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Rhopalostroma brevistipitatum sp. nov. from Thailand with an extended generic description for *Rhopalostroma*

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Abstract

Rhopalostroma species were collected from Northern Thailand and subjected to morph-molecular analysis**.** One species possessed small, clavate stromata, with short and stout stipes, asci with an amyloid apical apparatus and ellipsoidal, dark ascospores with germ slits. Morphology and combined phylogenetic analysis of ITS, LSU, β-tubulin and RPB2 sequence data, showed it to be a new *Rhopalostroma* species introduced herein as *R. brevistipitatum.* A nodulisporium-like asexual morph was produced in culture. A morphological description and photographs of *R. brevistipitatum* are provided in this paper, with amendments to the generic description.

Key words: apical apparatus, *Nodulisporium*, phylogeny, taxonomy, Xylariaceae

Introduction

Hawksworth (1977) introduced the genus *Rhopalostroma* for species characterized by stipitate, carbonaceous stromata with expanded convex heads, dark brown to black internal flesh without concentric zones, in which the perithecia are immersed and arranged in a single layer. Asci are reported to be evanescent in the majority of the species, but when present the asci are inamyloid (Hawksworth 1977, Hawksworth *et al.* 1979, Whalley & Thienhirun 1996, Whalley *et al.* 1998, Stadler *et al.* 2010, Daranagama *et al.* 2014). *Rhopalostroma* currently comprises ten species and two varieties (Index Fungorum 2015). Hawksworth (1977) transferred four species previously disposed in various genera of Xylariaceae into the genus and described *R. indicum* D. Hawksw. & Muthappa as the type species. An additional five new species have since been described, three from Thailand and two from India (Hawksworth and Whalley 1985, Vaidya *et al.* 1991, Whalley & Thienhirun 1996, Whalley *et al.* 1998). *Rhopalostroma angolense* (Welw. & Curr.) D. Hawksw. is the only species restricted to Africa (Patil *et al.* 2012). Thus it is believed the genus is exclusively African and Asian.

Rhopalostroma gracile D. Hawksw. & Whalley (Hawksworth & Whalley 1985) and *R. hawksworthii* Vaidya *et al.* (Vaidya *et al.* 1991) were grown in culture and produced nodulisporium-like asexual morphs. Stadler *et al.* (2010) and Daranagama *et al.* (2014) have described nodulisporium-like asexual morphs in culture from *R. angolense* and *R. lekae* Whalley *et al*.

The phylogenetic affinities of *Rhopalostroma* were shown by Stadler *et al.* (2004, 2010) and Daranagama *et al.* (2014). In this study we introduce a new species of *Rhopalostroma* from Northern Thailand. *Rhopalostroma brevistipitatum* contains certain characters that have not previously been observed in other *Rhopalostroma* species. Therefore we have observed the type, *Rhopalostroma indicum* from IMI in order to facilitate a better morphological comparison with our new species.

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Materials and methods

Sample collection and specimen examination

Specimens of *Rhopalostroma* were collected in Chiang Rai Province, Northern Thailand in November 2014 and macroscopic and microscopic characters were recorded. A Motic SMZ-168 dissecting microscope was used to observe the structure of stromata and perithecia. Formation of stromatal pigments was observed by placing a small piece of stroma (from both head and stipe) in a few drops of 10% KOH (Ju & Rogers 1996). The color designations were determined following Rayner (1970). A Nikon ECLIPSE 80i compound microscope was used to observe asci and ascospore characters, the reaction of ascal apical rings were tested using Melzer's reagent. Measurements of stromata (n=10), ascomata (n=10), asci (n=20) and ascospores (n=40) were made from materials mounted in water and the mean values were used in the descriptions. Photomicrography was carried out using a Canon 450D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems Inc). Herbarium material is deposited in Mae Fah Luang University, Thailand (MFLU) and Kunming Institute of Botany Herbarium (KUN), China and cultures at Mae Fah Luang University Culture Collection, Thailand and the Kunming Institute of Botany Culture Collection (KIBCC), China. Faces of fungi numbers and Index Fungorum numbers are as explained in Jayasiri *et al.* (2015) and Index Fungorum (2015).

Description of cultures and asexual morph

Pure cultures were obtained from single spores following the method detailed by Chomnunti *et al.* (2014). The cultures were grown in Malt and Yeast Extract Agar media (Malt extract 6 g/L, yeast extract 0.6 g/L, dextrose 4 g/l) and incubated at 25–28°C for 2–4 days. After 2–4 days, hyphal tips were cut and transferred to fresh Difco Oatmeal Agar (OA) media. The cultures were incubated at 25–28°C for one month. After 2–3 weeks, cultures on OA were checked for asexual structures. Conidiogenous structures (conidiophores, conidiogenous cells and conidia) were observed and measured by phase contrast microscopy under 400–1000 × optical magnification. Asexual morphs are classified based on Stadler *et al* (2013).

DNA isolation, PCR and sequencing

DNA was extracted from isolates grown on Malt and Yeast Extract Agar media overlaid with sterilized cellophane for 5 days at 25°C (Murali *et al.* 2006). DNA isolation was carried out according to Udayanga *et al.* (2012) with modifications. Precipitated DNA was recovered by centrifugation of 12,000 rpm for 10 min and three washings with 70 % ethanol, air dried, dissolved in 50 µl of sterilized distilled water and stored at –20 °C until use for amplification reactions.

Four loci were sequenced including ITS, LSU, RPB2 and β-tubulin. The primers and PCR protocols follow Daranagama *et al.* (2015). The DNA fragments were amplified using an automated thermal cycler (DongShen EDC-810- Eastwin, LifeSciences). The total volume of 50 µl reaction mixture [10×PCR buffer, 0.25 mM dNTP, 0.4 µM of each primer; 1.5 mM $MgCl_2$, Taq Polymerase and 10 ng template DNA (1:10 diluted)], was used for PCR with adjustments of components' volumes and concentration when needed. The PCR products were visualized on 1% agarose mucilaginous stained with Goldview (Geneshun Biotech, China) with D2000 DNA ladder (Realtimes Biotech, Beijing, China). All the PCR products were purified according to the company protocols and DNA sequencing was performed using the same primers in an Applied Biosystem 3730 DNA analyzer at Sinogenomax Company, Beijing, China. The sequences derived from this study are deposited in GenBank.

Sequence alignment and phylogenetic analysis

To reveal the phylogenetic position of *R. brevistipitatum,* 51 strains from representative xylariaceous species from reliable studies were downloaded from GenBank and included in the analysis (Table 1), with *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. as the outgroup taxon. The respective sequences from this study are deposited in GenBank (see Table 1). Phylogenetic analysis was performed using a combined ITS–LSU–RPB2–β–tubulin sequence data matrix.

TABLE 1. Isolates used in this study and GenBank accession numbers.

Species	Culture collection/specimen number	Type status	Reference	GenBank accession numbers			
				ITS	RPB2	β-tubulin	LSU
Amphirosellinia fushanensis	**HAST Isolate 91111209**	Ex-type	Hsieh *et al.* 2010	GU339496	GQ848339	GQ495950	–
A. nigrospora	**HAST Isolate 91092308**	Ex-type	Hsieh *et al.* 2010	GU322457	GQ848340	GQ495951	–
Anthocanalis sparti	**MFLUCC 14–0010**	Ex-type	Daranagama *et al.* 2015	KP297394	KP340522	KP406605	KP340536
A. sparti	**MFLUCC 14–0557**	Ex-paratype	Daranagama *et al.* 2015	KP297395	KP340523	KP406606	KP340537
Annulohypoxylon thailandicum	**MFLUCC 13-0118**	Ex–type	Liu *et al.* 2015	KP744434	–	–	KP744476
A. nitens	**MFLUCC 12-0823**	Authentic	Daranagama *et al.* 2015	KJ934991	KJ934994	KJ934993	KJ934992
A. stygium	**MFLUCC 13-0826**	Authentic	Daranagama *et al.* 2015	KJ940870	KJ940868	KJ940867	KJ940869
Anthostomella forlicesenica	**MFLUCC 14–0007**	Ex-type	Daranagama *et al.* 2015	KP297396	KP340524	KP406607	KP340538
A. forlicesenica	**MFLUCC 14–0558**	Ex-paratype	Daranagama *et al.* 2015	KP297397	KP340525	KP406608	KP340539
A. formosa	**MFLUCC 14–0170**	Authentic	Daranagama *et al.* 2015	KP297403	KP340531	KP406614	KP340544
A. helicofissa	**MFLUCC 14–0173**	Ex-type	Daranagama *et al.* 2015	KP297406	KP340534	KP406617	KP340547
A. obesa	**MFLUCC 14–0171**	Ex-type	Daranagama *et al.* 2015	KP297405	KP340533	KP406616	KP340546
A. rubicola	**MFLUCC 14–0175**	Authentic	Daranagama *et al.* 2015	KP297407	KP340535	KP406618	KP340548
Astrocystis bambusae	**HAST Isolate 89021904**	Authentic	Hsieh *et al.* 2010	GU322449	GQ844836	GQ495942	–
A. mirabilis	**HAST Isolate 94070803**	Authentic	Hsieh *et al.* 2010	GU322448	GQ844835	GQ495941	–
A. concavispora	**MFLUCC 14–0174**	Ex-type	Daranagama *et al.* 2015	KP297404	KP340532	KP406615	KP340545
Biscogniauxia arima	**WSP 122**	Ex-isotype	Hsieh *et al.* 2010	EF026150	GQ304736	AY951672	–
B. mediterranea	**YMJ 147**	Authentic	Hsieh *et al.* 2010	EF026134	GQ844765	AY951684	–
B. marginata	**MFLUCC 12-0740**	Authentic	Daranagama *et al.* 2015	KJ958407	KJ958409	KJ958406	KJ958408
Brunneiperidium gracilentum	**MFLUCC 14–0011**	Ex-type	Daranagama *et al.* 2015	KP297400	KP340528	KP406611	KP340542
B. gracilentum	**MFLUCC 14–0559**	Ex-paratype	Daranagama *et al.* 2015	KP297401	KP340529	KP406612	KP340549
B. involucratum	**MFLUCC 14-0009**	Ex-type	Daranagama *et al.* 2015	KP297399	KP340527	KP406610	KP340541
Collodiscula japonica	**CBS 124266**	Authentic	Jaklitsch and Voglmayr, 2011	JF440974	–	–	JF440974
Daldinia concentrica	**CBS 113277**	Authentic	Kuhnert *et al.* 2013	AY616683	–	KC977274	–
D. decipiens	**CBS 122879**	Authentic	Hsieh *et al.* 2005	JX658441	–	AY951694	–
D. loculata	**BCRC 34117**	Authentic	Hsieh *et al.* 2005	EF026145	–	AY951698	–
Hypoxylon fendleri	**MFLUCC 12-0816**	Authentic	Daranagama *et al.* 2015	KM017563	KM017566	KM017564	KM017565

...Continued on next page

TABLE 1. (Continued)

Species	Culture collection/specimen number	Type status	Reference	GenBank accession numbers			
				ITS	RPB2	β-tubulin	LSU
H. fragiforme	**MUCL 51264**	Authentic	Triebel *et al.* 2005/LSU β–tubulin and RPB2 sequenced in this study	KM186294	KM186296	KM186301	KM186295
H. lenormandii	**MFLUCC 13-0120**	Authentic	Daranagama *et al.* 2015	KM039135	KM039137	KM039138	KM039136
H. monticulosum	**MFLUCC 12-0818**	Authentic	Daranagama *et al.* 2015	KM052716	KM052719	KM052718	KM052717
Kretzschmaria clavus	**YMJ 114**	Authentic	Hsieh *et al.* 2010	EF026126	GQ844789	EF025611	–
K. guyanensis	**HAST 89062903**	Authentic	Hsieh *et al.* 2010	GU300079	GQ844792	GQ478214	–
K. neocaledonica	**HAST 94031003**	Authentic	Hsieh *et al.* 2010	GU300078	GQ844788	GQ478213	–
Lunatiannulus irregularis	**MFLUCC 14–0014**	Ex-type	Daranagama *et al.* 2015	KP297398	KP340526	KP406609	KP340540
Phylacia poculiformis	**MUCL 51706**	Authentic	Stadler *et al.* 2010	FN428830			
Podosordaria mexicana	**WSP 176**	Authentic	Hsieh *et al.* 2010	GU324762	GQ853039	GQ844840	–
P. muli	**WSP 167**	Ex-type	Hsieh *et al.* 2010	GU324761	GQ853038	GQ844839	–
Poronia pileiformis	**WSP 88113001**	Ex-epitype	Hsieh *et al.* 2010	GU324760	GQ853037	GQ502720	–
Pyriformiascoma trilobatum	**MFLUCC 14–0012**	Ex-type	Daranagama *et al.* 2015	KP297402	KP340530	KP406613	KP340543
Rhopalostroma brevistipitatum	**MFLUCC 15-0007**	Ex–type	This study	KT253585	KT359352	–	KT305986
R. brevistipitatum	**MFLUCC 15-0011**	Ex–isotype	This study	KT253586	KT359353	–	KT305987
R. angolense	**CBS 126414**	Authentic	Stadler *et al.* 2010a/ Daranagama *et al.* 2015	FN821965	KM186297	KM186299	KM186298
R. lekae	**MFLUCC 13–0123**	Authentic	Daranagama *et al.* 2014	KJ472428	KJ472427	–	KJ472429
Rosellinia buxi	**JDR 99**	Authentic	Hsieh *et al.* 2010	GU300070	GQ844780	GQ470228	–
R. lamprostoma	**HAST 89112602**	Authentic	Hsieh *et al.* 2010	EF026118	GQ844778	EF025604	–
R. necatrix	**HAST 89062904**	Authentic	Hsieh *et al.* 2010	EF026117	GQ844779	EF025603	–
Rostrohypoxylon terebratum	**CBS 119137**	Ex-type	Fournier *et al.* 2010	DQ631943	DQ631954	DQ840097	DQ840069
Sordaria fimicola	CBS 723.96	–	Tang *et al.* 2009	AY681188	DQ368647	DQ840087	AF132330
Thamnomyces camerunensis	**MUCL 51396**	Authentic	Stadler *et al.* 2010c	FN428828	–	–	–
Xylaria adscendens	**JDR 865**	Authentic	Hsieh *et al.* 2010	GU322432	GQ844818	GQ487709	–
X. hypoxylon	**CBS 122620**	Ex-epitype	Stadler *et al.* 2014/ Daranagama *et al.* 2015	AM993141	KM186302	KM186300	KM186301

Ex-types and authentic strains are in **bold.** Abbreviations: **AT**: Taxa collected and identified by Alvin M. C. Tang; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **BCRC**: Bioressource Collection and Research Centre, Taiwan; **HAST**: Herbarium, Research Centre for Biodiversity, Academia Sinica, Taipei; **JDR**: Herbarium of Jack D. Rogers; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **MUCL**: Mycothèque de l'Université catholique de Louvain, Germany; **YMJ**: Herbarium of Yu Ming Ju; **WSP**: Washington State University, USA.

Sequence data were aligned either with MUSCLE v.3.6 (Edgar 2004) or Bioedit 7.1.3.0 (Hall 1999) and further implemented with Clustal X v1.83 (Thompson *et al.* 1997) and manually aligned where necessary. All characters were assessed to be unordered and equally weighed. Gaps were treated as missing data. Phylogenetic analyses were performed using RAxML v7.0.3 (Stamatakis & Alachiotis 2010) as implemented in RAxML GUI 0.95 (Silvestro & Michalak 2012). The search strategy was set to rapid bootstrapping and the analysis carried out using the GTR model of nucleotide substitution. The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). The bootstrap analysis for each ML tree was performed with 1000 fast bootstrap replicates with the same parameter settings using the GTR substitution model selected by MrModel Test. Model parameters were calculated separately for four different gene regions included in the combined analyses. The resulting trees were viewed using the Tree View application (Page 1996).

Results

Molecular phylogenetic analysis

The combined ITS, LSU, β-tubulin and RPB2 phylogeny (Fig. 1) was generated from sequences derived from authentic isolates including ex-type, ex-isotype, ex-paratype and ex-epitype strains (Table 1). The combined data matrix used 51 taxa including the outgroup taxon. A total number of 3680 characters (including gaps) were evaluated in the Maximum Likelihood analysis using GTR model determined by MrModeltest 2.2. The best scoring ML tree was used in the study to infer the phylogenetic placement of new *R. brevistipitatum* strain (Fig.1).

The combined data matrix provides a better resolution of the terminal clades than any other individual data set (data not shown) with high bootstrap support. The phylogenetic analyses showed that all species separated into two well-supported groups; Hypoxyloideae and Xylarioideae (Fig. 1). According to the phylogenetic data presented in Fig. 1, they are resolved as two monophyletic groups with high bootstrap support (82%), which is similar to the results obtained in Daranagama *et al.* (2015), Maharachchikumbura *et al.* (2015) and Senanayake *et al.* 2015.

Twenty-five taxa, representing *Annulohypoxylon, Anthocanalis, Anthostomella, Biscogniauxia, Daldinia, Hypoxylon, Rhopalostroma, Rostrohypoxylon* and *Thamnomyces* species clustered in Hypoxyloideae clade and were well-resolved except for *Hypoxylon*, which appeared as singletons. The new species *R. brevistipitatum* clustered within the Hypoxyloideae and formed a well-supported monophyletic clade, with other *Rhopalostroma* species (*R. lekae* and *R. angolense*) with 88% bootstrap support. *Rhopalostroma brevistipitatum* clustered with *R. lekae* and *R. angolense* with 96% bootstrap support confirming its placement in *Rhopalostroma*. *Rhopalostroma* and *Thamnomyces* represent a monophyletic clade with 88% bootstrap support and formed a separate clade with *Anthocanalis* supported with 96% bootstrap support. *Daldinia* appeared as the sister clade to *Anthocanalis, Rhopalostroma* and *Thamnomyces* with 86% bootstrap support.

Xylarioideae taxa including *Amphirosellinia, Astrocystis, Brunneiperidium, Collodiscula, Kretzschmaria, Lunatiannulus, Podosodaria, Poronia, Rosellinia* and *Xylaria* formed a monophyletic clade. *Podosodaria* and *Poronia* resolved as basal clades to the other xylarioid genera, but with a low bootstrap support. *Amphirosellinia, Astrocystis*, *Collodiscula* and *Lunatiannulus* formed a separate clade, while *Brunneiperidium, Kretzschmaria, Rosellinia* and *Xylaria* grouped together. However, the bootstrap support for this separation was lower than 50%.

Barrmaelia and *Pyriformiascoma* clustered as the basal lineages to both the Hypoxyloideae and Xylarioideae clades with 82% and 78% bootstrap support.

Taxonomy

Rhopalostroma indicum D. Hawksw. & Muthappa, in Hawksworth, Kew Bull. 31(3): 426 (1977). Fig.2

Saprobic on decorticated *Ficus retusus*. **Sexual morph:** *Stromata* aggregated, erumpent, stipitate, head (6–)7–8(–9) × (2.7–)3–5(–5.2) mm ($\bar{x}$ = 7.6 × 4.1 mm), globose to subglobose, carbonaceous, purplish black, stipe (4–)5–7(–7.4) × (1–)1.5–2.5(–2.7) mm ($\bar{x}$ = 5.9 × 1.8 mm), black, fragile, carbonaceous, KOH extractable pigments present, vinaceous-purple (101) to vinaceous-grey (116). *Perithecia* (–327)400–700(–745) × (278–)300–500(–525) µm ($\bar{x}$ = 523 × 460 µm), subglobose to globose, immersed in stromatal tissue. *Paraphyses* (1.8–)2–3.5(–3.7) µm diam. ($\bar{x}$ = 2.6 µm), much longer than asci, filiform, remotely septate, hyaline, few. *Asci* (82–)90–130(–138) × (3–)4–5(5.5) µm ($\bar{x}$ = 120 × 4.6 µm), 8–spored, cylindrical, stipitate, deliquescent, unitunicate, ascal apical ring not bluing in Melzer's reagent, . *Ascospores* (5.5–)7–9(–9.8) × (3.7–)4–5.5(–6) µm ($\bar{x}$ = 7.8 × 5.1 µm), uniseriate dark brown, unicellular, equilateral ellipsoidal, with a longitudinal germ slit running spore length (Fig.2a–m).

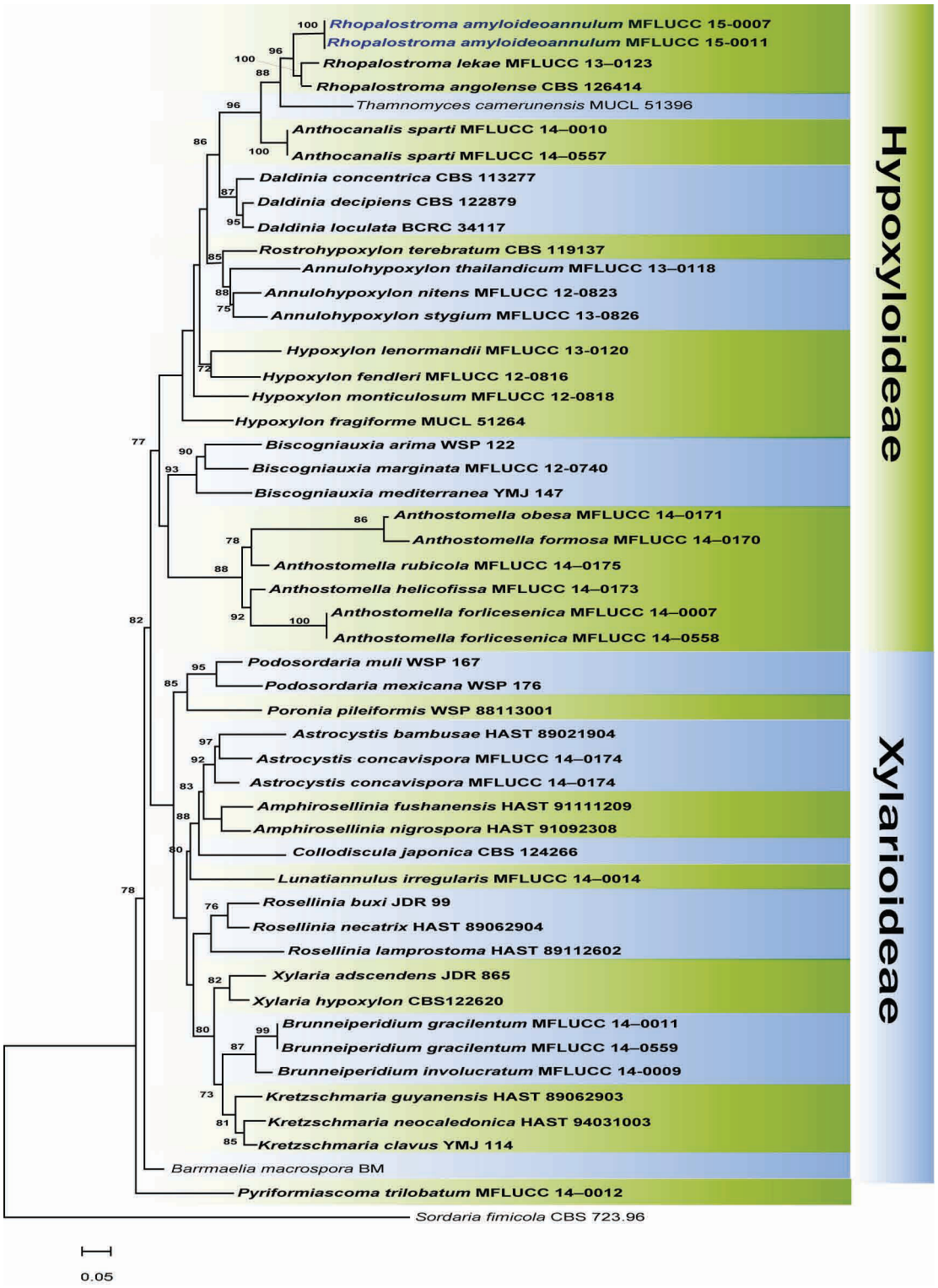

FIGURE 1. The phylogram inferred from likelihood analysis of members of Xylariaceae using combined ITS-LSU-β–tubulin-RPB2 sequence data. Strain/culture numbers are given following the taxon names; Type specimens, ex/iso/para/epi-type strains and authentic specimens and strains are highlighted in bold. The new sequences generated in this study are in blue. The bootstrap support values from likelihood analysis >50% from 1000 RAxML replicates are shown above or below the branches. The tree is rooted with *Sordaria fimicola* (CBS723.96).

Material examined: INDIA, Karnataka state, Central Coffee Research Institute, on dead bark of *Ficus retusus* L. (Moraceae), 25 November 1974, Muttappa (holotype IMI 190605!).

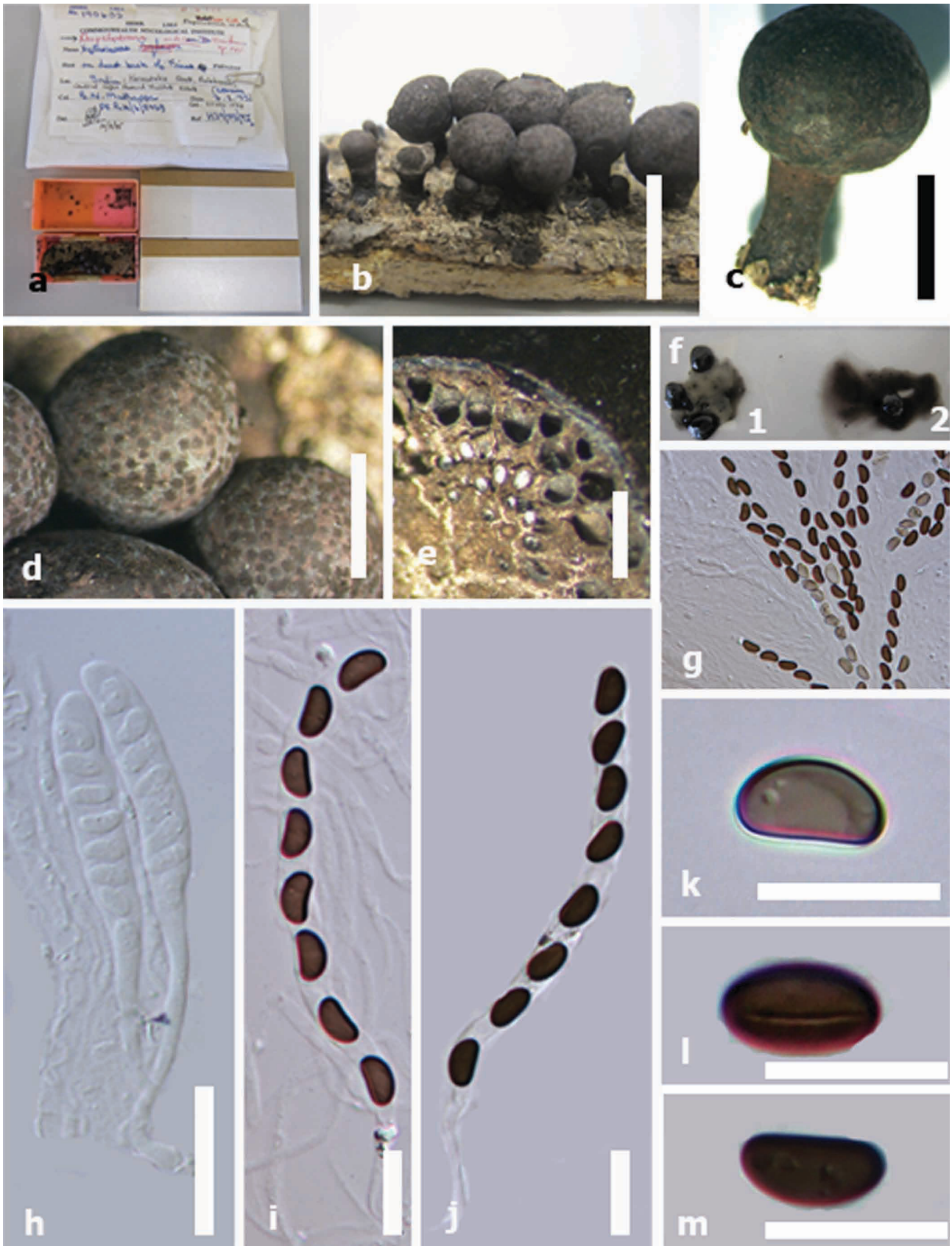

FIGURE 2. *Rhopalostroma indicum* (Holotype) a: Herbarium specimens, b, c: Stromata in wood, d: Black ostioles in stromatal surface, e: Cross section of stromata showing perithecia encased in stromatal tissue, f: KOH extractable pigments (1-head, 2-stipe) g: Paraphyses, h: Immature asci, i, j: Mature asci, k: Immature ascospore l: Ascospores showing the germ slit, m: Mature ascospore. *Scale bars:* b = 10 mm, c, d = 5 mm, e = 1000 µm, h-m = 10 µm.

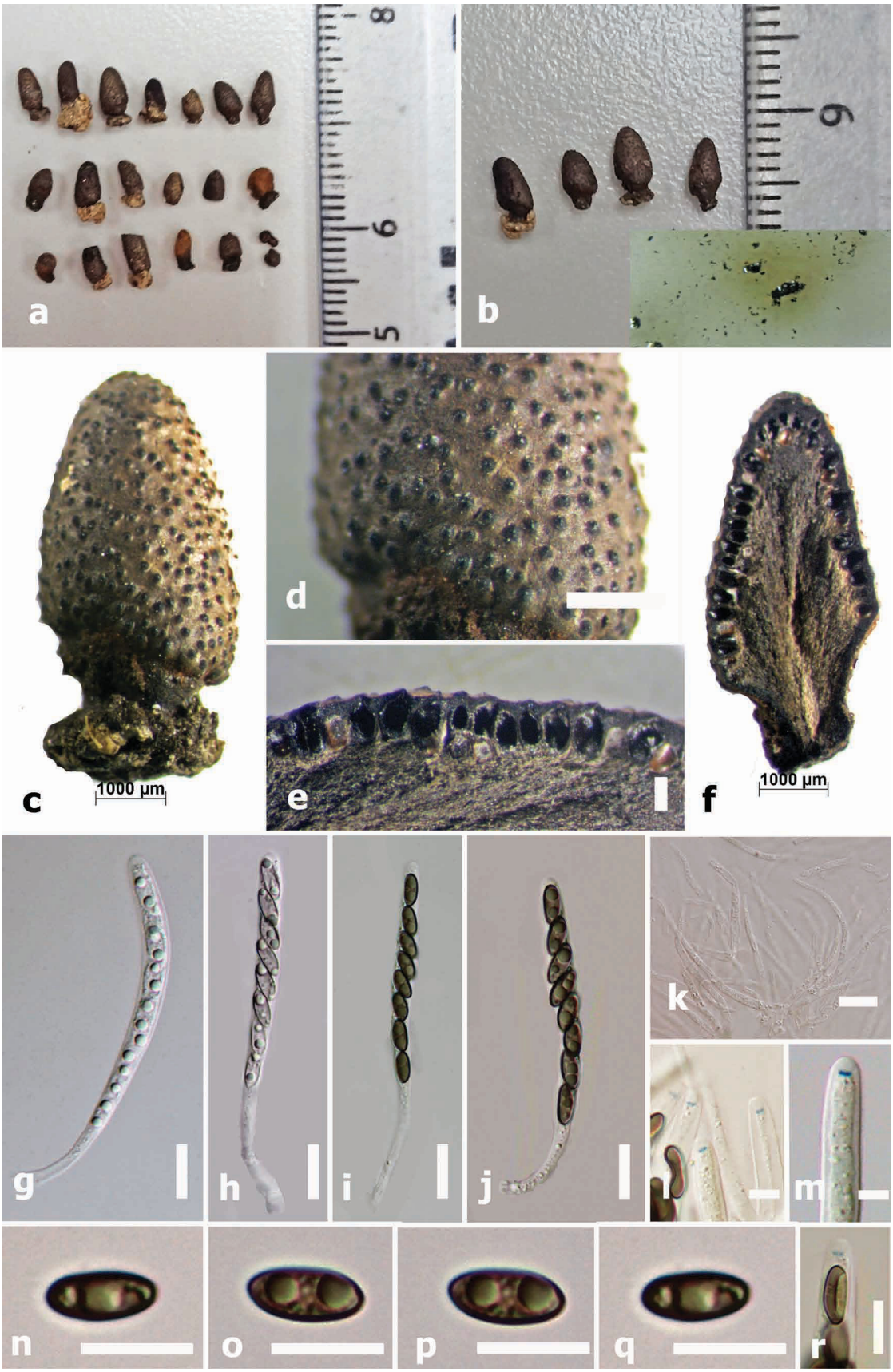

FIGURE 3. *Rhopalostroma brevistipitatum* (Holotype). a: Developmental stages of stromata. b: Separated mature stromata and KOH extractable pigments. c: Individual stroma showing expanded head and short, stout stipe. d: Stromatal surface with ostoles. e: Longitudinal section of stroma showing perithecia alignment in the periphery of stroma. f: Cross section of the stroma showing perithecia. g-h: Asci in water. k: Paraphyses. l and m: Asci in Melzer's reagent, Note the presence of an apical apparatus. n-q: Mature ascospores in water. r: Ascospores with germ slit. *Scale bars*: c = 1000 µm; d = 500 µm; e = 200 µm; f = 1000 µm; g-j = 10 µm; k-m = 5 µm; n-r = 10 µm.

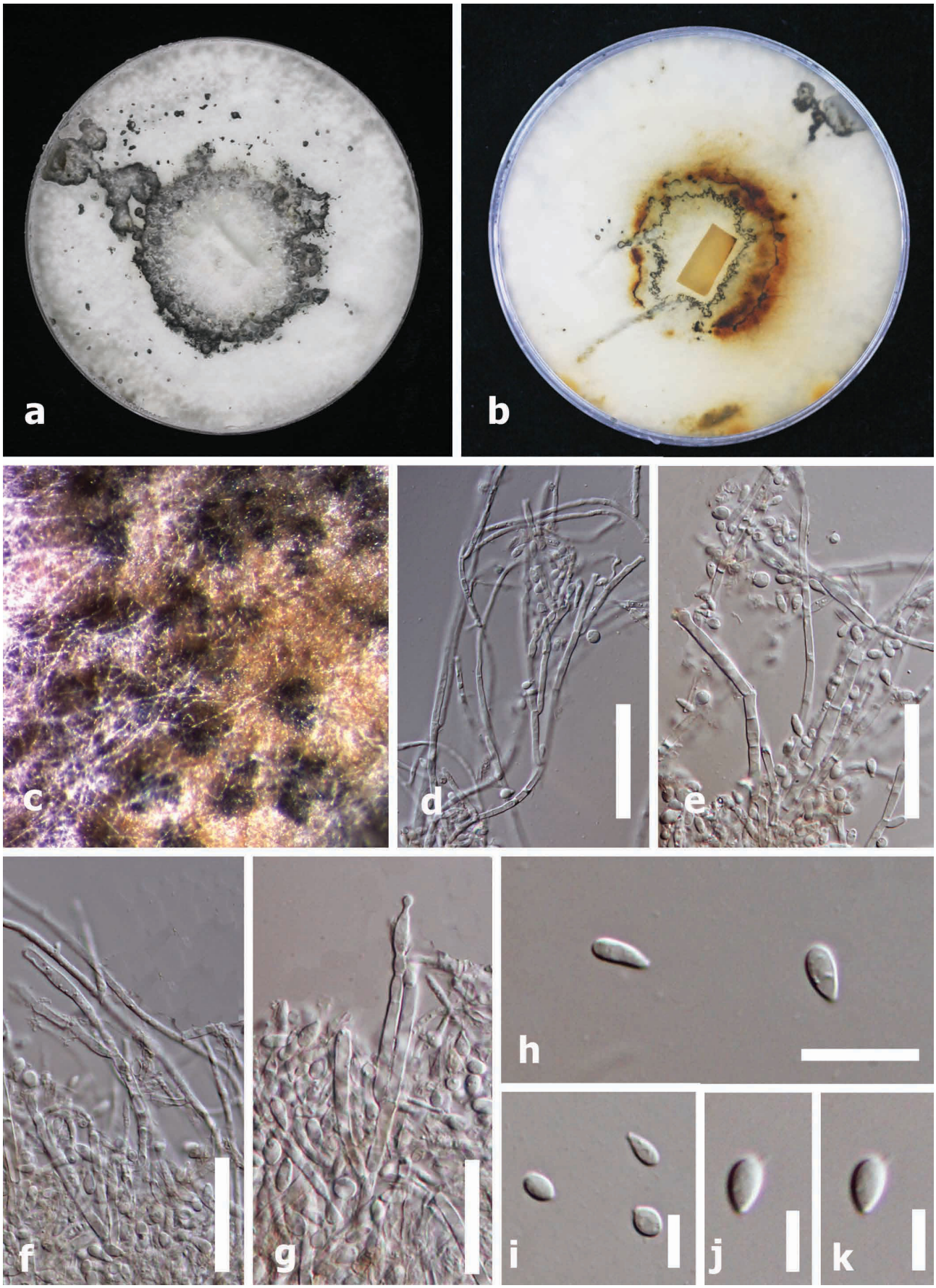

FIGURE 4. *Rhopalostroma brevistipitatum* in OA after 2 weeks (MFLUCC 15-0007-ex-type). a: From above. b: From below. c: Development of coniomata in the culture. d-g: Conidiophores from simple to more complex structure. h-k: Conidia. *Scale bars:* d-g = 40 µm; h = 10 µm; i-k = 5 µm.

Rhopalostroma brevistipitatum Daranagama & K. D. Hyde, *sp. nov.* Figs 3–4.

Index Fungorum number: IF 550815; *Facesof Fungi number:* FOF00656

Etymology:—Refers to small, short and stout stipe in stromata.

Diagnosis:—Differs from other species in *Rhopalostroma* by having small and solitary stromata with short and stout stipe and cylindrical asci with J+, discoid, apical apparatus.

Saprobic on decaying bark. **Sexual morph:** *Stromata* (2.3–)2.5–6.4(–6.8) mm high ($\bar{x}$ = 4.9 mm), erumpent through bark, widely scattered, simple, solitary, never clustered, not fused and not branched, light brown to purplish brown, carbonaceous, stipe (1–)1.3–1.8(–2) × (1.8–)2–3.2(–3.5) mm ($\bar{x}$ = 1.5 × 2.7 mm), compact, short and stout, head (3.8–)4–4.8(–5) × (1.7–)2–3.7(–3.7) mm ($\bar{x}$ = 4.5 × 2.9 mm), expanded, clavate, flesh of head greyish black and continuous with that of the stipe, stromatal pigments present, light green. *Ascomata* (153–)186–393(–398) × (160–)179–226(–234) µm ($\bar{x}$ = 284× 204 µm), immersed, arranged in a single layer below the convex layer of the head, encased in carbonaceous tissue, individual perithecia hemispherical. *Ostioles* appear as minute shiny black dots, papillate. *Paraphyses* at base (2.7–)3–4.2(–4.5) µm wide ($\bar{x}$ = 3.7 µm), slightly longer than asci, filamentous, aseptate. *Asci* (80.6–)85.2–102.5(–112.3) µm ($\bar{x}$ = 98.6 µm) in total length, spore bearing part (60.2–)62.5–83(–85.8) × (4.3)4.5–5(–5.1) µm ($\bar{x}$ = 77.5 × 4.8 µm), stipe (12.3–)18.5–33.5(–38.2) µm ($\bar{x}$ = 27.5 µm), 8-spored, cylindrical, stipitate, with an amyloid apical apparatus, discoid, (0.8–)1.2–2(–2.1) × (1.6–)2–2.2 (2.4) µm ($\bar{x}$ = 1.5 × 2.0 µm). *Ascospores* (9.6–)10.6–11.3(–11.5) × (3.3–)4–4.5(–4.8) µm ($\bar{x}$ = 11 × 4.3 µm), uniseriate, olivaceous, equilaterally ellipsoidal, with broadly rounded ends, epispore smooth, perispore indehiscent in 10% KOH, germ slit straight, spore length on the convex side (Fig. 3a–r). **Asexual morph:** *Conidiophores* (70–)80–97(–110) × (1.5–)2–2.5(–2.8) µm ($\bar{x}$ = 95.5 × 2.3 µm), simple to complex, hyaline, dichotomously branched, with nodulisporium-like branching pattern. *Conidiogenous cells* (15–)20–25(–35) × (0.7–)1–1.5(–1.6) µm ($\bar{x}$ =23.4 × 1.3 µm), developing terminally, cylindrical, hyaline, apically aggregated scars. *Conidia* (4.2–)5–6.7(–6.9) × (2.3–)2.5–3.2(–3.5) µm ($\bar{x}$ = 5.5 × 2.8 µm), hyaline, single, ellipsoidal, with one pointed end and one blunt end (Fig. 4d–k).

Culture characteristics:—Colonies on OA at 25°C reaching 5 cm in 7 days, at first whitish developing melanized pigments around the center within 5–10 days, azonate with diffuse margins, reverse at first, whitish and turning brownish orange at the center. Distinct sporulating regions observed after 10 days, black (Fig. 3a–c).

Material examined:—Thailand, Chiang Rai Province, Mae Fah Luang University Park, on decaying bark, 15 November 2014, D. A. Daranagama, (holotype MFLU 15-2245!) living cultures, MFLUCC 15-0007, KIBCC**;** *ibid* (isotype KUN!), ex-isotype, MFLUCC 15-0011.

Notes:—*Rhopalostroma brevistipitatum* is a new species of *Rhopalostroma* based on its unique morphology and phylogenetic analysis. The new species has a faint amyloid apical apparatus; this has not previously been observed in the genus. This species also has comparatively small stromata. *R. brevistipitatum* differs from other species in the genus by having a short, but stout stipe, as well as asci with J+ apical apparatus which has not been reported in the genus before. The asexual morph was identified as nodulisporium-like which is characteristic of the genus (Hawksworth & Whalley 1985, Stadler *et al.* 2010, Daranagama *et al.* 2014).

Discussion

Rhopalostroma brevistipitatum is herein reported as a new species discovered during the field collections in northern Thailand. According to the records (i.e. Whalley & Thienhirun 1996, Whalley *et al.* 1998, Daranagama *et al.* 2014) it is evident that *Rhopalostroma* species distribution is limited to Northern region within Thailand. *Rhopalostroma brevistipitatum* is another example of this.

With addition of *Rhopalostroma brevistipitatum,* the total number of species that belong to this genus increases up to eleven. *Rhopalostroma* species have received less attention due to the minute and fragile nature of the stromata. *Rhopalostroma brevistipitatum* has extremely minute stroma as compared with other *Rhopalostroma* species, which in turn looks like the immature stromata of *Xylaria* species. However morphological examination and molecular analysis confirmed this as a species belonging to *Rhopalostroma*. Unlike all the other *Rhopalostroma* species, *R. brevistipitatum* has an apical apparatus that blues in the presence of iodine reagents (Fig. 2). This has not been reported in other species. It is noteworthy that many species in *Rhopalostroma* have deliquescent asci, thus it was not possible to check the presence of amyloid apical apparatus in these species (Whalley & Thienhirun 1996, Whalley *et al.*1998, Patil *et al.* 2012). Hawksworth (1977) mentioned *R. indicum,* the type species was the only species with observable asci of all five species he observed.

TABLE 2: Morphological comparison of *Rhopalostroma* species.

Species name	Stromata	KOH extractable pigments	Ascomata	Hamathecium	Asci	Ascospores
Rhopalostroma africanum	Erect, singly arisen, in groups, head expanded, 2.5–4 mm, stipe 2–4 × 1.5–2 mm	Olivaceous-grey to isabelline or fawn	Hemispherical, monostichous, narrow necks, not papillate, 0.4–0.6 × 0.3–0.4 mm	Not reported	Not seen, evanescent at early stage.	Elongate–ellipsoidal, dark brown, longitudinal germ slit, 9–10 × 4–5 µm
Rhopalostroma brevistipitatum	Erect, widely scattered, simple, solitary, **not clustered, not fused and not branched, stipe compact, short and stout**,1.3–1.8 × 2–3.2 mm, head expanded, **clavate,** 4–4.8 × 2–3.7 mm.	**Light green**	Hemispherical, monostichous, **papillate ostioles**, 186–393 × 179–226 µm	Comprising **filamentous, aseptate**, hyaline paraphyses, 3.0–4.2 µm wide at base	Cylindrical, stipitate, 8-spored, **with an amyloid apical ring**, 85.2–102.5 × 4.5–5.0 µm	Equilaterally ellipsoidal, with broadly rounded ends, **olivaceous**, germ slit straight, 10.6–11.3 × 4.0–4.5 µm
Rhopalostroma angolense	Erect, gregarious, unbranched, head 0.8–1.3 cm high × 2.6–4 mm diam, stipe 1.5–1.8 mm diam.	Stipe–dark brick Head–olivaceous	Lanceolate, monostichous, 0.65–0.75 mm high × 0.2–0.25 mm diam.	Comprising deliquescent paraphyses, 5–6 µm at base	Cylindro-clavate to cylindrical, stipitate, 8–spored, without apical apparatus bluing in Melzer's reagent, 130–150 × 7.5–8.5 µm.	ellipsoid-inequilateral with broadly rounded ends, dark brown, a straight germ slit, 13–17 × 6–7 µm
Rhopalostroma dennisii	Erect, aggregated into dense tuft, unbranched or 1–2 branched, parallel to one another, stipe 8–13 × 1–1.5 mm, head globose, expanded, 2–4 mm diam.	Isabelline to honey	Lanceolate, monostichous, extending down to the upper part of the stipe, 0.5–0.6 × 0.25–0.4 mm	Not reported	Not seen, evanescent at maturity	Ellipsoidal, dark brown, longitudinal germ slit, 7–8.5 × 3–5 µm
Rhopalostroma gracile	Erect, arising from balck crust like subiculum, aggregated into small groups, not dense, stipe 8–16 × 1–1.8 mm, head subglobose, 1.5–2.5 mm diam.	weakly purple	Hemispherical, polystichous, unevenly arranged, lacking a distinct neck, non papillate, 0.25–0.35 × 0.2–0.3 mm	Not reported, evanescent at an early stage	Subcylindrical, stipitate, 8–spored, without apical apparatus bluing in Melzer's reagent, 65 × 6 µm	Ellipsoidal, one side more convex, dark brown, longitudinal germ slit, 9–11.5 × 4.5–7 µm

...Continued on next page

TABLE 2. (Continued)

Species name	Stromata	KOH extractable pigments	Ascomata	Hamathecium	Asci	Ascospores
Rhopalostroma indicum	Erect, aggregated, stipe 5–7 × 1.5–2.5 mm, head globose–subglobose, 5–7 × 1.5–2.5 mm diam.	vinaceous-purple to vinaceous-grey	Hemispherical, immersed in stromatal tissue, 400–700 × 300–500 µm diam.	Comprising filliform, remotely septate, hyaline paraphyses, 2–3.5µm diam.	cylindrical, stipitate, 8–spored, deliquescent, ascal apical ring not bluing in Melzer's reagent, 90–130 × 4–5 µm	equilateral ellipsoidal, dark brown, with a longitudinal germ slit, 7–9 × 4–5.5 µm
Rhopalostroma kanyae	Erect, solitary, widely scattered, clustered together, not fused, stipe 1–1.5 × 0.5–1 mm, head subglobose–hemispherical, 1.5–2.5 mm diam.	olivaceous-grey to isabelline or fawn	Lanceolate, monostichous, without a distinct neck, 0.3–0.6 × 0.2–0.4 mm	Not reported	Not seen, evanescent at maturity	Ellipsoidal, dark brown, straight germ slit, 8.5–10 × 3.5–5 µm
Rhopalostroma lekae	Erect, mostly solitary or rarely clustered, but not fused and rarely branched, stipe 3–5 × 0.5–1.5 mm, head globose–subglobose, 1–1.5 × 2–4 mm	Dark purple–purplish grey	Hemispherical, monostichous, lacking a distinct neck, 0.7–0.9 × 0.3–0.4 mm	Not present	Cylindrical, long–stipitate, 8–spored, ascal apical ring not bluing in Melzer's reagent, 140–160 × 6–7.2 µm	ellipsoidal to kidney bean–shaped, with broadly rounded ends, dark brown, straight germ slit, 7.5–10.4 × 3.5–5 µm
Rhopalostroma luzonense	Erect, aggregated into small groups, stipe 3–5 × 1.5–2 mm, head abruptly expanded, 2–4 mm diam.	–	Lanceolate, monostichous, non papillate, opening through a thin layer, 0.5–0.6 × 0.2–0.3 mm	Not reported	Not seen, evanescent at maturity	Elongate ellipsoidal, dark brown, longitudinal germ slit, 9–11 × 3–4 µm
Rhopalostroma sphaerocephalum	Erect, arising in dense tufts, stipe 1.2–2 × 1.2–2.5 mm, head abruptly expanded, convex, 2–3 mm diam.	–	Hemispherical, monostichous, non papillate, lacking narrow necks, 0.5 × 0.3 mm	Comprising filiform, septate, hyaline paraphyses, 2 µm wide at the base	Not seen, evanescent at maturity	Elongate ellipsoidal, dark brown, distinct longitudinal germ slit, 14–16 × 4–5 µm

* *Rhopalostroma hawksworthii* – A detailed morphological description of this species was not available, thus it was not included in the above table.
* The salient features of the new species are highlighted in bold.

Rhopalostroma brevistipitatum produced a nodulisporium-like asexual morph in the culture similar to those observed in *R. angolense* and *R. lekae* (Hawksworth 1977, Stadler *et al.* 2010, Daranagama *et al.* 2014). Having a nodulisporium-like asexual morph confirms the inclusion of *R. brevistipitatum* in Hypoxyloideae. According to our phylogenetic analysis *R. brevistipitatum* clustered in the Hypoxyloideae group with other *Rhopalostroma* species (Fig. 1). *Rhopalostroma* species usually exhibit a color reaction in the presence of KOH. As detailed by Stadler *et al.* (2010) and Daranagama *et al.* (2014) different species tend to produce different colors and is a taxonomically important character. We have observed a light green color in *R. brevistipitatum* in the presence of KOH. However the color is different from the oilvaceous tone reported in *R. angolense* (Stadler *et al.* 2010).

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