Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
8(3): 351–359 (2018) ISSN 2229-2225

www.creamjournal.org **Article**

Doi 10.5943/cream/8/3/6

New Ascomycetous fungi in the family Aigialaceae from Andaman Islands, India

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Niranjan M, Sarma VV 2018 – New Ascomycetous fungi in the family Aigialaceae from Andaman Islands, India. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 8(3), 351–359, Doi 10.5943/cream/8/3/6

Abstract

The examination of fallen, decaying twigs of different plants for a study on the diversity of saprobic, filamentous ascomycetous fungi from Andaman Islands, India, revealed 3 new species in the genera *Fissuroma* and *Neoastrosphaeriella*. These new taxa are introduced as *Fissuroma kavachabeejae* sp. nov., *F. microsporum* sp. nov. and *Neoastrosphaeriella alankrithabeejae* sp. nov. in the family Aigialaceae. *Fissuroma kavachebeejae* is distinct from other species in having a white colored slit instead of brown or black slit in addition to differing in dimensions of ascomata, asci and ascospores. *Fissuroma microsporum* differs from other species of *Fissuroma* in having smaller ascospores. *Neoastrosphaeriella alankrithabeejae* is identical from the type *N. krabiensis* (in having larger asci and ascospores. These new species are described in this paper supported by photomicrographs and are compared with closely related species, and a synopsis of important characters of the related species is provided in a table.

Key words – 3 new species – Dothideomycetes – morphology – Pleosporales – Taxonomy

Introduction

Pleosporales is the largest order in the Dothideomycetes, which comprises 55 families, 255 genera and more than 4700 species (Wijayawardene et al. 2014, Liu et al. 2017) and presently 75 families have been accepted in this order (Wijayawardene et al. 2018). The taxa belonging to this order occur in a wide variety of ecological regions and many of them thrive as saprobes on dead leaves and stems in terrestrial and aquatic environments (Zhang et al. 2009a, b, 2012), in addition to being plant, animal and human pathogens (Hyde et al. 2013, Seyedmousavi et al. 2013), or occurring on animal dung (Kruys & Wedin 2009) or as endophytes (Bhagat et al. 2012) or epiphytes on living plants (Liu et al. 2011). Many species also inhabit sea grasses and marine sponges (Sakayaroj et al. 2010, Paz et al. 2010).

The family Aigialaceae (Suetrong et al. 2009) mainly comprises saprobes colonizing submerged bark or wood of mangrove trees. Sexual morphs show that ascostromata are dark brown, immersed beneath or deeply immersed in the host epidermis, subglobose to conical, or hemisphaerical, coriaceous or carbonaceous, ostiolate. Ostioles usually have a slit-like opening. Hamathecium comprises trabeculate pseudoparaphyses, embedded in a hyaline gelatinous matrix. Asci 8-spored, bitunicate, fissitunicate, cylindrical, apically rounded, with a non-amyloid ocular chamber, persistent, short-pedicellate. Ascospores overlapping uni- to bi-seriate, hyaline to brown,

Submitted 3 February 2018, Accepted 25 April 2018, Published 27 June 2018
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ellipsoidal to fusiform, septate to muriform, with a mucilaginous sheath or cap (Suetrong et al. 2009, Hyde et al. 2013).

Suetrong et al. (2009) introduced the new family Aigialaceae to accommodate the three marine fungal genera viz. *Aigialus*, *Ascocratera* and *Rimora* (Suetrong et al. 2009, Hyde et al. 2013). Multi-gene analyses indicated that *Astrosphaeriella aggregata* was not related to the *Astrosphaeriella sensu stricto*, but clustered with the family Aigialaceae (Schoch et al. 2009, Zhang et al. 2012). Thus, Liu et al. (2011) established a new genus *Fissuroma* in Aigialaceae. Many species described in *Astrosphaeriella* were transferred into Aigialaceae due to their similarities based on morphological and molecular characterization. *Astrosphaeriella* was originally introduced with the type species *A. fusispora* Syd. & P. Syd. Based on phylogenetic analyses, Liu et al. (2011) introduced two new genera, *Fissuroma* and *Neoastrosphaeriella* in Aigialaceae to accommodate species with immersed ascostromata with slit-like ostioles, which were previously accommodated in *Astrosphaeriella*.

The genus *Fissuroma* was introduced to accommodate some lophiostoma-like species (Liu et al. 2011, Phookamsak et al. 2015, Tennakoon et al. 2018). Mostly, *Fissuroma* species grow as saprobes on bamboo or palms appearing as dome shaped, darkened areas on the host with slit-like ostioles, ascostromata dark brown to black, scattered to clustered, immersed beneath host epidermis, solitary to aggregated, becoming raised, hemispherical domes, uni-loculate, rarely bi-loculate joined at the base, glabrous, coriaceous or carbonaceous, ostiole a central, slit-like opening. Hamathecium composed of dense, trabeculate, anastomosing, pseudoparaphyses, embedded in a hyaline gelatinous matrix. Asci bitunicate, fissitunicate, obclavate to cylindrical, pedicellate, apically rounded, with an ocular chamber. Ascospores overlapping uni-to tri-seriate, hyaline, fusiform with narrowed ends, 1-septate, slightly constricted at the central septum, surrounded by a distinct sheath (Liu et al. 2011). *Fissuroma maculans* (Liu et al. 2011) is the type species. These species previously belonged to *Astrosphaeriella* and Liu et al. (2011) had found that they were not congeneric with *Astrosphaeriella sensu stricto*. Molecular phylogenetic analyses show that *Fissuroma* formed a robust clade in Aigialaceae (Phookamsak et al. 2015).

Neoastrosphaeriella was a new genus introduced by Liu et al. (2011) with *Neoastrosphaeriella krabiensis* as the type species. Both *Fissuroma* and *Neoastrosphaeriella* share common morphological characteristics such as shape of asci, colour, shape and surface of ascospores (Liu et al. 2011). The genus *Neoastrosphaeriella* however differs from *Fissuroma* in having smaller obclavate asci and brown, verrucose ascospores, while *Fissuroma* has cylindro-clavate asci and hyaline ascospores. *Neoastrosphaeriella* is also closely related to *Aigialus*, *Ascocratera* and *Rimora*, in Aigialaceae having similarities such as carbonaceous, apapillate ascomata, trabeculate pseudoparaphyses in hamathecium, cylindrical asci and ascospores with a sheath (Suetrong et al. 2009). Even molecular phylogeny also reveals a sister relationship with these three genera.

We are investigating the diversity of saprobic, filamentous ascomycetous fungi colonizing the dead and decomposing twigs of different plants fallen on the floor in the forests of Andaman Islands, India. In our recent collections from this region, we have encountered 3 new species that fit in the genera *Fissuroma* (2 species) and *Neoastrosphaeriella* (1 species). The new species *Fissuroma kavachabeejae*, *F. microsporum* and *Neoastrosphaeriella alankrithabeejae* are introduced based on differences in morphological features with other species in these genera. A synopsis of the important features of the related species in these genera is provided in a table.

Material and Methods

Dead and decaying twig samples fallen on the forest floor in the reserved forests of South, Middle and North Andaman Islands, India were collected and transferred into zip-lock plastic bags, air dried overnight, and packed into new plastic bags for shipment to the laboratory for further processing. Before undertaking the microscopic examination, the twigs were placed individually into plastic bread boxes lined with sterile tissue paper, rehydrated by sprinkling sterile water and incubated. The samples were then examined under a Stereo Zoom microscope (Optika SZM-LED,

Italy) to locate the fungal fruiting structures. Hand sections were taken wherever necessary. The fruit bodies were cut with a razor blade and the spore constituents were transferred to a microslide, mounted with stains like Lacto phenol, Lacto phenol cotton blue, Lougal's reagent and India ink. These slides were then examined under the Nikon ECLIPSE TiU upright microscope with DIC objectives fitted with Nikon DS-Fi2 digital camera, Japan to take photomicrographs. Measurements were taken with Nikon NIS-Elements-Imaging Software version 4.4 program. Photoplates were made with Microsoft power point and Adobe Photoshop version 7.0. The herbarium materials of the holotype were deposited at Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute (ARI), Pune, India. The newly described species are compared with the existing species http://www.indexfungorum.org/Names/Names.asp and http://www.mycobank.org/quicksearch.aspx

Results and Discussion

Taxonomy

Fissuroma kavachabeejae M. Niranjan and V.V. Sarma sp. nov. Fig. 1

Mycobank number: MB824347; Facesoffungi number: FoF04775

Etymology – In reference to the mucilaginous sheath surrounding the ascospores in Sanskrit (kavacha means cover or shield referring to the sheath around ascospores; beejae means spores).

Classification – Aigialaceae, Pleosporales, Dothideomycetes.

Saprobic on *Calamus andamanicus* Kurz (*Arecaceae*) twigs Sexual morph: *Pseudothecia* 170–200 × 600–660 µm, perithecoid, carbonaceous, mostly single, rarely grouped, superficial, immersed in host epidermal layer and raised like concave shape, flat apical surface, smooth, shining, surface with a centrally located long slit, base flat with thin cell layers of peridium, *Peridium* 40 µm wide, thick brown, with cells of textura angularis, apical layer covered by host cell layer. *Hamathecium.* **n**umerous pseudoparaphyses 0.8-1µm wide, trabeculate, persistent, anastomosing in a gelatinous matrix. *Asci* 142–167 × 15–20 µm ($\overline{x}$ = 153.3 × 16.5 µm, n = 23), bitunicate, fissitunicate, cylindrical, rounded ocular chamber, smooth-walled, persistent, short pedicellate. *Ascospores* 37.3–47.4 × 4.7–6.7 (–8.1) µm ($\overline{x}$ = 42.1 × 6.2 µm, n = 26), 8-spored, hyaline, biseriate, fusiform, smooth-walled, 1-septate with a central constriction, tapering acutely towards bipolar ends, surrounded by a mucilaginous sheath, sheath is uniformly thick throughout when young, thickening confined to apical ends at maturity with sheath becoming thinner on the sides, one cell wider than the other cell, mature spores become elongated with a decrease in width, occasionally guttulate. Asexual morph**:** Undetermined.

Known distribution – India.

Material examined – INDIA, Andaman and Nicobar Islands, North Andaman, 7 Kilometre away from Mohan Nagar, (12˚54'12.3"N 92˚51'4.6"E), recorded from a *Calamus andamanicus* twig, 6 January 2017, M. Niranjan M and V.V. Sarma, PUFNI-17498 (AMH-9963, holotype)

Notes – Currently six species are accepted in the genus *Fissuroma* (http://www.speciesfungorum.org/names/names). Most of the *Fissuroma species* have ascostromata smaller than 800 µm in width excepting *F. maculans*. *Fissuroma kavachabeejae* has smaller ascostromata similar to *F. maculans*, but differs in having curved ascostromata, a white colored coating along the slit. It has a thin sheath around ascospores at maturity, which is similar to *F. aggregata, F. fissuristoma* and *F. neoaggregata*. *Fissuroma kavachabeejae* is distinct from *F. aggregata* by having smaller asci and ascospores with the absence of appendages (see Table 1). *Fissuroma kavachabeejae* is also distinct from *F. fissuristoma* in having 1-septate ascospores. It varies from *F. neoaggregata* in not having appendages. *F. kavachabeejae* can be distinguished from *F. thailandicum* and *F. bambusae* in having shorter and smaller ascostromata (see Table 1). *Fissuroma kavachabeejae* is unique in having white colored ostiolated slit in contrast to a brown or black slit in all existing species. Our attempts to isolate culture have failed and hence we could not carry out molecular analyses. A synopsis on important characters of different species belonging to

Fissuroma and *Neoastrosphaeriella* is presented in table 1. Based on the above morphological differences among the existing species of *Fissuroma*, a new species, *F. kavachabeejae* has been proposed to be accommodated in the genus *Fissuroma*.

Fissuroma microsporum M. Niranjan and V.V. Sarma sp. nov. Fig. 2

Mycobank number: MB824348; Facesoffungi number: FoF04774

Etymology – With reference to the smaller ascospores when compared to other species of the genus *Fissuroma*

Classification – Aigialaceae, Pleosporales, Dothideomycetes.

Saprobic on *Borassus flabellifer*.L (Arecaceae). Sexual morph: *Ascostromata* 210–250 × 640–700 µm perithecial, carbonaceous, mostly single to grouped, smooth-walled, immersed in host periderm, raising apically in association with host cell layers, long central slit. *Peridium* 38–42 µm wide, consisting 2 layers, outer thick carbonaceous layer and inner brown layer consisting of *textura angularis* cells. *Hamatheicium* pseudoparaphyses 1–1.6 µm wide, septate, trabeculate, longer than the asci, unbranched, unevenness in width, anastomosing in a gelatinous matrix. *Asci* (75.9–) 80.3–103.6 × 7.4–8.7 µm ($\bar{x}$ = 94.0 × 8.4 µm, n = 25)**,** bitunicate, cylindrical, cylindric-oblong, 8-spored, an ocular chamber in the apex, smooth-walled, short pedicellate. *Ascospores* 14.6–21.8 × 3.5–4 µm ($\bar{x}$ = 18.7 × 3.2 µm, n = 26)**,** 8-spored, hyaline, overlapping uniseriate, biseriate at base, smooth-walled, fusiform with 1-septate, centrally constricted, 2–3 pseudosepta, acute ends, with a thick mucilaginous sheath and polar appendages. Asexual morph: Undetermined.

Known distribution – India.

Material examined – INDIA, Andaman and Nicobar Islands, North Andaman, Mayabunder, Panihati (12˚53’29.8”N 92˚51’28.4”E), on decaying rachis of *Borassus flabellifer*, 4 February 2016, M. Niranjan and V.V. Sarma, PUFNI-444 (AMH-9962, holotype).

Notes – *Fissuroma microsporum* is distinct from all the existing species of *Fissuroma* and the newly proposed *F. kavachabeejae* in having smaller ascospores. All the existing *Fissuroma* species were reported from bamboo plants excepting *F. fissuristoma*. *Fissuroma microsporum* in the present study is recorded from a palm. Based on smaller size of the ascospores present in the new taxon when compared to all other species of the genus, *F. microsporum* is introduced to be accommodated in the genus *Fissuroma*.

Neoastrosphaeriella alankrithabeejae M. Niranjan and V.V. Sarma sp. nov. Fig. 3

Mycobank number: MB 824349; Facesoffungi number: FoF04773

Etymology – With reference to the ornamented ascospores in Sanskrit – alantkritha means ornamented and beejae means spores.

Classification – Aigialaceae, Pleosporales, Dothideomycetes.

Saprobic on *Calamus andamanicus* Kurz (Arecaceae) twig. Sexual morph: *Ascomata* 210–300- × 670–820 µm, immersed in host periderm, raised, black, carbonaceous, perithecoid, associated with several host layers and covered up to apical end, smooth, shining surface, curved, ostiolar slit black, flat base, thin or lack of perithecial tissue. *Peridium* 45–50 µm thick, consists two layers, outer black carbonaceous layer with *textura globosa* cells and inner hyaline *textura angularis* cells. *Hamatheicium* **p**esudoparaphyses 1.1–1.6 µm wide, trabeculate, anastomosing, septate, interconnected, attached top to bottom. *Asci* 132–154.5 × (19.2–) 21.1–32.5 µm ($\bar{x}$ = 143.5 × 25.4 µm, n = 25), 8-spored, overlapping uniseriate to triseriate basally, cylindrical, matured asci obclavate, with a small apical chamber, rounded apically, smooth-walled, broader at basal part. *Ascospores* 40.2–46.7 × 8.5–9.3 (–10) µm ($\bar{x}$ = 44.2 × 9.0 µm, n = 25), hyaline to pale-brown at maturity, broad-fusiform with narrow ends, 1-septate, constricted at the septum, two pseudo-septate, becoming fully fusiform at maturity, guttulate, verrucose, with a thin mucilaginous sheath. Asexual morph: Undetermined.

Known distribution – India.

Material examined – INDIA, Andaman and Nicobar Islands, Middle Andaman, Bharatpur (12˚29’58.5”N 92˚52’53.0”E), *Calamus andamanicus* twig, 3 February 2016, M. Niranjan M and V.V. Sarma, PUFNI-379 (AMH-9961, holotype).

Notes – *Neoastrosphaeriella* is established recently as a new genus based on morphological and molecular phylogenetic characteristics with *N. krabiensis* J.K. Liu., E.B.G. Jones & K.D. Hyde as the type species. *Fissuroma* and *Neoastrosphaeriella* were described as new genera (Liu et al. 2011) due to their distinct morphological and molecular phylogenetic evidences. *Neoastrosphaeriella* is distinct from *Fissuroma* in having verrucose ascospores that become brown at maturity. *Neoastrosphaeriella alankrithabeejae* has larger asci and ascospores when compared to *N. krabiensis* in addition to having sharp ended and thin sheathed ascospores. Based on morphological differences with the existing species of the genus, a new species *N. alankrithabeejae* is proposed to be accommodated in the genus *Neoastrosphaeriella*.

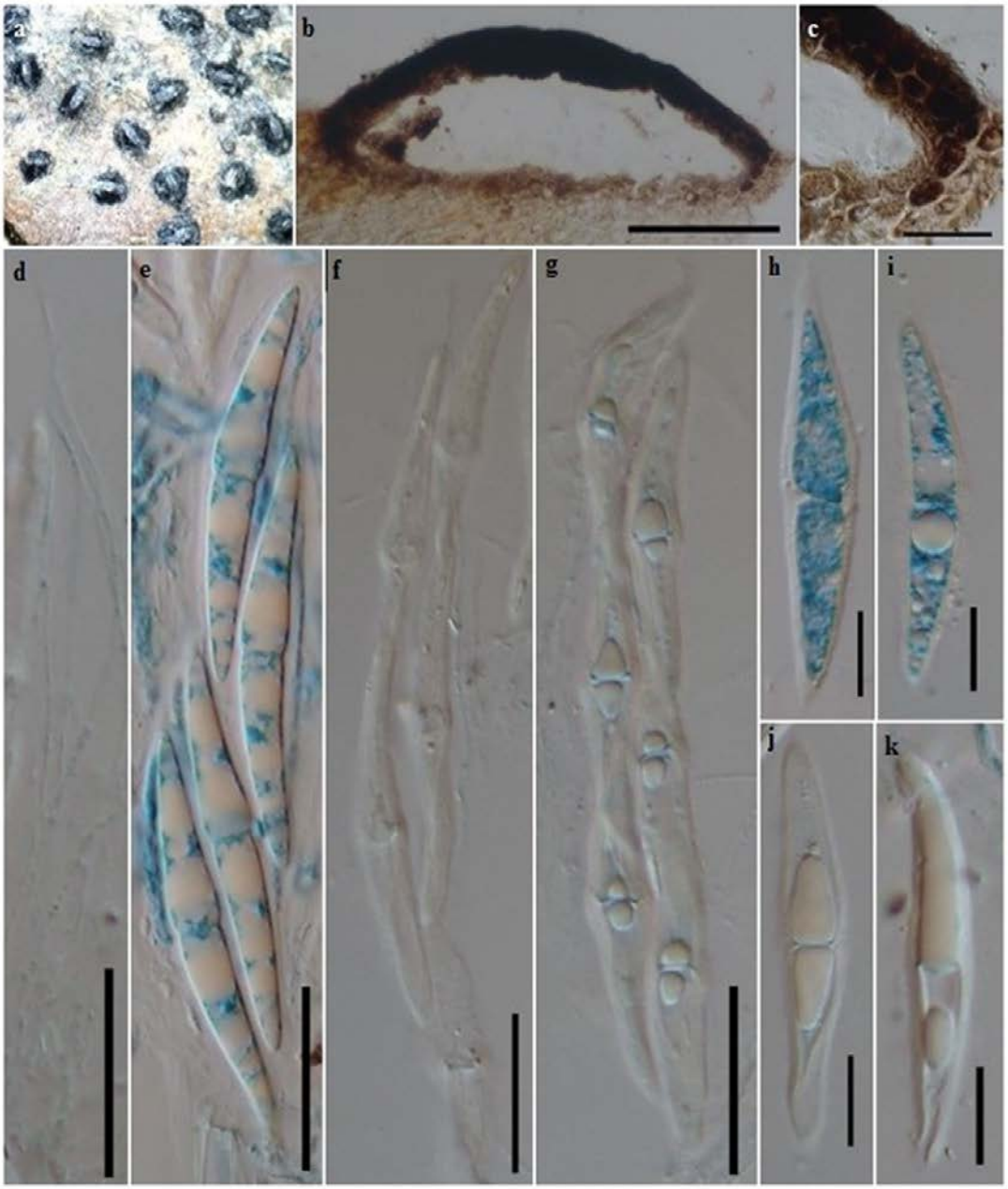

Fig. 1 – *Fissuroma kavachabeejae* (holotype). a Ascomata. b Vertical section. c Peridium. d Pesudoparaphyses. e–g Asci. h–k Ascospores. Scale bars: b = 200 µm c = 50 µm, d–g = 20 µm, h–k = 10 µm.

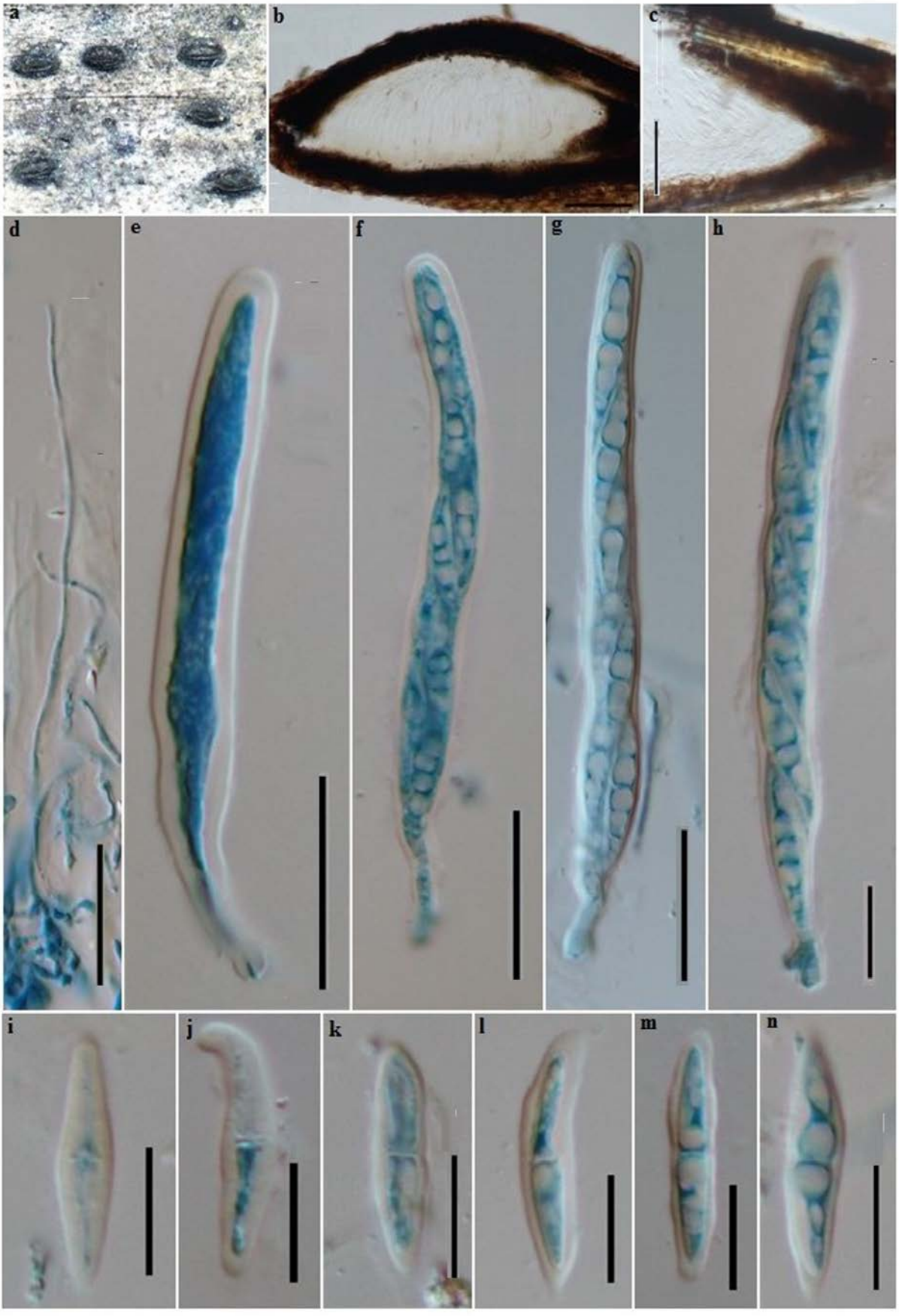

Fig. 2 – *Fissuroma microsporum* **(**holotype). a Ascomata. b Vertical section of ascoma. c Peridium d Paraphyses. e–h asci. i–n ascospores. Scale bars: b = 100 c = 50 µm, d–g = 20 µm, h–n = 10 µm.

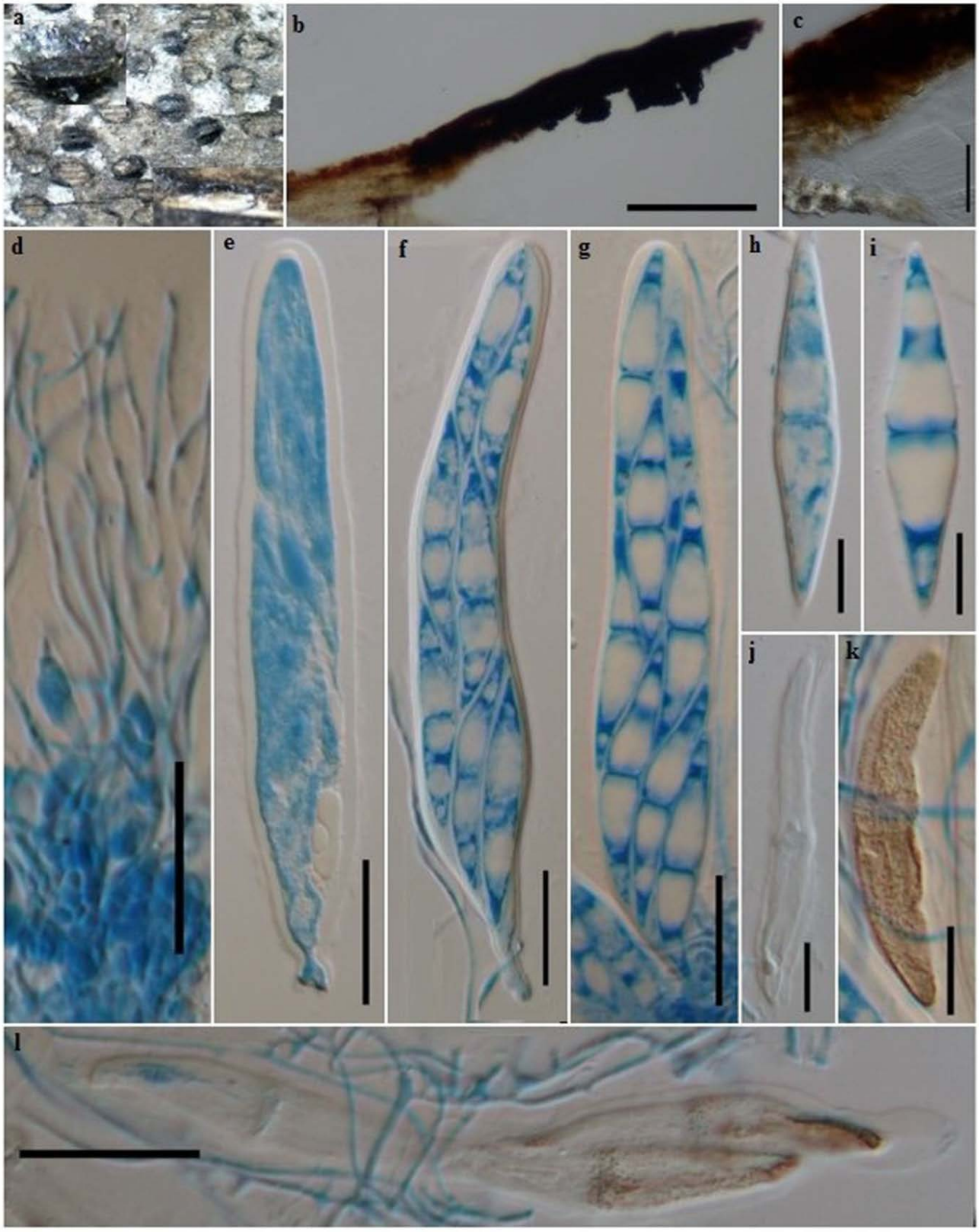

Fig. 3 – *Neoastrosphaeriella alankrithabeejae* (holotype). a Ascomata. b Vertical section of ascoma. c Peridium. d Pseudoparaphyses. e–g, l Asci. h–k Ascospores. Scale bars: b = 200 µm, c = 50 µm, e–g, l = 20 µm, h–k = 10 µm.

Table 1 A synopsis of ascomata, asci and ascospore dimensions of different species of *Fissuroma* and *Neoastrosphaeriella*.

No.	Fungi	Ascostromata	Asci	Ascospores	Reference
1.	*F. aggregata*	200–300 × 500–1000 µm.	155–197 × 15–18.5 µm ($\bar{x}$ = 177 × 16.5 µm)	38.5–54 × 7–10.5 µm ($\bar{x}$ = 47.5 × 8.5 µm)	Liu et al. 2011
2.	*F. bambusae*	250–400 × 750–1050 µm	(150–)170–187(–194) × (15–)17–19(–22) µm ($\bar{x}$ = 178.1 × 18.5 µm)	(40–)45–47(–52) × 6–8(–9) µm	Phookamsak et al. 2015
3.	*F. fissuristoma*	300–390 × 750–1030 µm	(124–)130–150(–166) × (16–)18–19(–26) µm ($\bar{x}$ = 144.1 × 19.3 µm)	(43–)45–50(–55) × 7–9µm	Phookamsak et al. 2015
4.	***F. kavachabeejae***	170–200 × 600–660 µm	142.2–167.5 × 14.9–20 µm ($\bar{x}$ =153.3 × 16.53)	37.3–47.4×4.7–6.7 (8.1) µm ($\bar{x}$ = 42.1 × 6.2)	This study
5.	*F. maculans*	300–450 × 450–700 µm	65–125×10–17 µm ($\bar{x}$ =85×13 µm)	29–38 × 4–8 µm ($\bar{x}$ = 30×6.5 µm)	Liu et al. 2011
6.	***F. microsporum***	210–250 × 640–700 µm	(75.9–)80.3–103.6.5 × 7.4–8.7 µm ($\bar{x}$ = 94.0 × 8.4)	14.6–21.8 × 3.5–4 µm ($\bar{x}$ = 18.7 × 3.2)	This study
7.	*F. neoaggregata*	290–410 × 870–1100µm	(155–)160–190(–197)×15–17(–18) µm ($\bar{x}$ = 177 × 16.5)	(39–)(41–)47–50(–54) × 7–9 µm	Phookamsak et al. 2015
8.	*F. thailandicum*	210–390 × 650–1050µm	(150–)170–190(–204)×15–18(–19.5) µm ($\bar{x}$ = 176.9 × 17)	(40–)43–46(–52) × 6–7(–9) µm	Phookamsak et al. 2015
9.	***N. alankrithabeejae***	210–300 × 670–820 µm	132.2–154.5 × (19.2–)21.1–32.5 ($\bar{x}$ = 143.5 × 25.4)	40.2–46.7 × 8.5–9.3(–10) ($\bar{x}$ = 44.2 × 9.0)	This study
10.	*N. krabiensis*	115–260 × 450–860 µm	85–135 × 15–23 µm	32–40 × 6–9 µm ($\bar{x}$ = 35.5 × 7)	Liu et al. 2011.

Acknowledgements

Authors are thankful to the Science and Engineering Research Board (SERB), Ministry of Science and Technology, Govt. of India for funding the project SERB/SB/SO/PS/18/2014 dt.19.5.2015. Niranjan is thankful to SERB, DST, Govt. of India for the award of the fellowship. Thanks are due to the forest department of Andaman and Nicobar Islands, India and District Forest Offices of North, Middle and South Andaman districts for providing permission to collect the research samples from their respective jurisdictions. The Department of Biotechnology, Pondicherry University is thanked for providing the facilities.

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