



## ***Marthomamyces* gen. nov. (Asterinales, Lembosiaceae) from Southern Western Ghats, India**

**Lini K M<sup>1\*</sup>, Jacob T<sup>2</sup> and Neeta N N<sup>3</sup>**

<sup>1</sup>Department of Botany, St. Thomas College, Kozhencherry-689103, Kerala, India. email: linikmathew1985@gmail.com

<sup>2</sup>Department of Botany, Mar Thoma College, Thiruvalla-689103, Kerala, India. email: jacobnthomas@gmail.com

<sup>3</sup>Department of Botany, Mar Thoma College, Thiruvalla-689103, Kerala, India. email: neeta.sanjeev@yahoo.com

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### **Abstract**

During a survey and study of black mildew in the Western Ghat's forests of Malabar Wildlife Sanctuary in Kerala State, the plant *Vateria indica* (Dipterocarpaceae) was seen to be infected with black mildew causing ectoparasitic foliicolous fungus. A microscopic study of this fungus revealed that it belongs to the Lembosiaceae family. Based on the non-appressoriate mycelia with peculiar nutritional hyphae (haustoria) surrounding the stomata and elongated thyriothecia with longitudinal dehiscence, the present collection has been placed under a new genus *Marthomamyces*. Based on the morphological characters, it was revealed that the fungus infecting leaves of *Vateria indica* (Dipterocarpaceae) is *Marthomamyces vateriae* nom. nov. et stat. nov. Lini K. Mathew, Jacob Thomas and Neeta N. Nair. *Echidnodella vateriae* Hosag. and Kamar is the synonym of this species.

**Key words** – Ascomycetes – Asterinales – Black Mildew fungi – Dipterocarpaceae – *Vateria indica*

### **Introduction**

During a survey and study of black mildew causing fungi in the Western Ghat's forests of Malabar Wildlife Sanctuary in Kerala State, the plant *Vateria indica* L. (Dipterocarpaceae), was seen to be infected with a fungus. A microscopic study of this fungus revealed that it belongs to the Lembosiaceae family. The fungus revealed non-appressoriate brown to black mycelium and longitudinally dehisced elongated thyriothecia. These are the characters of the genus *Echidnodella*.

Thyriothecious ascomycetes are found to be associated with a number of hosts, ranging from living leaves, twigs, stems and fruits to dead and decaying plant material and also on other fungi. They act as plant parasites, saprotrophs or mycoparasites with a worldwide distribution and exhibit diversity in tropical and subtropical regions (Hofmann 2009). As of now, forty species of *Echidnodella* are described from twenty-four host families, including angiosperms and pteridophytes (Stevens & Ryan 1939, Hosagoudar 2012, 2013, Mohammed & Thomas 2021). Among them, nine species were reported from Peninsular India. The present taxon compared with all these *Echidnodella* species and the present collection revealed that it is distinct from all other extant species. The non-appressoriate mycelia with peculiar nutritive hyphae (haustoria) surrounding the stomata, elongated thyriothecia with longitudinal dehiscence are the key

characteristic features of *Marthomamyces*. Based on the morphological comparisons and host specificity, we proposed it as a new genus *Marthomamyces* with description, illustrations, microphotographs, and scientific measurements.

## Materials & Methods

Infected plant parts were selected in the field, including twigs with leaves, photographed (plant habit and fungal infections), collected after thorough evaluation, and brought to the laboratory. Date of collection, locality (name and geographical coordinates), elevation, scientific and vernacular names of the host, nature of fungal colonies, vegetative characteristics of the host such as leaf odour, presence or absence of latex, glands and stipules, phyllotaxy, and parts affected etc. were noted down in the field book.

In the laboratory, samples were divided into two sets: one for the preparation of microscopic slides and the second for the preservation of the herbarium specimen. For herbarium preparation, the infected parts were dried by the usual pressing method between thick blotting papers using a wire press. After drying, they were examined carefully under zoom stereomicroscope (Magnus, India) to study colony characteristics and avoid hyperparasites. The nail polish technique (Hosagoudar & Kapoor 1985) was adopted to study the morphological and structural characters of fungi. A drop of transparent nail polish was applied and carefully thinned with the help of a fine brush or a glass rod without disturbing the selected colonies and kept in a dust-free chamber for it to get dried. After drying, a thin, colourless film was flipped off with slight pressure on the opposite side of the leaves and just below the colonies in the case of soft host parts. For the hard host parts, the flip was eased off with the help of a razor or scalpel. The lifted flip was mounted directly in dibutyl phthalate polystyrene xylene (DPX), labelled and dried.

Detailed taxonomic description of the specimen was written by studying the micro-morphological characters using different magnifications of the compound microscope Olympus (CX21iLED) with MagVision image analyzer software for the final confirmation of the identity of this foliicolous fungus. Biometric data of micro-morphological structures were based on at least 10 measurements. The colour photographs made with Magcam DC10 CMOS camera of 10 megapixels and illustrations are provided with legends. Using the appropriate literature, the isolates were identified and assigned to respective genera and species.

The identifications and descriptions of the fungus were made with the help of various books, monographs, reviews and indices (Hosagoudar 2008, 2012, 2013, Hosagoudar et al. 2012, 2013, Hosagoudar & Riju 2013, Thimmaiah et al. 2013, Thomas 2015). Those taxa having significant and considerable morphological variations than the earlier described one was treated as new species. The identified fungal specimen was deposited in regional herbarium Mar Thoma College Herbarium, Tiruvalla (MTCHT), Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram (TBGT) for easy access in future. The detailed description and illustration of the newly described species were deposited in Index Fungorum, and the accession number is given.

## Results

*Marthomamyces* Lini K. Mathew, Jacob Thomas and Neeta N. Nair, gen. nov. Figs 1, 2

Index Fungorum number: IF558390; Faces of fungi Number: FoF 09833

Etymology – named for honouring the research institution.

*Parasites* on leaves. *Mycelium* ectophytic, branching opposite at acute to wide angles, reticulate. *Haustoria* forming at the tip of the hyphal branches, reach the stomata, enlarge and divide, lack appressoria. **Sexual morph:** *Thyriothecia* oval, ellipsoidal, X or Y-shaped, elongated with radiating cells, astomatous, dehisce longitudinally at the center. *Asci* oval, octosporous, bitunicate. *Ascospores* brown, conglobate, uniseptate.

## Key to the genera of *Lembosiaceae*

1. Appressoria present.....2

1. Appressoria absent ..... 3
2. Appressoria intercalary ..... *Cirsosia*
2. Appressoria lateral ..... *Lembosia*
3. Conidia present ..... 4
3. Conidia absent ..... 5
4. Conidia 1-3 septate ..... *Eupelte*
4. Conidia many septate ..... *Maheshwaramyces*
5. Haustoria present around the stomata ..... *Marthomyces*
5. Haustoria absent around the stomata ..... 6
6. Hypostroma present ..... *Echidnodes*
6. Hypostroma absent ..... *Echidnodella*

**Type species:** *Marthomyces vateriae* Lini K. Mathew, Jacob Thomas and Neeta N. Nair

Figs 1, 2

### Description to the species

*Marthomyces vateriae* nom. nov. et stat. nov. (Hosag. and Kamar) Lini K. Mathew, Jacob Thomas and Neeta N. Nair

≡ *Echidnodella vateriae* Hosag. and Kamar. in Hosag., Zoos' Print J. 17: 945, 2002.

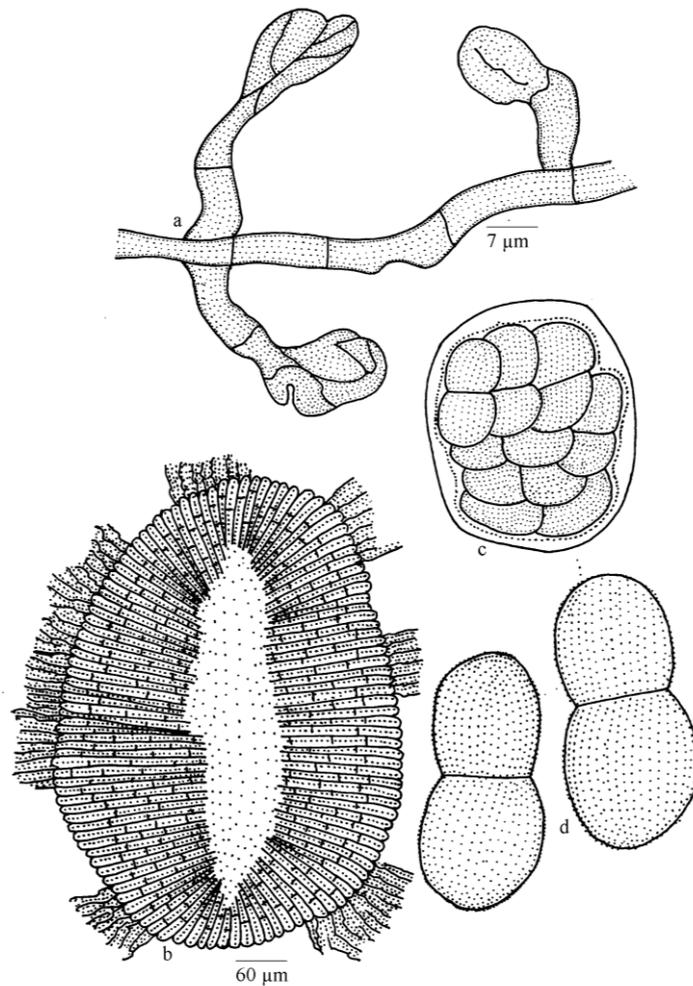
Index Fungorum number: IF558391; Faces of fungi Number: FoF 09834

Colonies hypophyllous, thin to subdense, subvelvety, spreading, up to 5 mm in diameter, confluent. *Hyphae* substraight to crooked, branching opposite at acute to wide angles, loosely to closely reticulate to form a mycelial mat, cells 18–28 × 6–8 μm ( $\bar{x}$  = 24.25 × 7.42 μm, n = 10). *Appressoria* absent. *Haustoria* forming at the tips of lateral hyphal branchlets, enlarged, ovate to globose, slightly divided like a slit at the centre, 10–13 × 7–12 μm ( $\bar{x}$  = 11.5 × 9.5 μm, n = 10), stalk cells one to three in number, cylindrical, 9–30 × 5–7 μm ( $\bar{x}$  = 20.25 × 6 μm, n = 10). **Sexual morph:** *Thyriothecia* orbicular, oval, ellipsoidal to elongated, dehisce longitudinally at the centre, 350–800 × 300–450 μm ( $\bar{x}$  = 575 × 375 μm, n = 10), margin fimbriate, fringed hyphae run parallel, compact. *Asci* oval to globose, octosporous, bitunicate, 60–85 μm ( $\bar{x}$  = 72.5 μm, n = 10) in diameter. *Ascospores* conglobate, brown, uniseptate, strictly constricted at the septum, 32–44 × 18–26 μm ( $\bar{x}$  = 38 × 22 μm, n = 10), wall punctate to echinulate. **Asexual morph:** *Pycnothyria* many, similar and smaller than thyriothecia. *Pycnothyriospores* deep brown, globose to pyriform, slightly papillate, 9–12 × 8–11 μm ( $\bar{x}$  = 10.5 × 9.5 μm, n = 10).

Type:— INDIA, Kerala: Thiruvananthapuram, Ponmudy, on leaves of *Vateria indica* L. (*Dipterocarpaceae*), 26 July, 2001, M. Kamarudeen (HCIO 44321, holotype; TBGT 668, Isotype); INDIA, Kerala: Kozhikode, Malabar Wildlife Sanctuary, Peruvannamuzhy, on the leaves of *Vateria indica* (*Dipterocarpaceae*), 26 December 2014, Lini K. Mathew, (MTCHT 99; MTCHT 127; TBGT 6978).

### Discussion

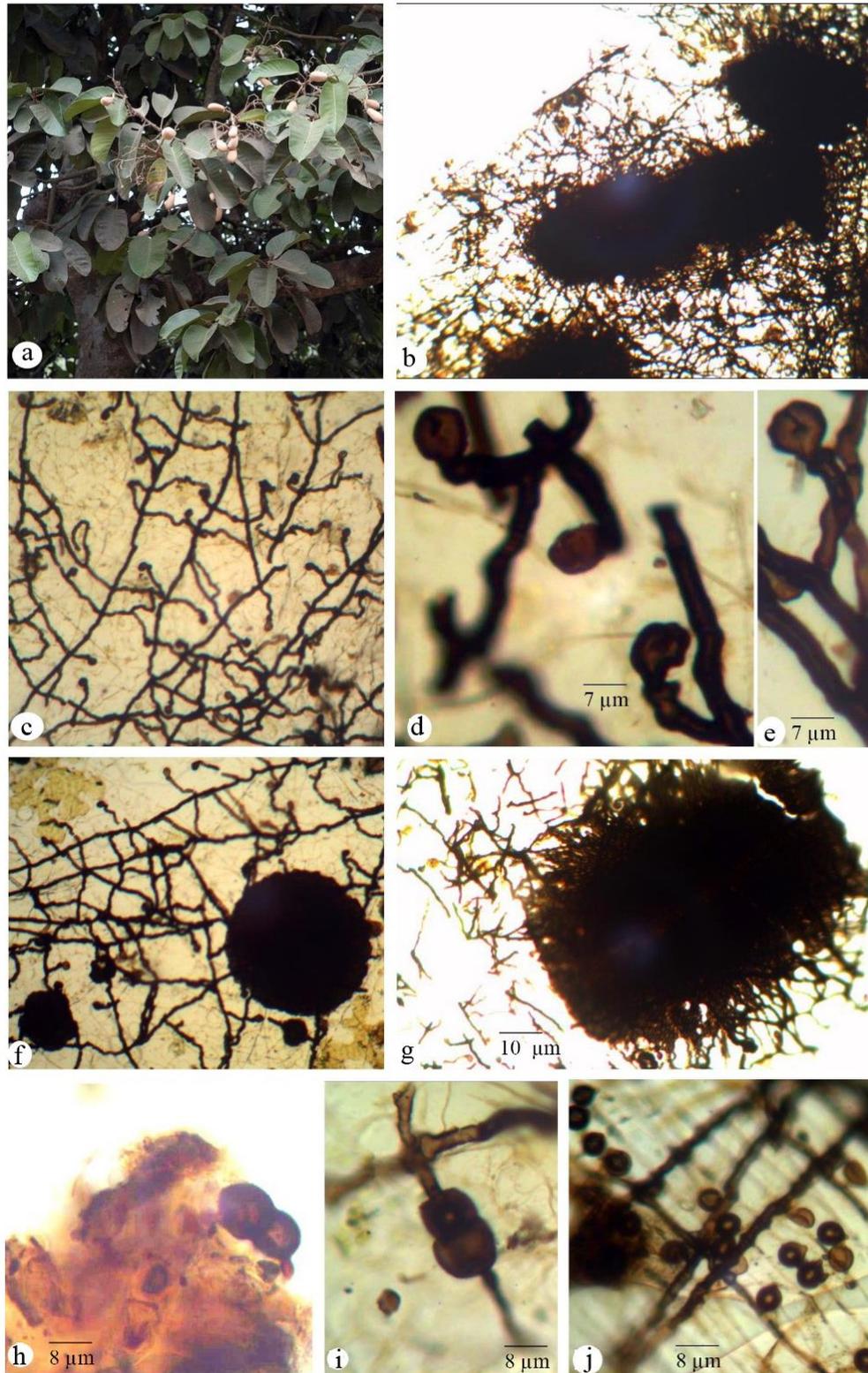
Molecular data are generally unavailable for most members of *Lembosiaceae* such as *Echidnodella*, *Echidnodes*, *Cirsosia*, *Maheshwaramyces*, *Eupeltae*, and the taxonomy of most genera in this family to date relies mainly on morphological data. The *Lembosiaceae* is a family of small, obligately biotrophic ascomycetes associated with living leaves of a broad range of plants from tropical and subtropical regions (Kirk et al. 2001, Barr & Huhndorf 2001, Taylor et al. 2005, Hofmann et al. 2010, Hyde et al. 2013). The important features of *Lembosiaceae* are the superficial, black, web-like colonies that form on the upper and lower surface of leaves, and hyphae with appressoria, forming haustoria that infect host tissue. The thyriothecia are closely attached to the host plant cuticle and comprise the scutellum, an upper dark wall, which is one cell layer thick and composed of radiating cells. The thyriothecia open at maturity with lateral slits (von Arx & Müller 1975, Kirk et al. 2001). The interascal hamathecium or pseudoparaphyses are often inconspicuous, deliquesce early, or are lacking (Hofmann et al. 2010). Ascospores are mostly conglobate, 2-celled, hyaline when young and become brown at maturity.



**Fig. 1.** *Marthomamyces vateriae* (MTCHT 99). a. Non-appressoriolate mycelium with haustoria, b. Dehiscent thyriothechia, c. Ascus, d. Ascospores

*Lembosiaceae* and *Asterinaceae* species are similar in that they are also obligately biotrophic, produce appressoria that obtain nutrients via haustoria that penetrate the host surface, and produce similar thyriothechia (Hosagoudar et al. 2001). *Lembosia* differs from *Asterina* in that the thyriothechia are elongate, which dehisce to open by a longitudinal or X- or Y-shaped slits (Hosagoudar 1991).

Species of *Aulographaceae* differ from *Asterinaceae* and *Lembosiaceae* species based on linear or X- or Y-shaped thyriothecium, hyphae without appressoria, and usually hyaline ascospores in *Aulographaceae* while globose thyriothechia, hyphae with appressoria, and hyaline, immature ascospores that change to brown at maturity in *Asterinaceae*. Molecular data indicate that *Aulographaceae* is not a member of *Asterinales*, and is excluded from *Asterinales* based on morphology and phylogeny. Genera included in *Lembosiaceae* are *Lembosia* (type genus), *Cirsosia*, *Echidnodes*, *Echidnodella*, *Eupeltae* and *Maheswaramyces*. These genera all comprised species with superficial thyriothechia with linear openings, mostly globose to subglobose or broadly clavate asci, and hyaline to brown, 1-septate ascospores. Although *Echidnodes*, *Echidnodella*, *Eupeltae* and *Maheswaramyces* lack appressoria but considered typical of *Lembosiaceae* in having colonies spread on host surface, superficial thyriothechia, globose asci and 1-septate ascospores. Genera transferred to *Aulographaceae* are *Aulographum*, *Lembosiella*, *Lembosina*, *Morenoina* and *Thyriopsis*. These genera have elongate thyriothechia, opening of slit-like, X- or Y-shaped fissures and lack appressoria. In the molecular analyses, the family *Aulographaceae* and *Lembosiaceae* were found in different groups (Hongsanan et al. 2014).



**Fig. 2.** *Marthomyces vateriae* (MTCHT 99). **a.** Infected host leaves of *Vateria idica*, **b.** Colony with thyriotheceia, **c.** Branching pattern of mycelia, **d-e.** Haustoria plugged around host stomata, **f.** Developing thyriotheceia, **g.** Mature thyriotheceium, **h.** Asci and ascospores, **i.** A germinating ascospore, **j.** Pycnidiospores

The genus *Echidnodella* was established by Theissen & Sydow (1917) with the type species *E. linearis* under the family *Asterinaceae*. Later the genus was transferred to the family *Lembosiaceae* (Hosagoudar 2012). *Echidnodella* are leaf inhabiting, host-specific, ectophytic

obligate biotrophs characterized by ectophytic mycelium without appressoria and hypostroma having oval, ellipsoidal, 'X' or 'Y' shaped thyriothecia which dehisce longitudinally at the center with oval, bitunicate, octosporous asci containing brown, conglobate and uniseptate ascospores (Hosagoudar et al. 1996, Hosagoudar 2012).

In addition to these typical characters, the mycelia originated from the main hyphae, plugged around and entered into the host through stomata, forming a peculiar nutritive hypha (Arx & Muller 1962, 1975, Hosagoudar et al. 2001). Hence, to accommodate such fungi, the genus *Marthomamyces* is proposed here. This is a transitional genus between *Ecdnodes* and *Echidnodella* (Hosagoudar et al. 1996, Hosagoudar 2012, Hofmann 2014, Hongsanan et al. 2014). In *Marthomamyces*, the tip of the hyphal branches reach the stomata, enlarge and divide and the haustoria formed through the stomata and hence it is nutritional cells. Initially, thyriothecia were orbicular but elongated at maturity. However, longitudinal dehiscence is consistent

Based on the non-appressoriolate mycelia with peculiar haustoria surrounding the stomata and elongated thyriothecia with longitudinal dehiscence, the present collection has been placed under a new genus *Marthomamyces* with *M. vateriae* Lini K. Mathew, Jacob Thomas and Neeta N. Nair. as the type species. Here it is proposed to elevate the species status of *Echidnodella vateriae* Hosag. & Kamar. to the newly proposed genus *Marthomamyces* as the type species. As molecular data are important for species identification, it is recommended to produce molecular data for these genera in *Asterinales* and deposit them in public databases.

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