

Taxonomic Descriptions of New Records of Microfungi from Kubah & Gunung Gading National Parks in Sarawak.

Lateef A. A.^{1,2}, Sepiah, M.¹, and Bolhassan, M. H.¹

¹ Department of Plant Science and Environmental Ecology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.

² Department of Plant Biology, Faculty of Life Science, University of Ilorin, Nigeria.

* Corresponding author: Lateef A.A. lateef.aa@unilorin.edu.ng, +(60)146902928.

ABSTRACT

During a survey of microfungi on leaves from Kubah and Gunung Gading National Parks, some microfungi species were isolated for the first time on the studied host plants and some new records for Sarawak. The host plants studied includes *Shorea macrophylla*, *Macaranga* sp., *Syzygium* sp., *Licuala bidentata*, *Eusideroxylon zwageri* and *Baccaurea* sp. Taxonomic descriptions and illustrations of 10 microfungi species which includes *Aspergillus nomius*, *Bartalania robillardoides*, *Chalara pteridina*, *Chaetospermum camelliae*, *Circinotrichum fertile*, *Wieneriomyces laurinus*, *Pestalotiopsis biciliata*, *Phalangispora* sp., *Cryptophiale* spp. and *Subulispora procurvata*, are provided in this report. This discovery further extends the distribution of the microfungi in Sarawak & on the new host plants.

Keywords: *Shorea macrophylla*, *Macaranga*, *Licuala*, *Aspergillus*, *Pestalotiopsis*

INTRODUCTION

Studies on microfungi diversity and distribution are important opportunities to map microfungi occurrences, which in turn becomes a useful resource for microbial drug screenings, biodiversity assessments, host-species relationships and many other environmental applications. In the understanding of microfungi diversity and distribution, “not everything is everywhere” (Fenchel & Finlay, 2004; Pérez-del-Olmo, Fernández, Raga, Kostadinova, & Morand, 2009; Thorsten Lumbsch, Buchanan, May, & Mueller, 2008) as microfungi occurrences are determined by several factors such as the host plant phylogeny, isolation methods, environmental factors, substrate nutrient composition, interspecific competition and other factors still not well understood.

Taxonomic documentation of microfungi encountered during surveys are also important for proper species delimitation and comparison (Bickford et al., 2007; Schlick-Steiner, Seifert, Stauffer, Christian, Crozier, & Steiner, 2007).

In this present study, the taxonomic descriptions and illustrations of 10 new records of microfungi from two national parks in Sarawak are provided with illustrations as well as comparison with other well-known species of microfungi.

MATERIALS AND METHODS

Sample collection

Leaf samples (with leaf litters) were collected from various places in Kubah National Park (KNP) and Gunung Gading National Park (GNP) (Table 1). The collected leaves were labelled and taken to the laboratory for endophytic and saprophytic microfungi isolation following the procedure used by Lateef, Sepiah, & Bolhassan (2014).

The leaves were plated on water agar and malt extract agar and the microfungi growing on the leaves were identified, slides were prepared and pictures were taken with an hand-held Samsung ES91 camera and an Olympus microscope BX50. Permanent slides & dried specimens were labelled and stored in the Mycology laboratory, Universiti Malaysia Sarawak. The taxonomic details of the species were observed and recorded.

Table 1. Details of the host plants sampled in this study.

	Plant collected	Collection site	GPS co-ordinate	Elevation
1.	<i>Macaranga triloba</i> (Family Euphorbiaceae)	KNP	N 01° 35 325 E 110° 11 508	796 m
2	<i>Eusideroxylon zwageri</i> (Belian tree) (Family Lauraceae)	KNP	N 01° 36 760 E 110° 11 794	127 m
3.	<i>Licuala bidentata</i> (Family Arecaceae)	KNP	N 01° 36 721 E 110° 11 743	163 m
4.	<i>Shorea macrophylla</i> (Family Dipterocarpaceae)	KNP	N 01° 36 757 E 110° 11 750	175 m
5.	<i>Baccaurea</i> sp (Family Phyllanthaceae)	GNP	N 01° 41 510 E 110° 50 704	635 m
6.	<i>Macaranga</i> sp 2 (Family Euphorbiaceae)	GNP	N 01° 41 510 E 109° 50 704	635 m
7.	<i>Syzygium</i> sp (Family Myrtaceae)	GNP	N 01° 41 625 E 109° 50 573	657 m

RESULTS AND DISCUSSION

In this report, the taxonomic descriptions of ten microfungal taxa comprising of 7 Hyphomycetes and 3 Coelomycetes are provided. The following symbols are used as:

† to represents a new record on the host plant.

‡ to represents a new record in Sarawak.

1. ‡ †*Aspergillus nomius* Kurtzman, Horn, & Hesselstine, (1987), *Antonie van Leeuwenhoek* 53:147-158 (1987).

The genus *Aspergillus* is an abundant soil microfungi. *Aspergillus* spp. are however not dominant leaf microfungi. The genus *Aspergillus* is found in almost all environment conducive for fungal growth. This genus was first described by Micheli in 1792 (Samson et al., 2014) with *A. glaucus* as the type species.

Aspergillus is characterised by having a globe-like spore bearing structure (the conidiophore) suspending the conidia. Variations in the shape of the conidiophore, the arrangement, size and colour of the conidia is an important identification characteristic in this genus (Samson et al., 2014).

A. nomius (Figure 1 A) is a member of the *A. flavus* section with similar morphological characteristics with *A. flavus* and *A. parasiticus* which has often lead to its misidentification as *A. flavus* or *A. parasiticus*. *A. nomius* is different from the other species in the *A. flavus* section by having a uniseriate conidial head and a rough conidia surface. In pure cultures, *A. nomius* is different from *A. flavus* in production of indeterminate sclerotia and a lower temperature optimum for growth (Kurtzman et al., 1987). Molecular characterization and physiological characteristics such as growth at different temperatures has been emphasized for adequate identification of *Aspergillus*. Molecular identification of this fungus was confirmed in this study with the GenBank accession number KR737578. *A. nomius* was isolated from leaves of *Eusideroxylon zwageri* (Belian tree) from Kubah National Park.

2. [†] *Bartalinia robillardoides* Tassi *Bulletin Labor. Orto Bot. de R. Univ. Siena* 3: 4 (1900) (Index fungorum, 2015).

The genus *Bartalinia* was established based on the type species *Bartalinia robillardoides*. This genus is characterised by black to dark brown conidiomata that is pycnidial to indeterminate in shape, fully or partly immersed in the colony. The conidia are generally fusiform to cylindrical, straight or slightly curved with 3-4 septate, bearing 2-3 apical appendage and mostly 1 basal appendage. The genus is separated from *Pestalotiopsis*, *Seimatosporium*, *Truncatella* and other closely related genera mainly based on their conidial appendages (Nag Raj, 1993). Currently, 22 species are formally recognised (Index fungorum, 2015) with *B. pondoensis* being the most recent addition to the genus (Marincowitz, Gryzenhout, & Wingfield, 2010). Interestingly, *B. robillardoides* has been reported to produce Taxol, an anti-cancer drug (Gangadevi & Muthumary, 2008).

B. robillardoides (Figure 1 B) has the following characteristics: **Conidiomata** Pycnidial to indeterminate, black, fully or partially immersed. No conidiomata seen at the observe surface at 2 weeks of growth in pure culture. At 1 month, 4-6 conidiomata appeared. **Conidia** fusiform, straight or slightly curved, $20.81 \pm 3.38 \times 3.54 \pm 0.33 \mu\text{m}$, 4 septate with the third cell from top longer, $5.78 \times 0.56 \mu\text{m}$, than the other cells (1st cell $3.76 \times 0.64 \mu\text{m}$; 2nd cell $4.62 \times 0.75 \mu\text{m}$), 2-3 apical, straight or sometimes bent (towards the middle) appendages present $10.84\text{-}23.22 \mu\text{m}$ long arising from the apical cell $1.84\text{-}4.44$ (Mean= 3.04 ± 0.64) μm . Basal appendage $6.64 \pm 1.79 \mu\text{m}$ long, single, straight, arising from one-side tip of the basal conical cell $3.60 \pm 1.22 \mu\text{m}$. Molecular identification of this fungus was confirmed in this study with the GenBank accession number KT337382. *B. robillardoides* was isolated on green leaves of *Macaranga triloba* from Kubah National Park.

3. [†] *Chaetospermum camelliae* Agnihothrudu *Mycopath. Mycol. Appl.* 16:115, 1962.

This genus was first described under the genus *Tubercularia* by Patouillard in 1888 from a decaying leaf litter, then in 1892, Saccardo renamed the fungus as *Chaetospermum tubercularioides* under a new genus *Chaetospermum*, then Smith & Ramsbottom (1913) renamed it to *C. chaetosporum* (De Fonseka, 1960). The genus *Chaetospermum* is characterised by cylindrical to ellipsoidal conidia, hyaline, aseptate, obtuse at both ends, with straight filiform & flexuous apical or sub apical appendages at both ends. Species in this genus are differentiated based on length & width of the conidia, in addition to the positions of

the appendages. The type species for this genus is *C. chaetosporum*. Five species are currently accepted in this genus which are *C. chaetosporum*, *C. artocarpi*, *C. camelliae*, *C. gossypinum* (Nag Raj, 1993), and *C. indicum* (Tangthirasunun et al., 2014) while *C. carneum* and *C. gelatinosum* are considered *nomen dubium* (i.e. doubtful names) and synonym to another species respectively (Nag Raj, 1993; Tangthirasunun et al., 2014).

Chaetospermum camelliae (Figure 1 C) has the following characteristics: **Conidiomata** white to light brown on leaf substrate, waxy when dry. **Conidia** Cylindrical, obtuse at both ends, 18.00–22.47 μm (Mean $\pm$ SD= 19.83 $\pm$ 1.37 μm) $\times$ 5.86–8.15 μm (Mean $\pm$ SD= 6.25 $\pm$ 1.15 μm), 2-4 apical to sub-apical appendages 9.72–18.26 μm (Mean $\pm$ SD=14.08 $\pm$ 3.11 μm). The dimensions of this isolate overlaps that of *C. camelliae* (Nag Raj, 1993) and differs from that of *C. artocarpi* mainly in the number of appendages, and length of the appendages. *C. camelliae* was found on green and senescent leaves of *Baccaurea* sp and *Macaranga* sp from Gunung Gading National Park, Sarawak.

4. [†]***Cryptophiale*** Pirozynski *Canadian J. of Botany* 46: 1123, 1968 (Pirozynski, 1968).

The Genus *Cryptophiale* was introduced by Pirozynski in 1968 with two species, *C. kakombensis* Pirozynski and *C. udagawae* Pirozynski & Ichinoe. *C. kakombensis* is the type species of this genus. This genus is characterised by having two rows of lateral phialides, one on each side of the setiform conidiophores, obscured by shield-shaped outgrowths of sterile cells located in the subapical region of the conidiophore, thus the name *Cryptophiale* meaning hidden phialide in Latin (Kuthubutheen & Sutton, 1985; Pirozynski, 1968). This genus, *Cryptophiale*, differs from similar genera, *Cryptophialoidae* (erected by Kuthubutheen & Nawawi, (1987)) and *Zanclospora* (erected by Hughes & Kendrick, (1965)) in its two rows of phialide and the shield-plate on its conidiophore, unlike *Cryptophialoidae* with only one row of phialide on one side of the conidiophore only and with no shield-plate, and unlike *Zanclospora* with vertical phialides and no shield-plate obscuring the phialides.

At present, there are 22 species of *Cryptophiale* described (Index fungorum, 2015). In this study, 3 species of *Cryptophiale* (Figure 1 D-E) were recorded which includes *C. udagawae*, *C. minor* and *C. kakombensis*. *Cryptophiale* spp were found on leaves of *Macaranga* sp and *Licuala bidentata* in Kubah National Park and also on *Syzygium* sp from Gunung gading National Park.

5. [†]***Chalara pteridina*** Syd. & P. Syd., *Annls mycol.* 10(5): 450 (1912) (Index fungorum, 2015).

Chalara was initially erected by Corda (1838) with the type species *Chalara fusidioides* as a subgenus of *Torula* (Pers.) Link and was later raised by Rabenhorst (1844) to the genus level. The genus was introduced with *C. fusidioides* described from an herbaceous plant. *Chalara* is characterised by having sessile or stalked phialides (called phialophores) enlarged at the base with a long collarete and a deep-seated conidiogenous locus. The conidia (called phialoconidia) are usually cylindrical, hyaline, less frequently subcylindrical, subhyaline or pale brown, unicellular or septate (Nag Raj and Kendrick, 1975). *Chalara*-like phialides are characteristic of three other genera: *Sporoschisma* Berk. & Br., *Chaetochalara* Sutton & Pirozynski, and *Fusichalara* (Nag Raj & Hughes, 1974). *Sporoschisma* differs from *Chalara* in possessing capitate hyphae and coloured phragmoconidia and *Chaetochalara* is distinct from *Chalara* in possessing ancillary sterile structures like setae. Unlike *Chalara*, *Fusichalara* has a convex thickening of the inner wall of the phialide at the point of transition from venter to collarete and produces two kinds of conidia, the first-formed conidium being

cylindrical and about twice as long as and with about twice as many septa as the subsequent fusiform and straight or slightly sigmoid conidia. Currently, there are 144 (Index fungorum, 2015) and 150 (on MycoBank) records of *Chalara* species in the fungi electronic databases.

Chalara pteridina (Figure 1 F) has the following characteristics: **Phialides** solitary, brown to dark brown, cylindrical to subcylindrical, septate, with a wide basal venter 70–120 µm long at base 30–33 µm. Phialoconidia cylindrical to subcylindrical, 1-septate or no septate, hyaline, smooth-walled. *C. pteridina* was found on green leaves and senescent leaves of *E. zwageri* from Kubah National Park.

6. †*Phalangispora* sp Nawawi & Webster *Tran . Br. mycol. Soc.* 79:65-68, 1982

This genus was erected with the description of *P. constricta* as the type species from pure cultures, isolated from river foam in Ulu Gombak, Malaysia. Before Nawawi & Webster's full description of the type species of this genus, spores of *Phalangispora* sp has been reported from Nigeria (Ingold, 1959), Ghana (Dixon, 1959), Sierra Leone (Le'John, 1965), Japan (Miura, 1974 In Nawawi & Webster, 1982) and Indonesia (Nayo, 1975 In Nawawi & Webster, 1982) but was neither identified nor obtained into pure culture for full description. The genus *Phalangispora* is differentiated from other genera with similar isthmospores like *Weisneriomyces*, *Abgliophragma* R.Y. Roy & S. Gujarati and *Gliophragma* Subram. & B.C. Lodha, in that their spores are branched laterally. Although, *Abgliophragma* R.Y. Roy & S. Gujarati and *Gliophragma* Subram. & B.C. Lodha are considered as synonyms of *Weisneriomyces* (Matsushima, 1971; Pirozynski, 1972 In Suetrong et al., 2014). *Phalangispora* is characterised by sporodochial conidiomata on which there is a mass of spores, 3-5 septate, long, dark setae. The conidia (Figure 1 G) are in branched chains with cells connected by a narrow isthmus. The genus currently has only three species described and accepted (Index fungorum, 2015), both from terrestrial and aquatic environments. *Phalangispora* sp was isolated from leaf litter of *Syzygium* sp from Gunung Gading National Park.

7. †*Circinotrichum fertile* Pirozynski & Hodges Jr. (1973). New Hyphomycetes from South Carolina. *Can. J. Bot.* 51: 157-173. (Pirozynski & Hodges Jr, 1973).

The genus *Circinotrichum*, with type species *Circinotrichum maculiformes* Nees ex Pers., is characterised by having unbranched, brown, solitary, sterile setae, surrounded at the base by clusters of aseptate, hyaline conidiogenous cells (Seifert *et al.* 2011 In Crous et al., 2014). Presently, there are 26 species of *Circinotrichum* recorded in the Index Fungorum database.

C. fertile was found on leaves of *Macaranga* sp, *Eusideroxylon zwageri* and *Syzygium* sp from Kubah and Gunung Gading National Park.

8. †*Subulispora procurvata* Tubaki, K. and Yokoyama, T. (1971). Notes on the Japanese hyphomycetes V. *Trans. Of the Mycological Society of Japan* 12: 18-28.

This genus was based on the description of two species, *S. procurvata* and *S. rectilineata* with *S. procurvata* as the type species. The genus is characterised by macronematous, brownish, thick-walled conidiophores with hyaline to pale fuscous, subuliform, conoid, fusoid or bottle-shaped conidia, with or without terminal appendage. Presently, there are 16 species listed in Index fungorum although, only seven species were recognised by Marvanová & Laichmanová (2007). This is as a result of the heterogeneity of the genus. Thorough morphological and molecular studies for this genus have been recommended (Marvanová &

Laichmanová, 2007). *S. procurvata* (Figure 1 H) was found on leaves of *Macaranga* sp from Kubah National Park.

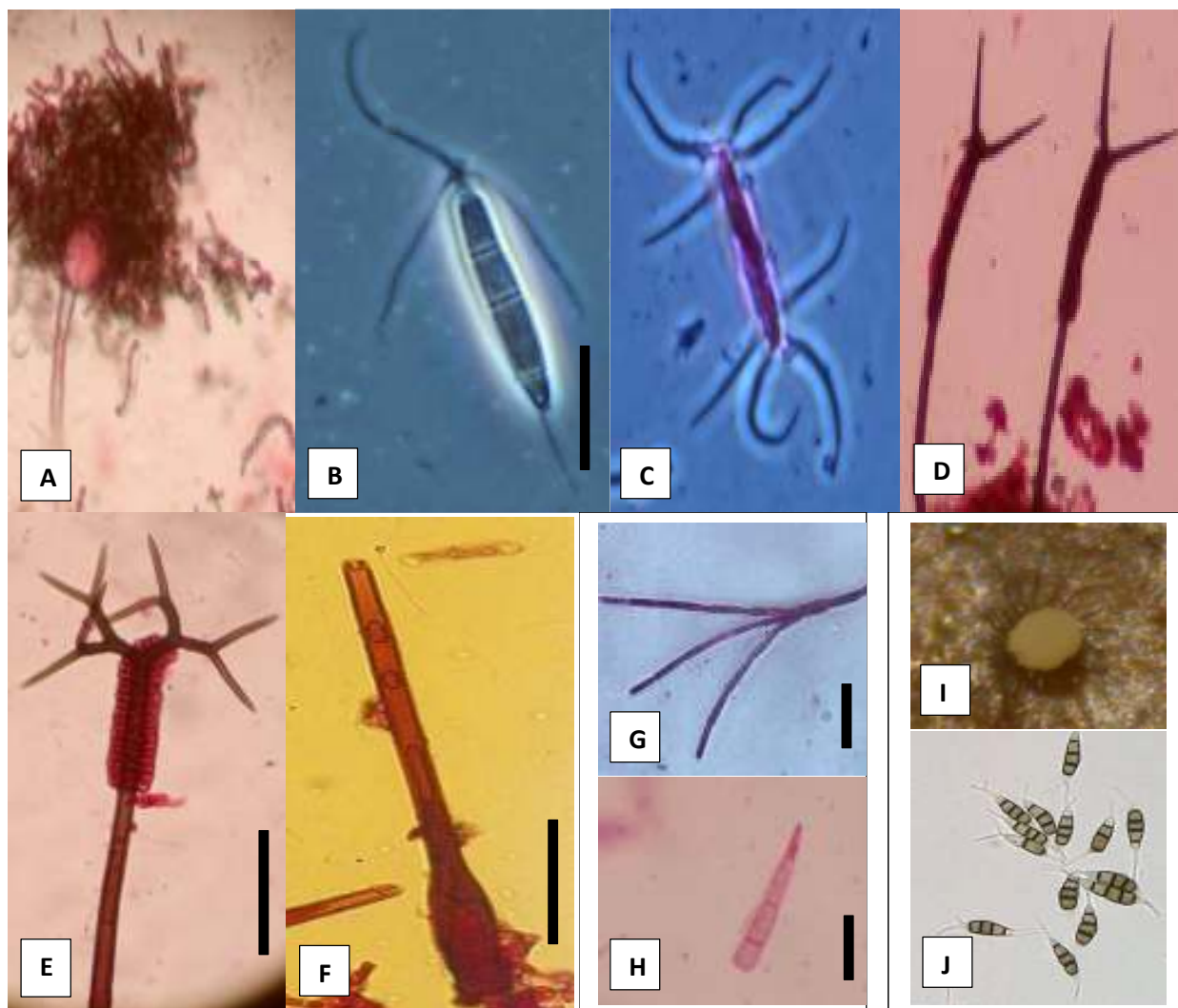


Figure 1. A. Conidiophore of *Aspergillus nomius* Mag X 1000. B. Conidia of *Bartalinia robillardoides*. C. Conidia of *Chaetospermum camelliae*. D. *Cryptophiale minor*. E. *Cryptophiale udagawae*. F. *Chalara pteridina*. G. Conidia of *Phalangispora*. H. Conidia of *Subulispora procurvata* Mag X 1000. I. *Wiesneriomyces laurinus* D. Conidia of *Pestalotiopsis biciliata*. Scale bars= 50 µm.

9. *†Wiesneriomyces laurinus* (Tassi) P.M. Kirk, 1984. *Transactions of the British Mycological Society* 82: 748–749.

The genus *Wiesneriomyces* is an asexual genus with no known sexual stage. This genus has been recently elevated to a higher taxonomic rank, the family *Weisneriomycetaceae* under the class *Dothideomycetes* (Suetrong et al., 2014). There are only 2 known and accepted species in this genus so far. These 2 species are *W. laurinus* and *W. conjuctosporus* (Index fungorum, 2015; Kuthubutheen & Nawawi, 1988; Suetrong et al., 2014).

The genus *Wiesneriomyces*, with type species *W. laurinus*, is characterised by a conidiomata with conspicuous setae raising upwards, with conidia mass in between or surrounding the setae, conidia aseptate, hyaline, in uniseriate chains (in a single row) connected together by a narrow isthmi. This genus, is differentiated from similar genus *Phalangispora* by having a single row of conidial chains, whereas *Phalangispora* has branched chains of conidia. The genera *Abgliophragma* R.Y. Roy & S. Gujarati, and *Gliophragma* Subram. & B.C. Lodha are ultimately synonyms to *Weisneriomyces* (Suetrong et al., 2014; Whitton et al., 2012). Only 1 species, *W. laurinus* (Figure 1 I), was recorded in this study on leaves of *Syzygium* sp, *Macaranga* sp and *Baccaurea* sp from Gunung gading National Park

10. *Pestalotiopsis biciliata* Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, *Stud. Mycol.* 79: 156 (2014).

The genus *Pestalotiopsis* was initially splitted from *Pestalotia* De Not in 1949 by Steyaert, with type species *Pestalotiopsis guepinii* (Index fungorum, 2015) to accommodate species with 5-celled conidia different from *Truncatella* with 4-celled conidia (Maharachchikumbura et al., 2014). Recently, the genus *Pestalotiopsis* Steyaert was reviewed which resulted in two other genera being separated from *Pestalotiopsis*, namely, *Neopestalotiopsis* and *Pseudopestalotiopsis* (Maharachchikumbura et al., 2014).

P. biciliata (Figure 1 J) was isolated from green leaves of *Shorea macrophylla* from Kubah National Park and is hereby described: **Conidiomata** pycnidial, globose, black to dark brown, immersed or sub-immersed; exuding black to dark brown mass of conidia. **Conidiophores** indistinct, reduced to conidiogenous cells. **Conidiogenous cells** discrete, cylindrical to sub-cylindrical, hyaline, thin-walled, smooth. **Conidia** fusoid or sometimes subcylindrical, straight to slightly curved, 4-septate, 16.69-25.02 × 4.16-6.41 µm, mean ± SD = 19.89 ± 2.15 × 5.23 ± 0.54 µm; basal cell conical to obconic, hyaline, thin-walled, smooth, 3.5-5.8 µm long, mean ± SD = 4.4 ± 0.68 µm; the three median cells together 9.65-15.24 µm long, mean ± SD = 12.25 ± 1.40 µm, concolourous, light brown with septa darker than the rest of the cells (second cell from base 2.74-6.11 µm, mean ± SD = 4.15 ± 0.69 µm; third cell 3.07-5.04 µm, mean ± SD = 3.98 ± 0.54 µm; fourth cell 3.13-5.45 µm, mean ± SD = 4.12 ± 0.56 µm); apical cell 2.0-4.83 µm long, mean ± SD = 3.23 ± 0.76 µm, conical to sub-cylindrical, hyaline; 2-3 (mostly 3) tubular apical appendages, unbranched, flexuous, 5.80-16.50 µm long, mean ± SD = 10.39 ± 2.46 µm; basal appendage present, two (one long, pointing directly downwards, the other short, points at an angle from the first), tubular, the long one 4.49-9.92 µm long, mean ± SD = 6.50 ± 1.38 µm.

CONCLUSION

This study has provided taxonomic details of newly recorded microfungi from two national parks in Sarawak, establishing the occurrences and host affinities of these fungi in Sarawak. More studies of this kind on the documentation of Sarawak's fungi are essential in order to fully appreciate Sarawak's biodiversity richness.

ACKNOWLEDGMENTS

The first author is grateful to Universiti Malaysia Sarawak (UNIMAS) for the Zamalah scholarship awarded. We are also grateful to the Sarawak government and to Sarawak Forestry Co-operation (SFC) for permission to collect samples from the National Parks.

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