosis:** *Synnemata* determinate, consisting of brown, apically splaying conidiophores. *Conidiogenous cells* terminal and intercalary, with a single or several aggregated swellings, subcircular to somewhat oblong in outline, later often collapsing, becoming cupulate, mono- to polytretic. *Conidia* solitary, dictyosporous, globose, subglobose, cruciately to obliquely septate, pale to medium dark olivaceous brown, verruculose to verrucose.

**Type species:** *Braunomyces dictyosporus* Melnik & Crous 2014.

**Description:** Hyphomycete (asexual morph of ascomycetes). *Conidiomata* synnematosus, on leaf debris, saprobic, scattered, dark brown to blackish. *Synnemata* determinate, erect, straight, composed of tightly appressed filaments, forming a firm subcylindrical, dark brown stipe, widened and foot-like at the base, and a loose capitulum of apically divergent conidiophores. *Conidiophores* individually filiform, simple

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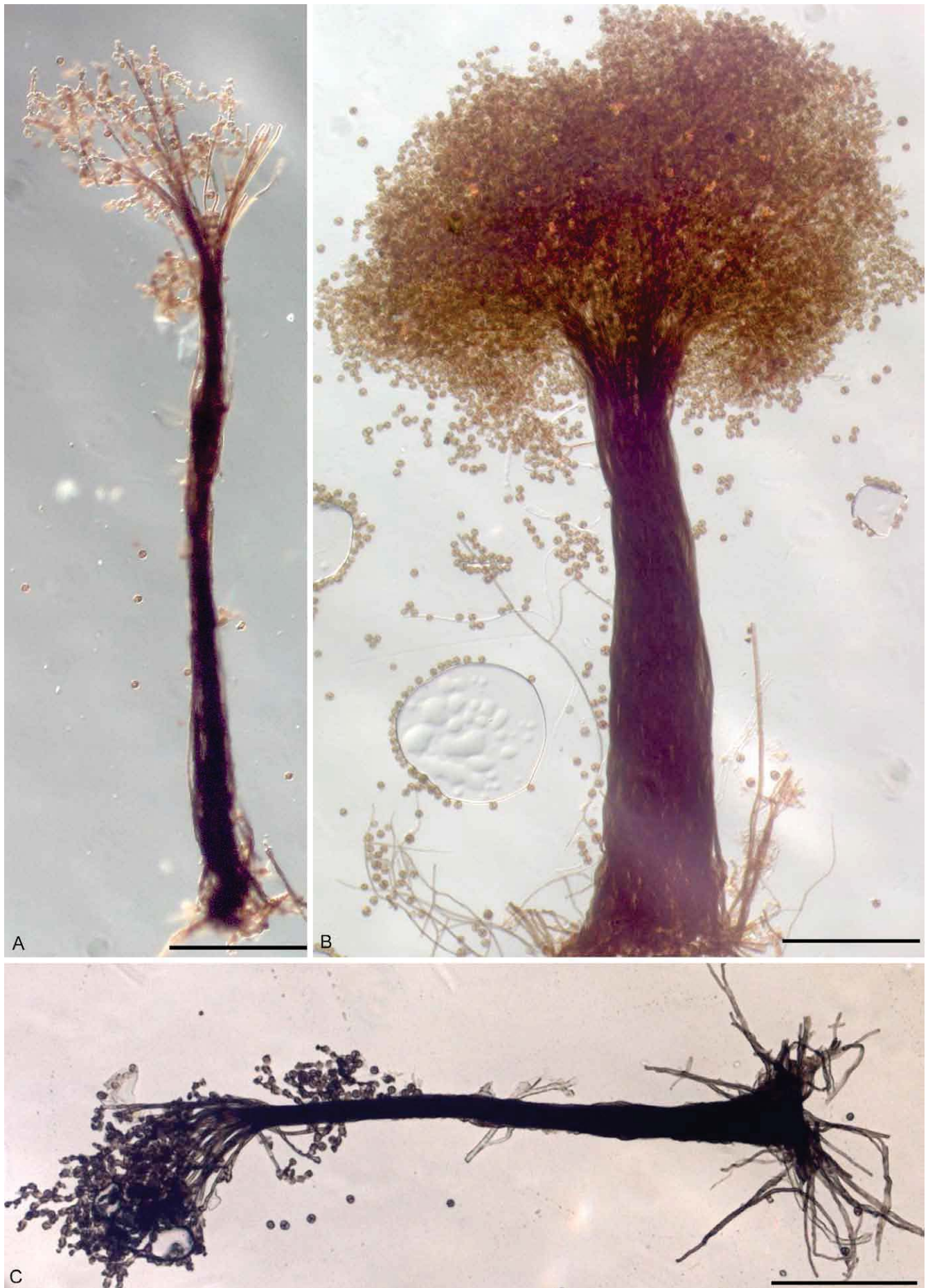
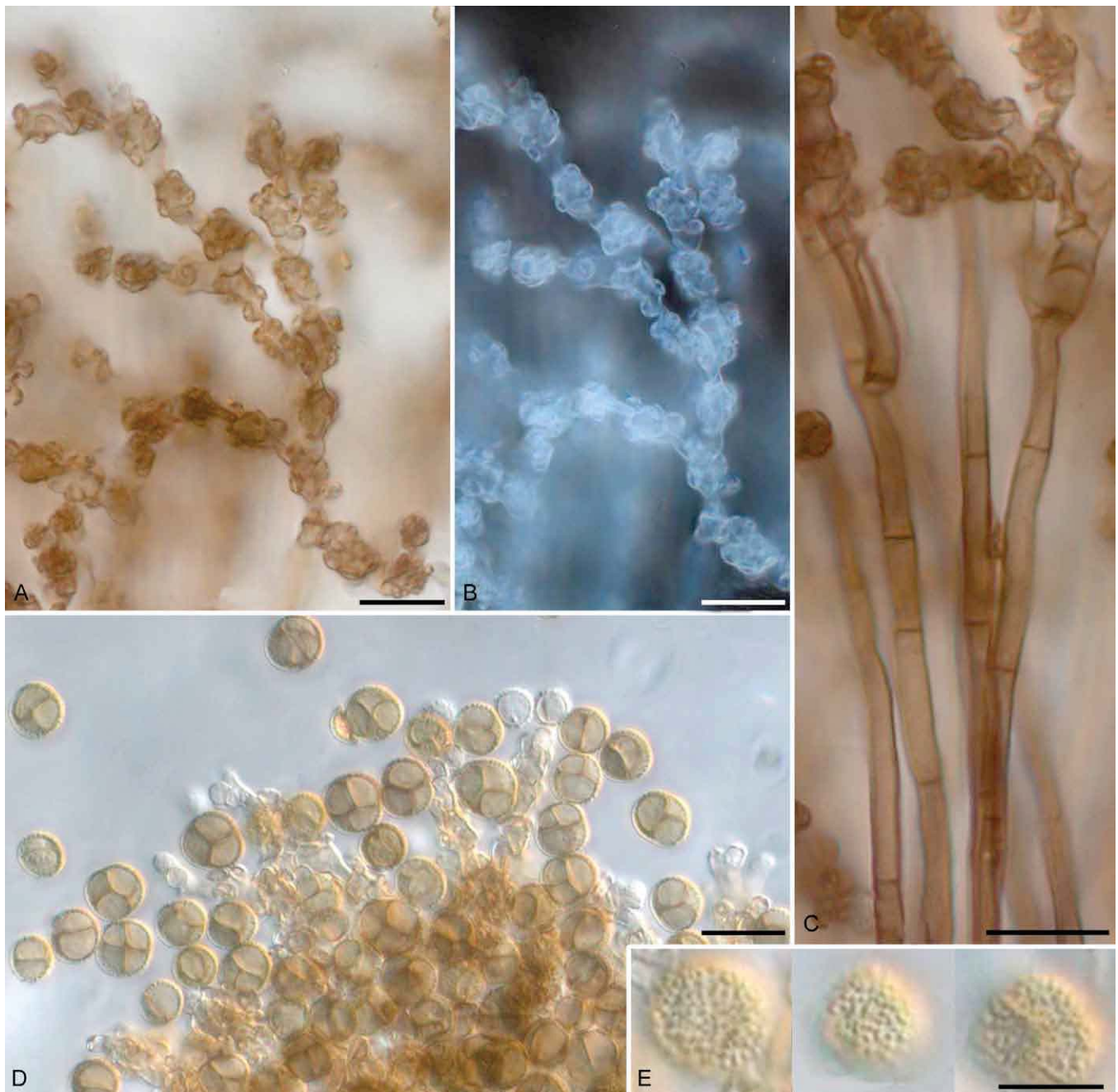


Fig. 1. *Brauomyces dictyosporus* (HAL 2606). A–B. Synnemata. C. Synnema with rhizoid-like base. Bars = 100 µm.



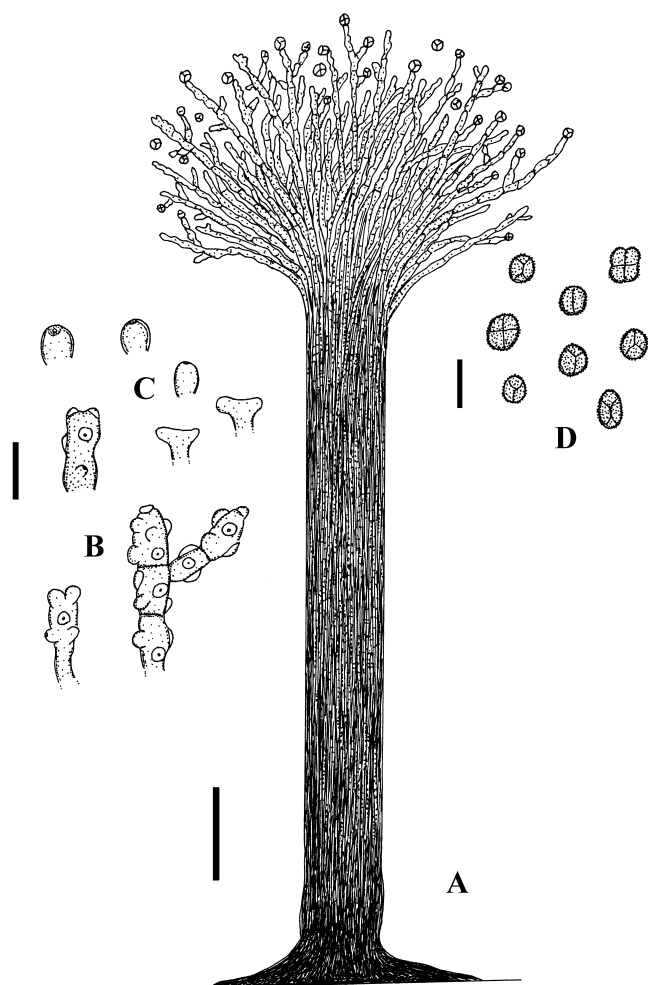
**Fig. 2.** *Braunomyces dictyosporus* (HAL 2606). **A–B.** Monochasial branching of conidiogenous cells (B in dark field). **C.** Threads of the synnema stipe with terminal conidiogenous cells. **D.** Conidia (focused on conidial septa). **E.** Conidia (focused on ornamentation). Bars: A–D = 10  $\mu$ m, E = 5  $\mu$ m.

or sparingly branched, pluriseptate, brown, wall thin to slightly thickened, smooth. *Conidiogenous cells* integrated, terminal and intercalary, irregularly shaped due to vesicular conidiogenous loci, pale to medium brown, thin-walled, smooth, with a single or several aggregated swellings, subcircular to somewhat oblong in outline, later often collapsing, becoming cupulate, with a minute pore surrounded by a slightly darkened-refractive halo or halo sometimes lacking, conidiogenesis tretic (mono- to mostly polytretic). *Conidia* solitary, dictyosporous, globose, subglobose to slightly angular in outline, cruciately to obliquely septate, occasionally slightly constricted at the septa, wall relatively thin, pale to medium dark olivaceous brown, verruculose to verrucose.

*Notes:* Although we have no sexual structures or accompanying DNA phylogenetic data, we conclude that *Braunomyces* is a genus of ascomycetes, chiefly based on its tretic conidiogenous cells, further suggesting it to be affiliated to *Dothideomycetes*.

***Braunomyces dictyosporus* Melnik & Crous, sp. nov.**  
Mycobank MB807594  
(Figs 1–3)

*Etymology:* Epithet derived from the dictyoseptate conidia.



**Fig. 3.** *Braunomyces dictyosporus* (HAL 2606). **A.** Synnema. **B.** Conidiogenous cells. **C.** Conidiogenous vesicles. **D.** Conidia. Bars: A = 50  $\mu\text{m}$ , B–D = 10  $\mu\text{m}$ .

**Diagnosis:** *Synnemata* determinate, 400–650  $\mu\text{m}$  long, consisting of brown, apically splaying conidiophores. *Conidiogenous cells* terminal and intercalary, 6–20  $\times$  2–7  $\mu\text{m}$ , with a single or several aggregated swellings, 1.5–3.5  $\mu\text{m}$  diam, subcircular to somewhat oblong in outline, later often collapsing, becoming cupulate, mono- to polytretic, loci about 0.8–1.5  $\mu\text{m}$  diam. *Conidia* solitary, dictyosporous, globose, subglobose to broadly ellipsoidal, consistently aseptate (amerosporous), dark brown and thick-walled (Moore 1984, Bhat 1985, Dominik 1970, Seifert *et al.* 2011) – in contrast to the tretic, dictyosporous Vietnamese fungus. Because of the collapsing, cupulate conidiogenous cells and globose, aseptate, dark, thick-walled conidia, the non-synnematous hyphomycete genus *Lemkea* is morphologically similar to *Paathramaya*, but differs in that the conidiogenous cells are discrete, appearing vesicle-like (Morgan-Jones & Sinclair 1983). Similar subglobose dictyosporous conidia are formed in *Neopericonia* (Kamal *et al.* 1983), but that genus is readily distinguishable by its mononematous conidiophores, discrete terminal conidiogenous cells, mono- to polyblastic conidiogenesis, and conidia formed singly or in short basipetal chains. Globose conidiogenous cells that finally collapse and become cupulate are also known in the mononematous

**Type: Vietnam:** *Dong Nai Prov.:* Cat Tien National Park, Nam Cat Tien Sector, polydominant monsoon tropical forest, on dry leaves of an unidentified broadleaved tree, 16 Nov. 2011, Yu. Novozhilov (HAL 2606 F – holotype; LE 263985, CBS H-21489 – isotypes).

**Description:** *Conidiomata* synnematos, scattered, dark brown to blackish. *Synnemata* determinate, erect, straight, 400–650  $\mu\text{m}$  long, composed of tightly appressed filaments, forming a firm subcylindrical, dark brown stipe, 20–40(–50)  $\mu\text{m}$  wide, widened and foot-like at the very base, to

130  $\mu\text{m}$  diam, with a loose capitulum of apically divergent conidiophores, free terminal portions of the filaments 80–300  $\mu\text{m}$  long. *Conidiophores* individually filiform, simple or occasionally branched, 1.5–3  $\mu\text{m}$  wide, pluriseptate, brown, wall thin to slightly thickened, smooth. *Conidiogenous cells* integrated, terminal and intercalary, about 6–20  $\times$  2–7  $\mu\text{m}$ , irregularly shaped due to vesicular conidiogenous loci, pale to medium brown, thin-walled, smooth, with a single or several aggregated swellings (“conidiogenous vesicles”), 1.5–3.5  $\mu\text{m}$  diam, subcircular to somewhat oblong in outline, later often collapsing, becoming cupulate, loci about 0.8–1.5  $\mu\text{m}$  diam, with minute pore surrounded by a slightly darkened refractive halo or halo sometimes lacking, conidiogenesis tretic (mono- to mostly polytretic). *Conidia* solitary, conidium initials globose, colourless or very pale, aseptate, in this stage rough wall ornamentation already evident, at first forming a single median septum, followed by one or mostly two additional oblique to perpendicular septa, pigmentation setting in with septation and gradually proceeding, mature conidia dictyosporous, globose, subglobose to slightly angular in outline, (4–)5–8(–9)  $\mu\text{m}$  diam, 2- to 4-celled, cruciately to obliquely septate, occasionally slightly constricted at the septa, wall relatively thin (up to 0.8  $\mu\text{m}$ ), pale to medium dark olivaceous-brown, verruculose to verrucose.

## DISCUSSION

Using the key to synnematos hyphomycete genera by Seifert & Okada (in Seifert *et al.* 2011), all accepted genera with dictyoconidia proved to be distinct from, and not applicable to, this species. Among other synnematos hyphomycete genera, there is only one which is superficially similar, *Paathramaya*, established by Subramanian (1956) for a single Indian species, *P. sundara*, based on a collection on dead twigs. Later, Subramanian & Nair (1966) introduced another genus, *Panchanania*, which was reduced to synonymy with *Paathramaya* by Bhat (1985). The conidiomata of the latter genus are also synnematos, determinate, and the conidiogenous cells are equipped with similar conidiogenous vesicles that collapse with age and become cupulate, but the conidiogenesis is holoblastic and the conidia of all species assigned to this genus (*Paathramaya drewsii*, *P. indica*, *P. jaipurensis*, *P. sundara*, and *P. suttonii*) are globose, subglobose to broadly ellipsoidal, consistently aseptate (amerosporous), dark brown and thick-walled (Moore 1984, Bhat 1985, Dominik 1970, Seifert *et al.* 2011) – in contrast to the tretic, dictyosporous Vietnamese fungus. Because of the collapsing, cupulate conidiogenous cells and globose, aseptate, dark, thick-walled conidia, the non-synnematous hyphomycete genus *Lemkea* is morphologically similar to *Paathramaya*, but differs in that the conidiogenous cells are discrete, appearing vesicle-like (Morgan-Jones & Sinclair 1983). Similar subglobose dictyosporous conidia are formed in *Neopericonia* (Kamal *et al.* 1983), but that genus is readily distinguishable by its mononematous conidiophores, discrete terminal conidiogenous cells, mono- to polyblastic conidiogenesis, and conidia formed singly or in short basipetal chains. Globose conidiogenous cells that finally collapse and become cupulate are also known in the mononematous

hyphomycete genera *Bahuchashaka* and *Dwayabeeja*, but the conidiogenous cells in these genera are discrete, and the conidia are phragmosporous and moniloid (Seifert *et al.* 2011). *Dictyopolyschema* is a polyschema-like genus with monotretic conidiogenous cells forming dictyoconidia, but conidiophores are lacking, i.e. conidiogenous cells are formed on supporting hyphae directly (Ellis 1976, Seifert *et al.* 2011). In summary, although there are some hyphomycete genera with single or several traits similar to those of the Vietnamese fungus, none of them is sufficiently similar to justify classification of our species within them. Therefore, we introduce the new generic name *Braunomyces*, for this synnematous hyphomycete.

## ACKNOWLEDGEMENTS

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## Foliicolous fungi from *Arctostaphylos pungens* in Mexico

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**Abstract:** *Arctostaphylos pungens* “Manzanita” is an important shrub in the southwestern USA, and northern and central Mexico. Manzanita bears apple-like fruit that is utilised for a range of edible products. Over the past two years, several foliar disease problems were noted on this host in the San José de Gracia region of Mexico. The aim of the present study was to elucidate their identity through the analysis of morphological characters and DNA phylogeny (based on the large subunit nuclear ribosomal RNA gene and the ITS spacers and the intervening 5.8S rRNA gene of the nrDNA operon) of the fungi associated with these disease symptoms. Three species are newly described: *Phaeococcomyces mexicanus* sp. nov., a presumed epiphyte, and two species associated with leaf spots and defoliation, namely *Coccomyces arctostaphyloides* sp. nov. and *Passalora arctostaphyli* sp. nov. A fourth species is also associated with leaf spots and tip dieback is *Harknessia arctostaphyli*, for which an epitype is designated. All species can co-occur on the same shrub, which adds to the stress experienced by the plant, leading to further defoliation and dieback.

### Key words:

*Coccomyces*  
*Harknessia*  
ITS  
LSU  
*Passalora*  
*Phaeococcomyces*  
systematics

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## INTRODUCTION

*Arctostaphylos pungens* (Ericaceae), or “Pointleaf Manzanita”, is native to the Southwestern USA and to northern and central Mexico. It grows in chaparral and woodland habitats, where it forms erect, spreading shrubs about 1–3 m in height (Márquez-Linares *et al.* 2005). The fruit is minute and apple-like, 5–8 mm diam, which is eaten by many birds and wildlife, and harvested for a multitude of uses. The common name “Manzanita” is Spanish for “little apple” referring to the small apple-shaped fruit. The fruit can be eaten raw or cooked, or is made into a type of jam in Mexico. Fruits may be dried and ground into a powder and then used as flavouring in soups. Leaves have also been used in the treatment of diarrhoea and to relieve itching and pain caused by poison oak and poison ivy. Tea made from the leaves and berries has also been used to treat bronchitis and urinary tract problems (Berg 1974, Weise *et al.* 1991).

Over the past two years, several severe foliar disease problems were noted on *A. pungens* plants growing in San José de Gracia region, Mexico (Fig. 1). Although not much is known about diseases of *Arctostaphylos* spp., the USDA website (nt.ars-grin.gov.) lists several plant pathogenic fungi as occurring on this host. Information about diseases linked to *Arctostaphylos* spp. occurring in Mexico, however, is sparse. The aim of the present study was therefore to characterise

and identify the fungi associated with the various foliar disease symptoms found on this host.

## MATERIALS AND METHODS

### Isolates

Symptomatic leaves were placed in damp chambers for 1–2 d. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing 2 % malt extract agar (MEA; Crous *et al.* 2009c) as described earlier (Crous *et al.* 1991). Colonies were sub-cultured onto potato-dextrose agar (PDA), oatmeal agar (OA), and MEA (Crous *et al.* 2009c), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains were deposited at the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands.

### DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA) according to the manufacturer’s protocol. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify the nuclear rDNA operon spanning the 3’ end of the 18S rRNA gene, the first internal transcribed spacer,

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