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# *Marthamyces manglicola*, a new species (Ascomycota, Marthamycetaceae) on black mangrove from Cozumel Island, Mexico

## *Marthamyces manglicola*, una especie nueva (Ascomycota, Marthamycetaceae) sobre mangle negro de la Isla de Cozumel, México

Tania Raymundo<sup>1</sup> , Yenitze García-Martínez<sup>2</sup> , Michelle Martínez-Pineda<sup>1</sup> , Ricardo Valenzuela<sup>1,3</sup> 

### Abstract:

**Background and Aims:** The genus *Marthamyces* is characterized by its orbicular to polygonal apothecia, immersed within the host tissue, erumpent from the substrate surface, by the hymenium with pruinose appearance due to the presence of small crystals on its surface and amongst the paraphyses, and by filiform, 0- to 3-septate ascospores. Members of this genus grow on leaves of plants throughout the world, mainly in the tropics and subtropics. The aims of this study are to describe and illustrate *Marthamyces manglicola* as a new species that grows on *Avicennia germinans* (black mangrove) from Cozumel Island and to elaborate a key to all species of the genus *Marthamyces* described until now.

**Methods:** Specimens were collected on fallen leaves of black mangrove in Punta Sur Ecological Park, Cozumel Island Biosphere Reserve. Field data and morphological characteristics were recorded using the traditional techniques in mycology. The material was deposited in the ENCB herbarium of the Escuela Nacional de Ciencias Biológicas of the Instituto Politécnico Nacional.

**Key results:** *Marthamyces manglicola* is characterized by its apothecia opening by 3-5 pruinose and yellowish white to pale yellow flaps, a hymenial surface greyish blue to dull blue in young specimens, fading to bluish white to pale blue or pale yellow in mature apothecia. Microscopically, it has paraphyses swelling slightly near the apex, with several short, finger-like projections and straight to slightly curved ascospores on release. A key to all described species of *Marthamyces* is presented.

**Conclusions:** *Marthamyces manglicola* on *Avicennia germinans* (black mangrove) is the only species of this genus described from mangroves.

**Key words:** *Avicennia germinans*, Chaetomellales, Leotiomyces, Mexican Caribbean.

### Resumen:

**Antecedentes y Objetivos:** El género *Marthamyces* se caracteriza por sus apotecios orbiculares a poligonales inmersos en el tejido del hospedero y errumpentes en la superficie del sustrato, por el himenio con apariencia pruinoso debido a la presencia de cristales sobre su superficie himenial y entre las paráfisis, y por las ascosporas filiformes, con 0 a 3 septos. Miembros de este género crecen sobre hojas de plantas en todo el mundo, principalmente en los trópicos y subtropicos. Los objetivos de este estudio son describir e ilustrar a *Marthamyces manglicola* como especie nueva que crece sobre *Avicennia germinans* (mangle negro) de la Isla de Cozumel y elaborar una clave de todas las especies del género *Marthamyces* descritas hasta el momento.

**Métodos:** Los especímenes fueron recolectados en hojas caídas de mangle negro en el Parque Ecológico Punta Sur de la Reserva de la Biosfera Isla Cozumel. Los datos de campo y las características morfológicas fueron registrados usando las técnicas tradicionales en micología. El material fue depositado en el herbario ENCB de la Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional.

**Resultados clave:** *Marthamyces manglicola* se caracteriza por sus apotecios que abren en 3-5 solapas pruinosas, variando en coloración de blanco-amarillentos a amarillo pálido, una superficie himenial azul-grisácea a azul opaca en especímenes jóvenes, decolorando de blanco-azulado a azul pálido o amarillo pálido en apotecios maduros. Microscópicamente, este tiene paráfisis ligeramente ensanchadas cerca del ápice, con varias proyecciones cortas semejantes a dedos y ascosporas rectas a ligeramente curvadas. Se presenta una clave para todas las especies descritas de *Marthamyces*.

**Conclusiones:** *Marthamyces manglicola* crece sobre *Avicennia germinans* (mangle negro) y es la única especie de este género descrita de manglares.

**Palabras clave:** *Avicennia germinans*, Caribe mexicano, Chaetomellales, Leotiomyces.

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## Introduction

*Marthamyces* was described by Minter (2003) with *M. emarginatus* (Cooke & Massee) Minter as the type species. It was set up to accommodate species with filiform ascospores placed in *Propolis* (Fr.) Corda sensu Sherwood (1977) and growing on dead leaves. *Marthamyces* includes species with ascomata on abaxial and adaxial surface of plants throughout the world, but mainly in the tropics and subtropics (Johnston, 2006). Macroscopically, apothecia are subepidermal, orbicular to polygonal, resembling small pustules, erumpent from the substrate surface, not associated with bleaching of surrounding substrate, and lacking associated zone lines (Minter, 2003; Johnston, 2006). They are deeply immersed within the host tissue, and the surface of the hymenium often has a pruinose appearance due to the presence of small crystals amongst the paraphyses (Johnston, 2006). Microscopically, ascospores are filiform, 0- to 3-septate, with apical and basal gelatinous caps and the paraphyses have short branches near apex, tapering or swelling, tangled or with several short, finger-like projections, either often intermixed with crystals, or coated with crystals, the tips of the paraphyses sometimes being embedded in brownish gel (Sherwood, 1977; Johnston, 2006). *Marthamyces* is the type genus of the family Marthamyceaceae, order Chaetomellales, class Leotiomycetes of the phylum Ascomycota (Ekanayaka et al., 2019).

The genus *Marthamyces* has 18 described species. Of these, 13 species are registered from Australia and New Zealand, five from the Americas, three from Europe, two from Asia and one from Africa. Only two have previously been reported from Mexico, *Marthamyces quadrifidus* (Lév.) Minter (as *Propolis quadrifida* (Lev.) Mont.) from Oaxaca (Sherwood, 1977), and *M. coronadoae* Raymundo, R. Valenz. & Esqueda described on *Fagus grandifolia* Ehrh. subsp. *mexicana* (Martínez) A.E. Murray in the municipality of Zacualtipán, Hidalgo state (Raymundo et al., 2016). In 2019, during a visit to the Cozumel Island Biosphere Reserve, several fallen leaves of *Avicennia germinans* (L.) L. (black mangrove) were collected in the Punta Sur Ecological Park in mangroves. They had small pustules of erumpent apothecia of a fungus which did not correspond to any known species. The aims of this study are to describe and illustrate *Marthamyces manglicola* as a new

species that grows on *Avicennia germinans* (black mangrove) on Cozumel Island and to elaborate a key to all species of the genus.

## Materials and Methods

Punta Sur Ecological Park, located in the Cozumel Island Biosphere Reserve, Quintana Roo, Mexico, has an area of 1114 ha, with coastal dunes and mangroves as a principal type of vegetation (Fig. 1). Dominant plant species are *Rhizophora mangle* L. (Rhizophoraceae), *Laguncularia racemosa* (L.) C.F. Gaertn. (Combretaceae), *Conocarpus erectus* L. (Combretaceae) and *Avicennia germinans* (Acanthaceae).

Our specimens are deposited in the Herbarium ENCB of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico. Longitude and latitude coordinates were obtained with GPS eTrex (Garmin, Olathe, USA). Colors were coded according to Kornerup and Wanscher (1978). Morphological examinations were conducted using protocols outlined by Johnston (2006) and Raymundo et al. (2016). Measurements of anatomical characters were taken from rehydrated tissues in 5% aqueous KOH and Melzer's reagent with camera optical microscope (CX-31, Olympus, Tokyo, Japan). The macroscopic pictures were taken with a Nikon D7000 (Nikon, Tokyo, Japan) and the microscopic pictures with a Sony DSC-WX350 (Nikon, Tokyo, Japan). The morphological terms are based on the dictionary of Ulloa and Hanlin (2012).

A key of all described species to the genus *Marthamyces* is presented as a tool to differentiate the new species from the other species. The identification key can be applied globally given the broad distribution of the host (*Avicennia* L.) in different parts of the world. The following literature was used to elaborate this key: De Notaris (1845), Sherwood (1977), Johnston (1986, 1991, 2006), Gu et al. (2015), Raymundo et al. (2016), Crous et al. (2019), Johnston and Park (2019).

## Results

### Taxonomy

#### Ascomycota



Letiomyces

Chaetomellales

Marthamycetaceae

***Marthamyces manglicola*** Raymundo, García-Martínez, Martínez-Pineda & R. Valenz., sp. nov. Figs. 2, 3.

TYPE: MEXICO. Quintana Roo, municipio Cozumel, Reserva de la Biosfera Isla de Cozumel, Parque Ecológico Punta Sur, 28°16'48"N, 86°58'44.39"W, 16.X.2019, *T. Raymundo* 8324 (holotype: ENCB!); Mycobank: MB839088.

*Marthamyces manglicola* differs from *M. quadrifidus* in having apothecia opening by 3-5 pruinose and yellowish

white to pale yellow flaps, a hymenial surface greyish blue to dull blue in young specimens, fading to bluish white to pale blue or pale yellow in mature apothecia; paraphyses swelling slightly near apex, with several short, finger-like projections and ascospores  $50\text{--}60 \times 1\text{--}2.4 \mu\text{m}$ , straight to slightly curved, hyaline, 0- to 1-septate.

Apothecia 0.7-1 mm diameter, on abaxial and adaxial surfaces of dead leaves, subepidermal, resembling small pustules, visible initially as round brown patches on the leaf surface, becoming raised as ascomata mature, erumpent from the substrate surface, not associated with bleaching of host tissue, without zone lines, polygonal, some triangular, quadrangular to rhomboid, others irregular in shape, superficial layer brown to beige in young apothecia, covering the hymenium, opening by 3-5 prominent, irregu-

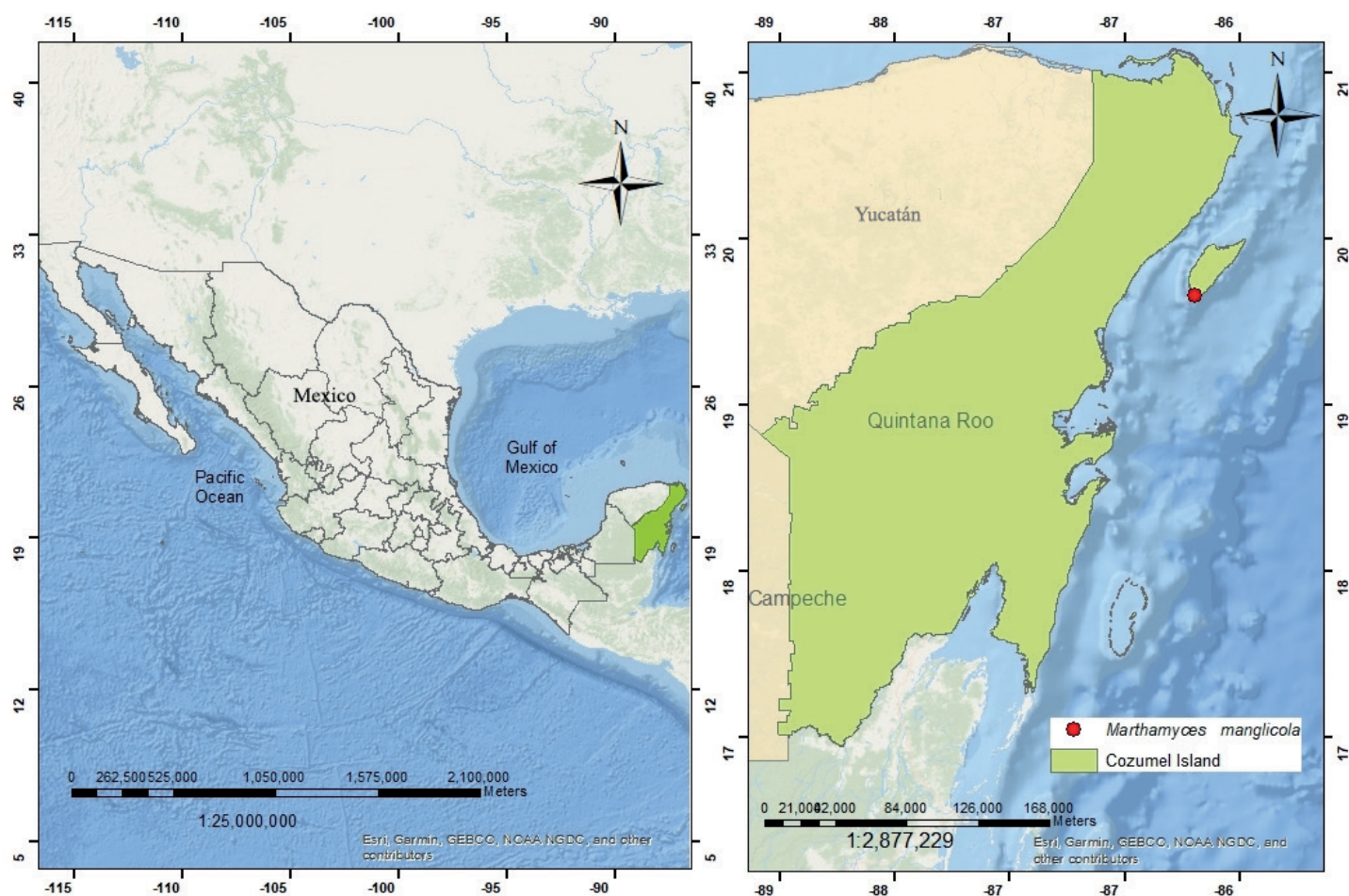
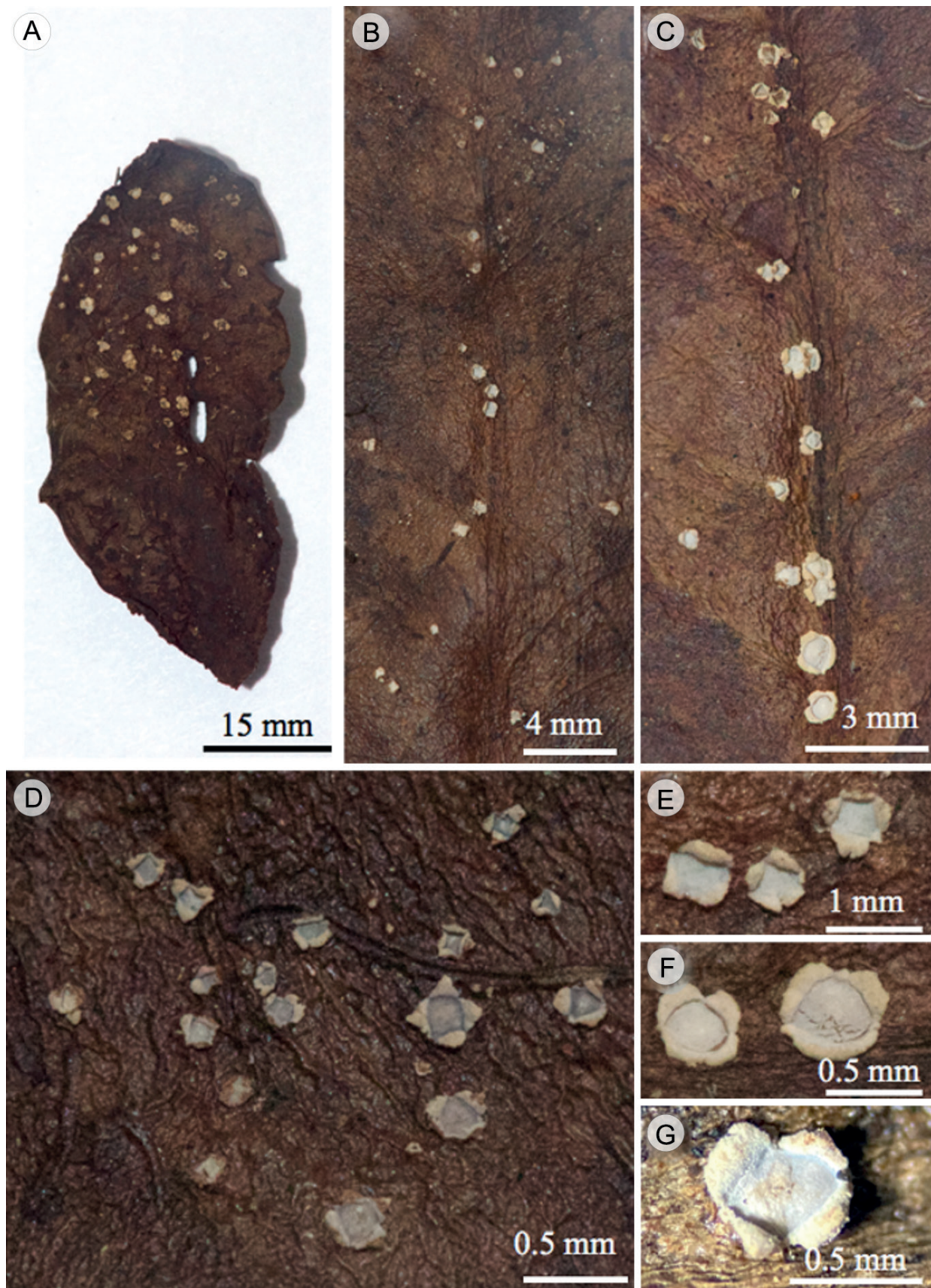


Figure 1: Map of the locality of *Marthamyces manglicola* Raymundo, García-Martínez, Martínez-Pineda & R. Valenz.







**Figure 2:** *Marthamyces manglicola* Raymundo, García-Martínez, Martínez-Pineda & R. Valenz. A. ascomata growing on leaves of *Avicennia germinans* L.; B. gregarious apothecia on abaxial surface; C. gregarious apothecia on adaxial surface; D. apothecia with exposed hymenium; E. polygonal hypothecia; F. quadrangular and triangular apothecia; G. pruinose hymenium.

lar, pruinose and yellowish white (4A2) to pale yellow (4A3) flaps; hymenium somewhat depressed below level of the substrate, pruinose, greyish blue (22B4) to dull blue (22D5) in young specimens, fading to bluish white (22A2) to pale blue (22A3) or pale yellow (4A3) in several parts of mature apothecia, perimeter line absent; paraphyses up to 90 µm long and 2 µm diameter, swelling slightly near apex, up to 3 µm diameter, with several short, finger-like projections, coated with crystals at the apex, crystals soluble in KOH; asci 65-80 × 6-8 µm, subcylindric to subfusoid, tapering at apex, wall undifferentiated at the apex, 8-spored; ascospores parallel to subparallel in the ascus, 50-60 × 1-2.4 µm, filiform, straight to slightly curved on release, colorless in KOH, thin walled, 0- to 1-septate, apical and basal gelatinous caps, globose, 2-2.5 µm diameter, gelatinous caps soluble in KOH.

Habit and habitat: growing on fresh fallen leaves and dead leaves of *Avicennia germinans* in mangrove forest.

Etymology: referring to the common name in Spanish (manglar) of habitat where the species is growing.

Additional material examined: MEXICO. Quintana Roo, municipio Cozumel, Reserva de la Biosfera Isla de Cozumel, Parque Ecológico Punta Sur, 28°16'48"N, 86°58'44.39"W, 16.X.2019, T. Raymundo 8323 (ENCB), 8338 (ENCB), 8341 (ENCB).

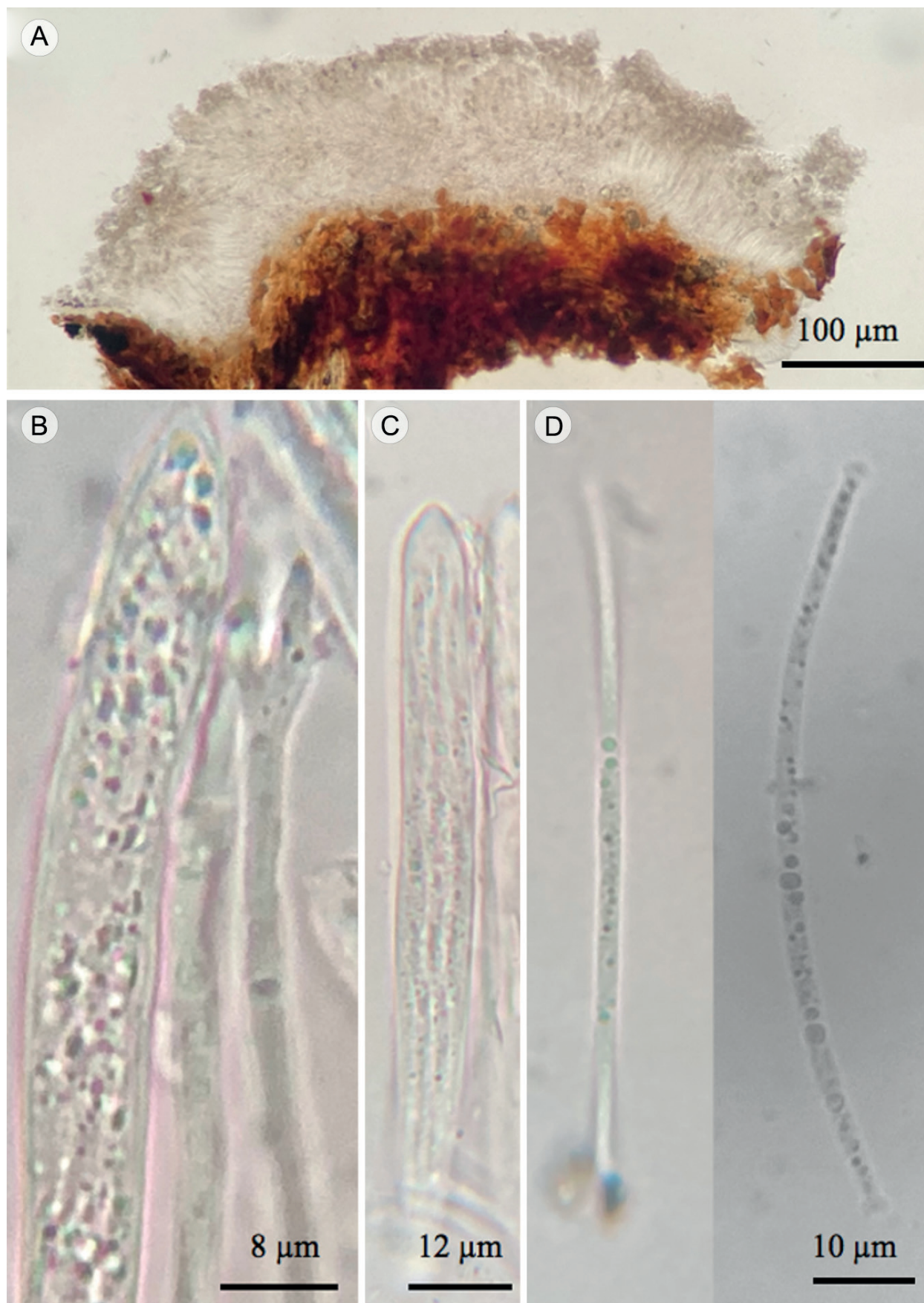
Taxonomy notes: *Marthamyces manglicola* is characterized by its polygonal to irregular apothecia, the young apothecia erumpent from a brown covering layer, opening by 3-5 prominent irregular and yellowish white to pale yellow flaps, and by its straight to slightly curved on release and 0- to 1-septate ascospores.

### Key to the world species of *Marthamyces* based on literature

- 1a. Apothecia growing on several Monocots: *Carex* L. and *Ficinia* Schrad. (Cyperaceae), *Dendrobium* Sw. (Orchidaceae), *Andropogon* L. and *Panicum* Lam. (Poaceae), *Phormium* J.R. Forst. & G. Forst (Xanthorrhoeaceae) ... 2
- 1b. Apothecia growing on several Eudicots and some Magnoliids: *Avicennia* L. (Asterides, Acanthaceae), *Aetoxicum* Ruiz & Pav. (Superasterids, Aetoxicaceae), *Fissistigma* Griff. (Magnoliids, Annonaceae), *Arctostaphylos* Adans. (Asterids, Ericaceae), *Fagus* L. and *Quercus* L. (Rosids, Fagaceae), *Eucalyptus* L' Hér. and *Metrosideros* Banks ex Gaertn. (Rosids, Myrtaceae), *Olea* L. (Asterids, Oleaceae), *Hakea* Schrad. and *Orites* R. Br. (Eudicots, Proteaceae) ..... 6
- 2a. Apothecia opening by single longitudinal slits ..... *Marthamyces desmoschoeni* (P.R. Johnst.) Minter
- 2b. Apothecia opening in several radial or irregular slits ... 3
- 3a. Only growing on *Carex firma* Host. from Austria ..... *Marthamyces foliicola* Nogrased & Matzer
- 3b. Species growing on *Andropogon*, *Dendrobium*, *Panicum* or *Phormium* ..... 4
- 4a. Apothecia round, opening by radial slits, growing on *Dendrobium* from New Zealand ..... *Marthamyces dendrobi* (P. R. Johnst.) Minter
- 4b. Apothecia not round, opening by irregular slits, growing on *Andropogon*, *Panicum* or *Phormium* from USA and New Zealand ..... 5
- 5a. Apothecia narrow elliptical to elongate, no flaps ruptured, hymenium pale blue, 37-42 × 2-3 µm, 1-septate, straight, on *Andropogon* and *Panicum* from USA ..... *Marthamyces culmigenus* (Ellis & Langlois) P. R. Johnst.
- 5b. Apothecia irregular in shape, splitting irregularly, flaps not defined, hymenium pale yellow to greenish yellow, ascospores 55-78 × 3.5-4.5 µm, 1-septate, straight to slightly curved, on *Phormium tenax* J. R. Forst. & G. Forst. from New Zealand ..... *Marthamyces harakeke* P.R. Johnst.
- 6a. Ascospores 0- to 1-septate ..... 7
- 6b. Ascospores 3- to 5-septate ..... 16
- 7a. Ascospores straight to slightly curved ..... 8
- 7b. Ascospores curved, sigmoid or coiled ..... 14
- 8a. Paraphyses simple, not branched, becoming clavate ..... *Marthamyces johnstonii* Crous & Carnegie
- 8b. Paraphyses branched at the apex ..... 9
- 9a. Paraphyses swelling near the apex, with several short finger-like projections ..... 10
- 9b. Paraphyses tapering toward the apex, with several short narrow branches at apex ..... 11







**Figure 3:** *Marthamyces manglicola* Raymundo, García-Martínez, Martínez-Pineda & R. Valenz. A. apothecium; B. asci and paraphyse apex; C. asci; D. ascospores.

- 10a. Apothecia round to irregular, hymenium dark green, without crystals, opening by 2-3 irregular white flaps, ascospores (65-)75-85(-90)  $\times$  2-2.5  $\mu$ m, on *Orites acicularis* (R. Br.) Roem. & Schult. from Tasmania ..... *Marthamyces oritis* P.R. Johnst.
- 10b. Apothecia polygonal to irregular, hymenium bluish white to pale blue, pruinose by the crystals, opening by 3-5 yellowish white to pale yellow flaps, ascospores 50-60  $\times$  1-2.4  $\mu$ m, on *Avicennia germinans* L. from Mexico ..... *Marthamyces manglicola* Raymundo, García-Martínez, Martínez-Pineda & R. Valenz.
- 11a. Growing on *Eucalyptus* L. Her., flaps dark brown, 3-5 in the apothecia ..... *Marthamyces emarginatus* (Cooke & Massee) Minter
- 11b. Growing on *Metrosideros*, flaps white to pale yellow, (2-)3-4(-5) in the apothecia ..... 12
- 12a. Apothecia angular to elongate ..... *Marthamyces renga* P. R. Johnst.
- 12b. Apothecia orbicular to angular, not elongate ..... 13
- 13a. Hymenium white, ascospores 45-85  $\times$  2-3  $\mu$ m, growing on *M. robusta* A. Cunn. from New Zealand ..... *Marthamyces metrosideri* P. R. Johnst.
- 13b. Hymenium pale yellow to grey, ascospores 50-70  $\times$  1.5-2  $\mu$ m, growing on *M. collina* (J. R. Forst. & G. Forst.) A. Gray from the Cook Islands ..... *Marthamyces maccormackii* P. R. Johnst.
- 14a. Ascospores (95-)110-135(-160)  $\times$  2-2.5  $\mu$ m ..... *Marthamyces hakeae* P. R. Johnst.
- 14b. Ascospores 60-74(-80)  $\times$  1.2-2.4  $\mu$ m ..... 15
- 15a. Apothecia triangular to pentagonal, occasionally elliptical, hymenium white, opening by 3-5 grey flaps, ascospores 60-72  $\times$  1.2-1.6  $\mu$ m, on *Fissistigma* from China ..... *Marthamyces chinensis* Y. R. Lin & H. L. Gu
- 15b. Apothecia orbicular to polygonal, opening by 4-5 brownish beige flaps, hymenium pale grey to pale yellow, ascospores 60-74(-80)  $\times$  1.6-2.4  $\mu$ m, on *Fagus grandifolia* subsp. *mexicana* from Mexico ..... *Marthamyces coronadoae* Raymundo, R. Valenz. & Esqueda
- 16a. Ascospores 5-septate, 75-105  $\times$  2.5-3  $\mu$ m, on *Olea* from Italy ..... *Marthamyces panizzei* (De Not.) Minter
- 16b. Ascospores 3-septate, 50-75  $\times$  1.5  $\mu$ m, on *Aetoxicum*, *Arctostaphylos* and *Quercus* from Chile, Sweden and USA ..... 17
- 17a. Apothecia angular, hymenium pale gray ..... *Marthamyces quadrifidus* (Lev) Minter
- 17b. Apothecia orbicular, hymenium white to pale yellow ..... 18
- 18a. Growing on *Quercus*, ascospores 50-65  $\times$  1.5  $\mu$ m ..... *Marthamyces quercifolius* (Cooke & Ellis) Minter
- 18b. Growing on *Arctostaphylos uva-ursi* (L.) Spreng., ascospores 60-75  $\times$  1.5-2.0  $\mu$ m ..... *Marthamyces phacidioides* (Fr.) Minter

## Discussion

The principal features that can distinguish species of *Marthamyces* are color and presence of pruinose material in flaps of the ascomata, color of the hymenial surface and size, shape and number of septa of ascospores. In addition, we consider that the plant with which each *Marthamyces* species is associated can be an important ecological character to separate species. Besides, other authors who have described species of *Marthamyces* have found a narrow range of hosts (Gu et al., 2015; Raymundo et al., 2016; Johnston and Park, 2019). *Marthamyces coronadoae* and *M. emarginatus* are close to *M. manglicola* by having 0- to 1-septate ascospores but differ by ascospore size and associated plant identity; the first species has larger ascospores (60-75  $\times$  1.6-2.4  $\mu$ m) and grows on *Fagus* L. (Raymundo et al., 2016), while in the second ascospores are (60-)65-75(-85)  $\times$  2(-2.5)  $\mu$ m and the fungus grows on *Eucalyptus* (Sherwood, 1977; Johnston, 2006). Other American species are *M. phacidioides* (Fr.) Minter, *M. quercifolius* (Cooke & Ellis) Minter and *M. quadrifidus*, but they are separated by 3-septate and larger ascospores (Sherwood, 1977; Cabarro-Hernández et al., 2014).

Species of *Marthamyces* have been quoted as biotrophs (Gu et al., 2015; Johnston and Park, 2019) and saprobes (Minter, 2003; Raymundo et al., 2016) and always as foliicolous fungi. Ascomata of *Marthamyces manglicola* were found on freshly fallen leaves. Hyde and Soyong (2008), Promputtha et al. (2010) and De Silva et al. (2016) suggest that this is evidence of an endophytic life strategy, growing on living leaves, followed by the production of sporophores as saprobes after leaf death, during decomposition. For mangroves this process is very important because they generate a large amount of litter which fungi





can decompose into simpler organic matter which is then exported to the neighboring ecosystem such as seagrasses and coral reefs.

Another important aspect is the host identity of the associated plant. *Marthamyces* species show a preference for colonizing particular plants (Johnston and Park, 2019). Although *M. manglicola* was found in a mangrove forest with four mangrove species: *Avicennia germinans*, *Rhizophora mangle* L., *Laguncularia racemosa* C.F.Gaertn. and *Conocarpus erectus* L. (Téllez-Valdés et al., 1989), it was found only on *A. germinans*. The factors likely to determine host specificity are physicochemical characteristics of the leaves, such as the amount of tannins, degree of lignification and the presence of salt. Compared with other mangrove species, *A. germinans* is known to have less tannins than *R. mangle* and more salt than the other taxa mentioned, due to its ability to exclude salt through leaf glands (Clough, 2013). *Marthamyces manglicola* can therefore be considered a halotolerant and able to tolerate conditions particular to mangrove forest such as waterlogging and high solar radiation.

## Conclusions

Most *Marthamyces* species have been reported from temperate and tropical forest (Minter, 2003; Johnston and Park, 2019). *Marthamyces manglicola* is the first species of this genus found in mangrove ecosystems, and is manglicolous. This is important because only around 300 species of manglicolous fungi are known globally (Raghukumar, 2017). *Marthamyces manglicola* is presented as a new species growing on fresh fallen and dead leaves of *Avicennia germinans* in mangrove forests in Cozumel Island.

## Author contributions

TR and RV conceived and designed the study. TR carried out the collections and TR, RV, YAGM and MMP made the determinations of the species. The photos of the figures were taken by TR. TR y RV drafted the manuscript with the help of MMP and YAGM. All authors contributed to the discussion, review, and approval of the final manuscript.

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