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New species and new records of *Astrosphaeriellaceae* from Andaman Islands, India

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ABSTRACT

Andaman and Nicobar Islands of India are relatively underexplored for fungal diversity. From the studies conducted from Andaman region, a few new species and new records have been documented. *Astrosphaeriellaceae* is characterized by superficial ascomata, or beneath the host periderm, thick carbonaceous walls with central ostioles, brown fusiform ascospores, hyphomycetous or ceolomycetous anarmophs. In the present paper, we introduce two new species, viz. *Astrosphaeriella uniseptata* and *Pithomyces hyalosporae* and report two new records i.e *Astrosphaeriella stellata* and *Astrosphaeriella tornata*.

Key words: *Astrosphaeriellaceae, Pleosporales, Dothideomycetes*, new records, new species.

INTRODUCTION

Astrosphaeriellaceae belongs to the order *Pleosporales* (*Dothidiomycetes*) and contains 5 genera (Zhang *et al*. 2012; Wijayawardene *et al*., 2018), namely *Astrosphaeriella* Syd. & P. Syd., *Astrosphaeriellopsis* Phook. *et al*., *Javaria* Boise, *Pithomyces* Berk. & Broome and *Pteridiospora* Penz. & Sacc. *Astrosphaeriellaceae* has been established by Phookamsak & K.D. Hyde (Phookamsak *et al*., 2015). *Astrosphaeriella* is a type genus of *Astrosphaeriellaceae*, and it consists of more than 50 species as mentioned in Index Fungorum, 2019. However, Wijayawardene *et al*. (2017a) accepted only 10 species. Other genera such as *Astrosphaeriellopsis, Javaria* and *Pteridiospora* contain two species each, while *Pithomyces* has 30 species. *Astrosphaeriellaceae* has morphological similarities and sister relation in phylogeny with *Aigialaceae*. The species belonging to *Astrosphaeriella* were mostly found on bamboo, palms and stout grasses and are distributed in subtropical or temperate regions (Zhou *et al*., 2003 and Phookamsak *et al*., 2015). *Astrosphaeriella* species are reported as saprobic or parasitic from diverse environments such as aquatic, submerged or terrestrial habitats, and some of the dead parts of dicotyledons (Phookamsak *et al*., 2015). A key was proposed for the *Astrosphaeriellaceae* by Phookamsak *et al*. (2015). *Astrosphaeriella* consists of yellowish brown to reddish brown ascospores, while hyaline to brown ascospores characterize *Pteridiospora*. Recently, Wanasinghe *et al*. (2018) proposed a key to species of *Astrosphaeriellaceae*. The genus *Javaria* was introduced by Boise (1984) and it is similar to *Astrosphaeriella* (Hyde and Fröhlich, 1998). The taxa belonging to this genus contain hyaline ascospores that are surrounded by mucilaginous sheaths and it is considered as a synonym of *Astrosphaeriella*.

To begin with the genus *Astrosphaeriellopsis* Phookamsak, J.K. Liu & K.D. Hyde, was placed in order *Pleosporales*, family *Incertae sedis* (Phookamsak *et al*., 2015). Later it was transferred to family *Astrosphaeriellaceae*. The genus *Astrosphaeriellopsis* is characterized by immersed ascomata beneath the host epidermis, cylindric-clavate asci with apical rounded ends containing an apical chamber; ascospores hyaline to pale brown, 1-septate, surrounded by a mucilaginous sheath. Although Barr (1987, 1990) placed the genus *Javaria* with *J. samuelsii* as the type species in *Platystomaceae*, however, it was later transferred to *Melanommataceae* by Tian *et al*. (2015). Recently *Astrosphaeriellopsis* has been transferred to *Astrosphaeriellaceae* by Wijayawardene *et al*. (2017a; 2018). Boise (1984) described this genus as having erumpent ascomata, conical, carbonaceous peridium, lageniform to cylindrical asci and ellipsoid to fusoid hyaline ascospores.

Astrosphaeriellaceae members are characterized by superficial ascomata; ascostromata solitary to gregarious, erumpent to superficial, carbonaceous; Peridium poorly developed at the base, asci bitunicate, fissitunicate, cylindrical to cylindric-clavate, with an ocular chamber, or J- subapical ring; ascospores hyaline to pale brown, or reddish brown, with or without appendages and mucilaginous sheath and are saprobic or parasitic on bamboo, palm or stout grasses. *Pseudoastrosphaeriellaceae* was raised as a new family by Phookamsak *et al*. (2015) by transferring some species of *Astrosphaeriella*. *Pseudoastrosphaeriella* is defferent from *Astrosphaeriella* and *Astrosphaeriellopsis* in having hemispherical ascostromata, cylindrical asci, phylogenetic variations and morphological differences such as a long neck. Taxa with shorter necks in ascomata were transferred to the new genus *Pseudoastrosphaeriella*. *Pithomyces* is the largest genus in the *Astrosphaeriellaceae* as it consists of 30 species (Da Cunha *et al*., 2014; Phookamsak *et al*., 2015; Wanasinghe *et al*., 2018). It is characterized by mammiform to conical ascomata, cylindrical asci and brown multiseptate ascospores. Also it is morphologically distinct from other genera in having the hyphomycetous asexual states (Wijayawardene *et al*., 2017b) than coelomycetous asexual states.

MATERIAL AND METHODS

Samples of dead and decomposing twigs fallen on the forest floor in the Andaman Islands, India, were collected in large polyethylene bags. They were rinsed with tap water to remove debris, dried overnight and packed in new plastic bags for shipment to the laboratory for further processing. Before performing the microscopic examination, the twigs were placed individually in plastic bread boxes, lined with sterile tissue paper, rehydrated by spraying sterile distilled water and incubated for a week to 3 months. Then, the samples were examined under a Stereo Zoom microscope (Optika SZM - LED, Italy) to locate the fungal fruiting structures. Hand

sections were cut with a razor blade and mounted on the slide in lactophenol or cotton blue + lactophenol. The slides were examined under the compound microscope (Olympus CH20i, Japan) for morphological characteristics. Nikon ECLIPSE TiU vertical microscope with DIC lenses equipped with Nikon DS - Fi2 digital camera, Japan, was used to take photomicrographs. The measurements were carried out with the Nikon NIS - Elements - Imaging Software version 4.4 program. The photo plates were prepared with the help of Microsoft Power Point and Adobe Photoshop version 7.0. The morphological identification was made by consulting the recent literature (Chen and Hsieh, 2004; Fröhlich and Hyde, 2004; Tanaka and Harada, 2005; Phookamsak *et al*., 2015; Wijayawardene *et al*., 2018; Wanasinghe *et al*. 2018). Herbarium specimens were deposited at the Ajrekar Mycological Herbarium (AMH), Pune and the Department of Biotechnology, University of Pondicherry.

RESULTS AND DISCUSSION

Taxonomy

1. ***Astrosphaeriella stellata*** (Pat.) Sacc. *Sylloge Fungorum* **24** (2): 938 (1928). **Figs. 1. a-e**

Saprobic on unidentified Bamboo culms. **Teleomorph:** Ascostromata numerous, erumpent to superficial, opaque, inverted conical, carbonaceous, immersed in host epidermis, uniloculate, glabrous, brittle, ostiole central, with a small pore-like opening. Peridium varying in thickness, poorly developed at the base, laterally composed of several layers of thick, dark opaque and melanized cells, arranged in textura angularis to textura prismatica. **Hamathecium:** pseudoparaphyses numerous, anastomosing, septate, branched. **Ascospores** 45-50 × 7.5-8.7 µm, brown, 1-septate, multiguttulate, apical cells wider and longer than the basal cells, septum centrally constricted, with crescent germ slit, straight to slightly curved, smooth walled. **Anamorph:** Undetermined.

Material examined: INDIA, Andaman and Nicobar Islands, Middle Andaman, Nimbudera (12˚43'40"N 92˚53'1.9"E). Recorded on unidentified Bamboo culms, February 3, 2016, M. Niranjan & V.V. Sarma (PUFNI 301). Additional specimens examined: South Andaman, Port Blair, Chidiya Tapu (11˚31'7" N 92˚42'52"E) (T101F1) on an unidentified twig, February 7, 2016.

Remarks: Currently there are 10 species accepted in *Astrosphaeriella* (Wijayawardane *et al*., 2017a). It has inverted conical ascomata with smooth surface, different from the other genera of bambusícolous fungi. Our collection has ascospores that are of similar in size as that of the type (45-50 × 7.5-8.7 µm vs. (36-) 38-45 (-47) (-53) × 5-7 µm). We were unable to locate the intact asci in the specimens that had ascomata that were fully mature and dry.

2. ***Astrosphaeriella tornata*** (Berk. & M.A. Curtis) D. Hawksw. & Boise, *Sydowia* **38**: 119 (1986). **Figs.2. a-n**

Saprobic on a *Calamus andamanicus* rachis. **Teleomorph:** Ascomata 560-825 × 623-700 µm perithecial, scattered,

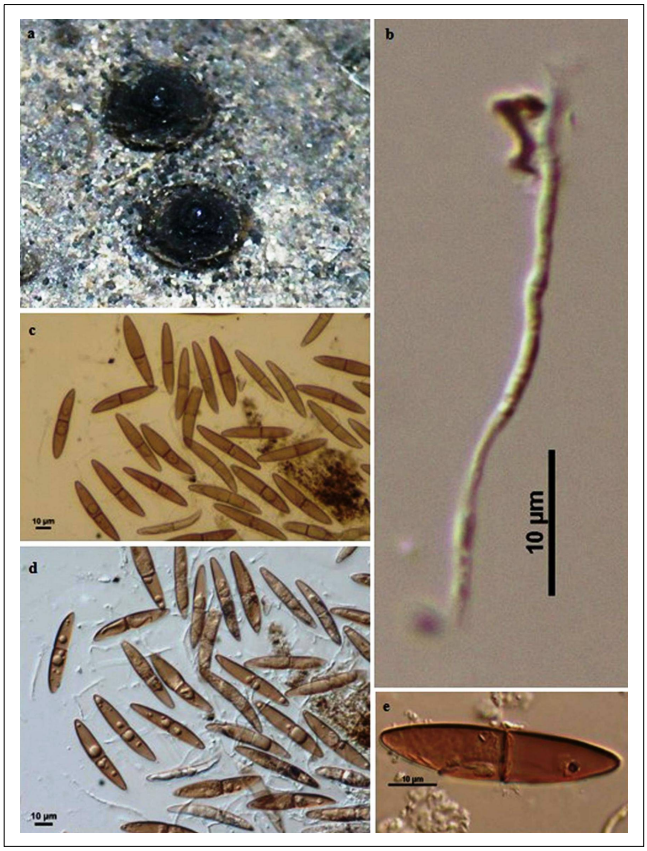

Fig.1. *Astrosphaeriella stellata* (PUFNI 301): **a.** Ascostromata; **b.** Pseudoparaphyses; c-e. Ascospores.

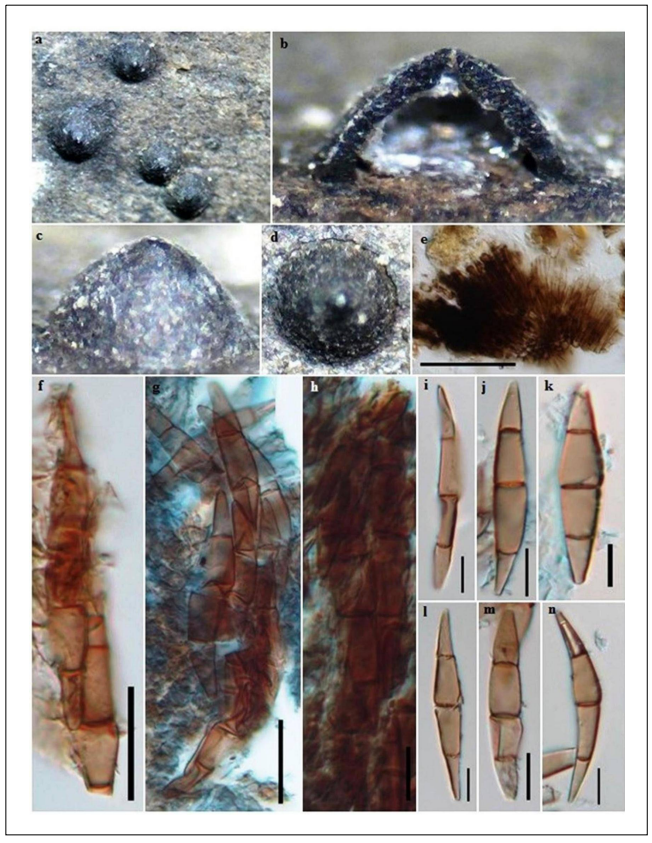

Fig.2. *Astrosphaeriella tornata* (PUFNI 174103): **a-d.** Ascostromata; **e.** Textura prismatica; **f-h.** Asci; **i-n.** Ascospores (**Scale bars**: **e** =50µm **f, g** =20µm **h-o**= 10 µm).

superficial, inverted conical or pyramidal, black carbonaceous, brittle, central papillate. Asci unitunicate, cylindrical. **Ascospores** 37.5-55 × 4-8.7 µm (= 45.7 × 7.4, n=25), 8-spored, bi or tri striate, brown, fusiform, 3- septate, with strong central constrictions, polar septa slight constricted, acute apical ends, sometimes wider, straight to slightly curved, smooth walled. **Anamorph:** Undetermined.

Known distribution: Brazil (Vitória *et al*., 2016), India (In our collection), Mexico (San Martin and Lavin, 1999), Surinam (Hyde and Fröhlich, 1998) and Thailand (Phookamsak *et al*., 2015).

Material examined: INDIA, Andaman and Nicobar Islands, North Andaman, Diglipur, Mohanpur (13˚11'25.5"N 92˚53'23.7"E). Recorded on a *Calamus andamanicus* rachis, January 2, 2017, M. Niranjan & V.V.Sarma (PUFNI 174103). Additional specimens examined: South Andaman, Mount Harriet, Gun Point (11°72'23"N 92°73'40"E) on unidentified twig (T12F1), December 7, 2017); North Andaman, Diglipur, Ganadabla (12˚55'33" N 92˚50'32"E) on *Calamus andamanicus* (T373F1), January 6, 2017.

3. ***Astrosphaeriella uniseptata*** M. Niranjan & V.V. Sarma sp. nov. **Figs. 3. a-o**

Index Fungorum Number: IF557069

Etymology: The specific epithet uniseptata refers to the presence of single septate ascospores.

Diagnosis: The new species has hyaline, one-septate ascospores that are slightly constricted.

Saprobic on *Miliusa tectona*. **Teleomorph:** Ascomata perithecial, solitary to scattered, conical, lignicolous, black, thick, carbonaceous, unipartite. Peridium, brittle. Cells of textura prismatica that are verruculose. **Hamathecium:** pseudoparaphyses 1-1.5 µm in width, anastomosing, filamentous, septate, branched, loosely connected, longer than asci. Asci 175-262 × 20-30µm (= 220.2 × 24.7, n=19), bitunicate, fissitunicate, 8- spored, overlapping uni-biseriate, cylindrical, with an ocular chamber in apical apices, short pedicellate, smooth-walled, persistent. **Ascospores** 52.5-60 × 7.5-10 (12.5)µm (= 55.80 × 10.1, n=17), hyaline, verruculose, 1- septate, with a mucilaginous sheath. **Anamorph:** Undetermined.

Material examined: INDIA, Andaman and Nicobar Islands, North Andaman, Panihutti (13˚21'24"N 92˚55'57"E). Recorded on *Miliusa tectona* twig, February 4, 2016, M. Niranjan & V.V. Sarma (PUFNI 439).

Remarks: Sixty six *Astrosphaeriella* species are documented in Index Fungorum of which 10 species are accepted in a recent article (Wijayawardane *et al*. 2017a). *Astrosphaeriella vesuvius* has superficial ascomata similar to our collection, but is distinct in having 3-5 septate ascospores that become brown at maturity. *A. aosimensis* is the only species considered to have 1-septate hyaline ascospores in the genus *Astrospheriella* (Hawksworth and Bois, 1985). Phookamsak *et al*. (2015) added two new species to this genus. Hyde and Fröhlich (1998) considered *A. aosimensis* to be a synonym of *A. bakeriana*. Liu *et al*. (2011) constructed a phylogeny for *A. bakeriana* which showed that it has a distinct clade from the known species of *Astrosphaeriella*. *A. bakeriana* has therefore been transferred to a new genus *Astrosphaeriellopsis* (*A. bakeriana*). *A. bakeriana* is distinct from our species in that the ascomata are erumpent and covered by host periderm and ascospores have a thick sheath. Hyde and Fröhlich (1998) included 10 new species in *Astrosphaeriella* and provided a pictorial key to the species. Among these *A. angustispora* has stromata closely related to *Astrosphaeriella uniseptata*. However, *A. uniseptata* is distinct from *A. angustispora* in having wider asci (175-262.5 × 20-30 µm vs. 85-110 × 5-5.8 µm) and larger ascospores (52.5-60 × 7.5-10 µm vs. 25-30 × 2.3-2.8µm) with constricted septa. Therefore, we introduce a new species *A. uniseptata* to be accommodated in *Astrosphaeriella* based on the morphological differences discussed above.

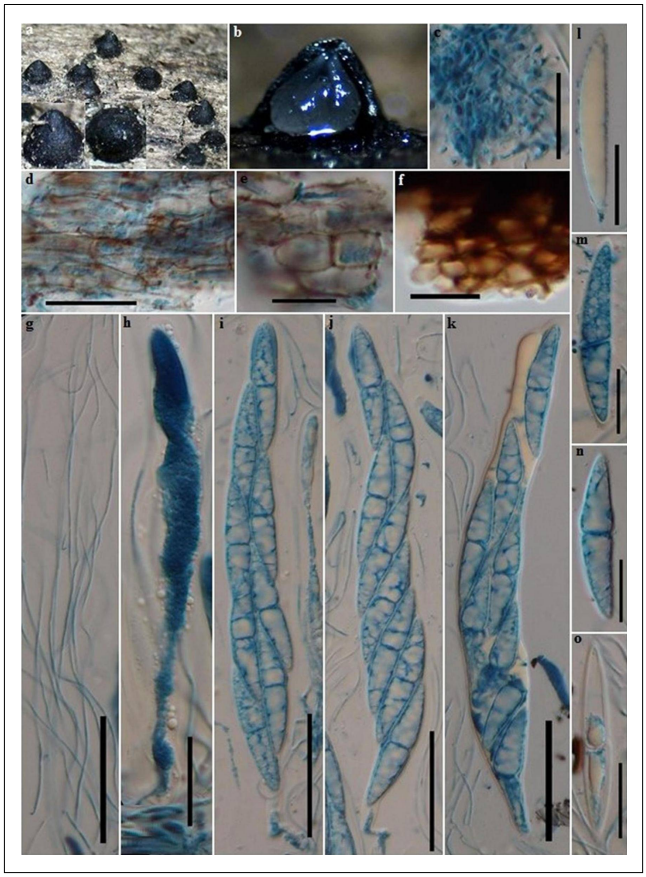

Fig. 3. *Astrosphaeriella uniseptata* **(**PUFNI 439 Holotype**): a.** Ascomata; **b.** Vertical section of ascoma; **c.** Hamathecium; **d.** Textura prismatica; **e-f.** Textura angularis; **g.** Pseudoparaphyses; **h-k.** Asci; **i-o.** Ascospores (**Scale bars**: **i-k** =50 µm**c, d, g, h, l-o** =20 µm **e, f**=10 µm).

4. ***Pithomyces hyalosporae*** M. Niranjan & V.V. Sarma sp.nov. **Figs. 4: a–n**

Index Fungorum Number: IF557068

Etymology: The specific epithet hyalosporae refers to the presence of hyaline ascospores.

Diagnosis: The new species is distinct in having the

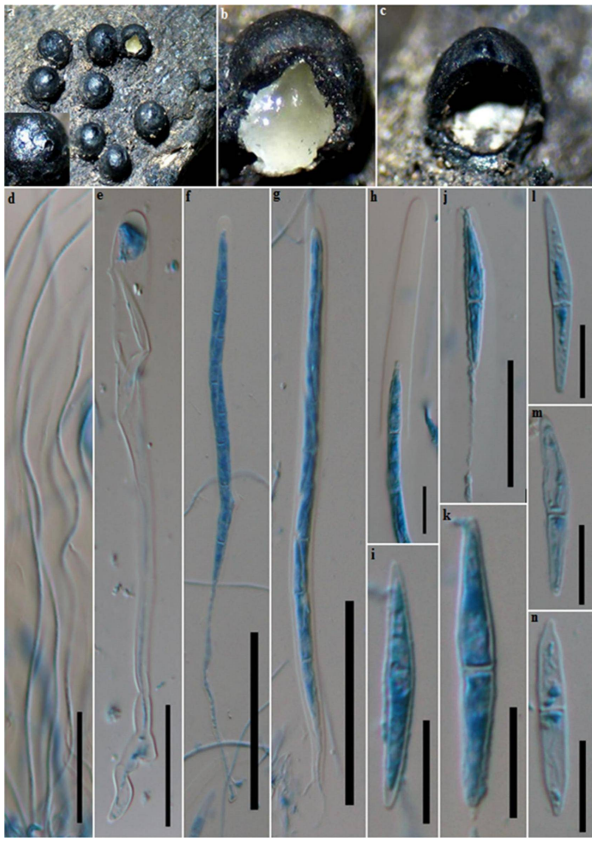

Fig. 4. *Pithomyces hyalosporae* (PUFNI 17456): **a.** Ascomata on host; **b-c.** Vertical section; **d.** Paraphyses; **e-h.** Asci; **j.** Apical ring; **i, k-n.** Ascospores (**Scale bars**: **f, g** =50 µm **d,e,j** =20µm **h**= 10 µm **h,i, kn** =10 µm).

mammiform ascomata, cylindrical asci and hyaline fusiform ascospores.

Saprobic on unknown decaying twig. **Teleomorph:** Ascomata perithecial, scattered, superficial, mammiform, thick carbonaceous wall obtuse towards the apex, with central apical pore, having hyaline ascomatal contents. Peridium 45 µm thick, consists of thick carbonaceous wall and inner hyaline layer. **Haimathecium:** pseudoparaphyses septate, unbranched, 1.7 µm wide. Asci 92-120 × 4.5-7.5 µm (= 104.1 × 5.4, n=25), bitunicate, fissitunicate, 8-spored, long cylindrical, rounded apical ends with an ocular chamber, short pedicellate. **Ascospores** 22.5-27.5 × 2.5-3.5(4.5) µm (= 23.7 × 3.1, n=25), hyaline, fusiform, overlapping biseriate, 1-septate, slightly constricted at the septum, acute apices, smooth walled. **Anamorph:** Undetermined.

Material examined: INDIA, Andaman and Nicobar Islands, South Andaman, Port Blair, Chidiya Tapu (11˚29'23"N 92˚42'36"E). Recorded on unidentified twig, January 8, 2017, M. Niranjan & V.V. Sarma (PUFNI 17456).

Remarks: *Pithomyces hyalosporae* is similar to genera *Astrosphaeriella* and *Javaria* (Hyde and Fröhlich 1998; Phookamsak *et al*., 2015) in having superficial carbonized ascomata and hyaline ascospores that are surrounded by mucilaginous sheaths. Though the present taxon fits in the genus *Astrosphaeriellopsis* based on the colour and septation of the ascospores, the ascomatal characters of *P. hyalosporae*, however, are distinct from *Astrosphaeriellopsis* in having mammiform ascomata, which bring it closer to *Pithomyces* species (Wanasinghe *et al*., 2018). Fusiform ascospores are found in *Astrosphaeriella, Astrosphaeriellopsis, Javaria* and *Pithomyces*. Superficial ascomata are commonly found in *Javaria* and *Pithomyces*. Mammiform ascomata, cylindrical asci and fusiform ascospores present in our taxon *P. hyalosporae* are common features with the genus *Pithomyces*. *P. hyalosporae* is, however, distinct from other species of this genus in having hyaline ascospores. Hence, based on the morphological differences presented above, a new species *P. hyalosporae,* is introduced in the genus *Pithomyces*.

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