



## New records of lichenized fungi in the family Trypetheliaceae from Andaman Islands, India

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### Abstract

Trypetheliaceae is a family of lichenized fungi in the phylum Ascomycota and it comprises seventeen genera with most of the species being found in neotropical regions in addition to countries in the Paleotropics such as India. Recent exploration of filamentous ascomycetous fungi from Andaman Islands, India, revealed two new records of lichenized fungi in the family *Trypetheliaceae*. These are *Marcelaria benguelensis* new to Andaman and Nicobar Islands and *Viridothelium solomonense* new to India. These fungi are illustrated with photomicrographs and are compared with closely related species in this paper.

**Key words** – 2 new records – Dothideomycetes – Taxonomy – Trypetheliaceae

### Introduction

D.D. Awasthi, considered as the “Father of Indian lichenology” could be credited to have implanted the seeds of research in lichen taxonomy in India. Subsequently many other Lichenologists including P.G. Patwardhan, Urmila Makhija, P.P. Sethy, Ajay Singh, D.K. Upreti, K.P. Singh, and G.P. Sinha have explored the Indian lichens. In recent years, the exploration of lichens in Andaman and Nicobar Islands (A & N Islands), has been mainly carried out by T.A.M. Jagadeesh Ram (Ram 2014, 2016, Sethy et al 2012, Ram & Sinha 2016). Worldwide there are 19,387 lichenized fungi that have been recorded (Lücking et al 2016). The Botanical survey of India maintains the list of fungi from India and has names of 14400 fungi from India ([http://www.bsienvi.nic.in/Database/Fungi\\_in\\_India\\_26113.aspx](http://www.bsienvi.nic.in/Database/Fungi_in_India_26113.aspx)). Out of these 2532 are lichens ([http://www.bsienvi.nic.in/Database/Lichen\\_22590.aspx](http://www.bsienvi.nic.in/Database/Lichen_22590.aspx)). India is one of the countries where Lichen diversity has been explored considerably particularly the order Trypetheliales (Aptroot & Luecking 2016). We have recently compiled the data on the list of fungi from A & N Islands, which shows that there are 169 lichenized fungi being reported from the Islands (Niranjan & Sarma 2018).

The order Trypetheliales contains two divergent families (Wijayawardene et al 2018). Polycoccaceae is a lichenicolous fungal family that consists of only 1-septate ascospores, whereas *Trypetheliaceae* is a family of lichenized fungi that predominantly contains phragmosporous and muriform ascospores producing taxa (Ertz et al 2015). Aptroot & Luecking (2016) revised the *Trypetheliaceae* family and have provided a key to the genera and species belonging to this family based on morphological, molecular and chemotaxonomic characters. *Trypetheliaceae* is a diverse

family and has a wide host range and is mainly found in the tropical habitats. *Trypetheliaceae* is characterized by the presence of an endoperidermal thallus, a cortex and a unique association of pigment chemistry. Ascomata of *Trypetheliaceae* vary from one genus to another in the composition of xanthenes and anthraquinone chemical substances, in the distance between two adjacent ascomata, ostiolar orientation, ascomatal emergence and thallus covering range; ascospores containing a gelatinous sheath or not.

Eight genera of *Trypetheliaceae* family produce muriform ascospores. The ascospores are thin and contain a gelatinous sheath in *Bogoriella* and *Novomicrothelia* with spores becoming brown at maturity. In *Pseudopyrenula* the ascospores are predominantly phragmosporous and rarely muriform. In *Aptrootia* the ascospores are brown and muriform. *Marcelaria* species have a thallus which is corticated except in the ostiolar region, a medulla, yellow pigments, immersed, pseudostromatic ascomata, an interspersed hamathecium, and hyaline, muriform ascospores that have diamond-shaped lumina.

## Material and methods

Dead and decaying twig samples fallen on the forest floor in the reserved forests of South Andaman Islands, India during 2016–2017, 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 for one week or up to 3 months. 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 razorblade and the sections were transferred to a microslide, mounted with stains such as Lactophenol, Lactophenol Cotton Blue, Lugol'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 prepared with the help of Microsoft power point, and Adobe Photoshop version 7.0. Morphological identification was carried out by referring to various monographs and individual publications (Pande 2008, Hyde et al. 2013, Aptroot & Luecking 2016). The Vouchers are maintained in our fungal biotechnology laboratory, Department of Biotechnology, Pondicherry University, India.

## Results

### Taxonomy

#### ***Marcelaria benguelensis* (Müll. Arg.) Aptroot, Nelsen & Parmen *Glia* 5(2): 4 (2013)**

Classification – Trypetheliaceae, Trypetheliales, Dothideomycetes.

Epiphytic on *Planchonia andamanica* twigs. Telomorph – *Thallus* crustose, corticolous, continuous, yellow and black spotted pruinose, crust with white yellow cartilaginous medulla in the middle, scattered photobiont cells close to peridium. *Thallus chemistry* K–ve, C–ve, KC–ve and UV +ve. *Ascomata* perithecial, situated in epiperidermal layer, covered by the thallus except at the black ostiolar area, yellow vertical warts, UV+ yellow, single or multiloculate, 2–3, immersed in thallus, erumpent through globose to subglobose ascomata. *Ostiole*: ostiolar neck centrally oriented, protruding out with prominent black spots on the twigs indicating location of ostioles, apically thickened, 175–229 µm high, tightly packed within thallus. *Peridium* two layered, an outer carbonaceous layer and medulla consisting of brown cells of *textura angularis*, 19–23.7 µm wide. *Hamathecium*: *Pseudoparaphyses* filamentous, numerous, anastomosing, 1.8 µm wide, with oil droplets, embedded in a gelatinous matrix. *Asci* 212.5–350 × 45–62.5 ( $\bar{X}$  = 256.25 × 50.70) µm (n=16), bitunicate, cylindro-clavate, fissitunicate, persistent, apically rounded with an apical

chamber, outer wall thickened at the apex and thin laterally down to the base, short pedicellate. *Ascospores* 70–90 × 17.5–22.5 µm ( $\bar{X}$ = 74.32 × 19.91 µm) (n=26), remain hyaline in Lugol's reagent, biseriate in the apical region, uniseriate below, fusiform with transverse septa when young, fusiform–ellipsoid becoming muriform with 17–20 transverse rings at maturity, each ring consisting of 14 cells in the middle of the ascospores, smooth-walled, individual cells having diamond-shaped lumina at senescence, obtuse apical ends, with a thick sheath around the spore, 4–10 µm.

Known distribution – Cambodia, India, Myanmar and Thailand.

Material examined – INDIA, Andaman and Nicobar Islands, South Andaman, Shoal Bay–10 (11°31'21.8"N 92°43'8.3"E), recorded on *Planchonia andamanica* twig, 7 February 2016, identified by Niranjana M & Sarma. V.V.

Notes – The genus *Marcelaria* has been recently established by Aptroot et al. (2013). It has a sister relation with *Trypethelium* (Aptroot & Luecking 2016), with pseudostromatic ascomata and muriform ascospores. *Marcelaria* is characterized by large ascomata covered by yellow to orange or red warts, split between inner wall and covering tissues, having yellow to orange or red pigments. The genus *Marcelaria* currently has three species. In *M. purpurina* the ascomata are UV negative and the ascospores are larger in size; *M. cumingii* is characteristic with UV+ ascomata and non-pruinose thallus, broad ostioles and two ascospores per ascus. The third species *M. benguelensis* has ascomata in yellow pruinose thallus, UV+ and eight ascospores per ascus that are larger in size than *M. cumingii* (Figs 1a, 1b). This is the new record to Andaman and Nicobar Islands.

### ***Viridothelium solomonense* Aptroot**

#### **The Lichenologist 48 (6): 637 (2016)**

Classification – Trypetheliaceae, Trypetheliales, Dothideomycetes.

Epiphyte on an unidentified twig. Teleomorph – *Thallus* crustose, numerous around the ascomata except at the apical end, smooth surface, composed of 3 layers, an outer crust layer with hyaline cells, a middle photobiont cell layer with globose to subglobose cells and a medulla layer with parenchymatic cells. *Thallus chemistry* K–ve, C–ve, KC–ve, I–ve, UV–ve. Pseudostromata clustered, immersed in raised thallus warts, ascomata 890–1000 µm high, perithecioid, multi-loculate, globose to subglobose, small tubular apical portion raised above, epicentral ostiole, *Peridium* with thick walls, black, carbonaceous, 40–50 µm in width. *Hamathecium Pseudoparaphyses* paraphysoid, filamentous, longer than asci, anastomosing, numerous, embedded in a gelatinous matrix. *Asci* 145–282.5 × 27.5–60 µm ( $\bar{X}$ =208.32 × 38.05 µm) (n=15), bitunicate, broadly cylindrical, fusiform to clavate, apically rounded with an ocular chamber, smooth surface, shortly pedicellate. *Ascospores* 57.5–87.5 × 12.7–17.5 µm ( $\bar{X}$ =68.25 × 14.85 µm) (n=20), hyaline, I–ve, overlapping triseriate, fusiform, mature ascospores transversely septate, each segment biguttulate, ends acute with thick caps, 17–19 septate, constricted at individual septa, individual segments showing a rectangular lumina.

Known distribution – Argentina, Brazil, Paraguay and India (In present study).

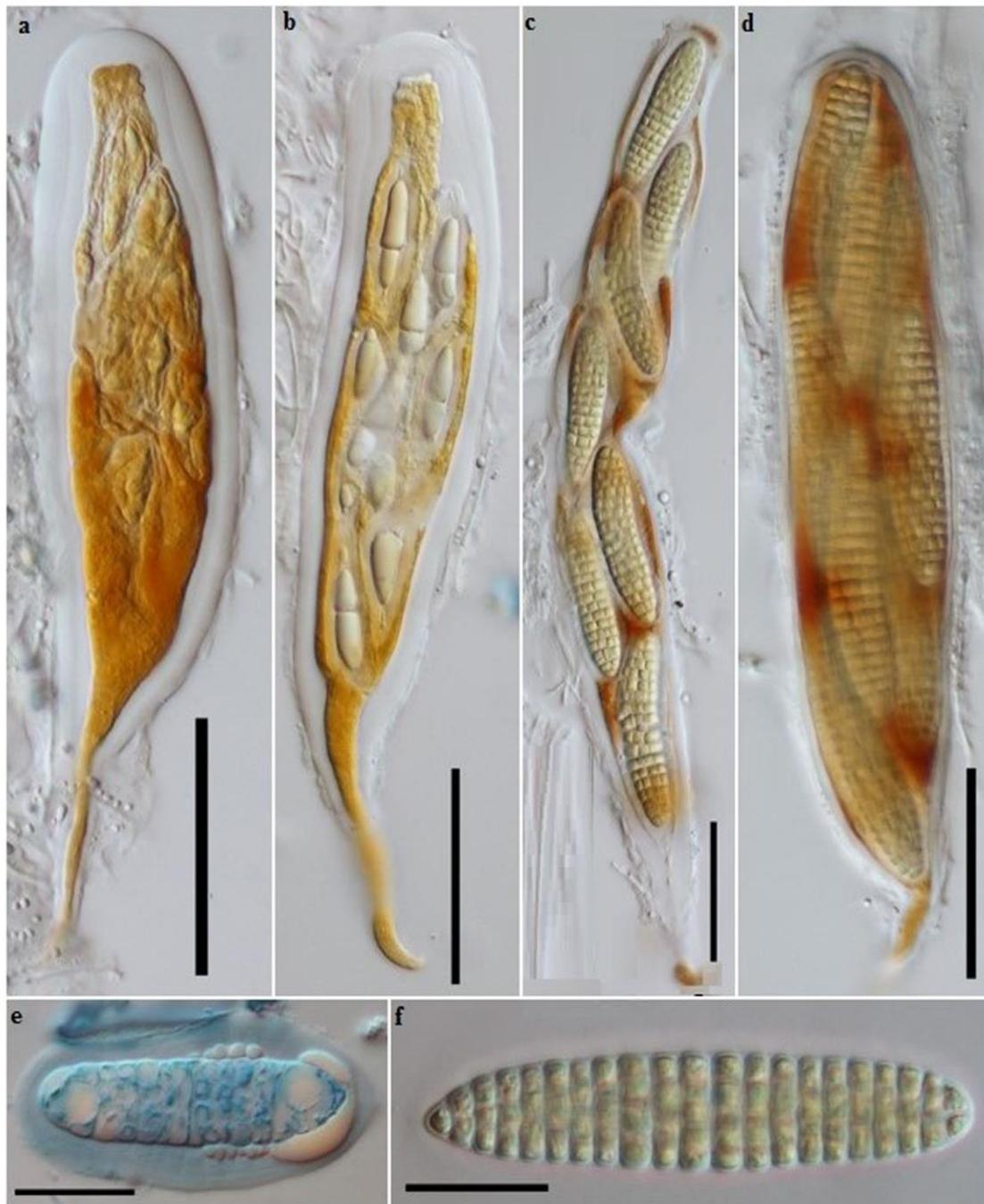
Material examined – INDIA, Andaman and Nicobar Islands, South Andaman, Manarghat. (12°53'29.8"N 92°51'28.4"E). Recorded on a *Pterocarpus dalbergioides* twig, 4 January, 2017, identified by Niranjana M. & Sarma V.V.

Notes – *Viridothelium* was revised recently by Aptroot & Luecking (2016). *V. virens* (Tuck. ex Michener) Lücking et al. is the type species. The genus contains over 11 species and these have recently been redescribed along with a key (Aptroot & Luecking 2016, Da Silva Cáceres & Aptroot 2017). *Viridothelium* is characterized by the presence of pseudostromatic ascomata and hyaline, multiseptate ascospores with rectangular lumina. Similar characters are also found in other genera such as *Astrothelium* and *Trypethelium*, but these genera have a distinct morphology from *Viridothelium*. *Viridothelium solomonense* (Fig. 2) is closely related to *V. cinereoglaucescens* but differs from it in having ascospores with 17–20 septa, when compared to 7–11 septa present in the

latter species. *V. solomense* reported from Andaman and Nicobar Islands in this paper is a new record to India. *Astrothelium purpurascens* has similar ascomata but its spores are muriform and are larger in size. Similarly, *A. megaspermum* has similar ascomata but has 4 ascospores per ascus that are muriform. In *Astrothelium infuscatulum* though the ascomata are similar, the ascospores are 3-septate. *Astrothelium diplocarpum* differs in having ascomatal aggregations in pockets and the ascospores are 9-septate.



**Fig. 1a** – *Marcelaria benguelensis* (PUFNI-353 Isotype). a Ascomata. b Vertical section of ascoma. c Peridium. d Thallus. e Neck. f Paraphysoids. g Apical thickening. i shedding of ascospore. h, j Horizontal and vertical views of ascospores. k Paraphyses. l-n Asci. o-r Ascospores. Scale Bars: b=200 $\mu$ m, c-f, k-n = 50  $\mu$ m, g-l, o-r =20  $\mu$ m, j transverse section =10  $\mu$ m and spore =20  $\mu$ m.

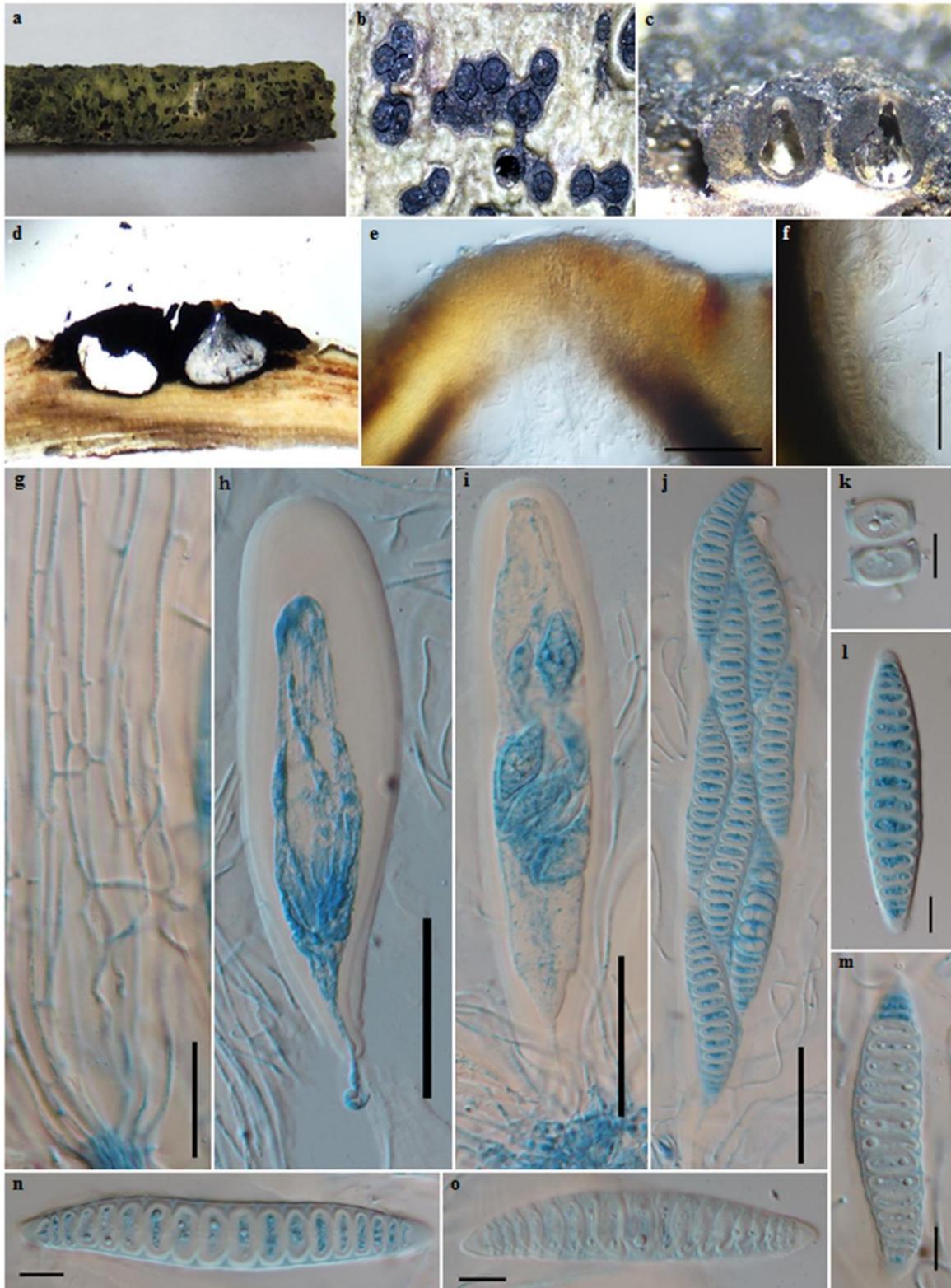


**Fig. 1b** – *Marcelaria benguelensis*. a–d Asci in Lugol’s reagent. e Immature ascospore in a sheath. f Mature ascospore. Scale Bars: a–d =50  $\mu$ m, e, f = 10  $\mu$ m.

### Discussion

In a recent check-list of fungi from Andaman and Nicobar Islands we found that there are 446 fungi reported from these Islands. Three groups of fungi were predominantly found in the list. These are Meliolales, marine fungi and lichenized fungi. Out of the 446 fungi 169 belong to lichenized fungi (Niranjan & Sarma 2018). The few studies that reported Trypetheliaceae from A & N Islands were mainly contributions by Makhija et al. (1999) on *Coccocarpia*, Makhija & Patwardhan (1993, 1998) on *Trypethelium*, Upreti (1990, 1991) on *Pyrenula*, Singh (1969, 1970a,b, 1971, 1973, 1978) on foliicolous lichens. Studies on lichenized fungi in A & N Islands is still at a rudimentary stage and still a lot more needs to be explored. In this paper, we are reporting two new records of lichenized fungi in the Trypetheliaceae. So far, there was no *Viridithelium* species have been reported from the A & N Islands. While *Marcelaria benguelensis* is new record to A & N

Island as well as India, *Viridithelium solomonense* is reported for the first time from A and N islands, India thus extending their geographic range. In addition, these fungi were also reported from new hosts thus extending their host range. There seem to be a huge hidden diversity of lichenized fungi in these A and N Islands warranting concerted efforts for further exploration.



**Fig. 2** – *Viridithelium solomonense* (PUFNI-17451 Isotype) a Ascomata. c, d Vertical section of perithecia. e Ostiolated neck. f Peridium. g Paraphyses. h–j Asci. k Individual cells of ascospore. l–o Ascospores. Scale Bars: e, f, h–j =50  $\mu$ m, g=20  $\mu$ m, k–o=10  $\mu$ m.

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