รายงานวิจัยฉบับสมบูรณ์

ความหลากหลายทางชีวภาพ วงศ์วานวิวัฒนาการ และบทบาทของราเอนโดไฟต์บน *Rhizophora apiculata* และ *Nypa fruticans*

(Biodiversity, phylogeny and role of fungal endophytes on above parts of *Rhizophora apiculata* and *Nypa fruticans*)

โดย รศ. ดร. เควิน เดวิด ไฮด์ และคณะ

ตั้งแต่วันที่ 16 มิ.ย. 2559 ถึงวันที่ 15 มิ.ย. 2562

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สนับสนุนโดยสำนักงานกองทุนสนันสนุนการวิจัย

และมหาวิทยาลัยแม่ฟ้าหลวง

<u>บทคัดย่อ</u>

"ป่าชายเลนครอบคลุมพื้นที่ชายฝั่ง 1 ใน 4 ของโลก และเป็นระบบนิเวศที่ถูกคุกคามมากที่สุดในปัจจุบัน" ซึ่งจากข้อมูลนี้ ถูกนำมาเป็นที่มาของข้อมูลพื้นฐานของการศึกษาวิจัยในปัจจุบัน และความสำคัญด้านการพัฒนาป่าชายเลนเพิ่มมากขึ้น รายงานฉบับสมบูรณ์นี้ เป็นส่วนหนึ่งของโครงการเรื่อง "ความหลากหลายทางชีวภาพ วงศ์วานวิวัฒนาการ และบทบาทของราเอนโดไฟต์บน *Rhizophora apiculata* และ *Nypa fruticans* " ได้นำเสนอและสรุปผลการวิจัยของโครงการในระยะเวลา 3 ปี ตั้งแต่วันที่ 16 มิถุนายน พ.ศ. 2560 ถึง 15 มิถุนายน 2562 การศึกษาวิจัยนี้ ได้ศึกษาทางด้านความหลากหลายทางชีวภาพ วงศ์วานวิวัฒนาการของเชื้อราระดับโมเลกุล และบทบาทของเชื้อราเอนโดไฟต์ บนพืช *Rhizophora apiculata* และ *Nypa fruticans* ในป่าชายเลน ประเทศไทย คณะวิจัยได้ทำการออกพื้นที่เพื่อเก็บตัวอย่าง บริเวณป่าชายเลนของแต่ละจังหวัดในประเทศไทย ได้แก่ จันทบุรี, ระยอง, ตราด, เพชรบุรี, ภูเก็ต, ประจวบคีรีขันธ์, กระบี่, สมุทรสงคราม และ สมุทรสาคร ซึ่งสามารถพบลักษณะของเชื้อราได้ทั้งในลักษณะเชื้อราย่อยสลาย, เชื้อราสาเหตุโรคพืช และเชื้อราแฝง ซึ่งในช่วงของการดำเนินการการศึกษาวิจัยในช่วงสามปี ได้ทำการเก็บตัวอย่างเชื้อรา จำนวนมากกว่า 200 ตัวอย่าง จากพืช *Rhizophora apiculata* และ *Nypa fruticans* ในป่าชายเลน และตัวอย่างทั้งหมดได้ทำการศึกษา ณ มหาวิทยาลัยแม่ฟ้าหลวง เพื่อศึกษาลักษณะของเชื้อรา รวมทั้งการแยกเชื้อรา และตัวอย่างเชื้อราดังกล่าวได้เก็บรักษาและฝากใน Herbarium-MFLU มหาวิทยาลัยแม่ฟ้าหลวง และเส้นใยของเชื้อราฝากไว้กับ MFLUCC มหาวิทยาลัยแม่ฟ้าหลวง และศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ (BIOTEC) และประมาณมากกว่า 100 ตัวอย่างได้ส่งไปยังประเทศจีนเพื่อการสกัดดีเอ็นเอและการวิเคราะห์ทางด้านชีวโมเลกุล เพื่อหาความสัมพันธ์ของสายวิวัฒนาการในระดับที่แตกต่างกัน โดยใช้การจัดหมวดหมู่บนพื้นฐานของข้อมูลลำดับดีเอ็นเอ และสัณฐานวิทยา เพื่อเป็นผลการวิจัยในการเผยแพร่ และในช่วงระยะเวลาสามปีของการดำเนินโครงการวิจัย ทางคณะผู้วิจัยได้นำข้อมูลผลงานบางส่วนไปนำเสนอในการประชุมวิชาการระดับนานาชาติ 15th International Marine and Freshwater Mycology Symposium (IMFMS) ครั้งที่ 15 ที่ มณฑลเซียะเหมิน, ประเทศจีน อีกทั้งผลการวิจัยจากโครงการได้มีการตีพิมพ์งานวิจัยในวารสารระดับนานาชาติ จำนวน 38 ฉบับ และอีก 3 ฉบับ ที่อยู่ในกระบวนการเรียบเรียงเพื่อตีพิมพ์เผยแพร่ข้อมูล โดยได้ระบุงานวิจัยทั้งหมดได้รับการสนับสนุน โดยสำนักงานกองทุนสนับสนุนการวิจัย(สกว.) และมหาวิทยาลัยแม่ฟ้าหลวง เลขที่สัญญา RSA5980068 โครงการเรื่อง ความหลากหลายทางชีวภาพ วงศ์วานวิวัฒนาการ และบทบาทของราเอนโดไฟต์บน *Rhizophora apiculata* และ *Nypa fruticans* " ทุนการศึกษาวิจัยนี้ยังเป็นส่วนหนึ่งของการศึกษาของ นักศึกษาปริญญาเอก จำนวน 2 คน ที่ได้ร่วมทำโครงการ ซึ่งโครงการจะดำเนินต่อไปในอีก 6-12 เดือนข้างหน้า หรือจนกว่านักศึกษาจะจบการศึกษา อย่างไรก็ตาม จากข้อมูลทั้งหมดวัตถุประสงค์ของโครงการได้ดำเนินการครบตามวัตถุประสงค์ของการศึกษา และได้ดำเนินการตามเป้าหมาย

ABSTRACT

Mangroves cover a quarter of the world's coastlines and are one of the most threatened ecosystems. Recent studies on mangrove fungi have provided information on frequency of occurrence, and host-substratum specificity. However, our lack of knowledge is pronounced for specific fungal populations, such as those on the aerial parts of the mangrove trees. Our study focused on the biodiversity, phylogeny and role of fungal endophytes on above parts of *Rhizophora apiculata* and *Nypa fruticans*. By selecting two hosts and accurately accessing the entire biodiversity of endophytes, saprobes and pathogens present, we have examined several important hypotheses with findings resulting in high impact publications. The duration of our study was three years. Within the first two and a half years, we collected specimens near coastlines in southern Thailand, and obtained about two hundred isolates, with outcomes of identified taxa and a number of relevant publications. We published a new endophytic fungus from *Rhizophora apiculata* and a saprobic fungus from the aerial part of the same, *Neopestalotiopsis alpapicalis* and *Rhytidhysteron mangrovei*, respectively. In particular, we deposited and published many fungal taxa from these two hosts. The pure cultures are kept at Mae Fah Luang Culture Collection (MFLUCC) and herbarium specimens are deposited at MFLU (Herbarium of Mae Fah Luang University) at Centre of Excellence in Fungal Research, Mae Fah Luang University. Along with these, 41 publications with the authors acknowledging the Thailand Research Fund (TRF) grant no RSA5980068 entitled Biodiversity, phylogeny and role of fungal endophytes on aerial parts of *Rhizophora apiculata* and *Nypa fruticans* have been published or submitted to top tier publications in Mycology and Plant Science journals. Our data regarding to evolutionary analyses and new taxa was presented at the 15th International Marine and Freshwater Mycology Symposium (IMFMS), Xiamen, China. With the help of the grant we managed to answer various hypotheses, such as using ITS sequence data to identify fungal endophytes from *Rhizophora apiculata*, showed that some groups of fungi may have jumped hosts and even to new ecosystems and provided divergence time estimations for the marine fungal groups. These results provide additional insights into the fungal community and their roles in the above parts as pathogens, saprobes or endophytes on *Rhizophora apiculata* and *Nypa fruticans*.

EXCUTIVE SUMMARY

The project involved investigating the fungi from *Rhizophora apiculata* and *Nypa fruticans* to answer several hypotheses pertaining to their life cycles. In the first two and a half years, we have collected specimens near coastlines in southern Thailand, and obtained about two hundred isolates, with outcomes of identified taxa and a number of relevant publications. In the last six months, we continued to write up papers and deposited data to herbaria. New taxa isolated from the two mangrove host plants were described and documented: a new endophyte *Neopestalotiopsis alpapicalis* was isolated from healthy leaves of *Rhizophora apiculata*, and a new species *Rhytidhysteron mangrovei* was discovered (see from Appendix X, published papers 2019_06, 2019_07). In addition, an asexual fungus *Savoryella nypae* was recognized, and a pleosporalean family Striatiguttulaceae that accommodates fungi isolated from rachides or petioles of *Nypa fruticans*, was established based on morphology and evolutionary phylogenetic analysis (see from Appendix X, published paper 2019_11 and submitted papers). Several new taxa have also been identified and written up as papers (see from Appendix X, published papers 2019_01, 2019_03, 2019_08). Here we would like to provide the final report for this project, which contains the work of the past six months as mentioned above, and our answers corresponding to research objectives, as well as our outcomes, especially those related publications.

Herein we are also answer various hypotheses that have been raised in this grant. In our case study of using ITS sequence data to identify fungal endophytes from *Rhizophora apiculata*, we showed that there may be 75% or more new species from a single host in Thailand. During the survey of fungal diversity of aerial parts of *Rhizophora apiculata* and *Nypa fruticans*, we found some groups of fungi may have jumped hosts even to new ecosystems and speciation occurred. For example, *Acuminatispora palmarum* was found from *Nypa fruticans* and *Phoenix paludosa*, while *Fasciatispora nypae* has been considered as host-specific for mangrove palm *Nypa fruticans*, but a new *Fasciatispora* sp. resembles *Fasciatispora nypae*, was found from a *Cocos nucifera* near mangroves. Cryptic species from these two hosts were also found to exist. Based on the sequence data, we found some species may occupy more than one life mode. For example, *Lasiodiplodia theobromae* and *Pestalotiopsis* have been found with saprobic and pathogenic life modes, *Colletotrichum* sp. and *Phomopsis* sp. have been isolated from healthy leaves as endophytes, and also been isolated from leaves with lesions. We hypothesised that mangrove fungi are slowly evolving as compared to those on host crops and that the fungi on mangroves are basal. We found some lineages comprising traditional mangrove or aquatic fungi, that are basal based in our evolutionary phylogenetic analysis. For example, Savoryellaceae, which diverged from ancestral Sordariomycetes, was ranked as a subclass Savoryellomycetidae around 213 MYA (198–303). However, there are also some exceptions. The family Striguttulaceae belonging to Dothideomycetes comprised taxa isolated from aerial parts of mangrove hosts, diversified approximately 60 (35–91) MYA. Based on our research, there is not enough evidence to prove the hypothesis about mangrove fungi evolving slowly.

The three years' research resulted in 41 publications (38 published and three submitted) with the authors acknowledging Thailand Research Fund (TRF) grant no RSA5980068 entitled

Biodiversity, phylogeny and role of fungal endophytes on aerial parts of *Rhizophora apiculata* and *Nypa fruticans*. Those research papers published in international academic journals that thank this grant are listed in Appendix X. Our data regarding to evolutionary analyses and new taxa were presented at the 15th International Marine and Freshwater Mycology Symposium (IMFMS), Xiamen, China (see from Appendix X). Two PhD students were trained as a result of this grant and will complete their PhDs within 12 months and publish several more papers acknowledging this grant. Our results have achieved the research objectives of the project, which include

- To establish the biodiversity of fungi on the aerial parts of two mangrove species.

  We collected samples from Cha-am, Chanthaburi, Krabi, Phang-Nga, Phetchaburi, Phuket, Prachuap Khiri Khan, Ranong and Samut Songkhram, Trat, the provinces in Thailand. The isolates and sequenced strains are listed in Appendix II (Tables 1, 2).
- To establish the percentage of new species as compared to known species and to describe all new species.

  We did a case study of isolate fungal endophytes from *Rhizophora apiculata* in Thailand. One hundred and fifty-four isolates were obtained, and the results from Blast searches and ITS phylogeny revealed 15 genera and one unidentified genus. Twenty-five of the 30 isolates could not be identified and thus an estimated 20 isolates are likely to be new species and one a new genus. This is remarkable, as endophytes of a single host in Thailand, may yield 75% or more of new species (as mentioned in published paper 2017_04). We also studied morphology and phylogeny of fungi that were isolated from leaves, rachides and petioles from the two mangrove host plants, and discovered a number of new species. The photo plates, descriptions, as well as phylogenetic trees are listed in Appendix III (Figures 1–37, Tables 3–10).
- To identify endophytic isolates and establish their phylogenetic relationships at different taxonomic levels based on DNA sequence data.

  Endophytic fungi are widely distributed in mangrove ecosystems and are integral contributors to global biodiversity. We recognized a new endophytic species *N. alpapicalis*, and a combined dataset of ITS, β-tublin and TEF1 genes was used to infer the phylogenetic placement of the new species (as mentioned in Appendix IV, 38–42).
- To prepare herbarium material of all collections for future reference.

  The strains isolated in this project were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Guizhou Culture Collection (GZCC). Herbarium specimens were deposited at the herbaria of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and Biotec Bangkok Herbarium (BBH), Bangkok, Thailand, and Kunming Institute of Botany Academia Sinica (KUN), Kunming, China. The herbarium material deposit information was listed in Appendix V (Table 11).
- To sequence appropriate genes of phylogenetically well-studied genera to identify cryptic species within species complex.

  Sequence similarity comparison and phylogenetic analysis of the ITS regions were used to identify taxa, especially the cryptic species within species complex. We found that ITS sequence data is reliable to assign isolates at the generic rank, and can be useful to

identify taxa to species level in a small number of fungal genera. For example, in the case of pestaloid fungi we found that all species were new to science (we have written up one paper and already submitted to journal). It cannot generally be used to determine specific species in most genera. We mentioned in Appendix VI (Table 12, 13, 14; Figures 43–46) and the published paper 2017_04.

- To investigate evolutionary relationships of unidentified endophytes, poorly studied genera and new species based on a polyphasic approach.

  The taxonomic ranking of fungi at higher levels (class, subclass, order, family, genus) has always been contentious and prone to subjectivity, and the estimation of divergence times using molecular clock methods have been used as objective evidence for higher ranking of taxa. A series papers were published which carried out evolutionary phylogenetic analysis. As mentioned in Appendix VII (Table 15; Figures 47–51), and the published papers in Appendix X: 2017_05, 2017_07, 2017_08, 2019_01, 2019_09, 2019_11.

- To establish if any species occupy more than one life mode (e.g. saprobic, endophytic, pathogenic).

  Sequence-based analyses have shown that a specific group of endophytic strains can act as saprotrophs in the laboratory or in the terrestrial environment. In general, leaf decomposition is a process taking several months where succession of different groups of decomposers occurs, endophytes are considered within the group of primary decomposers. However, fungal endophytes can have a significant effect on leaf decomposition, whether participating in it or not. Based on the sequences data, we found some species may occupy more than one life mode. From Appendix VIII (Table 16), we can see that *Lasiodiplodia theobromae* and *Pestalotiopsis* sp. present saprobic and pathogenic life modes, while *Colletotrichum* sp. and *Phomopsis* sp. have been isolate from health leaves as endophyte, and also been isolated from leaves with lesions.

- To obtain cultures of accurately identified mangrove taxa for novel medicinal compound discovery research and future phylogenetic studies.

  Fungi can produce medically significant metabolites or can be induced to produce such metabolites using biotechnology. Fungi derived from marine sources, such as mangroves, are considered to represent a huge reservoir of secondary metabolites, many of which are biologically active and are produced e.g. by multifunctional enzyme complexes such as polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS). Marine (mangrove) fungi are highly potent producers of bioactive substances with antifungal, antibacterial, antiviral, cytotoxic and immunosuppressive activity. The various biological activities make them a valuable source for pharmaceutical applications. In this study, all the obtained cultures were deposited in culture collection, and could be used for novel medicinal compound discovery research and future phylogenetic studies. Appendix IX (Figures 52–55).

RESEARCH CONTENTS

ความหลากหลายทางชีวภาพ วงศ์วานวิวัฒนาการ และบทบาทของราเอนโดไฟต์บน *Rhizophora apiculata* และ *Nypa fruticans*

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Introduction to the research problem and its significance:

Biodiversity and fungal species numbers

Fungi are thought to be the second most diverse organisms on earth (behind insects) with approximately 3.8 million species being the generally accepted figure. However, it is thought that only 15% of fungi are presently known. The fungi occurring in intertidal habitats of mangroves have been relatively well-researched (especially by the co-investigators), are specialized salt tolerant species, and there are thought to be more than 300 intertidal species in Malaysia alone. The fungi in the aerial parts of mangroves (i.e. aerial in this document mean plant parts that are never inundated by saline waters) are poorly known worldwide. In this study we will therefore investigate the biodiversity of aerial fungi of two mangrove tree species, based on fresh collection of saprobes and pathogens, and isolation of endophytes and fungi that cause dieback and cankers. The ability to accurately identify fungi has advanced significantly with the use of molecular data. Previously, mycologists identified species based on morphology and naming of species was very much subjective and, in most cases, involved clumping species in morphological similar species, now known as species complexes. It is now possible to identify genera and species, including cryptic species, by DNA sequence analyses of single spore isolates of the taxa collected. Sequence data can also directly be obtained from the fungi, especially from basidiomycetes; with microfungi it is more difficult but is possible if enough material is available. As long as one is aware that GenBank sequences are mostly wrongly named (e.g. 86% wrongly named *Colletotrichum gloeosporioides*), and that analyses must therefore use DNA sequence data from type strains, it is relatively easy to identify species in the better studied and important genera. Genera and or groups (*Bipolaris, Botryosphaeriaceae, Curvularia, Colletotrichum, Diaporthe, Fusarium, Pestalotiopsis, Phomopsis* and *Xylariaceae*) that would be common in the mangrove hosts are now relatively well-studied at the molecular level. The mangrove hosts should therefore contain cryptic species if fungal estimates are correct. Therefore, by studying two trees in a poorly studied habitat we can establish whether previous estimates of fungal diversity are realistic. If as estimated, only 15% of the fungi are known, then we should discover at least 85% of new species in the aerial parts of the two trees under investigation. If we find a much lower percentage of new species, then we can suggest that fungal estimates are too high.

Phylogeny and Evolution

With the use of molecular data, important pathogenic genera have been shown to comprise several species complexes that comprise cryptic species. This is particularly true in

general crops, fruit orchards and forest plantations, which are planted in large areas in single variety plantations. It is important that the fungi evolve rapidly so that they infect and rapidly colonize these newly planted areas which occupy large land areas and are nutritious habitats for the fungal growth and reproduction. Fungi have therefore probably rapidly evolved to occupy these new ecological niches or have jumped hosts to become serious pests. Mangroves on the other hand have been around for millennia, with *Nypa fruticans* being a very ancient plant, and therefore there are likely to be less selective pressures for species evolution. We would therefore expect to find fewer cryptic species in the various species complexes found in mangrove hosts and the species resolved should be basal in the species complexes. There may also be novel species outside the various species complexes that are only found on these unique mangrove hosts. Our findings will provide evidence as to whether mangrove fungi are slowly evolving as compared to those on host crops and indicate if the fungi on mangroves are basal. In addition, it is highly likely that a number of endophytes isolated will exist as "*mycelia sterilia*" and therefore cultural studies alone will be insufficient to identify them. DNA based phylogenies will unravel their evolutionary relationships and indicate whether these endophytes represent a unique or diverse lineage as well as help in their identification.

Role of endophytes

Microfungi are a heterogeneous group of micro-organisms that have diverse lifestyles. The type of association established with their host plant represents a major life history strategy that differs greatly among species and may be saprotrophism, parasitism and/or endophytism, or some other less common association, such as being predatory. However, these life strategies are not fixed, and it has been shown that certain fungi may switch from being endophytes to pathogens or saprobes. In this study we will therefore 2) compare the "terrestrial" endophytes and saprobes of two mangrove tree hosts, as well as the pathogens causing twig dieback, cankers and tree death. In this way we will establish if the species which are endophytes are also able to become saprobes on dead canopy wood, and/or cause tree disease.

In this study we will use morphology combined with sequence data to identify aerial species in two mangrove trees. All new species can be described, and the percentage of new species can be used to increase our understanding of fungal numbers. Cryptic species will be resolved using molecular data to establish if speciation is occurring more rapidly in crops than in the relatively unaltered mangrove hosts. We will also establish whether the same species are capable of being saprobes, endophytes and/or pathogens. Literature review: Fungal Biodiversity Accurate details of the numbers of organisms worldwide are important because only then can we gauge what biodiversity is being lost as result of habitat.

Literature review

Fungal Biodiversity

Accurate details of the numbers of organisms worldwide are important because only then can we gauge what biodiversity is being lost as result of habitat loss, global warming and other changes in our environment. Without such figures we would have no idea whether humans are moving towards sustainable living. In the case of fungi, knowledge of numbers is of particular importance because fungi are nutrient recyclers and an integral part of all food chains (Kendrick 2003). They are economically important plant pathogens and are important

biosecurity organisms (KoKo et al. 2011a; Wang et al. 2012), and yet they also have untapped potential in discovering new food sources (Klomklung et al. 2012), novel medicinal compounds (Aly et al. 2011; Debbab et al. 2012; De Silva et al. 2012a.b) and biofertilizers (Kaewchai et al 2009). Fungi are the most diverse organisms on earth, second to insects with approximately 1.5 million species being the generally accepted figure (Hyde, 2001). Only 15% of fungi are probably presently known (Hyde 2001). It is therefore important that research is directed towards establishing fungal numbers worldwide (Hyde 2001).

The fungi of the tropics are less well known than those or temperate region and it is expected that studies would results in numerous new species being discovered (Hyde 2001). Therefore if any new habitat in a tropical country is studied one would expect to discover novelty. This novelty then has potential to be exploited in various biotechnological applications (Aly et al. 2011; Jeewon et al. 2013). The fungi occurring in intertidal habitats of mangroves have been relatively well-researched in Malaysia and Brunei (especially by the co-investigators – e.g Hyde et al. 1999; Hyde and Alias 2000; Pang et al. 2010; Alias et al. 2010); these are specialized salt tolerant species, and there are thought to be more than 300 intertidal species in Malaysia alone (Alias et al 2010). The fungi in the aerial parts of mangroves (i.e. those in the canopy) are poorly known worldwide.

Phylogeny and evolution

The ability to accurately identify fungi has advanced significantly in recent years with the use of molecular data (Jeewon et al. 2009; KoKo et al. 2011b). Previously, mycologists identified species based on morphology and naming of species was very much subjective and, in most cases, involved clumping species into morphologically similar species (Hyde et al. 2009; Jeewon et al. 2002; Udayanga et al 2011, 2012), now known as species complexes (KoKo et al. 2011b). It is now possible to identify genera and species, including cryptic species, by sequencing single spore isolates of the taxa collected (Boonmee et al. 2012; Maharachchikumbura et al. 2012). For example, anthracnose of post harvest fruits were mainly thought to be caused by *Colletotrichum acutatum* and *C. gloeosporioides*. Phoulivong et al. (2010) however showed that neither of these species causes fruit rots in Thailand and that several other cryptic species were responsible. Such data has important implications on disease causal agents, disease control and to a lesser extent, international movement of fruits.

As molecular sequence data has become more readily available, it is commonly used to classify the fungi in order to develop a natural classification system (Chomnunti et al 2011, 2012). Sequence data can directly be obtained from the fungi, especially from basidiomycetes (mushrooms), although with microfungi it is more difficult, but is possible if enough material is available (Jeewon and Hyde 2007). This has resulted in a better understanding of fungal classification at the higher taxonomic levels, and has also shown that morphologically similar fungi (species complexes) comprise many cryptic species. Endophytes that were previously almost impossible to name to species or genera can now be named by blasting DNA sequence data in GenBank. However, many researchers have made the mistake of believing the names of fungi in GenBank although there is absolutely no control on what is place in this public database. Therefore, many of the papers published today are highly erroneous. KoKo et al (2011b) explained that researchers must use sequences from type strains in their analyses if they want to identify the fungi accurately.

As long as one is aware that GenBank sequences are mostly wrongly named (e.g. 86% wrongly named *Colletotrichum gloeosporioides*, Cai et al. 2009), and that analyses must therefore use sequence data from type strains, it is relatively easy to identify species in the better studied and important genera (Cai et al. 2011). Genera and or groups (*Bipolaris, Botryosphaeriaceae, Curvularia, Colletotrichum, Diaporthe, Fusarium, Pestalotiopsis, Phomopsis* and *Xylariaceae*) that would be common in the mangrove hosts are now relatively well-studied at the molecular level (e.g. Liu et al. 2012; Manamgoda et al. 2012; Udayanga et al 2012). The mangrove hosts should therefore contain cryptic species if fungal estimates are correct. Therefore, by studying two trees in a poorly studied habitat we can establish whether previous estimates of fungal diversity are realistic. If as estimated, only 15% of the fungi are known, then we should discover at least 85% of new species in the aerial parts of the two trees under investigation. If we find a much lower percentage of new species, then we can suggest that fungal estimates are too high. With the use of molecular data, important pathogenic genera have been shown to comprise several species complexes that comprise cryptic species (Damm et al. 2012; Weir et al. 2012). This is particularly true in general crops, fruit orchards and forest plantations, which are planted in large areas in single variety plantations. It is important that the fungi evolve rapidly so that they infect and rapidly colonize these newly planted areas which occupy large land areas and are nutritious habitats for the fungal growth and reproduction. Fungi have therefore probably rapidly evolved to occupy these new ecological niches or have jumped hosts to become serious pests. Mangroves on the other hand have been around for millennia and therefore there are likely to be less selective pressures for species evolution. We would therefore expect to find fewer cryptic species in the various species complexes found in mangrove hosts and the species resolved should be basal in the species complexes. There may also be novel species outside the various species complexes that are only found on these unique mangrove hosts. Our findings will provide answer to the above questions.

Role of endophytes

Microfungi are a heterogeneous group of micro-organisms that have diverse lifestyles. Plant-infecting fungi in particular affect their host plant in many ways, ranging from mutualistic to parasitic interactions (Purahong and Hyde 2011). The type of association established with their host plant represents a major life history strategy that differs greatly among species and may be saprotrophism, parasitism and/or endophytism, or some other less common association, such as being predatory. However, these life strategies are not fixed and it has been shown that certain fungi may switch from being endophytes to pathogens or saprobes. In this study we will therefore compare the “terrestrial” endophytes and saprobes of two mangrove tree hosts, as well as the pathogens causing twig dieback, cankers and tree death. In this way we will establish if the species which are endophytes are also able to become saprobes on dead canopy wood, and/or cause tree disease.

Some previous studies have tried to provide evidence to link the various life styles of fungi. Brown et al (1998) showed that many endophyte species were also pathogens and provided evidence that many endophytes were latent plant pathogens. Further evidence was provided by Photita et al. (2004), who did pathogenicity testing with endophytes of banana and found that some endophytes could cause disease. Promputtha et al. (2007) analyzed DNA

sequence data from endophytes and saprobes and demonstrated that the same species could have endophytic or saprobic lifestyles, while Promputtha et al. (2010) showed that endophytes can produce the enzymes for a saprobic lifestyle, implicating that they need these when they convert life styles from an endophyte to a saprobe. Purahong and Hyde (2011) discussed the role of endophytes in grass decay indicating that endophytes had a major role, while Vázquez de Aldana et al. (2013) showed that grass endophytes were responsible for decay of grasses and that their spores were important in causing allergic disease. In the most recent study, Delaye et al (2013) has shown by analysis of molecular data that there have been many shifts between saprobic to necrotrophic (pathogenic) life styles, whereas once a species becomes a biotroph then it does not revert back to an endophyte.

Objective

1. To establish the biodiversity of fungi on the aerial parts of two mangrove species.
2. To establish the percentage of new species as compared to known species and to describe all new species.
3. To identify endophytic isolates and establish their phylogenetic relationships at different taxonomic levels based on DNA sequence data.
4. To prepare herbarium material of all collections for future reference.
5. To sequence appropriate genes of phylogenetically well-studied genera to identify cryptic species within species complex.
6. To investigate evolutionary relationships of unidentified endophytes, poorly studied genera and new species based on a polyphasic approach.
7. To establish if any species occupy more than one life mode (e.g. saprobic, endophytic, pathogenic).
8. To obtain cultures of accurately identified mangrove taxa for novel medicinal compound discovery research and future phylogenetic studies.

Methodology

Field observations

Bi-annual visits to mangroves were made in southern Thailand. During the first two years of the study and samples were collected from the two hosts *Nypa fruticans* and *Rhizophora apiculata*. Saprobes occurring on dead aerial twigs and branches attached to the living trees, were broken from the trees, or cut from the larger trunks. Aerial diseased samples with dieback, cankers, tree decline and seedling disease were collected in a similar way and symptoms were photographed. Healthy leaves and twigs were collected for endophyte isolation. Data for all specimens were recorded and specimens were numbered and placed in plastic bags and returned to the laboratory for observation.

Morphological study

Diseased specimens including those with leaf spots, die back, cankers, butt rots and other diseases were examined under a stereomicroscope to detect fungi present. If fungi were not present, then samples were incubated in moist chambers to induce sporulation or fungi were isolated from the edges of disease tissue using standard plant pathological techniques (Pang et al. 2010). If sporulation occurs, then the fine forceps were used to remove one or two fruiting structures, and been mounted in sterile seawater (Pang et al. 2010). Morphology of the asci and ascospores will be documented using various microscopes.

Single spore isolation

The objective of this part is to get axenic culture of all fungi that causing disease of mangrove trees, as well as all saprobes. Isolations were made from single spores, conidia or fruiting bodies when they can be found. Fruiting bodies were cut through horizontally and the contents transferred to a drop of sterile water on a flamed microscope slide. The spore suspension was spread over a few square centimeters of a Petri plate containing 2 % water agar (WA) and incubated at 25°C overnight. The next day individual germinating spores could be transferred to fresh PDA media following the method of Chomnunti et al. (2011). All cultures were deposited in the culture collection.

Method for canker and dieback isolations

Isolations from cankers were done from wood tissue at the advancing zone of the lesion after surface disinfestation in 70% ethyl alcohol for 30 s; rinse twice in sterile distilled water and blotted dry on sterile filter paper (Zhu et al. 2013). Disinfested tissue was placed on 2% MEA and incubated at room temperature (~20°C) for 1 week. Colonies were subcultured using hyphal typing technique to a fresh 2% MEA plate for further investigation (Pérez et al. 2010).

Branches with symptomatic wood (e.g. dieback, canker, necrosis) were isolated. Wood pieces (2 3 2 mm) from the margin between necrotic and apparently healthy tissue were carried out surface sterilization (1 min in 3.5% NaOCl and 30 s in 70% ethanol), then were placed on potato-dextrose agar (2% PDA, Biolab, Midrand, South Africa, supplemented with 100 mg/L streptomycin sulfate and 100 mg ampicillin) and on synthetic nutrient agar medium (SNA, Nirenberg 1976) supplemented with 100 mg penicillin G, 50 mg streptomycin sulphate, 10 mg chlortetracycline hydrochloride, pH 6 and incubated under cool fluorescent white light at 25 C. Additional isolates were derived from pycnidia on the bark of pruning debris. Single conidial

isolates were obtained from all strains for further morphological and molecular characterization (Ulrike 2007).

Isolation of endophytes

Living healthy, random leaves and small branches or twigs were collected from the aerial parts of the mangrove plants following the methods of Kjer et al. (2010). Plant samples were collected and placed in sterile bags. At every study site, samples were collected and returned to the laboratory for processing. Healthy plant tissues were cut into discs and surface sterilized using 4% bleach and 70% ethanol after which they were placed on PDC (Potato Dextrose - Chloramphenicol) agar plates (Vieira et al. 2014). Chloramphenicol in PDA inhibits the growth of bacteria, thus only fungi will grow in them. After incubating for one week, colonies that different in color or other culture characters were transferred to new Potato Dextrose Agar plates and incubated for 7-10 days at 25º C. (Wikee et al. 2013)

DNA extraction

Selected fungal isolates were grown on PDA for 21 d at 28 °C in the dark. Genomic DNA were extracted from the fresh mycelium using Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) according to the manufacturer's protocol (Hangzhou, P.R. China).

PCR amplification and sequencing

The methods for PCR amplification and sequencing followed those used in previous studies by the authors (Jeewon et al. 2002; Pang et al 2010). DNA amplification were performed by polymerase chain reaction (PCR). Primer pairs NS1 and NS4 were used to amplify a region spanning the small subunit rDNA. LROR and LR5 primer pairs were used to amplify a segment of the large subunit rDNA. Primer pairs ITS5 and ITS4 were used to amplify the internal transcribed spacers. Other primers for gene regions were used when necessary. The amplifications were performed in a 50 µl reaction volume as follows: 1X PCR buffer, 0.2 mMd'NTP, 0.3 µM of each primer; 1.5 mM $MgCl_2$, 0.8 units Taq Polymerase and 5–10 ng DNA (Cai et al. 2009). The amplification conditions were used as follows: initial denaturation of 5 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 48 °C and 90 s at 72 °C, and a final extension period of 10 min at 72 °C. The PCR products were checked on 1 % agarose electrophoresis gels stained with ethidium bromide. PCR products then would be purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27–9602–01). DNA sequencing were carried out by reliable selected company (Tao et al. 2013).

Phylogenetic analysis

The methods for phylogenetic analysis followed those used in previous studies by the authors (Jeewon et al. 2002; Pang et al 2010). Sequences generated from different primers were analyzed with other sequences obtained from GenBank. A Blast search was performed to reveal the closest matches with taxa in the various fungal groups. In addition, fungal members from different families and related orders were included in the analyses. Sequences were aligned using Bioedit and ClustalX v. 1.83 (Doilom et al. 2013). The alignments were visually checked and improved manually where necessary. Phylogenetic analyses were performed by using

PAUP v. 4.0b10 for Maximum-parsimony (MP) and Neighbour-joining (NJ) analyses, MrBayes v. 3.0b4 for Bayesian analyses and MEGA 5 or RAMxL where needed. Trees were rooted to appropriate outgroup taxa. Maximum-parsimony analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability were assessed by using bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa. The model of evolution was estimated by using MrModeltest 2.2. Posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4. Six simultaneous Markov chains were runned for 1 000000 generations and trees were sampled every 100th generation (resulting in 10000 total trees). The first 2000 trees, representing the burn-in phase of the analyses, were discarded and the remaining 8000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree. Phylogenetic trees were viewed in Treeview. Sequences derived in this study were deposited in GenBank, and the alignments used for analyses were submitted in TreeBASE (www.treebase.org)

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Schedule for the entire project and expected outputs

Activity	Months 1-12												Months 13-24												Months 25-36											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Collection of samples to obtain biodiversity of fungi on the aerial parts of two mangrove species																																				
Laboratory work (Microscopy), cultural studies and isolation of fungi																																				
Preparation of herbarium material of all collections																																				
Isolation of genomic DNA and RNA, PCR amplification and purification, DNA Sequencing and sequence alignment																																				
Identification of endophytic isolates and establishment of																																				

Activity	Months 1-12												Months 13-24												Months 25-36											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
their phylogenetic relationships at different taxonomic levels based on DNA sequence data																																				
Evolutionary relationships study of unidentified endophytes, poorly studied genera and new species based on a polyphasic approach																																				
Establishment of life mode/style of isolates (saprobic/ endophytic/pathogenic)																																				
Presentation of data, report writing and publication																																				

OUTPUTS

1. Published papers which acknowledge the grant

Thirty-eight publications were published in SCI and three manuscripts submitted with process to publish with the authors acknowledging the Thailand Research Fund (TRF) grant no RSA5980068 entitled Biodiversity, phylogeny and role of fungal endophytes on aerial parts of *Rhizophora apiculata* and *Nypa fruticans*. A list of publications with attached first pages and acknowledgement of publications are provided in Appendix X.

2. Number of PhD (by research) students

Three PhD students

1. Shengnan Zhang
   - Doctor of philosophy of Chiang Mai university, Thailand
   - Topic: Taxonomy and phylogeny of microfungi from selected palms
2. Kumar Vinit
   - Doctor of philosophy of Chiang Mai university, Thailand
   - Topic: Taxonomy and phylogeny of microfungi from *Rhizophora apiculata*
3. Chada Norphanphoun
   - Doctor of philosophy of Mae Fah Luang university, Thailand
   - Topic: Ascomycete on mangroves in thailand

3. Specific or potential applications

As we mentioned in the excutive summary results above and appendixs. Some of result from this project were mentioned below with the future utilization.

1. This project have surveyed the biodiversity disease of mangroves in Thailand. This contributes to knowledge of biodiversity, phylogeny and role of fungal endophytes on aerial parts of *Rhizophora apiculata* and *Nypa fruticans* from Thailand.

2. A modern natural classification of fungi causing disease on aerial parts of *Rhizophora apiculata* and *Nypa fruticans* will be established and this will be a very important document and used by researchers worldwide.

3. This project will provide baseline data of biodiversity, phylogeny and role of fungal endophytes on aerial parts of *Rhizophora apiculata* and *Nypa fruticans* disease cause by fungi in Thailand and provide a small booklet for disease identification

4. The results will be published and be useful to systematics, plant health practitioners, organic chemists, quarantine experts, and all users of fungi.

5. This project has done with development and promotion of utilization of science, technology, research and development and innovation.

In the future, if any novel compounds are discovered during research additional it may have potential applications.

APPENDIX

Contents

Figure 46: Phylogram generated from maximum likelihood analysis (RAxML) based on ITS sequence data in *Pseudopithomyces* species.

Table 12: Cultural characteristics of the 20 morphotypes from *Rhizophora apiculata* on potato dextrose agar (PDA) at 25 °C in the dark.
Table 13: Closest match for endophytes following MegaBLAST search of NCBI GenBank nucleotide database. Max identity and max score are shown.
Table 14: Details of representative strains of isolated endophytic fungi from *Rhizophora apiculata*.

Appendix VII To investigate evolutionary relationships of unidentified endophytes, poorly studied genera and new species based on a polyphasic approach.

Figure 47: RAxML tree of Pleosporales based on analysis of combined LSU, SSU, TEF1α and RPB2 sequence data.
Figure 48: Maximum clade credibility (MCC) tree with divergence times estimates for Pleosporales and selected groups in Dothideomycetes.
Figure 49: Morphology of *Striatiguttula nypae*.
Figure 50: Morphology of *Striatiguttula phoenicis*.
Figure 51: Morphology of *Longicorpus striataspora*.

Table 15: Divergence time estimates of Pleosporales and selected lineages of Dothideomycetes obtained from a Bayesian approach (BEAST) on basis of three calibrations.

Appendix VIII To establish the biodiversity of fungi and also establish if any species occupy more than one life mode (e.g. saprobic, endophytic, and pathogenic).

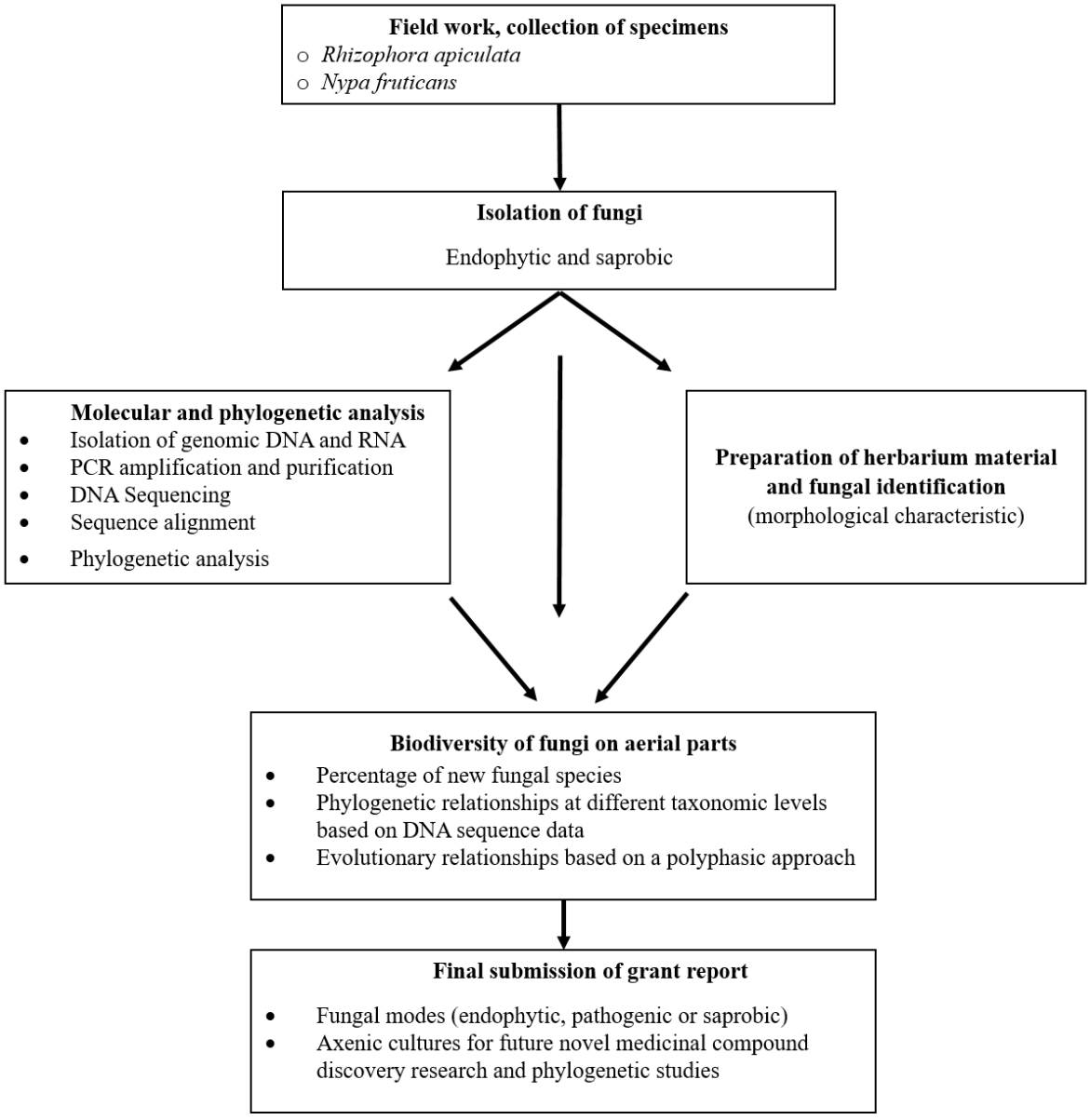
Table 16: List of isolate fungi with deposit information and identification based on sequence data.

Appendix IX To obtain culture of accurately identified mangrove taxa for novel medicinal compound discovery research and future phylogenetic studies.

Figure 52: Cultures of fungi isolated from *Nypa fruticans*.
Figure 53: Culture characteristics observation.
Figure 54: Colonies on PDA of *Acuminatispora palmarum*.
Figure 55: Culture characters observation.

Appendix X Research published with international academic journals and conections.

Appendix I Flow chart of research activities.



Appendix II To establish the biodiversity of fungi on the aerial parts of two mangrove species.

Table 1. Endophytic fungi isolated from *Rhizophora apiculata* and *Nypa fruticans* collected in this research.

MFLUCC	Original Code	Species Name	Habitat	Host	Collection Date	Isolation Method
	RYE1/2A	*Phyllosticta* spp.	Leaf spot symptom	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE1/2C	*Phyllosticta* spp.	Leaf spot symptom	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE1/2D	Unknown	Leaf spot symptom	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE2/1A	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE2/1C	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
17-0030	RYE3/2A	*Phyllosticta* spp.	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE3/2B	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE3/2C	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE3/4A	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE3/4B	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE4/1	*Phyllosticta* spp.	Asymptomatic petiole	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE4/2A	Unknown	Asymptomatic petiole	*Rhizophora apiculata*	06-07-16	Tissue transplanting
17-0031	RYE5/1A	*Phyllosticta* spp.	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
17-0032	RYE5/1B	*Phyllosticta* spp.	Asymptomatic leaf	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE6/1	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE6/2A	Unknown	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE6/3A	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE6/3B	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE6/3C	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE6/3D	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE6/4A	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE6/4B	Unknown	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
17-0033	RYE6/4C	Unknown	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
17-0034	RYE6/4D	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE7/1A	Unknown	Leaf spot symptom	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE7/1B	Unknown	Leaf spot symptom	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE7/1C	Unknown	Leaf spot symptom	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE7/1D	Unknown	Leaf spot symptom	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE8/1A	Unknown	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting

MFLUCC	Original Code	Species Name	Habitat	Host	Collection Date	Isolation Method
	RYE8/1B	Unknown	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE8/2A	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
17-0035	RYE8/2B	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE8/2C	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE8/2D	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE8/3A	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE8/3B	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	RYE8/3C	*Diaporthe* sp.	Asymptomatic aerial root	*Rhizophora apiculata*	06-07-16	Tissue transplanting
	CBE9/3A	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE9/3B	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE9/3D	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
17-0036	CBE12/1A	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE12/1B	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE12/2A	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE12/3A	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE13/2C	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE13/2D	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE13/3B	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE13/3C	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE14/1A	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE15/1A	*Phyllosticta* spp.	Asymptomatic petiole	*Rhizophora apiculata*	07-07-16	Tissue transplanting
17-0037	CBE15/2A	*Phyllosticta* spp.	Asymptomatic petiole	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE15/2B	*Phyllosticta* spp.	Asymptomatic petiole	*Rhizophora apiculata*	07-07-16	Tissue transplanting
	CBE16/1B	Unknown	Asymptomatic petiole	*Rhizophora apiculata*	07-07-16	Tissue transplanting
17-0002	SRNE1AL	*Phanerochaete* sp.	Asymptomatic leaf	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0003	SRNE1BL	*Stagonosporopsis cucurbitacearum*	Asymptomatic leaf	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0004	SRNE1DL	*Colletotrichum* spp.	Asymptomatic leaf	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0005	SRNE1EL	*Nemania diffusa*	Asymptomatic leaf	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0006	SRNE1AP	*Phyllosticta* spp.	Asymptomatic petiole	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0007	SRNE1BP	*Rigidoporus vinctus*	Asymptomatic petiole	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
	SRNE7R	*Pestalotia* sp.	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0021	SRNE9AR	Unknown	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting

MFLUCC	Original Code	Species Name	Habitat	Host	Collection Date	Isolation Method
	SRNE9BR	Unknown	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0022	SRNE9CR	*Preussia* sp.	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0023	SRNE9DR	*Preussia* sp.	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0024	SRNE13AR	*Pestalotiopsis* spp.	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0025	SRNE13BR	*Pestalotiopsis* spp.	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
17-0026	SRNE13CR	*Pestalotiopsis* spp.	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
	SRNE13DR	*Pestalotiopsis* spp.	Asymptomatic root	*Rhizophora apiculata*	30/11/2016	Tissue transplanting
	NNSE1AL1	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
	NNSE1AL2	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
	NNSE4AL	*Hypoxylon lechatii*	Asymptomatic leaf	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
	NNSE4BL	*Colletotrichum* spp.	Asymptomatic leaf	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
17-0027	NNSE4CL	*Hypoxylon griseobrunneum*	Asymptomatic leaf	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
	NNSE4DL	Unknown	Asymptomatic leaf	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
17-0020	NNSE4EL	*Hypoxylon* sp.	Asymptomatic leaf	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
17-0028	NNSE6AR	Unknown	Asymptomatic root	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
17-0029	NNSE6BR	*Penicillium citrinum*	Asymptomatic root	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
	NNSE6CR	*Schizophyllum commune*	Asymptomatic root	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
	NNSE6CRA2	*Schizophyllum commune*	Asymptomatic root	*Rhizophora apiculata*	2/12/2016	Tissue transplanting
	RNNP1AP	*Skeletocutis diluta*	Asymptomatic petiole	*Nypa fruticans*	5/12/2016	Tissue transplanting
	RNNP2AL	*Grammothele lineata*	Asymptomatic leaf	*Nypa fruticans*	5/12/2016	Tissue transplanting
	RNNP2BL	Unknown	Asymptomatic leaf	*Nypa fruticans*	5/12/2016	Tissue transplanting
	RNNP3AP	Unknown	Asymptomatic petiole	*Nypa fruticans*	5/12/2016	Tissue transplanting
	RNE2AR	Unknown	Asymptomatic root	*Rhizophora apiculata*	4/12/2016	Tissue transplanting
	RNE2BR	Unknown	Asymptomatic root	*Rhizophora apiculata*	4/12/2016	Tissue transplanting
	RNE7R	Unknown	Asymptomatic root	*Rhizophora apiculata*	4/12/2016	Tissue transplanting
	MCD 044	Unknown	Submerged wood	*Rhizophora apiculata*	6/12/2016	Single spore isolation
	MCD 046	Unknown	Submerged wood	*Rhizophora apiculata*	6/12/2016	Single spore isolation
	MCD 052	Unknown	Submerged wood	*Rhizophora apiculata*	6/12/2016	Single spore isolation
	MCD 053	Unknown	Submerged wood	*Rhizophora apiculata*	6/12/2016	Single spore isolation
	MCD 054	Unknown	Submerged wood	*Rhizophora apiculata*	6/12/2016	Single spore isolation
	MCD 055	Unknown	Submerged wood	*Rhizophora apiculata*	6/12/2016	Single spore isolation
	MCD 060	Unknown	Submerged wood	*Rhizophora apiculata*	6/12/2016	Single spore isolation
	MCD 085	Unknown	Submerged wood	*Rhizophora apiculata*	2/12/2016	Single spore isolation

MFLUCC	Original Code	Species Name	Habitat	Host	Collection Date	Isolation Method
	MCD 087	Unknown	Submerged wood	*Rhizophora apiculata*	2/12/2016	Single spore isolation
	MCD 088	Unknown	Submerged wood	*Rhizophora apiculata*	2/12/2016	Single spore isolation
	MCD 090	*Hysterium angustatum*	Submerged wood	*Rhizophora apiculata*	2/12/2016	Single spore isolation
	MCD 091	Unknown	Submerged wood	*Rhizophora apiculata*	2/12/2016	Single spore isolation
	MCD 092	Unknown	Submerged wood	*Rhizophora apiculata*	2/12/2016	Single spore isolation
	MCD 021	Unknown	Submerged wood	*Rhizophora apiculata*	4/12/2016	Single spore isolation
	MCD 022	Unknown	Submerged wood	*Rhizophora apiculata*	4/12/2016	Single spore isolation
	MCD 023	Unknown	Submerged wood	*Rhizophora apiculata*	4/12/2016	Single spore isolation

Table 2. Endophytic fungi isolated from Rhizophora apiculata and Nypa fruticans used for sequencing

Original Code	Suspected/Family/ Genus/ Species	Sample recived date	Assigned code	CTAB	ITS	TEF
					ITS5 & ITS4	983 & 2218R
SRNE1AL	*Phanerochaete* sp.	24-02-17	J04	used	ITS5 & ITS4 success	
SRNE1BL	*Stagonosporopsis cucurbitacearum*	24-02-17	J05	used	ITS5 & ITS4 success	
SRNE1DL	*Colletotrichum* spp.	24-02-17	J03	used	ITS5 & ITS4 success	
SRNE1EL	*Nemania diffusa*	24-02-17	J07	used	ITS5 & ITS4 success	
SRNE1BP	*Rigidoporus vinctus*	24-02-17	J02	used	ITS5 & ITS4 success	
SRNE4DL	*Colletotrichum* sp.	24-02-17	J12	used	ITS5 & ITS4 success	
SRNE6AP	Unknown	24-02-17	J14	used	ITS5 & ITS4	
SRNE7R	*Pestalotia* sp.	24-02-17	J06	used	ITS5 & ITS4 success	
SRNE9CR	*Preussia* sp.	24-02-17	J16	used	ITS5 & ITS4 success	
SRNE9DR	*Preussia* sp.	24-02-17	J15	used	ITS5 & ITS4 success	
SRNE13AR	*Pestalotiopsis* spp.	24-02-17	J17	used	ITS5 & ITS4 success	
SRNE13BR	*Pestalotiopsis* spp.	24-02-17	J20	used	ITS5 & ITS4 success	
SRNE13CR	*Pestalotiopsis* spp.	24-02-17	J18	used	ITS5 & ITS4 success	
SRNE13DR	*Pestalotiopsis* spp.	24-02-17	J19	used	ITS5 & ITS4 urgent wrong	
NNSE4AL	*Hypoxylon* sp.	24-02-17	J34	used	ITS5 & ITS4 success	
NNSE4BL	*Colletotrichum* spp.	24-02-17	J35	used	ITS5 & ITS4 success	
NNSE4CL	*Hypoxylon griseobrunneum*	24-02-17	J36	used	ITS5 & ITS4 success	

Original Code	Suspected/Family/ Genus/ Species	Sample recived date	Assigned code	CTAB	ITS ITS5 & ITS4	TEF 983 & 2218R
NNSE4EL	*Hypoxylon* sp.	24-02-17	J33	used	ITS5 & ITS4 success	
NNSE6AR	Unknown	24-02-17	J38	used	ITS5 & ITS4 success	
NNSE6BR	*Penicillium citrinum*	24-02-17	J39	used	ITS5 & ITS4 success	
NNSE6CR	*Schizophyllum commune*	24-02-17	J37	used	ITS5 & ITS4 success	
NNSE6CRA2	*Schizophyllum commune*	24-02-17	J40	used	ITS5 & ITS4 success	
RNNP1AP	*Skeletocutis diluta*	24-02-17	J01	used	ITS5 & ITS4 success	
RNNP2AL	*Grammothele lineata*	24-02-17	J29	used	ITS5 & ITS4 success	
RYE3/2A	*Phyllosticta* spp.	24-02-17	J23	used	ITS5 & ITS4 success	EF1-728F& EF2
RYE3/4B	*Colletotrichum* spp.	24-02-17	J24	used	ITS5 & ITS4 success	
RYE4/1	*Phyllosticta* spp.	24-02-17	J25	used	ITS5 & ITS4 success	
RYE5/1	*Phyllosticta* spp.	24-02-17	J26	used	ITS5 & ITS4 success	EF1-728F& EF2
RYE6/3A	Unknown	24-02-17	J27	used	ITS5 & ITS4 unsuccess	
RYE8/3B	*Diaporthe* spp.	24-02-17	J28	used	ITS5 & ITS4 success	
CBE9/3B	Unknown	24-02-17	J21	used	ITS5 & ITS4 success	
CBE14/1A	Unknown	24-02-17		used	ITS5 & ITS4 please ignor	
CBE17/2B	Unknown	24-02-17	J22	used	ITS5 & ITS4 urgent wrong	
SRNE9BR	Unknown	01-04-17	J42	used	ITS5 & ITS4 urgent	
RYE8/2B	*Diaporthe* sp.	01-04-17	J45	used	ITS5 & ITS4 success	
CBE12/1B	Unknown	01-04-17	J46	used	ITS5 & ITS4 success	
CBE14/1A	Unknown	01-04-17	J43	used	ITS5 & ITS4 urgent	
NNSE1AL1	Unknown	01-04-17	J44	used	ITS5 & ITS4 urgent	
NNSE1AL2	Unknown	01-04-17	J41	used	ITS5 & ITS4 success	

Appendix III To establish the percentage of new species as compared to known species and describe all new species.

In this study, we isolated fungal endophytes from *Rhizophora apiculata* in Thailand and established how many can be identified to species level based on ITS sequence data. Endophytic fungi were isolated from leaves, petioles and aerial roots of *R. apiculata* in four provinces of Thailand. One hundred and fifty-four isolates were obtained and initially grouped into 20 morphotypes based on cultural characteristics. Nine were sporulating morphotypes, which were assigned to seven genera (*Colletotrichum*, *Cytospora*, *Diaporthe*, *Hypoxylon*, *Neopestalotiopsis*, *Neodevriesia*, *Pestalotiopsis*, *Phyllosticta*, *Pseudopestalotiopsis*), and eleven morphotypes were non-sporulating mycelia sterilia. The results from Blast searches and ITS phylogeny of the 15 genera and the one unidentified genus are discussed. Twenty-five of the 30 isolates could not be identified and thus an estimated 20 isolates are likely to be new species and one a new genus. This is remarkable, as endophytes of a single host in Thailand, may yield 75% or more of new species.

We also studied morphology and phylogeny of fungi that were isolated from leaves, rachides and petioles form the two mangrove host plants. The photo plates, descriptions are provided as below, as well as some rough phylogenetic trees.

Table 3. Microfungi from *Nypa fruticans* Collection

Date	Site/Province	Quantity
6 July, 2016	Thang Kwian	5
7 July, 2016	Chanthaburi	1
2 Dec. 2016	Prachuap Khiri Khan	6
3-7 Dec. 2016	Ranong	35
25 April 2017	Chanchaburi	11
27 April 2017	Koh Chang	5
Total		63

Note: Some collections without fungi are excluded. Isolated fungi from 51 specimens and obtained 36 germinated stains.

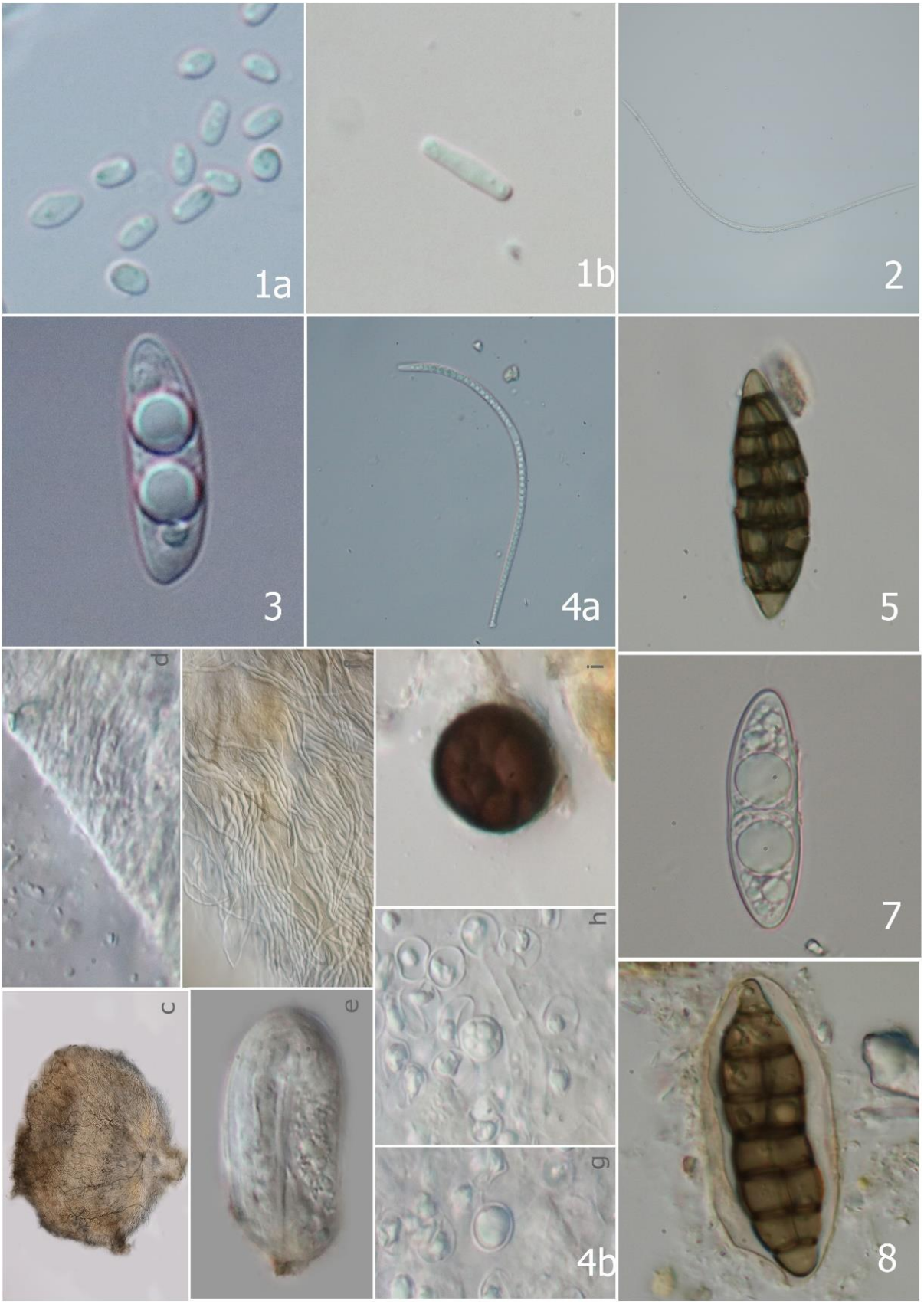


Figure 1 – 1–8 Spores of marine fungi from submerged wood in mangrove forest.

Figure 2 – 10–31 Spores of marine fungi from submerged wood in mangrove forest.

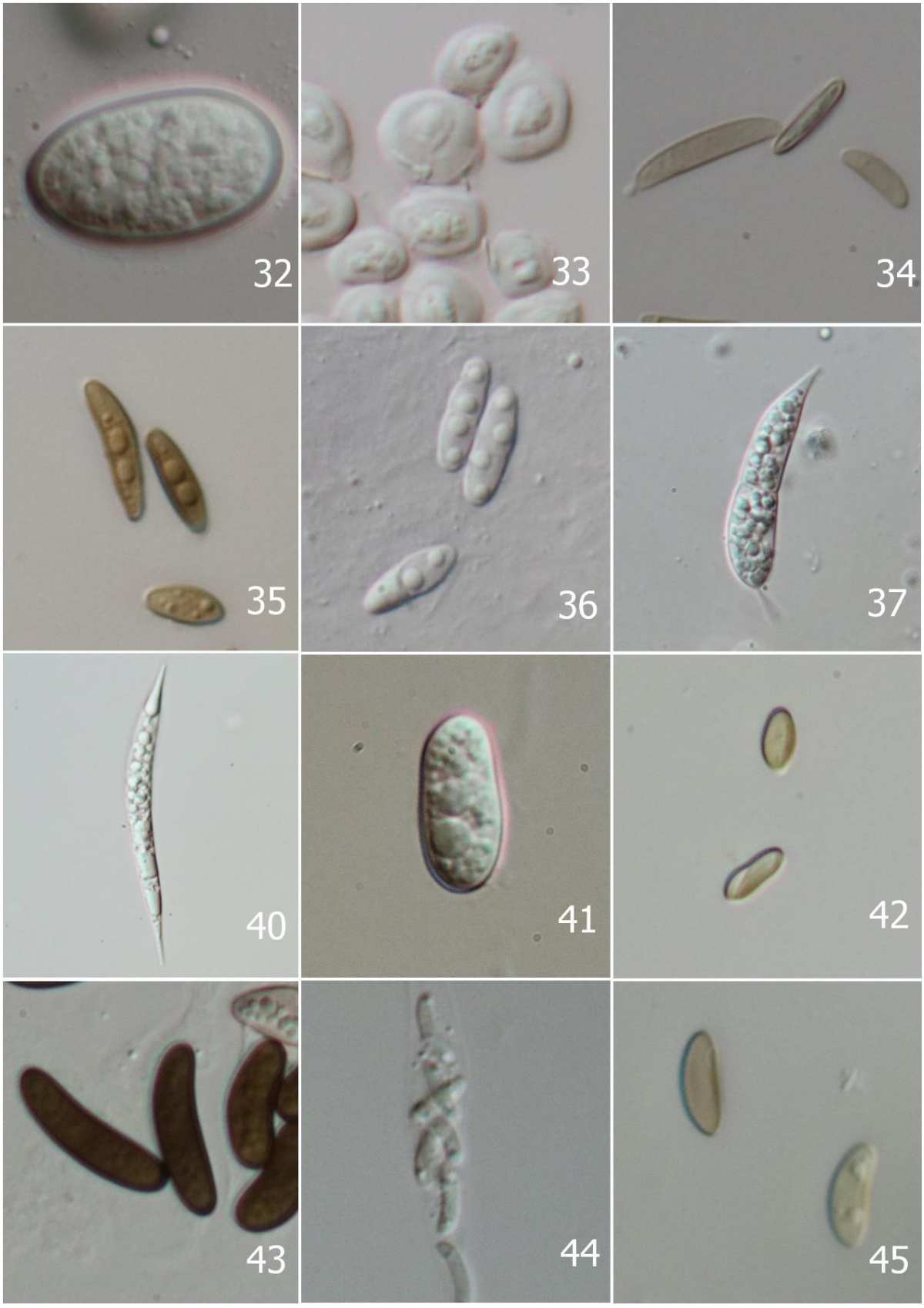


Figure 3 – 32–45 Spores of marine fungi from submerged wood in mangrove forest.

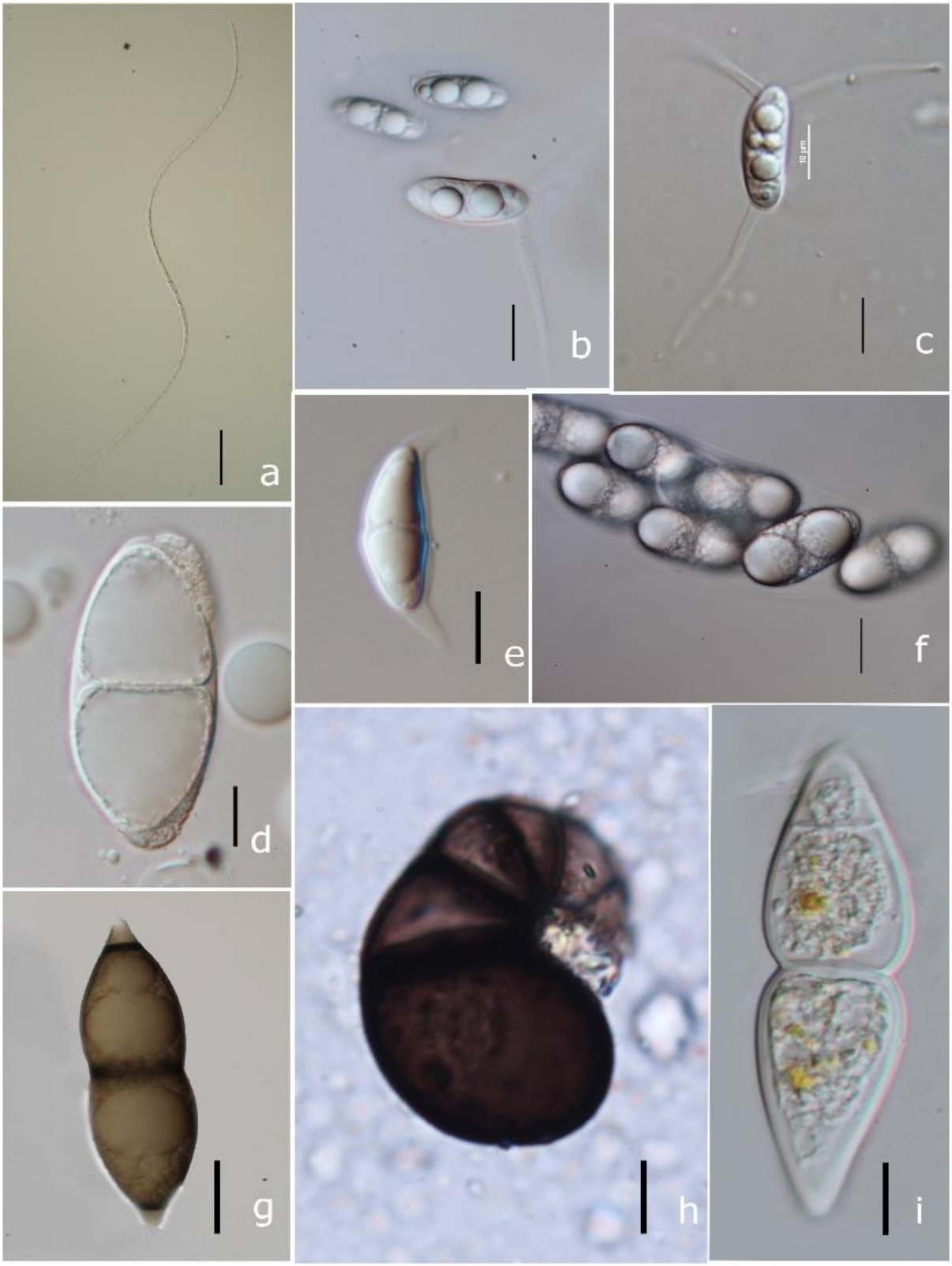


Figure 4 – Spores and ascus of some common micro-fungi found in marine habitat. a) Lulworthia sp. b,c) Halosphaeriaceae sp. d) Saagaromyces sp. e) Vaginatispora sp. f) Halosphaeriaceae sp. g) Savoryella sp. h) Hydea sp. i) unverified fungal spore from Pleosporales. Scale bars: a,b,c,f) 10 µm d,e,g,h,i) 5 µm.

We carried out phylogenetic analysis for new taxa and some of them have been written into papers (see from no 3). The Figures 5,6,7,8 are associate with *Rhizophora apiculata*, and Figures 9, 10, 11, 12, 13, 14 are the fungi associate with *Nypa fruticans*.

Acuminatispora S.N. Zhang, K.D. Hyde & J.K. Liu **gen. nov.**

Etymology: Name refers to the ascospores with acute or narrowly pointed ends.

Saprobic in mangrove habitats. **Sexual morph**: *Ascomata* black, subglobose, solitary, scattered, immersed, with an erupment short neck. Ostiole central, periphysate, cylinder-like opening. *Peridium* composed several brown outside layers and inter layers with hyaline cells of textura angularis. *Hamathecium* comprising numerous, filamentous, hyaline, branched, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 8-spored, cylindrical, slightly curved, with overlapping biseriate to triseriate ascospores, long pedicellate, apically rounded with an ocular chamber. *Ascospores* hyaline to brown, fusiform with acute or narrowly pointed ends, 1-(rarely 3) septate, constricted at the central septa, the upper cell broader, each cell with one large guttule and sometimes several small ones, smooth-walled. **Asexual morph**: Undetermined.

Type species: *Acuminatispora palmarum* S.N. Zhang, K.D. Hyde & J.K. Liu

Phylogenetic analyses

The BLAST search of LSU gene in NCBI showed that *Acuminatispora palmarum* is related to taxa in Pleosporales, and the closer species are regarding to the family *Testudinaceae*, as well as a putatively named strain of *Pseudotrichia guatopoensis*. To clarify the relationship of the newly described species within Pleosporales, we have carried out phylogenetic analysis with most of the families (Liu et al. 2017) in Pleosporales and some taxa Pleosporales family *Incertae sedis* which are referred to Hashimoto et al. (2017), which included most of taxa with molecular data available to represent families of the order. We conducted phylogenetic analyses using gene regions of LSU, SSU, TEF and RPB2 individually (not shown) and combined of LSU (1–902 bp), SSU (903–2129 bp), TEF (2130–3032 bp) and RPB2 (3033–3980 bp) (Figure 5) sequences data. The final dataset consisted of 126 taxa with total 3980 characters after the alignment, of which 2462 characters were constant and 1239 characters were parsimony informative, 279 variable characters were parsimony uninformative. After a heuristic search using PAUP, 575 equally most parsimonious trees were obtained (tree length = 9053 steps, CI = 0.277, RI = 0.614, RC = 0.170, HI = 0.723).

The best-sorting RAxML tree (Figure 5) showed that the two suborders Massarineae and Pleosporineae are well-supported, and have the similar topology in the family assignment to previous studies (Hyde et al. 2013, Ariyawansa et al. 2015, Liu et al. 2017). The new collections of *Acuminatispora palmarum* is close to Astrosphaeriellaceae Phook. & K.D. Hyde 2015 and Caryosporaceae Huang Zhang, K.D. Hyde & Ariyaw. 2015, while the genus *Acrocordiopsis* Borse & K.D. Hyde 1989 which was assigned into Caryosporaceae formed a distinct clade from the type genus *Caryospora* and showed closely phylogenetic relationship with Astrosphaeriellaceae and *Astrosphaeriellopsis*.

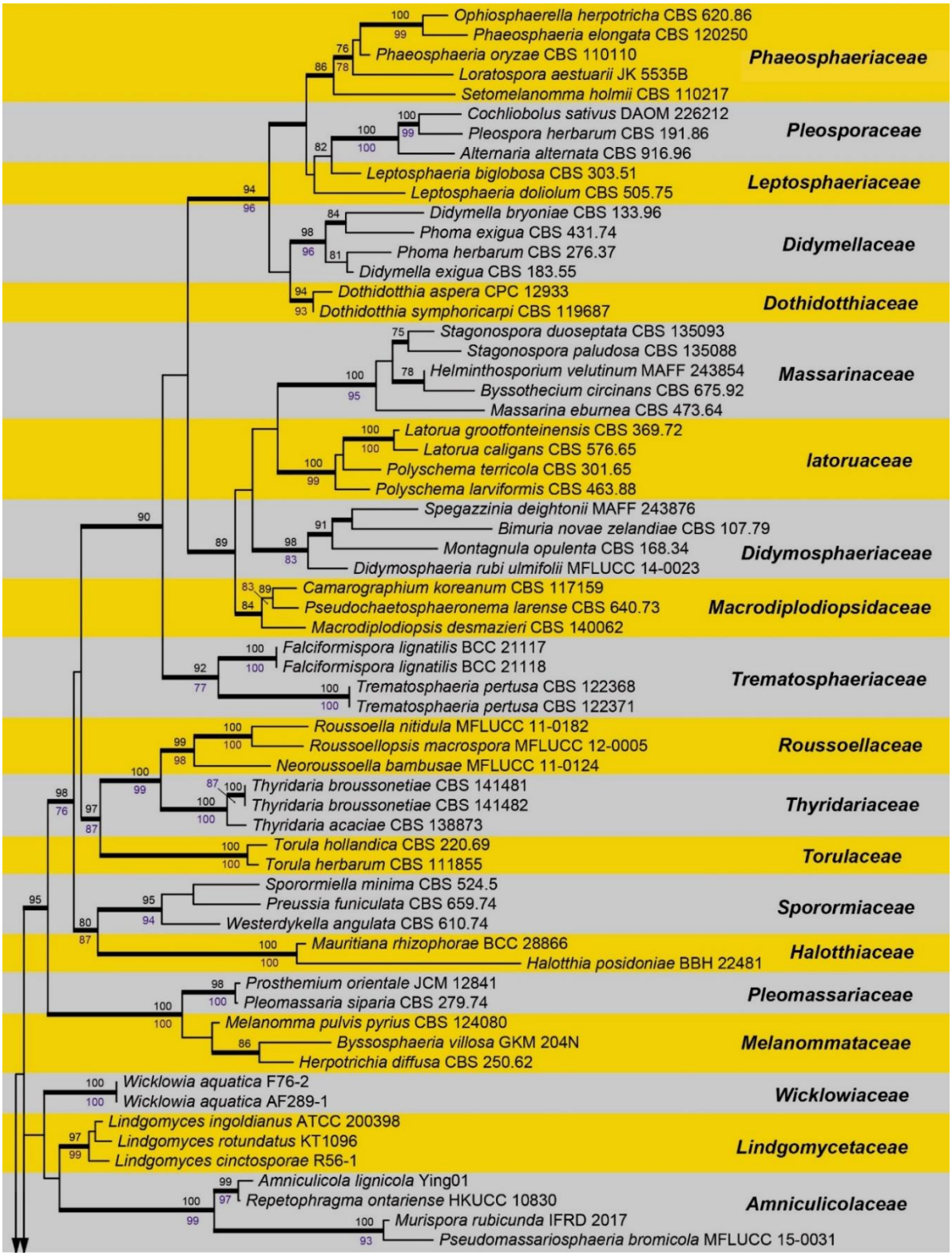


Figure 5 – RAxML tree of Pleosporales based on analysis of combined LSU, SSU, TEF1 and RPB2 sequences data. Bootstrap values for ML and MP equal to or greater than 75 are placed above and below the branches respectively. Branches with Bayesian posterior probabilities (PP) from MCMC analysis equal or greater than 0.95 are in bold. Newly generated sequences are indicated in blue. The tree is rooted with *Mytilinidion mytilinellum* and *Mytilinidion andinense*.

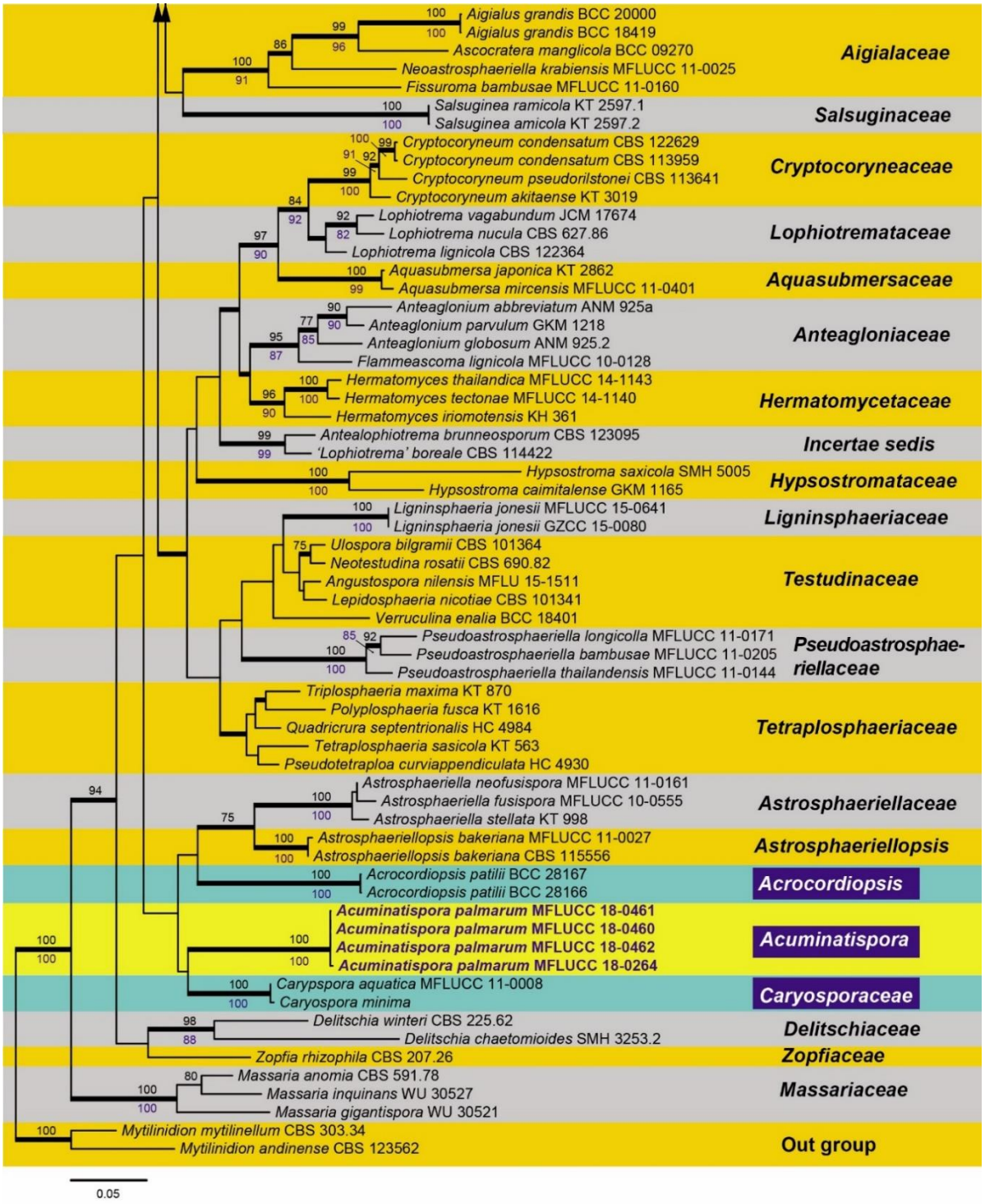


Figure 5 – Continued

Acuminatispora palmarum S.N. Zhang, K.D. Hyde & J.K. Liu **sp. nov.**

Etymology: The epithet refereeing to the host on which the fungus was collected.

Saprobic on mangrove palms. **Sexual morph**: *Ascomata* 430–580 µm high (including neck), 360–430 µm diam. ($\bar{x}$ = 315.8 × 387.6 µm, n = 5), black, subglobose, solitary, scattered, immersed, with an erumpent short neck. Ostiole 72–85 µm diam., central, periphysate,

cylinder-like opening. *Peridium* 10–20 µm wide, composed several brown outside layers and inter layers with hyaline cells of textura angularis. *Hamathecium* up to 2.5 µm wide, comprising numerous, filamentous, hyaline, branched, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 93–125 × 13–22 µm, ($\bar{x}$ = 109.5 × 16 µm, n = 15), 8-spored, cylindrical, slightly curved, with overlapping biseriate to triseriate ascospores, long pedicellate, apically rounded with an ocular chamber. *Ascospores* 24–30 × 7–10 µm ($\bar{x}$ = 27.2 × 8.1 µm, n = 30), hyaline to brown, fusiform with acute or narrowly pointed ends, 1-(rarely 3) septate, strongly constricted at the central septa, the upper cell broader, each cell with one large guttule and sometimes several small ones, smooth-walled. **Asexual morph**: Undetermined.

Culture characteristics: Ascospores germinating on PDA within 24 hours at room temperature in natural light. Germ tubes produced from each end. Colonies growing well on both PDA and MEA media and attaining a diameter of 1.5 cm on PDA after 21 days at room temperature, slow growing, obverse olive to grey-green or light grey-green, tufted colony center elevated, reverse dark green. Mycelium 2.5–3.5 µm wide, hyaline to pale brown, aerial, septate, ornamented, branched and anastomosing, producing Chlamydospores.

Material examined: THAILAND, Ranong, Ranong Mangrove Forest Research Center, on decayed rachis of *Phoenix paludosa* (Arecaceae Roxb.), immersed mangrove mud and water, 6 December 2016, S.N. Zhang SNT53 (MFLU, holotype, HKAS, isotype), ex-type living culture MFLUCC 18-0264 = TBRC; Ibid., Koh Chang, on decayed rachis of *Phoenix paludosa* immersed mangrove mud, 27 April 2017, S.N. Zhang SNT107 (MFLU, paratype), living culture MFLUCC 18-0460; Ibid., Koh Chang, on decayed petiole of *Nypa fruticans* (Arecaceae Wurmb), immersed mangrove mud, 27 April 2017, S.N. Zhang SNT111 (MFLU, paratype), living culture MFLUCC 18-0461; Ibid., Chanthaburi, on decayed petiole of *Phoenix paludosa*, immersed mangrove mud, 25 April 2017, S.N. Zhang SNT133 (MFLU, paratype), living culture MFLUCC 18-0462.

Notes: This monotypic genus *Acuminatispora* is characterized by having immersed ascomata with an erumpent short neck, and a central, periphysate, cylinder-like opening ostiole, cellular pseudoparaphyses, cylindrical, slightly curved, long pedicellate asci, and brown, fusiform ascospores with acute or narrowly pointed ends, 1-(rarely 3) septate, strongly constricted at the central septa.

Acuminatispora palmarum shares similar morphological characters to *Coronopapilla mangrovei* (K.D. Hyde) Kohlm. & Volkm.-Kohlm. 1991 (≡ *Caryospora mangrovei* K.D. Hyde 1989), which was considered to be conspecific of *Coronopapilla avellina* Kohlm. & Volkm.-Kohlm.1990, and having almost total immersed ascomata merges into clypeus around the ostiole and brown, 1-3-septate ascospores with median septum constricted. However, the relatively thinner peridium, cellular pseudoparaphyses and fusiform ascospores with acute or narrowly pointed ends of *Acu. palmarum* are distinct from *Cor. mangrovei* and *Cor. avellina* . The genus *Coronopapilla* was placed in the family Zopfiaceae G. Arnaud ex D. Hawksw. 1992 (Lumbsch & Huhndorf 2007, 2010, Wijayawardene et al. 2018) based on the morphology and there is no phylogenetic evidence to support this treatment. While based on such morphology distinction, in this study we introduce a new genus *Acuminatispora* to accommodate the new fungus *Acuminatispora palmarum*.

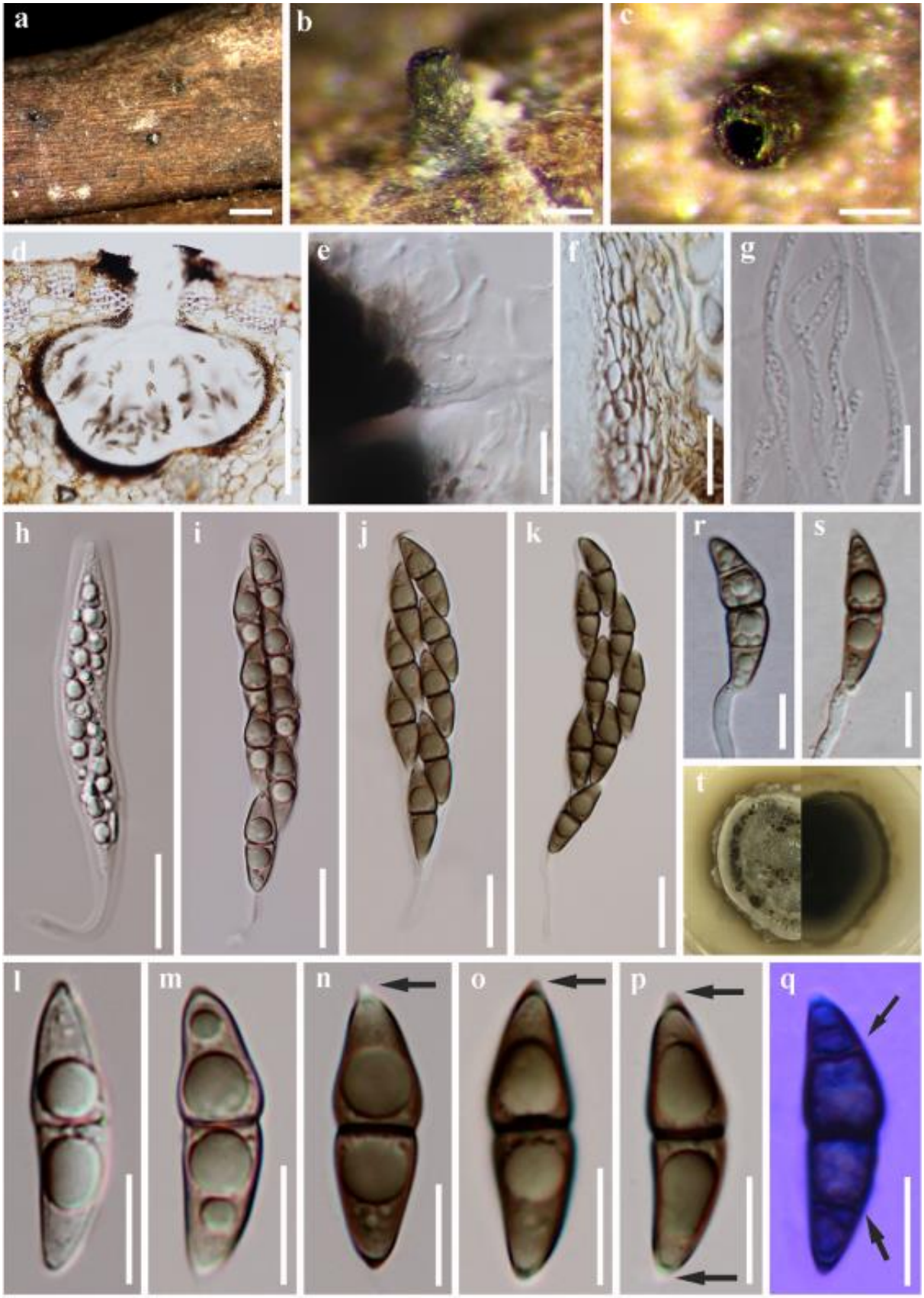


Figure 6 – *Acuminatispora palmarum* (MFLU, holotype). a-c Appearance of ascomata on host surface. d Vertical section of ascomata. e Ostiolar with periphyses. f Structure of peridium. g

Cellular pseudoparaphyses. h-k Asci. l-q Ascospores. q, 3-septate ascospore in lactophenol cotton blue reagent with acute ends clearly. s-r Germinating ascospores. t Colony on PDA. Sacle bars: a = 500 µm, b-c = 100 µm, d = 200 µm, f, h-k = 20 µm, e, g, l-s = 10 µm.

Akanthomyces Lebert, Z. Wiss. Zool. 9: 449 (1858)

Index Fungorum Fnumber: IF7083

Type species: *Akanthomyces aculeatus* Lebert, Z. Wiss. Zool. 9: 449 (1858)

Akanthomyces muscarius (Petch 1932), Spatafora, Kepler & B. Shrestha (2017)

Index Fungorum Fnumber: IF484535, Facesoffungi number: FoF04377

Synonyms, see (Zare & Gams 2001).

Endophyte on leaves of *Nypa fruticans* Wurmb. Asexual morph: Colonies reaching 2–2.5 cm diam. after 10 days at 26°C on PDA, circular, compact, with reverse cream to pale yellow (rarely yellow). Mycelium composed of septate, branched, hyaline, smooth hyphae. Conidiophores macronematous, mononematous, erect, flexuous, septate, branched, smooth, 13.6–28.4 x 1.8–2.5µm ($\overline{x}$ = 22.6 × 2.3 µm, n = 9). Conidiogenous cells 10–28 × 1.5–2.8 µm ($\overline{x}$ = 15 × 1.6 µm, n = 11), phialidic, with a minute collarette, develop at the tip of prostrate or on secondary branches of conidiophores, singly or in verticels, swollen at the base, partly-tapered toward the tip, smooth. Conidia solitary 2.4–7.2 × 1.7–2.6 µm ($\overline{x}$ = 4.5 × 1.8 µm, n = 25), slimy, ellipsoid, subcylindrical to cylindrical with attenuate base, 1-celled, variable in size, shape, smooth hyaline to subhyaline. Sexual morph: Undetermined (Zare & Gams 2001).

Culture characteristics: Endophytic, isolations were done, by transferring growing hyphae on a PDA plate. Colony on PDA, above white, fluffy and regular, bottom yellowish, hyphae, septate, branched, and smooth, after 10 days.

Material examined: THAILAND, Krabi Province, isolated from leaves of Nypa fruticans (Arecaceae), 11 Sept. 2017, Vinit K., E104 (MFLU xxxx, culture: MFLUCC 17-2540).

Notes: *Akanthomyces muscarius* is cosmopolitan with a wide range of hosts and has been synonymized several times (See Zare & Gams 2001). Phylogenetically this species clearly differs from all other species used in this analysis such as *A. lecanii*, *A. tuberculatus*, *L. antillanum*, *L. aphanocladii*, *L. aranearum*, *A. attenuates*. Our isolate is very similar to *A. muscarius*, and hence we have added it as a new host record. The basic morphological comparison between similar taxa and their distribution are listed. Conidiogenous cells, phialides and conidia were selected morphs used to compare among mentioned taxa (Zare & Gams 2001, 2008).

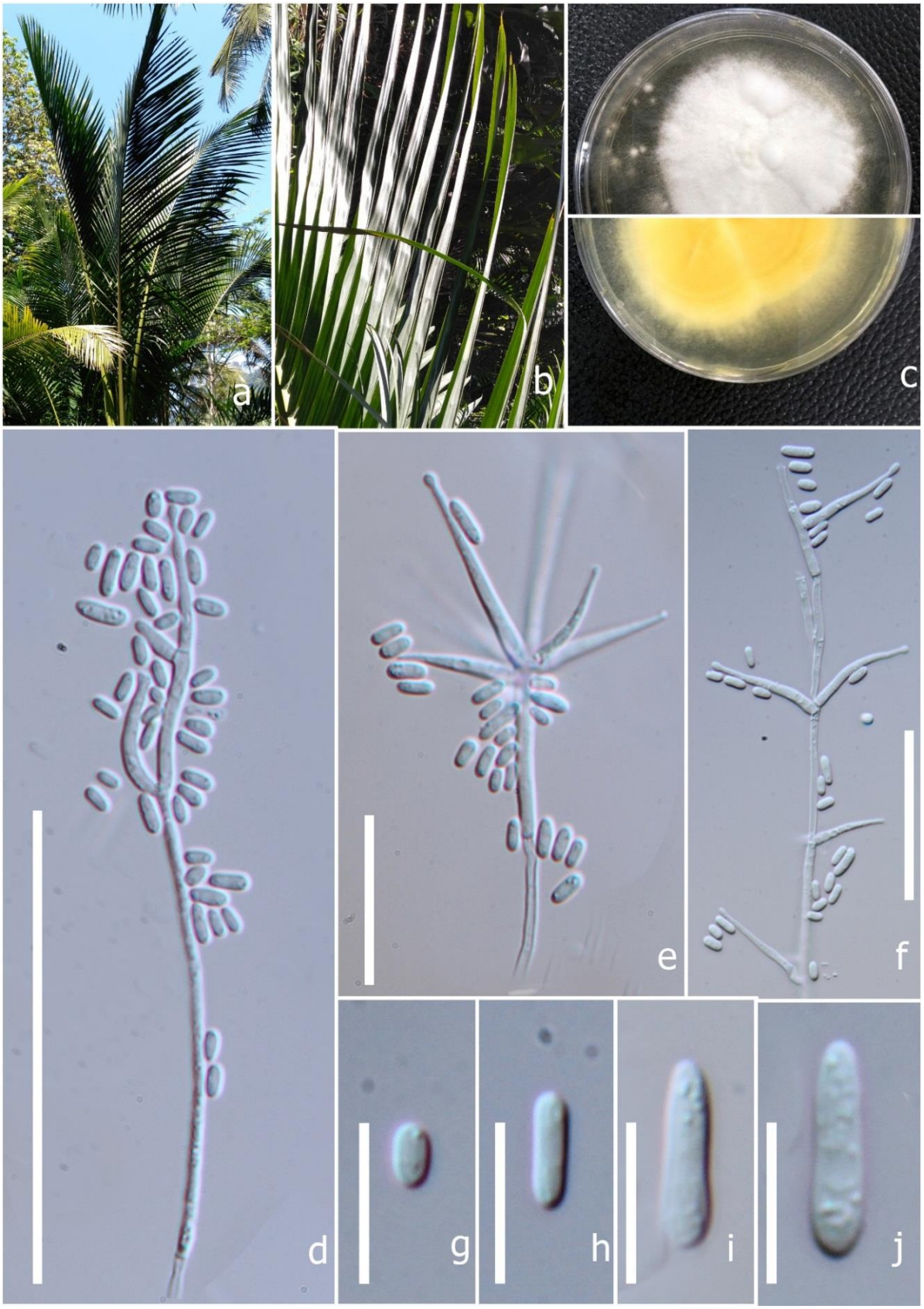


Figure 7 – *Akanthomyces muscarius* (MFLU-CC 17-2540). a,b Fresh leaf of Nypa fruticans., c Culture plate d Conidiogenesis, e, f Conidiophore, Conidiogenous cells and phialides. g – j Conidia. Scale bars = d = 50 µm, e,f = 20 µm, g,h = 5 µm.

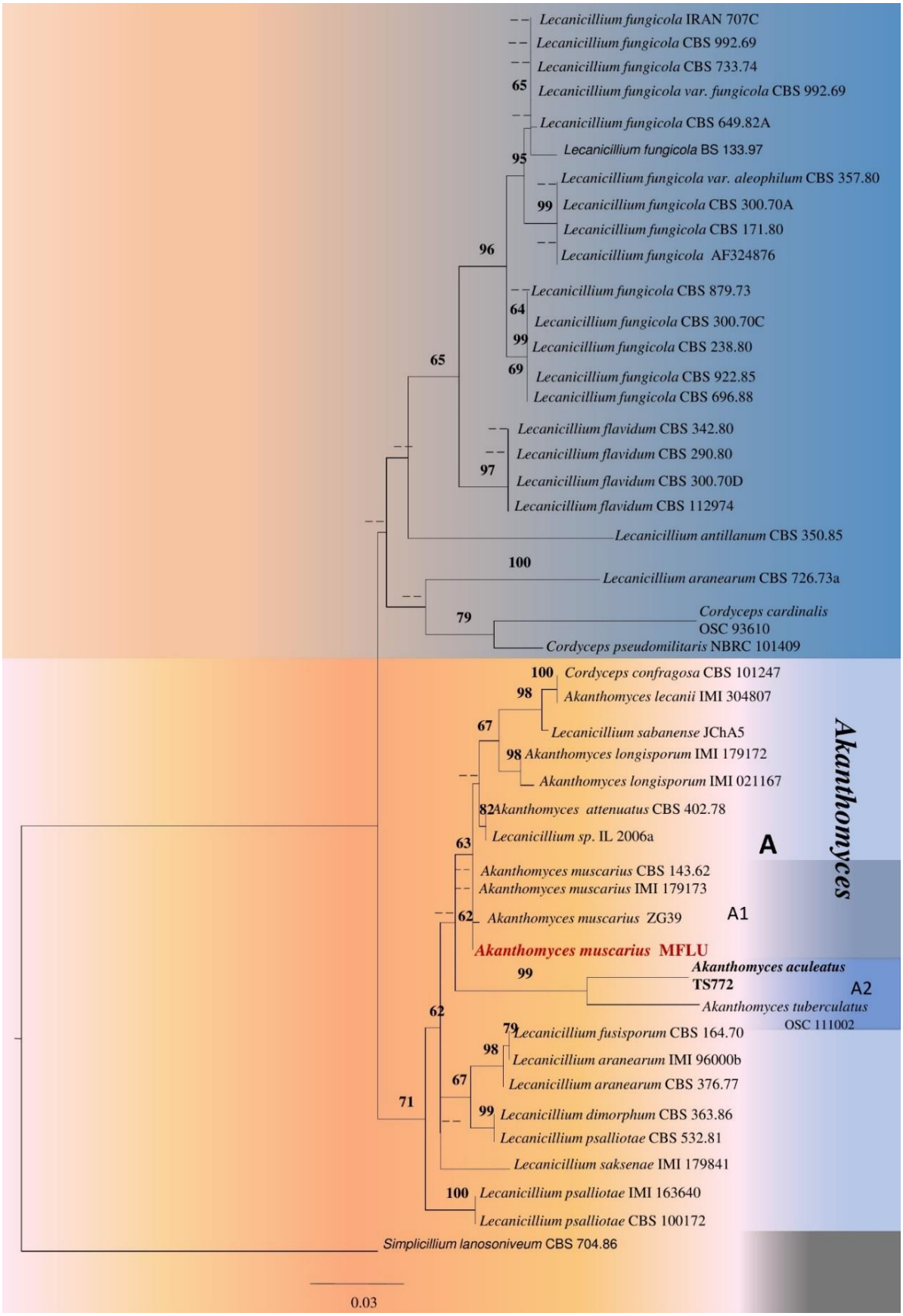


Figure 8 – Maximum likelihood majority rule consensus tree for the isolates based on ITS sequence data. Bootstrap support values for maximum likelihood (ML) is greater than 50% are

indicated above the nodes as MLBS. The scale bar represents the expected number of changes per site. The tree is rooted with *Simplicillium lanosoniveum* CBS704.86. The strain numbers are noted after the species names. The new strain is in red bold.

Cytospora Ehrenb., Sylv. mycol. berol. (Berlin): 28 (1818)

Index Fungorum Number: IF7904

Type species: *Cytospora chrysosperma* (Pers.) Fr., Syst. mycol. (Lundae) 2(2): 542 (1823)

Phylogenetic analysis of combined ITS, LSU, ACT and RPB2 sequences

The combined alignment of ITS, LSU, ACT, and RPB2 sequences comprised 86 taxa, including our strains, with *Diaporthe eres* (CBS 183.5) as the outgroup taxon. The total length of the dataset was 2037 characters including alignment gaps (1–199, 200–357, 358–518, 519–1056, 1057–1296 and 1297–2037 corresponding to ITS1, 5.8S, ITS2, LSU, ACT and RPB2, respectively). The combined dataset contained 1426 constant, 144 parsimony uninformative and 467 parsimony informative characters. The result from the partition homogeneity test (PHT) was not significant (level 95%), indicating that the individual datasets were congruent and could be combined. The combined dataset was analyzed using MP, ML and Bayesian analyses. The trees generated under different optimality criteria were essentially similar in topology and did not differ significantly (data not shown). The descriptive statistics of the phylogram generated from MP analysis based on the combined dataset of ITS, LSU, ACT and RPB2 were TL = 2418, CI = 0.375, RI = 0.650, RC = 0.244, HI = 0.625. The best scoring likelihood tree selected with a final value for the combined dataset = -14466.797686. The aligned sequence matrix of the ITS1+ITS2 dataset comprising 19 taxa had 279 constant, 23 parsimony uninformative and 57 parsimony informative characters. The descriptive statistics of the most parsimonious tree were TL = 2418, CI = 0.375, RI = 0.650, RC = 0.244, HI = 0.625. The best scoring likelihood tree obtained for the ITS1+ITS2 dataset had a log-likelihood of= -1276.782916.

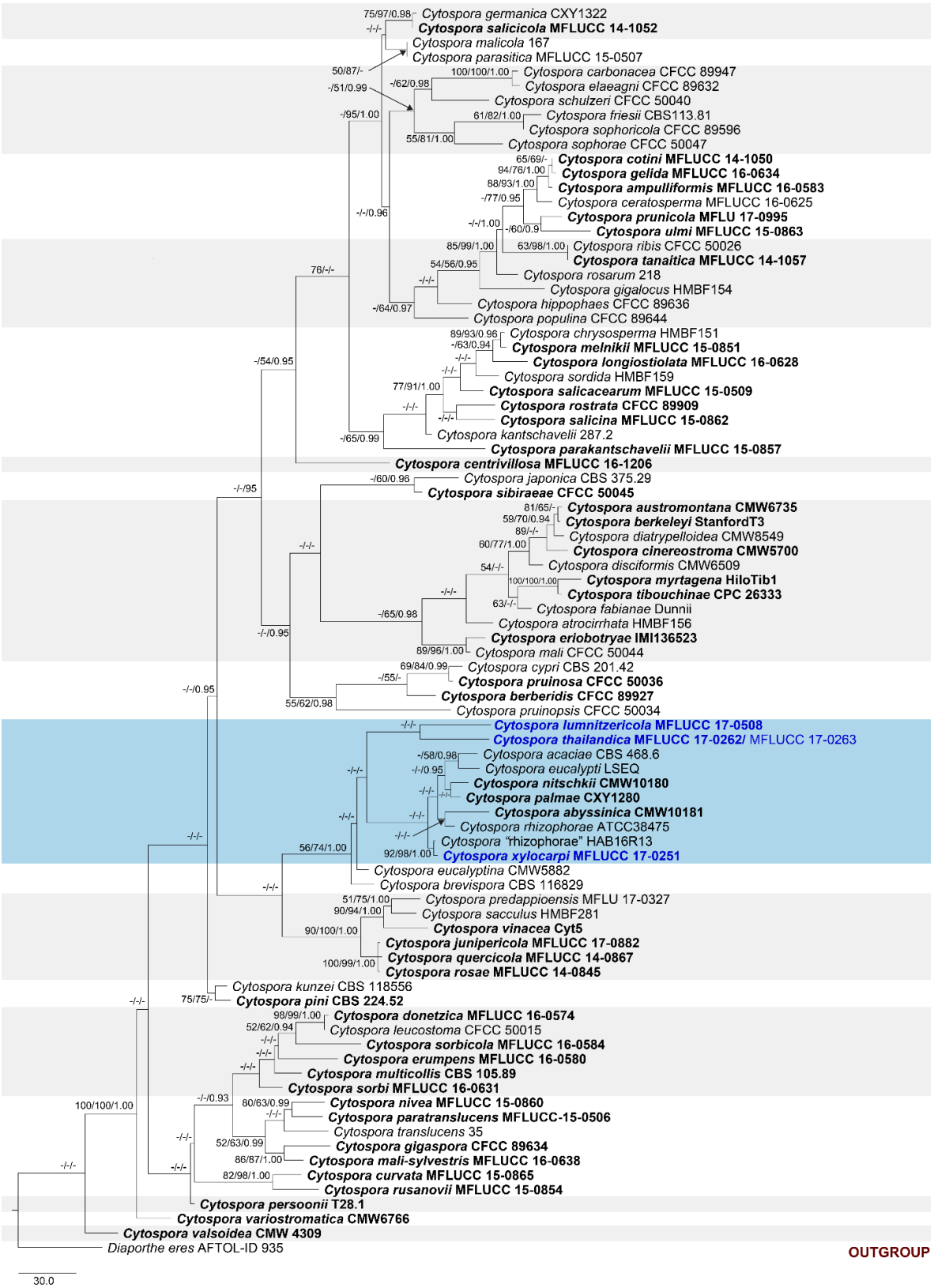


Figure 9 – Phylogram generated from maximum parsimony analysis based on analysis of a combined ITS, LSU, ACT and RPB2 sequence data. The tree is rooted to *Diaporthe eres* (AFTOL-ID 935). Maximum parsimony and maximum likelihood bootstrap values ≥50%, Bayesian posterior probabilities ≥0.90 (MPBS/MLBS/PP) are given at the nodes. The species obtained in this study are in blue font. Ex-type taxa from other studies are in black bold.

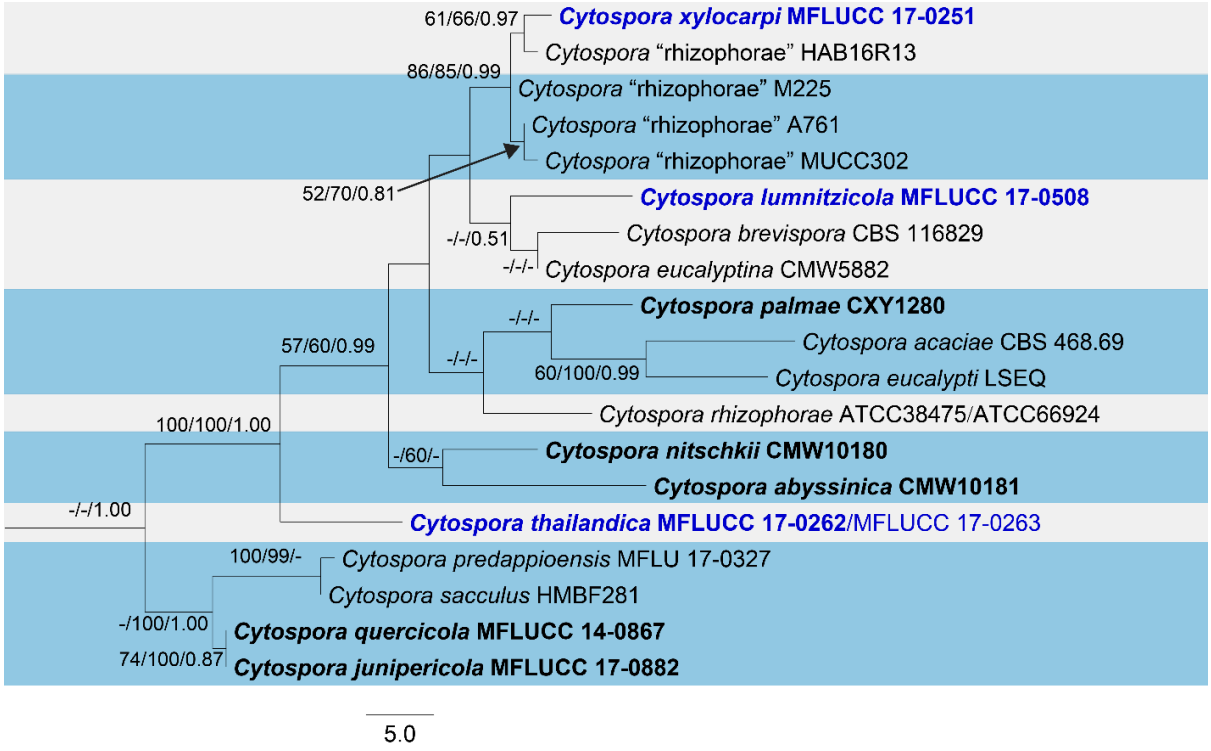


Figure 10 – Maximum parsimony phylogenetic tree inferred from ITS1 and ITS2 sequence data. Maximum parsimony and maximum likelihood bootstrap values ≥50%, Bayesian posterior probabilities ≥0.90 (MPBS/MLBS/BIPP) are given at the nodes. The species obtained in this study are in blue font. Ex-type taxa from other studies are in black bold.

Taxonomy

Cytospora lumnitzericola Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF554778; Facesoffungi number: FoF 04603 Fig. 11

Etymology: refers to the host where the fungus was isolated.

Holotype: MFLU 18-1227

Isolated from *leaf spot* of *Lumnitzera racemosa*. Culture characteristic: Colonies on MEA reaching 5–6 cm diameter after 2 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairy fluffy, white to gray from above, light yellow to green from below; not producing pigments in agar. **Asexual morph:** *Conidiogenous cells* (8–)8.5–14 × 0.6–1.4(–1.6) µm ($\bar{x}$ = 8.4 × 1.4, n = 15), blastic, enteroblastic, flask-shaped, phialidic, hyaline, and smooth-walled. *Conidia* (3.7–)4–4.5 × 1–1.3(–1.5) µm ($\bar{x}$ = 4 × 1.2 µm, n = 30), unicellular, subcylindrical, hyaline, smooth-walled.

Material examined: THAILAND, Phetchaburi, the Sirindhorn International Environmental Park, on leaf spot of *Lumnitzera racemosa*, 30 November 2016, Norphanphoun Chada NNS23-2a (MFLU 18-1227 dried culture, **holotype**; PDD, isotype); ex-type-living culture, MFLUCC 17-0508.

Notes: *Cytospora lumnitzericola* is phylogenetically based on combine gene region closely related to *Cytospora thailandica*. Although conidial sizes of both species are similar but differ among nucleotide positions: ITS (26 nt), ACT (22 nt), and RPB2 (53 nt). The

phylogenetic tree of *C. lumnitzericola* independent lineage is close to *C. brevispora* CBS 116829 and *C. eucalyptina* CMW5882. In future more collections are needed to confirm whether life cycle of *C. lumnitzericola* is saprobe, endophyte or pathogen.

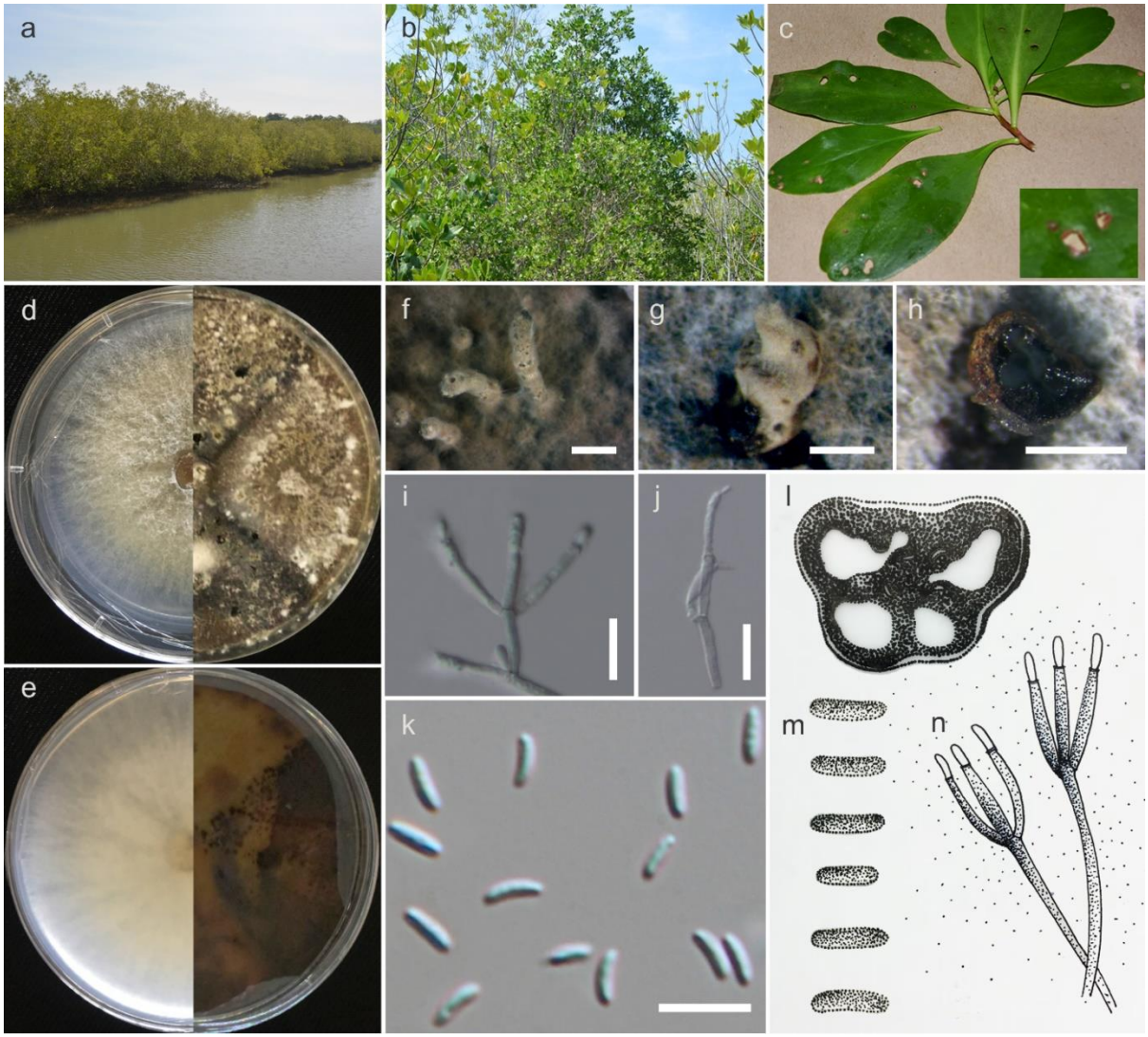


Figure 11 – *Cytospora lumnitzericola* (MFLUCC 17-0508, from culture). a Mangrove collecting site b, c *Lumnitzera racemosa* in mangroves forest d, e Colonies on MEA after 6 days (left) and 30 days (right) (d-from above, e-from below) f, g Conidiomata produced on MEA h, l Transverse sections of conidioma i, j, n Conidiogenous cells with attached conidia k, m Conidia. Scale bars: f = 1000 µm, g, h = 500 µm, i, j = 10 µm, k = 5 µm.

Cytospora thailandica Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF554779; Facesoffungi number: FoF 04605 Fig. 12

Etymology: refers to the country where the fungus was collected.

Holotype: MFLU 17-0709

Associated with twigs and branches of *Xylocarpus moluccensis*. **Sexual morph:** Stromata immersed in bark. *Ascostromata* 400–1000 × 70–250 µm diameter, semi-immersed in host tissue, scattered, erumpent, uni- or multi-loculate, with ostiolar neck. *Ostiole* 70–150 µm diameter, numerous, dark brown to black, at the same level as the disc, occasionally area below disc a lighter entostroma. *Peridium* comprising several layers of cell of *textura angularis*, with inner most layer thick, brown, outer layer dark brown. *Hamathecium* comprising long cylindrical, cellular, anastomosed paraphyses. *Asci* (21–)23–25 × 4.1–4.7(–5) µm ($\bar{x}$ = 22 × 4.3 µm, n = 15), 6–8-spored, unitunicate, clavate to elongate obovoid, with a J-, refractive apical ring. *Ascospores* (5.6–)6–6.8 × 1.3–1.5(–2) µm ($\bar{x}$ = 6.6 × 1.5 µm, n = 20), biseriate, elongate-allantoid, unicellular, hyaline, smooth-walled. **Asexual morph:** *Conidiomata* 400–1200 × 180–380 µm diameter, semi-immersed in host tissue, solitary, erumpent, scattered, discoid, circular to ovoid, with multi-loculate, pycnidial, embedded in stromatic tissue, with ostiole. *Ostioles* 230–300 µm long, with an ostiolar neck. *Peridium* comprising few layers of cells of *textura angularis*, with inner most layer thin, pale brown, outer layer brown to dark brown. *Conidiophores* unbranched or occasionally branched at the bases, formed from the inner most layer of pycnidial wall, with conidiogenous cells. *Conidiogenous cells* (3.3–)6–9.1 × 1–1.3(–1.7) µm ($\bar{x}$ = 6 × 1.3 µm, n = 15), blastic, enteroblastic, flask-shaped, phialidic, hyaline, and smooth-walled. *Conidia* (3.3–)3.8–4 × 1–1.3(–1.5) µm ($\bar{x}$ = 3.8 × 1.3 µm, n = 30), unicellular, subcylindrical, hyaline, smooth-walled.

Material examined: THAILAND, Ranong Province, Ngao Mangrove Forest, on branches of *Xylocarpus moluccensis*, 6 December 2016, Norphanphoun Chada NG02a (MFLU 17-0709, **holotype**; PDD, isotype); ex-type-living cultures, MFLUCC 17-0262, MFLUCC 17-0263, ICMP.

Notes: *Cytospora thailandica* was collected from branches of *Xylocarpus moluccensis*. The new species resembles some other to *Cytospora* species, but is characterized by uni- or multi-loculate ascomata/conidiomata with unicellular, subcylindrical, and hyaline spores in both morphs. *Cytospora* species associated with *Xylocarpus granatum* is also reported in this study as *C. xylocarpi* (MFLUCC 17-0251). *Cytospora xylocarpi* is similar to *C. thailandica* in its conidiomata being multi-loculate and in the length of conidia in the asexual morph (*C. xylocarpi*: conidia 3 × 1.1 µm versus 3.8 × 1.3 µm in *C. thailandica*). However, *C. thailandica* differs from *C. xylocarpi* in having shorter ostiolar necks, and larger asci and ascospores. Phylogenetic analysis of our combined gene also reveals *C. thailandica* is closely related to *C. lumnitzericola*, which the nucleotide differences were mentioned in notes of *C. lumnitzericola*. The individual ITS1+ITS2 phylogenetic tree, *C. thailandica* is formed separately from other species with good support (Fig. 10).

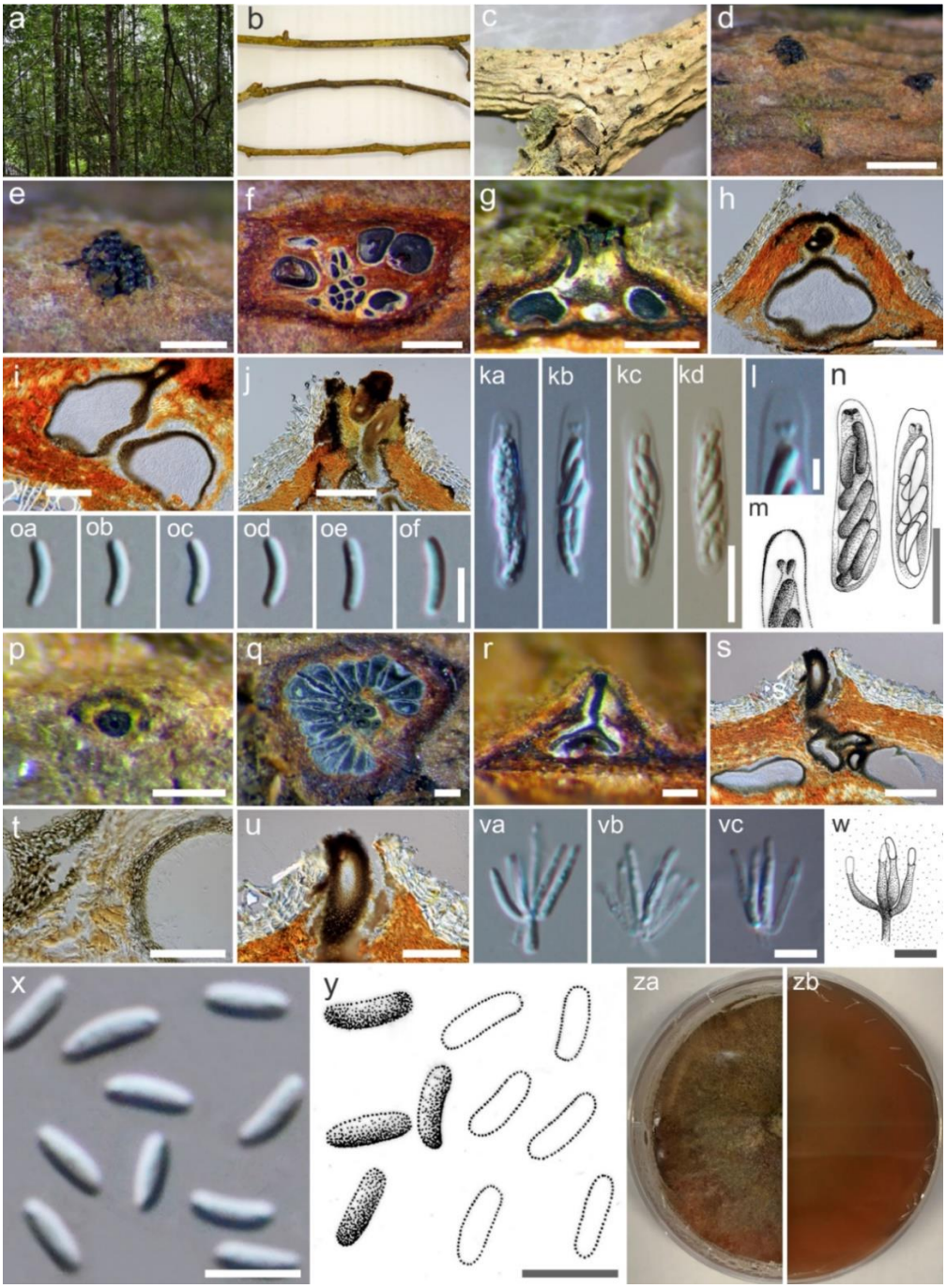


Figure 12 – *Cytospora thailandica* (MFLU 17-0709, holotype). a *Xylocarpus moluccensis* b Branch of *Xylocarpus moluccensis* c Ascostromata on host substrate d, e Surface of ascomata f Transverse sections through ascostroma to show distribution of locules g–h Longitudinal sections through ascostroma to show distribution of locules i Peridium j Ostiolar neck ka–kd, n Asci l, m Apical ring oa–of Ascospores p Surface of conidioma q Transverse sections through conidioma to show distribution of locules r, s Longitudinal sections through conidioma to show distribution of locules t Peridium u Ostiolar neck va–vc, w Conidiogenous cells with attached conidia x, y Conidia za, zb Colonies on MEA (za-from above, zb-from below). Scale bars: d = 1000 µm, e–g = 400 µm, h, j, p–s = 200 µm, i, u = 100 µm, ka–kd, n = 10 µm, l, m = 2 µm, oa–of, va–vc, w = 5 µm, t = 50 µm, x, y = 4 µm.

Cytospora xylocarpi Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF554810; Facesoffungi number: FoF 04604 Fig. 13

Etymology: refers to the host genus that fungus was collected.

Holotype: MFLU 17-0708

Associated with *Xylocarpus granatum* branches. **Sexual morph:** Stromata immersed in bark. *Ascostromata* 230–600 × 90–250 µm diameter, semi-immersed in host tissue, scattered, erumpent, multi-loculate, with ostiolar neck. *Ostiole* 160–200 µm diameter, numerous, dark brown to black, at the same level as the disc, occasionally area surrounded with white hyphae. *Peridium* comprising several layers of cells of *textura angularis*, with inner most layer thick, pale brown, outer layer dark brown to black. *Hamathecium* comprising long cylindrical, cellular, anastomosed paraphyses. *Asci* (22–)24–28.8 × 3.6–4.8(–5.1) µm ($\bar{x}$ = 26 × 4 µm, n = 15), 6–8-spored, unitunicate, clavate to elongate obovoid, with a refractive,, J-, apical ring. *Ascospores* (5.5–)6–6.5 × 1.7–1.8(–2) µm ($\bar{x}$ = 5.7 × 1.8 µm, n = 20), biseriate, elongate-allantoid, unicellular hyaline, smooth-walled. **Asexual morph:** *Conidiomata* 700–1200 × 400–480 µm diameter, semi-immersed in host tissue, solitary, erumpent, scattered, multi-loculate, with ostiole. *Ostioles* 200–250 µm long, with 1–2 ostiolar necks. *Peridium* comprising several layers of cells of *textura angularis*, with inner most layer brown, outer layer dark brown to black. *Conidiophores* unbranched or occasionally branched at the bases, formed from the inner most layer of pycnidial wall, with conidiogenous cells. *Conidiogenous cells* (6.3–)7.9–10 × 0.9–1.4(–1.6) µm ($\bar{x}$ = 8.5× 1.4 µm, n = 15), blastic, enteroblastic, flask-shaped, phialidic, hyaline, and smooth-walled. *Conidia* (2.4–)3–3.1 × 0.8–1 (–1.2) µm ($\bar{x}$ = 3 × 1 µm, n = 30), unicellular, subcylindrical, hyaline, smooth-walled.

Material examined: THAILAND, Ranong Province, Ngao Mangrove Forest, on branches of *Xylocarpus granatum*, 6 December 2016, Norphanphoun Chada NG09b (MFLU 17-0708, **holotype**; PDD); ex-type-living cultures, MFLUCC 17-0251, ICMP

Notes: The asexual morph of *C. xylocarpi* studied here is most similar to *C. rhizophorae* from dead roots of *Rhizophora mangle* L. in Guatemala, in having multi-loculate conidiomata and allantoid, slightly curved, hyaline and 3–6 × 1.1–1.5 µm conidia (Kohlmeyer and Kohlmeyer 1971). However, in the phylogenetic tree, *C. xylocarpi* belonged to a clade distinct from *C. rhizophorae* (ATCC 38475), a strain from *Rhizophora mangle* that was identified by Kohlmeyer, the author of the species (Fig. 10). The two species differ by 25 substitutions in ITS1+ITS2 and were collected from different hosts. Therefore, the collection in the present study is designated as a new species.

Our phylogeny also indicates a close relationship to unpublished sequences from GenBank. The similarity in the ITS1 and ITS2 sequence between our strain and the sequences from GenBank (HAB16R13, M225, A761, MUCC302) are presented in Table 5. Those sequences were collected from difference hosts (Table 4), and together with our strain, show substantial variation and the positions of nucleotides differences in the ITS1 and ITS2 (Table 5). More collections are needed to further study morphological and genetic variation in this group and better delineate the species.

Figure 13 – *Cytospora xylocarpi* (MFLU 17-0708, holotype). a *Xylocarpus granatum*. b Branch of *Xylocarpus granatum* c Ascostromata on host substrate d Surface of ascomata e Transverse sections through ascostroma to show distribution of locules f, g Longitudinal sections through ascostroma to show distribution of locules h Peridium i–l, n Asci m, o Ascospores p Germinating spore q, r Colonies on MEA (q-from above, r-from below) s Transverse sections through conidioma to show distribution of locules t Longitudinal sections through conidioma to show distribution of locules u, v Conidiogenous cells with attached conidia w Mature conidia. Scale bars: c = 2000 µm, d–f = 500 µm, g = 200 µm, h = 20 µm, i, p = 10 µm, j–o, u–w = 5 µm, s, t = 400 µm.

Table 4. Synopsis of species of *Cytospora* discussed in the paper.

Taxon	Sexual morph				Asexual morph				References
	Ascostoma	**Ostiolar neck**	**Asci**	**Ascospores**	**Conidiomata**	**Ostiolar neck**	**Conidiogenous cell**	**Conidia**	
C. lumnitzericola	-	-	-	-	-	-	8.4 × 1.4	4 × 1.2	In this study
C. rhizophorae	-	-	-	-	370–500 × 100–310	30 × 10–25	13–20 × 1–1.8	3–6 × 1.1–1.5	Kohlm. and Kohlm. (1971)
C. thailandica	400–1000 × 70–250	70–150	22 × 4.3	6.6 × 1.5	400–1200 × 180–380	230–300	6 × 1.3	3.8 × 1.3	In this study
C. xylocarpi	230–600 × 90–250	160–200	26 × 4	5.7 × 1.8	700–1200 × 400–480	200–250	8.5× 1.4	3 × 1	In this study

Table 5. GenBank BLAST search from ITS1 and ITS2 of *Cytospora xylocarpi* (MFLUCC 17-0251) with sequence from GenBank identified as *Cytospora rhizophorae*.

Toxon	**Strain**	**Host**	**Country**	**Accessions**	**ITS1**	**ITS2**	**ITS1+ITS2**	**Identities (I), Query cover (QC)**	**References**
C. "rhizophorae"	HAB16R13	*Cinnamomum porrectum*	Malaysia	HQ336045	213/215	167/169	380/384	I=98.9%, QC=99%	Harun et al. (2011)
C. "rhizophorae"	M225	*Rhizophora mucronata*	Philippines	KR056292	213/217	167/169	380/386	I=98.4%, QC=100%	Unpublished
C. "rhizophorae"	A761	*Morinda officinalis*	China	KU529867	213/217	166/169	379/386	I=98.2%, QC=100%	Unpublished
C. "rhizophorae"	MUCC302	*Eucalyptus grandis*	Australia	EU301057	213/217	164/169	377/386	I=97.7%, QC=100%	Unpublished
C. rhizophorae	ATCC38475	*Rhizophora mangle*	LA, USA	DQ996040	187/202	156/166	343/368	I=93.2%, QC=100%	He et al. (2003)

Table 6. Nucleotide differences in the ITS1+ITS2 of *Cytospora xylocarpi* (MFLUCC 17-0251) with sequence from GenBank identified as *Cytospora rhizophorae*.

Taxon	Strain	**ITS1**																	
		14	16	18	30	92	93	96	99	102	103	104	105	113	115	118	119	135	154
C. xylocarpi	MFLUCC 17-0251	-	G	A	C	C	C	C	G	G	G	C	G	C	T	T	C	A	G
C. rhizophorae	ATCC38475	G	A	C	T	G	A	T	A	T	T	T	A	T	-	C	T	-	T
C. "rhizophorae"	HAB16R13	?	G	A	C	C	C	C	G	G	G	C	G	C	T	T	C	A	T
C. "rhizophorae"	M225	?	G	A	T	C	C	C	G	G	G	C	G	C	T	T	C	A	T
C. "rhizophorae"	A761	?	G	A	T	C	C	C	G	G	G	C	G	C	T	T	C	A	T
C. "rhizophorae"	MUCC302	?	G	A	T	C	C	C	G	G	G	C	G	C	-	T	C	A	T

Taxon	Strain	**ITS2**											
		13	24	40	46	47	50	51	75	111	112	115	123
C. xylocarpi	MFLUCC 17-0251	C	C	A	T	-	T	T	C	A	A	C	T
C. rhizophorae	ATCC38475	T	T	-	T	-	-	T	C	G	T	A	T
C. "rhizophorae"	HAB16R13	C	T	A	T	-	T	T	C	A	A	C	C
C. "rhizophorae"	M225	C	T	A	T	-	T	T	C	A	A	C	T
C. "rhizophorae"	A761	C	T	A	T	T	-	T	C	A	A	C	T
C. "rhizophorae"	MUCC302	C	T	A	-	-	-	-	T	A	A	C	T

Table 7. Nucleotides differences in the ITS, ACT and RPB2 sequences of *Cytospora lumnitzericola*, *C. thailandica*, and *C. xylocarpi*.

Taxon	Strain	**ITS**																	
		29	88	91	92	93	94	96	97	99	101	102	103	104	105	106	107	108	111
C. lumnitzericola	MFLUCC 17-0508	T	C	T	T	T	T	C	T	C	G	G	A	C	T	A	T	A	G
C. thailandica	MFLUCC 17-0262	T	-	T	-	-	-	T	C	T	C	A	G	-	-	A	C	G	C
C. thailandica	MFLUCC 17-0263	T	-	T	-	-	-	T	C	T	C	A	G	-	-	A	C	G	C
C. xylocarpi	MFLUCC 17-0251	C	C	C	-	-	C	C	C	C	G	G	G	-	-	G	C	G	G

Taxon	Strain																		
		119	120	121	122	123	124	125	134	157	389	396	404	405	412	413	414	415	420
C. lumnitzericola	MFLUCC 17-0508	T	T	C	-	-	-	-	-	T	T	A	A	-	-	-	-	T	G
C. thailandica	MFLUCC 17-0262	C	T	T	C	-	G	G	-	T	T	G	T	T	-	-	-	-	A
C. thailandica	MFLUCC 17-0263	C	T	T	C	-	G	G	-	T	T	G	T	T	-	-	-	-	A
C. xylocarpi	MFLUCC 17-0251	T	C	T	C	C	G	G	A	G	C	A	A	A	C	T	T	T	G

Taxon	Strain						**ACT**												
		439	468	485	487	488	74	78	80	92	95	96	97	107	122	125	129	136	137
C. lumnitzericola	MFLUCC 17-0508	T	T	C	T	A	G	C	A	T	T	-	-	C	T	A	G	A	A
C. thailandica	MFLUCC 17-0262	T	T	T	C	T	T	G	A	A	T	-	-	T	C	T	G	A	G
C. thailandica	MFLUCC 17-0263	T	T	T	C	T	T	G	A	A	T	-	-	T	C	T	G	A	G
C. xylocarpi	MFLUCC 17-0251	C	C	C	T	T	G	C	T	A	C	C	C	T	C	A	A	G	A

Taxon	Strain																		
		139	146	147	148	149	150	152	159	165	198	209	210	212	215	216	217	218	223
C. lumnitzericola	MFLUCC 17-0508	A	A	G	C	T	C	C	G	T	C	T	C	G	A	A	A	C	A
C. thailandica	MFLUCC 17-0262	A	G	-	-	T	T	T	T	T	T	T	C	A	A	A	-	C	A
C. thailandica	MFLUCC 17-0263	A	G	-	-	T	T	T	T	T	T	T	C	A	A	A	-	C	A
C. xylocarpi	MFLUCC 17-0251	G	G	-	-	A	A	C	T	C	C	A	T	A	T	G	-	A	-

Taxon	Strain								**RPB2**										
		224	225	231	234	242	245	246	4	18	33	42	57	84	85	96	102	108	120
C. lumnitzericola	MFLUCC 17-0508	C	G	C	-	-	A	A	T	T	C	T	C	C	T	T	C	G	A
C. thailandica	MFLUCC 17-0262	T	T	C	T	G	T	G	T	C	A	T	C	T	C	T	C	A	G
C. thailandica	MFLUCC 17-0263	T	T	C	T	G	T	G	T	C	A	T	C	T	C	T	C	A	G
C. xylocarpi	MFLUCC 17-0251	T	T	A	C	G	T	A	C	T	C	C	T	T	C	C	A	A	G

Taxon	Strain	123	126	129	144	153	171	174	177	204	210	213	216	222	231	237	243	246	279
C. lumnitzericola	MFLUCC 17-0508	C	G	C	G	T	G	C	C	G	C	T	C	T	T	C	T	C	T
C. thailandica	MFLUCC 17-0262	T	A	T	A	C	G	T	C	G	T	C	C	C	T	T	T	T	C
C. thailandica	MFLUCC 17-0263	T	A	T	A	C	G	T	C	G	T	C	C	C	T	T	T	T	C
C. xylocarpi	MFLUCC 17-0251	C	A	C	A	T	A	T	T	C	C	C	T	C	G	C	C	C	T

Taxon	Strain	282	294	306	309	336	339	342	351	352	357	378	390	393	396	402	405	435	441
C. lumnitzericola	MFLUCC 17-0508	C	A	T	C	T	C	G	T	C	G	A	C	C	G	T	T	C	T
C. thailandica	MFLUCC 17-0262	T	G	C	T	C	A	A	C	T	C	G	C	T	A	T	C	C	T
C. thailandica	MFLUCC 17-0263	T	G	C	T	C	A	A	C	T	C	G	C	T	A	T	C	C	T
C. xylocarpi	MFLUCC 17-0251	T	A	C	C	T	C	G	T	C	C	A	T	T	A	C	T	T	G

Taxon	Strain	456	465	468	492	498	510	516	517	543	561	570	576	603	612	613	615	627	633
C. lumnitzericola	MFLUCC 17-0508	C	T	C	G	T	T	A	T	T	A	A	G	T	T	C	C	C	G
C. thailandica	MFLUCC 17-0262	C	C	G	C	C	C	A	T	C	A	G	A	C	C	T	G	C	G
C. thailandica	MFLUCC 17-0263	C	C	G	C	C	C	A	T	C	A	G	A	C	C	T	G	C	G
C. xylocarpi	MFLUCC 17-0251	T	T	T	G	T	C	G	C	C	G	G	G	T	C	T	G	G	A

Taxon	Strain	651	663	675	678	690	693	699	702	711	732
C. lumnitzericola	MFLUCC 17-0508	T	A	C	T	T	G	T	C	C	T
C. thailandica	MFLUCC 17-0262	C	G	T	C	G	A	C	T	C	C
C. thailandica	MFLUCC 17-0263	C	G	T	C	G	A	C	T	C	C
C. xylocarpi	MFLUCC 17-0251	C	A	T	C	T	A	C	C	T	T

All isolates are new taxa in this study; “-” gap (insertion/deletion); “?” missing data.

Hysterium Pers., Tent. disp. meth. fung. (Lipsiae): 5 (1797)

Index Fungorum Number – IF2464

Type species: *Hysterium pulicare* Pers., Neues Mag. Bot. 1: 85 (1794)

Hysterium, a genus belongs to Hysteriaceae fmily, with characterized by having hysterothecium ascomata which are thick-walled, navicular, characteristically dehiscing by an invaginated slit or sulcus (Zogg 1962, Boehm et al. 2009). However, recent molecular data shows the potential for morphology to be difficult to interpret, and even unhelpful in phylogenetic inference and reconstruction for this group of fungi (Schoch *et al.* 2006, Boehm *et al.* 2009, Mugambi & Huhndorf 2009, Boehm et al. 2009).

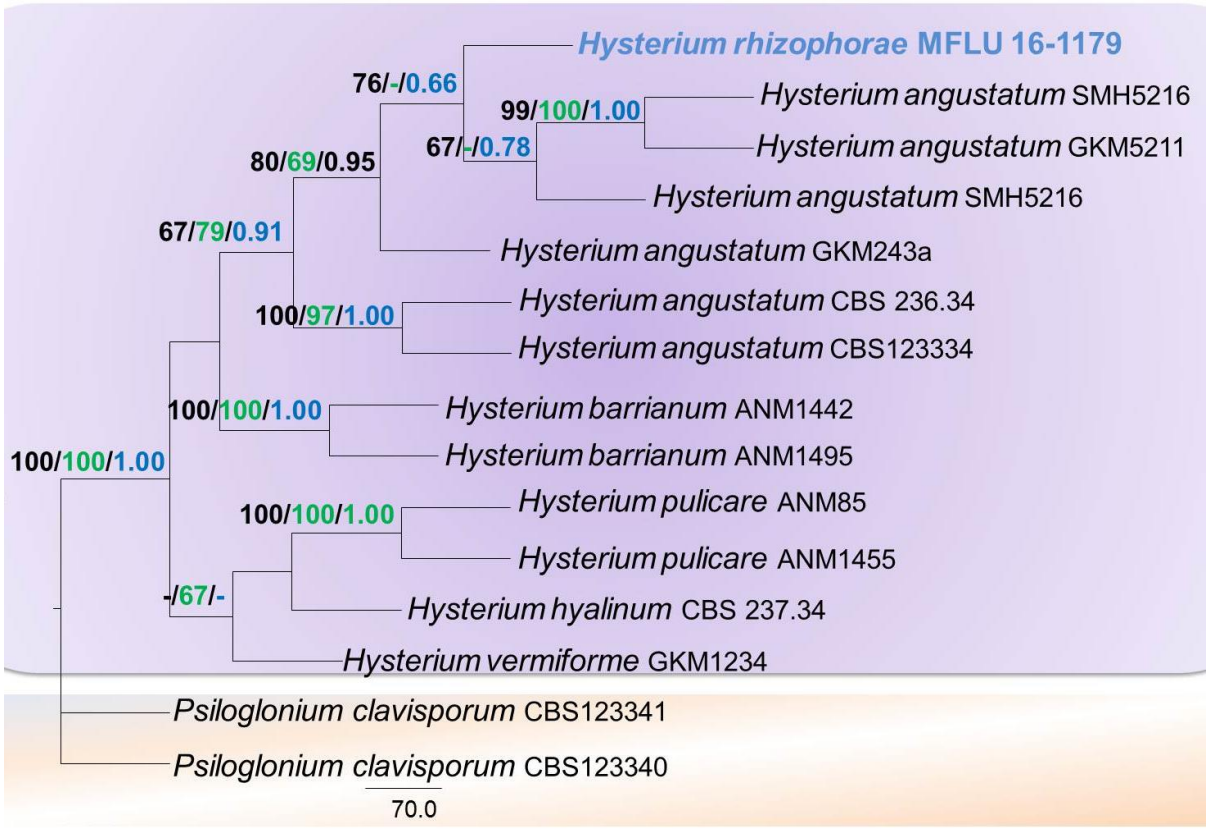


Figure 14 – Phylogram generated from maximum parsimony analysis of combined LSU, SSU and TEF1 sequence data of selected taxa. Maximum parsimony bootstrap (black) values > 60%, maximum likelihood bootstrap (green) values > 60% and Bayesian posterior probabilities (blue) > 0.90%) are given above the nodes. The new isolates are in blue bold. The tree is rooted with *Psiloglonium clavisporum*.

Hysterium rhizophorae Dayarathne & K. D. Hyde

Facesoffungi number: FoF 02911 Fig. 15

Saprobic on bark attached to the living plant of Rhizophora apiculata, attached to the living plant. Sexual morph Ascomata hysterothecial, 1500–2500 µm long, 200–250 µm high, 170–200 µm wide, erumpent to superficial, with base immersed, solitary to gregarious, straight to flexuous, ellipsoid or elongate, with pointed ends, opening by a depressed longitudinal slit, in vertical section subglobose to globose, carbonaceous, black,. Peridium 42–64 µm wide, carbonaceous, comprising an outer layer of dark brown cells of textura globosa and inner layer of hyaline to pale brown cells of textura globosa. Pseudoparaphyses 1–1.5 µm (n = 30) wide, cellular, septate, flexuous, branched. Asci 45–50 × 3.5–5.5 µm ($\overline{x}$ = 48 × 4 µm, n = 10), 8-spored, bitunicate, cylindric to claviform, short pedicellate. Ascospores 14–18 × 4–6 µm ($\overline{x}$ = 16 × 5 µm, n = 10), overlapping biseriate, light brown, ellipsoidal, slightly curved, with 3 transverse septa, often slightly constricted at medium septum, with 1 guttule in cell. Asexual morph: Undetermined.

Culture characteristics: Colonies on MEA slow growing at 25–28 C reaching 2 cm in 14 days, yellow at first, becoming ash when mature and reverse yellow.

Material examined: THAILAND, Phetchaburi Province, Hat Chao Samran, on bark attached to the living plant of *Rhizophora apiculata* (Rhizophoraceae), 28 July 2015, Monika Dayarathne, CHAM 013 (MFLU 16-1179, holotype), ex-type living culture, MFLUCC 15-0950, ICMP

Notes: *Hysterium rhizophorae* differs from *H. angustatum* Alb. & Schwein., the most similar species, mainly by relatively smaller hysterothecial ascomata (1500–2500 × 200–250 × 170–200 µm viz 3000–2000 × 250–300 × 200–300 µm), asci (45–50 × 3.5–5.5 µm viz 64–120 × 8–14 µm) and ascospores (14–18 × 4–6 µm viz 16–30 × 4–8.5 µm) (http://fungi.myspecies.info/all-fungi/hysterium-angustatum). Ascomata, asci and ascospores sizes of *H. angustatum* relies on wide range such as, Lohman (1933) and Zogg (1962) described larger asci (105–120 × 10–16 µm and 100–120 × 11–14 µm, respectively), while Ellis & Everhart (1892) reported wider asci with similar lengths (75–80 × 12–15 µm). Ellis & Everhart (1892) and Lohman (1933) described *H. angustatum* with relatively larger ascospores (15–22 × 6–7 µm and 22–26 × 6.5–8(9) µm, respectively). Therefore, our new species is clearly distinct from *H. angustatum* by sizes of asci, ascospores. Combined LSU, SSU and TEF1 phylogenetic analyses demonstrated that *H. rhizophorae* is closely related to *H. angustatum* but form a separate lineage (76%MP, 60%ML) within *Hysterium*. Strains of *H. angustatum* showed different placements within the genus hence, species complex.

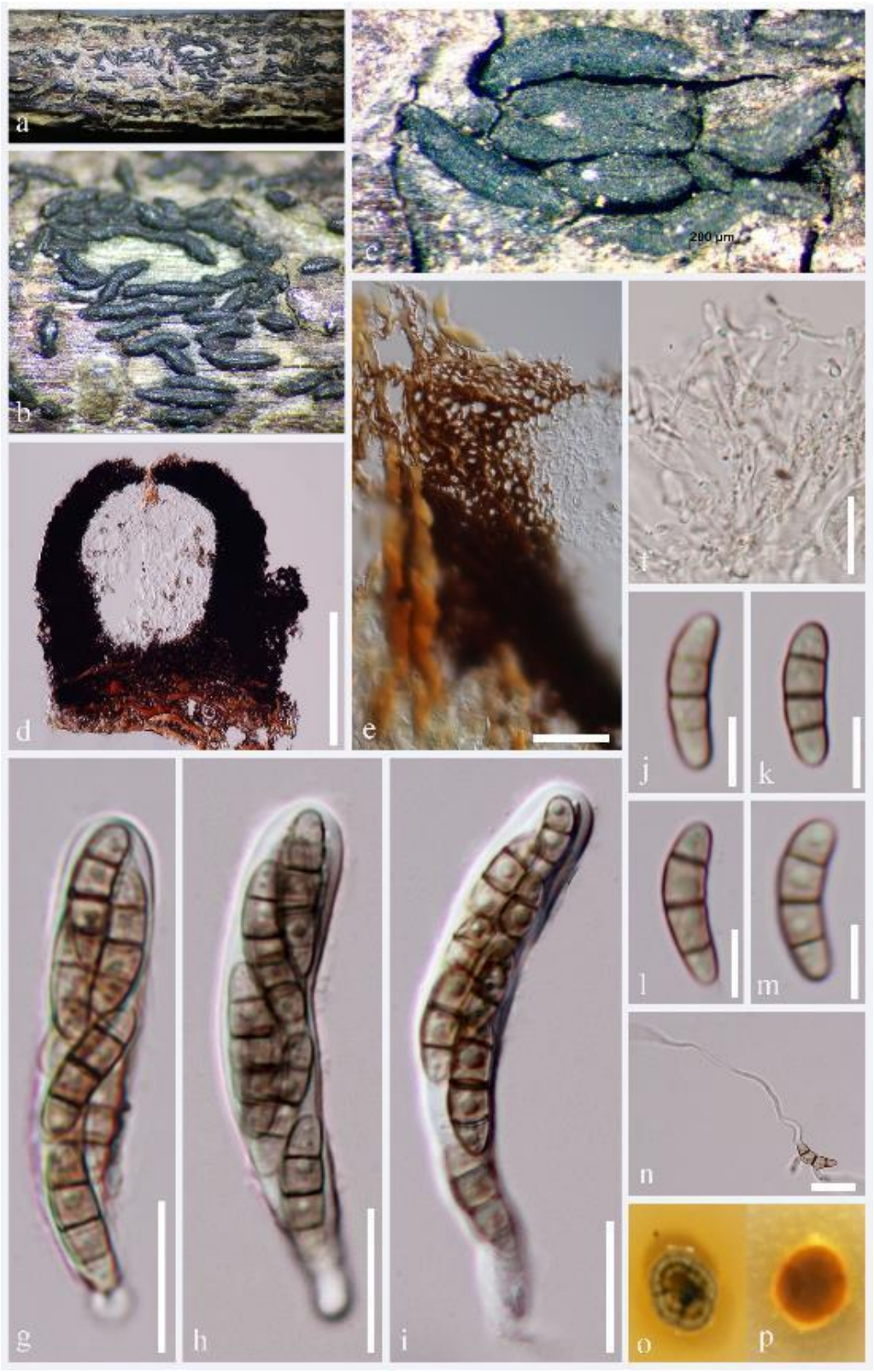


Figure 15 – *Hysterium rhizophorae* (MFLU 16-1179) a Host. b, c Appearance of hysterothecia on host. d Vertical section through hysterothecium. e Peridium. f Pseudoparaphyses. g–i Asci. j–m Ascospores. n Germinating ascospores. o,p Culture on MEA (o upper, p lower). Scale bars: c = 200 µm, d = 100 µm, e, f = 20 µm, g–i = 10 µm, j–n = 5 µm.

Kamalomyces R.K. Verma, N. Sharma & Soni, Forest Fungi of Central India: 196 (2008)

Index Fungorum Number – IF512509

Type species: *Kamalomyces indicus* R.K. Verma, N. Sharma & Soni, Forest Fungi of Central India: 196 (2008)

Kamalomyces mangrovei Dayarathne & K.D. Hyde **sp. nov.**

Index Fungorum Number – IF555385, Facesoffungi Number: FoF 04946 Fig. 17

Etymology – Epithet derived from the mangrove habitat that species found

Holotype – MFLU 18-1691

Saprobic on decaying wood in a mangrove strand. **Sexual morph**: *Ascomata* 280–315 × 250–300 µm, superficial, solitary to gregarious, embedded in a subiculum of crowded, black, septate, thickwalled hyphae, superficial, solitary, gregarious, globose to subglobose, glabrous, short stalked, apapillate, indistinct ostiolate. *Peridium* 30–45 µm wide, comprising light brown cells of textura angularis, and inwardly small, subhyaline cells of textura prismatica. *Hamathecium* comprising numerous, 1.5–2 µm wide, filiform, septate, branched, hyaline pseudoparaphyses. *Asci* 148–180 × 16–20 µm ($\overline{x}$ = 165 × 18 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short pedicellate, apically rounded, with an ocular chamber. *Ascospores* 52–67 × 6.5–8.5 µm ($\overline{x}$ = 62 × 7.5 µm, n = 30), 2–3-seriate, hyaline becoming light brown when mature, elongate cylindrical to fusiform-clavate, tapering towards the lower cells, enlarged at the 4rd and 5th cell, straight or slightly curved, 8–9 septa, distoseptate.

Culture characteristics: Colonies on PDA reaching 3 cm diam. after 30 days at 25°C, circular, smooth margin white at first, dark gray to black after 6 weeks, flat on the surface, without aerial mycelium, reverse brownish black.

Material examined: THAILAND, Ranong province, Amphoe Maung, Mu 4 Tombol Ngao, Ranong Mangrove Research Center (GPS: 9°43' to 9°57'N; 98°29' to 98°39'E) on decaying wood of Rhizopora sp., 07 December 2016, Monika C. Dayarathne, MCD 053 (MFLU 18-1691 holotype), ex-type living culture, MFLUCC 17-0407, TBRC.

Notes: *Kamalomyces mangrovei* is characterized by having solitary to gregarious, globose to subglobose, short stalked ascomata, lacking ostioles and embedded in a subiculum of black hyphae, bitunicate, cylindrical to clavate asci and hyaline, elongate cylindrical to fusiform-clavate, septate ascospores. *Kamalomyces mangrovei* is morphologically highly similar to the genus *Kamalomyces* (Verma et al. 2008, Dubey & Neelima 2013, Boonmee et al. 2011, 2014, Phookamsak et al. 2018, Lu et al. 2018b). *Kamalomyces mangrovei* closely resembles K. bambusicola Y.Z. Lu & K.D. Hyde and *K. thailandicus* Phook., Y.Z. Lu & K.D. Hyde due to its ascomatal characters and asci, ascospore shape. However, *K. mangrovei* differs significantly from *K. bambusicola* and *K. thailandicus* ascospore by having fewer ascospore septa (8-9 vs 27–30 and 33–36 septa), being swollen at the 4th and 5th cells In contrast, ascospores of *K. bambusicola* are swollen at the 3rd or 5th cell and 7th cell of *K. thailandicus*. Further, novel species is also different by its occurrence on Mangrove sp. while *K. bambusicola* and *K. thailandicus* have been reported from Bamboo. This is the first record of Tubeufiaceae fungi reported from a marine habitat. According to our phylogenetic analyses with concatenated LSU, ITS and TEF sequence data, our new species clustered within the genus *Kamalomyces* with high statistical support (84 % ML, 75 % MP, 0.99 PP).

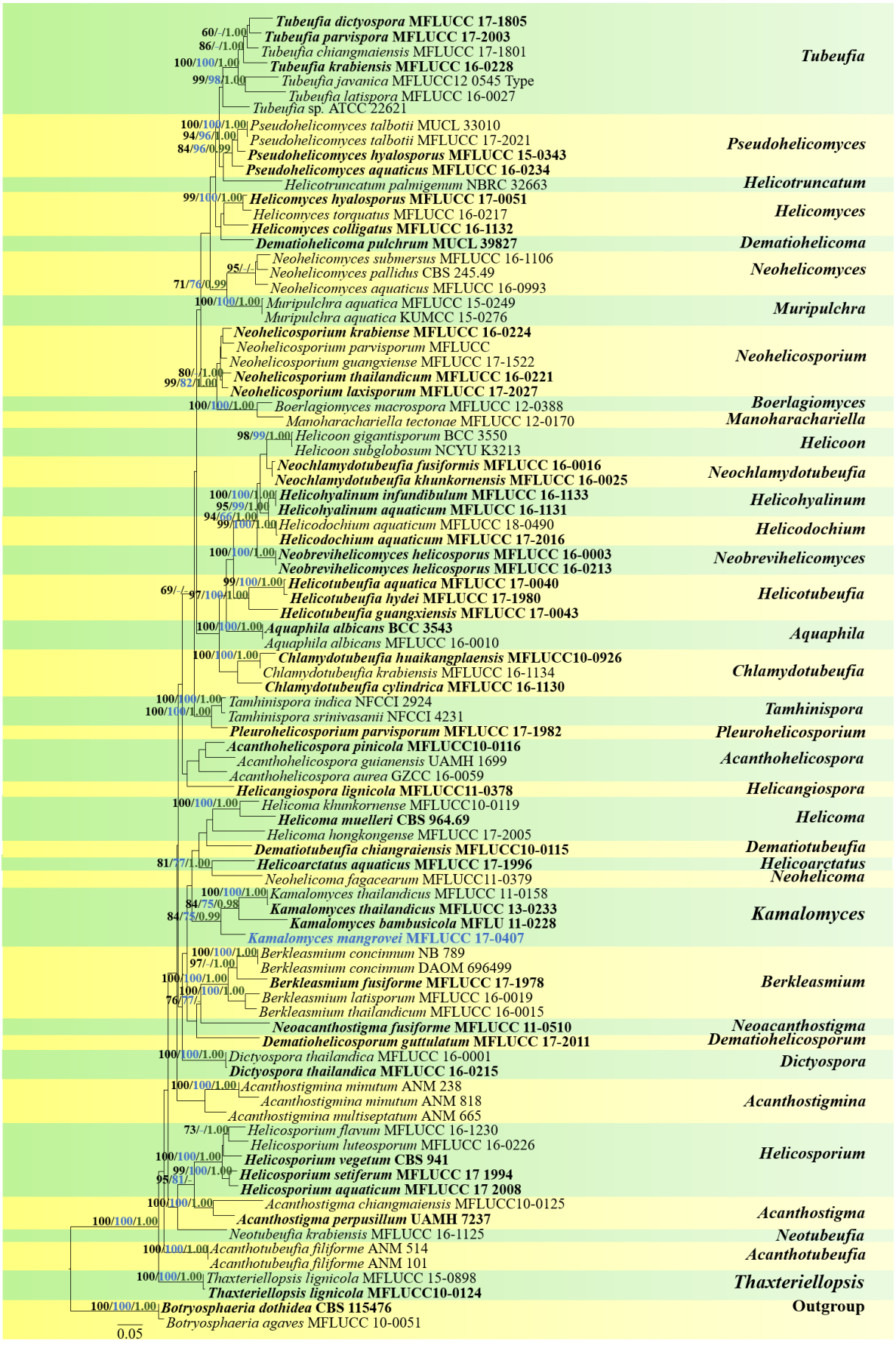

Tubeufia dictyospora MFLUCC 17-1805
Tubeufia parvispora MFLUCC 17-2003
Tubeufia chiangmaiensis MFLUCC 17-1801
Tubeufia krabiensis MFLUCC 16-0228
Tubeufia javanica MFLUCC12 0545 Type
Tubeufia latispora MFLUCC 16-0027
Tubeufia sp. ATCC 22621
Tubeufia
Pseudohelicomyces talbotii MUCL 33010
Pseudohelicomyces talbotii MFLUCC 17-2021
Pseudohelicomyces hyalosporus MFLUCC 15-0343
Pseudohelicomyces aquaticus MFLUCC 16-0234
Pseudohelicomyces
Helicotruncatum palmigenum NBRC 32663
Helicotruncatum
Helicomyces hyalosporus MFLUCC 17-0051
Helicomyces torquatus MFLUCC 16-0217
Helicomyces colligatus MFLUCC 16-1132
Helicomyces
Dematiohelicoma pulchrum MUCL 39827
Dematiohelicoma
Neohelicomyces submersus MFLUCC 16-1106
Neohelicomyces pallidus CBS 245.49
Neohelicomyces aquaticus MFLUCC 16-0993
Neohelicomyces
Muripulchra aquatica MFLUCC 15-0249
Muripulchra aquatica KUMCC 15-0276
Muripulchra
Neohelicosporium krabiense MFLUCC 16-0224
Neohelicosporium parvisporum MFLUCC
Neohelicosporium guangxiense MFLUCC 17-1522
Neohelicosporium thailandicum MFLUCC 16-0221
Neohelicosporium laxisporum MFLUCC 17-2027
Neohelicosporium
Boerlagiomyces macrospora MFLUCC 12-0388
Boerlagiomyces
Manoharachariella tectonae MFLUCC 12-0170
Manoharachariella
Helicoon gigantisporum BCC 3550
Helicoon subglobosum NCYU K3213
Helicoon
Neochlamydotubeufia fusiformis MFLUCC 16-0016
Neochlamydotubeufia khunkornensis MFLUCC 16-0025
Neochlamydotubeufia
Helicohyalinum infundibulum MFLUCC 16-1133
Helicohyalinum aquaticum MFLUCC 16-1131
Helicohyalinum
Helicodochium aquaticum MFLUCC 18-0490
Helicodochium aquaticum MFLUCC 17-2016
Helicodochium
Neobrevihelicomyces helicosporus MFLUCC 16-0003
Neobrevihelicomyces helicosporus MFLUCC 16-0213
Neobrevihelicomyces
Helicotubeufia aquatica MFLUCC 17-0040
Helicotubeufia hydei MFLUCC 17-1980
Helicotubeufia guangxiensis MFLUCC 17-0043
Helicotubeufia
Aquaphila albicans BCC 3543
Aquaphila albicans MFLUCC 16-0010
Aquaphila
Chlamydotubeufia huaikangplaensis MFLUCC10-0926
Chlamydotubeufia krabiensis MFLUCC 16-1134
Chlamydotubeufia cylindrica MFLUCC 16-1130
Chlamydotubeufia
Tamhinispora indica NFCCI 2924
Tamhinispora srinivasanii NFCCI 4231
Tamhinispora
Pleurohelicosporium parvisporum MFLUCC 17-1982
Pleurohelicosporium
Acanthohelicospora pinicola MFLUCC10-0116
Acanthohelicospora guianensis UAMH 1699
Acanthohelicospora aurea GZCC 16-0059
Acanthohelicospora
Helicangiospora lignicola MFLUCC11-0378
Helicangiospora
Helicoma khunkornense MFLUCC10-0119
Helicoma muelleri CBS 964.69
Helicoma hongkongense MFLUCC 17-2005
Helicoma
Dematiotubeufia chiangraiensis MFLUCC10-0115
Dematiotubeufia
Helicoarctatus aquaticus MFLUCC 17-1996
Helicoarctatus
Neohelicoma fagacearum MFLUCC11-0379
Neohelicoma
Kamalomyces thailandicus MFLUCC 11-0158
Kamalomyces thailandicus MFLUCC 13-0233
Kamalomyces bambusicola MFLU 11-0228
Kamalomyces mangrovei MFLUCC 17-0407
Kamalomyces
Berkleasmium concinnum NB 789
Berkleasmium concinnum DAOM 696499
Berkleasmium fusiforme MFLUCC 17-1978
Berkleasmium latisporum MFLUCC 16-0019
Berkleasmium thailandicum MFLUCC 16-0015
Berkleasmium
Neoacanthostigma fusiforme MFLUCC 11-0510
Neoacanthostigma
Dematiohelicosporum guttulatum MFLUCC 17-2011
Dematiohelicosporum
Dictyospora thailandica MFLUCC 16-0001
Dictyospora thailandica MFLUCC 16-0215
Dictyospora
Acanthostigmina minutum ANM 238
Acanthostigmina minutum ANM 818
Acanthostigmina multiseptatum ANM 665
Acanthostigmina
Helicosporium flavum MFLUCC 16-1230
Helicosporium luteosporum MFLUCC 16-0226
Helicosporium vegetum CBS 941
Helicosporium setiferum MFLUCC 17 1994
Helicosporium aquaticum MFLUCC 17 2008
Helicosporium
Acanthostigma chiangmaiensis MFLUCC10-0125
Acanthostigma perpusillum UAMH 7237
Acanthostigma
Neotubeufia krabiensis MFLUCC 16-1125
Neotubeufia
Acanthotubeufia filiforme ANM 514
Acanthotubeufia filiforme ANM 101
Acanthotubeufia
Thaxteriellopsis lignicola MFLUCC 15-0898
Thaxteriellopsis lignicola MFLUCC10-0124
Thaxteriellopsis
Botryosphaeria dothidea CBS 115476
Botryosphaeria agaves MFLUCC 10-0051
Outgroup
0.05

Figure 16

Figure 16 – Phylogram generated from maximum likelihood analysis based on combined LSU, ITS and TEF1 sequence data of selected taxa. Related sequences were obtained from GenBank. Data set comprises 2530 characters including gaps. Single gene analyses were carried out and compared with each species, to compare the topology of the tree and clade stability. Tree was rooted with Botryosphaeria agaves (MFLUCC 10-0051) and Botryosphaeria dothidea (CBS 115476). Tree topology of the ML analysis was similar to the MP and BI. The best scoring RAxML tree with a final likelihood value of -27950.184903 is presented. The matrix had 1137 distinct alignment patterns, with 26.87% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.234676, C = 0.258513, G = 0.266669, T = 0.240142; substitution rates AC = 1.053687, AG = 4.072971, AT = 3.048439, CG = 0.739670, CT = 8.539737, GT = 1.000000; gamma distribution shape parameter α = 0.712704. The maximum parsimonious dataset consisted of constant 1587, 768 parsimony-informative and 175 parsimony-uninformative characters. The parsimony analysis of the data matrix resulted in the maximum of two equally most parsimonious trees with a length of 5615 steps (CI = 0.273, RI = 0.584, RC = 0.160, HI = 0.727) in the first tree Maximum parsimony bootstrap (MPBT, blue) values > 65%, Bayesian posterior probabilities (PP, green) > 0.80% and maximum likelihood bootstrap (ML, black) values > 65%) are given above the nodes. The scale bar indicates 0.05 changes. The ex-type strains are in bold and new isolates in blue bold.

Neopestalotiopsis Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 135 (2014)

Index Fungorum number: IF809759

Type species: *Neopestalotiopsis protearum* (Crous & L. Swart) Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 147 (2014)

The alignment of *Neopestalotiopsis* (Fig. 18) comprised 52 taxa, with the outgroup taxon, *Pestalotiopsis diversiseta* (MFLUCC 12-0287). The total length of the dataset was 1358 characters including alignment gaps, 1–324, 325–482, 483–700, 701–886, 887–1054 and 1055–1358 corresponding to ITS1+ITS2, 5.8S, β-tubulin (exon), β-tubulin (intron), EF1α (exon) and EF1α (intron), respectively. The combined dataset contained 1001 constant, 175 parsimony uninformative and 182 parsimony informative characters. The combined dataset was analyzed using MP, ML and BI. The trees generated under different optimality criteria were essentially similar in topology and did not differ significantly (data not shown). The descriptive statistics generated from MP analysis based on the combined dataset of ITS1+ITS2, 5.8S, β-tubulin (exon), β-tubulin (intron), EF1α (exon), and EF1α (intron) were TL = 643, CI = 0.673, RI = 0.670, RC = 0.451, HI = 0.327. The best scoring likelihood tree selected with a final value for the combined dataset = -5197.543923.

Figure 17 – *Kamalomyces mangrovei* (MFLU 18-1691). A,B. Ascomata. C. Section of ascomata. D. Section through peridium. E–G Asci and pseudoparaphyses. H–K. Ascospores. L. Germinating ascospores, M,N Cultures on PDA (M-upper, N-lower). Scale bars: A = 500 µm, C = 100 µm, E–G = 50 µm, H–L = 20 µm.

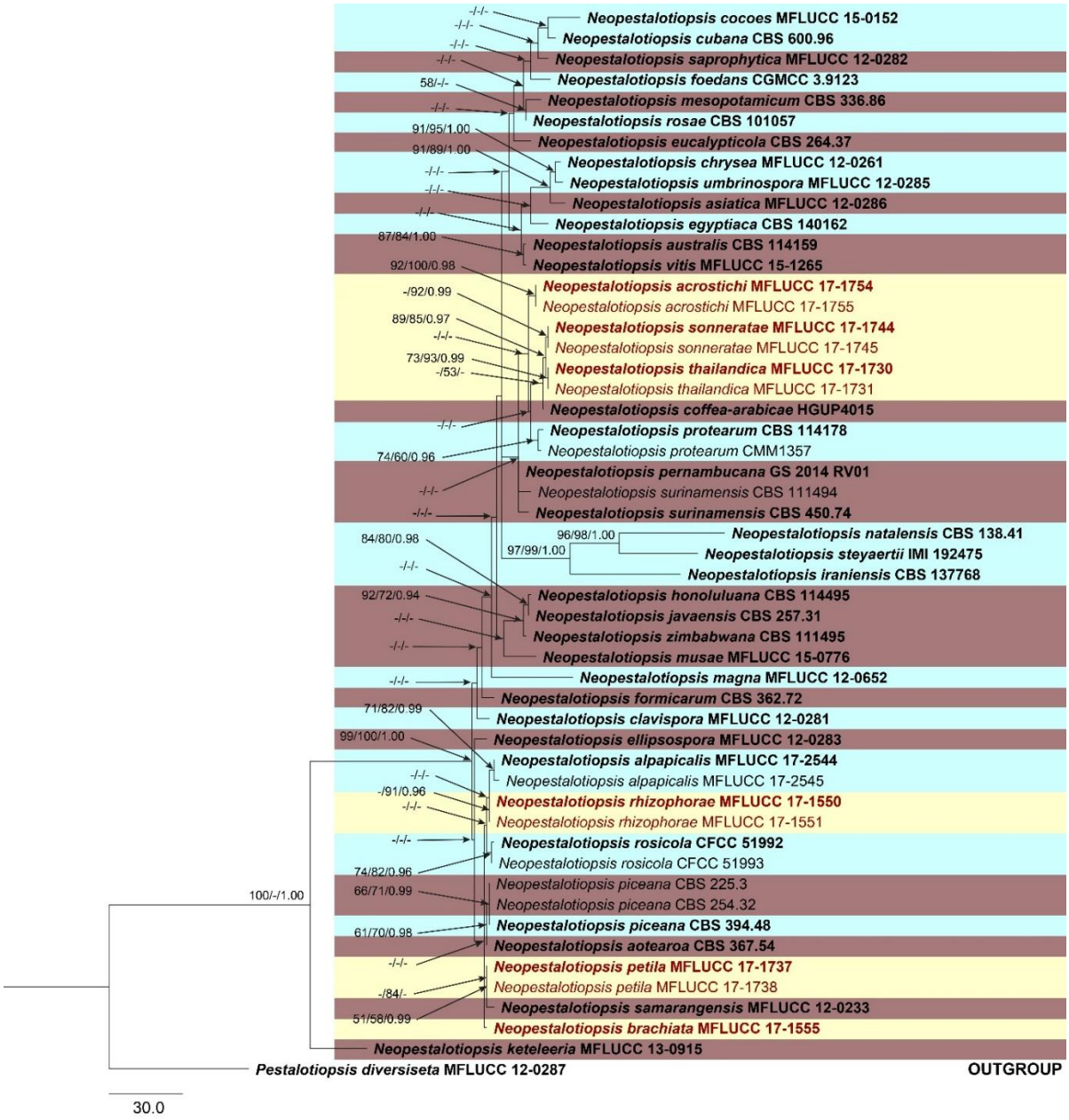


Figure 18 – One of the 1000 most parsimonious trees obtained from a heuristic search of combined ITS, β-tubulin and EF1α sequence data for the genus *Neopestalotiopsis*. The tree is rooted to *Pestalotiopsis diversiseta* (MFLUCC 12-0287). Maximum parsimony and maximum likelihood bootstrap values ≥ 50% , Bayesian posterior probabilities ≥ 0.90 (MPBS/MLBS/PPBY) are given at the nodes. The species obtained in this study are in red. Ex-type taxa from other studies are in black bold.

Neopestalotiopsis acrostichi Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556434; Facesoffungi number: FoF 05780 Fig. 19

Etymology – refers to the host from which the fungus was isolated, *Acrostichum aureum* L.

Holotype – MFLU 19-0774

Associated with leaf spots of *Acrostichum aureum* L. *Symptoms* sub circular to irregular shape, pale brown, appear on adaxial surface leaves of *A. aureum*, which later expand outwards. Small brown spots appeared initially and then gradually enlarged, changing to pale brown circular spots with a black border. They were usually 4–5 circular spots occurred on a single affected leaf . Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth, thin-walled, proliferating once percurrently, collarette present and not flared, 10–25 × 2–5 μm. *Conidia* (22–)23–26(–27) × (5–)5.5–6.5(–7) μm, (mean ± SD = 24.3 ± 1.3 × 6 ± 0.6 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (4.5–)5–6(–7) μm long (mean ± SD = 5.5 ± 0.8 μm); three median cells (14–)15–16(–18) μm long (mean ± SD = 15.9 ± 1 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–6.5) μm long (mean ± SD = 5.6 ± 0.6 μm); third cell brown, 4.5–6 μm long (mean ± SD = 4.9 ± 0.5 μm); fourth cell brown, (4–)4.5–5(–6.5) μm long (mean ± SD = 5.4 ± 0.6 μm); apical cell (3–)4.5–5(–6) μm long (mean ± SD = 4.9 ± 0.8 μm), hyaline, conic to acute, with 3–5 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (16–)19–28.5(–33.5) μm long (mean ± SD = 24.5 ± 4.7 μm); single basal appendage, tubular, unbranched, centric, (4.5–)5–7(–12) μm long, (mean ± SD = 7.1 ± 2 μm).

Culture characteristics – Colonies on PDA reaching 6–8 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Chanthaburi Province, leaf spots of *Acrostichum aureum* L., 25 April 2017, Norphanphoun Chada JT12-1 (MFLU 19-0774 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1754, TNCC. THAILAND, Chanthaburi Province, leaf spots of *Acrostichum aureum* L., 25 April 2017, Norphanphoun Chada JT12-2 (MFLU 19-0775 dried culture, paratype); ex-type-living cultures, MFLUCC 17-1755.

Notes – *Neopestalotiopsis acrostichi* was isolated from a leaf spot of *Acrostichum aureum*. The new species resembles several other *Neopestalotiopsis* species. The combined phylogenetic tree indicated that *N. acrostichi* is sister to *N. protearum* (CBS 114178) and *N. surinamensis* (CBS 450.74) (Fig. 18), but they differ in morphology. *Neopestalotiopsis acrostichi* have larger conidia than *N. protearum* (*N. protearum*: (14–)16–17(–18) × (6.5–)8–9(–10)). *Neopestalotiopsis acrostichi* is very much similar to *N. surinamensis* (Crous et al. 2011, Maharachchikumbura et al. 2014). However, *N. acrostichi* differs from this species by having smaller conidia (*N. surinamensis*: (23–)24–28(–29) × (7–)7.5–9(–9.5)) with four apical tubular appendages (*N. surinamensis*: (15–)18–27(–28)) (Table 8, Maharachchikumbura et al. 2014). This is the first report of *Neopestalotiopsis* on *A. aureum*.

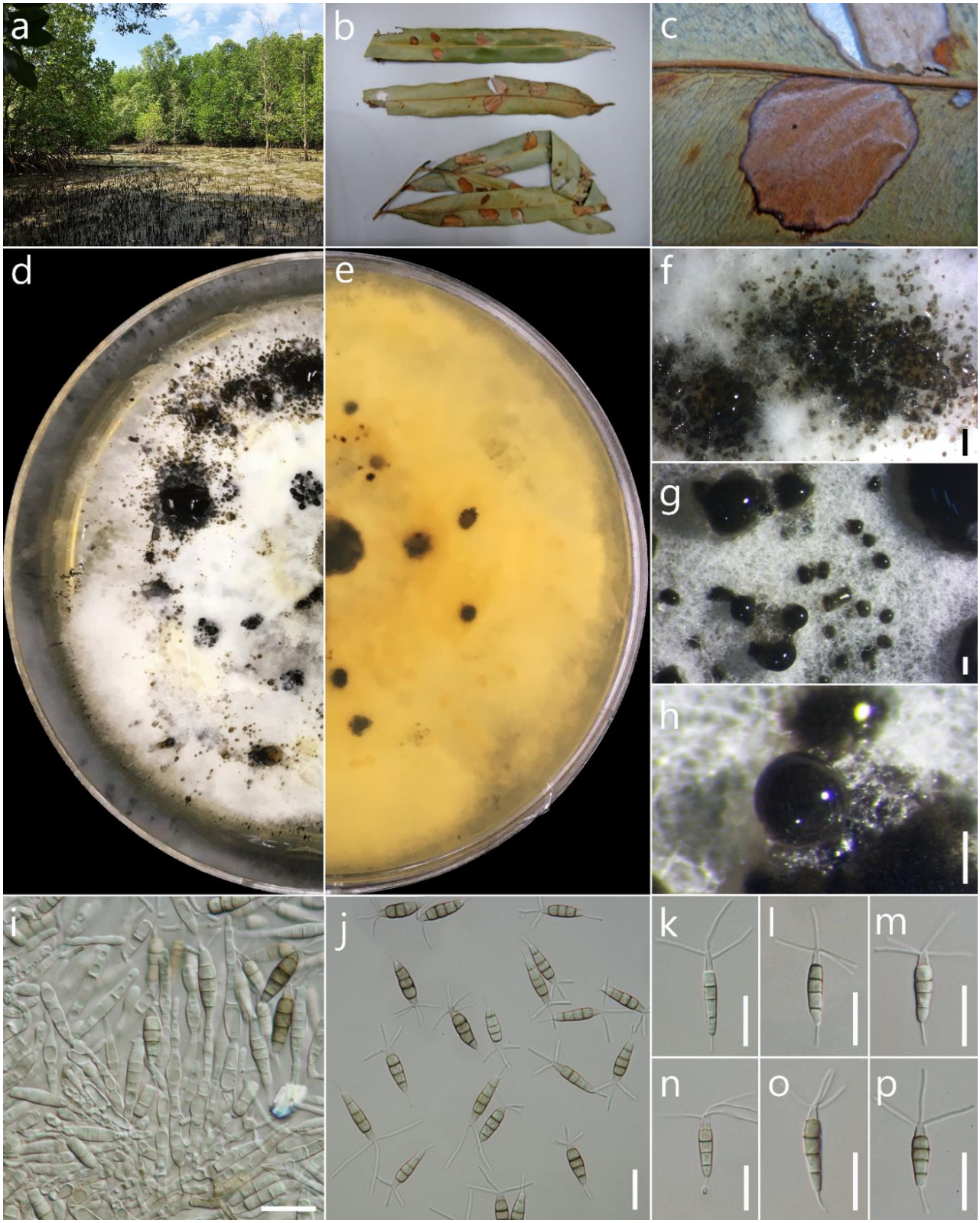


Figure 19 – *Neopestalotiopsis acrostichi* (MFLU 19-0774, holotype)**.** a Habitat**.** b, c Leaf spots on *Acrostichum aureum***.** d, e Culture on PDA (d**-**above, e**-**reverse)**.** f–h Colony sporulating on PDA**.** i Conidiogenous cells giving rise to conidia**.** j–p Conidia**.** Scale bars**:** f–h = 200 µm, i–p = 20 µm.

Neopestalotiopsis brachiata Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556435; Facesoffungi number: FoF 05771 Fig. 20

Etymology – the specific epithet "brachiata" refers to the character of apical appendages (Latin 'brachiata' means 'branches').

Holotype – MFLU 19-0776

Associated with leaf spots of *Rhizophora apiculata* Blume. *Symptoms* circular or sub circular shape, grayish brown, slightly sunken spots appear on adaxial surface leaves of *R. apiculata*, which later expand outwards. Small auburn spots appeared initially and then gradually enlarged, changing to grayish brown circular ring spots with a dark brown border. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spreaded evenly on the leaves. Asexual morph: *Conidiomata* pycnidial, globose, brown, semi- immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to ampulliform to lageniform, hyaline, smooth- and thin- walled, simple, proliferating 1–2 times percurrently, collarette present and not flared, 5–10 × 5–8 μm. *Conidia* (18–)18.5–25(–26) × (5–)5.5–6(–6.5) μm (mean ± SD = 21 ± 2.2 × 5.5 ± 0.6 μm), fusiform to clavate, straight to slightly curved, 4- septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth- walled, (3–)3.5–4(–5) μm long (mean ± SD = 3.8 ± 0.7 μm); three median cells (10–)10.5–15(–16) μm long (mean ± SD = 12.7 ± 1.7 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (3–)4–5(–6) μm long (mean ± SD = 4.4 ± 0.8 μm); third cell brown, (3.5–)4–5(–6) μm long (mean ± SD = 4.2 ± 0.7 μm); fourth cell brown, (3–)4–5(–5.5) μm long (mean ± SD = 4.1 ± 0.5 μm); apical cell (4–)4.5–5(–6) μm long (mean ± SD = 4.5 ± 0.7 μm), hyaline, conic to acute; with 1–3 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, branched, flexuous, (8.5–)9.5–33(–34) μm long (mean ± SD = 20 ± 7.6 μm); single basal appendage, tubular, unbranched, centric, (3.5–)4–9(–10) μm long (mean ± SD = 6.5 ± 1.6 μm).

Culture characteristics – Colonies on PDA reaching 6–7 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Ngao, Ranong Province, Ngao Mangrove Forest Research Centre, leaf spots of *Rhizophora apiculata*, 6 December 2016, Norphanphoun Chada NG33 (MFLU 19-0776 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1555, TNCC.

Notes – *Neopestalotiopsis brachiata* is similar to *N. rosicola* (strain CFCC 51992) in conidial size (*N. brachiata* (18–)18.5–25(–26) × (5–)5.5–6(–6.5) μm *vs. N. rosicola*: (19−)20−25.5(−26) × (5−)5.5−8(−8.5) μm) but phylogenetically distinct (Jiang et al. 2018). In the multigene phylogenetic analysis presented here, the new species formed a sister clade to *N. aotearoa* (strain CBS 367.54), *N. piceanae* (strain CBS 394.48) and *N. petila* (in this study), which appear to be phylogenetically distinct (Fig. 18). The conidia of *N. brachiata* are different from *N. aotearoa* ((19.5–)21–28(–29) × (6–)6.5–8.5(–9) μm), *N. piceanae* ((19–)19.5–25(–26) × (7–)7.5–9(–9.5) μm) and *N. petila* ((20–)21–26.5(–27.5) × (5.5–)6–7(–8) μm) and also differs by having branched, flexuous apical tubular appendages (Maharachchikumbura et al. 2014).

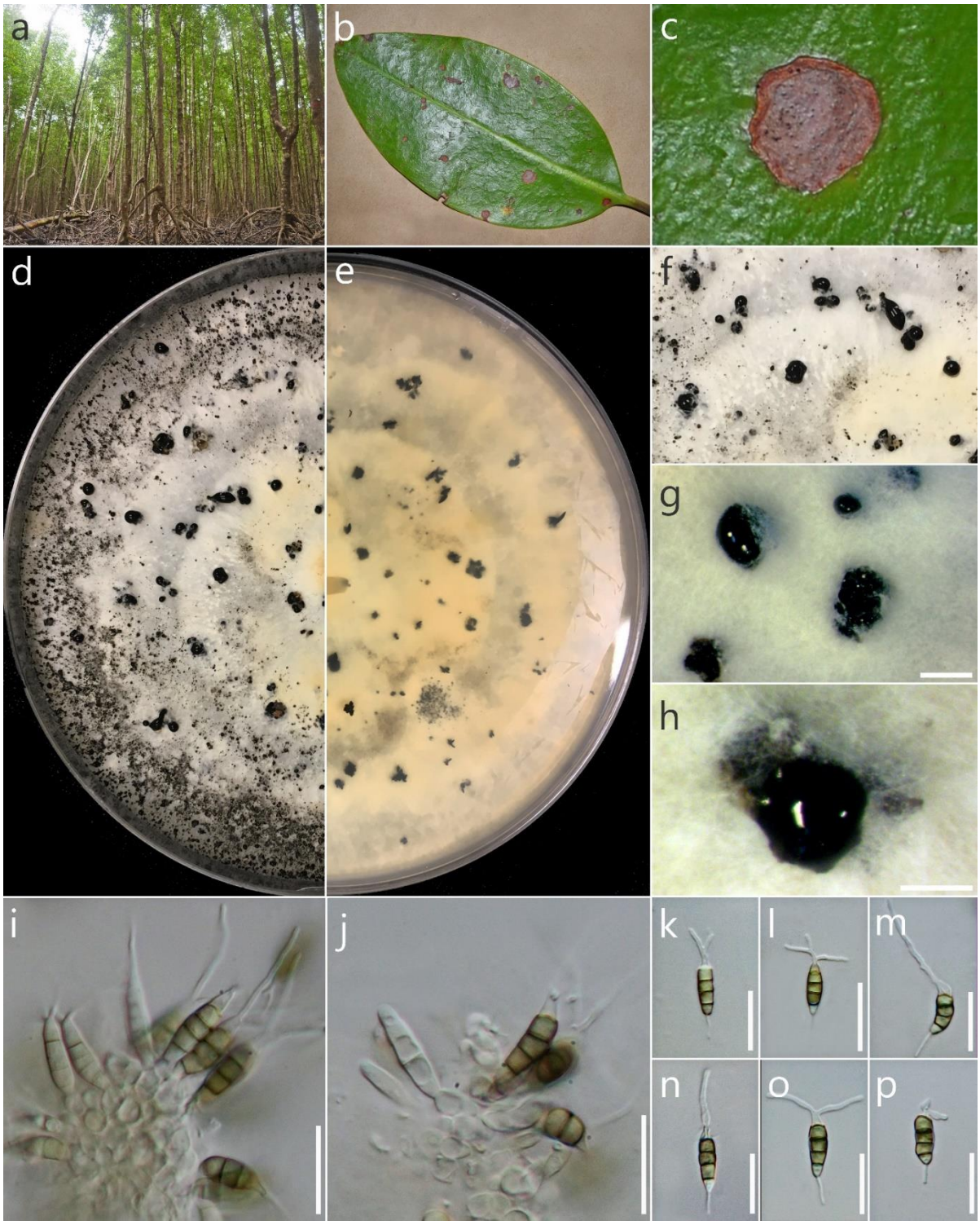


Figure 20 – *Neopestalotiopsis brachiata* (MFLU **19-0776**, holotype)**.** a Habitat**.** b, c Leaf spots on *Rhizophora apiculata***.** d, e Culture on PDA (d**-** above, e**-** reverse)**.** f–h Colony sporulating on PDA**.** i Conidiogenous cells giving rise to conidia**.** j–p Conidia**.** Scale bars**:** g = 1000 µm, h = 500 µm, i–p = 20 µm**.**

Neopestalotiopsis petila Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556436; Facesoffungi number: FoF 05772 Fig. 21

Etymology – the latin "petilus" meaning slander/ slim, which refers to the shape of conidia.

Holotype – MFLU 19-0777

Associated with leaf spots of *Rhizophora mucronata* Lam. *Symptoms* subcircular to the irregular shape, pale brown, slightly sunken spots appear on adaxial surface leaves of *R. mucronata*, which later expand outwards. Small auburn spots appeared initially and then gradually enlarged, changing to pale-auburn circular ring spots with a dark auburn border. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves. Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* distinct. *Conidiogenous cells* discrete to integrated, hyaline, smooth- and thin- walled, proliferating one time percurrently, collarette present and not flared. *Conidia* (20–)21–26.5(–27.5) × (5.5–)6–7(–8) μm (mean ± SD = 24.5 ± 2.0 × 6.7 ± 0.7 μm), fusiform to clavate, straight to slightly curved, 4- septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (3–)4–5.5(–6) μm long (mean ± SD = 4.52 ± 0.8 μm); three median cells (12.5–)13.5–15(–17) μm long (mean ± SD = 15.3 ± 1.0 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–7) μm long (mean ± SD = 5.2 ± 0.5 μm); third cell brown, (3.5–)4–5(–5.5) μm long (mean ± SD = 4.8 ± 0.5 μm); fourth cell brown, (4.5–)5–5.5(–6) μm long (mean ± SD = 5.3 ± 0.5 μm); apical cell (3–)4–5(–7) μm long (mean ± SD = 4.6 ± 1.0 μm), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (21–)22–29(–33) μm long (mean ± SD = 25.5 ± 3.1 μm); single basal appendage, tubular, unbranched, centric, (2–)3–8(–9) μm long (mean ± SD = 6 ± 2.1 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Kor Chang, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC05-1 (MFLU 19-0777 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1737, TNCC. THAILAND, Kor Chang, Trat Province, leaf spots of *R. mucronata*, 27 April 2017, Norphanphoun Chada KC05-2 (MFLU 19-0778 dried culture, paratype); living cultures, MFLUCC 17-1738.

Notes – Based on multigene analyses, *Neopestalotiopsis petila* is closely related to *N. aotearoa* (strain CBS 367.54), *N. piceana* (strain CBS 394.48), *N. brachiata* (in this study) and *N. samarangensis* (strain MFLUCC 12-0233) (Fig. 18). However, *N. petila* differs from *N. aotearoa* in having long apical appendages (*N. petila*: (21–)22–29(–33) *vs. N. aotearoa*: (3–)5–12(–13)) and differs from *N. piceana* by having 2–3 apical appendages (*N. piceana* containing only 3 appendages) and short basal appendage (*N. petila*: (2–)3–8(–9) *vs. N. piceana*: 6–23) and larger conidia than *N. samarangensis* (*N. petila*: (20–)21–26.5(–27.5) × (5.6–)6–7(–7.8) *vs. N. samarangensis*: 18–21 × 6.5–7.5) (Maharachchikumbura et al. 2014, 2013). The morphological differences between *N. petila* and *N. brachiata* have been mentioned above as notes of *N. brachiata*. Thus, *N. petila* is considered a novel species.

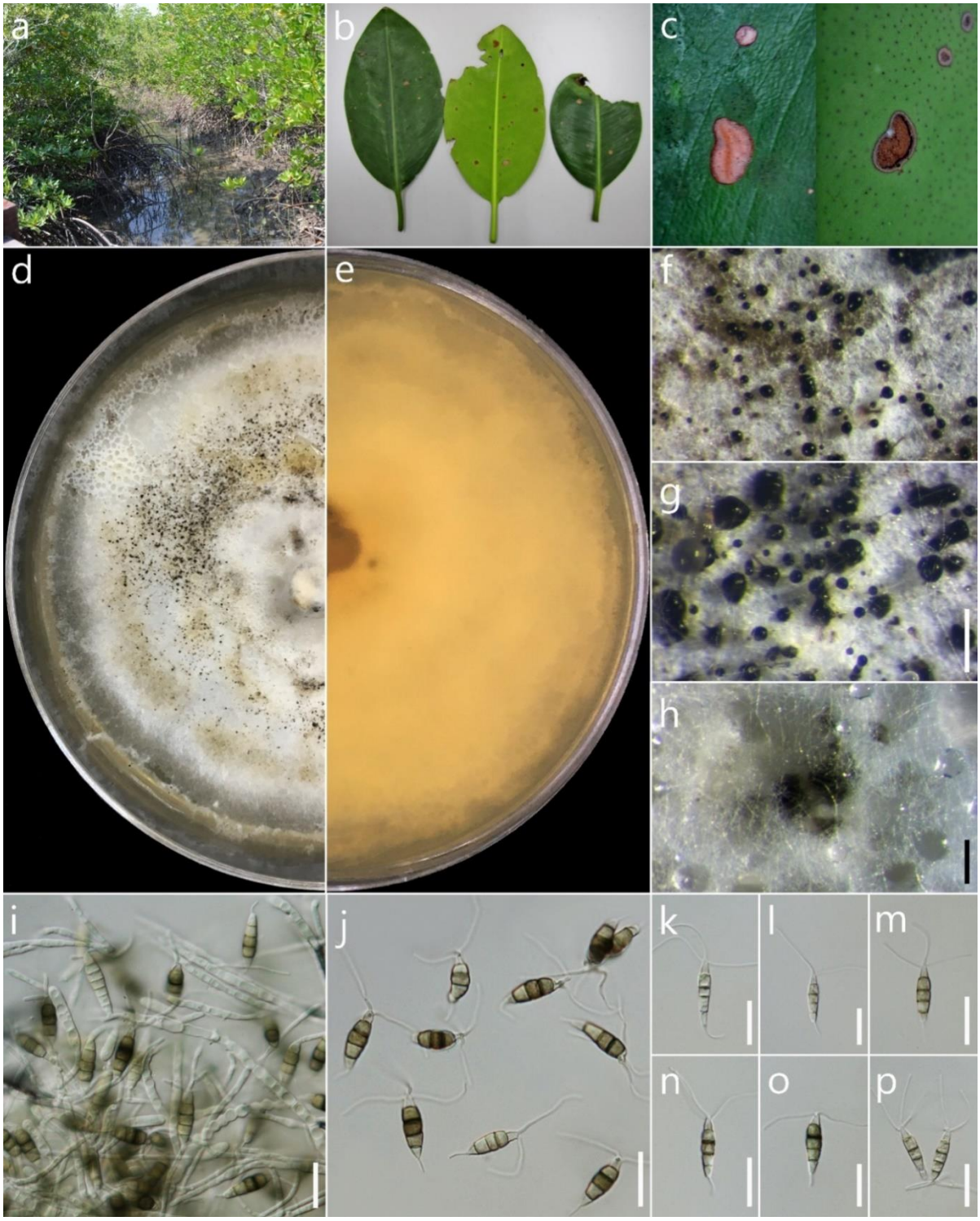


Figure 21 – *Neopestalotiopsis petila* (MFLU 19-0777, holotype). a Habitat. b, c Leaf spots on *Rhizophora mucronata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g–h = 200 µm, i–p = 20 µm.

Neopestalotiopsis rhizophorae Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556437; Facesoffungi number: FoF 05773 Fig. 22

Etymology – refers to the host from which the fungus was collected, *Rhizophora mucronata* Lam.

Holotype – MFLU 19-0779

Associated with leaf spots of *Rhizophora mucronata* Lam. *Symptoms* irregular to subcircular shape, brown, slightly sunken spots appear on adaxial surface leaves of *R. mucronata*, which later expand outwards on the surface of the leaves. Small auburn spots appeared initially and then gradually enlarged, changing to tawny circular ring spots with a dark mahogany border and jagged edge. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves. Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* distinct. *Conidiogenous cells* discrete or integrated, ampulliform, clavate or subcylindrical, hyaline, smooth, 8–20 × 4–8 μm. *Conidia* (20–)20.5–27(–27.5) × (6–)6.5–7.5(–8) μm (mean ± SD = 24.5 ± 0.3 × 7.2 ± 0.6 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (2–)3–4(–5) μm long (mean ± SD = 3.7 ± 0.6 μm); three median cells (14–)15.5–17(–19.5) μm long (mean ± SD = 16.7 ± 1.4 μm), brown, septa and periclinal walls darker than restof the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–7) μm long (mean ± SD = 5.9 ± 0.7 μm); third cell brown, (4–)5–7(–10) μm long (mean ± SD = 5.2 ± 0.6 μm); fourth cell brown, (4–)5–5.5(–6.5) μm long (mean ± SD = 5.5 ± 0.6 μm); apical cell (3–)3.5–4(–5.5) μm long (mean ± SD = 4.1 ± 0.7 μm), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (6–)12.5–22(–24) μm long (mean ± SD = 17.5 ± 4.4 μm); single basal appendage, tubular, unbranched, centric, with or without knob (2.5–)3–9.5(–10) μm long (mean ± SD = 5.2 ± 1.8 μm).

Culture characteristics – Colonies on PDA reaching 7–8 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Ngao, Ranong Province, Ngao Mangrove Forest Research Centre, leaf spots of *Rhizophora mucronata*, 6 December 2016, Norphanphoun Chada NG16a (MFLU 19-0779 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1550, TNCC. THAILAND, Ngao, Ranong Province, Ngao Mangrove Forest Research Centre, leaf spots of *Rhizophora mucronata*, 6 December 2016, Norphanphoun Chada NG16b (MFLU 19-0780 dried culture, paratype); ex-type-living cultures, MFLUCC 17-1551.

Notes – The new species *Neopestalotiopsis rhizophorae* (MFLUCC 17-1550, MFLUCC 17-1551) is isolated from *R. mucronata*, from Ranong province in Thailand. *Neopestalotiopsis rhizophorae* is most similar to *N. petila* and *N. thailandica* (this study) in its conidial size (*N. petila*: (20–)21–26.5(–27.5) × (5.5–)6–7(–8) μm, *N. thailandica*: (20–)21–25(–25.5) × (5.5–)6–7(–7.5) μm) (Table 8). However, based on combined gene phylogenetic analysis, *N. rhizophorae* is separated from *N. petila* and *N. thailandica*, and nested between these two species; *N. alpapicalis* (MFLUCC 17-2544, MFLUCC 17-2545), the collections from *R. mucronata* in Phuket, Thailand and *N. rosicola* (strain CFCC 51992, CFCC 51993), the collections from *Rosa chinensis* in China (Fig. 18). *Neopestalotiopsis rhizophorae* can be

distinguished from *N. alpapicalis* by larger conidia and longer appendages (*N. alpapicalis*: conidial size 14–22.5 × 5–7 µm, apical appendages 5.5–15 µm, basal appendages 3–6.5 µm, Table 8) with polymorphic nucleotide differences of β-tubulin (5- bp) and EF1α (3- bp) sequence data. It differs from *N. rosicola* by ITS (4-bp) and EF1α (4-bp) sequence data.

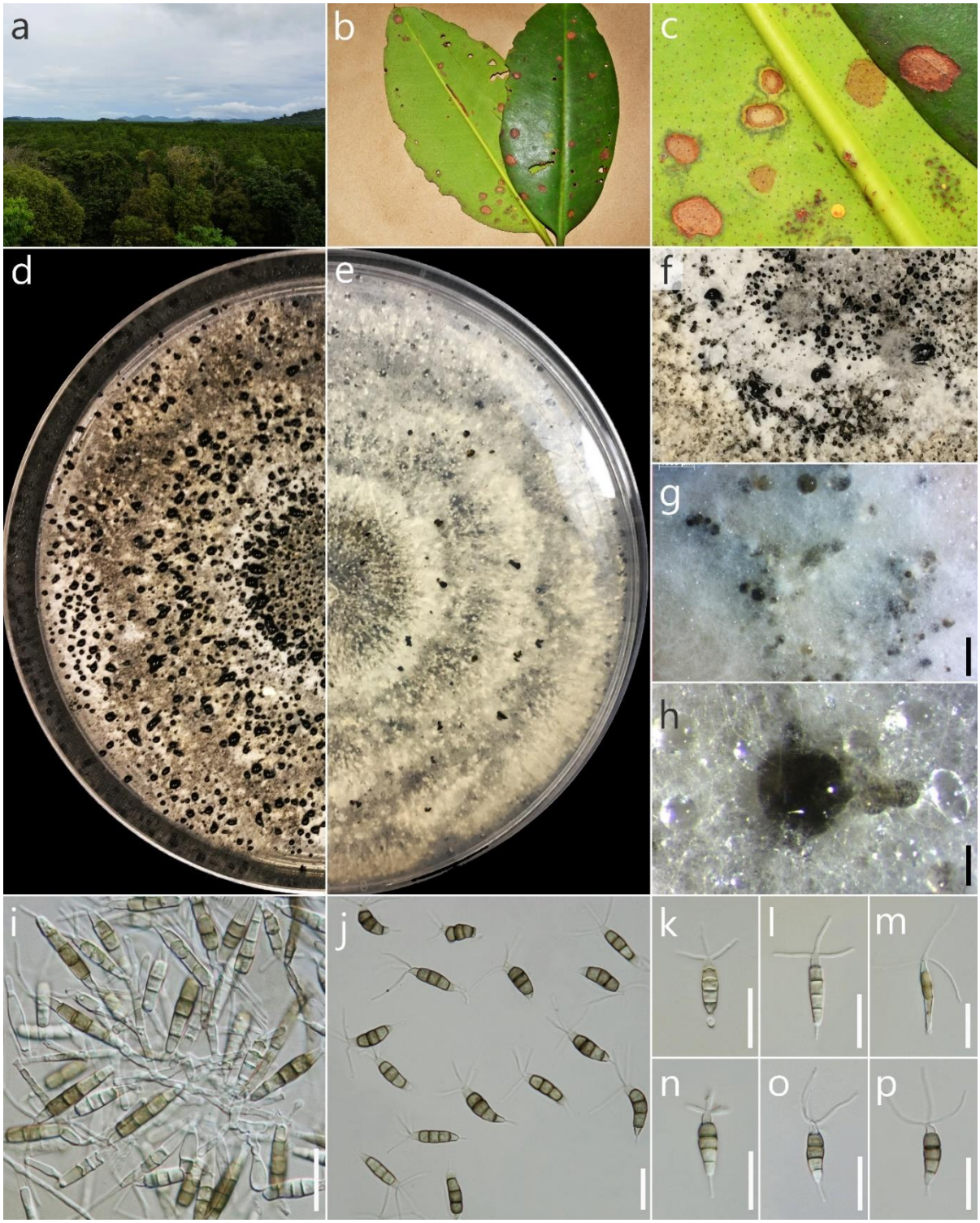


Figure 22 – *Neopestalotiopsis rhizophorae* (MFLU 19-0779, holotype)**.** a Habitat**.** b, c Leaf spots on *Rhizophora mucronata***.** d, e Culture on PDA (d- above, e- reverse)**.** f– h Colony

sporulating on PDA**.** i Conidiogenous cells giving rise to conidia**.** j–p Conidia**.** Scale bars**:** g = 1000 μm, h = 100 μm, i = 50 μm, j–p = 20 μm**.**

Neopestalotiopsis sonneratae Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556438; Facesoffungi number: FoF 05774 Fig. 23

Etymology – refers to the host from which the fungus was isolated, *Sonneronata alba* L.

Holotype – MFLU 19-0781

Associated with leaf spots on *Sonneronata alba* L. *Symptoms* small circular shape, auburn, slightly spots appear on adaxial surface leaves of *S. alba*, which later expand outwards on the surface of the leaves. Small auburn spots appeared initially and then gradually enlarged, changing to dark brown circular spots and border with blurred edge. They were usually > 5 circulars, which occurred on a single affected leaf. Asexual morph: *Conidiomata* pycnidial, 200–400 μm diam, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, 3–8 × 2–6 μm, proliferating one time percurrently, collarette present and not flared. *Conidia* (21.5–)24–26(–28) × 7–7.5(–8), (mean ± SD = 24 ± 1.6 × 7.51 ± 0.4 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (2–)3–3.5(–4) μm long (mean ± SD = 3 ± 0.6); three median cells (14.5–)15–16.5(–17.5) μm long (mean ± SD = 15.8 ± 0.9), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–7) μm long (mean ± SD =5.6 ± 0.8 μm); third cell brown, (4–)5–5.5(–6) μm long (mean ± SD = 5.2 ± 0.7 μm); fourth cell brown, (4–)5–6(–7) μm long (mean ± SD = 5.1 ± 0.7 μm); apical cell (3.5–)4–4.5(–5) μm long (mean ± SD =4 ± 0.6 μm), hyaline, conic to acute; with 1–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (5.5–)7–8(–14) μm long (mean ± SD = 8.5 ± 2); single basal appendage, tubular, unbranched, centric, (2.5–)3–4(–5) μm long (mean ± SD = 3.4 ± 0.9) long.

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), moderate-to-strongly fluffy, fluffy to floccose, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Kor Chang, Trat Province, leaf spots of *Sonneronata alba*, 27 April 2017, Norphanphoun Chada KC01-1 (MFLU 19-0781 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1744, TNCC. THAILAND, Kor Chang, Trat Province, leaf spots of *Sonneronata alba*, 27 April 2017, Norphanphoun Chada KC01-2 (MFLU 19-0782 dried culture, paratype); ex-type-living cultures, MFLUCC 17-1745.

Notes – *Neopestalotiopsis sonneratae* was isolated from a leaf spot of *Sonneronata alba* in Thailand. In the phylogenetic analyses of combined genes, *N. sonneratae* forms a sister group to *N. coffea-arabicae* (strain HGUP4015) and *N. thailandica* (in this study) (Fig. 18). However, there are significant differences in morphological characteristics; *N. coffea-arabicae* and *N. thailandica* conidia are smaller (*N. coffea-arabicae*: 16–20 × 5–7 μm, *N. thailandica*: (20–)21–25(–25.5) × (5.7–)6–7(–7.3) μm) with both species containing longer apical appendages (*N. coffea-arabicae*: 11–16 μm, *N. thailandica*: (30–)32.5–38(–40) μm) and *N. thailandica* having two apical appendages (Song et al. 2013).

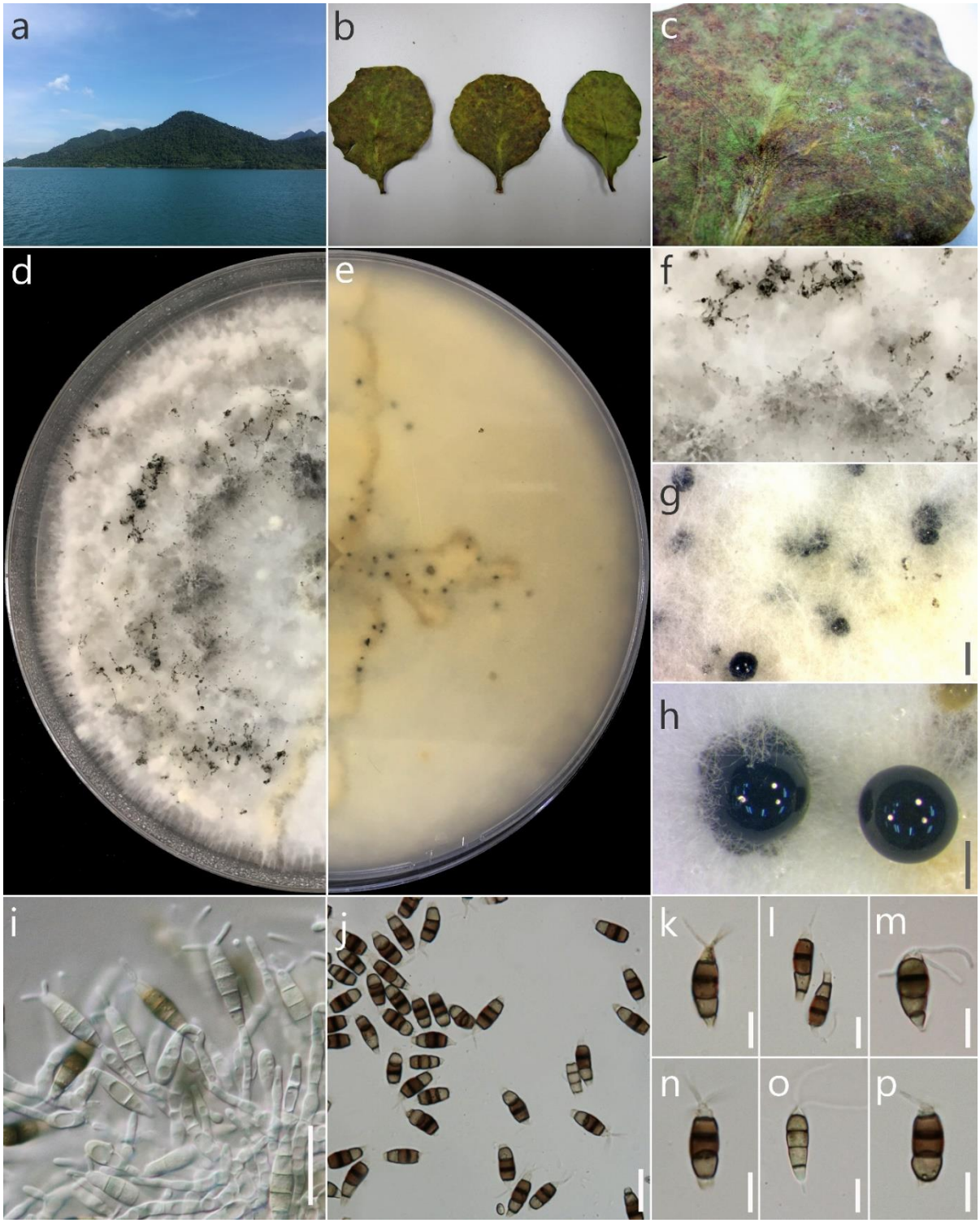


Figure 23 – *Neopestalotiopsis sonneratae* (MFLU 19-0781, holotype)**.** a Habitat**.** b, c Leaf spots on *Sonneronata alba***.** d, e Culture on PDA (d**-**above, e**-**reverse)**.** f–h Colony sporulating on PDA**.** i Conidiogenous cells giving rise to conidia**.** j–p Conidia**.** Scale bars**:** g = 1000 µm, h = 500 µm, i, j = 20 µm, k–p = 10 µm**.**

Neopestalotiopsis thailandica Norphanphoun & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556439; Facesoffungi number: FoF 05775 Fig. 24

Etymology – refers to the country where the fungus was collected, Thailand.

Holotype – MFLU 19-0783

Associated with leaf spots of *Rhizophora mucronata* Lam. *Symptoms* small irregular to subcircular shape, brown, slightly sunken spots appear on adaxial surface leaves of *R. mucronata*, which later expand outwards on the surface of the leaves. Small auburn spots appearedinitially and then gradually enlarged, changing to tawny circular ring spots with a dark mahogany border and jagged edge. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves. Asexual morph: *Conidiomata* pycnidial, globose, brown, semi- immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* distinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin- walled, proliferating 1–2 times percurrently, collarette present and not flared. *Conidia* (20–)21–25(–25.5) × 6–7(–7.5) μm (mean ± SD = 22.6 ± 1.3 × 6.6 ± 0.5 μm), fusiform to clavate, straight to slightly curved, 4(–7)-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (2.5–)3–4(–4.5) μm long (mean ± SD = 3.7 ± 0.5 μm); threemedian cells (12–)12.5–15(–16) μm long (mean ± SD = 14.2 ± 0.9 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4–)4.5–5(–5.5) μm long (mean ± SD = 4.6 ± 0.3 μm); third cell brown, (3.5–)11–26(–27.5) μm long (mean ± SD = 4.5 ± 0.4 μm); fourth cell brown, (4–)5–5.5(–6) μm long (mean ± SD = 5.1 ± 0.6 μm); apical cell (3.5–)4–5.5(–6) μm long (mean ± SD = 4.7 ± 0.6 μm), hyaline, conic to acute; with 1–2 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (30–)32.5–38(–40) μm long (mean ± SD = 34.5 ± 3.7 μm); single basal appendage, tubular, unbranched, centric, (3–)6–9(–10) μm long (mean ± SD = 7.6 ± 2.2 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Kor Chang, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC11-1 (MFLU 19-0783 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1730, TNCC.THAILAND, Kor Chang, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC11-2 (MFLU 19-0784 dried culture, paratype); ex-type-living cultures, MFLUCC 17-1731.

Notes – The collections (MFLUCC 17-1730, MFLUCC 17-1731) were observed and introduced as *N. thailandica*. The new species is introduced with the type from *Rhizophora mucronata* from Trat Province in Thailand. *Neopestalotiopsis thailandica* is most similar to *N. petila* and *N. rhizophorae* (both species are introduced in this study) with its conidial size. The morphological differences between *N. petila* and *N. rhizophorae* have been mentioned above as notes of *N. rhizophorae* (Table 8). However, based on the combined gene phylogenetic analysis, *N. thailandica* is separated from other species in the genus and is sister to *N. coffea-arabicae* (HGUP4015, Song et al. 2013), which was isolated from leaves of *Coffea arabica* L. and *N. sonneratae* (in this study) from leaf spots of *Sonneronata alba* (Fig. 18). However, their morphology is different. *Neopestalotiopsis thailandica* differs from *N. coffea-arabicae* by having larger conidia (*N. thailandica*: (20–)21–25(–25.5) × 6–7(–7.5) *vs. N. coffea-arabicae*:16–20 × 5–7)), and longer apical appendages (*N. thailandica*:(30–)32.5–38(–40) *vs.*

N. coffea-arabicae:11–16) (Table 4). The morphological differences between *N. thailandica* and *N. sonneratae* are mentioned above under the notes of *N. sonneratae*. Thus, based on morphology and phylogeny it is considered that *N. thailandica* is a novel species.

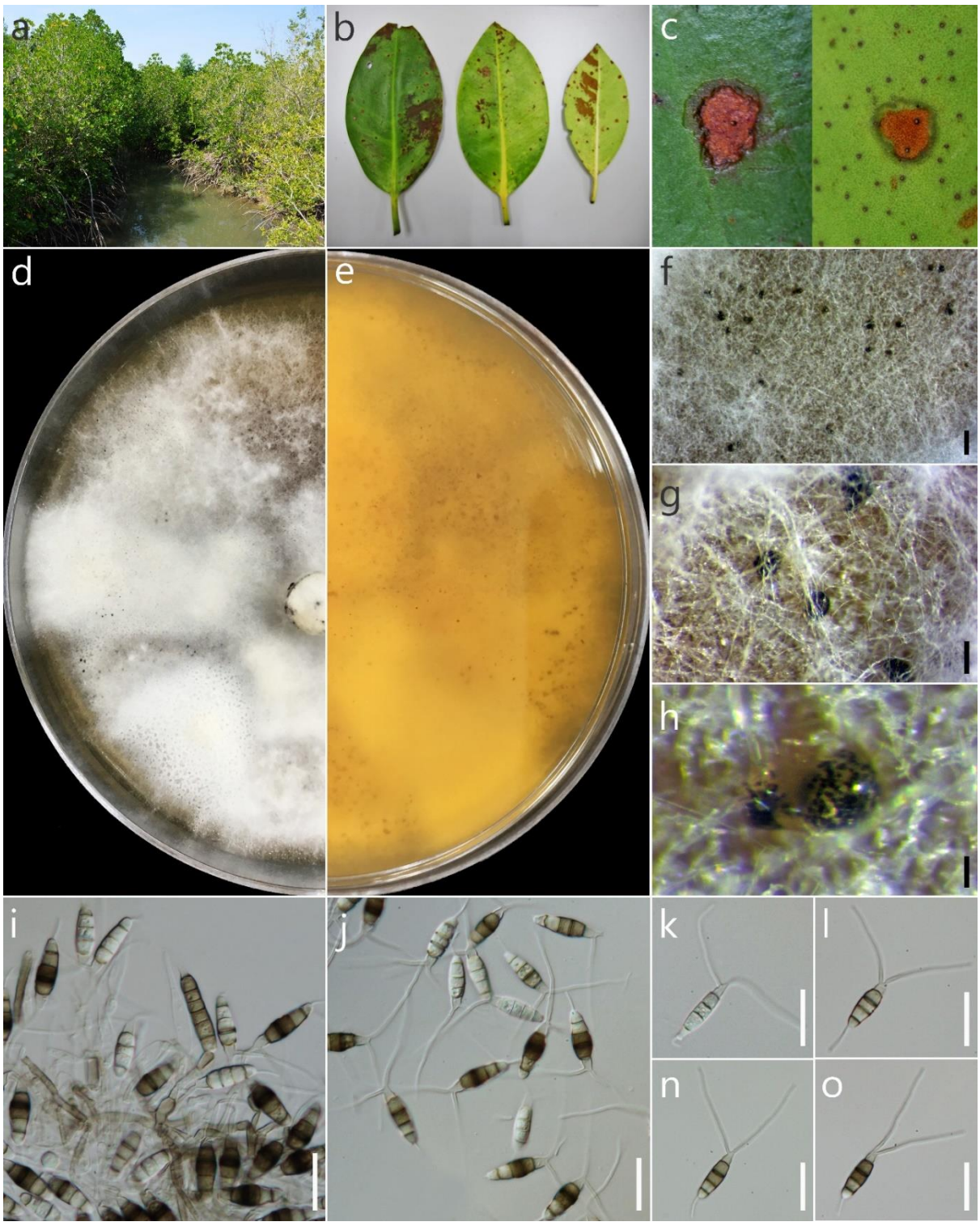


Figure 24 – *Neopestalotiopsis thailandica* (MFLU 19-0783, holotype)**.** a Habitat**.** b, c Leaf spots on *Rhizophora mucronata***.** d, e Culture on PDA (d**-** above, e**-** reverse)**.** f–h Colony sporulating on PDA**.** i Conidiogenous cells giving rise to conidia**.** j–o Conidia**.** Scale bars**:** f = 500 µm, g = 200 µm, h = 50 µm, i–o = 20 µm.

Pestalotiopsis Steyaert, Bull. Jard. bot. État Brux. 19: 300 (1949)

Index Fungorum number: IF9272

Type species: *Pestalotiopsis guepinii* (Desm.) Steyaert [as 'guepini'], Bull. Jard. bot. État Brux. 19(3): 312 (1949)

The phylogenetic tree, Fig. 25 (*Pestalotiopsis*) comprised 70 taxa with the outgroup, *Neopestalotiopsis saprophytica* (MFLUCC 12-0282). The total length of the dataset was 1440 characters including alignment gaps, 1–380, 381–538, 539–779, 780–979, 980–1147, and 1148–1440 corresponding to ITS1+ITS2, 5.8S, β-tubulin (exon), β-tubulin (intron), EF1α (exon), and EF1α (intron), respectively. The combined dataset contained 887 constant, 174 parsimony uninformative and 379 parsimony informative characters. The combined dataset was analyzed using MP, ML and BI. The trees generated under different optimality criteria were essentially similar in topology and did not differ significantly (data not shown). The descriptive statistics generated from MP analysis based on the combined dataset of ITS1+ITS2, 5.8S, β-tubulin (exon), β-tubulin (intron), EF1α (exon), and EF1α (intron) were TL = 1664, CI = 0.510, RI = 0.685, RC = 0.349, HI = 0.490. The best scoring likelihood tree selected with a final value for the combined dataset = -10362.397645.

Pestalotiopsis rhizophorae Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556440; Facesoffungi number: FoF 05781 Fig. 26

Etymology – refers to the host from which the fungus was isolated, *Rhizophora apiculate* Blume.

Holotype – MFLU 19-0785

Associated with leaf spots of *Rhizophora apiculata* Blume. *Symptoms* small irregular spots shape, rufous, slightly sunken spots adaxial surface leaves of *R. apiculata*, which later expand outwards on the surface of the leaves. Small rufous spots appeared initially and then gradually enlarged, changing to tawny circular ring spots with a dark brown border and smooth edge. They were usually few circulars, which occurred on a single affected leaf. Asexual morph: *Conidiomata* 20–70 μm diam, pycnidial, globose, brown, semi-immersed on PDA, rreleasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, 10–20 × 1–2 μm, proliferating 1–2 times percurrently, collarette present and not flared. *Conidia* (17–)17.5–23(–23.5) × (5.5–)6–6.5(–7) μm (mean ± SD = 20 ± 1.6 × 6.3 ± 0.5 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (2–)3–3.5(–5) μm long (mean ± SD = 3.7 ± 0.7 μm); three median cells (11–)11.5–14(–14.5) μm long (mean ± SD = 13.2 ± 1.2 μm), brown, septa and periclinal walls darker than rest of the cell, concolorous, wall rugose; second cell from base pale brown, (3.5–)4–5(–5.5) μm long (mean ± SD = 4.6 ± 0.6 μm); third cell brown, (3–)4–4.5(–5) μm long (mean ± SD = 4.1 ± 0.6 μm); fourth cell brown, (3.5–)4–5(–5.5) μm long (mean ± SD = 4.6 ± 0.5 μm); apical cell (1.8–)2–3(–4.5) μm long (mean ± SD = 3.1 ± 0.5 μm), hyaline, conic to acute; with 1–2 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (7.5–)8–13(–14.5) μm long (mean ± SD = 11.4 ± 2.4 μm); single basal appendage, tubular, unbranched, centric, 1.5–4.5(–5) μm long (mean ± SD = 2.5 ± 1.1 μm).

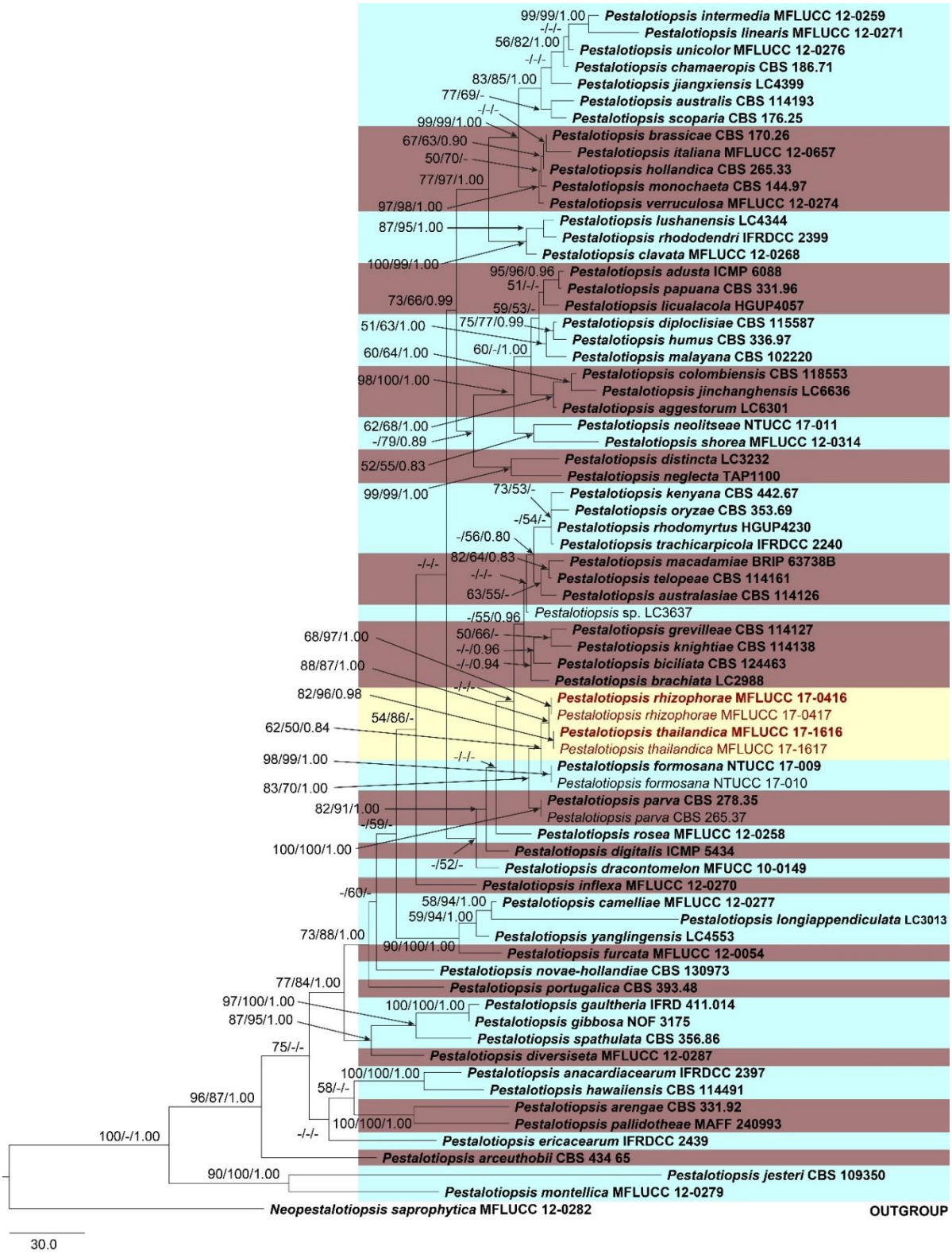


Figure 25 – One of the 1000 most parsimonious trees obtained from a heuristic search of combined ITS, β-tubulin and EF1α sequence data for the genus *Pestalotiopsis*. The tree is rooted to *Neopestalotiopsis saprophytica* (MFLUCC 12-0282). Maximum parsimony and maximum likelihood bootstrap values ≥ 50%. Bayesian posterior probabilities ≥ 0.90 (MPBS/MLBS/PPBY) are given at the nodes. The species obtained in this study are in red font. Ex-type taxa from other studies are in black bold.

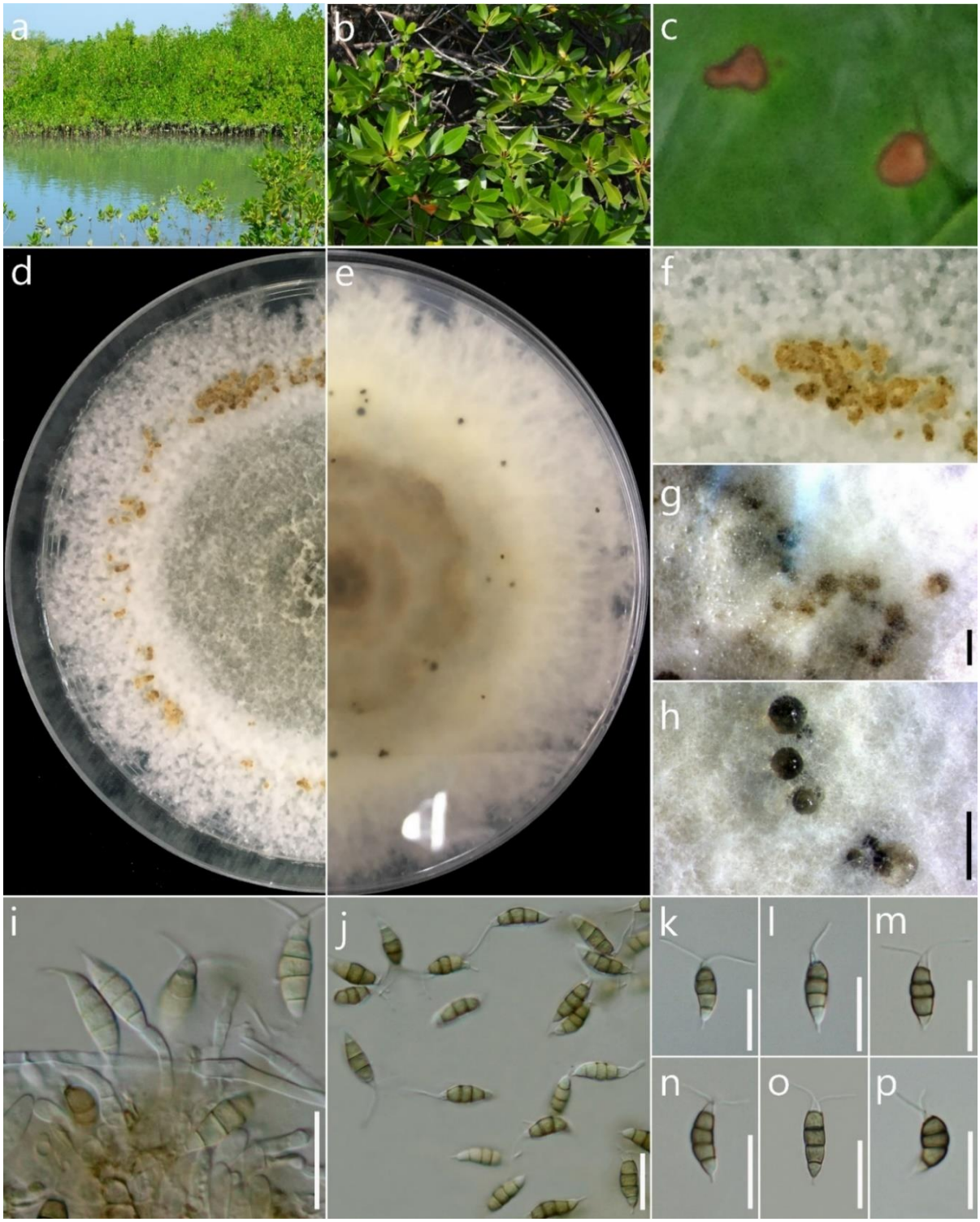


Figure 26 – *Pestalotiopsis rhizophorae* (MFLU 19-0785, holotype). a Habitat b, c Leaf spots of *Rhizophera apiculata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f–h = 100 µm, i–p = 20 µm.

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium

dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, The Sirindhorn International Environmental Park, Cha-am District, Phetchaburi Province, leaf spots on *Rhizophora apiculata*, 30 November 2016, Norphanphoun Chada NNS28a (MFLU 19-0785, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-0416, TNCC. THAILAND, The Sirindhorn International Environmental Park, Cha-am District, Phetchaburi Province, leaf spots on *Rhizophora apiculata*, 30 November 2016, Norphanphoun Chada NNS28b (MFLU 19-0786, paratype); living cultures, MFLUCC 17-0417.

Notes – *Pestalotiopsis rhizophorae* formed a distinct clade in the multi-locus tree and is sister to *P. formosana* (strain NTUCC 17-009), *P. parva* (strain CBS 265.37) and *P thailandica* (in this study) (Fig. 25). *Pestalotiopsis rhizophorae* differs from *P thailandica* and *P. formosana* by shorter conidia (*P. rhizophorae*: (17–)17.5–23(–23.5) µm *vs. P thailandica*: (17–)17.5–28(–29) µm, *P. formosana*: (15–)18–22(–26) × (5–)6–7 µm); shorter apical appendages (*P. rhizophorae*: (7.5–)8–13(–14.5) µm *vs. P thailandica*: (5.5–)11–34(–38) µm) and basal appendages (*P. rhizophorae*: (1.3–)1.5–4.5(–5) µm *vs. P thailandica*: (2–)2.5–9.5(–10) µm, *P. formosana*: (2–)3–5(–6) µm) (Table 9, Ariyawansa and Hyde 2018, Maharachchikumbura et al. 2014). *Pestalotiopsis rhizophorae* is similar to *P. parva* in conidial size (*P. parva*: (16–)16.5–20(–21) × 5–7(–7.5) µm) but differs by having 1–2 apical appendages (Table 9, Ariyawansa and Hyde 2018, Maharachchikumbura et al. 2014). The two species (*P. rhizophorae*, *P. thailandica*) are also different in four base pairs in ITS, one base pair in β-tubulin and EF1α. Therefore, the collection in the present study is designated as a new species.

Pseudopestalotiopsis Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 180 (2014)

Index Fungorum number: IF809753

Type species: *Pseudopestalotiopsis theae* (Sawada) Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 183 (2014)

The phylogenetic tree, Fig. 27 comprised 27 taxa, with *Neopestalotiopsis natalensis* (CBS 138.41) as the outgroup taxon. The total length of the dataset was 1404 characters including alignment gaps, 1–335, 336–493, 494–742, 743–925, 926–1093 and 1094–1404 corresponding to ITS1+ITS2, 5.8S, β-tubulin (exon), β-tubulin (intron), EF1α (exon), and EF1α (intron), respectively). The combined dataset contained 1122 constant, 193 parsimony uninformative and 89 parsimony informative characters. The combined dataset was analyzed using MP, ML and BI. The trees generated under different optimality criteria were essentially similar in topology and did not differ significantly (data not shown). The descriptive statistics generated from MP analysis based on the combined dataset of ITS1+ITS2, 5.8S, β-tubulin (exon), β-tubulin (intron), EF1α (exon), and EF1α (intron) were TL = 386, CI = 0.832, RI = 0.823, RC = 0.685, HI = 0.168. The best scoring likelihood tree selected with a final value for the combined dataset = -3905.071762.

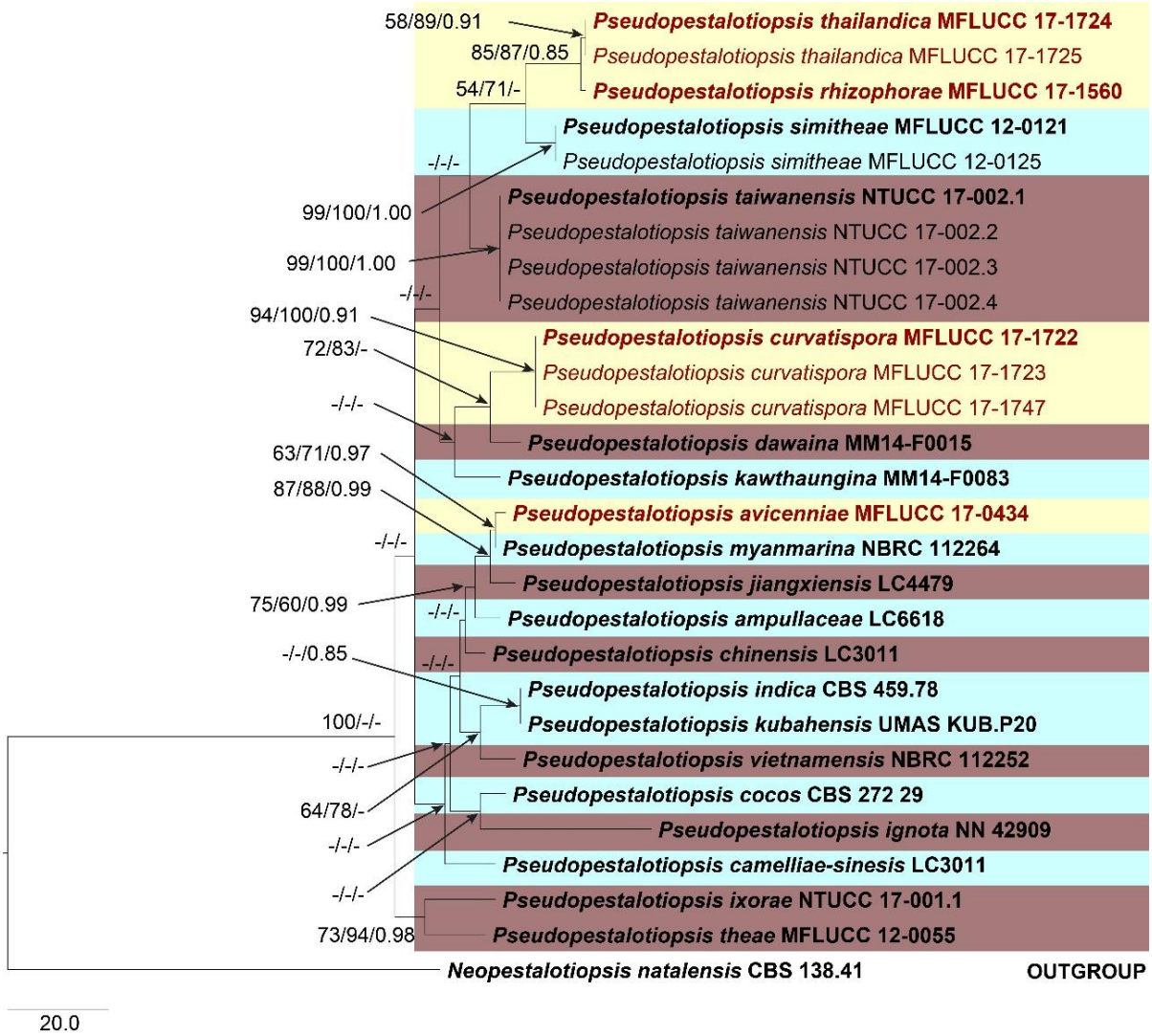


Figure 27 – One of the 93 most parsimonious trees obtained from a heuristic search of combined ITS, β-tubulin and EF1α sequence data of the genus *Pseudopestalotiopsis***.** The tree is rooted with *Neopestalotiopsis natalensis* (CBS 138.41). Maximum parsimony and maximum likelihood bootstrap values ≥ 50**%** , Bayesian posterior probabilities ≥ 0.90 (MPBS/MLBS/PPBY) are given at the nodes. The species obtained in this study are in red font. Ex-type taxa from other studies are in black bold.

Pseudopestalotiopsis avicenniae Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556442; Facesoffungi number: FoF 05776 Fig. 28

Etymology – refers to the host from which the fungus was isolated, *Avicennia marina* (Forssk.) Vierh.

Holotype – MFLU 19-0789

Associated with leaf spots of *Avicennia marina* (Forssk.) Vierh. *Symptoms* circular to irregular, grayish-brown, slightly sunken spots adaxial surface leaves of *A. marina*, which later expand outwards on the surface of the leaves (Fig. 12c). Small black spots appeared initially and then gradually enlarged, changing to beige circular ring spots with a black border and smooth edge. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves (Fig. 12b). Asexual morph: *Conidiomata* 300–1000 μm diam, pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, 3–6 × 2–56 μm, proliferating 1–2 times percurrently, collarette present and not flared. *Conidia* (22–)22.5–26.5(–27) × (5–)5.5–6(–6.5) μm (mean ± SD = 23.9 ± 1.4 × 6 ± 0.4 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (3–)4–4.5(–6) μm long (mean ± SD = 4 ± 0.7 μm); three median cells (15–)15.5–17(–18) μm long (mean ± SD = 16.3 ± 0.8 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–6.5) μm long (mean ± SD = 5.5 ± 0.5 μm); third cell brown, (4.5–)5–5.5(–6) μm long (mean ± SD = 4.9 ± 0.4 μm); fourth cell brown, (5–)5.5–6(–7) μm long (mean ± SD = 5.9 ± 0.5 μm); apical cell (2–)2.5–4(–5) μm long (mean ± SD = 3.5 ± 1.0 μm), hyaline, conic to acute; with 1–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (14–)15.5–28.5(–35.5) μm long (mean ± SD = 21.3 ± 5.9 μm); single basal appendage, tubular, unbranched, centric, (2–)3–4(–4.5) μm long (mean ± SD = 3.1 ± 0.7 μm).

Culture characteristics – Colonies on PDA reaching 5–7 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, The Sirindhorn International Environmental Park, Cha-am, Cha-am District, Phetchaburi Province, leaf spots of *Avicennia marina*, 30 November 2016, Norphanphoun Chada NNS05-1 (MFLU 19-0789, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-0434, TNCC.

Notes – The combined phylogenetic tree showed that *Ps. avicenniae* is sister to *Ps. jiangxiensis* (strain LC4479) and *Ps. elaeidis* (=*Ps. myanmarina*, strain NBRC 112264) (Fig. 27, Liu et al. 2017, Nozawa et al. 2017). *Pseudopestalotiopsis avicenniae* is morphologically similar to *Ps. jiangxiensis*, but phylogenetically clearly distinct as an independent lineage. *Pseudopestalotiopsis avicenniae* is phylogenetically closer to *Ps. elaeidis* (strain NBRC 112264), which was collected from *Averrhoa carambola* in Myanmar, but can be distinguished by its larger conidia (*Ps. elaeidis*: 31–38.5 × 6.5–9 μm) and 2–3 apical appendages (Table 10, Nozava et al. 2017).

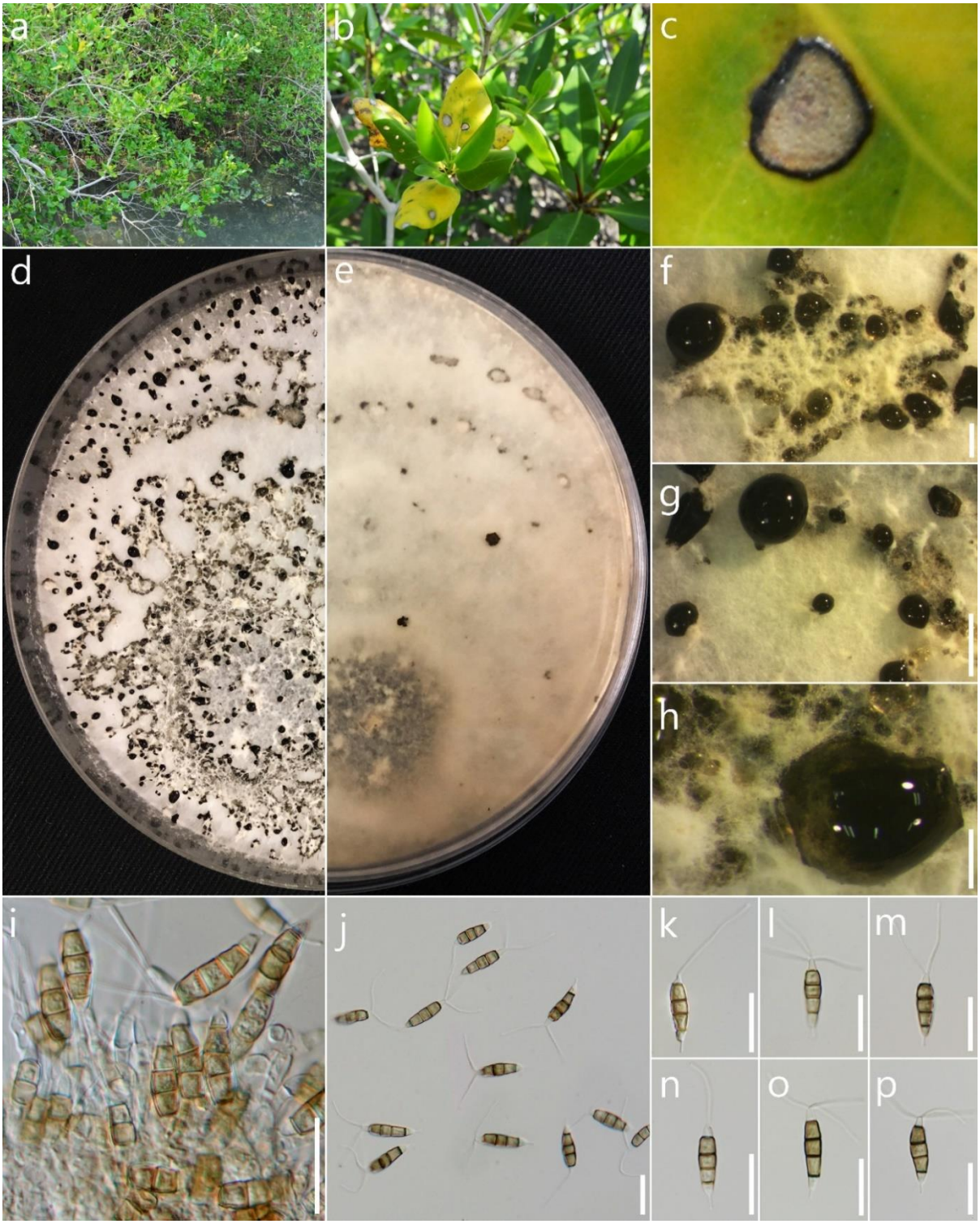


Figure 28 – *Pseudopestalotiopsis avicenniae* (MFLU 19-0789, holotype). a Habitat. b, c Leaf spots on *Avicennia marina*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f = 1000 µm, g = 200 µm, h = 500 µm, i–p = 20 µm.

Pseudopestalotiopsis curvatispora Norphanphoun, T.C. Wen & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556443; Facesoffungi number: FoF 05777 Fig. 29

Etymology – in reference to the Latin word "curvatus" in reference to the shape of the conidia.

Holotype – MFLU 19-0790

Associated with leaf spots of *Rhizophora mucronata* Blume. *Symptoms* small subcircular, grayish brown, slightly sunken spots adaxial surface leaves of *R. mucronata*, which later expand outwards on the surface of the leaves. Small brown spots appeared initially and then gradually enlarged, changing to beige circular ring spots with a dark brown border and smooth edge. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves. Asexual morph: *Conidiomata* 200–400 μm diam, pycnidial, globose, brown, semi-immersed on PDA, rreleasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, 5.5–11 × 3–5.5 μm, proliferating 1–2 times percurrently, collarette present and not flared. *Conidia* (18.5–)22–25(–26.5) × (6–)6.5–7 μm (mean ± SD = 23.5 ± 2 × 6.6 ± 0.4 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (3–)3.5–4(–5) μm long (mean ± SD = 4.3 ± 0.7 μm); three median cells (11–)13–14(–15) μm long (mean ± SD = 13.8 ± 1.1 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, 4–5 μm long (mean ± SD = 4.5 ± 0.5 μm); third cell brown, (3.5–)4–5(–5.5) μm long (mean ± SD = 4.4 ± 0.6 μm); fourth cell brown, (3.5–)4–5(–5.5) μm long (mean ± SD = 4.6 ± 0.6 μm); apical cell (3.5–)4–6(–7) μm long (mean ± SD = 5.1 ± 1.1 μm), hyaline, conic to acute; with 1–2 tubular appendages on an apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched or branched irregularly along their length resulting in 1–2 branches, flexuous, (10–)20–29(–35) μm long (mean ± SD = 22.7 ± 8.1μm); single basal appendage, tubular, unbranched, centric, (5.5–)9–12(–13.5) μm long (mean ± SD = 9.2 ± 2 μm).

Culture characteristics – Colonies on PDA reaching 3–4 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC12-1, (MFLU 19-0791, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1722, TNCC. THAILAND, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC12-2, (MFL 19-0792, paratype); living cultures, MFLUCC 17-1723. THAILAND, Trat Province, leaf spots of *Rhizophora apiculata*, 27 April 2017, Norphanphoun Chada KC18-2 (MFLU 19-0790, paratype); ex-type-living cultures, MFLUCC 17-1747.

Notes – The new species *Pseudopestalotiopsis curvatispora* is introduced, which was isolated from a leaf spot on *Rhizophora mucronata*, with the morphology of curved conidia and flexuous branched apical appendages. Based on combined gene phylogenetic analyses, it showed that *Ps. curvatispora* is nested in between *Ps. simitheae* (MFLUCC 12-0121) and *Ps. thailandica* (in this study), which are morphologically different (Table 10). *Pseudopestalotiopsis simitheae* is (MFLUCC 12-0121), distinct from other species by forming a well-separated clade (Fig. 27). This is also supported by morphological differences (larger conidia (22–30 μm) and 2–4 tubular, shorter apical and basal appendages (apical appendages: 14.5–26.5 μm, basal appendages: 4–6.5 μm, Song et al. 2014)). *Pseudopestalotiopsis thailandica* is different from *Ps. simitheae* by having larger conidia ((24–)24.5–30(–30.5) μm),

longer apical appendages ((26.5–)28–36(–39.5) µm) and shorter basal appendages ((3.5–)4.5–5(–6.5) µm) (Table 6, Song et al. 2014).

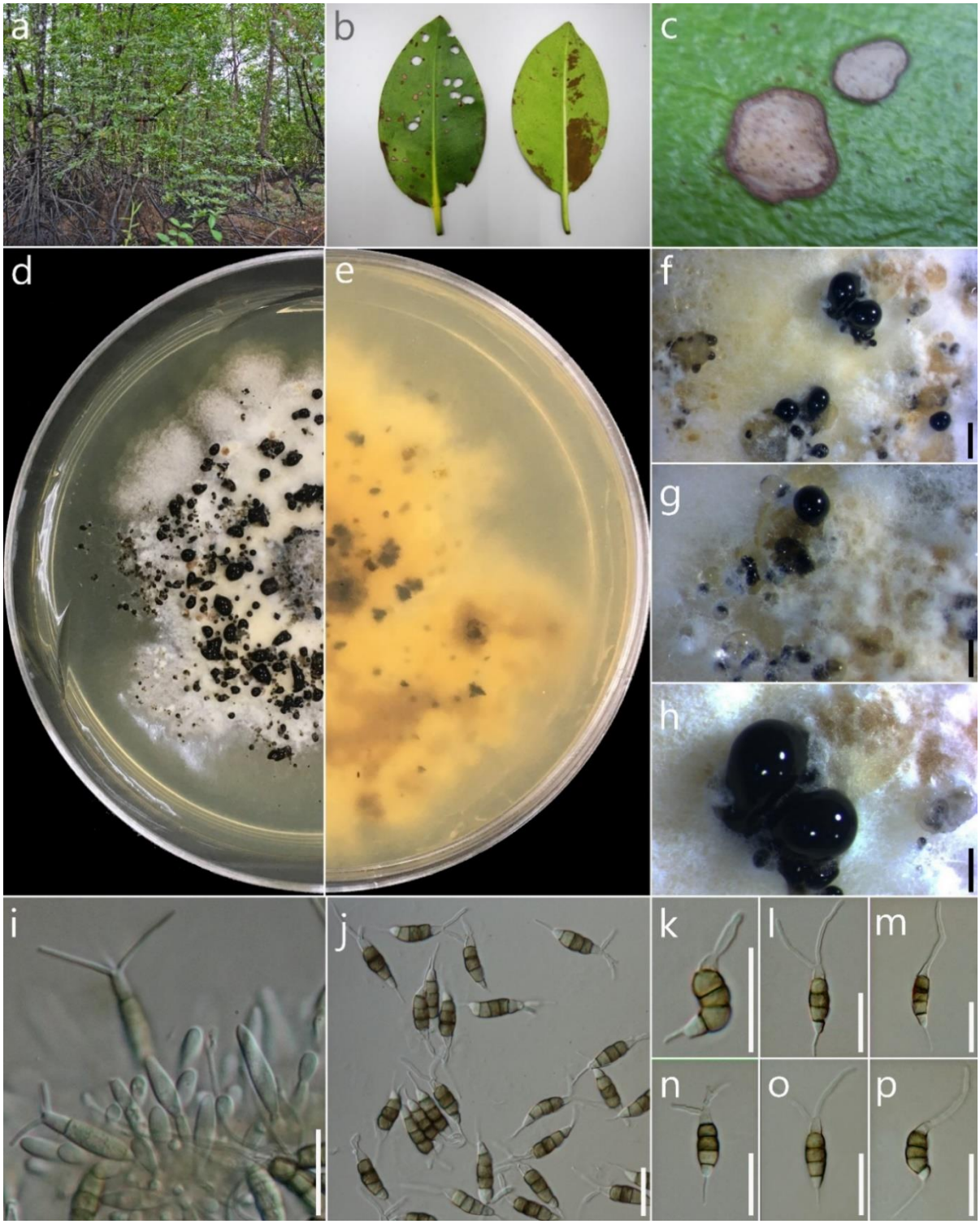


Figure 29 – *Pseudopestalotiopsis curvatispora* (MFLU 19-0791, holotype). a Habitat. b, c Leaf spots of *Rhizophera mucronata*. d, e Culture on PDA (d- above, e- reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f = 1000 µm, g, h = 500 µm, i–p = 20 µm.

Pseudopestalotiopsis rhizophorae Norphanphoun, T.C. Wen & K.D. Hyde**, sp. nov.**

Index Fungorum number: IF556444; Facesoffungi number: FoF 05778 Fig. 30

Etymology – refers to the host from which the fungus was isolated, *Rhizophora apiculata* Blume.

Holotype – MFLU 19-0793

Associated with leaf spots of *Rhizophora apiculata* Blume. *Symptoms* irregular shape, pale brown, slightly sunken spots adaxial surface leaves of *R. apiculata*, which later expand outwards on the surface of the leaves. Small brown spots appeared initially and then gradually enlarged, changing to pale brown irregular spots with auburn border. They were usually >5 circular spots occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves with defected leaves. Asexual morph: *Conidiomata* 100–200 μm diam, pycnidial, globose, brown, semi- immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, 5–8 × 5–6 μm, proliferating 1–2 times percurrently, collarette present and not flared. *Conidia* (23–)24–29.5(–30) × 5–5.5(–6) μm (mean ± SD = 26 ± 2.5 × 5.4 ± 0.4 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (3–)4–5(–6) μm long (mean ± SD = 4.5 ± 0.9 μm); three median cells (14.5–)15–19(–19.5) μm long (mean ± SD = 16.4 ± 2 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4–)5–5.5(–7) μm long (mean ± SD = 5.8 ± 1 μm); third cell brown, 4–5(–5.5) μm long (mean ± SD = 4.7 ± 0.4 μm); fourth cell brown, (4.5–)5.5–6(–7.5) μm long (mean ± SD = 5.9 ± 1.0 μm); apical cell (3.5–)4–5(–7) μm long (mean ± SD = 5.2 ± 0.8 μm), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (16.5–)18–25(–26) μm long (mean ± SD = 22 ± 3.1 μm); single basal appendage, tubular, unbranched, centric, (3–)3.5–6(–7) μm long (mean ± SD = 5 ± 1.2 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Ngao, Ranong Province, Ngao Mangrove Forest Research Centre, leaf spots of *Rhizophora apiculata*, 6 December 2016, Norphanphoun Chada NG38a (MFLU 19-0793, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1560, TNCC.

Notes – *Pseudopestalotiopsis rhizophorae* formed an independent branch in the phylogeny presented here (Fig. 27) and is closely related to *Ps. dawaina* (MM14-F0015) and *Ps. kubahensisa* (UMAS KUB-P20). *Pseudopestalotiopsis rhizophorae* differs from *Ps. dawaina* and *Ps. kubahensisa* in length of appendage in both ends: shorter apical appendage (*Ps. dawaina*: 20.5–33.5 μm, *Ps. kubahensisa*: 16–29.5 μm) and longer basal appendage (*Ps. dawaina*: 2.5–6.5 μm, *Ps. kubahensisa*: 3–6 μm) (Table 10). Thus, *Ps. rhizophorae* is introduced as a new species.

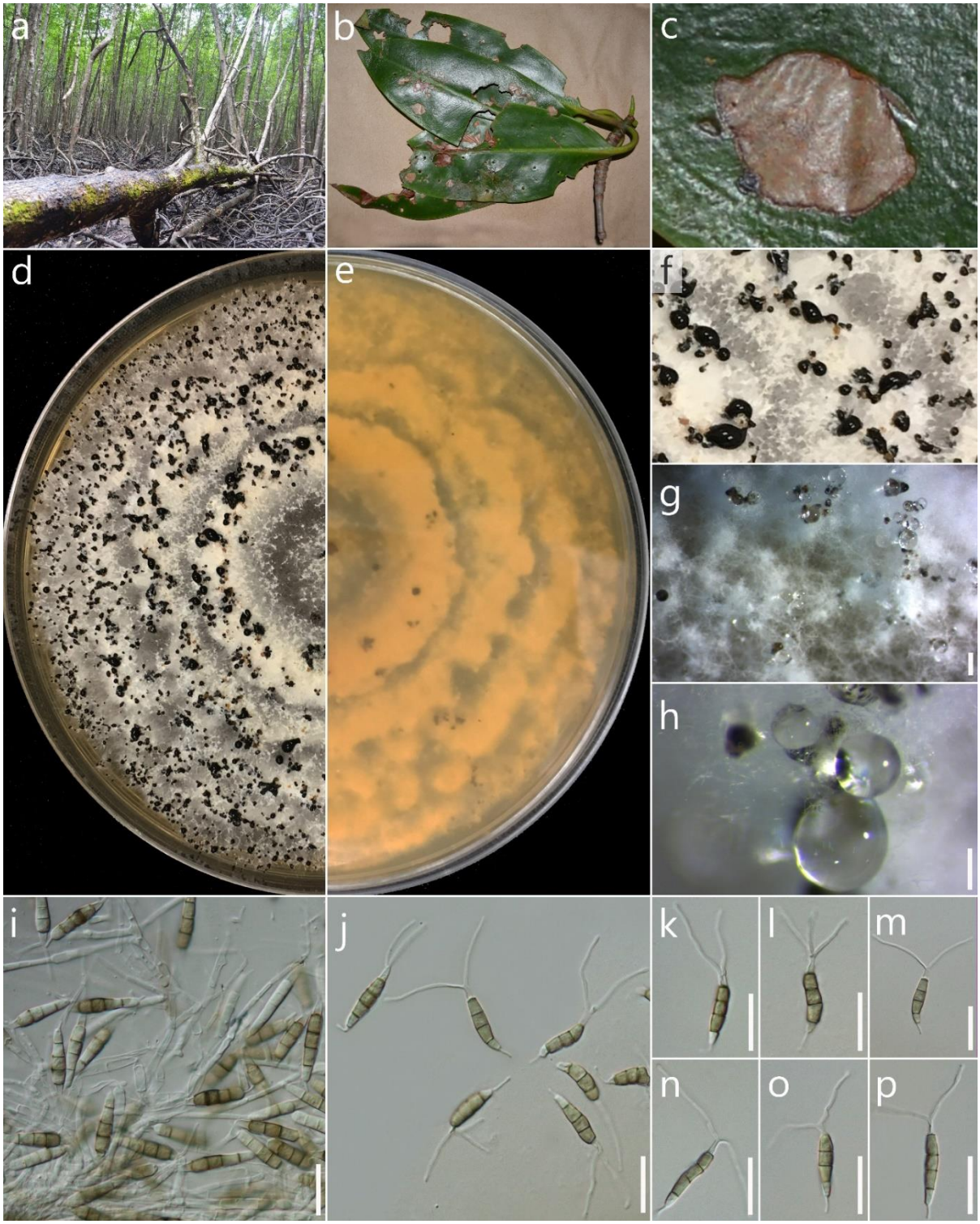


Figure 30 – *Pseudopestalotiopsis rhizophorae* (MFLU 19-0793, holotype). a Habitat. b, c Leaf spots of *Rhizophora apiculata*. d, e Culture on PDA (d- above, e- reverse). f– h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g = 500 µm, h = 200 µm, i–p = 20 µm.

Pseudopestalotiopsis thailandica Norphanphoun & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556445; Facesoffungi number: FoF 05779 Fig. 31

Etymology – refers to the country where the fungus was collected, Thailand.

Holotype – MFLU 19-0794

Associated with leaf spots of *Rhizophora mucronata* Blume. *Symptoms* subcircular to the irregular shape, pale brown, slightly sunken spots adaxial surface leaves of *R. mucronata*, which later expand outwards on the surface of the leaves. Small auburn spots appeared initially and then gradually enlarged, changing to pale-auburn circular ring spots with a dark auburn border. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves. Asexual morph: *Conidiomata* 250–500 μm diam, pycnidial, globose, brown, semi- immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin- walled, proliferating 1–2 times percurrently, collarette present and not flared. *Conidia* (24–)24.5–30(–30.5) × (5–)5.5–6(–6.7) μm (mean ± SD = 26.6 ± 2.2 × 5.9±0.3 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth- walled, (3.5–)4–5(–6.6) μm long (mean ± SD = 4.4 ± 1 μm); three median cells (13.5–)16–18(–19) μm long (mean ± SD = 17.2 ± 1.5 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (5–)5.5–6(–7.5) μm long (mean ± SD = 5.8 ± 0.9 μm); third cell brown, (5–)5.5–6(–6.2) μm long (mean ± SD = 5.6 ± 0.5 μm); fourth cell brown, (5.5–)6–6.5(–7) μm long (mean ± SD = 6.2 ± 0.6 μm); apical cell (3.5–)4.5–5(–7) μm long (mean ± SD = 4.5 ± 1.1 μm), hyaline, conic to acute; with 1–2 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (26.5–)28–36(–39.5) μm long (mean ± SD = 31.3 ± 3.9μm); single basal appendage, tubular, unbranched, centric, (3.5–)4.5–5(–6.5) μm long (mean ± SD = 4.8 ± 1 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Chanthaburi Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC21-1 (MFLU 19-0794, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1724, TNCC. THAILAND, Chanthaburi Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC21-12 (MFLU 19-0795, paratype); ex-type-living cultures, MFLUCC 17-1725.

Notes – The new species, *Pseudopestalotiopsis thailandica* was isolated from a leaf spot from *Rhizophora mucronata* from Chanthaburi Province, Thailand. Based on the combined gene phylogenetic analysis, it showed that *Ps. thailandica* is sister to *Ps. curvatispora* (in this study), and morphology differences as mentioned in the notes of *Ps. curvatispora*. Thus, it is considered that *Ps. thailandica* is a novel species.

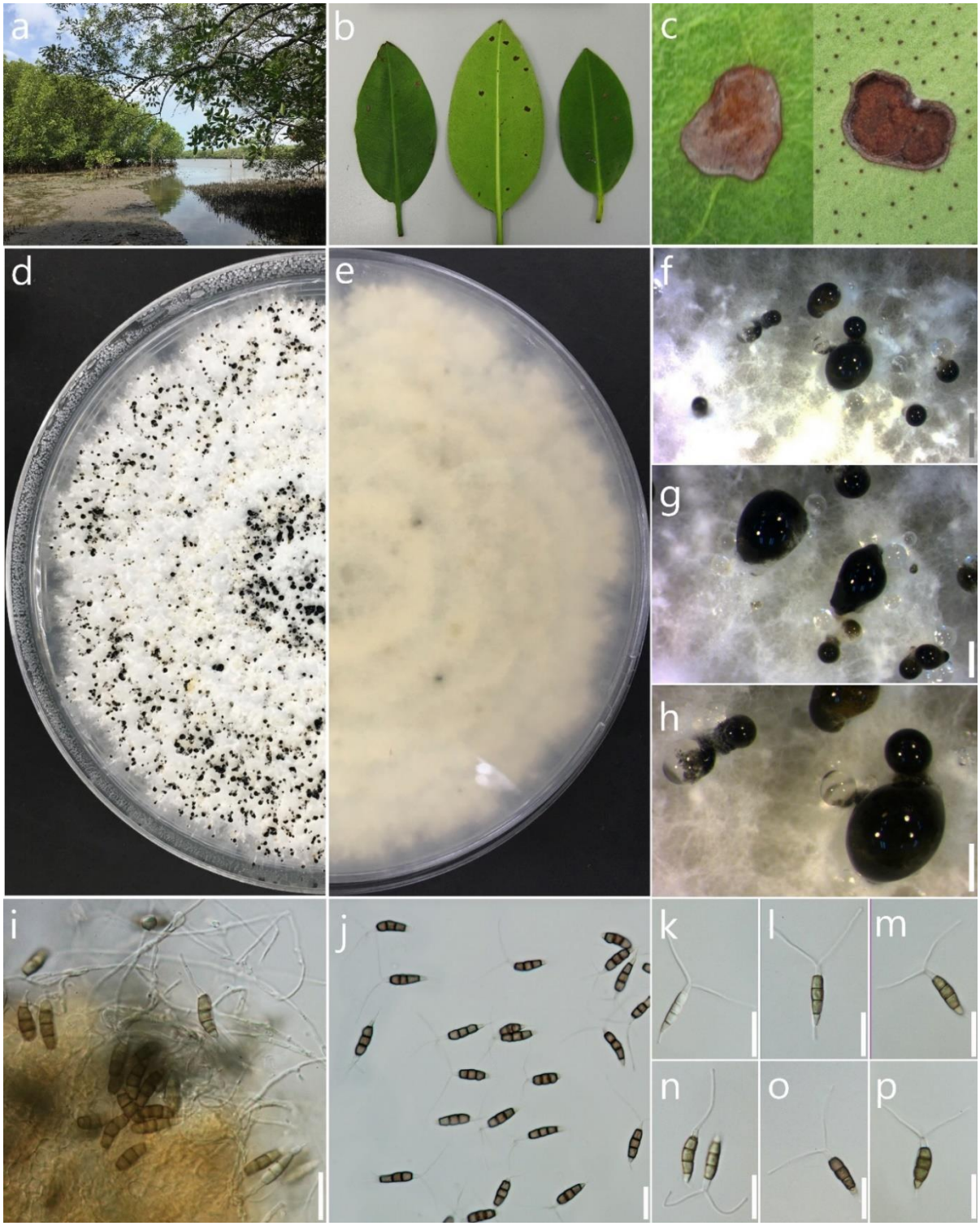


Figure 31 – *Pseudopestalotiopsis thailandica* (MFLU 19-0794, holotype). a Habitat. b, c Leaf spots on *Rhizophora mucronata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f = 1000 µm, g, h = 500 µm, i–p = 20 µm.

Table 8. Comparison of conidia of *Neopestalotiopsis* species related to this study.

Species	Strain	Conidial size (µm)	Apical appendages		Basal appendage Length (µm)
			Number	Length (µm)	
N. acrostichi	MFLUCC 17-1754/ MFLUCC 17-1755	(22–)23–26(–27.2) × (5–)5.5–6.5(–7.1)	3–4 (4)	(16–)19–65(–69.5)	(7–)12.5–36(–37)
N. alpapicalis[g]	MFLUCC 17-2544	14–22.3 × 5–6.8	1–4(1, 2)	5.6–15	3.1–6.4
N. aotearoa[d]	CBS 367.54	(19.5–)21–28(–29) × (6–)6.5–8.5(–9)	2–3	(3–)5–12(–13)	1.5–4
N. coffea-arabicae[f]	HGUP4015	16–20 × 5–7	2–4	11–16	3–5
N. musae[b]	MFLUCC 15-0776	18.6–25 9 4.1–5	2–3	16.3–25	4.6–10.3
N. piceana[d]	CBS 394.48	(19–)19.5–25(–26) × (7–)7.5–9(–9.5)	3	(19–)21–31(–33)	6–23
N. protearum[a]	CBS 114178	(14–)16–17(–18) × (6.5–)8–9(–10)	2–4	(10–)15–17(– 22)	(2–)3–3.5(–5)
N. brachiata	MFLUCC 17-1555	(18–)18.5–25(–26) × (4.7–)5.5–6(–6.3)	1–3	(8.5–)9.5–33(–34)	(3.5–)4–9(–10)
N. rhizophorae	MFLUCC 17-1550/ MFLUCC 17-1551	(20–)20.5–27(–27.5) × (6–)6.5–7.5(–8.2)	1–4 (3)	(6–)12.5–22(–24)	(2.5–)4–9.5(–10)
N. rosicola[c]	CFCC 51992	(18.9−)20.2−25.5(−26.2) ×(5−)5.5−8(−8.5)	2–4 (2, 3)	(16.5−)17−22.8(−25.9)	2−9.5
N. samarangensis[e]	MFLUCC 12-0233	18–21 × 6.5–7.5	3	12–18	3.5–5.2
N. sonneratae	MFLUCC 17-1744/ MFLUCC 17-1745	(21.6–)24–26(–28.2) × (6.8–)7–7.5(–8.1)	1–3	(5.3–)7–8(–13.8)	(2.5–)3–4(–4.7)
N. surinamensis[d]	CBS 450.74	(23–) 24–28(–29) × (7–)7.5–9(–9.5)	2–3	(15–)18–27(–28)	Up to 5–7
N. thailandica	MFLUCC 17-1730/ MFLUCC 17-1731	(20–)21–25(–25.5) × (5.7–)6–7(–7.3)	1–2	(30–)32.5–38(–40)	(3–)6–9(–10)
N. petila	MFLUCC 17-1737/ MFLUCC 17-1738	(20–)21–26.5(–27.5) × (5.6–)6–7(–7.8)	2–3	(21–)22–29(–33)	(2–)3–8(–9)

Strains in this study are in bold.

[a]Crous et al. (2011); [b]Hyde et al. (2016); [c]Jiang et al. (2018); [d]Maharachchikumbura et al. (2014); [e]Maharachchikumbura et al. (2013); [f]Song et al. (2013); [g]Kumar et al. (2019).

Table 9. Comparison of conidia of *Pestalotiopsis* species related to this study.

Species	Strain	Conidial size (µm)	Apical appendages (µm)		Basal appendage (µm)
			Number	Length	
P. formosana [a]	NTUCC 17-009	(15–)18–22(–26) × (5–)6–7	2–3	(8–)11–16(–20)	(2–)3–5(–6)
P. parva [b]	CBS 265.37	(16–)16.5–20(–21) × 5–7(–7.5)	2–3	(6–)6.5–12(–13)	2–4
P. rhizophorae	MFLUCC 17-0416/ MFLUCC 17-0417	(17–)17.5–23(–23.5) × (5.5–)6–6.5(–7)	1–2	(7.5–)8–13(–14.5)	(1.3–)1.5–4.5(–5)
P. thailandica	MFLUCC 17-1616/ MFLUCC 17-1617	(17–)17.5–28(–29) × (4.9–)5.5–6.5(–7.1)	1–2	(5.5–)11–34(–38)	(2–)2.5–9.5(–10)

Strains in this study are in bold.
[a]Ariyawansa & Hyde (2018); [b]Maharachchikumbura et al. (2014).

Table 10. Comparison of conidia of *Pseudopestalotiopsis* species related to this study.

Species	Strain	Conidial size (µm)	Apical appendages (µm)		Basal appendage (µm)
			Number	Length	
Ps. ampullacea[b]	LC6618	21–31.5 × 6.5–9	2–3	17–25	3.5–7
Ps.avicenniae	MFLUCC 17-0434	(22–)22.5–26.5(–27) × (5–)5.5–6(–6.4)	1–3	(14–)15.5–28.5(–35.5)	(2–)3–4(–4.5)
Ps. chinensis[b]	LC3011	25.5–35.5 × 6–9	2–3	24–41	5–12
Ps.rhizophorae	MFLUCC 17-1560	(18.5–)22–25(–26.5) × (6.2–)6.5–7(–7.2)	1–2	(10.2–)20–29(–35)	(5.5–)9–12(–13.5)
Ps. dawaina[d]	MM14-F0015	22–31 × 8–9.5	3	20.5–33.5	2.5–6.5
Ps. elaeidis (=*Ps. myanmarina*) [c]	NBRC 112264	31–38.5 × 6.5–9	2–3	22.5–38.5	unbranched
Ps. jiangxiensis[b]	LC4479	22–29 × 6–9	2–4(3)	16.5–32	6.5–19.5
Ps. kawthaungina[d]	MM14-F0083	29.5–34.5 × 7–9	3	28–41	4.5–9
Ps. kubahensis[a]	UMAS KUB-P20	(26–)27–30(–33) × 5.6–7.3	2–4(3)	15.9–29.4	3.1–6.0
Ps.curvatispora	MFLUCC 17-1722/ MFLUCC 17-1723	(18.6–)19–26(–26.4) × (5.5–)6–7(–7.4)	2–3	(5.5–)6–24(–26.6)	(5.8–)5–11(–12.2)
Ps. simitheae[e]	MFLUCC 12-0121	22–30 × 5–6.5	2–4	14.5–26.5	4–6.5
Ps. taiwanensis[f]	NTUCC 17-002.1	21–26 × 6–7	2–5	16–25	3–7
Ps. thailandica	MFLUCC 17-1724/ MFLUCC 17-1725	(24–)24.5–30(–30.5) × (5–)5.5–6(–6.7)	1–3	(26.5–)28–36(–39.5)	(3.5–)4.5–5(–6.6)

Strain in this study are in bold.
[a]Lateef et al. (2015); [b]Liu et al. (2017); [c]Nozawa et al. (2017); [d]Nozawa et al. (2018); [e]Song et al. 2014; [f]Tsai et al. (2018).

Supplementary Table (1). Nucleotides differences in the ITS, β-tubulin and EF1α sequences of *Neopestalotiopsis* discussed in the paper.

Taxon	Strain	ITS										β-tubulin			
		68	85	100	370	372	373	374	375	449	487	163	169	201	205
Neopestalotiopsis acrostichi	MFLUCC 17-1754	T	A	T	T	C	A	T	T	T	T	C	T	T	A
N. alpapicalis	MFLUCC 17-2544	-	A	T	T	-	-	A	T	T	T	-	-	-	-
N. aotearoa	CBS 367.54	T	G	T	T	-	-	A	T	T	T	C	T	C	T
N. brachiata	MFLUCC 17-1555	T	A	T	T	-	-	A	T	T	T	T	T	C	T
N. coffea-arabicae	HGUP4015	T	A	T	T	-	-	A	T	T	T	-	-	-	-
N. ellipsospora	MFLUCC 12-0283	T	A	T	C	-	-	G	G	T	T	-	-	-	-
N. petila	MFLUCC 17-1737	T	A	T	T	-	-	A	T	T	T	T	T	C	T
N. piceana	CBS 394.48	T	G	T	T	-	-	A	T	T	T	T	T	C	T
N. protearum	CBS 114178	T	A	T	C	-	-	G	G	T	C	C	T	T	A
N. rhizophorae	MFLUCC 17-1550	T	A	T	T	-	-	A	T	T	T	T	T	C	T
N. rosicola	CFCC 51992	T	A	T	T	-	-	T	A	C	T	T	T	C	T
N. samarangensis	MFLUCC 12-0233	T	A	T	T	-	-	A	T	T	T	-	-	-	-
N. sonneratae	MFLUCC 17-1744	T	A	T	T	-	-	A	T	T	T	C	T	T	A
N. surinamensis	CBS 450.74	T	A	T	T	-	-	T	T	T	T	C	A	T	A
N. thailandica	MFLUCC 17-1730	T	A	C	T	-	-	A	T	T	T	C	T	T	A

Taxon	Strain	β-tubulin													
		218	341	355	364	369	370	373	374	375	376	378	379	382	383
Neopestalotiopsis acrostichi	MFLUCC 17-1754	G	C	C	C	G	G	T	G	T	T	C	C	T	C
N. alpapicalis	MFLUCC 17-2544	-	-	C	T	G	G	T	G	T	A	T	C	T	C
N. aotearoa	CBS 367.54	T	C	T	C	G	G	T	G	T	A	T	C	T	C
N. brachiata	MFLUCC 17-1555	T	C	T	C	G	G	T	G	T	A	T	C	T	C
N. coffea-arabicae	HGUP4015	-	-	-	-	A	A	C	A	C	A	C	G	A	T
N. ellipsospora	MFLUCC 12-0283	-	-	C	T	G	G	T	G	T	A	T	C	T	C
N. petila	MFLUCC 17-1737	T	C	T	C	G	G	T	G	T	A	T	C	T	C
N. piceana	CBS 394.48	T	C	T	C	G	G	T	G	T	A	T	C	T	C
N. protearum	CBS 114178	G	C	C	C	G	G	T	G	T	T	C	C	T	C
N. rhizophorae	MFLUCC 17-1550	T	C	T	C	G	G	T	G	T	A	T	C	T	C
N. rosicola	CFCC 51992	T	C	T	C	G	G	T	G	T	A	T	C	T	C
N. samarangensis	MFLUCC 12-0233	-	-	C	T	G	G	T	G	T	A	T	C	T	C
N. sonneratae	MFLUCC 17-1744	T	T	C	C	G	G	T	G	T	T	C	C	T	C
N. surinamensis	CBS 450.74	T	C	C	C	G	G	T	G	T	T	C	C	T	C
N. thailandica	MFLUCC 17-1730	T	T	C	C	G	G	T	G	T	T	C	C	T	C

Supplementary Table (1). Continued.

Taxon	Strain	β-tubulin													
		384	385	386	393	402	428	437	438	441	446	459	469	471	472
Neopestalotiopsis acrostichi	MFLUCC 17-1754	T	G	C	A	C	C	A	C	-	A	G	G	T	A
N. alpapicalis	MFLUCC 17-2544	T	G	C	A	A	C	T	C	-	T	C	G	T	G
N. aotearoa	CBS 367.54	T	G	C	A	A	C	T	C	-	T	C	G	T	G
N. brachiata	MFLUCC 17-1555	T	G	C	A	A	C	T	C	-	T	C	G	T	G
N. coffea-arabicae	HGUP4015	C	T	G	-	A	C	A	C	T	A	G	A	G	A
N. ellipsospora	MFLUCC 12-0283	T	G	C	A	A	T	T	C	-	T	C	G	T	G
N. petila	MFLUCC 17-1737	T	G	C	A	A	C	T	C	-	T	C	G	T	G
N. piceana	CBS 394.48	T	G	C	A	A	C	T	C	-	T	C	G	T	G
N. protearum	CBS 114178	T	G	C	A	C	C	A	A	-	A	G	G	T	A
N. rhizophorae	MFLUCC 17-1550	T	G	C	A	A	C	T	C	-	T	C	G	T	G
N. rosicola	CFCC 51992	T	G	C	A	A	C	T	C	-	T	C	G	T	G
N. samarangensis	MFLUCC 12-0233	T	G	C	A	A	C	T	C	-	T	C	G	T	G
N. sonneratae	MFLUCC 17-1744	T	G	C	A	A	C	A	C	T	A	G	A	T	A
N. surinamensis	CBS 450.74	T	G	C	A	C	C	A	C	-	A	G	G	T	A
N. thailandica	MFLUCC 17-1730	T	G	C	A	A	C	A	C	T	A	G	A	G	A

Taxon	Strain	β-tubulin					EF1α								
		543	544	571	674	683	70	81	84	85	86	87	88	89	90
Neopestalotiopsis acrostichi	MFLUCC 17-1754	A	C	T	T	C	T	C	C	A	T	-	-	-	-
N. alpapicalis	MFLUCC 17-2544	T	T	C	T	C	-	-	C	A	T	-	-	-	-
N. aotearoa	CBS 367.54	T	T	C	T	C	T	C	C	A	T	-	-	-	-
N. brachiata	MFLUCC 17-1555	T	T	C	T	C	T	C	C	A	T	-	-	-	-
N. coffea-arabicae	HGUP4015	A	C	C	T	T	T	C	C	A	T	-	-	-	-
N. ellipsospora	MFLUCC 12-0283	T	T	C	T	C	T	A	C	A	T	-	-	-	-
N. petila	MFLUCC 17-1737	T	T	C	C	C	T	C	C	A	T	-	-	-	-
N. piceana	CBS 394.48	T	T	C	T	C	T	C	C	A	T	-	-	-	-
N. protearum	CBS 114178	A	C	T	T	C	T	C	-	-	-	-	-	-	-
N. rhizophorae	MFLUCC 17-1550	T	T	C	T	C	T	C	C	A	T	-	-	-	-
N. rosicola	CFCC 51992	T	T	C	T	C	T	C	C	A	T	-	-	-	-
N. samarangensis	MFLUCC 12-0233	T	T	C	C	C	T	C	C	A	T	-	-	-	-
N. sonneratae	MFLUCC 17-1744	A	C	C	T	T	T	C	C	A	T	C	A	T	C
N. surinamensis	CBS 450.74	A	C	T	T	C	C	C	C	A	T	-	-	-	-
N. thailandica	MFLUCC 17-1730	A	C	C	T	T	T	C	C	A	T	C	A	T	C

Supplementary Table (1). Continued.

Taxon	Strain	EF1α													
		91	92	93	97	104	105	112	113	114	118	120	124	133	167
Neopestalotiopsis acrostichi	MFLUCC 17-1754	-	-	-	C	C	A	C	A	T	A	T	T	T	T
N. alpapicalis	MFLUCC 17-2544	-	-	-	C	C	A	-	-	-	A	T	C	T	T
N. aotearoa	CBS 367.54	-	-	-	C	C	A	C	A	T	A	T	C	T	T
N. brachiata	MFLUCC 17-1555	-	-	-	C	C	A	C	A	T	A	A	C	T	T
N. coffea-arabicae	HGUP4015	-	-	-	C	C	A	C	A	T	A	T	C	T	T
N. ellipsospora	MFLUCC 12-0283	-	-	-	C	C	A	C	A	T	A	T	C	T	T
N. petila	MFLUCC 17-1737	-	-	-	C	C	A	C	A	T	A	T	C	T	T
N. piceana	CBS 394.48	-	-	-	C	C	A	C	A	T	A	T	C	T	G
N. protearum	CBS 114178	-	-	-	C	C	A	C	A	T	A	T	C	T	T
N. rhizophorae	MFLUCC 17-1550	-	-	-	C	C	A	-	-	-	A	T	C	T	T
N. rosicola	CFCC 51992	-	-	-	C	C	A	C	A	T	A	T	C	T	T
N. samarangensis	MFLUCC 12-0233	-	-	-	C	G	A	C	A	T	A	T	C	T	T
N. sonneratae	MFLUCC 17-1744	C	C	C	C	C	A	C	A	T	A	T	C	T	T
N. surinamensis	CBS 450.74	-	-	-	T	C	T	C	G	T	G	T	C	C	T
N. thailandica	MFLUCC 17-1730	C	C	C	C	C	A	C	A	T	A	T	C	T	T

Taxon	Strain	EF1α													
		169	186	210	246	287	290	407	456	462	480	485			
Neopestalotiopsis acrostichi	MFLUCC 17-1754	-	G	G	G	A	T	T	C	A	A	A			
N. alpapicalis	MFLUCC 17-2544	-	G	G	G	A	T	C	T	A	A	G			
N. aotearoa	CBS 367.54	-	G	G	G	A	T	C	C	A	T	A			
N. brachiata	MFLUCC 17-1555	-	G	G	G	A	T	C	C	A	T	A			
N. coffea-arabicae	HGUP4015	-	G	G	G	A	T	C	C	A	T	A			
N. ellipsospora	MFLUCC 12-0283	-	A	A	A	A	T	C	C	A	T	A			
N. petila	MFLUCC 17-1737	-	G	G	G	A	T	C	C	A	T	A			
N. piceana	CBS 394.48	-	G	G	G	A	T	C	C	A	T	A			
N. protearum	CBS 114178	T	G	G	G	A	T	C	C	A	T	A			
N. rhizophorae	MFLUCC 17-1550	-	G	G	G	A	T	C	C	A	A	G			
N. rosicola	CFCC 51992	-	G	G	G	A	T	C	C	A	A	A			
N. samarangensis	MFLUCC 12-0233	-	G	G	G	A	T	C	C	T	A	A			
N. sonneratae	MFLUCC 17-1744	C	G	G	G	A	C	C	C	T	A	A			
N. surinamensis	CBS 450.74	-	G	G	T	G	T	C	C	T	A	A			
N. thailandica	MFLUCC 17-1730	C	G	G	G	A	C	C	C	T	A	A			

Supplementary Table (2). Nucleotides differences in the ITS, β-tubulin and EF1α sequences of *Pestalotiopsis* discussed in the paper.

Taxon	Strain	ITS											β-tubulin		
		14	23	162	164	433	491	539	547	551	558	559	14	36	38
Pestalotiopsis rhizophorae	MFLUCC 17-0416	A	T	C	A	A	A	T	T	T	T	G	A	T	C
P. thailandica	MFLUCC 17-1616	A	T	C	G	G	-	C	T	T	T	G	T	T	C
P. formosana	NTUCC 17-009	-	-	T	G	A	-	C	T	-	G	T	-	-	-
P. parva	CBS 278.35	T	A	T	G	A	-	C	C	-	T	G	A	G	G
Taxon	**Strain**	**β-tubulin**													
		39	43	130	131	132	153	174	260	360	368	372	403	428	430
Pestalotiopsis rhizophorae	MFLUCC 17-0416	C	C	A	A	G	C	C	C	C	T	A	G	A	T
P. thailandica	MFLUCC 17-1616	C	C	A	A	G	C	C	C	C	T	A	G	A	T
P. formosana	NTUCC 17-009	-	-	-	-	-	-	-	-	-	-	C	G	A	T
P. parva	CBS 278.35	A	C	-	-	-	G	T	A	T	C	A	A	T	C
Taxon	**Strain**		**EF1α**												
		762	33	34	36	56	57	58	71	81	123	126	226	261	434
Pestalotiopsis rhizophorae	MFLUCC 17-0416	A	T	C	C	C	C	A	C	C	A	C	T	C	G
P. thailandica	MFLUCC 17-1616	G	T	C	C	C	C	A	C	C	A	C	T	C	G
P. formosana	NTUCC 17-009	A	T	C	C	-	-	-	G	T	G	A	C	C	A
P. parva	CBS 278.35	A	A	T	T	T	C	A	G	C	A	C	T	T	A

Supplementary Table (3). Nucleotides differences in the ITS, β-tubulin and EF1α sequences of *Pseudopestalotiopsis* discussed in the paper.

Taxon	Strain	ITS													
		30	39	75	119	147	409	410	482	527	545	546	549	553	554
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	A	T	A	G	A	-	-	T	T	G	A	A	G	C
Ps. curvatispora	MFLUCC 17-1722	A	T	A	G	G	-	-	C	T	G	A	A	G	C
Ps. dawaina	MM14 F0015	T	A	A	T	G	-	-	C	T	G	A	A	G	C
Ps. jiangxiensis	LC4479	-	-	-	G	A	-	-	T	T	G	A	A	G	C
Ps. kawthaungina	MM14 F0083	T	A	A	G	A	-	-	T	T	G	A	A	G	C
Ps. myanmarina	NBRC 112264	T	A	A	G	A	-	-	T	T	G	A	A	G	C
Ps. rhizophorae	MFLUCC 17-560	A	T	A	G	G	A	A	C	T	G	A	A	G	C
Ps. simitheae	MFLUCC 12-0121	T	A	A	G	A	-	-	T	-	-	-	G	A	T
Ps. taiwanensis	NTUCC 17-002.1	T	A	A	G	A	-	-	T	-	-	-	-	-	-
Ps. thailandica	MFLUCC 17-1724	A	T	A	G	G	A	A	C	T	G	A	A	G	C

Supplementary Table (3). Continued.

Taxon	Strain	ITS	β-tubulin												
		556	22	107	125	134	148	149	150	151	153	158	183	192	194
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	G	G	T	A	C	T	-	-	-	T	C	A	A	A
Ps. curvatispora	MFLUCC 17-1722	G	G	G	C	C	G	C	A	G	C	C	G	G	A
Ps. dawaina	MM14 F0015	G	-	-	-	-	-	-	-	-	-	-	-	-	-
Ps. jiangxiensis	LC4479	G	G	T	A	C	T	-	-	-	T	C	A	A	A
Ps. kawthaungina	MM14 F0083	G	-	-	-	-	-	-	-	-	-	-	-	-	-
Ps. myanmarina	NBRC 112264	G	-	-	-	-	-	-	-	-	-	-	-	-	-
Ps. rhizophorae	MFLUCC 17-560	G	A	T	C	T	G	C	A	G	T	A	A	G	G
Ps. simitheae	MFLUCC 12-0121	C	-	-	-	-	-	-	-	-	-	-	-	-	-
Ps. taiwanensis	NTUCC 17-002.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ps. thailandica	MFLUCC 17-1724	G	A	T	C	T	G	-	-	-	T	A	A	G	G

Taxon	Strain	β-tubulin													
		198	206	223	225	250	302	330	355	361	362	363	364	365	372
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	A	G	A	G	C	G	A	C	G	T	A	T	G	A
Ps. curvatispora	MFLUCC 17-1722	T	A	G	A	T	G	G	C	G	T	A	T	G	A
Ps. dawaina	MM14 F0015	-	-	-	-	-	-	-	-	G	T	A	T	G	A
Ps. jiangxiensis	LC4479	A	G	A	G	C	G	A	C	G	T	A	T	G	A
Ps. kawthaungina	MM14 F0083	-	-	-	-	-	-	-	-	G	T	A	T	G	A
Ps. myanmarina	NBRC 112264	-	-	-	-	-	-	-	-	-	-	-	-	-	A
Ps. rhizophorae	MFLUCC 17-560	T	A	A	A	T	A	A	C	G	T	A	T	G	A
Ps. simitheae	MFLUCC 12-0121	-	-	-	-	-	-	-	-	A	G	T	C	A	-
Ps. taiwanensis	NTUCC 17-002.1	-	-	-	-	-	-	-	T	G	T	A	T	G	A
Ps. thailandica	MFLUCC 17-1724	T	A	A	A	T	G	A	C	G	T	A	T	G	A

Taxon	Strain	β-tubulin													
		376	413	434	435	445	453	459	505	538	539	540	558	559	560
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	A	T	T	G	C	-	G	T	-	-	-	C	A	T
Ps. curvatispora	MFLUCC 17-1722	A	T	T	A	A	G	G	T	C	C	C	T	C	C
Ps. dawaina	MM14 F0015	A	T	T	A	A	G	G	T	C	C	C	T	C	C
Ps. jiangxiensis	LC4479	A	T	T	G	C	-	G	T	-	-	-	C	A	C
Ps. kawthaungina	MM14 F0083	A	T	T	A	A	G	G	T	C	C	C	T	C	C
Ps. myanmarina	NBRC 112264	A	T	T	G	C	-	G	T	-	-	-	C	A	T
Ps. rhizophorae	MFLUCC 17-560	C	C	G	A	A	G	G	C	T	T	C	C	A	C
Ps. simitheae	MFLUCC 12-0121	-	C	G	A	A	G	G	T	T	T	C	C	A	C
Ps. taiwanensis	NTUCC 17-002.1	C	C	G	A	A	G	G	T	T	T	C	C	A	C
Ps. thailandica	MFLUCC 17-1724	C	C	G	A	A	G	A	C	T	T	C	C	A	C

Supplementary Table (3). Continued.

Taxon	Strain	β-tubulin										EF1α			
		571	572	573	581	625	628	691	715	739	772	88	89	93	95
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	T	T	G	G	T	C	T	C	T	C	A	C	T	T
Ps. curvatispora	MFLUCC 17-1722	T	C	A	T	T	C	C	T	C	T	A	T	T	C
Ps. dawaina	MM14 F0015	T	C	A	T	T	C	C	T	C	T	A	C	T	T
Ps. jiangxiensis	LC4479	T	T	G	G	T	C	T	C	T	C	A	C	T	T
Ps. kawthaungina	MM14 F0083	T	C	A	T	T	C	C	T	C	T	A	C	T	T
Ps. myanmarina	NBRC 112264	T	T	G	G	T	C	T	C	T	C	A	C	T	T
Ps. rhizophorae	MFLUCC 17-560	T	C	A	T	C	G	C	T	C	T	C	C	T	T
Ps. simitheae	MFLUCC 12-0121	C	C	A	T	T	C	C	T	C	T	G	C	A	T
Ps. taiwanensis	NTUCC 17-002.1	C	C	A	T	T	C	C	T	C	T	A	C	T	T
Ps. thailandica	MFLUCC 17-1724	T	C	A	T	C	C	C	T	C	T	C	C	T	T

Taxon	Strain	EF1α													
		99	100	102	105	114	115	123	125	127	128	134	137	140	150
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	T	C	A	G	-	-	A	C	T	C	C	G	C	C
Ps. curvatispora	MFLUCC 17-1722	T	G	T	A	A	A	A	T	C	C	A	A	T	C
Ps. dawaina	MM14 F0015	C	-	-	A	-	-	T	C	C	C	C	A	T	A
Ps. jiangxiensis	LC4479	T	C	A	G	-	-	A	C	T	C	C	G	C	C
Ps. kawthaungina	MM14 F0083	T	C	A	G	-	-	A	C	T	C	C	G	C	C
Ps. myanmarina	NBRC 112264	T	C	A	G	-	-	A	C	T	C	C	G	C	C
Ps. rhizophorae	MFLUCC 17-560	T	C	T	A	-	-	G	C	C	C	C	A	T	C
Ps. simitheae	MFLUCC 12-0121	T	C	T	A	-	-	G	C	C	C	C	A	T	C
Ps. taiwanensis	NTUCC 17-002.1	T	C	T	A	A	-	A	C	T	T	C	G	T	C
Ps. thailandica	MFLUCC 17-1724	T	C	T	A	-	-	G	C	C	C	C	A	T	C

Taxon	Strain	EF1α													
		151	152	157	167	172	176	186	252	267	268	269	270	271	272
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	C	A	A	C	A	G	T	A	C	A	G	C	A	A
Ps. curvatispora	MFLUCC 17-1722	-	-	C	T	A	C	C	A	C	A	G	C	A	A
Ps. dawaina	MM14 F0015	G	A	A	T	A	C	C	A	C	A	G	C	A	A
Ps. jiangxiensis	LC4479	C	A	A	T	A	C	T	T	C	A	G	C	A	A
Ps. kawthaungina	MM14 F0083	C	N	A	T	A	C	T	A	C	A	G	C	A	A
Ps. myanmarina	NBRC 112264	C	A	A	T	A	C	T	A	C	A	G	C	A	A
Ps. rhizophorae	MFLUCC 17-560	G	A	A	T	C	C	C	A	-	-	-	-	-	-
Ps. simitheae	MFLUCC 12-0121	G	A	A	T	C	C	C	A	-	-	-	-	-	-
Ps. taiwanensis	NTUCC 17-002.1	A	A	A	T	A	C	T	A	C	A	G	C	A	A
Ps. thailandica	MFLUCC 17-1724	G	A	A	T	C	C	C	A	-	-	-	-	-	-

Supplementary Table (3). Continued.

Taxon	Strain	EF1α													
		273	274	275	276	277	278	279	280	281	282	283	286	290	304
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	C	-	-	-	C	A	T	G	C	A	C	C	T	A
Ps. curvatispora	MFLUCC 17-1722	C	-	-	-	C	A	T	G	C	A	C	T	T	A
Ps. dawaina	MM14 F0015	C	T	A	C	C	A	T	G	C	A	C	C	T	A
Ps. jiangxiensis	LC4479	C	-	-	-	C	A	T	G	C	A	C	C	T	A
Ps. kawthaungina	MM14 F0083	C	-	-	-	C	A	T	G	C	A	C	C	T	A
Ps. myanmarina	NBRC 112264	C	-	-	-	C	A	T	G	C	A	C	C	T	A
Ps. rhizophorae	MFLUCC 17-560	-	-	-	-	-	-	-	-	-	-	-	C	C	A
Ps. simitheae	MFLUCC 12-0121	-	-	-	-	-	-	-	-	-	-	-	C	T	C
Ps. taiwanensis	NTUCC 17-002.1	C	-	-	-	C	A	T	G	C	A	C	C	T	A
Ps. thailandica	MFLUCC 17-1724	-	-	-	-	-	-	-	-	-	-	-	C	C	A

Taxon	Strain	EF1α													
		306	308	312	318	321	343	475	476	478	479	480	484	487	488
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	T	A	C	C	C	C	G	T	C	C	T	C	G	C
Ps. curvatispora	MFLUCC 17-1722	C	A	C	C	C	C	T	T	C	C	T	A	A	C
Ps. dawaina	MM14 F0015	C	A	C	C	C	C	T	T	C	T	T	C	A	C
Ps. jiangxiensis	LC4479	T	A	C	C	C	C	G	T	C	C	T	C	G	C
Ps. kawthaungina	MM14 F0083	C	A	C	C	C	C	T	T	A	C	T	C	G	T
Ps. myanmarina	NBRC 112264	T	A	C	C	C	C	G	T	C	C	T	C	G	C
Ps. rhizophorae	MFLUCC 17-560	C	G	C	T	A	T	T	C	C	T	C	C	G	C
Ps. simitheae	MFLUCC 12-0121	C	A	C	C	C	C	T	C	C	T	T	C	G	C
Ps. taiwanensis	NTUCC 17-002.1	C	A	T	C	C	C	T	T	C	C	C	C	A	C
Ps. thailandica	MFLUCC 17-1724	C	G	C	T	A	T	T	C	C	T	C	C	G	C

Taxon	Strain	EF1α													
		491	492	493	496	500	501	504	510	512	521	524			
Pseudopestalotiopsis avucenniae	MFLUCC 17-0434	A	G	C	C	G	A	T	T	-	T	C			
Ps. curvatispora	MFLUCC 17-1722	A	G	C	C	G	C	T	C	-	T	T			
Ps. dawaina	MM14 F0015	A	G	T	C	G	A	T	T	T	T	T			
Ps. jiangxiensis	LC4479	A	G	C	C	G	A	T	T	-	T	C			
Ps. kawthaungina	MM14 F0083	A	A	C	C	G	A	G	T	-	T	C			
Ps. myanmarina	NBRC 112264	A	G	C	C	G	A	T	T	-	T	C			
Ps. rhizophorae	MFLUCC 17-560	T	C	C	C	G	A	T	T	-	C	C			
Ps. simitheae	MFLUCC 12-0121	A	C	G	C	A	A	T	T	-	C	C			
Ps. taiwanensis	NTUCC 17-002.1	A	G	C	T	G	A	T	T	-	T	G			
Ps. thailandica	MFLUCC 17-1724	T	C	C	C	G	A	T	T	-	C	C			

Savoryella E.B.G. Jones & R.A. Eaton, Trans. Br. mycol. Soc. 52(1): 161 (1969)

Index Fungorum number: IF 4870; Facesoffungi number: FoF 02131

Emended description: Asexual morph: Colonies effuse, black, glistening, punctiform. Mycelium subhyaline to pale brown. *Conidiophores* micronematous, mononematous, smooth, thin-walled, hyaline to pale brown. *Conidiogenous cells* holoblastic, determinate, integrated, terminal and intercalary, cylindrical. *Conidia* solitary or aggregated, pyriform to oboviod, thick-walled, 2−5-septate, septa thick and band-like, dividing the conidium into unequal cells, the upper cell being largest and dark brown, middle cells brown or paler, and the basal cell subhyaline or pale brown.

Savoryella nypae (K.D. Hyde & Goh) S.N. Zhang, K.D. Hyde & J.K. Liu, **comb. nov.**

≡ Trichocladium nypae K.D. Hyde & Goh, Mycological Research 103(11): 1420 (1999)

Index Fungorum number: IF 556269; Facesoffungi number: FoF 05833 Fig. 33

Family: Savoryellaceae

Saprobic on submerged rachides of mangrove palm (*Nypa fruticans*). **Sexual morph**: Undetermined. **Asexual morph**: Colonies effuse, black, glistening, punctiform distributed on substrates. Mycelium 1.5–2.5 µm, subhyaline to pale brown, septate, branched. *Conidiophores* inconspicuous or micronematous, mononematous, hyaline to pale brown, smooth, thin-walled. *Conidiogenous cells* holoblastic, determinate, integrated, terminal becoming intercalary, cylindrical. *Conidia* 15–21(–25) × 9−13(–18) ($\bar{x}$ = 18.6 × 11.6 µm, n = 25), solitary or aggregated, rhexolytic, pyriform to obovoid, broadly rounded at the apex, straight or slight curved, thick-walled, 2(−3)-septate, septa thick and band like, dividing the conidium into unequal cells, the upper cell being largest and dark brown, 10–17 µm long and 10–15 µm wide, middle cells brown or paler, and the basal cell subhyaline or pale brown.

Culture characteristics: Colonies growing well on PDA, and attaining a diameter of 12 mm after 21 days at 25ºC, obverse olive to grey-green or light grey-green, tufted colony center elevated, reverse dark green. Mycelium in culture up to 5.5 µm wide, hyaline to brown, septate, branched. Chlamydospore-like structures are apparent in culture and developed to conidia. Conidia in culture 14–22(–24) × 9−16(–18) ($\bar{x}$ = 18.5 × 13.6 µm, n = 50).

Material examined: THAILAND, Ranong, on submerged petiole of *Nypa fruticans* Wurmb. (Arecaceae), 4 December 2016, S.N. Zhang, SNT97 (MFLU 19-0011, HKAS 102604); living culture: MFLUCC 18-1570.

Notes: Morphological characters of *Savoryella nypae* resemble several genera, such as *Bactrodesmiastrum*, *Bactrodesmium* and *Trichocladium*, but *S. nypae* is phylogenetically distinct from species in these genera (Hernández-Restrepo et al. 2013, 2015, 2017, Tanaka et al. 2015, Dayarathne et al. 2019, Wang et al. 2019). However, the new collection of *Savoryella nypae* is identical to *Trichocladium nypae* K.D. Hyde & Goh (1999), which was found from the same host *Nypa fruticans*. The dimension of conidia in *Savoryella nypae* is 15–21(–25) × 9−13(–18) µm, in which 15–21 × 9−13 contains 92% of the number of measured conidia and extreme values are given in parentheses. The dimension of conidia of S. nypae is slightly larger but also comparable (15–21(–25) × 9−13(–18) µm vs. 15–20 × 10–13(–15) µm) to *Trichocladium nypae*. We identify our new collection as the same species of *Trichocladium nypae*, and the multi-gene phylogenetic analyses showed that *Savoryella nypae* as an asexual

species nested in the genus *Savoryella*. Therefore, we synonymize *Trichocladium nypae* as *Savoryella nypae*, and provide a reference specimen with living culture in this study.

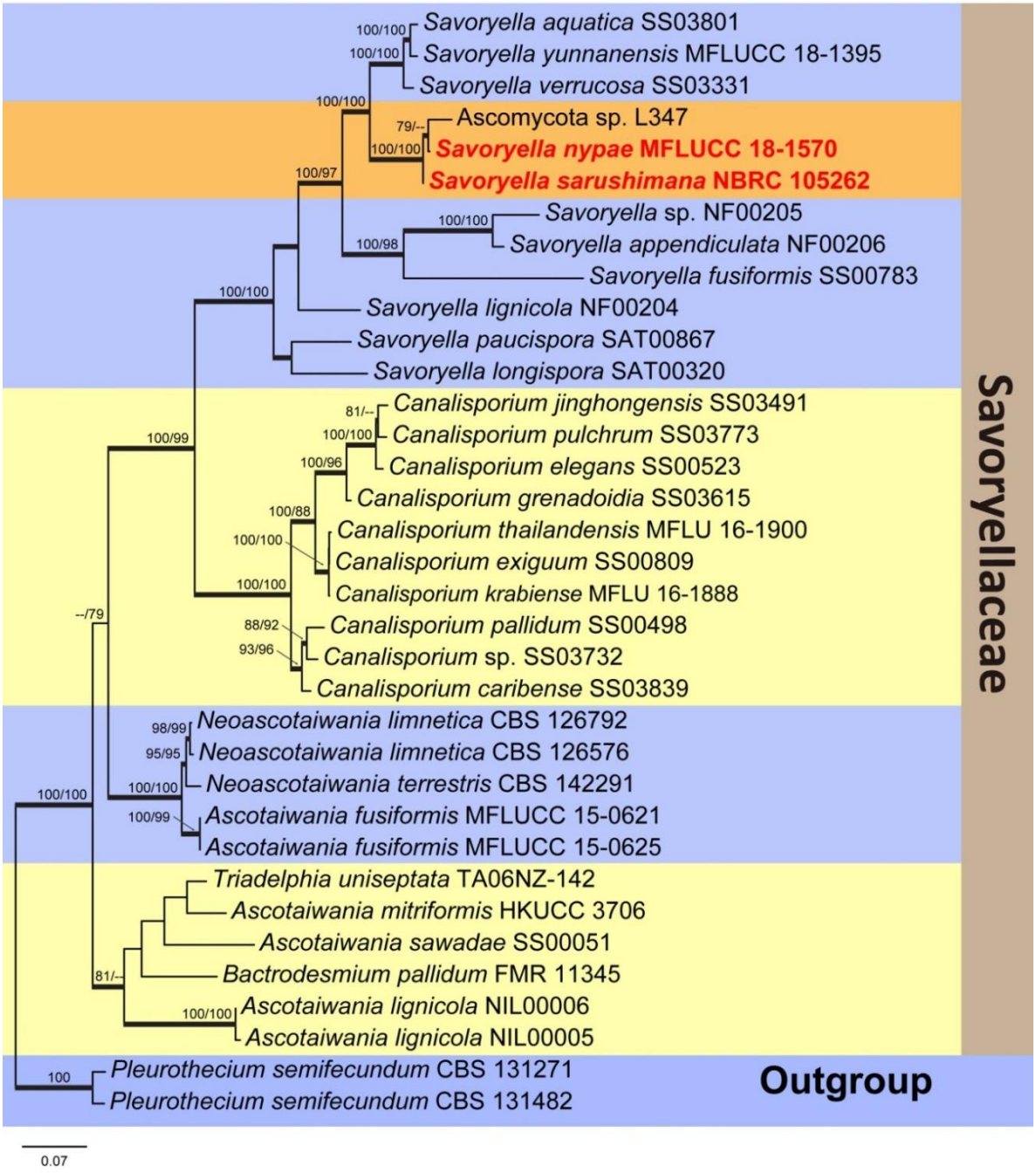


Figure 32 – RAxML tree of Savoryellaceae based on analysis of ITS, LSU, SSU, TEF1α and RPB2 gene region sequnces data. Bootstrap values for ML and MP equal to or greater than 75 are placed (ML/MP) above the branches respectively. Branches with Bayesian posterior probabilities (PP) from MCMC analysis equal or greater than 0.95 are in bold. Newly generated sequences are indicated in red. The tree is rooted with *Pleurothecium semifecundum* (CBS 131482 and CBS 131271).

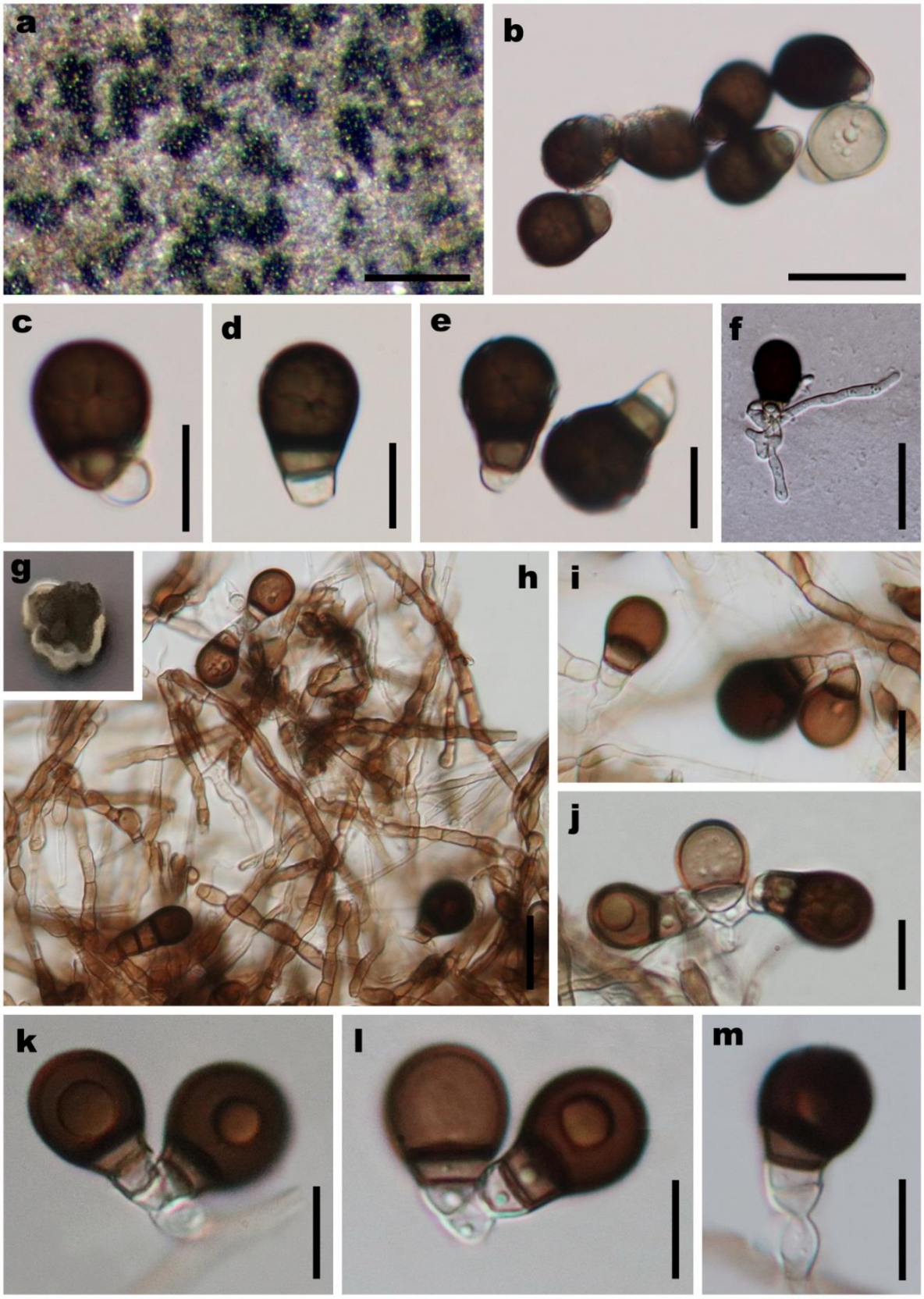


Figure 33 – *Savoryella nypae* (MFLU 19-0011) a Colonies on host substrate. b–e Conidia. f Germinated conidium. g Colony of conidia growing on PDA media. h–m Sporulating conidia in culture. Scale bars: a = 200 µm, b,f,h = 20 µm, c–e, i–m = 10 µm.

Tirisporella E.B.G. Jones, K.D. Hyde & Alias, Can. J. Bot. 74(9): 1489 (1996)

Index Fungorum Number: IF27659

Type species: *Tirisporella beccariana* (Ces.) E.B.G. Jones, K.D. Hyde & Alias, in Jones, Hyde, Read, Moss & Alias, Can. J. Bot. 74(9): 1490 (1996)

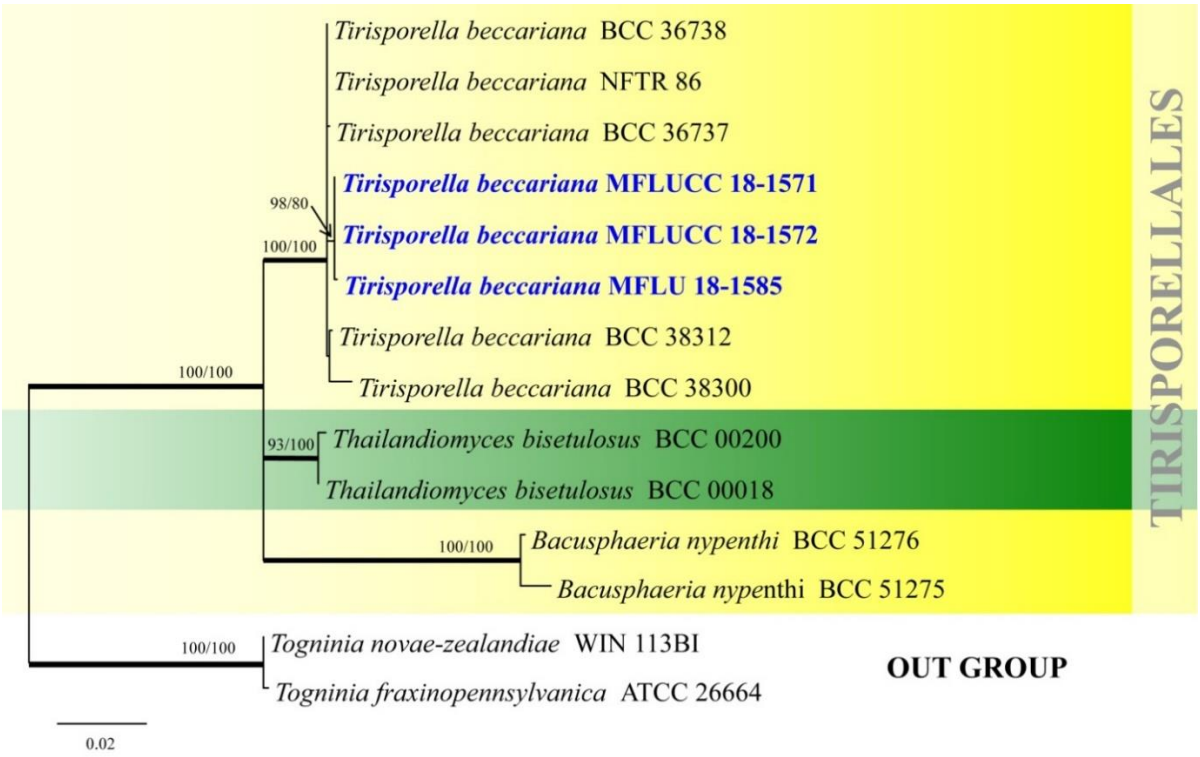


Figure 34 – RAxML tree of Tirisporellales and closely related groups based on analysis of combined LSU and SSU rDNA sequences data. Bootstrap values for ML and MP equal to or greater than 75 are placed above and below the branches respectively. Branches with Bayesian posterior probabilities (PP) from MCMC analysis equal or greater than 0.95 are in bold. Newly generated sequences are indicated in bold and red. The tree is rooted with *Togninia novae-zealandiae* (WIN 113BI) and *Tonginia fraxinopennsylvanica* (ATCC 26664).

Tirisporella beccariana (Ces.) E.B.G. Jones, K.D. Hyde & Alias Fig. 35

Saprobic on *Nypa fruticans* petiole, collected in mangrove forest. **Sexual morph**: *Ascomata* 590–930 µm black, globose to subglobose, solitary to scattered, uniloculate, coriaceous, carbonaceous, superficial, with a short neck and central ostiole. *Peridium* 95–125 µm wide, an outer layer of textura angularis of thick-walled, black to reddish brown cells, and a narrow inner layer of hyaline, thin-walled, elongate cells of textura prismatica. *Paraphyses* 4.5–9 µm wide, hyaline, unbranched, septate, constricted at the septa and tapering. *Asci* 125–220 × 15–21 µm ($\bar{x}$ = 175.5 × 18.3 µm, n = 10), 8-spored, bitunicate, fissitunicate, cylindrical, short pedicellate, apex flattened, with an apical appendage. *Ascospores* 28–45 × 6–11 µm, ($\bar{x}$ = 37.6 × 7.6 µm, n = 20), brown, 4-septate, falcate to lunate, verrucose, apical cell appendaged,

basal cell pointed and hyaline. The first septum formed in the ascospores is near the base and delimits the light-coloured basal cell. **Asexual morph**: *Phialophora* cf. *olivacea*.

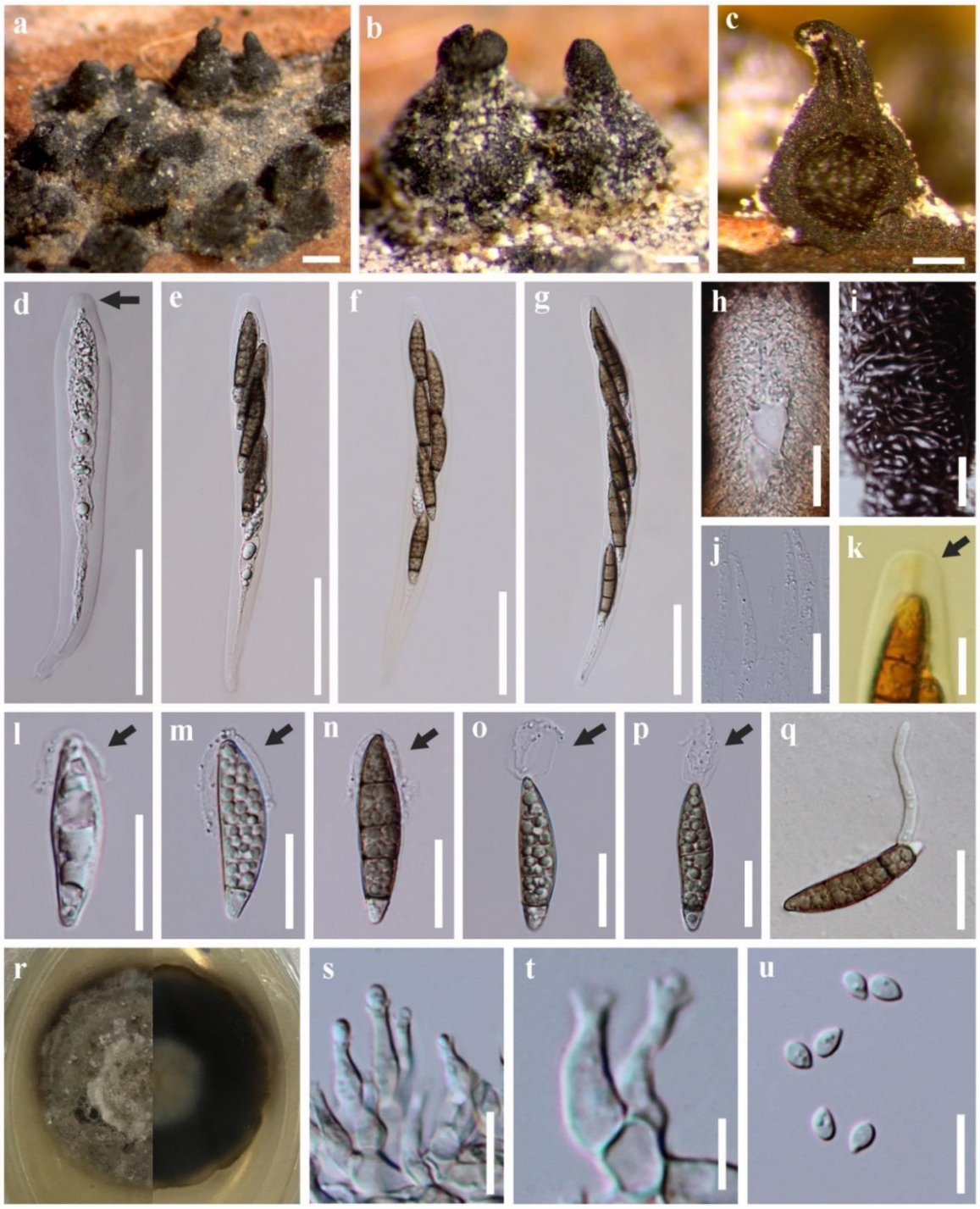


Figure 35 – *Tirisporella beccariana* (MFLU 18-1582, MFLU 18-1585). a, b Appearance of ascomata on host surface with ostioles. c Vertical section through the ascoma. d–g Asci. h Ostiole with periphyses. i Structure of peridium. j Paraphyses. k Apex of ascus in Lugol's iodine, with a J-, apical ring. l–q Ascospores. q Germinating spore. r Colony on PDA. s–u Asexual morph structure in culture. Scale bars: a = 500 µm, b, c = 200 µm, d–g = 50 µm, h–j, l–q = 20 µm, k, s, u = 10 µm, t = 5 µm.

Vaginatispora K.D. Hyde, Nova Hedwigia 61(1-2): 234 (1995)

Index Fungorum Number: IF27644

Type species: *Vaginatispora aquatica* K.D. Hyde, Nova Hedwigia 61(1-2): 235 (1995)

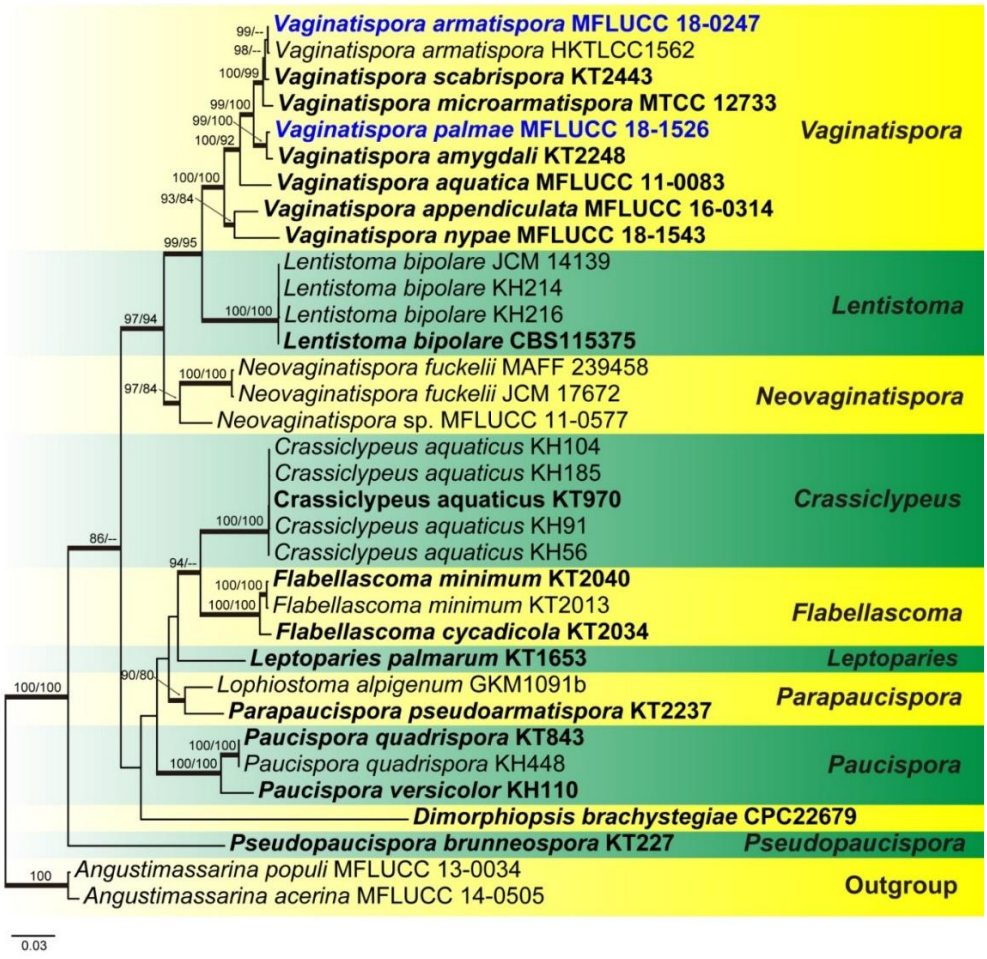


Figure 36 – Phylogram generated from maximum likelihood analysis based on combined ITS, LSU, SSU, TEF1α and RPB2 sequence data for *Vaginatispora* species and several closely related genera in Lophiostomataceae. Related sequences were referred to Thambugala et al. (2015); Wanasinghe et al. (2016); Devadatha et al. (2017) and Hashimoto et al. (2018). Thirty-four strains are included in the combined genes sequence analyses which comprise total 4243 characters (605 characters fro ITS, 832 characters for LSU, 895 characters for SSU, 894 characters for TEF1, 1017 characters for RPB2) after alignment. *Angustimassarina populi* MFLUCC 13–0034 and *Angustimassarina acerina* MFLUCC 14-0505 (Amorosiaceae, Pleosporales) are used as the out group taxa. Single gene analyses are carried out and the topology of each tree with clade stability. Tree topology of the maximum likelihood analysis is similar to the maximum parsimony analysis and the Bayesian analysis. The best sorting RaxML tree with a final likelihood value of –17911.101212 is presented. Bootstrap values for maximum likelihood (ML) and maximum parsimony (MP) equal to or greater than 75 are placed above and below the branches respectively. Branches with Bayesian posterior probabilities (BYPP) equal or greater than 0.95 are in bold. The ex-type strains are in bold and newly generated sequences are indicated in bold and blue.

Vaginatispora palmae S.N. Zhang, J.K. Liu & K.D. Hyde **sp. nov.**

Index Fungorum Number: IF556316; Facesoffungi Number: FoF 05089 Fig. 37

Etymology: The epithet reflects the family of host plant.

Holotype: MFLU 18–1586

Saprobic on immersed rachis of *Nypa fruticans*. **Sexual morph***: Ascomata* in vertical section 250–340 µm high, 215–385 µm diam. ($\overline{x}$ = 310.2 × 325.9 µm, n = 10), dark brown to black, scattered, semi-immersed, erumpent, subglobose to elongated, base flatted, coriaceous to carbonaceous. Ostiole crest-like, variable in shape, central papillate. *Peridium* 15–38 µm wide, wider at the apex and thinner at the base, composed of several pale brown to brown cells of textura angularis, cells towards the inside lighter, at the outside, darker, somewhat flattened, fusing and with the host tissues. *Pseudoparaphyses* 1–2.5 µm wide, hypha-like, numerous, septate, rarely branched and anastomosed, tapering towards the apex. *Asci* 89–115 × 12–20 µm ($\overline{x}$ = 100.5 × 16.0 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindric-clavate, with a short bulbous pedicel, rounded at the apex, with an ocular chamber. *Ascospores* 23–45 × 6–9 µm, ($\overline{x}$ = 35.3 × 7.5 µm, n = 30), hyaline or rarely pale brown, uniseriate or overlapping to biseriate, 1-septate, occasionally producing pseudosepta, slightly constricted at the central septum, cell above central septum swollen, guttulate, smooth-walled, surrounded by a narrow mucilaginous sheath and drawn out towards each end to form tapering appendages, 6–8 µm long. **Asexual morph**: Undetermined.

Culture characteristics: Ascospores germinating on PDA within 24 h. Colonies growing on PDA reaching 2.0 cm diam. after 21 days at 25°C, the off-white hyphae in first one week, then becoming grayish blue and dark bluish, composed of brown to dark brown, septate, smooth or verrucose hyphae.

Material examined: Thailand, Ranong, on immersed rachis of *Nypa fruticans* Wurmb (Arecaceae), 03 December 2016, S.N. Zhang, SNT92 (MFLU 18–1586, holotype); ibid. (HKAS 102207, isotype), ex-type living culture MFLUCC 18–1526.

GenBank numbers: ITS: MK085055; LSU: MK085059; SSU: MK085057; TEF1α: MK087657.

Notes: Most species assigned to the genus *Vaginatispora* are in tropical regions and commonly occur in fresh water and marine environments. Morphologically, almost all the known *Vaginatispora* species have 1-septate ascospores with terminal appendages or sheath. But the detailed characters of ascospores are useful for distinguishing taxa in species level, and molecular sequence data is the key for the identification of taxa in this group. In this study, the multi-gene database included all species within this genus and, the result indicated that the new isolate *Vaginatispora palmae* clustered with *V. amygdali*. *V. palmae* differs from *V. amygdali* because the latter speciespresent a lateral pad-like structure within the sheath and an internal chamber at both ends of ascospores, while *V. palmae* lacks those structures or that is not clearly observed. They are also different in the width of peridium (15–38 µm vs. 37.5–62.5 µm), the size of asci (mean: 100.5 × 16.0 µm vs. 115.0 × 18.5 µm) and ascospores (mean: 35.3 × 7.5 µm vs. 30.6 × 8.8 µm). In addition, the polymorphic nucleotides comparison showed that these two strains are differed in eight positions of ITS1 and ITS2 regains including two gaps, and differed in eight positions of TEF1α. The difference in molecular sequence data also distinguishes *V. palmae* from *V. amygdali*, therefore, we introduce a new species *V. palmae* in this study.

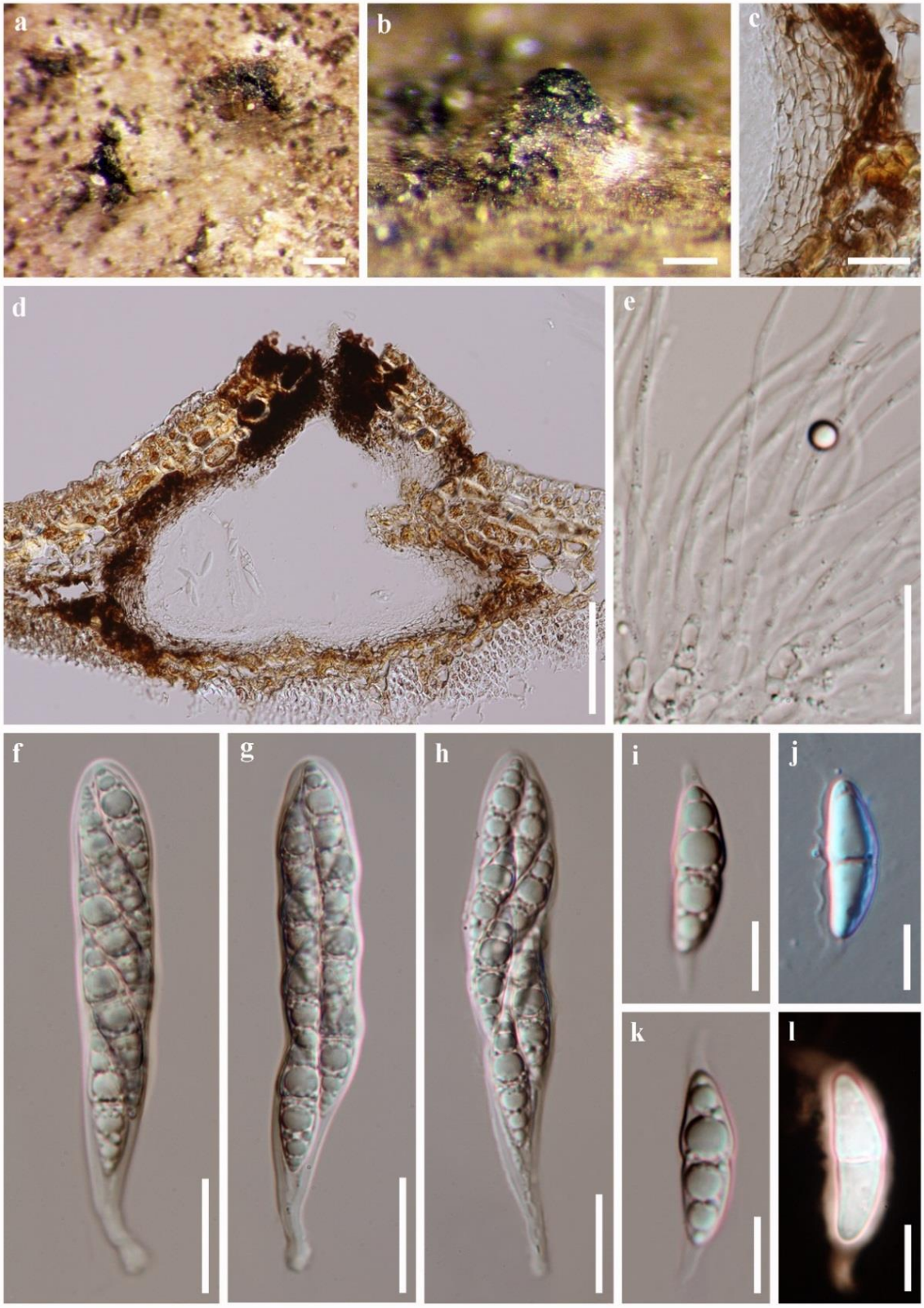


Figure 37 – *Vaginatispora palmae* (MFLU 18–1586, Holotype). a-b Appearance of stromata on host surface. c Structure of peridium. d Vertical section through the stromata with ascomata. e Pseudoparaphyses. f-h Ascus. i-l Ascospores, l Ascospore in India Ink, with clearly terminal appendages and narrow sheath. Sacle bars: a = 200 µm, b = 500 µm, c,e = 20 µm, d = 100 µm, f-h = 20 µm, i-l =10 µm.

Appendix IV To identify endophytic isolates and establish their phylogenetic relationships at different taxonomic levels based on DNA sequence data.

Mangroves are highly complex habitats sustaining a diverse array of terrestrial and aquatic fungal species. Endophytic fungi are widely distributed in mangrove ecosystems and are integral contributors to global biodiversity. *Neopestalotiopsis* and *Pestalotiopsis* species occur as endophytes, saprobes and opportunistic pathogens of many plant hosts. Herein, two strains of novel species, *Neopestalotiopsis alpapicalis* and *Pestalotiopsis thailandica* clustered together and have a close affinity to other species as we have mentioned in species notes. Based on a combined dataset of ITS, β-tub and TEF1 genes was used to infer the phylogenetic placement of the new species and morphology. Some endophyte species for this study were mentioned in Appendix VI, which an estimated 20 morphotypes in this study based on ITS sequence data and cultural characteristic as well as support with available morphological study of sporulating species.

Neopestalotiopsis Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 135 (2014)

Index Fungorum number: IF809759

Type species: *Neopestalotiopsis protearum* (Crous & L. Swart) Maharachch., K.D. Hyde & Crous, in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 147 (2014)

See the phylogenetic tree from Appendix III, Fig 18

Neopestalotiopsis alpapicalis Vin. Kumar, Gentekaki & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556161, Facesoffungi number: FoF 05753 Fig. 34

Etymology: Refers to small size of the apical cell, 'alpa' = small (Sanskrit), apicalis = apical (Latin).

Holotype: MFLU 19-0405

Endophyte isolated from healthy leaves of Rhizophora mucronata and symptomatic leaves of R. apiculata. **Asexual morph**: Conidiomata (on PDA) pycnidial, globose to clavate, solitary or confluent, embedded or semi-immersed to erumpent, dark brown, 200–450 µm diam., exuding globose, dark brown to black conidial masses. Conidiophores indistinct often reduced to conidiogenous cells. Conidiogenous cells discrete, subcylindrical to ampulliform, hyaline, 8–15 × 1.5–2.5 µm. Conidia fusoid, ellipsoid, straight to slightly curved, 4-septate, 14–22.3 × 5–6.8 µm ($\bar{x}$ = 18.5 × 6.0 µm, n = 20); basal cell conic with a truncate base, hyaline, rugose and thin-walled, 2.7–6.5 µm long; three median cells doliiform, 12–15 µm long ($\bar{x}$ = 12.50 µm), wall smooth, versicoloured, septa darker than the rest of the cell (second cell from the base pale brown, 3.5–5.5 µm long; third cell honey brown, 3.4–5.6 µm long; fourth cell brown, 3.2–6.0 µm long); apical cell 0.75–2.3 ($\bar{x}$ = 1.25 µm) µm long, hyaline, subcylindrical, rugose, thin- and smooth-walled; with 1–4 tubular apical appendages (mostly 1 and 2, seldom 3 and 4), arising from the apical crest, unbranched, filiform, 5.6–15 µm ($\bar{x}$ = 9.3 µm); basal appendage single, tubular, unbranched, centric, 3.1–6.4 µm ($\bar{x}$ = 4.7 µm) long. **Sexual morph**: Undetermined.

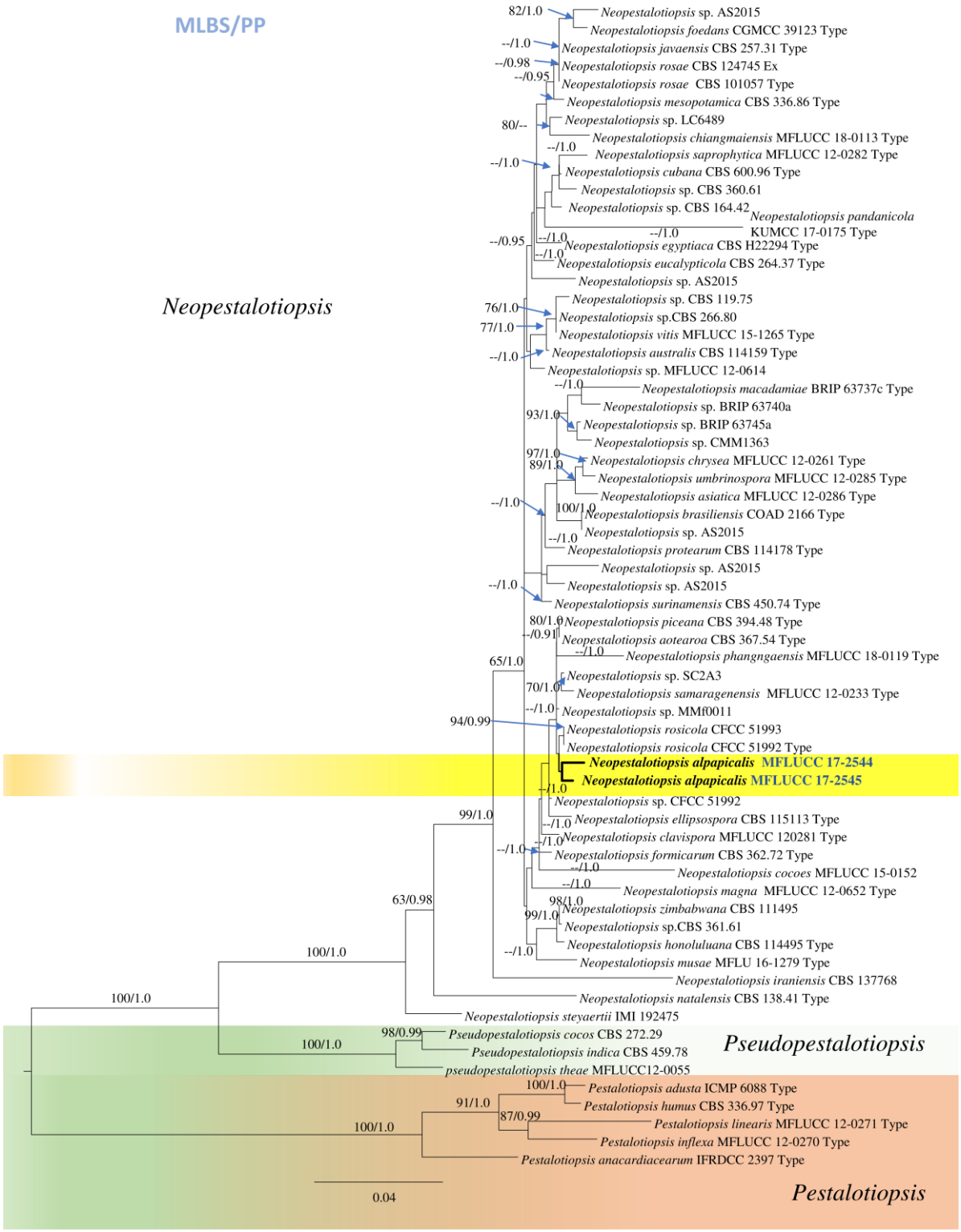


Figure 38 – Maximum-likelihood phylogenetic tree inferred from combined ITS, β-tub and TEF1 gene sequences of 65 taxa. The GTR+ I model of nucleotide evolution was used. Strains of the newly described species are depicted in bold lettering. Values at tree nodes indicate bootstrap support and posterior probabilities in that order. Only bootstrap values above 60 and posterior probabilities above 0.9 are shown.

Culture characteristics: Colonies on PDA attaining 25–30 mm diameter after 7 days at 26 ± 1 °C, with undulate edge, white, sparse aerial mycelium on the surface with black, gregarious conidiomata; reverse pale yellow.

Habitat: Endophytic on leaves of *Rhizophora mucronata* and *R. apiculata*.

Material examined: THAILAND, Krabi Province, fresh asymptomatic leaves of *Rhizophora mucronata* (Rhizophoraceae), 22 Sept. 2017, Vinit Kumar, RM5 (MFLU 19-0405), holotype, ex-type culture MFLUCC 17-2544), ibid. BBH isotype.

Notes: *Neopestalotiopsis alpapicalis* (MFLUCC 17-2544) falls within *Neopestalotiopsis* and forms a separate branch with a sister strain, MFLUCC 17-2545. *N. alpapicalis* is morphologically similar to *N. samarangensis*. However, *N. samarangensis* has 3 tubular apical appendages, which are long and thin, while *N. alpapicalis* has 1–4 short and thick apical appendages (Maharachchikumbura et al. 2013a). Along with this, *N. alpapicalis* has smaller apical cell as compared to other species within *Neopestalotiopsis* (Maharach. et al. 2014). When comparing the 472 TEF1 nucleotides of *N. alpapicalis* with other *N. rosicola* in the clade, there are only 6 bp differences, 5 bp differences in ITS, and 2 bp difference in β-tub. Morphological characters of the two strains of *N. alpapicalis* (MFLUCC 17-2544 and MFLUCC 17-2545), such as, the size of conidia, are often overlapping, but differ based on the size of apical cells, 0.75–2.3 ($\bar{x}$ = 1.25 µm) in MFLUCC 17-2544 and 2.0–4.0 ($\bar{x}$ = 2.75 µm). The sister taxon, *N. rosicola* (CFCC 51992, CFCC 51993), is different from *N. alpapicalis* in terms of conidial size and in the number and size of apical appendages (1–4 in *N. alpapicalis* and 2–4 in *N. rosicola*), while the size of apical appendages varies from 17–22.8 µm ($\bar{x}$ = 20.5 µm) in *N. rosicola* and 5.6–15 µm ($\bar{x}$ = 9.3 µm) in *N. alpapicalis* (Crous et al. 2012, Maharach. et al. 2014, Jiang et al. 2018). Dashes indicate BP/BI with less than 50/0.9. The tree is artificially rooted to *Pestalotiopsis humus* (CBS 336.97), *P. anacardiacearum* (IFRDCC 2397), *P. adusta* (ICMP 6088), *P. linearis* (MFLUCC 12-0271) and *P. inflexa* (MFLUCC 12-0270).

Pestalotiopsis Steyaert, Bull. Jard. bot. État Brux. 19: 300 (1949)

Index Fungorum number: IF9272

Type species: *Pestalotiopsis guepinii* (Desm.) Steyaert, Bull. Jard. bot. État Brux. 19(3): 312 (1949)

See the phylogenetic tree from Appendix III, Fig 25

Pestalotiopsis thailandica Norphanphoun, Doilom & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF556441; Facesoffungi number: FoF 05782 Fig. 40

Etymology – refers to the country where the fungus was collected, Thailand.

Holotype – MFLU 19-0787

Isolated from asymptomatic leaf of *Rhizophora apiculata* Blume.Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, proliferating 1–2 times percurrently, collarette present and not flared. *Conidia* (17–)17.5–28(–29) × (4.9–)5.5–6.5(–7.1) µm (mean

Figure 39 – *Neopestalotiopsis alpapicalis* (holotype MFLU 19-0405) on healthy leaf of *Rhizophora mucronata* and symptomatic leaf of *R. apiculata*. b, c Colony on PDA (above and below). d Conidiomata on PDA. e, f Conidia germinating from mycelia. g Conidiogenous cell. h–l Conidia. Scale bars: e–h = 10 µm, i–l = 5 µm.

± SD = 23.3 ± 3.0 × 5.8 ± 0.5 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (1.8–)2–4(–6) μm long (mean ± SD = 3.9 ± 1.3 μm); three median cells (12–)12.5–16(–18) μm long (mean ± SD = 15.3 ± 1.5 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4–)4.5–6(–7) μm long (mean ± SD = 5.6 ± 0.8 μm); third cell brown, (3.5–)4–4.5(–5.5) μm long (mean ± SD = 4.6 ± 0.5 μm); fourth cell brown, (3.5–)4–5(–6.5) μm long (mean ± SD = 5.1 ± 0.7 μm); apical cell (2–)3.5–4(–6) μm long (mean ± SD = 4.2 ± 1.0 μm), hyaline, conic to acute; with 1–2 tubular appendages on apical cell, inserted at same loci at the apex of the apical cell, unbranched, flexuous, (5.5–)11–34(–38) μm long (mean ± SD = 22.2 ± 8.5 μm); single basal appendage, tubular, unbranched, centric, (2–)2.5–9.5(–10) μm long (mean ± SD = 4 ± 2.0 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, The Sirindhorn International Environmental Park, Cha-am, Cha-am District, Phetchaburi Province, asymptomatic leaf of *Rhizophora apiculata*, 30 November 2016, Mingkwan Doilom NNSE03AL (MFLU 19-0787, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1616, TNCC. THAILAND, The Sirindhorn International Environmental Park, Cha-am, Cha-am District, Phetchaburi Province, asymptomatic leaf of *Rhizophora apiculata*, 30 November 2016, Mingkwan Doilom NNSE03DL (MFLU 19-0788, paratype); living cultures, MFLUCC 17-1617.

Notes – *Pestalotiopsis thailandica* isolated as an endophyte from a living leaf of *Rhizophora apiculata* is introduced here as a new species. In the phylogenetic analyses based on combined genes, the species appeared as a distinct species represented by two strains and is sister to *P. rhizophorae* (in this study) (Fig. 2). Although these two species are found on the same host and location, *P. thailandica* differs by having larger conidia (*P. rhizophorae*: (17–)17.5–23(–23.5) × (5.5–)6–6.5(–7) μm) and longer apical appendages (*P. rhizophorae*: (7.5–)8–13(–14.5) μm) than *P. rhizophorae* (Table 5) (see notes under *P. rhizophorae*). In the phylogenetic analyses, *P. rhizophorae* is also related to *P. formosana* (NTUCC 17-009) and *P. parva* (CBS 265.37). However, *Pestalotiopsis formosana* and *P. parva* are different from *P. thailandica* by having smaller conidia (*P. formosana*: (15–)18–22(–26) μm, *P. parva*: (16–)16.5–20(–21) μm) and shorter apical appendages (*P. formosana*: (8–)11–16(–20) μm, *P. parva*: (6–)6.5–12(–13) μm) with 2–3 tubular apical appendages (Table 5, Ariyawansa & Hyde 2018, Maharachchikumbura et al. 2014).

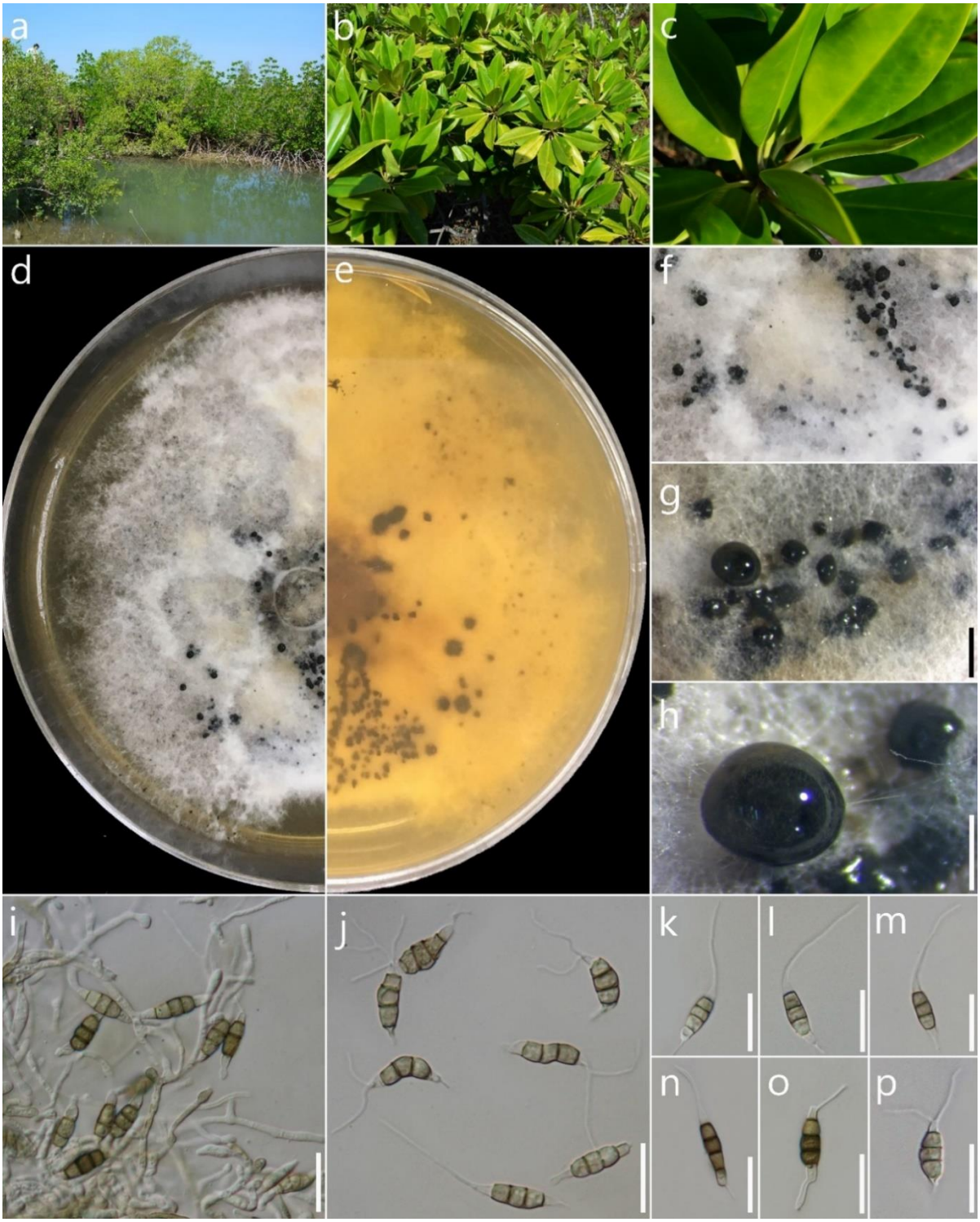


Figure 40 – *Pestalotiopsis thailandica* (MFLU 19-0787, holotype). a Habitat. b, c Leaf of *Rhizophera apiculata*. d, e Culture on PDA (d- above, e- reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g = 1000 µm, h = 100 µm, i–p = 20 µm.

Rhytidhysteron Speg, Anal. Soc. cient. argent. 12(4): 188 (1881)

Index Fungorum number: IF4740

Type species: *Rhytidhysteron brasiliense* Speg., Anal. Soc. cient. argent. 12(4): 188 (1881)

Rhytidhysteron mangrovei Vin. Kumar & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF555374, Facesoffungi number: FoF 04883 Fig. 41

Etymology: The specific epithet refers to the mangrove plant in Latin on which the fungus was collected.

Holotype: MFLU 18-1894.

Saprobic on decaying wood of standing mangrove tree. **Sexual morph**: *Ascomata* 0.93–1.98 long, 0.78–0.91 wide and 0.5–0.52 mm high ($\overline{x}$ = 0.94 × 0.8 × 0.5 mm, n = 10), apothecioid, crowded to aggregate, superficial to semi-immersed, subiculum, brown-black, with exposed, lenticular to irregular, brown-black disc, folded along the margins, compressed at the apex, perpendicularly striate along the axis. Exciple 65–90 µm wide ($\overline{x}$ = 85, n = 10), composed of dark brown to black, thin-walled cells of textura angularis. *Hamathecium* comprising 1.8–2.8 µm wide, hymenium turns blue in Melzer's reagent, J+, comprised dense, septate pseudoparaphyses, compressed at the septa, hyaline, unbranched and forming a dark epithecium above the asci, at the apex and enclosed in a gelatinous matrix. *Asci* 110–150 × 9.4–10 µm ($\overline{x}$ = 146 × 9.5, n = 20), (2–6–)8-spored, bitunicate, cylindrical, with short pedicel, rounded at the apex, with distinct ocular chamber. *Ascospores* 21–28 × 7.5–8.5 µm ($\overline{x}$ = 23 × 8.3, n = 30), uniseriate, slightly overlapping, hyaline to lightly pigmented when immature, becoming reddish-brown when mature, ellipsoidal to fusiform, straight or curved, rounded to slightly pointed at both ends, (1–)3-septate, guttulate, smooth wall. **Asexual morph**: Undetermined.

Material examined: THAILAND, Cha-am District, Phetchaburi Province, on dead twigs of mangrove tree, 11 January 2018, Vinit Kumar (MFLU 18-1894, holotype); ibid. (BBH isotype), ex-type living culture (MFLUCC 18-1113).

Notes: The new species, *Rhytidhysteron mangrovei* is different from the other *Rhytidhysteron* species based on morphological and molecular data. *Rhytidhysteron mangrovei* is characterized by large, conspicuous ascomata with coloured pruina, and fits well within the species concept of *Rhytidhysteron*. However, *R. mangrovei* differs in size of exciple (65–90 vs. 72–130 µm), appearance of ascomata (perpendicularly rough-striate vs. rough-without striations) and ascospore septations and size (1–3 vs. 3-septate, 21–28 × 7.5– 8.5 vs. 20–31 × 7.5–12 µm, respectively) from *R. thailandicum*. *R. mangrovei* is phylogenetically close to *R. thailandicum* (MFLUCC 12-0530, MFLUCC 14-0503) (100% MLBS/1.00 PP; 100% MLBS/1.00 PP). Apart from this, we compared the genetic distance of *R. thailandicum* and *R. mangrove*i for gene regions of ITS, LSU and TEF (Jeewon & Hyde 2016). R. mangrovei has 24 (ITS), 12 (LSU), and 27 (TEF) base-pair differences with *R. thailandicum*). Hereby, we established *R. mangrovei* as a new species following the recommendations from Jeewon & Hyde (2016).

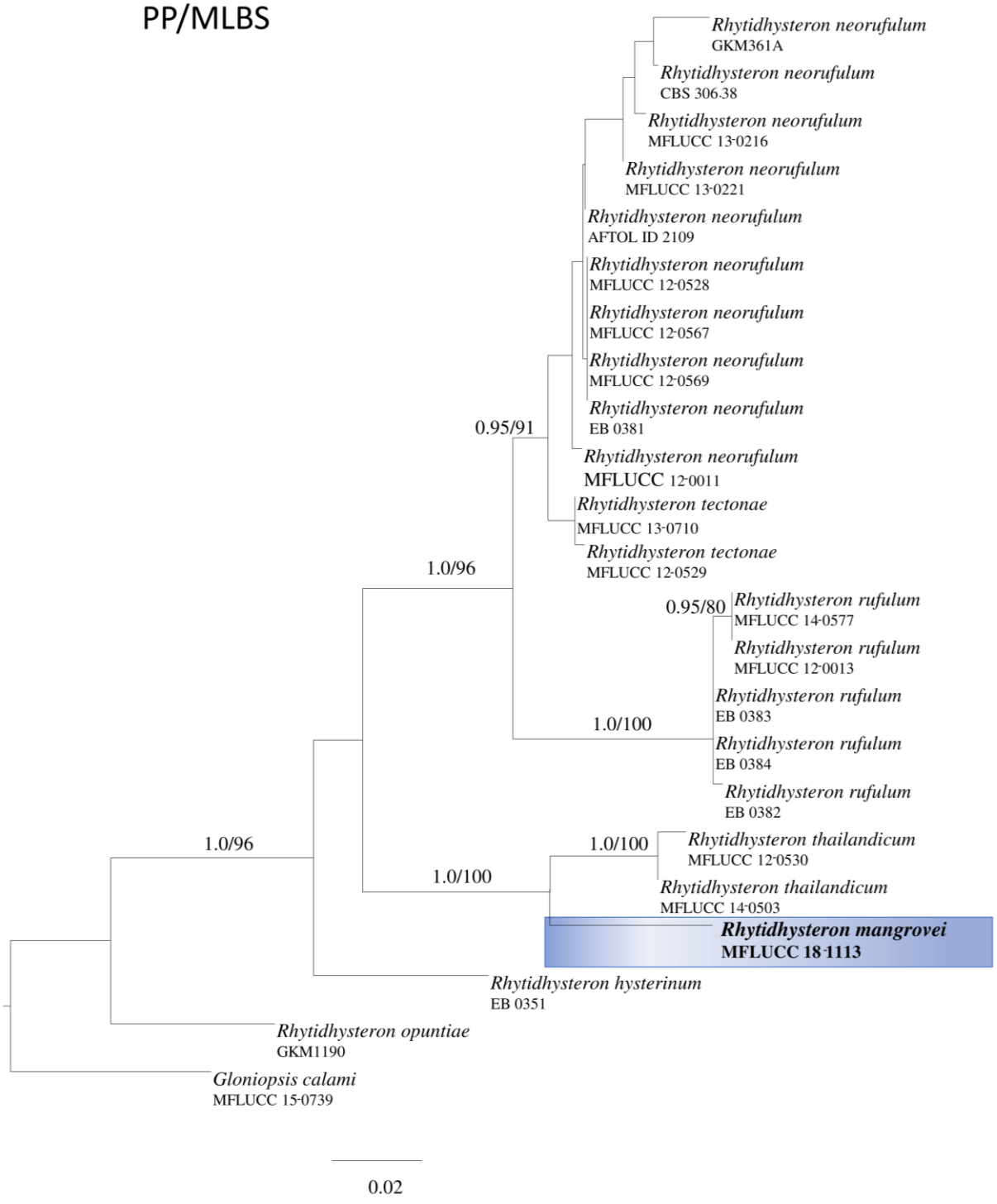


Figure 41 – Simplified phylogram generated from maximum likelihood (RAxML) analysis, based on combined LSU, ITS and TEF sequence dataset including 23 taxa from *Rhytidhysteron*. The tree is rooted in *Gloniopsis calami* (MFLUCC 15-0739). Maximum likelihood bootstrap values (MLBS) ≥70 % are defined as MLBS above or below the nodes. The new species is in bold font.

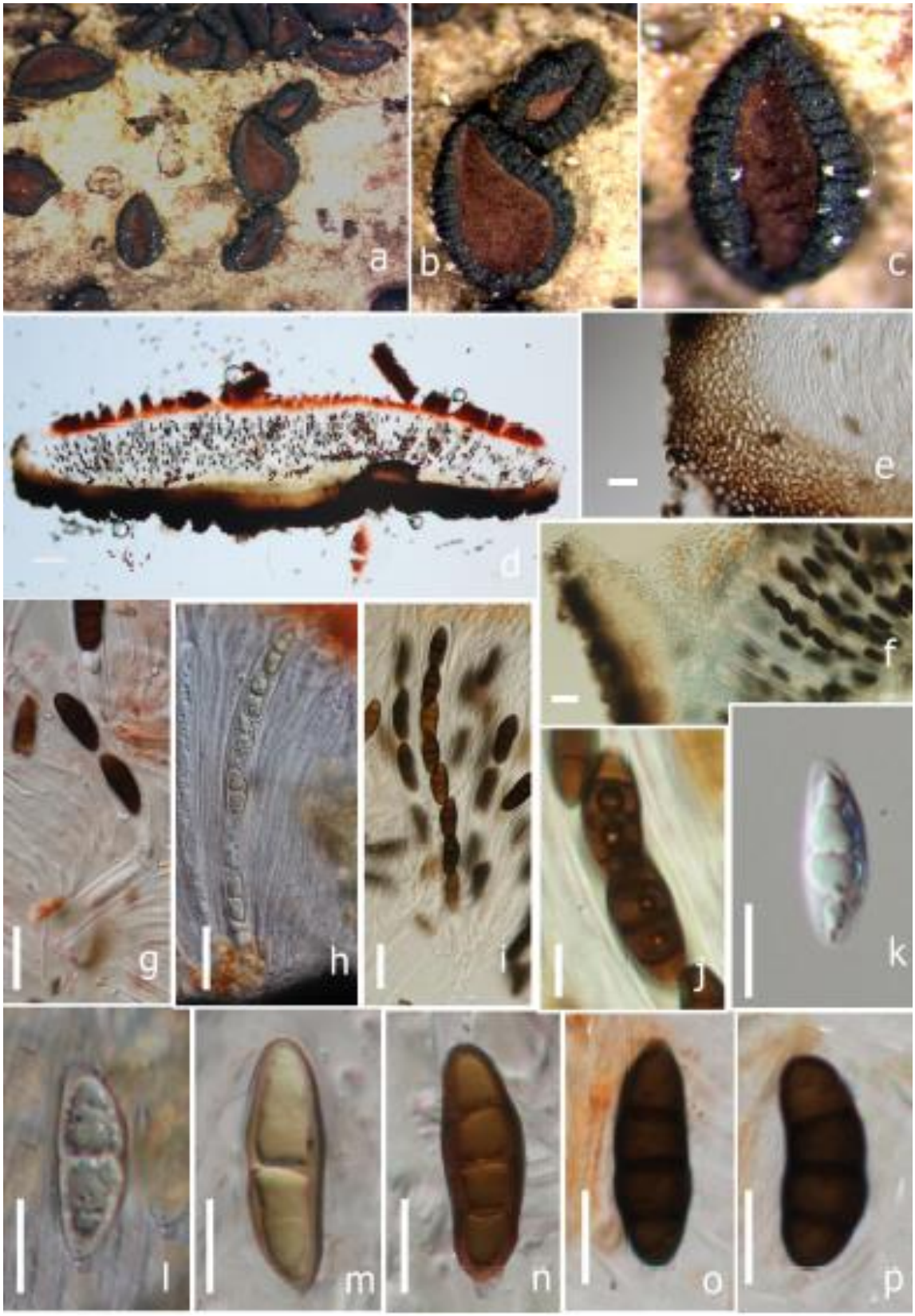


Figure 42 – *Rhytidhysteron mangrovei* (MFLUCC 18-1113, holotype). a Appearance of ascomata on the host substrate. b, c Close up of Hysterothecium. d Section of ascoma. e Exciple. f J+ hymenium. g–i Asci. j Ascospore with guttules. k–p Ascospores. Scale bars: d = 100 µm, e–i = 20 µm, j–p = 10 µm.

Appendix V To prepare herbarium material of all collections for future reference.

The strains isolated in this project were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Guizhou Culture Collection (GZCC). Herbarium specimens were deposited at the herbaria of Mae Fah Luang University (MFLU), Chiang Rai, Thailand, Biotec Bangkok Herbarium (BBH), Bangkok, Thailand, and Kunming Institute of Botany Academia Sinica (KUN), Kunming, China.

Table 11. Herbarium material deposit information.

Taxa	Order	Class	Original code	Herbarium code	Culture collection (MFLUCC)	Host	Substrate	Life mode	Collection date	Collector
Linocarpon sp.	Chaetosphaeriales	Sordariomycetes	SNT01	HKAS105422	*	*Nypa fruticans*	rachis	saprobic	6-July-2016	M. Doilom
Xylariaceae sp.	Xylariales	Sordariomycetes	SNT05	GZCC 19-0112	16-1328	*N. fruticans*	rachis	saprobic	6-July-2016	M. Doilom
Neodeightonia sp.	Botryosphaeriales	Dothideomycetes	SNT06	HKAS105423	16-1329	*N. fruticans*	petiole	saprobic	6-Jul-2016	M. Doilom
Linocarpaceae sp.	Chaetosphaeriales	Sordariomycetes	SNT34	HKAS105436	*	*N. fruticans*	rachis	saprobic	2-Dec-2016	S.N. Zhang
Pestalotiopsis sp.	Amphisphaeriales	Sordariomycetes	SNT35	HKAS105437	18-1522	*N. fruticans*	rachis	saprobic	2-Dec-2016	S.N. Zhang
Neodeightonia sp.	Botryosphaeriales	Dothideomycetes	SNT36	HKAS105438	*	*N. fruticans*	petiole	saprobic	3-Dec-2016	S.N. Zhang
Linocarpaceae sp.	Chaetosphaeriales	Sordariomycetes	SNT37	HKAS105439	18-1472	*N. fruticans*	rachis	saprobic	3-Dec-2016	S.N. Zhang
Astrosphaeriellaceae sp.	Pleosporales	Dothideomycetes	SNT42	MFLU 19-0805	18-1439	*N. fruticans*	rachis	saprobic	3-Dec-2016	S.N. Zhang
Xylariaceae sp.	Xylariales	Sordariomycetes	SNT43	HKAS105442	18-1474	*N. fruticans*	rachis	saprobic	3-Dec-2016	S.N. Zhang
Striatiguttula nypae	Pleosporales	Dothideomycetes	SNT44	MFLU 18-1576	18-0265	*N. fruticans*	rachis	saprobic	3-Dec-2016	S.N. Zhang
Rhytismataceae sp.	Rhytismatales	Leotiomycetes	SNT45A	HKAS105443	18-1523	*N. fruticans*	rachis	saprobic	3-Dec-2016	S.N. Zhang
Linocarpaceae sp.	Chaetosphaeriales	Sordariomycetes	SNT45B	HKAS105444	*	*N. fruticans*	rachis	saprobic	3-Dec-2016	S.N. Zhang
Neopestalotiopsis piceana	Amphisphaeriales	Sordariomycetes	SNT46	MFLU 19-0806	18-1475	*N. fruticans*	leaves	saprobic	3-Dec-2016	S.N. Zhang
Xylariaceae sp.	Xylariales	Sordariomycetes	SNT80	HKAS105459	18-0459	*N. fruticans*	leaves	saprobic	2-Dec-2016	S.N. Zhang
Linocarpaceae sp.	Chaetosphaeriales	Sordariomycetes	SNT81	HKAS105460	*	*N. fruticans*	rachis	saprobic	4-Dec-2016	S.N. Zhang
Tirisporella beccariana	Tirisporellales	Sordariomycetes	SNT82	MFLU 18-1583	18-1572	*N. fruticans*	petiole	saprobic	7-Dec-2016	S.N. Zhang
Phaeoacremonium sphinctrophorum	Diaporthales	Sordariomycetes	SNT86	HKAS105462	*	*N. fruticans*	rachis	saprobic	6-Dec-2016	S.N. Zhang
Vaginatispora palmae	Pleosporales	Dothideomycetes	SNT92	MFLU 18-1586	18-1526	*N. fruticans*	rachis	saprobic	3-Dec-2016	S.N. Zhang
Longicorpus striataspora	Pleosporales	Dothideomycetes	SNT93	MFLU 18-1580	18-0267	*N. fruticans*	rachis and petiole	saprobic	4-Dec-2016	S.N. Zhang
Savoryella nypae	Savoryellales	Sordariomycetes	SNT97	MFLU 19-0011	18-1570	*N. fruticans*	petiole	saprobic	4-Dec-2016	S.N. Zhang
Longicorpus striataspora	Pleosporales	Dothideomycetes	SNT195	MFLU 18-1582	17-2515	*N. fruticans*	petiole	saprobic	30-Aug-2017	S.N. Zhang
Tirisporella beccariana	Tirisporellales	Sordariomycetes	SNT203	MFLU 18-1585	*	*N. fruticans*	petiole	saprobic	30-Aug-2017	S.N. Zhang

Taxa	Order	Class	Original code	Herbarium code	Culture collection (MFLUCC)	Host	Substrate	Life mode	Collection date	Collector
Striatiguttula nypae	Pleosporales	Dothideomycetes	SNT207	MFLU 18-1577	17-2517	*N. fruticans*	petiole	saprobic	30-Aug-2017	S.N. Zhang
Akanthomyces muscarius	Hypocreales	Sordariomycetes	E104	MFLU 18-1145	17-2540	*N. fruticans*	leaf	endophyte	11-Sep-2017	Vinit Kumar
Halocyphina sp.	Agaricales	Agaricomycetes	SS7b	*	*	*N. fruticans*	leaf sheath	saprobic	11-Jun-2018	Vinit Kumar
Oxydothis sp.	Xylariales	Sordariomycetes	SS7	*	*	*N. fruticans*	leaf sheath	saprobic	11-Jun-2018	Vinit Kumar
Tubeufia sp.	Tubeufiales	Dothideomycetes	VPH-12	*	*	*N. fruticans*	leaf sheath	saprobic	5-May-2019	Vinit Kumar
Peroneutypa scoparia	Xylariales	Sordariomycetes	KC-6	MFLU 19-0623	18-1111	*Rhyzophora apiculata*	aerial branch	saprobic	11-Jan-2018	Vinit Kumar
Peroneutypa sp.	Xylariales	Sordariomycetes	KC-4	*	18-1110	*Rh. apiculata*	aerial branch	saprobic	11-Jan-2018	Vinit Kumar
Rhytidhysteron mangrovei	Hysteriales	Dothideomycetes	KC-8	MFLU 18-1894	18-1113	*Rh. apiculata*	aerial branch	saprobic	11-Jan-2018	Vinit Kumar
Rhytidhysteron sp.	Hysteriales	Dothideomycetes	KC-3	*	18-1112	*Rh. apiculata*	aerial branch	saprobic	11-Jan-2018	Vinit Kumar
Neopestalotiopsis alpapicalis	Amphisphaeriales	Sordariomycetes	RM-5	MFLU 19-0405	17-2544	*Rh. apiculata*	fresh leaf	endophyte	22-Sep-17	Vinit Kumar
Neopestalotiopsis alpapicalis	Amphisphaeriales	Sordariomycetes	RM-1	*	17-2545	*Rh. apiculata*	fresh leaf	endophyte	22-Sep-17	Vinit Kumar
Diaporthe sp.	Diaporthales	Sordariomycetes	E103	*	17-2541	*Rh. apiculata*	fresh leaf	endophyte	11-Sep-2017	Vinit Kumar
Diaporthe sp.	Diaporthales	Sordariomycetes	E106	*	17-2543	*Rh. apiculata*	fresh leaf	endophyte	11-Sep-2017	Vinit Kumar
Halosarpheia sp.	Microascales	Sordariomycetes	VPH-17	*	*	*Rh. apiculata*	dead branch	saprobic	5-May-2019	Vinit Kumar
Lulworthia grandispora	Lulworthiales	Sordariomycetes	S-102	*	17-2539	*Rh. apiculata*	dead branch	saprobic	11-Sep-2017	Vinit Kumar
Hysterium sp.	Hysteriales	Dothideomycetes	VPH-2	*	*	*Rh. apiculata*	aerial branch	saprobic	5-May-2019	Vinit Kumar
Dictyosporium marinum	Pleosporales	Dothideomycetes	GJ357	BBH on process	*	*	*	saprobic	12-May-2017	M.C. Dayarathne
Boeremia exigua var. *maritima*	Pleosporales	Dothideomycetes	GJ389b	BBH on process	*	*	*	saprobic	20-Jun-2017	M.C. Dayarathne
Neocamarosporium artemisiae	Pleosporales	Dothideomycetes	GJ392	BBH on process	*	*	*	saprobic	20-Jun-2017	M.C. Dayarathne
Neocamarosporium maritima	Pleosporales	Dothideomycetes	GJ389a	BBH on process	*	*	*	saprobic	21-Jun-2017	M.C. Dayarathne
Nigrograna rhizophorae	Pleosporales	Dothideomycetes	MCD185	BBH on process	*	*	*	saprobic	18-jan-2018	M.C. Dayarathne
Periconia salina	Pleosporales	Dothideomycetes	GJ374	BBH on process	*	*	*	saprobic	12-May-2017	M.C. Dayarathne
Amarenographium ammophilicola	Pleosporales	Dothideomycetes	GJ448	BBH on process	*	*	*	saprobic	15-Oct-2017	M.C. Dayarathne
Salsuginea rhizophorae	Pleosporales	Dothideomycetes	MCD055	BBH on process	*	*	*	saprobic	7-Dec-2016	M.C. Dayarathne
Halotestudina muriformis	Pleosporales	Dothideomycetes	MCD163	BBH on process	*	*	*	saprobic	31-Aug-2017	M.C. Dayarathne
Asterodiscus mangrovei	Stigmatodiscales	Dothideomycetes	KLA025	BBH on process	*	*	*	saprobic	16-Dec-2015	M.C. Dayarathne
Diaporthe salinicola	Diaporthales	Sordariomycetes	MCD072	BBH on process	*	*	*	saprobic	6-Dec-2016	M.C. Dayarathne
Chaetopsina aurantisalinicola	Hypocreales	Sordariomycetes	MCD21Y	BBH on process	*	*	*	saprobic	2-Dec-2017	M.C. Dayarathne

Appendix VI To sequence appropriate genes of phylogenetically well-studies genera to identify cryptic species within species complex.

Culture-based studies have recovered fungal endophytes from numerous plant hosts, while direct examination of sporulating cultures has enabled identification. However, many endophytes cannot be identified due to the fact that they only form mycelia sterilia in culture. Although next generation sequencing (NGS), as well as ITS sequence analyses have been used to identify endophytes, identification is still rudimentary. In this study, we isolated fungal endophytes from *Rhizophora apiculata* in Thailand and established how many can be identified to species level based on ITS sequence data. Endophytic fungi were isolated from leaves, petioles and aerial roots of *R. apiculata* in four provinces of Thailand. One hundred and fifty four isolates were obtained and initially grouped into 20 morphotypes based on cultural characteristics. Nine were sporulating morphotypes, which were assigned to seven genera (*Colletotrichum*, *Diaporthe*, *Hypoxylon*, *Neopestalotiopsis*, *Neodevriesia*, *Pestalotiopsis* and *Phyllosticta*), and eleven morphotypes were non-sporulating mycelia sterilia. Sequence similarity comparison and phylogenetic analysis of the ITS regions were further used to identify taxa. While ITS sequence data is reliable to assign isolates at the generic rank, and can be useful to identify taxa to species level in a small number of fungal genera, it cannot generally be used to determine specific species in most genera. ITS analysis classified 30 representative isolates into 20 taxonomic units residing in 15 known genera: *Allophoma* sp., *Colletotrichum* spp., *Diaporthe* spp., *Hortaea werneckii*, *Hypoxylon griseobrunneum*, *Hypoxylon* sp., *Pestalotiopsis* sp., *Phanerochaete* sp., *Phyllosticta* spp., *Pseudopithomyces maydicus*, *Preussia* sp., *Nemania* sp., *Neodevriesia* sp., *Neopestalotiopsis* sp., *Rigidoporus vinctus*, *Schizophyllum* sp. and one unidentified genus. Of the morphotypes, four were identified to species.

Cultural characteristics

Based on cultural characteristics such as colony shape, colour of hyphae and surface, 154 isolates were grouped into 20 morphotypes. Of these, nine morphotypes sporulated on PDA and they were identified to seven genera viz. *Colletotrichum*, *Diaporthe*, *Hypoxylon*, *Neopestalotiopsis*, *Neodevriesia*, *Pestalotiopsis* and *Phyllosticta*. The remaining eleven groups were mycelia sterilia on PDA. Different endophytic taxa were selected to be potentially identified to assess our current taxonomic concept based on morphotypes. Growth rate and colony were observed and noted (Table 12, Figs 43, 44). These characters are provided as they may be useful for primary identification of endophytic fungi from mangroves and/or other host plants, at least for certain taxonomic groups. However, the colour of mycelium, and growth rates, can vary under different conditions such as media, light and temperature. Moreover, grouping of taxa into morphotypes does not reflect species phylogeny as morphotypes are not real taxonomic units (Guo et al. 2003). Surprisingly, some isolates having different cultural characters were found to belong to the same genus (Figs 44a, 44b). Two replicates of the same morphotype showed slightly different cultural characteristics (Figs 44g, 44h).

Phylogenetic analysis

The blast search results of ITS sequence data are shown in Table 5. The ITS alignments length including gaps comprised 901 and 708 characters for the two datasets investigated (Figs. 45, 46, respectively). The RAxML analysis resulted in a best scoring likelihood tree selected with a final value for the ITS dataset = –11994.67091, and –1963.727247 The likelihood of the final tree was evaluated and optimized under GAMMA model parameters, with 694 distinct alignment patterns and 38.29% of completely undetermined characters and gaps, and 167 distinct alignment patterns and 21.66% undetermined characters or gaps. The ITS phylogeny resulted in the detection of 20 taxonomic units residing in 15 known genera (*Allophoma*, *Colletotrichum*, *Diaporthe*, *Hortaea*, *Hypoxylon*, *Pestalotiopsis*, *Phanerochaete*, *Phyllosticta*, *Pseudopithomyces*, *Preussia*, *Neodevriesia*, *Neopestalotiopsis*, *Nemania*, *Rigidoporus*, *Schizophyllum*) and one unidentified ascomycete genus from 30 representative strains out of the 154 isolates. Thirteen of the genera belong to Ascomycota, while three genera, *Phanerochaete*, *Rigidoporus* and *Schizophyllum* are basidiomycetes. Basidiomycetous fungi such as *Polyporales* and *Rigidoporus* have also been found as endophytes in leaves of coniferous trees and *Paphiopedilum villosum* (Yoo & Eom 2012, Khamchatra et al. 2016). Details of representative strains of the determined endophytic fungal genera on *R. apiculata* are listed (Table 14). The results of Blast search and ITS phylogeny of these 16 genera as well as additional data and genes are discussed below.

Allophoma – MFLUCC 17-0003 had 100% matches with many isolates in the MegaBLAST search of NCBIs GenBank nucleotide database. The maximum score was 981, which is similar to e.g. *Stagonosporopsis cucurbitacearum* E-271 (Table 13). Our ITS based phylogeny does not clearly separate *Allophoma from Stagonosporopsis* (Fig 25: K). Strain MFLUCC 17-0003 is assigned to *Allophoma* as the ITS sequence was most similar to this genus. However, *S. cucurbitacearum* E-271 has not been formally published. A combined multi-locus phylogenetic analysis based on ITS, large subunit rDNA (LSU), second largest subunit of RNA polymerase II (RPB2) and β-tubulin (Tub2), and morphological studies are needed for a better generic delimitation for these closely related genera as has been the case for the Didymellaceae (Chen et al. 2015, 2017, Hyde et al. 2016).

Colletotrichum – Three representative isolates (MFLUCC 17-1943, MFLUCC 17-1944, MFLUCC 17-0004) were identified as Colletotrichum based on morphological and cultural characteristics. ITS sequence data of MFLUCC 17-1943 and MFLUCC 17-1944 were identical, but differed from MFLUCC 17-0004. Based on a MegaBLAST search, the ITS of MFLUCC 17-1943 matched 100% with many isolates of *Colletotrichum* and fungal endophytes, but had max score (1074) with C. gloeosporioides AAP-018 and Fungal sp. SF3 (Table 13). Isolate MFLUCC 17-0004 also shared 99% with many Colletotrichum species, but had a 1088 max score with *C. brevisporum* PC-1 (Table 13). However, it is known that many species of *Colletotrichum* cannot be distinguished reliably using ITS (Hyde et al. 2009, Weir et al. 2012), and hence the three representative isolates are named as *Colletotrichum* spp. Multi-locus gene regions would help determine the current species. Cai et al. (2009) provided detailed protocols for studying *Colletotrichum* species. Multilocus phylogenetic analyses have typically been used to resolve *Colletotrichum* species (e.g. Talhinhas et al. 2002, Weir et al. 2012, Damm

et al. 2013, Tao et al. 2013). Recommended genetic markers were suggested (Hyde et al. 2014). Genes and combination of genes that can be used for identification of the *Colletotrichum* species complexes were recommended (Cannon et al. 2012, Hyde et al. 2014) and have been summarized by Jayawardena et al. (2016). For example, a combined of ITS, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), chitin synthase 1 (CHS-1), histone H3 (HIS3), actin (ACT) and Tub2 sequences data was used to identify species in the *Colletotrichum acutatum* species complex (Jayawardena et al. 2016).

Diaporthe – Comparison of nucleotide polymorphism of the ITS sequences data between MFLUCC 17-1942 and MFLUCC 17-0035 showed the major differences over a span of c. 29 polymorphisms. These consist of two deletions, five insertions, 12 transitions and 10 tranversions. Based on the recommendations of Jeewon & Hyde (2016), we consider these two isolates to be distinct. Blast searches of the ITS, MFLUCC 17-0035 and MFLUCC 17-1942 are provided in Table 5. ITS phylogenetic analysis showed that MFLUCC 17-1942 grouped with *Phomopsis* sp. MA194 with weak bootstrap support, and next to Diaporthe arengae CBS 114979 (ex-type) with 95% ML and 0.95 PP (Fig 45: F). These three isolates grouped with Diaporthe arecae CBS 161.64 (ex-isotype) and MFLUCC 17-0035 with 100% ML and 1.00 PP. However, multiple gene analyses are required to improve the accuracy of species delimitation of these two isolates. Identification of *Diaporthe* species is not always appropriate using morphological characters, because of their plasticity and overlap between different species (Santos & Phillips 2009). ACT, calmodulin (CAL), HIS3, ITS, translation elongation factor 1-alpha (TEF) and Tub2 should be used in combined analyses with at least 4–5 genes with recommended primers (Udayanga et al. 2012, 2014a, b, Gomes et al. 2013, Hyde et al. 2014, Dissanayake et al. 2017). Santos et al. (2017) suggested that if only four loci can be sequenced these should be TEF, Tub2, CAL and HIS3; if three loci these should be TEF, Tub2 and CAL; if two loci TEF and Tub2; if only one locus TEF as TEF sequence data is the most informative for species separation. ITS sequence data is the least informative to resolve *Diaporthe* species (Santos et al. 2017). However multi-loci sequence analysis (MLSA) provides more accurate estimation of phylogeny and has less separation errors than single locus analysis, if reasonable loci are used (Gadagkar et al. 2005, Mirarab et al. 2016, Santos et al. 2017).

Hortaea – Based on a MegaBLAST search of NCBIs GenBank nucleotide database, the closest hit using the ITS sequence of MFLUCC 17-1940 matched 100% and had max score (966) with many isolates such as *Hortaea werneckii* MCw215 (Table 12). This latter isolate is from a sample of water, collected during the pre-monsoon summer season from mangroves at Santa Cruz, India (Nayak et al. 2012). The ITS phylogeny grouped MFLUCC 17-1940 with *H. werneckii* MCw215 and CBS 107.67 (ex-neotype) with 100% ML and 1.00 PP, and is therefore considered to be *Hortaea werneckii*.

Hypoxylon – ITS sequence data of MFLUCC 17-0020 was identical to MFLUCC 17-0027. The closest hits using a MegaBLAST search of the ITS DNA sequence data of MFLUCC 17-0020 had 100% similar to several strains, but had a 935 max score to *H. anthochroum* EGJMP20 and Xylariaceae sp. AT4 (unpublished in GenBank 2017) (Table 13). The types and

published strains of *H. griseobrunneum* were also included in the ITS phylogeny based on the information from Kuhnert et al. (2014a). Two isolates in this study grouped with *H. griseobrunneum* agrEK07, STMA06148, CBS 331.73 (ex-type), BCRC 34050 (as *H. anthochroum*), and *H. anthochroum* EGJMP20 with 93% ML and 0.99 PP support. MFLUCC 17-0020 and MFLUCC 17-0027 are identified as *H. griseobrunneum* based on similarity of ITS sequence data and morphology of the asexual morph being similar to *H. griseobrunneum* (Kuhnert et al. 2014a). Blast searches of another isolate MFLUCC 17-1945 are provided in Table 5. This isolate grouped sister to *H. lechatii* MUCL 54609 and CBS 123577 (ex-type) (Kuhnert et al. 2014b), and Xylariaceae sp. D11a4 with 87% ML and 0.97 PP. Comparison of nucleotide polymorphism of the ITS sequence data differs between our collection MFLUCC 17-1945 and *H. lechatii* CBS 123577 (ex-type) strain with eight polymorphisms consisting of five transitions, two deletions and one insertion. Thus, MFLUCC 17-1945 is considered to be *Hypoxylon* sp., however, it is necessary to compare the Tub2 gene to clarify species level reliably (Kuhnert et al. 2014a, b, Daranagama et al. 2017).

Nemania – MFLUCC 17-0005 was closely related to *Nemania diffusa* BCC 18754 (Okane et al. 2012) with 100% sequence similarity and 1003 max score based on a MegaBLAST search (Table 13). *Nemania diffusa* BCC 18754 has been reported as a xylariaceous endophytic fungus from Pteris decrescens in Thailand (Okane et al. 2012). There are many isolates of *N. diffusa* with ITS sequences data available in GenBank, but the type strain is not sequenced. MFLUCC 17-0005 had high ITS sequence similarity to *N. diffusa* BCC 18754. However, *N. diffusa* BCC 18754 (Okane et al. 2012) showed multiple nucleotide differences from other *N. diffusa* isolates GZ AT-F006 and FR AT-113 (Tang et al. 2007, 2009). MFLUCC 17-0005 is named as *Nemania* sp. The isolate should be compared with ex-type cultures for resolving the species name. An epitype for this species should be designated.

Neodevriesia – The ITS of MFLUCC 17-1939 was 99% similar to *Neodevriesia pakbia* CBS 139914 (ex-type) (Table 13). This isolate grouped close to, but was distinct from *N. pakbia* CBS 139914 (ex-type) with 100% ML and 1.00 PP. MFLUCC 17-1939 is determined to be Neodevriesia sp. LSU, TEF and Tub2 should be used for further species identification coupled with morphological characterization (Crous et al. 2014).

Neopestalotiopsis – Blast searches of the ITS for isolate MFLUCC 17-1941 is provided in Table 5. The ITS phylogeny suggests it is closely related to *Neopestalotiopsis aotearoa* CBS 367.54 (ex-type), *N. piceana* CBS 394.48 (ex-type), N. eucalypticola CBS 264.37 (ex-type), and Pestalotiopsis sp. LH162 (from blast search) with 90% ML and 1.00 PP, Morphological examination of characters also pointed to the genus *Neopestalotiopsis* and hence we refer isolate MFLUCC 17-1941 to *Neopestalotiopsis* sp. However, we refrain from assigning a species name because of morphological plasticity among those species (Jeewon et al. 2002, 2003, Maharachchikumbura et al. 2014). Analysis of combined sequence data of ITS, Tub2 and TEF gene regions is necessary to delimit the species according to Maharachchikumbura et al. (2014, 2016).

Pestalotiopsis – ITS sequence data of MFLUCC 17-0016, MFLUCC 17-0018, MFLUCC 17-0019, MFLUCC 17-0024, MFLUCC 17-0025, MFLUCC 17-0026 and MFLUCC 17-1941

were aligned together in MAFFT. All of these isolates shared identical sequences, except MFLUCC 17-1941. Isolate MFLUCC 17-0019 was selected as the representative strain of this group and blasted using the GenBank BLAST option. The ITS of MFLUCC 17-0019 had 100% similarity to several strains, and one of these was *Pestalotiopsis* sp. SC5A8 with 1075 max score (Table 5). MFLUCC 17-0016, MFLUCC 17-0018, MFLUCC 17-0019, MFLUCC 17-0024, MFLUCC 17-0025 and MFLUCC 17-0026 are named as *Pestalotiopsis* sp. as they were similar to *Pestalotiopsis* sp. based on morphological and cultural characteristics, and ITS phylogeny. Most of the key conidial characters are unstable for species level separation as they vary with host range, generation, culture and other environmental conditions (Jeewon et al. 2003, Hu et al. 2007). Naming of species based on morphological characters should be taken into account rather than host association (Jeewon et al. 2004, Maharachchikumbura et al. 2011). Maharachchikumbura et al. (2012) utilized ten gene regions (ACT, Tub2, CAL, GAPDH, glutamine synthetase (GS), ITS, LSU, largest subunit of RNA polymerase II (RPB1), SSU and TEF) to resolve cryptic *Pestalotiopsis* species, and showed that ITS, Tub2 and TEF were better markers. The other gene regions were less useful owing to poor success in PCR amplification and/or in their ability to determine species delimitation. TEF appeared to be an ideal candidate and functions well to determine species delimitation due to its better species resolution and PCR success. Tub2 showed fairly good differences among species. Combined ITS, Tub2 and TEF gene regions has given a high number of strongly supported nodes at the terminal clades as compared to single gene analysis (Maharachchikumbura et al. 2012, 2014).

Phanerochaete – ITS of MFLUCC 17-0002 had similarity (97% identity) with 946 max score to *Phanerochaete stereoides* He2309 (Table 12). ITS phylogeny of MFLUCC 17-0002 grouped sister to *P. stereoides* He2309 (Liu & He 2016) with bootstrap support of ML (96%) and PP (1.00). Isolate MFLUCC 17-0002 is therefore assigned to *Phanerochaete* sp. RPB1, RPB2, ITS and LSU were used to revised the taxonomy of *Phanerochaete* (Polyporales, Basidiomycota) (Floudas & Hibbett 2015).

Phyllosticta – Isolate MFLUCC 17-0030 had 92% similarity with many species of *Phyllosticta*, but showed 845 max score with *P. aristolochiicola* BRIP 53316a (Table 13). MFLUCC 17-0030 and MFLUCC 17-0031 grouped together with 100% ML and 1.00 PP, but separated from Guignardia sp. 1-3-5-1-3-1 with weak bootstrap support, and next to *P. styracicola* CGMCC 3.14985 (ex type) with 70% ML and weak support of PP. Another isolate, MFLUCC 17-1937 was 99% similar to many isolates, but had max score (1064) with Fungal sp. isolate 59815 (Table 5). MFLUCC 17-1937 grouped 100% ML and 1.00 PP with Fungal sp. isolate 59815 and *P. fallopiae* MUCC 0113. There are two species of *Phyllosticta* from *Rhizophora apiculata* in this study based on ITS sequence data. However multiple genes are required to clarify species names reliably. Wikee et al. (2013a) compared phylogenies of five genes analysis (ACT, GAPDH, ITS, LSU and TEF) with two genes analysis (ACT and ITS). They indicated that the combined ACT and ITS gene loci is sufficiently robust to distinguish most taxa in *Phyllosticta*, except those closely related to *P. capitalensis*. Wikee et al. (2013b) used analysis of combined ITS, ACT and TEF gene data to confirm the identity of all isolates of *P. capitalensis*. ITS gene and combined analyses of ITS, TEF, GAPDH and ACT sequence data are recommended for generic level and inter-specific delineation respectively (Hyde et al.

2014). A polyphasic approach including morphological, molecular and proteomic techniques were used to improved species identification and delimitation (Wulandari et al. 2009, Glienke et al. 2011, Wicht et al. 2012, Wong et al. 2012, Guarnaccia et al. 2017).

Preussia – The ITS sequences of MFLUCC 17-0022 and MFLUCC 17-0023 were identical. Isolate MFLUCC 17-0023 was selected to blast and it matched 99% with many isolates, but had max score (983) with *Preussia* sp. CY218 (Table 13). The ITS phylogeny, grouped MFLUCC 17-0022 and MFLUCC 17-0023 close to *Preussia* sp. CY218 with 100% ML and 0.97 PP, and sister to *P. persica* CBS 117680 (ex-type) with 100% ML and 1.00 PP. Nucleotide differences comparison of the ITS sequence data between our isolates and *P. persica* CBS 117680 (ex-type) strain reveal nine polymorphisms. Thus, the two isolates are described herein as Preussia sp. Additional genes and morphological characteristics are needed for more precise species delimitation. Arenal et al. (2007) used morphological characters and combined ITS, LSU and TEF loci to erect and identify new species of *Preussia*.

Pseudopithomyces – Isolate MFLUCC 17-0028 had the highest similarity (100%) to many isolates, but had max score (1040) with *Pithomyces maydicus* UTHSC 06-1549 (da Cunha et al. 2014) However, *Pithomyces maydicus* (Sacc.) M.B. Ellis was combined as *Pseudopithomyces maydicus* (Sacc.) J.F. Li, Ariyawansa & K.D. Hyde by Ariyawansa et al. (2015). The genus *Pseudopithomyces* comprises *P. chartarum* (Berk. & M.A. Curtis) M.B. Ellis, P. maydicus (Sacc.) M.B. Ellis, P. sacchari (Speg.) M.B. Ellis and some unidentified *Pithomyces* strains, which group together in Didymosphaeriaceae (Ariyawansa et al. 2015). The isolate in this study (MFLUCC 17-0028) grouped with *P. maydicus* UTHSC 06-1549 and UTHSC 06-3954 with high statistical support. *Pseudopithomyces* species can be separated based on ITS phylogeny in this study. Isolate MFLUCC 17-0028 is therefore considered as *P. maydicus*. A combined dataset of ITS, LSU, GAPDH and RPB2 has also been reported to resolve taxonomy of Pseudopithomyces species (Crous et al. 2016).

Rigidoporus – MFLUCC 17-0007 had a high sequence similarity (100%) identity and 1158 max score with *Rigidoporus vinctus* FRIM142 (Table 12). ITS phylogeny placed this isolate with *R. vinctus* FRIM142, PAPH04 and N_L7_E6 with 100% ML and 1.00 PP, and it is therefore considered to be *Rigidoporus vinctus*.

Schizophyllum – ITS sequence data of MFLUCC 17-1946 was selected to blast as it was identical to the ITS sequence of MFLUCC 17-1947. Isolate MFLUCC 17-1946 showed 99% sequence similarity and 1146 max score with *Schizophyllum commune* isolate UZ1552_14 (Table 13). Other published isolates and reference strains of *Schizophyllum* were also included in the ITS phylogeny (Siqueira et al. 2016). The isolates in this study could not be well separated from *S. commune* and *S. radiatum*. Therefore, the two isolates are determined to be *Schizophyllum* sp. Siqueira et al. (2016) noted that the phylogenetic analyses of the individual ITS and LSU genes were much conserved and did not discriminate well between the closely related species *S. commune* and *S. radiatum*. The LSU, TEF, and RPB2 markers showed consistency and were used to perform a concatenated study.

Unidentified – MFLUCC 17-1938 showed a sequence similarity (99%) and max score (983) with Mycosphaerellaceae sp. MA12 (Table 13). Isolate MA12 is an endophyte on a mangrove plant in the South of Thailand, and Buatong (2010) placed it in Mycosphaerellaceae and reported that the isolate displayed strong antifungal activity against *Cryptococcus neoformans* ATCC90112. ITS phylogeny groups our isolate (MFLUCC 17-1938) with Mycosphaerellaceae sp. MA12 with 100% ML and 1.00 PP. MFLUCC 17-1938 and Mycosphaerellaceae sp. MA12 are nested in between the genera Devriesia and Hortaea which are classified in the Teratosphaeriaceae. We could not observe morphological details on culture of MFLUCC 17-1938 as it did not produce spores. Thus, isolate MFLUCC 17-1938 remains an unidentified taxon.

The use of highest nucleotide similarity in MegaBLAST searches of NCBIs GenBank nucleotide database is a preliminary step towards determining fungal endophyte species. The top score match may not necessarily indicate the same species (Kang et al. 2010). It is difficult to make conclusions for isolates within or related to species complexes based on MegaBLAST searches and phylogenetic analysis of ITS sequences data. However, the ITS region is still useful in some cases for reconstruction of interspecific relationships (Cai et al. 2009). Although ITS has been formally proposed as the primary barcoding marker for fungi by the Consortium for the Barcode of Life, there is the possibility that supplementary barcodes may be developed for particularly narrowly circumscribed taxonomic groups (Schoch et al. 2012). It is better to use multigene analysis to accurately identify species of fungal endophytes (Guo et al. 2001, Huang et al. 2009, Ko et al. 2011, Sun et al. 2011). Moreover, most of the sequences named in GenBank are erroneously named, such as *Colletotrichum* and *Curvularia lunata* (Cai et al. 2009, 2011, da Cunha et al. 2013). A comparison of sequence data from fungal endophytes with ex-type cultures of named species must be considered (Dayarathne et al. 2016). This will prevent misidentification of endophytes isolated from various hosts and localities (Ko et al. 2011). Correct identification of fungi is important for understanding the biology, ecology, evolutionary relationships, for controlling plant diseases, and useful for future application in biotechnology (Santos & Phillips 2009, Ko et al. 2011, Udayanga et al. 2011, Hyde et al. 2014).

Comparison between culture-dependent and culture-independent techniques

ITS is the targeted region in culture-dependent or NGS analysis. In this study, we identified the fungal endophytes based on ITS sequence data using the culture-dependent approach, which is different from culture-independent methods (e.g. study using NGS that reads into operational taxonomic units (OTUs)). The identification of bacteria and fungi by culture-dependent methods has resulted in lower numbers of microorganisms than culture-independent methods (Carraro et al. 2011, Stefani et al. 2015). This is because culture conditions used (aeration, nutritional, temperature, etc.) can affect growth of organisms. Artificial medium usually allows growth of only a small fraction of the organisms (Carraro et al. 2011). Fast-growing species suppress growth of others, thus slow-growing organisms are out-competed (Nocker et al. 2007). Culture-independent methods can also reveal the community of unculturable organisms. Nevertheless, culture-dependent methods have higher taxonomical accuracy (discriminative power), and ease of performance and interpretation when compared to culture-independent methods ((denaturing gradient gel electrophoresis (DGGE)

and 454 Pyrosequencing) (Vaz-Moreira et al. 2011). Moreover, culturing is essential for future applications in agricultural, industrial, food and medicine.

New high-throughput methods, in the culture-independent approach, are a useful strategy to estimate diversity of the mycobiome from any substrate. However, methodological biases, limitations of the markers and bioinformatic analysis, may lead to misleading conclusions (Lindahl et al. 2013, Vaz et al. 2017). Operational taxonomic units are based on sequences with ≥ 97% similarity. However, several problems can be seen in this method. If the ITS sequences of two species are ≥ 97% similar they can be assigned into one OTU instead of two. Another problem is that the same species that has a similarity less than 97% can be assigned as two different OTUs. This can be observed clearly in species complexes such as Colletotrichum and Diaporthe. Also, in the OUT dataset, species that occur only once are considered as rare OTUs or singletons considering that they may have originated from sequencing errors. In most cases, the fungal identifications from both methods can be consistent. However, inconsistent identifications can occur due to the lower power of taxonomic assignment in the culture-independent (amplicon sequencing) as compared with culture dependent approaches. This will result in identifying the organisms to family, order or class level instead of species level.

Conclusions

We resolved four endophyte species out of an estimated 20 morphotypes in this study based on ITS sequence data and cultural characteristic as well as support with available morphological study of sporulating species. These were *Hortaea werneckii*, *Hypoxylon griseobrunneum*, *Pseudopithomyces maydicus* and *Rigidoporus vinctus* and accuracy depends on the fungal group. These four species are first reports on *Rhizophora apiculata*. Both Ascomycota and Basidiomycota genera were found from *Rhizophora apiculate*, with Ascomycota being the most abundant. ITS sequence data can be used to identify taxa that have identical nucleotide sequence data to those of ex-types of species. ITS can separate species or indicate new species when having nucleotide sequence clearly diverges from previous described species. Individual ITS can clearly discriminate Pseudopithomyces species. ITS sequence data are hardly enough to identify the species boundary for species complex such as *Colletotrichum* and *Diaporthe*, in fact for most genera isolated in this study, but can provide an idea for taxonomic groups at least to genus level in most cases. Additional data and analysis, especially from multiple gene loci, are required to identify fungal endophytes.

Table 12. Cultural characteristics of the 20 morphotypes from *Rhizophora apiculata* on potato dextrose agar (PDA) at 25 °C in the dark.

Morphotype	Representative strain	Size (mm) of colony after 7 days	Colour[a,b]		Form or Shape	Elevation	Margin	Density	Figure
			Above	Below					
1	MFLUCC17-0025	27–35	Orange white (6A2) mix with white (6A1)	Light brown (6D6)[a], Pale orange (5A3)[b]	Irregular	Raised to lower convex	Erose or dentate	Spare	1a
2	MFLUCC 17-1941	60–65	White (5A1)[a], light orange (5A4)[b]	Orange white (5A2)[a], light orange (5A5)[b]	Irregular	Convex with papillate surface	Undulate	Spare	1b
3	MFLUCC 17-0005	14–20	Brownish grey (7D2) mix with white (7D1)	Black mix with orange white (6A2)	Irregular	Flat	Erose or dentate	Spare	1c
4	MFLUCC 17-0020	37–45	Brown (7E5) mix with white (7A1)	Light brown (7D6) mix with white (7A1)	Irregular, punctiform	Raised	Undulate	Spare	1d
5	MFLUCC 17-1945	40–50	Brownish grey (6F2), brownish orange (6C4), alternate white (6A1)	Brownish grey (6F2), white (6A1)	Irregular to circular	Raised to convex with papillate surface	Undulate	Medium	1e
6	MFLUCC 17-0004	52–60	Greyish brown (6F3) to (6E3) alternate white (6A1)	Greyish brown (7F3), white (7D1) and greyish brown (7D3)	Circular	Flat or effuse to raise	Entire edge	Dense	1f
7	MFLUCC 17-1943	50–60	Brownish grey (7C2)[a], white (7A1)[b]	Brownish grey (7D2)[a], white (7A1)[b]	Circular	Raised to convex with papillate surface	Undulate	Medium	1g
8	MFLUCC 17-0035	70–80	Yellowish white (4A2)	Light yellow (4A4)	Irregular	Raised convex with papillate surface	Lobate, with concentric rings	Dense	1h
9	MFLUCC 17-1942	37–45	Pale yellow (4A3)[a], white (4A1)[b]	Yellowish brown (5F8)[a], pale yellow (4A3)[b]	Irregular	Raised to lower convex	Lobate to undulate	Dense	1i
10	MFLUCC 17-0003	22–31	Greyish orange (5B4)[a], white (5A1)[b]	Light brown (6D5)[a], white (6A1)[b]	Circular	Raised to lower convex	Entire edge	Medium	1j
11	MFLUCC 17-0028	40–45	Light brown (6D4) alternate brown (6E4)	Brown (6F4) alternate brown (6E5)	Circular	Raised to lower convex	Undulate	Medium	1k

Morphotype	Representative strain	Size (mm) of colony after 7 days	Colour[a,b]		Form or Shape	Elevation	Margin	Density	Figure
			Above	Below					
12	MFLUCC 17-0022	7–18	Pale orange (5A3)[a], orange white (5A2)[b]	Orange white (5A2)	Irregular	Flat	Entire edge	Spare	1l
13	MFLUCC 17-0030	10–15	Olive brown (4E2)[a], yellowish white (4A2)[b]	Greyish beige (4D2)[a], yellowish white (4A2)[b]	Irregular	Flat to lower convex	Crenated to lobate	Medium	2a
14	MFLUCC 17-1937	15–22	Olive grey (3F2)	Olive (3F3)	Irregular	Flat or effuse	Crenated to lobate	Medium	2b
15	MFLUCC 17-1938	8–13	Olive grey (2F2)	Olive grey (2F2)	Irregular	Raise	Undulate to Entire edge	Medium	2c
16	MFLUCC 17-1940	11–13	Olive grey (3F2)	Olive brown (4F3)	Irregular	Flat	Undulate	Spare	2d
17	MFLUCC 17-1939	7–10	Grey (3F1)	Olive grey (2F2)	Irregular	Lower convex	Undulate	Spare	2e
18	MFLUCC 17-0007	Completely covering the Petri-dish	White (4A1)	Yellowish white (4A2)	Circular	Raised	Entire edge	Dense	2f
19	MFLUCC 17-0002	60–70	Yellowish white (4A2)[a], violet brown (11F4)[b]	Yellowish white (4A2)[a], violet brown (11F4)[b]	Irregular	Flat or effuse	Crenated	Spare	2g, h
20	MFLUCC 17-1946	Completely covering the Petri-dish	White (4A1)	Yellowish white (4A2)	Circular	Raised, convex in center	Crenated	Medium dense	2i

[a]Center of culture, [b]edge of culture.

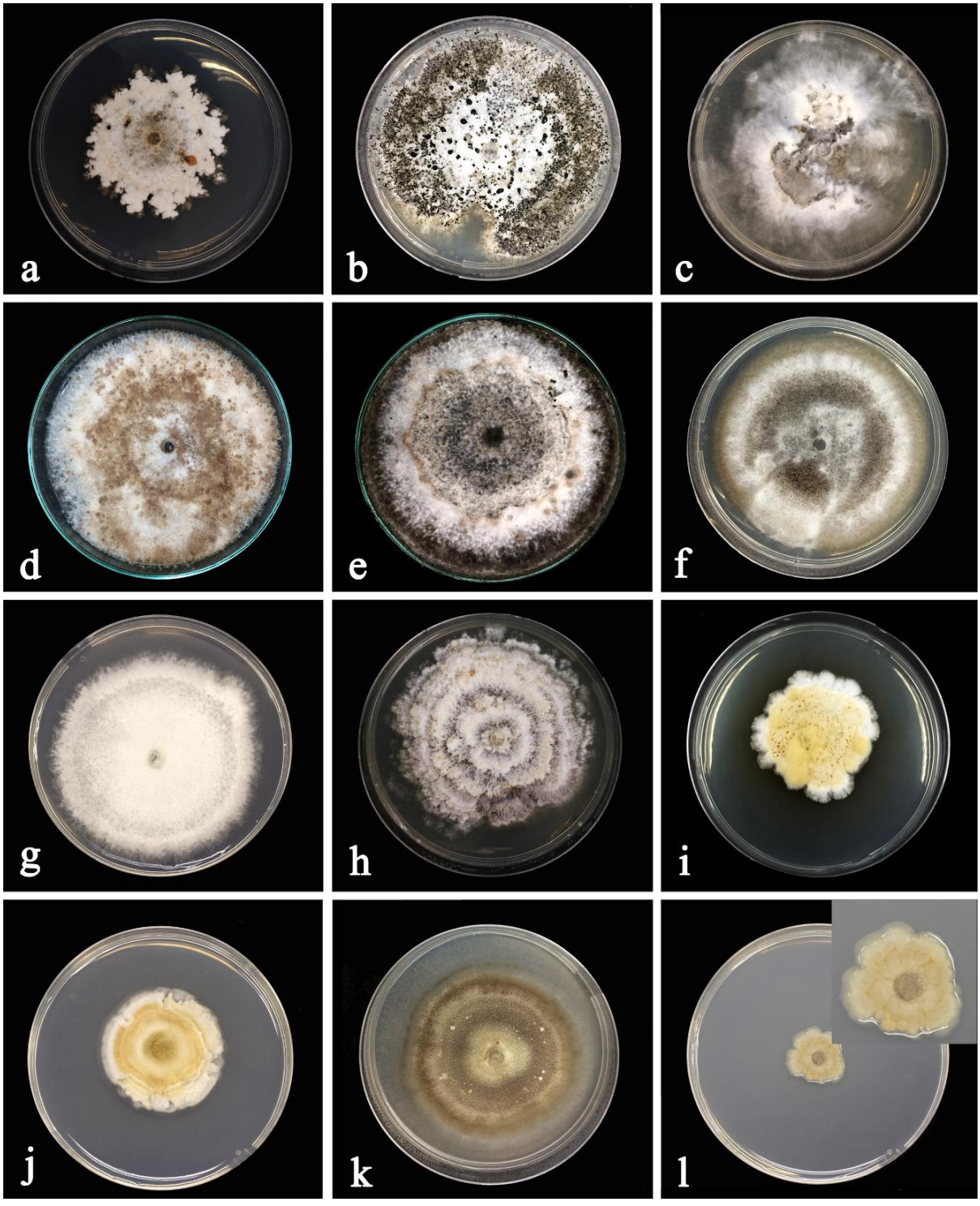


Figure 43 – Colony morphology of fungal endophytes on PDA, at 25 °C in the dark, isolated from Rhizophora apiculata. a Pestalotiopsis sp. MFLUCC 17-0025 (22 d). b Neopestalotiopsis sp. MFLUCC 17-1941 (30 d). c Nemania sp. MFLUCC 17-0005 (22 d). d Hypoxylon griseobrunneum MFLUCC 17-0020 (22 d). e Hypoxylon sp. MFLUCC 17-1945 (22 d). f Colletotrichum sp. MFLUCC 17-0004 (25 d). g Colletotrichum sp. MFLUCC 17-1943 (14 d). h Diaporthe sp. MFLUCC 17-0035 (5 d). i Diaporthe sp. MFLUCC 17-1942 (14 d). j Allophoma sp. MFLUCC 17-0003 (14 d). k Pseudopithomyces maydicus MFLUCC 17-0028 (25 d). l Preussia sp. MFLUCC17-0022 (14 d).

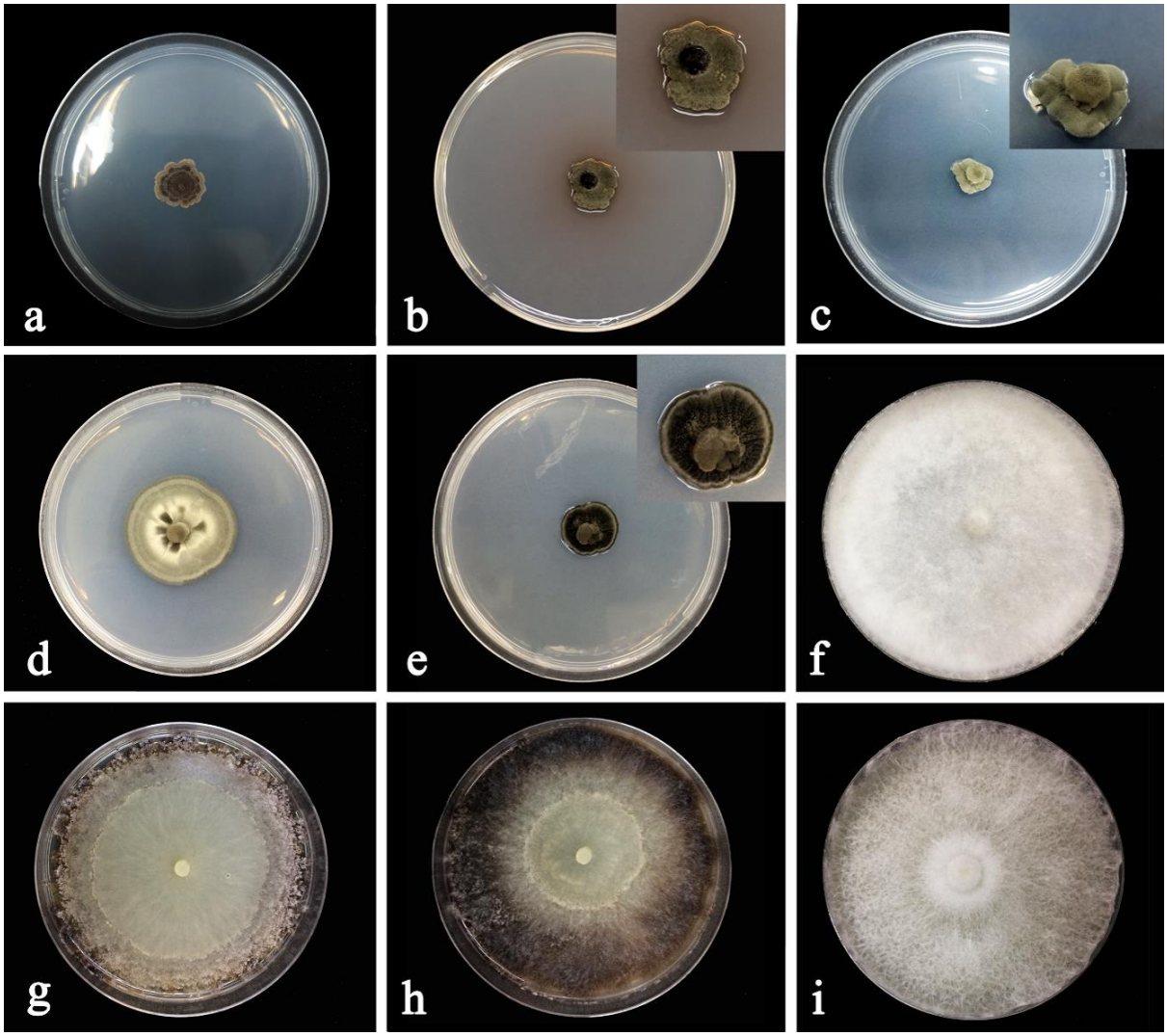


Figure 44 – Colony morphology of fungal endophytes on PDA, at 25 °C in the dark, isolated from Rhizophora apiculata. a Phyllosticta sp. MFLUCC 17-0030 (14 d). b Phyllosticta sp. MFLUCC 17-1937 (14 d). c Fungal endophyte (unidentified) MFLUCC 17-1938 (14 d). d Hortaea werneckii MFLUCC 17-1940 (30 d). e Neodevriesia sp. MFLUCC 17-1939 (30 d). f Rigidoporus vinctus MFLUCC 17-0007 (30 d). g, h Phanerochaete sp. MFLUCC 17-0002 (25 d). i Schizophyllum sp. MFLUCC 17-1946 (30 d).

Table 13. Closest match for endophytes following MegaBLAST search of NCBI GenBank nucleotide database. Max identity and max score are shown.

Isolate number in this study	Nearest match BLAST search result	Voucher/ Culture	ITS GenBank accession number	Max score	Max identity (%)
Ascomycota					
MFLUCC 17-0003	*Stagonosporopsis cucurbitacearum*	E-271	KU059901	981	531/531(100%)
	Stagonosporopsis cucurbitacearum	SE5	AB714984	981	531/531(100%)
	Didymella bryoniae	MA71	GU592001	981	531/531(100%)
MFLUCC 17-0004	*Colletotrichum brevisporum*	PC-1	KX756146	1088	602/608(99%)
MFLUCC 17-0005	*Nemania diffusa*	BCC 18754	AB625422	1003	543/543(100%)
	Pestalotiopsis sp.	SC5A8	KU252287	1075	582/582(100%)
MFLUCC 17-0019	*Pestalotiopsis* sp.	SC3A14	KU252282	1075	582/582(100%)
	Pestalotiopsis sp.	SC3A4	KU252277	1075	582/582(100%)
MFLUCC 17-0020	*Hypoxylon anthochroum*	EGJMP 20	KF192825	935	506/506(100%)
	Xylariaceae sp.	AT4	KX953392	935	506/506(100%)
MFLUCC 17-0023	*Preussia* sp.	CY218	HQ608038	983	539/542(99%)
MFLUCC 17-0028	*Pithomyces maydicus*	UTHSC 06-1549	HG933801	1040	563/563(100%)
MFLUCC 17-0030	*Phyllosticta aristolochiicola*	BRIP 53316a	NR_111791	845	574/627(92%)
MFLUCC 17-0035	*Diaporthe arecae*	CBS 161.64	KC343032	1026	560/562(99%)
MFLUCC 17-1937	*Fungal* sp.	59815	KP890364	1064	592/600(99%)
MFLUCC 17-1938	Mycosphaerellaceae sp.	MA 12	GU591997	983	542/547(99%)
MFLUCC 17-1939	*Neodevriesia pakbiae*	CPC 25044/ CBS 139914	KR476742	998	557/565(99%)
	Hortaea werneckii	MCw215	HQ711621	966	523/523(100%)
	Fungal endophyte	2789	KR015340	966	523/523(100%)
	Hortaea werneckii	RY 51	KM014604	966	523/523(100%)
MFLUCC 17-1940	*Hortaea werneckii*	JY 54	KM014589	966	523/523(100%)
	Hortaea werneckii	Hw5	JN997374	966	523/523(100%)
	Hortaea sp.	F47	FJ755827	966	523/523(100%)
	Hortaea werneckii	IFM 4988	AB087199	966	523/523(100%)
	Pestalotiopsis sp.	LH162	HQ832816	1003	547/549(99%)
MFLUCC 17-1941	*Pestalotiopsis* sp.	MA129	GQ254681	1003	547/549(99%)
	Pestalotiopsis sp.	MA165	GU592005	1003	547/549(99%)
	Pestalotiopsis sp.	14JAES	EF451799	1003	547/549(99%)
MFLUCC 17-1942	*Phomopsis* sp.	MA194	GU592007	1029	572/579(99%)
MFLUCC 17-1943	*Colletotrichum gloeosporioides*	AAP-018	KU534983	1074	581/581(100%)
	Fungal sp.	SF3	MF962538	1074	581/581(100%)
MFLUCC 17-1945	Xylariaceae sp.	D11a4	JQ341090	1068	604/616(98%)
	Hypoxylon lechatii	MUCL 54609	KF923407	1068	606/619(98%)
Basidiomycota					
MFLUCC 17-0002	*Phanerochaete stereoides*	He2309	KX212219	946	547/564(97%)
MFLUCC 17-0007	*Rigidoporus vinctus*	FRIM 142	HQ400710	1158	627/627(100%)
MFLUCC 17-1946	*Schizophyllum commune*	UZ1552_14	KP326577	1146	625/627(99%)

Note: When more than one isolate shared similarity of ITS sequence alignment then a representative isolate was blasted.

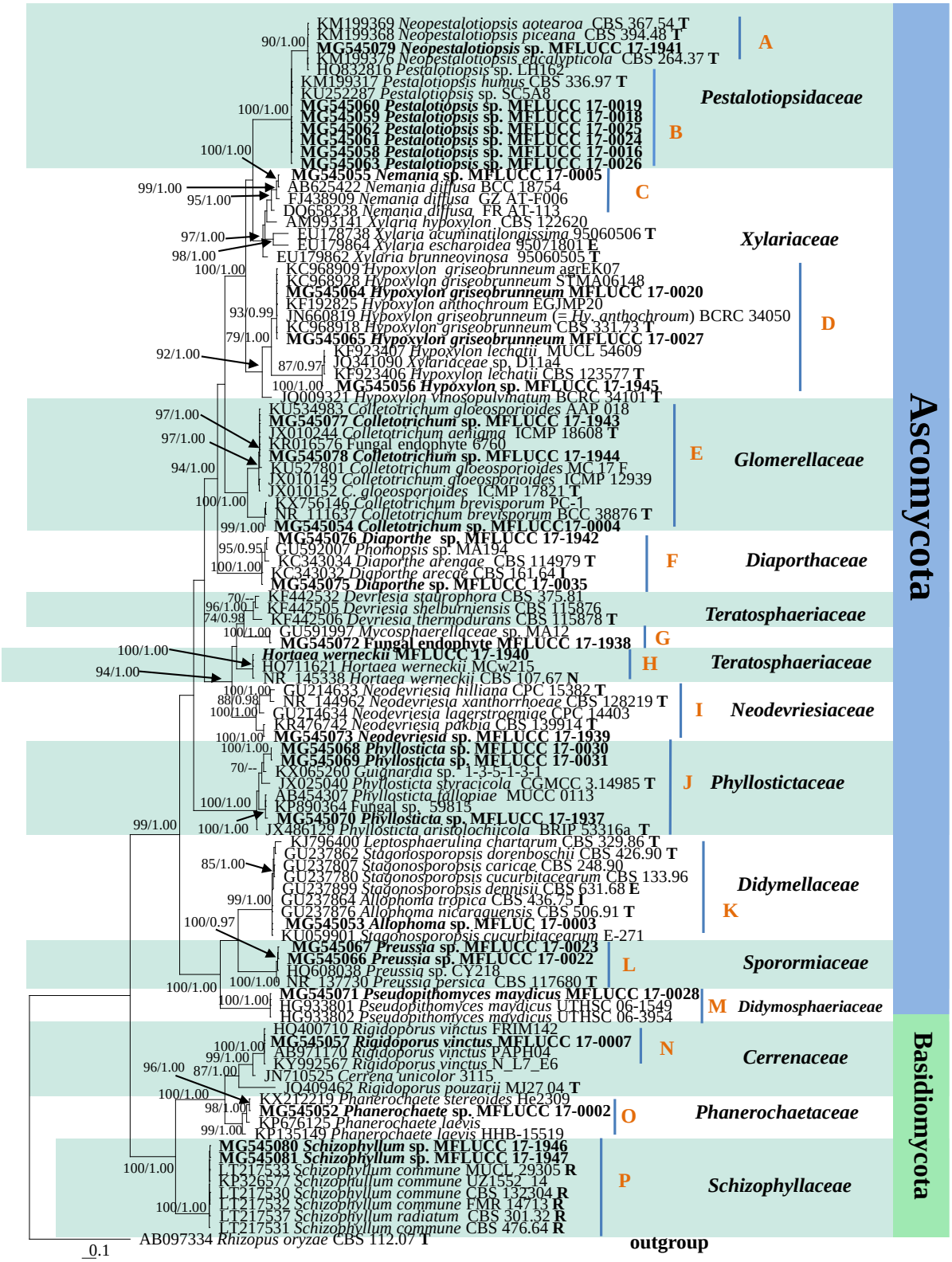


Figure 45 – Phylogram generated from maximum likelihood analysis (RAxML) based on ITS sequence data. The tree is rooted to Rhizopus oryzae CBS 112.07. Maximum likelihood bootstrap values (MLBS) ≥ 70% and Bayesian posterior probabilities ≥ 0.95, (MLBS/PP) are given at the nodes. Ex-epitype, ex-isotype, ex-neotype, ex-type and reference strains are marked with E, I, N, T and R, respectively. The new isolates are in bold.

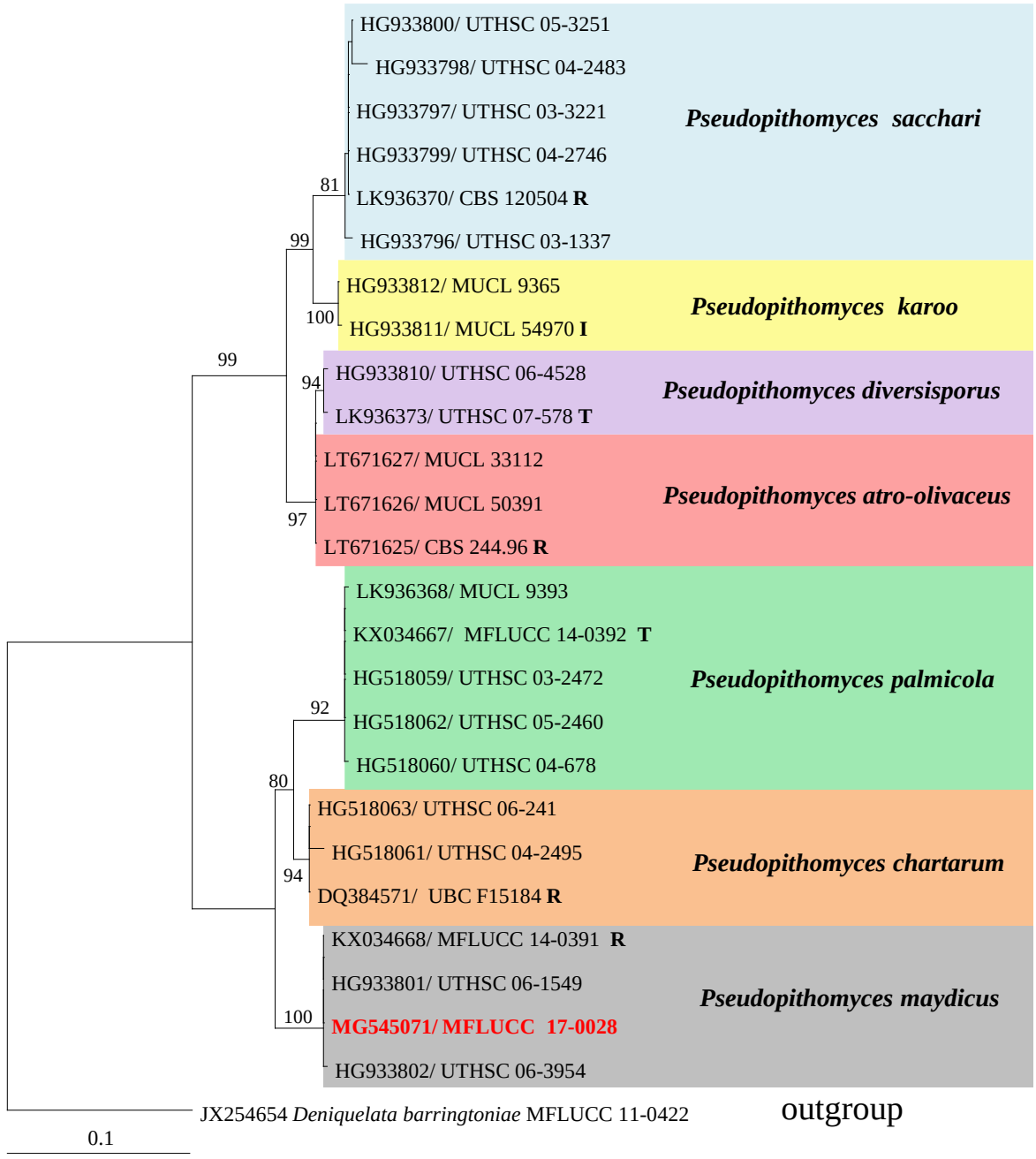


Figure 46 – Phylogram generated from maximum likelihood analysis (RAxML) based on ITS sequence data in Pseudopithomyces species. The tree is rooted to Deniquelata barringtoniae MFLUCC 11-0422. Maximum likelihood bootstrap values (MLBS) ≥ 70% are given at the nodes. Ex-isotype, ex-type and reference strains are marked with I, T and R, respectively. The new isolate is in red bold.

Table 14. Details of representative strains of isolated endophytic fungi from *Rhizophora apiculata*.

	Classification	**Strain no**	**Locality**	**Habitat**
Ascomycota, Dothideomycetes	Botryosphaeriales, Phyllostictaceae			
	Phyllosticta spp.	MFLUCC 17-0030	Kram Subdistrict, Kleang District, Rayong Province	Leaf
		MFLUCC 17-0031	Phra Samut Chedi Klang Nam, Mueang District, Rayong Province	Leaf
		MFLUCC 17-1937	Phra Samut Chedi Klang Nam, Mueang District, Rayong Province	Petiole
	Capnodiales, Neodevriesiaceae			
	Neodevriesia sp.	MFLUCC 17-1939	Sirinart rajini, Pak Nam Pran Sub district, Pranburi District, Prachuap Khiri Khan Province	Leaf
	Capnodiales, Teratosphaeriaceae			
	Hortaea werneckii	MFLUCC 17-1940	Ao Khung Kraben, Khlong Khut Sub district, Tha Mai District, Chanthaburi Province	Leaf
	Pleosporales, Didymellaceae			
	Allophoma sp.	MFLUCC 17-0003	Sirindhorn, Cha-am Subdistrict, Cha-am District, Phetchaburi Province	Leaf
	Pleosporales, Didymosphaeriaceae			
	Pseudopithomyces maydicus	MFLUCC 17-0028	Sirinart rajini, Pak Nam Pran Subdistrict, Pranburi District, Prachuap Khiri Khan Province	Aerial stilt root
	Pleosporales, Sporormiaceae			

	Classification	Strain no	Locality	Habitat
	Preussia sp.	MFLUCC 17-0022 and MFLUCC 17-0023	Sirindhorn, Cha-am Subdistrict, Cha-am District, Phetchaburi Province	Aerial stilt root
	Ascomycota genera, incertae sedis			
	Fungal endophyte (unidentified)	MFLUCC 17-1938	Ao Khung Kraben, Khlong Khut Sub district, Tha Mai District, Chanthaburi Province	Leaf
Ascomycota, Sordariomycetes	Amphisphaeriales, Pestalotiopsidaceae			
	Pestalotiopsis spp.	MFLUCC 17-0016, MFLUCC 17-0018, MFLUCC 17-0019, MFLUCC 17-0024, MFLUCC 17-0025 and MFLUCC 17-0026	Sirindhorn, Cha-am Subdistrict, Cha-am District, Phetchaburi Province	Aerial stilt root
	Neopestalotiopsis sp.	MFLUCC 17-1941	Sirindhorn, Cha-am Subdistrict, Cha-am District, Phetchaburi Province	Aerial stilt root
Ascomycota, Sordariomycetes	*Diaporthales*, Diaporthaceae			
	Diaporthe sp.	MFLUCC 17-0035	Kram Subdistrict, Kleang District, Rayong Province	Aerial stilt root
	Diaporthe sp.	MFLUCC 17-1942	Kram Subdistrict, Kleang District, Rayong Province	Aerial stilt root
	Glomerellales, Glomerellaceae			
	Colletotrichum sp.	MFLUCC 17-0004	Sirindhorn, Cha-am Subdistrict, Cha-am District, Phetchaburi Province	Leaf
	Colletotrichum sp.	MFLUCC 17-1943	Kram Subdistrict, Kleang District, Rayong Province	Leaf
	Colletotrichum sp.	MFLUCC 17-1944	Sirinart rajini, Pak Nam Pran	Leaf

	Classification	Strain no	Locality	Habitat
			Subdistrict, Pranburi District, Prachuap Khiri Khan Province	
	Xylariales, Xylariaceae			
	Hypoxylon griseobrunneum	MFLUCC 17-0020 and MFLUCC 17-0027	Sirinart rajini, Pak Nam Pran Subdistrict, Pranburi District, Prachuap Khiri Khan Province	Leaf
	Hypoxylon sp.	MFLUCC 17-1945	Sirinart rajini, Pak Nam Pran Subdistrict, Pranburi District, Prachuap Khiri Khan Province	Leaf
	Nemania sp.	MFLUCC 17-0005	Sirindhorn, Cha-am Subdistrict, Cha-am District, Phetchaburi Province	Leaf
Basidiomycota, Agaricomycetes	Agaricales, Schizophyllaceae			
	Schizophyllum sp.	MFLUCC 17-1946 and MFLUCC 17-1947	Sirinart rajini, Pak Nam Pran Subdistrict, Pranburi District, Prachuap Khiri Khan Province	Aerial stilt root
	Polyporales, Cerrenaceae			
	Rigidoporus vinctus	MFLUCC 17-0007	Sirindhorn, Cha-am District, Phetchaburi Province	Petiole
	Polyporales, Phanerochaetaceae			
	Phanerochaete sp.	MFLUCC 17-0002	Sirindhorn, Cha-am District, Phetchaburi Province	Leaf

Appendix VII To investigate evolutionary relationships of unidentified endophytes, poorly studied genera and new species based on a polyphasic approach.

The taxonomic ranking of fungi at higher levels (class, subclass, order, family, genus) has always been contentious and prone to subjectivity, since higher taxa are perceived as human constructs and not as natural entities, and their ranking is arbitrary, which in some instances has resulted in unnecessary personal attacks in the literature. Accurate and natural classifications should rely on clear scientific principles, with evidence from as many avenues as possible, and provide objective criteria for their use by others. Earlier classifications based on phenotype were certainly more subjective; however, remarkably many of these old arrangements have stood the test of time. More recently, phylogenetic analyses have provided evidence towards a natural classification, resulting in significant changes in many lineages. The classification arrangements however, are still subjective and dependent on the taxa analysed, resulting in different taxonomic interpretations and schemes, particularly when it comes to ranking. Thus, what have been considered as genera by some, have been introduced as families by others. More recently, estimation of divergence times using molecular clock methods have been used as objective evidence for higher ranking of taxa. To investigate evolutionary relationships of unidentified endophytes, poorly studies genera based on polyphasic approach, a series papers were published with thankful to this grant.

In the paper Hyde et al. 2017, we provided an overview over Ascomycota, showing how application of temporal banding could affect the recognition of higher taxa at certain rank levels. We then use Sordariomycetes as an example where we use divergence times to provide additional evidence to stabilize ranking of taxa below class level. We propose a series of evolutionary periods that could be used as a guide to determine the various higher ranks of fungi: phyla 550 MYA, subphyla 400–550 MYA; classes 300–400 MYA; subclasses 250–300 MYA, orders 150–250 MYA, and families 50–150 MYA. It is proposed that classification schemes and ranking of taxa should, where possible, incorporate a polyphasic approach including phylogeny, phenotype, and estimate of divergence times.

In the paper Hongsanan et al. 2017, Hongsanan and co-authors provide an updated phylogeny of Sordariomycetes and recommended changes based on both phylogenetic and molecular clock evidence.

In the paper Liu et al. (2017), Liu and coauthors use Dothideomycetes and Pleosporales as case studies for evidence from molecular clock data for ranking orders and families, respectively.

Recently, Phillips et al. (2019) selected families in Botrypsphaeriales, carried out morphological, phylogenetic and evolutionary study, and synonymizd three families under existing families based on evidence of divergence time analysis. Dayarathne et al. (2019) provided evolutionary phylogenetic analysis of Savoryellaceae, and ranking it as a subclass Savoryellomycetidae.

During our study of fungi from aerial parts of *Nypa fruticans*, a new family Striatiguttulaceae was established based on morphology and multi-gene phylogenetic evidence, and it diverged approximately 60 (35–91) MYA. The phylogenetic analysis and taxonomy notes are provided as below:

Phylogenetic results

The multi-gene dataset comprised 113 taxa and 4113 characters after alignment (LSU: 919 bp; SSU: 1245 bp; TEF1α: 929 bp; RPB2: 1020 bp) including gaps. RAxML, MP and Bayesian analyses were conducted and resulted in generally congruent topologies, and the familial assignments are similar to previous work (Hashimoto et al. 2017, Liu et al. 2017). Maximum parsimony analyses indicated that 2,302 characters were constant, 355 variable characters parsimony uninformative and 1,456 characters are parsimony-informative. A heuristic search yield four equally most parsimonious trees (TL = 10905, CI = 0.278, RI = 0.561, RC = 0.156, HI = 0.722). The combined dataset provided higher confidence values for the familial level than those of the individual gene trees (data not shown), and RAxML analysis based on LSU, SSU, TEF1α and RPB2 yielded a best sorting tree (Figure 47) with a final optimization likelihood value of –52455.532059.

The eight newly generated strains clustered together and positioned outside the two suborders (Massarineae and Pleosporineae) of Pleosporales, and formed a well-supported monophyletic clade and represented as a new linage of Pleosporales. The phylogeny also revealed that this clade is close to Ligninsphaeriaceae, Pseudoastrosphaeriellaceae, Testudinaceae and Tetraplosphaeriaceae, and can be recognized as a novel family (Striatiguttulaceae). Furthermore, the eight strains formed two well-supported monophyletic sub-clades, which can be identified as two new genera (*Longicorpus* and *Striatiguttula*) and three new species (*Longicorpus striataspora*, *Striatiguttula nypae* and *S. phoenicis*).

Divergence times estimates

The maximum clade credibility (MCC) tree with divergence estimates (Figure 48) obtained through BEAST was topologically identical to those recovered by Bayesian and ML procedures with regards to the placement Pleosporales and several major lineages within Dothideomycetes. The mean dates of Pleosporales crown is corroborate with reported estimates (Phukhamsakda et al. 2016, Liu et al. 2017, 2018). Divergence estimates for crowns of the newly generated family in Pleosporales and three calibration points used in this study are provided in Table 15.

Table 15. Divergence time estimates of Pleosporales and selected lineages of Dothideomycetes obtained from a Bayesian approach (BEAST) on basis of three calibrations. For each divergence, the median and the 95% highest posterior density (HPD) are provided. Divergence times are provided in millions of years (MYA).

Nodes	**Crown group**	**Divergence times**				
		This study		**Phukhamsakda et al. (2016)**	**Liu et al. (2017)**	**Liu et al. (2018)**
		Crown age	**Stem age**	**Crown age**		
1	Arthoniomycetes-Dothideomycetes	312 (220–413)	–	317	–	310~320
2	*Capnodiales*	195 (131–266)	269 (196–347)	147	216 (151–283)	~120
3	*Aigialus*	41 (35–56)	64 (44–91)	39	–	~50
4	Dothideomycetes	286 (210–369)	312 (220–413)	293 ~(210–370)	341 (257–425)	255 (166–344)
5	*Pleosporales*	206 (148–274)	221 (158–292)	211 ~(140–270)	204 (148–260)	195 (124–271)
6	*Striatiguttulaceae*	39 (20–63)	60 (35–91)	–	–	–

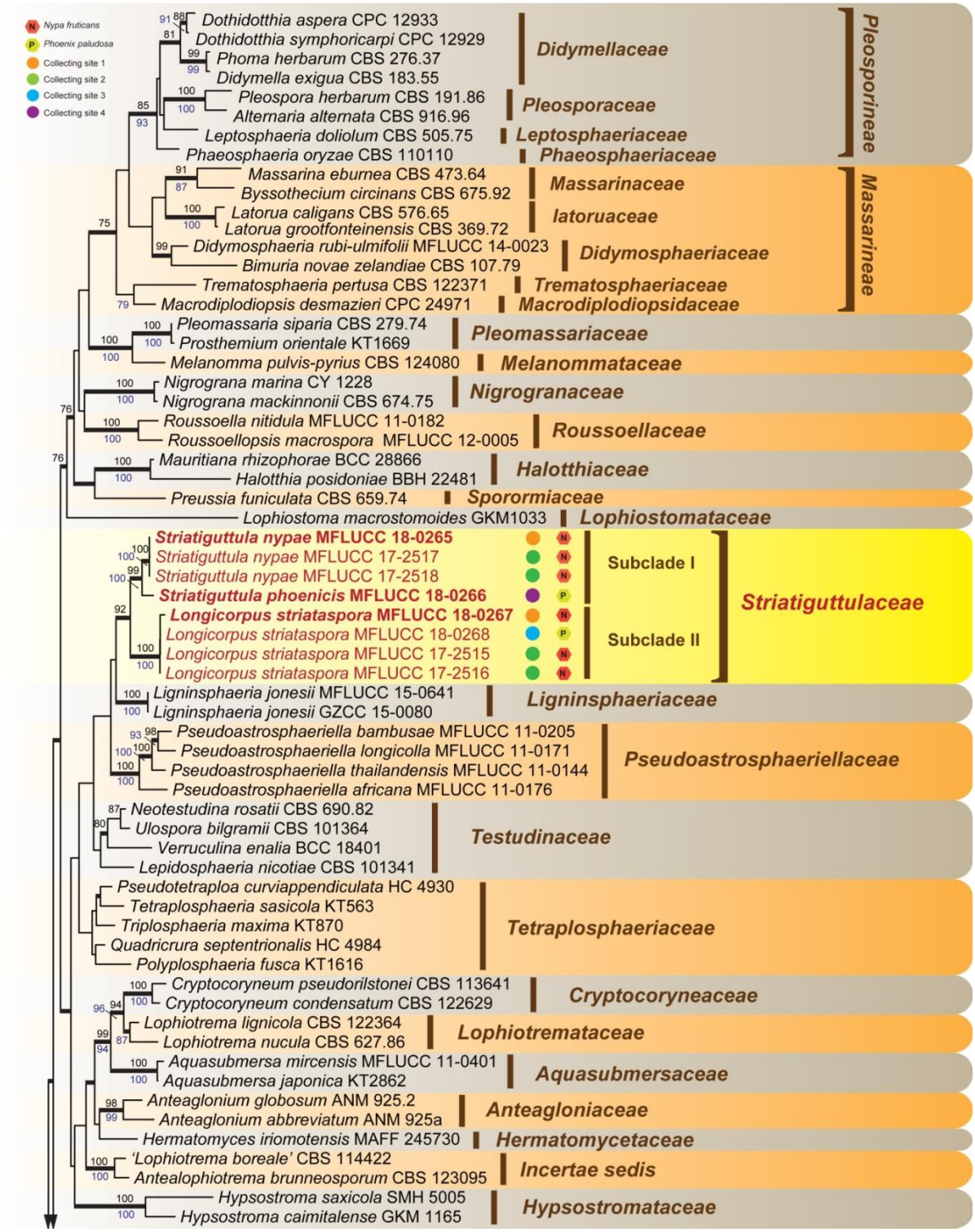


Figure 47 – RAxML tree of Pleosporales based on analysis of combined LSU, SSU, TEF1α and RPB2 sequence data. Bootstrap values for ML and MP equal to or greater than 75% are placed above and below the branches respectively. Branches with Bayesian posterior probabilities (PP) from MCMC analysis equal or greater than 0.95 are in bold. Newly generated sequences are indicated in red.

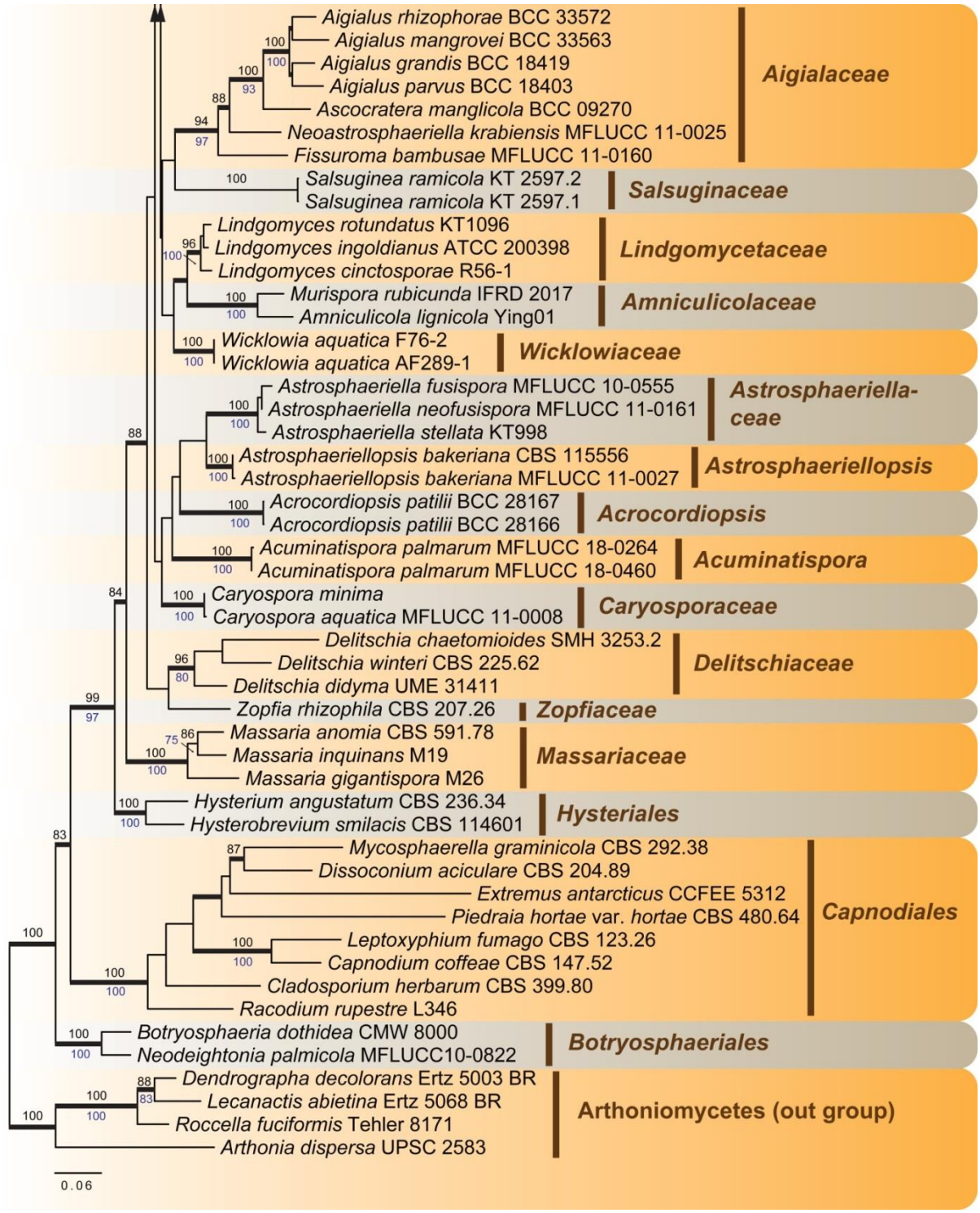


Figure 47 – Continued

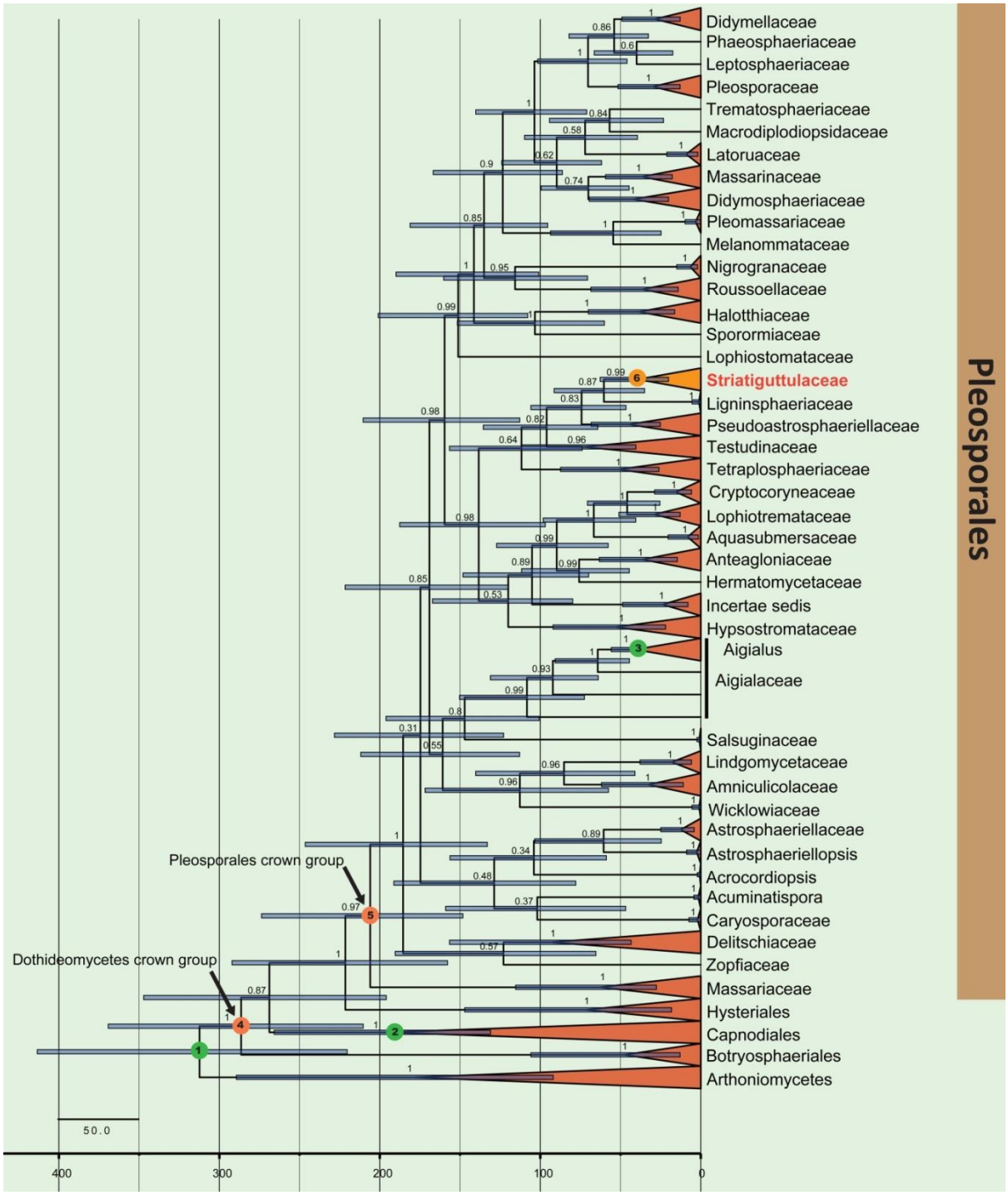


Figure 48 – Maximum clade credibility (MCC) tree with divergence times estimates for Pleosporales and selected groups in Dothideomycetes, obtained from a Bayesian approach (BEAST) using one secondary and two fossil calibrations. Numbers at nodes indicate posterior probabilities (pp) for node support; bars correspond to the 95% highest posterior density (HPD) intervals. Numbers inside green circles indicate nodes used for calibrations: 1) the split of Arthoniomycetes and Dothideomycetes; 2) Metacapnodiaceae; 3) *Margaretbarromyces dictyosporus*.

Taxonomy

Striatiguttulaceae S.N. Zhang, K.D. Hyde & J.K. Liu, **fam. nov.**

MycoBank: MB828272; Facesoffungi: FoF 05032

Etymology: Name refers to the name of the type genus.

Description: Saprobic on palms distributed in mangrove habitats. Sexual morph: *Stromata* black, scattered to gregarious, immersed beneath host epidermis, and erumpent to superficial, with a papilla or a short to long neck, ampulliform, subglobose or conical, uni-loculate or bi-loculate, coriaceous to carbonaceous, ostiolate, periphysate, papillate, clypeate or not clear, glabrous or somewhat interwoven pale brown hyphae or setae. *Peridium* composed of several brown to hyaline cell layers. Hamathecium of trabeculate pseudoparaphyses. *Asci* 8-spored, bitunicate, cylindric-clavate, pedicellate, apically rounded, with an ocular chamber. *Ascospores* hyaline to brown, uniseriate to biseriate or triseriate, fusiform or ellipsoidal, 1–3-septate, striate, guttulate, with paler end cells and surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Type genus: *Striatiguttula* S.N. Zhang, K.D. Hyde & J.K. Liu

Notes: The family Striatiguttulaceae is introduced to accommodate two new genera *Longicorpus* and *Striatiguttula*, characterized by the immersed, and erumpent to superficial stromata, with a papilla or a short to long neck, trabeculate pseudoparaphyses, bitunicate asci, and hyaline to brown, fusiform to ellipsoidal, striate, guttulate, 1–3-septate ascospores, with paler end cells and surrounded by a mucilaginous sheath. Members of Striatiguttulaceae are morphologically similar to the genera Leptosphaeria and Trematosphaeria, but they are phylogenetically distinct and also differ in ascospores characteristics. Multi-gene phylogenetic analysis revealed a close relationship of Striatiguttulaceae to Ligninsphaeriaceae and Pseudoastrosphaeriellaceae. However, Striatiguttulaceae differs from Pseudoastrosphaeriellaceae as the latter has narrowly fusiform, 1–3-septate or 2–5-septate ascospores. The slit-like ascomata and rather large ascospores in Ligninsphaeriaceae are distinct from those found in Striatiguttulaceae. Additionally, a divergence time estimate analysis indicated that the crown age 39 (20–63) MYA and stem age 60 (35–91) MYA of Striatiguttulaceae, match with the recommendations of using divergence times to recognize families in Liu et al. (2017). The morphological examination of both asexual and sexual morphs was also conducted in order to build comprehensive familial concept for Striatiguttulaceae, but unexpectedly, we did not obtain the asexual morphs. Further morphological investigations together with more molecular data are needed.

Striatiguttula S.N. Zhang, K.D. Hyde & J.K. Liu, **gen. nov.**

MycoBank: MB828273; Facesoffungi: FoF 05033

Etymology: Name refers to the striate and guttulate ascospores.

Description: Saprobic on palms which are distributed in mangrove habitats. **Sexual morph**: *Stromata* black, scattered to gregarious, immersed beneath host epidermis, and erumpent to superficial, with a papilla or a short to long neck, ampulliform, subglobose or conical, uni-loculate or bi-loculate, coriaceous to carbonaceous, ostiolate, periphysate, papillate, clypeate or not, glabrous or somewhat interwoven pale brown hyphae or setae, lying at apex of the neck. *Peridium* thin, composed of several pale brown to hyaline angular cells. Wall of the neck having elongated angular cells. *Hamathecium* filament thin, trabeculate pseudoparaphyses, septate, branched, anastomosing, embedded in a gelatinous matrix. *Asci* 8-

spored, bitunicate, cylindric-clavate, pedicellate, apically rounded, with an ocular chamber. *Ascospores* hyaline to brown, uniseriate to biseriate or triseriate, fusiform to ellipsoidal, 1–3-septate, constrict, the middle cells slightly swollen towards the central septa, striate, guttulate, end cells slightly paler or not, surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Type species: *Striatiguttula nypae* S.N. Zhang, K.D. Hyde & J.K. Liu

Striatiguttula nypae S.N. Zhang, K.D. Hyde & J.K. Liu, **sp. nov.**

MycoBank number: MB828274; Facesoffungi number: FoF 05034, Figure 49

Etymology: The epithet reflects the genus name of the host plant *Nypa fruticans*, from which the specimens were collected.

Type: THAILAND. Ranong: Ranong, on decayed rachis of Nypa fruticans, 3 December 2016, S.N. Zhang, SNT44 (holotype: MFLU 18-1576; isotype: HKAS 97480; ex-type living culture MFLUCC 18-0265 = GZCC 18-0005).

Description. Saprobic on mangrove palm *Nypa fruticans*. **Sexual morph**: *Stromata* in vertical section 240–380 µm high, 195–385 µm diameter, (mean = 318.2 × 289.0 µm, n = 15), black, scattered, gregarious, immersed beneath host epidermis, and erumpent to superficial, with a papilla or short to long neck up to 550 µm, subglobose or conical, uni-loculate or bi-loculate, coriaceous to carbonaceous, ostiolate, periphysate, papillate and clypeate, glabrous or somewhat interwoven pale brown hyphae or with setae, lying at apex of the neck. *Peridium* 9–16 µm, thin, composed of several pale brown to hyaline angular cells, compressed and pallid inwardly. Wall of the clypeus composed of cells of textura epidermoidea and host tissue. Wall of the neck with thicker and elongated angular cells. *Hamathecium* 1–2 µm wide, trabeculate pseudoparaphyses, septate, branched, filamentous, anastomosing, embedded in a gelatinous matrix. *Asci* 64–145 × 8–17 µm, (mean = 106.3 × 13.8 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindric-clavate, pedicellate, apically rounded, with an ocular chamber. *Ascospores* 18–26 × 4–6 µm, (mean = 22.2 × 5.3 µm, n = 50), hyaline to brown, uniseriate to biseriate or triseriate, fusiform, 1–3-septate, constricted at the central septum, the upper middle cell slightly swollen towards the central septum, straight or slightly curved, striate, guttulate, end cells slightly paler, surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA attaining 15 mm diam. within 21 days at 25 ºC under natural light, velvety, centrally raised, greenish grey or greyish olivaceous, reverse dull green or grey olivaceous, with a margin of translucent, milky white to hyaline mycelia.

Additional specimens examined: Thailand. Krabi: near Pali, Mueang Krabi District, on submerged decaying rachis of Nypa fruticans, 30 August 2017, S.N. Zhang, SNT207 (paratype: MFLU 18-1577; living culture MFLUCC 17-2517 = GZCC 18-0006); Thailand. Krabi: near Pali, Mueang Krabi District, on submerged decaying rachis of Nypa fruticans, 30 August 2017, S.N. Zhang, SNT208 (paratype: MFLU 18-1578; living culture MFLUCC 17-2518 = GZCC 18-0007).

Habitat and distribution: Inhabiting Thai mangrove forests, Andaman sea (west) coastline, Thailand.

Notes: *Striatiguttula nypae* varies in ascomatal appearance, mostly immersed beneath the plant surface, sometimes visible as a papilla or dome-shaped area on the plant surface, and becomes erumpent to superficial, with a papilla or a short to long neck. The typical morphological characters of *S. nypae* are the appearance of stromata, with interwoven pale

brown hyphae or setae at the apex of the neck, and the hyaline to brown, 1–3-septate, fusiform ascospores, striate, guttulate, with slightly paler end cells and a mucilaginous sheath. We have compared *Striatiguttula nypae* to previously encountered species on *Nypa fruticans*, and several morphologically similar mangrove fungal species. However, the striation of ascospores can be a reliable morphological character to distinguish *Striatiguttula nypae* from *Astrosphaeriella nipicola* (Hyde and Fröhlich 1998), *A. nypae* (Hyde 1992a) and Leptosphaeria spp. (Spegazzini 1881, Cribb and Cribb 1955, Hyde et al. 1999, Pang et al. 2011), which are characterized by one or three septa and hyaline or brown ascospores. The presence of erumpent to superficial stromata, the number of septa and size of ascospores in *S. nypae* are also different from Trematosphaeria spp., despite being quite similar in ascospore morphology. In addition, the phylogenetic analysis showed that the three isolates of *Striatiguttula nypae* clustered together and were distinct from S. phoenicis.

Striatiguttula phoenicis S.N. Zhang, K.D. Hyde & J.K.Liu, **sp. nov.**

MycoBank: MB828275; Facesoffungi: FoF 05035, Figure 50

Etymology: The epithet referring to the host on which the fungus was collected.

Type: THAILAND. Ranong: Amphoe Mueang Ranong, Tambon Ngao, on decayed rachis of *Phoenix paludosa*, 6 December 2016, S.N. Zhang, SNT51 (holotype: MFLU 18-1579; isotype: HKAS 97481; ex-type culture MFLUCC 18-0266 = GZCC 18-0008).

Description: Saprobic on mangrove date palm *Phoenix paludosa*. **Sexual morph**: Ascomata in vertical section 195–580 µm high, 135–390 µm diameter, (mean = 396.0 × 230.3 µm, n = 15), black, scattered, rarely gregarious, immersed, and erumpent through host epidermis by a papilla or a short neck, ampulliform, subglobose, uni-loculate, coriaceous to carbonaceous, ostiolate, periphysate, papillate, glabrous or somewhat interwoven pale brown hyphae or setae, lying around apex of the neck. Peridium 10–24 µm, composed of several pale brown to hyaline cells of textura angularis, compressed and pallid inwardly. Wall of the neck composed thick and elongated angular cells. Hamathecium of 1–2 µm wide, septate, branched, filamentous, anastomosing, trabeculate pseudoparaphyses, embedded in a gelatinous matrix. Asci 89–141 × 12–18 µm, (mean = 120.5 × 15.4 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindric-clavate, pedicellate, apically rounded, with an ocular chamber. Ascospores 20–29 × 6–10 µm, (mean = 24.5 × 7.8 µm, n = 40), hyaline to brown (all cells nearly concolorous), uniseriate to biseriate, fusiform to ellipsoidal, 1–3-septate, constricted at the central septum, the upper middle cell slightly swollen and larger, straight or slightly curved, striate, guttulate, surrounded by an irregular mucilaginous sheath**. Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA attaining 14 mm diam within 21 days at 25 ºC under natural light, velvety, centrally raised, greenish grey or greyish olivaceous, reverse dull olivaceous or grey, with a margin of translucent, milky white to hyaline mycelium.

Habitat and distribution. Inhabiting Thai mangrove forests, Andaman sea (west) coastline, Thailand.

Notes: The fusiform to ellipsoidal, 1–3-septate ascospores of Striatiguttula phoenicis is similar to those of Trematosphaeria mangrovis, associated with submerged roots of mangrove trees. However, Striatiguttula phoenicis differs from T. mangrovis (Kohlmeyer 1968) as the latter has larger ascospores and lacks striations. Striatiguttula phoenicis is morphologically different from S. nypae as it has ellipsoidal ascospores which are broader in width. Currently, the erumpent to superficial stromata have not been found in *S. phoenicis*. The phylogenetic

analysis also confirms that they are distinct species. There are 26 noticeable nucleotide differences across the 474 nucleotides of ribosomal ITS sequence data (strains: MFLUCC 18-0266 vs. MFLUCC 18-0265, MFLUCC 17-2517 and MFLUCC 17-2518).

Longicorpus S.N. Zhang, K.D. Hyde & J.K. Liu, **gen. nov.**

MycoBank: MB828276; Facesoffungi: FoF 05036

Etymology: Name refers to the elongated ascomata and ascospores.

Description: Saprobic on mangrove palms. Sexual morph: Ascomata black, scattered to gregarious, immersed, and erumpent through host epidermis by a papilla or a short to long neck, sometimes visible as a slightly raised, dome-shaped area, with a clypeus comprises host tissue and fungal hyphae, ampulliform, subglobose or conical, uni-loculate, coriaceous to carbonaceous, ostiolate, periphysate, papillate, glabrous or somewhat interwoven pale brown hyphae or setae. Peridium composing of pale brown or brown angular cells. Hamathecium of septate, branched, thin, anastomosing trabeculate pseudoparaphyses, embedded in a gelatinous matrix. Asci 8-spored, bitunicate, cylindric-clavate, pedicellate, apically rounded, with an ocular chamber. Ascospores uniseriate to biseriate, hyaline to brown, fusiform, 1–3-septate, the upper middle cell slightly swollen towards the central septum, and the end cells paler and smaller, striate, guttulate, surrounded by a mucilaginous sheath. Asexual morph: Undetermined.

Type species. *Longicorpus striataspora* (K.D.Hyde) S.N.Zhang, K.D.Hyde & J.K.Liu

Notes: *Longicorpus* differs from *Striatiguttula* in not having stromata and elongate, fusiform ascospores with relatively larger middle cells and paler end cells (Figures 49, 50, 51). Multi-gene phylogeny also strongly supports the establishment of two genera. Longicorpus is sister to Striatiguttula but forms a distinct phylogenetic sub-clade (Figure 47). There are noticeable differences (nucleotide substitutions) at specific positions in the large subunit nuclear ribosomal DNA: 51, 428, 436, 465 (T substituted by C); 53, 55, 102, 153, 163, 166, 251, 367, 369, 427, 435, 440, 446, 448, 466, 504, 550, 654 (C substituted by T); 130 (G substituted by A); 362, 406 (G substituted by T); 370 (C substituted by A); 547 (A substituted by C).

Longicorpus striataspora (K.D.Hyde) S.N.Zhang, K.D.Hyde & J.K.Liu, **comb. nov.**

Trematosphaeria striataspora K.D. Hyde, Botanical Journal of the Linnean Society 98(2): 142. 1988. *Astrosphaeriella striataspora* (K.D. Hyde) K.D. Hyde, Botanical Journal of the Linnean Society 110(2): 97. 1992. Type: North Sumatra. K.D. Hyde (holotype: IMI 312390).

MycoBank: MB828277; Facesoffungi: FoF 05037, Figure 51

Epitype: THAILAND. Ranong: Ranong, on decayed rachis of Nypa fruticans, 6 December 2016, S.N.Zhang, SNT93 (epitype designated here: MFLU 18-1580; epi-isotype designated here: HKAS 97479; ex-epitype living culture MFLUCC 18-0267 = GZCC 18-0009).

Description: Saprobic on mangrove palms. Sexual morph: Ascomata in vertical section (including short papilla) 300–500 µm high, 230–560 µm diameter, (mean = 405.3 × 376.6 µm, n = 15), long neck up to 1285 µm, black, scattered to gregarious, immersed, and erumpent through host epidermis by a papilla or a short to long neck, sometimes visible as a slightly raised, dome-shaped area, with a clypeus comprises host tissue and fungal hyphae,

ampulliform, subglobose or conical, uni-loculate, coriaceous to carbonaceous, ostiolate, periphysate, papillate, glabrous or somewhat interwoven pale brown hyphae or setae, lying at apex of the neck. Peridium 11–15 µm wide, composing of brown to pale brown angular cells, thicker at the rim towards the apex. Hamathecium comprising up to 1.5 µm wide, septate, branched, filamentous, trabeculate, anastomosing pseudoparaphyses, embedded in a gelatinous matrix. Asci 85–160 × 10–17 µm (mean = 122.7 × 13.7 µm, n = 22), 8-spored, bitunicate, cylindric-clavate, pedicellate, apically rounded, with an ocular chamber. Ascospores 24–45 × 7–8.8 µm, (mean = 34.2 × 7 µm, n = 40), uniseriate to biseriate, hyaline to brown, fusiform, 1–3-septate, the upper middle cell slightly swollen towards the central septate, middle cells larger and longer, end cells paler and smaller, straight or slightly curved, striate, guttulate, surrounded by a mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics: Colonies on PDA attaining 12 mm diameter within 21 days at 25 ºC under natural light, velvety, centrally raised, irregular to circular in shape, greenish grey and mixed with milky white mycelium at the edge of a colony, the reverse dull green or grey olivaceous.

Additional specimens examined: Thailand. Chanthaburi, 12°26′43″ N; 102°15′47″ E, on rachis of Phoenix paludosa, immersed mangrove mud and water, 25 April 2017, S.N.Zhang, SNT130 (epi-paratype MFLU 18-1581; living culture MFLUCC 18-0268 = GZCC 18-0010); Thailand. Krabi, near Pali, on decayed rachis of Nypa fruticans, immersed mangrove mud and water, 30 August 2017, S.N.Zhang, SNT195 (epi-paratype MFLU 18-1582; living culture MFLUCC 17-2515 = GZCC 18-0011; MFLUCC 17-2516 = GZCC 18-0012).

Habitat and distribution: Inhabiting in Thai mangrove forests, the Andaman sea (west) coastline and the Gulf of Thailand (east).

Notes*: Longicorpus striataspora* was found on two mangrove palm species, *Nypa fruticans* and *Phoenix paludosa*. The typical characteristics of *L. striataspora* are the deeply immersed, carbonaceous ascomata with a long neck, and the striate, guttulate, fusiform, 1–3-septate ascospores, with larger middle cells and relatively smaller and paler end cells, surrounded by a mucilaginous sheath. However, such characteristics are similar to *Trematosphaeria* spp., and match with *Trematosphaeria striataspora* (Hyde 1988), the holotype collected from intertidal wood of *Nypa fruticans* in North Sumatra. *Trematosphaeria striataspora* was later accommodated in Astrosphaeriella Syd. & P. Syd. (Hyde 1992a) with proposals for recollection and further phylogenetic studies (Liu et al. 2011, Phookamsak et al. 2015). We have compared the fresh collections of *Longicorpus striataspora* with the type material of *Trematosphaeria striataspora*, and concluded that the two are identical in morphology. On the other hand, the genus *Trematosphaeria* Fuckel has been assigned to the family Trematosphaeriaceae K.D. Hyde, Y. Zhang ter, Suetrong & E.B.G. Jones, based on molecular data of its type species T. pertusa Fuckel. Therefore, we follow Ariyawansa et al. (2014) and designate an epitype for *Longicorpus striataspora* in this study.

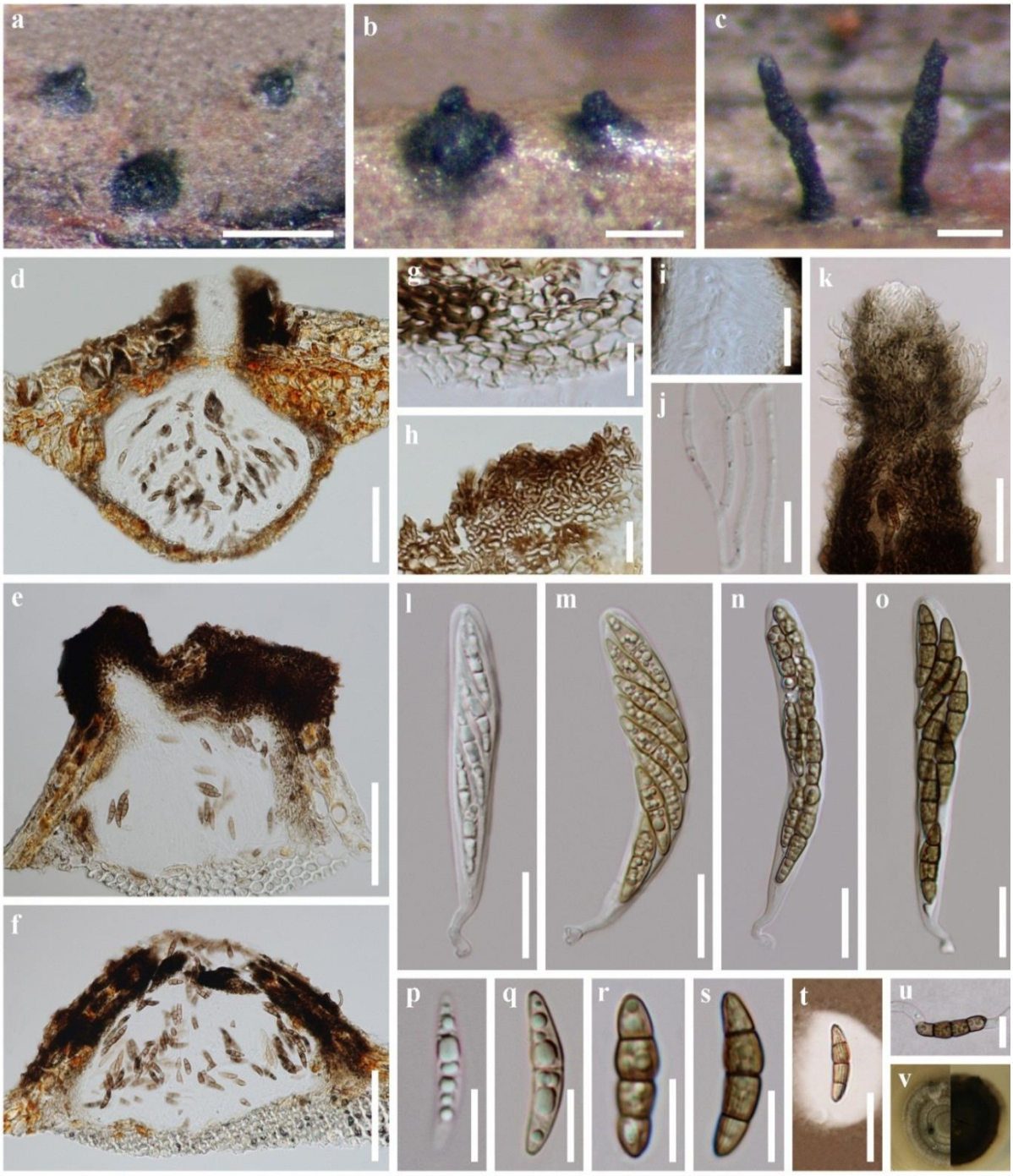


Figure 49 – *Striatiguttula nypae* (holotype MFLU 18-1576, paratype MFLU 18-1578). a-c Appearance of stromata on host surface. d-f Vertical section through a stroma. g Structure of peridium. c Structure of clypeus near the ostiole, composed of epidermoidea cells and host tissue. i Ostiole with periphyses. j Pseudoparaphyses. k Apex of the neck, with somewhat interwoven pale brown hyphae or setae. l-o Ascus. p-s Ascospores. t Ascospore in India ink and presenting a clear mucilaginous sheath. u Germinating ascospore. v Colony on PDA. Scale bars: a = 500 µm, b-c = 200 µm, d-f = 100 µm, g, p-s, u = 10 µm, h-i, l-o, t = 20 µm, k = 50 µm.

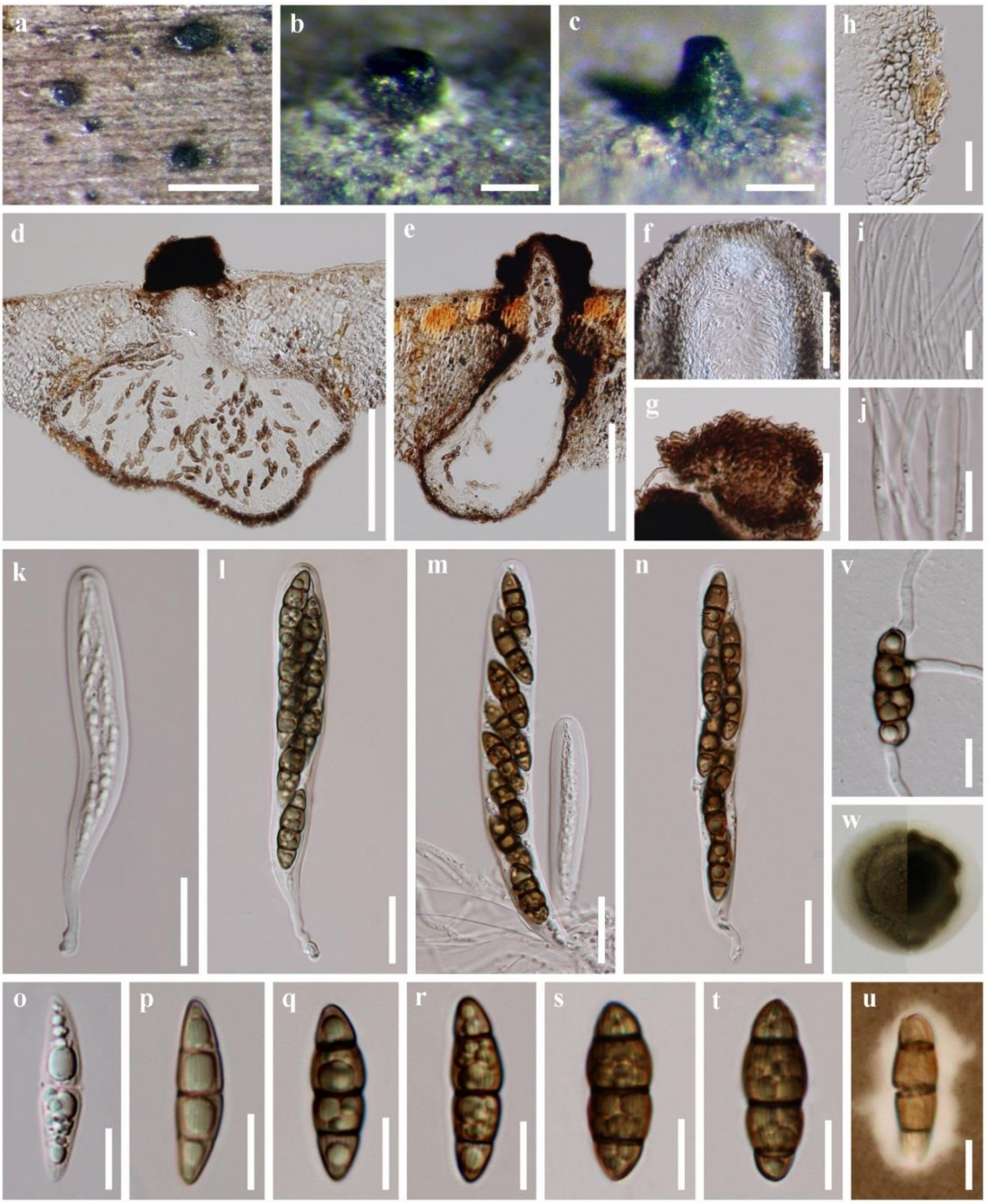


Figure 50 – *Striatiguttula phoenicis* (holotype MFLU 18-1579). a-c Appearance of ascoma on host surface. d-e Vertical section through an ascoma. f Ostiole. g Apex of the neck, with somewhat interwoven pale brown hyphae or setae. h Structure of peridium. i-j Pseudoparaphyses. k-n Asci. o-u Ascospores. u Ascospore in India ink and presenting a clear mucilaginous sheath. v Germinating ascospore. w Colony on PDA. Scale bars: a = 500 µm, b, c = 100 µm, d, e = 200 µm, f, g = 50 µm, h, k-n = 20 µm, i, j, o-v = 10 µm.

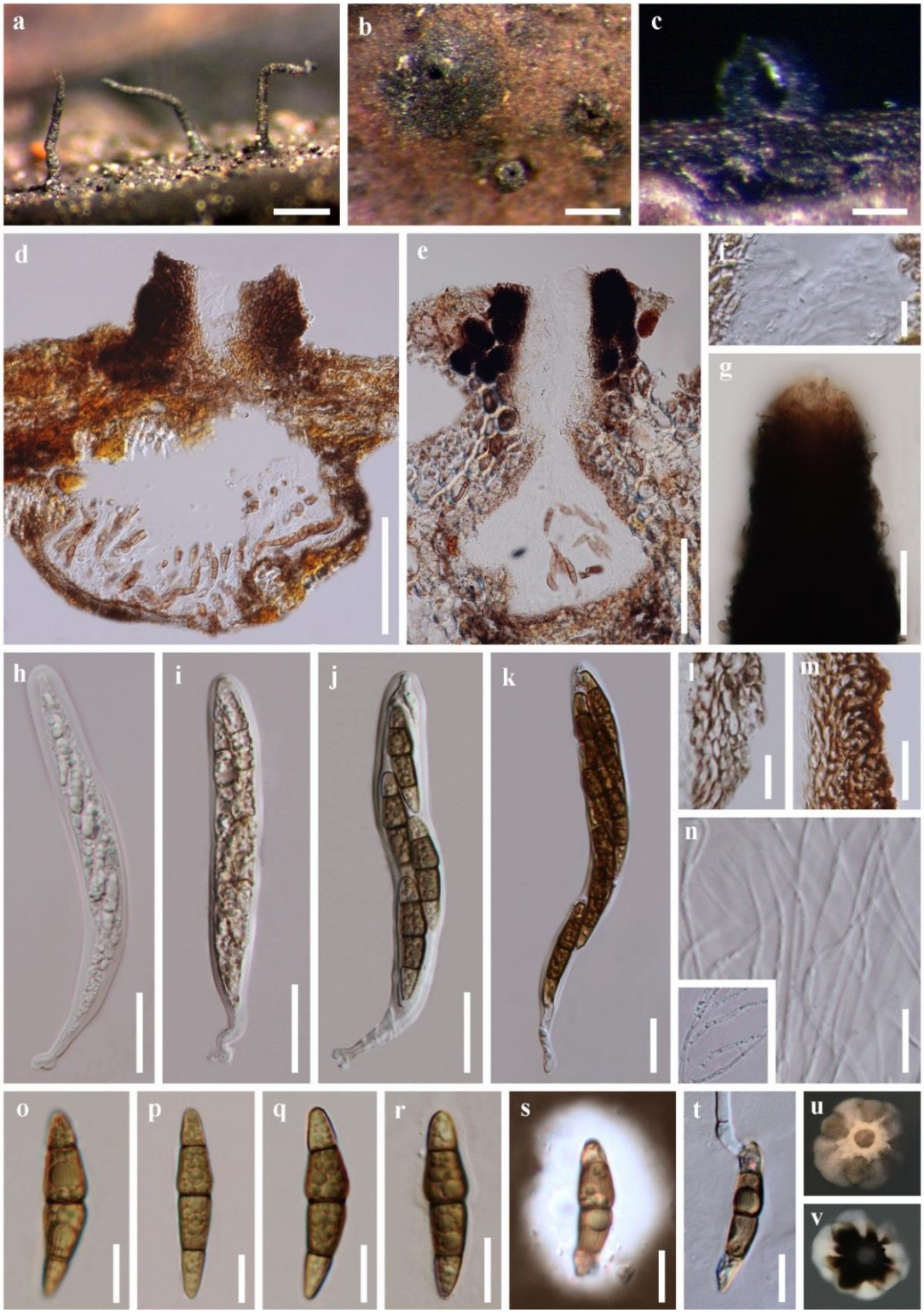


Figure 51 – *Longicorpus striataspora* (epitype MFLU 18-1580, epi-paratype MFLU 18-1582). a-b Appearance of ascoma on host surface. c-e Vertical section through an ascoma, with a clypeus near the ostiole. f Ostiole with periphyses. g Apex of the neck, with somewhat interwoven pale brown hyphae or setae. h-k Ascus. l Structure of peridium. m Structure of the neck, with thicker angular cells. n Pseudoparaphyses. o-r Ascospores. s Ascospore in India ink and presenting a clear mucilaginous sheath. t Germinating ascospore. u-v Colony on PDA. Scale bars: a = 500 µm, b = 200 µm, c-e = 100 µm, f, l, n-t = 10 µm, g = 50 µm, h-k, m = 20 µm.

Appendix VIII To establish the biodiversity of fungi and also establish if any species occupy more than one life mode (e.g. saprobic, endophytic, and pathogenic).

Table 16. List of isolate fungi with deposit information and identification based on sequence data

Original Code	Taxa (BLAST result based on ITS sequence data)	Substrate	Life Mode
E05-2	*Phyllosticta*	Leaves	Endophytic
E10A	*Hypoxylon monticulosum*	Leaves	Endophytic
E104	*Akanthomyces muscarius*	Leaves	Endophytic
E09A-1	*Neopestalopsis*	Leaves	Endophytic
E09A-2	*Phullosticta*	Leaves	Endophytic
E09B-3	*Phyllosticta*	Leaves	Endophytic
E12A	*Nigrospora sphaerica*	Leaves	Endophytic
E14	*Biscogniauxia uniapiculata*	Leaves	Endophytic
E07	*Physalospora*	Leaves	Endophytic
E11-2	*Nectria sinopica*	Leaves	Endophytic
E30-1	*Colletotrichum* sp.	Leaves	Endophytic
P01-1	*Colletotrichum gloeosporioides*	Leaves	Pathogenic
P01-3	*Colletotrichum gloeosporioides*	Leaves	Pathogenic
E29-1	*Phomopsis* sp.	Leaves	Endophytic
E29-2	*Phomopsis* sp.	Leaves	Endophytic
P01-4	*Phomopsis* sp.	Leaves	Pathogenic
P02-1	*Pseudopestalotiopsis*	Leaves	Pathogenic
P02-2	*Wardomyces sp.*	Leaves	Pathogenic
P02B	*Neopestalotiopsis*	Leaves	Pathogenic
P09B-2	*Phyllosticta*	Leaves	Pathogenic
P09H	*Neopestalotiopsis*	Leaves	Pathogenic
P10A-1	*Pestalotiopsis*	Leaves	Pathogenic
SNT35	*Pestalotiopsis*	rachis	Saprobic
SNT46	*Pestalotiopsis*	rachis	Saprobic
P11A-1	*Lasiodiplodia theobromae*	Leaves	Pathogenic
P11B-1	*Pestalotiopsis*/ *Neopestalotiopsis*	Leaves	Pathogenic
P11A-3	*Lasiodiplodia theobromae*	Leaves	Pathogenic
SNT21	*Lasiodiplodia theobromae*	Inflorescence	Saprobic

Sequence-based analyses have shown that a specific grou of endophytic strains can act as saprotrophs in the laboratory or in a terrestrial environment. In general, leaf decomposition is a process taking several months where succession of different groups of decomposers occurs, endophytes being considered within the group of primary decomposers. However, fungal endophytes can have a significant effect on leaf decomposition, whether participating in it or not.

Based on the sequences data, we found some species may occupy more than one life mode. From table 16, we can see that *Lasiodiplodia theobromae* and *Pestalotiopsis* appearant saprobic and pathogenic life modes, while *Colletotrichum* sp. and *Phomopsis* sp. have been isolate from health leaves as endophyte, and also been isolated from leaves with lesions.

Appendix IX To obtain culture of accurately identified mangrove taxa for novel medicinal compound discovery research and future phylogenetic studies.

Fungi can produce medically significant metabolites or can be induced to produce such metabolites using biotechnology. The range of medically active compounds that have been identified include antibiotics, anti-cancer drugs, cholesterol inhibitors, psychotropic drugs, immunosuppressants and even fungicides. Although initial discoveries centred on simple moulds of the type that cause spoilage of food, later work identified useful compounds across a wide range of fungi.

Fungi derived from marine sources, such as mangroves, are considered to represent a huge reservoir of secondary metabolites, many of which are biologically active and are produced e.g. by multifunctional enzyme complexes such as polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS). Marine (mangrove) fungi are highly potent producers of bioactive substances with antifungal, antibacterial, antiviral, cytotoxic and immunosuppressive activity. The various biological activities make them a valuable source for pharmaceutical applications. In this study, all the obtained cultures were deposited in culture collection, and could be use for novel medicinal compound discovery research and future phylogenetic studies.

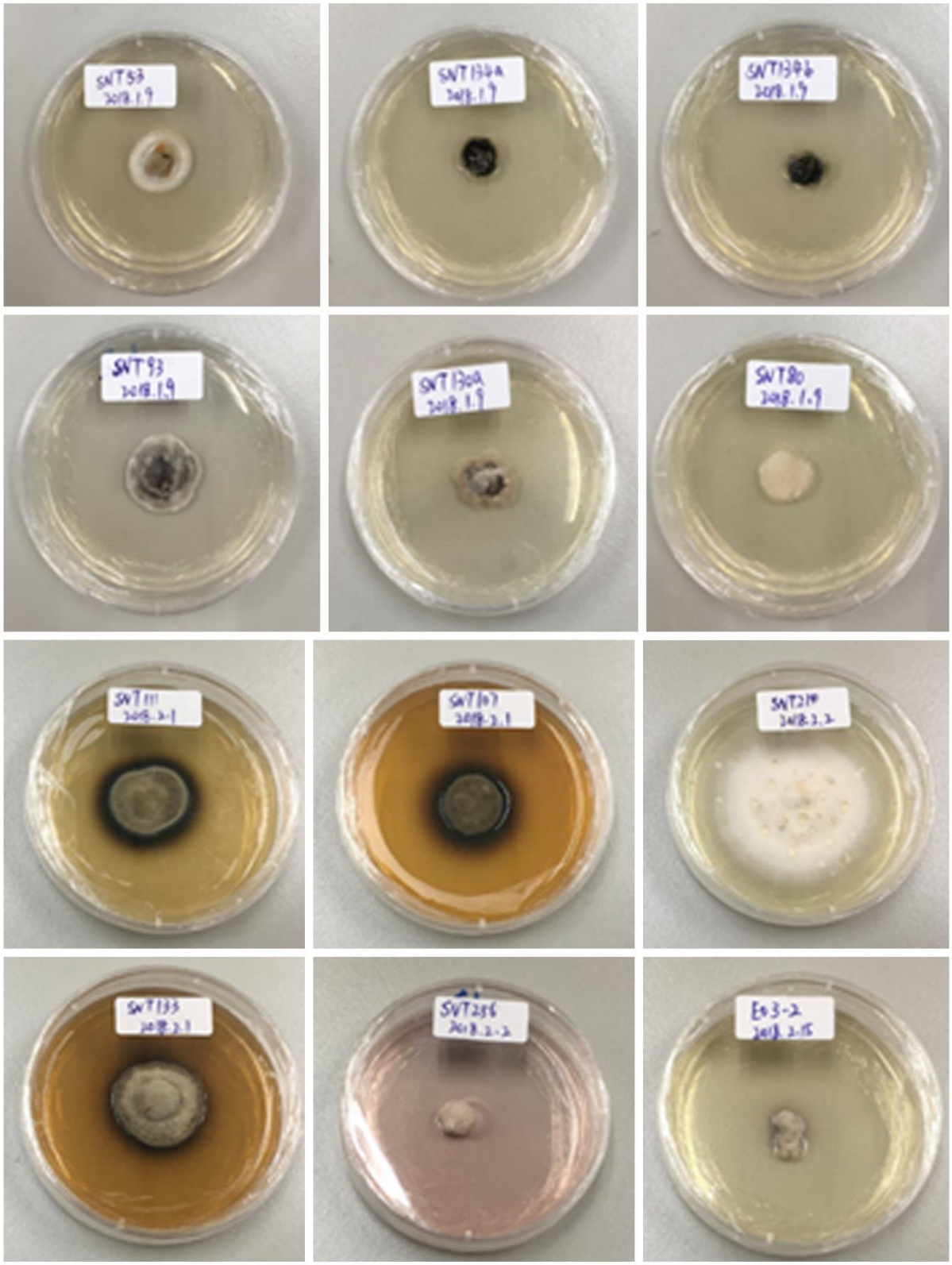


Figure 52 – Cultures of fungi isolated from *Nypa fruticans*.

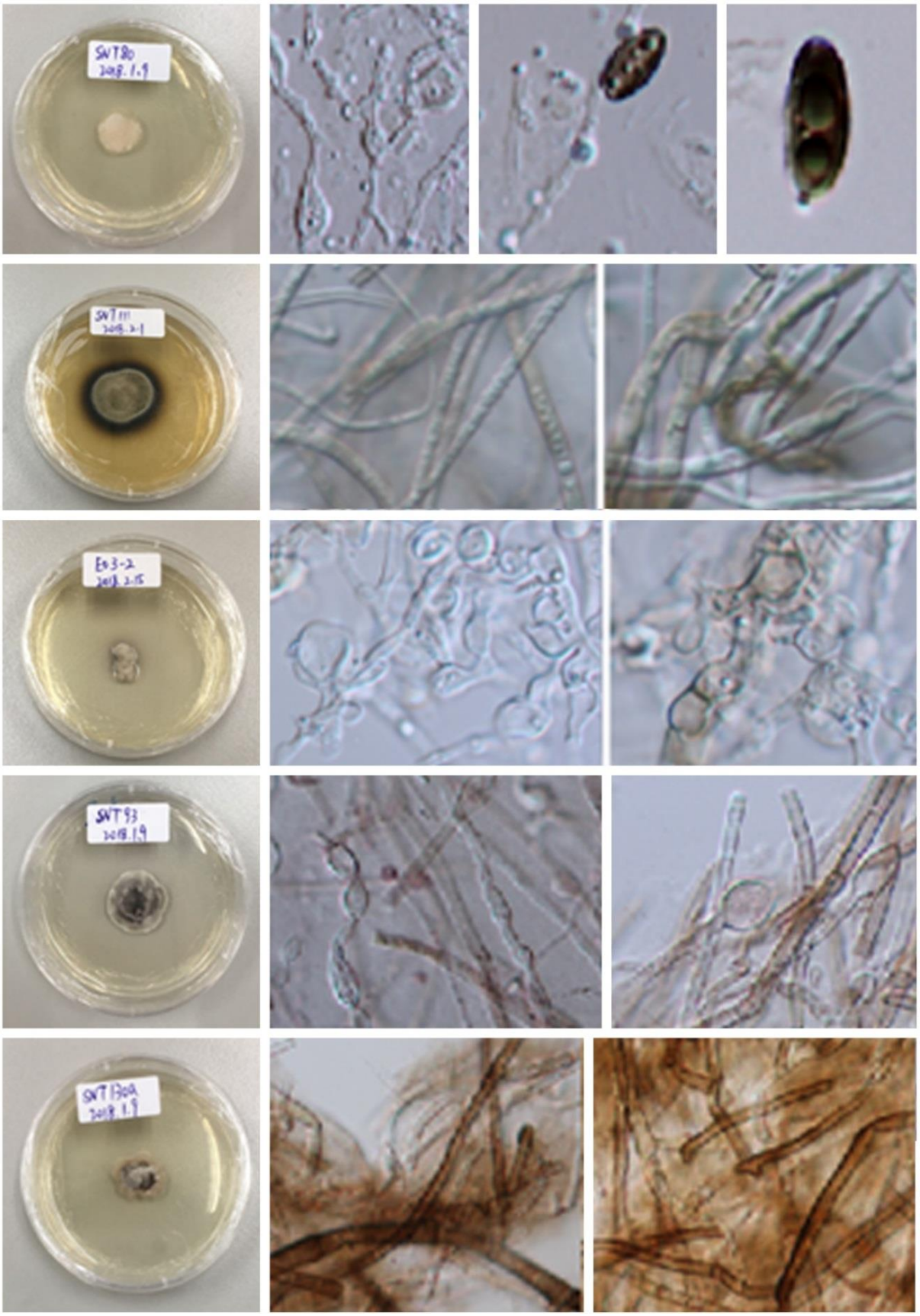


Figure 53 – Culture characteristics observation.

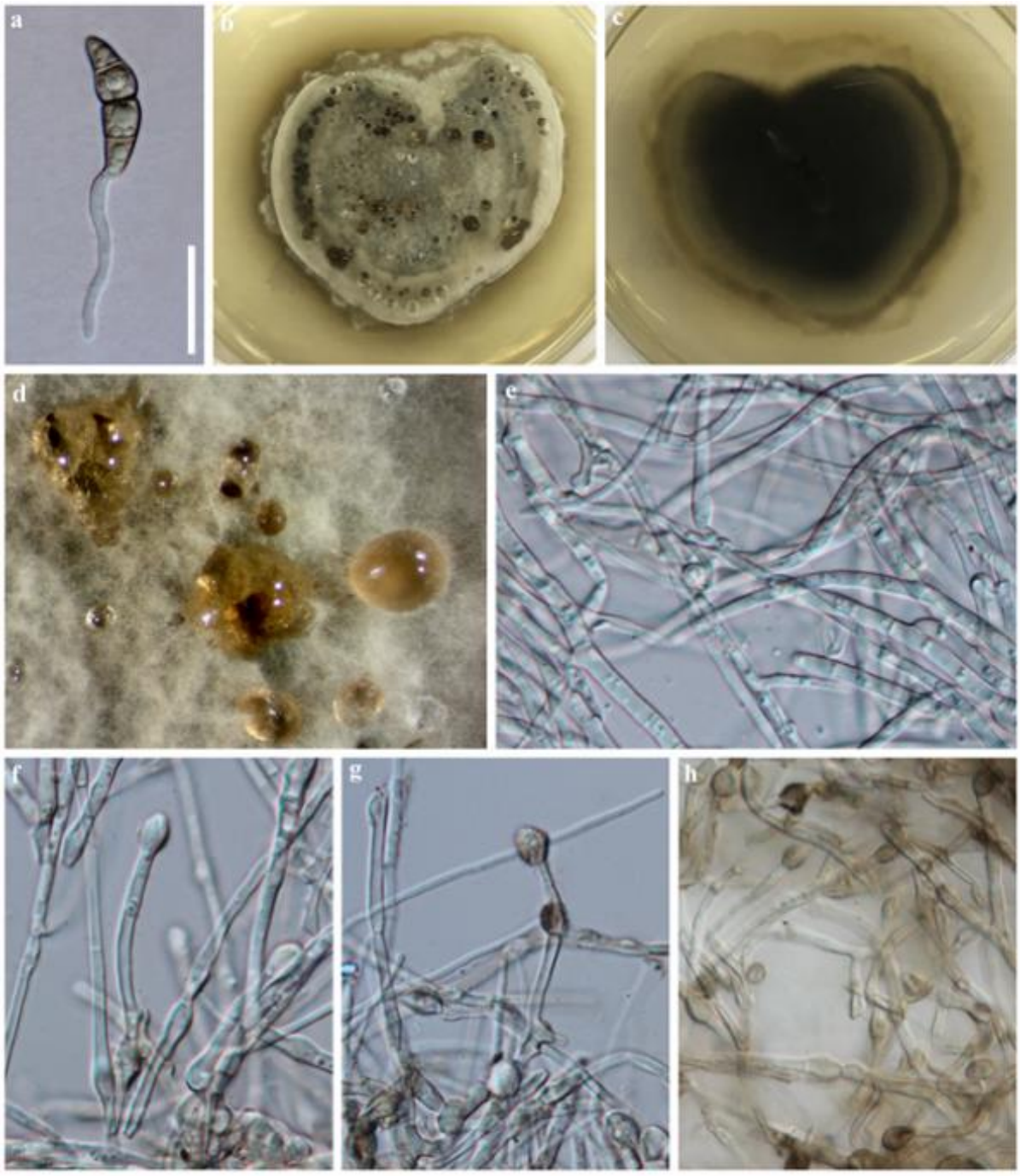


Figure 54 – *Acuminatispora palmarum*. a Germinating ascospore. b–c Colony on PDA, above (b) and below (c). d Mycelium on PDA showing aerial hyphae. e–h Mycelium growing on PDA after one month, f-h Mycelium with a chlamydospore. Scale bars: a = 20 µm.

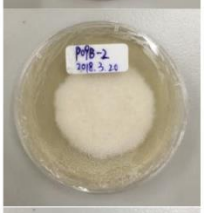
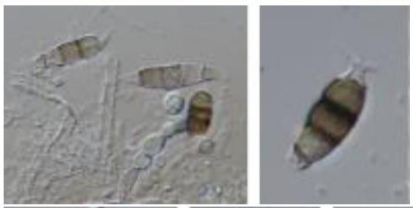
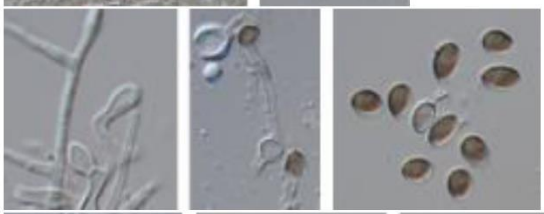
MFLUCC	Original Code	Special Features and Properties of The Strains	Plate photo
18-0966	P02-2		
18-0967	P04C-1		
18-0968	P04C-2		
18-0969	P09B-2		
18-0970	P09H		
18-0971	P10C-1		
18-0972	P11A-1		

Figure 55 – Culture characters observation.

Appendix X Research published with international academic journals, conections and conferences.

38 published papers were published and three manuascript have been submitted with the authors acknowledging Thailand Research Fund (TRF) grant no RSA5980068 entitled Biodiversity, phylogeny and role of fungal endophytes on aerial parts of *Rhizophora apiculata* and *Nypa fruticans*.

List of five submitted manuascripts and process to publish

Jones EBG, Devadatha B, Abdel-Wahab MA, Dayarathne MC, Zhang SN, Hyde KD et al. 2019 – New marine fungal genus and species. Botanica Marina (submitted)

Zhang SN, Abdel-Wahab MA, Jones EBG, Hyde KD, Liu JK 2019 – Additions to the genus Savoryella (Savoryellaceae), with the asexual morphs *Savoryella nypae* comb. nov. and S. sarushimana sp. nov. Phytotaxa (in press)

Zhang SN, Liu JK, Jones EBG, Cheewangkoon R. et al. 2019 – Morphology and Phylogeny of *Yunnanomyces phoenicis* sp. nov. (Sympoventuriaceae) from Thailand. Asian Journal of Mycology (ready to submit)

List of thirty-six publications since June 2017–June 2019

- **Papers published in 2017 (16 publications)**

2017_01

Dayarathne MC, Abeywickrama P, Jones EBG, Bhat DJ, Chomnunti P, Hyde KD 2017 – Multi-gene phylogeny of *Jattaea bruguierae*, a novel asexual morph from *Bruguiera cylindrica*. Studies in Fungi 2(1), 235–245.

2017_02

Dayarathne MC, Maharachchikumbura SSN, Jones, EBG, Goonasekara ID et al. 2017 – *Neophyllachora* gen nov. (Phyllachorales), three new species of *Phyllachora* from Poaceae and resurrection of Polystigmataceae (Xylariales). Mycosphere 8: 1598–1625.

2017_03

Devadatha B, Sarma VV, Wanasinghe DN, Hyde KD et al. 2017 – Introducing the new Indian mangrove species, *Vaginatispora microarmatispora* (Lophiostomataceae) based on morphology and multigene phylogenetic analysis. Phytotaxa 329: 139–149.

2017_04

Doilom M, Manawasinghe IS, Jeewon R, Jayawardena RS et al. 2017 – Can ITS sequence data identify fungal endophytes from cultures? A case study from *Rhizophora apiculata.* Mycosphere 8: 1869–1892.

2017_05

Hongsanan S, Maharachchikumbura SSN, Hyde KD, Samarakoon MC et al. 2017 – An updated phylogeny of Sordariomycetes based on phylogenetic and molecular clock evidence. Fungal Diversity 84: 25–41.

2017_06

Hyde KD, Norphanphoun C, Abreu VP, Bazzicalupo A et al. 2017 – Fungal diversity notes 603–708. Taxonomic and phylogenetic notes on genera and species. Fungal Diversity 87: 1–235.

2017_07

Hyde KD, Maharachchikumbura SSN, Hongsanan S, Samarakoon M. et al. 2017 – The ranking of fungi: a tribute to David L. Hawksworth on his 70th birthday. Fungal Diversity 84: 1–23.

2017_08

Liu JK, Hyde KD, Jeewon R, Phillips AJ, Maharachchikumbura SSN et al. 2017 – Ranking higher taxa using divergence times: a case study in Dothideomycetes. Fungal Diversity 84: 75–99.

2017_09

Norphanphoun C, Jeewon R, Mckenzie EHC, Wen TC, et al 2017 – Taxonomic position of *Melomastia italica sp. nov.* and phylogenetic reappraisal of *Dyfrolomyces.* Cryptogamie Mycologie 38: 507–525.

2017_10

Phookamsak R, Wanasinghe DN, Hongsanan S, Phukhamsakda C, et al. 2017 – Towards a natural classification of Ophiobolus and ophiobolus-like taxa; introducing three novel genera *Ophiobolopsis*, *Paraophiobolus* and *Pseudoophiobolus* in Phaeosphaeriaceae (Pleosporales). Fungal Diversity 87: 299–339.

2017_11

Senanayake IC, Crous PW, Groenewald JC, Maharachchikumbura SSN et al. 2017 – Families of Diaporthales based on morphological and phylogenetic evidence. Studies in Mycology 86: 217–296.

2017_12

Senwanna C, Phookamsak R, Doilom M, Hyde KD, et al. 2017 – Novel taxa of Diatrypaceae from Para rubber (*Hevea brasiliensis*) in northern Thailand; introducing a novel genus *Allocryptovalsa*. Mycosphere 8: 1835–1855.

2017_13

Tibpromma S, Hyde KD, Jeewon R, Maharachchikumbura SSN et al. 2017 – Fungal diversity notes 491–602: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 83: 1–261.

2017_14

Wanasinghe DN, Hyde KD, Jeewon R, Crous PW et al. 2017 – Phylogenetic revision of *Camarosporium* (Pleosporineae, Dothideomycetes) and allied genera. Studies in Mycology 87: 207–256.

2017_15

Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL et al. 2017 – Notes for genera: *Ascomycota*. Fungal Diversity 86: 1–594.

2017_16

Zhang SN, Hyde KD, Jones EBG, Cheewangkoon R et al. 2017 – *Novomicrothelia pandanicola* sp. nov, a non-lichenized Trypetheliaceae species from *Pandanus*. Phytotaxa 321: 254–264.

- **Papers published in 2018 (11 publications)**

2018_01

Dayarathne MC, Wanasinghe DN, Jones EBG, Chomnunti P, Hyde KD 2018 – A novel marine genus, *Halobyssothecium* (Lentitheciaceae) and epitypification of *Halobyssothecium obiones* comb. nov. Mycological Progress 17: 1161–1171.

2018_02

Doilom M, Hyde KD, Phookamsak R, Dai DQ et al. 2018 – Mycosphere Notes 225–274: types and other specimens of some genera of Ascomycota. Mycosphere 9(4): 647–754.

2018_03

Hyde KD, Chaiwan N, Norphanphou C, Boonmee S et al. 2018 – Mycosphere notes 169–224. Mycosphere 9: 271–430.

2018_04

Kumar V, Doilom M, Wanasinghe DN, Bhat DJ et al. 2018 – Phylogenetic placement of *Akanthomyces muscarius*, a new endophyte record from *Nypa fruticans* in Thailand. Current Research in Environmental & Applied Mycology 8(3): 404–417.

2018_05

Norphanphoun C, Raspé O, Jeewon R, Wen TC, Hyde KD 2018 – Morphological and phylogenetic characterization of novel *Cytospora* species associated with mangroves. MycoKeys 38: 93–120.

2018_06

Phookamsak R, Lu YZ, Hyde KD, Jeewon R et al. 2018 – Phylogenetic characterization of two novel *Kamalomyces* species in Tubeufiaceae (Tubeufiales). Mycological Progress 17: 647–660.

2018_07

Senanayake IC, Jeewon R, Chomnunti P, Wanasinghe DN et al. 2018 – Taxonomic circumscription of Diaporthales based on multigene phylogeny and morphology. Fungal diversity 93: 241–443.

2018_08

Tibpromma S, Hyde KD, McKenzie EHC, Bhat DJ et al. 2018 – Fungal diversity notes 840–928: micro-fungi associated with Pandanaceae. Fungal diversity 93: 1–160.

2018_09

Wanasinghe DN, Phukhamsakda C, Hyde KD, Jeewon R et al. 2018 – Fungal diversity notes 709–839: taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on Rosaceae. Fungal Diversity 89: 1–236.

2018_10

Wijayawardene NN, Hyde KD, Lumbsch HT, Liu JK et al. 2018 – Outline of *Ascomycota*: 2017 – Fungal Diversity 88: 167–263.

2018_11

Zhang SN, Hyde KD, Jones EBG, Cheewangkoon R, Liu JK 2018 – *Acuminatispora palmarum* gen. et sp. nov. from mangrove habitats. Mycological progress 17: 1173–1188.

- **Papers published in 2019 (11 publications)**

2019_01

Dayarathne MC, Maharachchikumbura SSN, Jones EBG, Dong w. et al. 2019 – Phylogenetic revision of Savoryellaceae and evidence for its ranking as a subclass. Frontiers in Microbiology 10: 840.

2019_02

Ekanayaka AH, Jones EBG, Hyde KD, Zhao Q. 2019 – A stable phylogeny for Dactylosporaceae. Cryptogamie Mycologie 40(3): 23–44.

2019_03

Hyde KD, Tennakoon DS, Jeewon R, Bhat DJ, Maharachchikumbura SSN et al. 2019 – Fungal diversity notes 1036-1150: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fugnal diversity 96: 1–242.

2019_04

Jiang HB, Hyde KD, Jayawardena RS, Doilom M, Xu JC, Phookamsak R. 2019 – Taxonomic and phylogenetic characterizations reveal two new species and two new records of *Roussoella* (Roussoellaceae, Pleosporales) from Yunnan, China. Mycological progress 18: 577–591.

2019_05

Jones EBG, Pang KL, Abdel-Wahab MA, Scholz B, Hyde KD et al. 2019 – An outline resource for marine fungi. Fungal diversity (accepted).

2019_06

Kumar V, Cheewangkoon R, Gentekaki E, Maharachchikumbura SSN, Brahmanage RS, Hyde KD et al. 2019 – *Neopestalotiopsis* alpapicalis sp. nov. a new endophyte from tropical mangrove trees in Krabi Province (Thailand). Phytotaxa 393(3): 251–262.

2019_07

Kumar V, Cheewangkoon R, Thambugala K, Jones EBG, Brahmanage RS, Doilom M, Jeewon R, Hyde KD 2019 – *Rhytidhysteron* mangrovei (Hysteriaceae), a new species from mangroves in Phetchaburi Province, Thailand. Phytotaxa 401(3): 166–178.

2019_08

Norphanphoun C, Jayawardena RS, Chen Y, Wen TC, Meepol W, Hyde KD. 2019 – Morphological and phylogenetic characterization of novel pestalotioid species associated with mangroves in Thailand. Mycosphere 10: 531–578.

2019_09

Phillips AJL, Hyde KD, Alves A, Liu JK 2019 – Families in Botryosphaeriales: a phylogenetic, morphological and evolutionary perspective. Fungal diversity 94: 1–22.

2019_10

Phookamsak R, Hyde KD, Jeewon R, Bhat DJ, Jones EBG et al. 2019 – Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi. Fungal diverisity 95: 1–273.

2019_11

Zhang SN, Hyde KD, Jones EBG, Jeewon R, Cheewangkoon R, Liu JK 2019 – Striatiguttulaceae, a new pleosporalean family to accommodate *Longicorpus* and *Striatiguttula* gen. nov. from palms. MycoKeys 49: 99–129.

Abroad connection and award

Prof. Kevin D. Hyde is Emeritus professor in Kunming Institute of Botany of the Chinese Academy of Sciences (KIB, CAS), China, and the Director of the Center of Excellence in Fungal Research, Mae Fah Luang University, Thailand. Prof. Kevin D. Hyde received an award from Thompson Reuters for being one of the World Most Influential Scientific Minds on 25 August 2016. In receiving the reward, he thanked the students he said - "the students did all the work - not me - so I have received this award because of my students" - "thank you students". He also thanked Mae Fah Luang University and The Thailand Research Fundation and other grants for the enormous support. Right now, he is ranked by Thompson Reuters as one of the top 3000 of the world 9 million estimated researchers.

An award of "2017 HIGHLY CITED RESEARCHER" also presented to Prof. Kevin D. Hyde on 11th January 2018, which is in recognition of ranking among the top 1% of researchers for most cited documents, in the area of Plant & Animal Science.

International conference and presentation

- Poster presentation in TRF conferences 2018 at Cha-am Province, Thailand

15th International Marine and Freshwater Mycology Symposium (IMFMS), Xiamen, China

Studies in Fungi 2 (1): 235–245 (2017) www.studiesinfungi.org ISSN 2465-4973
Article

Doi 10.5943/sif/ 2/1/27

Multi-gene phylogeny of *Jattaea bruguierae*, a novel asexual morph from *Bruguiera cylindrica*

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Dayarathne MC, Abeywickrama P, Jones EBG, Bhat DJ, Chomnunti P, Hyde KD 2017 – Multi-gene phylogeny of *Jattaea bruguierae*, a novel asexual morph from *Bruguiera cylindrica*. Studies in Fungi 2(1), 235–245, Doi 10.5943/sif/2/1/27

Abstract

During our survey on marine-based ascomycetes of southern Thailand, fallen mangrove twigs were collected from the intertidal zones. Those specimens yielded a novel asexual morph of *Jattaea* (*Calosphaeriaceae,* Calosphaeriales), *Jattaea bruguierae,* which is confirmed as a new species by morphological characteristics such as nature and measurements of conidia and conidiophores, as well as a multigene analysis based on combined LSU, SSU, ITS and β-tubulin sequence data. *Jattaea* species are abundantly found from wood in terrestrial environments, while the asexual morphs are mostly reported from axenic cultures. *Jattaea bruguierae* is the first documentation of an asexual morph species from marine- habitats.

Key words – marine fungi – morphology – phylogeny – taxonomy

Introduction

Berlese (1900) introduced *Jattaea* Berl. and *Wegelina* Berl. as morphologically similar genera with hyaline, allantoid, one-celled ascospores and clavate asci. They can be distinguished primarily by characters of perithecia and length of the ostiolar neck; papillate to short-beaked in *Jattaea* vs. cylindrical elongate necks in *Wegelina*. *Jattaea algeriensis* Berl. was designated as a respective lectotype by Clements & Shear (1931). Later, the generic name *Jattaea* was accepted, with *Wegelina* as its synonym, to include species with hyaline, allantoid to suballantoid ascospores in clavate, stipitate asci without an apical annulus borne on individual cells on ascogenous hyphae and phialophora-like asexual morphs produced in axenic culture (Réblová 2011). *Jattaea* species occur usually solitarily, scattered or in small irregular to valsoid groups on wood beneath the periderm, around old fungal stromata or margins of the peeled bark or are rarely immersed in decaying wood. Some species, however, have ascomata arranged in 1–2 vertical levels and in larger groups similar to *Calosphaeria* Tul. & C. Tul. The asci in *Jattaea* are oblong-clavate to clavate and short- to long-stipitate. Multigene analyses by Réblová (2011) also confirmed that septation of ascospores, a diagnostic feature used to separate calosphaeria-like fungi into the genus

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Discussion

The present study introduces a new species in the genus *Jattaea* (*Jattaea bruguierae),* provided with a morphological description, illustrations and combined analyses of LSU, SSU, ITS and β-tubulin sequence data. Recently, Réblová et al. (2015) has used combined LSU, SSU, ITS, RPB2 and β-tubulin genes in their phylogenetic reconstruction of Calosphaeriales. Unfortunately, we did not obtain RPB2 sequence data even after several attempts with different PCR temperature profiles by using the primers fRPB2-5F and fRPB2-7cR. However, the concatenated dataset of LSU, SSU, ITS and β-tubulin sequences reveals a phylogeny which is topologically congruent to Réblová et al. (2015). Hence, we are confident with our taxonomic arrangement of the novel taxon which is phylogenetically close to *Jattaea leucospermi* and *J. mookgoponga* with strong bootstrap support (100% ML/ 96% MP/ 1.00 PP, Fig. 1). Our species is morphologically different from all the other previously described species in having hyaline, elongated conidiophores and cylindrical conidia with a tapered base, while others have yellow brown conidiophores and cylindrical to ellipsoidal conidia with obtuse ends. Therefore, both morphological and phylogenetic support ensures that our species definition and justification for establishing a new species is scientifically valid within Calosphaeriaceae. *Jattaea* was recently revisited with 17 accepted species and asexual morphs linked to the genus comprise reduced, morphologically similar dematiaceous hyphomycetes with phialidic conidiogenous cells similar to *Phialophora* (Réblová et al. 2015). The asexual morphs of *Jattaea* have been experimentally established for nine of the 17 accepted species, i.e. *J. algeriensis* Berl., *J. aphanospora* Réblová & J. Fourn., *J. aurea* Réblová & J. Fourn., *J. discrete* (Berl.) Réblová, *J. leucospermi* Marinc., M.J. Wingf. & Crous, *J. mookgoponga* Damm & Crous, *J. ribicola* Réblová & Jaklitsch, *J. taediosa* (Sacc.) Réblová & Jaklitsch and *J. tumidula* (Sacc.) Réblová, by the previous studies of Damm et al. (2008), Réblová (2011) and Réblová et al. (2015). *Jattaea mookgoponga* and our novel species, *J. bruguierae* are known only as asexual morphs, while all the other asexual morphs have been linked with their sexual morphs (Réblová et al. 2015). Although the differences between asexual morphs of *Jattaea* based on their morphology they are distinctly different at the molecular phylogenetic level (Réblová 2011, Réblová et al. 2015) and as demonstrated in our study. Different authors have described colony characteristics of asexual morphs of *Jattaea* species in different culture media, such as normal PDA, MEA, potato-carrot agar (PCA, Gams et al. 1998), synthetic nutrient-poor agar medium (SNA, Nirenberg 1976). In our study, we described cultural characteristics of *J. bruguierae* on half-strength seawater PDA and half-strength seawater MEA media and it grew fast and sporulated well. However, we did not observe conidial structures on the host surface. Therefore, we designated a dry culture along with the herbarium material as the holotype. Our species also morphologically resembles asexual morphs of *Phaeoacremonium* spp. However, according to phylogenetic analysis it is confirmed that our novel species does not belong to *Phaeoacremonium*. *Jattaea mucronata,* introduced by Abdel-Wahab et al. (2017), is the first documentation of a sexual morph of *Jattaea* associated with a marine habitat while *J bruguierae* is the first record of an asexual morph from mangroves.

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Neophyllachora gen nov. (Phyllachorales), three new species of *Phyllachora* from Poaceae and resurrection of *Polystigmataceae* (Xylariales)

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Abstract

We collected six "tar spot" disease specimens from various hosts and these were subjected to morpho-phylogenetic studies. In this paper, a new genus, *Neophyllachora* is introduced to accommodate *N. cerradensis*, *N. myrciae*, *N. myrciariae*, *N. subcircinans* and *N. trucantispora*, which are related to *Phyllachora* species but constitutes an independent strongly supported monophyletic clade within *Phyllachoraceae* of the Phyllachorales. Three novel *Phyllachora* species; *P. chloridis*, *P. cynodonticola* and *P. panicicola* on Poaceae are also introduced. Phenotypic comparisons and phylogenetic analysis of partial SSU, LSU and ITS sequence data with homologous taxa, confirm the placement of the novel species in *Phyllachoraceae*. The family *Polystigmataceae* is re-established to accommodate *Polystigma* within the order Xylariales. The asexual morph of *Polystigma rubrum* was re-collected from Russia and is provided as a reference specimen with a description, illustrations and molecular data. Further studies with multiple gene analysis are recommended to provide a natural and stable classification system for members of Phyllachorales.

Key words – biotrophs – phylogeny – tar spots – taxonomy

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species) which were isolated from *Prunus* spp. should in any case be placed in the order Xylariales. Hence, the family *Polystigmataceae* is re-instated with *Po. rubrum* as the type species. We could not observe the sexual morph of this species as they occur in spring and our collection was made in the summer. Morphological characters of family *Polystigmataceae* are provided considering all representatives of genus *Polystigma* and a reference specimen for *Po. rubrum* is provided.

Phyllachora species have previously been identified based on morphology and there are only a few sequences present in GenBank for comparison. Similarly, most of the species only have ITS data and even most of them are short sequences. This indicates the taxonomical instability still present within family *Phyllachoraceae*. The majority of species belonging to this family has been introduced based on the host on which they occur. Illustrations, original descriptions and molecular data are inadequate for the genus and hence, it is rather controversial when introducing novel species (Parbery 1967, 1971, Gabel et al. 1999, Habibi et al. 2015, Santos et al. 2016, Dayarathne et al. 2016). Furthermore, there are no adequate phylogenetic studies to confirm their host specificities or phylogenetic placements to resolve species confusions in naming. Therefore, it is necessary to synonymize, epitipify or logically remove the controversial older species names to facilitate proper identification of new species within the genus (Dayarathne et al. 2016).

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Introducing the new Indian mangrove species, *Vaginatispora microarmatispora* (Lophiostomataceae) based on morphology and multigene phylogenetic analysis

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Abstract

Vaginatispora microarmatispora sp. nov. (Lophiostomataceae) is a new saprobic marine fungal species found on dead intertidal wood of *Aegiceras corniculatum* collected from Muthupet mangroves, Tamil Nadu, along the south-east coast of India. This species has characters similar to other species of the genus *Vaginatispora*, but differs in ascospore dimensions. Phylogeny inferred from a combined LSU, SSU, ITS and TEF1 sequence dataset reveal a close association of the new taxon to species of *Vaginatispora*, but as a distinct lineage. Hence, in this paper, we introduce the new species with illustrations, descriptions and comparison with related taxa. A synopsis of characters of all *Vaginatispora* species along with a dichotomous key are also provided.

Keywords: Ascomycetes, Marine, Pleosporales, Polar appendages, Taxonomy

Introduction

Indian marine fungal diversity has been documented mainly by Vittal and his students from the East Coast of India (Ravikumar & Vittal 1996; Sarma & Vittal 2000,2001, Sarma *et al*. 2001, Vittal & Sarma 2006, Sridhar 2009), while a comprehensive list of marine fungi from India was provided by Borse *et al.* (2013). Jones *et al.* (2009, 2015) listed seven genera with marine species in the family Lophiostomataceae. *Vaginatispora* was introduced by Hyde *et al.* (1995) to accommodate *Vaginatispora aquatica* K.D. Hyde, a new species from Australia and was placed in Massarinaceae. Based on both morphological characteristics and phylogenetic analysis, Thambugala *et al*. (2015) have placed *Vaginatispora* as a separate genus within Lophiostomataceae. *Vaginatispora* species are saprobic and have been reported from submerged wood in marine and freshwater habitats and terrestrial herbaceous plants. It is characterized by 'depressed globose ascomata, immersed beneath a blackened neck, with a slot-like ostiole, numerous filamentous pseudoparaphyses, cylindrical to clavate asci and narrowly ellipsoidal, hyaline, 1-septate ascospores with a mucilaginous collar around its equator, having large guttules in each cell, and a spreading papilionaceous sheath' (Hyde 1995, Zhang *et al*. 2014). However, some species in the genus show other characteristics such as hyaline appendages at the terminal ends of the ascospores (*Vaginatispora appendiculata* Wanas., E.B.G. Jones & K.D. Hyde, *V. armatispora* (K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones) Wanas., E.B.G. Jones & K.D. Hyde and *V. microarmatispora*), globose appendages (*V. fuckelii* (Sacc.) Thambugala, Wanas., Kaz. Tanaka & K.D. Hyde), with or without a mucilaginous sheath (*V. appendiculata*, *V. microarmatispora*). Currently, the genus comprises four species, namely *Vaginatispora appendiculata*, *V. aquatica*, *V. armatispora* and *V. fuckelii* (Tibpromma *et al.* 2017).

During collections and examination of marine fungi from Muthupet mangroves, we have isolated a taxon from Kaveri River Delta, Tamil Nadu (on the east coast of India), which belongs to the Lophiostomataceae based on our preliminary phylogenetic analyses. Subsequently, with further morphological and molecular characterization, we present an account of a new species in the genus *Vaginatispora*. This species is introduced with a comprehensive description supported with morphological differences with known taxa as well as phylogenetic inference from ribosomal RNA and protein-coding sequence based data.

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Discussion

Kohlmeyer and Kohlmeyer (1979) listed only 42 marine fungi occurring in mangroves as few studies had been undertaken. Subsequent studies have shown that mangrove plant substrata are one of the richest source of marine fungi (Jones & Alias 1997, Sarma & Hyde 2001). It is interesting to note that marine fungi occurring in mangroves are predominantly bitunicate species (Dothideomycetes), while unitunicate ascomycetes (particularly Halosphaeriales) are more dominant in other marine habitats, mostly colonizing the driftwood. Most marine Dothideomycetes occur as saprobes in the intertidal region in mangrove habitats with active discharge of their ascospores (Suetrong *et al.* 2009). More than 108 species of marine Dothideomycetes belonging to 64 genera, mainly in three orders, have been reported, and mostly from mangrove substrates (Jones *et al.* 2009). Among these, the Pleosporales has more representatives with 61 species in 25 genera (Jones *et al.,* 2009, 2015). There is a paucity of information on marine anamorphic states of marine dothideomycetes in contrast to freshwater fungi and terrestrial genera of the class (Shearer *et al.* 2009, Suetrong *et al.* 2009).

Vaginatispora was introduced by Hyde (1995) in Massarinaceae (Pleosporales) based on *Vaginatispora aquatica*. Pleosporalean fungi having a slot-like papillate ostiole in the ascomata, cylindro-clavate asci with a long pedicel and hyaline, 1-septate (euseptate) ascospores are a characteristic feature of the membes belonging to Lophiostomataceae e.g. *Lophiostoma* and *Lophiopoacea* (Zhang *et al.* 2009, 2014, Wanasinghe *et al.* 2016). Recent studies on the family placement of *Vaginatispora* have confirmed that it is a separate genus within Lophiostomataceae (Thambugala *et al.* 2015, Wanasinghe *et al.* 2016). This is based on morphological and multigene phylogenetic analyses, wherein it formed a distinct clade with high bootstrap and PP values and all the *Vaginatispora* species clustered together in a well-supported clade within the Lophiostomataceae (Wanasinghe *et al.* 2016). A similar multigene analysis performed with the *Vaginatispora microarmatispora* in the present study showed it clustered with other *Vaginatispora* species, and in particular is closely associated with *Vaginatispora armatispora* (Fig. 1).

Early studies showed that *Vaginatispora* to be mostly a fresh water genus but recent studies (Hyde *et al.* 1992, Wanasinghe *et al.* 2016, Tibpromma *et al.* 2017) in addition to the present study show that this genus is not restricted to freshwater and terrestrial environments, but also occurs in marine environments. While *V. appendiculata* and *V. fuckelii* were collected from dead twigs in terrestrial environments, *V. aquatica* is a saprobe on submerged wood in freshwater habitats, whereas *V. armatispora* and *V. microarmatispora* occur in marine environments.

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Can ITS sequence data identify fungal endophytes from cultures? A case study from *Rhizophora apiculata*

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Abstract

Culture-based studies have recovered fungal endophytes from numerous plant hosts, while direct examination of sporulating cultures has enabled identification. However, many endophytes cannot be identified due to the fact that they only form mycelia sterilia in culture. Although next generation sequencing (NGS), as well as ITS sequence analyses have been used to identify endophytes, identification is still rudimentary. In this study, we isolated fungal endophytes from *Rhizophora apiculata* in Thailand and established how many can be identified to species level based on ITS sequence data. Endophytic fungi were isolated from leaves, petioles and aerial roots of *R. apiculata* in four provinces of Thailand. One hundred and fifty four isolates were obtained and initially grouped into 20 morphotypes based on cultural characteristics. Nine were sporulating morphotypes, which were assigned to seven genera (*Colletotrichum*, *Diaporthe*, *Hypoxylon*, *Neopestalotiopsis*, *Neodevriesia*, *Pestalotiopsis* and *Phyllosticta*), and eleven morphotypes were non-sporulating mycelia sterilia. Sequence similarity comparison and phylogenetic analysis of the ITS regions were further used to identify taxa. While ITS sequence data is reliable to assign isolates at the generic rank, and can be useful to identify taxa to species level in a small number of fungal genera, it cannot generally be used to determine specific species in most genera. ITS analysis classified 30 representative isolates into 20 taxonomic units residing in 15 known genera: *Allophoma* sp., *Colletotrichum* spp., *Diaporthe* spp., *Hortaea werneckii*, *Hypoxylon griseobrunneum*, *Hypoxylon* sp., *Pestalotiopsis* sp., *Phanerochaete* sp., *Phyllosticta* spp., *Pseudopithomyces maydicus*, *Preussia* sp., *Nemania* sp., *Neodevriesia* sp., *Neopestalotiopsis* sp.,

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Table 3 Continued.

	Classification	Strain no	Locality	Habitat
Ascomycota, Sordariomycetes	***Diaporthales, Diaporthaceae***			
	Diaporthe sp.	MFLUCC 17-0035	Kram Subdistrict, Kleang District, Rayong Province	Aerial stilt root
	Diaporthe sp.	MFLUCC 17-1942	Kram Subdistrict, Kleang District, Rayong Province	Aerial stilt root
	Glomerellales, Glomerellaceae			
	Colletotrichum sp.	MFLUCC 17-0004	Sirindhorn, Cha-am Subdistrict, Cha-am District, Phetchaburi Province	Leaf
	Colletotrichum sp.	MFLUCC 17-1943	Kram Subdistrict, Kleang District, Rayong Province	Leaf
	Colletotrichum sp.	MFLUCC 17-1944	Sirinart rajini, Pak Nam Pran Subdistrict, Pranburi District, Prachuap Khiri Khan Province	Leaf
	Xylariales, Xylariaceae			
	Hypoxylon griseobrunneum	MFLUCC 17-0020 and MFLUCC 17-0027	Sirinart rajini, Pak Nam Pran Subdistrict, Pranburi District, Prachuap Khiri Khan Province	Leaf
	Hypoxylon sp.	MFLUCC 17-1945	Sirinart rajini, Pak Nam Pran Subdistrict, Pranburi District, Prachuap Khiri Khan Province	Leaf
	Nemania sp.	MFLUCC 17-0005	Sirindhorn, Cha-am Subdistrict, Cha-am District, Phetchaburi Province	Leaf
Basidiomycota, Agaricomycetes	***Agaricales, Schizophyllaceae***			
	Schizophyllum sp.	MFLUCC 17-1946 and MFLUCC 17-1947	Sirinart rajini, Pak Nam Pran Subdistrict, Pranburi District, Prachuap Khiri Khan Province	Aerial stilt root
	Polyporales, Cerrenaceae			
	Rigidoporus vinctus	MFLUCC 17-0007	Sirindhorn, Cha-am District, Phetchaburi Province	Petiole
	Polyporales, Phanerochaetaceae			
	Phanerochaete sp.	MFLUCC 17-0002	Sirindhorn, Cha-am District, Phetchaburi Province	Leaf

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An updated phylogeny of Sordariomycetes based on phylogenetic and molecular clock evidence

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Abstract The previous phylogenies of Sordariomycetes by M.E. Barr, O.E. Eriksson and D.L. Hawksworth, and T. Lumbsch and S. Huhndorf, were mainly based on morphology and thus were somewhat subjective. Later outlines by T. Lumbsch and S. Huhndorf, and Maharachchikumbura and co-authors, took into account phylogenetic evidence. However, even these phylogenetic driven arrangements for Sordariomycetes, were somewhat subjective, as the arrangements in trees depended on many variables, such as number of taxa, different gene regions and methods used in the analyses. What is needed is extra evidence to help standardize ranking in the fungi. Estimation of divergence times using molecular clock methods has been proposed for providing additional rational for higher ranking of taxa. Thus, in Sordariomycetes, a divergence period (i.e. 200–300 MYA) can be used as criteria to judge when a group of related taxa evolved and what rank they should be given. In this paper, we provide an updated classification of accepted subclasses, orders of Sordariomycetes and use divergence times to provide additional evidence to stabilize ranking of taxa in the class. We point out and discuss discrepancies where the phylogenetic tree conflicts with the molecular clock.

Keywords Class · Classification · Divergence times · Phylogenetics · Ranking

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Introduction

Sordariomycetes is an important class of ascomycetes, mainly characterized by non-lichenized, flask-shaped fruiting bodies (perithecia) and unitunicate asci (Lumbsch 2000; Zhang et al. 2006; Maharachchikumbura et al. 2015, 2016). However, this simple definition could change upon the growth form and habitat. Most members of Xylariomycetidae and some of Sordariomycetidae have dark perithecia, amyloid asci, true paraphyses and periphysate ostioles, while most taxa of Hypocreomycetidae have light coloured perithecia, nonamyloid ascal apical rings (when apical rings are present) and lack true paraphyses. Some groups of Sordariomycetes have cleistothecia (Zhang et al. 2006; Tang et al. 2007; Senanayake et al. 2015). The class Sordariomycetes has a cosmopolitan distribution and accommodates mostly terrestrial taxa, although several taxa can be found in aquatic habitats (Hyde and Jones 1989; Tsui et al. 2000; Ho et al. 2001; Cai et al. 2002; Jones et al. 2009a, b, 2015). They are also pathogens of plants, arthropods and mammals (Sung et al. 2007; Maharachchikumbura et al. 2012, 2015; Hyde et al. 2014, 2016) and have been isolated as endophytes from various plants (Guo et al. 2001;

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Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species

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Abstract
This is the sixth in a series of papers where we bring collaborating mycologists together to produce a set of notes of several taxa of fungi. In this study we introduce a new family *Fuscostagonosporaceae* in Dothideomycetes. We also introduce the new ascomycete genera *Acericola*, *Castellaniomyces*, *Dictyosporina* and *Longitudinalis* and new species *Acericola italica*, *Alternariaster trigonosporus*, *Amarenomyces dactylidis*, *Angustimassarina coryli*, *Astrocystis bambusicola*, *Castellaniomyces rosae*, *Chaetothyrina artocarpi*, *Chlamydotubeufia krabiensis*, *Colletotrichum lauri*, *Collodiscula chiangraiensis*, *Curvularia palmicola*, *Cytospora mali-sylvestris*, *Dictyocheirospora cheirospora*, *Dictyosporina ferruginea*, *Dothiora coronillae*, *Dothiora spartii*, *Dyfrolomyces phetchaburiensis*, *Epicoccum cedri*, *Epicoccum pruni*, *Fasciatispora calami*, *Fuscostagonospora cytisi*, *Grandibotrys hyalinus*, *Hermatomyces nabanheensis*, *Hongkongmyces thailandica*, *Hysterium rhizophorae*, *Jahnula guttulaspora*, *Kirschsteiniothelia rostrata*, *Koorchalomella salmonispora*, *Longitudinalis nabanheensis*, *Lophium zalerioides*, *Magnibotryascoma mali*, *Meliola clerodendri-infortunati*, *Microthyrium chinense*, *Neodidymelliopsis moricola*, *Neophaeocryptopus spartii*, *Nigrograna thymi*, *Ophiocordyceps cossidarum*, *Ophiocordyceps issidarum*, *Ophiosimulans plantaginis*, *Otidea pruinosa*, *Otidea stipitata*, *Paucispora kunmingense*, *Phaeoisaria microspora*, *Pleurothecium floriforme*, *Poaceascoma halophila*, *Periconia aquatica*, *Periconia submersa*, *Phaeosphaeria acaciae*, *Phaeopoacea muriformis*, *Pseudopithomyces kunmingnensis*, *Ramgea ozimecii*, *Sardiniella celtidis*, *Seimatosporium italicum*, *Setoseptoria scirpi*, *Torula gaodangensis* and *Vamsapriya breviconidiophora*. We also provide an amended account of *Rhytidhysteron* to include apothecial ascomata and a J+ hymenium. The type species of *Ascotrichella*

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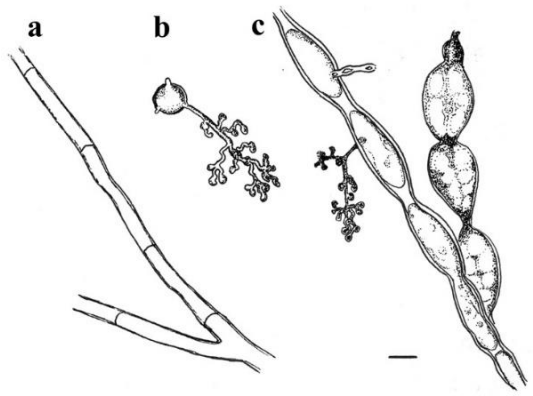

Fig. 161 Three types of hyphae. **a** Aerial hypha. **b** Substrate hypha emerging from a gemma. **c** Superficial hyphae with rhizoid-like branches. Scale bars 10 μm

sequence to any previously described taxa in this group is low: BS = 85% to *M. beljakovae*, BS = 85% to *M. paraensis*, BS = 83% to *M. gemmifera* and BS = 82% to *M. kuhlmanii*. The ITS sequence of *M. formicae* was the most similar (BS = 93%) to *Mortierella* strain CBS 109589. Its sequences were generated by Wagner et al. (2013) for phylogenetic studies and the name *M. formicicola* D.S. Clark & W. Gams was used for it in this paper. However, the species is not validly published and its morphological characteristic is not available. Nonetheless the strain CBS 109589 was isolated from infrabuccal pellet of *Camponotus pensylvanicus*. Although the host species of these two *Mortierella* species is different (*C. pensylvanicus* for *Mortierella* CBS 109589 and *Formica pratensis* for *M. formicae*), they form together well delimited ant-associated clade. All *Mortierella* strains marked in Fig. 159 with ant symbol were obtained from insects belonging to subfamily Formicinae, however the ants from genera *Camponotus* and *Formica* occupy different ecological niches.

The species is morphologically simple, without any sexual and asexual reproductive forms. The characteristic clusters of gemmae are often observed in some *Mortierella* species e.g. *M. zychae*, *M. parazychae*, *M. beljakovae*, *M. kuchlmanii* (Khalabuda 1973; Gams 1976; Domsch et al. 1993). Sometimes they are named chlamydospores (Gams 1976; Watanabe 2002). This term is inadequate, because only some gemmae, as well some hyphal segments develop thick cell wall and function as chlamydospores. Most of gemmae are rather a place of intensive metabolism. They are filled with oil drops, and they characterized by thin cell walls. Ogawa et al. (2012) show that some *Mortierella* strains could be used as a producers of various polyunsaturated fatty acids (PUFAs). The presence of endosymbiotic bacteria, and their metabolic activity in mycelium of *Mortierella* genus representatives were proven by several authors (Fujimura et al. 2014; Li et al. 2017b; Uehling et al. 2017). Gemmae of *M. formicae* are similar to those formed by *M. zychae, M. beljakovae* and *M. calciphila.* All of them form clusters. The commonly produced gemmae of the fourth closely related species *M. paraensis* are arranged in chains or are produced solitary. The unique character of *M. formicae* is formation of dense and thick layer or gemmae. Some of them are closely packed forming crust-like structure as it is shown in Fig. 160. Gemmae's dimensions of *M. calciphila* and *M. formicae* are similar. They reach usually approximately 18 μm. The gemmae of *M. beljakovae* are much bigger 20–45 (–60) μm, while gemmae of *M. zychae* and *M. paraensis* are rather smaller. In *M. calciphila* the cross walls are observed sporadically, while in *M. formicae,* they are regular, especially in aerial mycelium. The gemmae of *M. formicae* (as well as these of *M. calciphila* and *M. parazychae*) are usually completely rounded. Contrary in *M. zychae* the gemmae outline merges gradually into the connecting hyphal parts (Gams 1976). *Mortierella formicae* superficial hyphae segments sometimes are converted into elongated chlamydospores with oil drop and thick wall (Fig. 160). We put forward the hypothesis that gemmae are source of nutrition rich in fatty acids and sterols for insects.

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The ranking of fungi: a tribute to David L. Hawksworth on his 70th birthday

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Abstract The history of assigning ranks to fungi, as well as the relative importance of using divergence time estimates is reviewed. The paper pays tribute to the major mycological players, and especially to David Hawksworth on his 70th birthday and his contribution to fungal ranking in *Systema Ascomycetum* from 1982 to 1998. Following the conclusion of the latter series, the ranking continued with the *Outlines of Ascomycota* in 2007 and 2010 and more recently with specific classes in 'Towards an outline of Sordariomycetes' and 'Families of Dothideomycetes'. Earlier classifications based on phenotype were certainly more subjective; however, remarkably many of these old arrangements have stood the test of time. More recently, phylogenetic analyses have provided evidence towards a natural classification, resulting in significant changes in many lineages. The classification arrangements however, are still subjective and dependent on the taxa analysed, resulting in different taxonomic interpretations and schemes, particularly when it comes to ranking. Thus, what have been considered as genera by some, have been introduced as families by others. More recently, estimation of divergence times using molecular clock methods have been used as objective evidence for higher ranking of taxa. A divergence period (i.e. 200–300 MYA) can be used as a criterion to infer when a group of related taxa evolved and what rank they should be given. We compiled data on divergence times for various higher ranking taxa in the Kingdom Fungi. The kingdom evolved 1000–1600 MYA (Stenian–Calymmian), while the presently accepted phyla evolved between 358 and 541 MYA (Devonian–Cambrian). Divergence times for subphyla are generally between 358 and 485 MYA (Devonian–Ordovician), those of classes 145–358 MYA (Jurassic–Carboniferous),

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outcomes may be misleading, as has been shown in this case study on Sordariomycetes. It is very important that phylogenies are stable before any credence can be given to the applicability of divergence time estimations in ranking fungi. In addition, multi-gene analyses with a large number of genes and species will reduce statistical error in divergence time estimations (Kumar and Hedges 1998). If a lineage is poorly represented the crown age will be younger than that which should be expected. The age of the common ancestor (stem age) based proposed ranking is well suited for monophyletic clades, but provides unstable and doubtful divergences for ranking. Thus, uncertainty and misleading phylogenetic classification may lead to errors in divergence time estimations (Bromham et al. 1999, Bromham & Penny 2003). The other problem is that absolute divergence time estimations are dependent on fossil or secondary calibration. Therefore, new fossil findings, accurate calibration with an acceptable fossil, and possible multiple fossil calibrations are needed to obtain reliable absolute divergence times (Gandolfo et al. 2008; van Tuinen and Torres 2015). The proposed common divergence periods for ranking taxa in this study is presently based on few studies. We provide recommendations for ranking taxa with evidence for divergence times in Box 1.

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Ranking higher taxa using divergence times: a case study in Dothideomycetes

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Abstract The current classification system for the recognition of taxonomic ranks among fungi, especially at high-ranking level, is subjective. With the development of molecular approaches and the availability of fossil calibration data, the use of divergence times as a universally standardized criterion for ranking taxa has now become possible. We can therefore date the origin of Ascomycota lineages by using molecular clock methods and establish the divergence times for the orders and families of Dothideomycetes. We chose Dothideomycetes, the largest class of the phylum Ascomycota, which contains 32 orders, to establish ages at which points orders have split; and *Pleosporales*, the largest order of Dothideomycetes with 55 families, to establish family divergence times. We have assembled a multi-gene data set (LSU, SSU, TEF1 and RPB2) from 391 taxa representing most family groups of Dothideomycetes and utilized fossil calibration points solely from within the ascomycetes and a Bayesian approach to establish divergence times of Dothideomycetes lineages. Two separated datasets were analysed: (i) 272 taxa representing 32 orders of Dothideomycetes were included for the order level analysis, and (ii) 191 taxa representing 55 families of *Pleosporales* were included for the family level analysis. Our results indicate that divergence times (crown age) for most orders (20 out of 32, or 63%) are between 100 and 220 Mya, while divergence times for most families (39 out of 55, or 71%) are between 20 and 100 Mya. We believe that divergence times can provide additional evidence to support establishment of higher level taxa, such as families, orders and classes. Taking advantage of this added approach, we can strive towards establishing a standardized taxonomic system both within and outside Fungi. In this study we found that molecular dating coupled with phylogenetic inferences provides no support for the taxonomic status of two currently recognized orders, namely *Bezerromycetales* and *Wiesneriomycetales* and these are treated as synonyms of

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Table 2 continued

Family	Divergence times (crown age)	Divergence times (stem age)
Tetraplosphaeriaceae	66 (33–107)	121 (79–163)
Thyridariaceae	15 (3–38)	95 (60–138)
Torulaceae	15 (4–34)	140 (95–188)
Trematosphaeriaceae	65 (34–92)	88 (60–122)
Wicklowiaceae	2 (0–5)	91 (38–158)
Zopfiaceae	–	–

For each divergence, the median and the 95% highest posterior density (HPD) are provided. Divergence times are provided in millions of years (Mya)

Conclusions

We provide four examples where divergence times do not support the status of families and orders. Liu et al. (2016) also discussed the distinction of three genera as distinct families in *Botryosphaeriales* which was not supported in the MCC trees. These are examples where the distinctiveness of introduced higher taxa using phylogenies should be reinforced by further evidence e.g. divergence times.

Molecular clocks calibrated using fossils are important tools in estimating the timing of evolutionary events in fossil-poor groups. However, when fossil evidence is limited and there are considerable differences in substitution rates change between lineages, it is difficult to establish reliable divergence time estimates. The status of fossils and analysis, an awareness of the phylogeny, fossils and the clock will help to align expectations for fungal evolution. However, it will be many years before we can obtain natural classification for fungi. As more data and approaches become available and fossil and phylogenetic evidence becomes more reliable, we can obtain a better-characterized divergence dating with few limitations.

The major contribution of this paper is an updated phylogeny of the class Dothideomycetes to order level and the order *Pleosporales* to the family level. The addition of the maximum clade credibility (MCC) tree to support the phylogenetic conclusions show that some orders and some families are not supported and are therefore synonymized. A major conclusion is that any inference from phylogenetic trees depends on the taxa used. For example, Hyde et al. (2016) reported strain MFLUCC 15-1248 as a collection of *Neoacanthostigma septoconstrictum* as it had strong support in their phylogenetic tree. However, with the addition of ten extra strains in the genus, this strain clustered with a new species, *N. brownispora* with good support and the strain was renamed. Therefore, any interpretation of phylogenetic trees should be treated with caution as it depends entirely on the taxa chosen to build it. We predict that clade ages will improve the definitions (ranking) of higher taxa and understanding of phylogenetic relationships. Given that the rates of molecular evolution vary with the molecular markers used (ribosomal versus protein coding ones), future studies can show how this can affect calibration as well as estimate evolutionary rates for specific genes and their impact on dating. The results obtained in phylogenetic and molecular clock studies are currently the best hypotheses using present methodologies and data; however, additional data, taxa and/or new methodologies, may result in modified conclusions.

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Taxonomic position of *Melomastia italica sp. nov.* and phylogenetic reappraisal of Dyfrolomycetales

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Abstract – *Melomastia* is a genus of saprobic fungal species found on wood, with 29 species epithets listed in Index Fungorum. The classification of species in the genus has been a challenge due to a high degree of morphological overlap and a lack of DNA based phylogenies. The present study clarifies the phylogenetic placement of the genus and with an additional new species based on a fresh collection from Italy. The new species, *Melomastia italica*, is described based on morphological and relationships inferred from phylogenetic analyses of SSU and LSU sequence data. *Melomastia* is accommodated within the family *Pleurotremaceae* in the class Dothideomycetes. The phylogenetic relationships and intergeneric taxonomy within the family *Pleurotremaceae* are revisited, while *Dyfrolomyces maolanensis* is transferred to the genus *Melomastia*.

Genera *incertae sedis* / Dothideomycetes / Molecular analyses/ Morphology / Saprobic fungi

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strongly supported and consistent even when the same DNA dataset was subjected to different types of analyses (Figs 1, 2, 3). From a morphological perspective, all of these genera are characterized by thick-walled, non fissitunicate asci, and uniseriate, hyaline ascospores which are constricted at the septum, with a gelatinous sheath, which are typical of the *Pleurotremaceae*. In all phylogenies, all *Acrospermum* species constitute a well-supported independent lineage, which supports its generic rank in the family *Acrospermaceae*, sister to the *Pleurotremaceae*. In addition, results herein confirm that *Melomastia* should be accommodated in *Pleurotremaceae* (Barr, 1994; Hyde, 1992; Kang *et al.*, 1999; Lumbsch & Huhndorf, 2010; Maharachchikumbura *et al.*, 2015, 2016; Hyde *et al.*, 2016).

Our molecular study revealed interesting taxonomic relationships of the genus *Melomastia*. Both MP and BA derived phylogenies are congruent and indicate that *Melomastia* should be given generic status. Our species sampled herein cluster together with high support and are nested in between *Dyfrolomyces* species (Figs 2, 3). Analyses of the individual LSU datas*et als*o yielded similar topologies (results not shown). The only discrepancy we noted with respect to *Melomastia* was the placement of the two *Melomastia* species in the ML analyses (Fig 1). *Melomastia italica* segregates in an independent lineage with high support and a similar scenario is observed for *Dyfrolomyces maolanensis* which is nestled between *Melomastia italica* and other *Dyfrolomyces* species, albeit with weak support (Fig 1). Either this depicts a paraphyletic nature of *Melomastia* or simply an artifact during phylogenetic analyses. However, this anomaly reinforces our taxonomic assumption that *Melomastia italica* is a new taxon given its distinct phylogeny. On the other hand, it is worth mentioning that analyses of the LSU gene region only recovered similar phylogeny as the combined dataset (results not shown) and hence we rely mostly on the MP and BA for further taxonomic discussion. Despite a close affinity between *Dyfrolomyces maolanensis* and *Melomastia italica* in other analyses, we consider that *Melomastia italica* is a novel species given its distinct morphological features as compared to *Dyfrolomyces maolanensis*. The latter possesses, *Melomastia italica* is characterized by 2-septate, hyaline and contents granular of ascospores. In addition, comparison of the LSU nucleotides sequenced revealed striking differences in 16 base pairs that justifies it as a new taxon (Jeewon & Hyde, 2016).

There have also been some taxonomic irregularities with respect to the generic status of *Dyfrolomyces, Melomastia* and *Pleurotrema*. In fact, if we take a conservative approach we could combine *Dyfrolomyces* and *Pleurotrema* under the earlier *Melomastia.* However, despite some morphological overlap, our phylogeny supplemented with accurate morphological definitions, indicate that these should be considered as distinct genera. While our molecular data clearly indicates that *Dyfrolomyces* are phylogenetically apart, they are not significantly distinct in morphological characteristics such as septate, variously size ascospores (Fig. 6). However, we predict that many more taxa in these genera will be found and they will be supported as distinct in both phylogeny and morphology. Further studies with more *Pleurotremaceae* collections, especially of *Pleurotrema polysemum* are essential to resolve taxonomic relationships within this family.

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Towards a natural classification of *Ophiobolus* and ophiobolus-like taxa; introducing three novel genera *Ophiobolopsis*, *Paraophiobolus* and *Pseudoophiobolus* in *Phaeosphaeriaceae* (Pleosporales)

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Abstract
Ophiobolus is a large genus of *Phaeosphaeriaceae* comprising more than 350 possible species, most of which are saprobes on herbaceous plants in Europe and North America. *Ophiobolus* species are polyphyletic and the type of *Ophiobolus* is not represented in GenBank. Therefore, an increased taxon sampling of ophiobolus-like taxa and epitypification of the type species, *O. disseminans* is reported. Multigene phylogenetic analyses of combined LSU, SSU, TEF1-α and ITS sequence data position *O. disseminans* in a sister clade with *O. ponticus* and several *Entodesmium* species in *Phaeosphaeriaceae* with high support. Therefore, *Entodesmium* is synonymized under *Ophiobolus*. *Premilcurensis* with it type species, *P. senecionis* also clusters within the *Ophiobolus* clade and is synonymized under *Ophiobolus*. *Ophiobolus rossicus* sp. nov. is introduced and a reference specimen is designated for *O. ponticus*. Other ophiobolus-like taxa (*Ophiobolus sensu lato*) can be distinguished as three main groups, which are introduced as new genera. *Ophiobolopsis* is introduced to accommodate the new species, *Ophiobolopsis italica*. The new genus *Paraophiobolus* is introduced to accommodate *P. arundinis* sp. nov. and *P. plantaginis* comb. nov. This genus is characterized by hyaline to pale yellowish ascospores, some green-yellowish at maturity, with a swollen cell, terminal appendages and ascospores not separating into part spores. *Pseudoophiobolus* gen. nov. is introduced to accommodate six new species and two new combinations, viz. *Ps. achilleae*, *Ps. erythrosporus*, *Ps. galii*, *Ps. italicus*, *Ps. mathieui*, *Ps. rosae*, *Ps. subhyalinisporus* and *Ps. urticicola*. *Pseudoophiobolus* is characterized by subhyaline to pale yellowish or yellowish ascospores, with a swollen cell, lack of terminal appendages and ascospores that do not separate into part spores and is related to *Nodulosphaeria*. An updated tree for *Phaeosphaeriaceae* based on multigene analysis is also provided.

Keywords 7 new combinations · 11 new taxa · Dothideomycetes · Epitypification

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Studies in Mycology

Families of *Diaporthales* based on morphological and phylogenetic evidence

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Abstract: *Diaporthales* is an important ascomycetous order comprising phytopathogenic, saprobic, and endophytic fungi, but interfamilial taxonomic relationships are still ambiguous. Despite its cosmopolitan distribution and high diversity with distinctive morphologies, this order has received relativelyiaceae, *Macrohilaceae*, *Melanconidaceae*, *Pseudoplagiostomaceae*, *Schizoparmaceae*, *Stilbosporaceae* and *Sydowiellaceae*. Taxonomic uncertainties among genera are also clarified and recurrent discrepancies in the taxonomic position of families within the *Diaporthales* are discussed. An updated outline and key to families and genera of the order is presented.

Key words: Multi-gene DNA phylogeny, New taxonomic arrangement, Phytopathogenic fungi, Sordariomycetes, Systematics.

Taxonomic novelties: New families: *Apiosporopsidaceae* Senan. Maharachch. & K.D. Hyde, *Apoharknessiaceae* Senan. Maharachch. & K.D. Hyde, *Asterosporiaceae* Senan. Maharachch. & K.D. Hyde, *Auratiopycnidiellaceae* Senan. Maharachch. & K.D. Hyde, *Erythrogloeaceae* Senan. Maharachch. & K.D. Hyde, *Melanconiellaceae* Senan. Maharachch. & K.D. Hyde, *Prosopidicolaceae* Senan. & K.D. Hyde; **New genera:** *Marsupiomyces* Senan. & K.D. Hyde, *Microascospora* Senan., Camporesi & K.D. Hyde, *Phaeoappendicospora* Senan., Q.R. Li & K.D. Hyde, *Paradiaporthe* Senan., Camporesi, & K.D. Hyde, *Hyaliappendispora* Senan., Camporesi & K.D. Hyde, *Chiangraiomyces* Senan. & K.D. Hyde; **New species:** *Chiangraiomyces bauhiniae* Senan. & K.D. Hyde, *Coniella pseudokoreana* Senan., Tangthir. & K.D. Hyde, *Cytospora centrivillosa* Senan., Camporesi & K.D. Hyde, *Cytospora junipericola* Senan., Camporesi & K.D. Hyde, *Cytospora quercicola* Senan., Camporesi & K.D. Hyde, *Cytospora rosae* Senan., Camporesi & K.D. Hyde, *Cytospora fraxinigena* Senan., Camporesi & K.D. Hyde, *Diaporthe litoricola* Senan., E.B.G. Jones & K.D. Hyde, *Ditopella biseptata* R.H. Perera, Senan., Camporesi & K.D. Hyde, *Gnomoniopsis agrimoniae* Senan., Camporesi & K.D. Hyde, *Hyaliappendispora galii* Senan., Camporesi & K.D. Hyde, *Marsupiomyces epidermoidea* R.H. Perera, Senan., Bulgakov & K.D. Hyde, *Marsupiomyces quercina* Senan., Camporesi & K.D. Hyde, *Melanconis italica* Senan., Camporesi & K.D. Hyde, *Microascospora rubi* Senan., Camporesi & K.D. Hyde, *Paradiaporthe artemisiae* Senan., Camporesi & K.D. Hyde, *Phaeoappendicospora thailandensis* Senan., Q.R. Li & K.D. Hyde, *Plagiostoma jonesii* Senan., & K.D. Hyde, *Plagiostoma salicicola* Senan., Camporesi & K.D. Hyde, *Sydowiella urticicola* Senan., Camporesi & K.D. Hyde, *Tubakia thailandensis* Senan., Tangthir., K.D. Hyde; **New combinations:** *Coryneum arausiaca* (Fabre) Senan., Maharachch. & K.D. Hyde, *Microascospora fragariae* (F. Stevens & Peterson) Senan., Maharachch. & K.D. Hyde.

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INTRODUCTION

The *Diaporthales* is a distinct order in the subclass *Diaporthomycetidae* (*Sordariomycetes*) and it includes pathogens, saprobes and endophytes, with no known coprophilous, hypersaprobes or mycophylic species (Barr 1978, Rossman *et al.* 2007, Vasilyeva *et al.* 2007, Maharachchikumbura *et al.* 2015, 2016). Taxa of this order inhabit a wide diversity of hosts and substrates, including most economically and ecologically important trees and crops, soil and living animal and human tissues (Barr 1978, Gryzenhout *et al.* 2006c). Species in *Diaporthales* form solitary or aggregated, immersed to erumpent, rarely superficial, orange, brown to black perithecial ascomata, with short or long necks, that are located in stromatic tissues or substrates, with a centrum (or hamathecium) lacking or with few paraphyses (Alexopoulos & Mims 1978, Barr 1978, Castlebury *et al.* 2002). Asci are unitunicate with a conspicuous refractive ring (Hawksworth *et al.* 1995, Rossman *et al.* 2007). Ascospore morphology is diverse, ranging from short to elongate and aseptate or septate with hyaline or pigmented walls. The asexual morphs of *Diaporthales* are generally coelomycetous (Rossman *et al.* 2007), producing acervuli or pycnidial conidiomata, with or without a well-developed stroma. Conidiogenesis is phialidic or rarely annellidic and conidia are usually unicellular or 1-septate (Rossman *et al.* 2007).

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(Continued).

Sirococcus Preuss
Spataporthe Bronson *et al.*
Uniseta Ciccar
Valsalnicola D.M. Walker & Rossman
Harknessiaceae Crous
Dwiroopa Subram. & Muthumary
Harknessia Cooke
Juglanconidaceae Voglmayr & Jaklitsch
Juglanconis Voglmayr & Jaklitsch
Lamproconiaceae C. Norphanphoun *et al.*
Hercospora Fr.
Lamproconium (Grove) Grove
Macrohilaceae Crous
Macrohilum H.J. Swart
Melanconidaceae G. Winter
Melanconis Tul. & C. Tul.
Melanconiellaceae Senan. *et al.*
Dicarpella Syd.
Greeneria Scribn. & Viala
Melanconiella Sacc.
Microascospora Senan. & K.D. Hyde
Tubakia B. Sutton
Prosopidicolaceae Senan. & K.D. Hyde
Prosopidicola Crous & C.L. Lennox
Pseudoplagiostomataceae Cheew. *et al.*
Pseudoplagiostoma Cheew. *et al.*
Schizoparmaceae Rossman DF *et al.*
Coniella Höhn.
Stilbosporaceae Link
Crinitospora B. Sutton & Alcorn
Stegonsporium Corda
Stilbospora Pers.
Sydowiellaceae Lar.N. Vassiljeva
Alborbis Senan. & K.D. Hyde
Breviappendix Senan. & K.D. Hyde
Cainiella E. Müll
Calosporella J. Schröt
Chapeckia M.E. Barr
Italiomyces Senan. *et al.*
Hapalocystis Auersw. ex Fuckel
Lambro Racib.
Paragnomonia Senan. & K.D. Hyde
Ranulospora Senan. *et al.*
Rossmania Lar.N. Vassiljeva
Sillia P. Karst.
Sydowiella Petr.
Tenuiappendicula Senan. *et al.*
Tortilispora (Sacc.) Senan. & K.D. Hyde

Diaporthales* genera *incertae sedis

Anisomycopsis I. Hino & Katum.

(*continued on next page*)

(Continued).

Caudospora Starbäck
Chadefaudiomyces Kamat *et al.*
Cryptascoma Ananthap.
Cryptoleptosphaeria Petr.
Cytomelanconis Naumov
Dictyoporthe Petr.
Ditopellina J. Reid & C. Booth
Durispora K.D. Hyde
Fremineavia Nieuwl.
Hypodermina Höhn.
Hypophloeda K.D. Hyde & E.B.G. Jones
Kapooria J. Reid & C. Booth
Keinstirschia J. Reid & C. Booth
Lollipopaia Inderbitzin
Macrodiaporthe Petr.
Maculatipalma J. Fröhlich & K.D. Hyde
Massariovalsa Sacc.
Mebarria J. Reid & C. Booth
Melanamphora Lafl.
Melanconiopsis Ellis & Everh.
Natarajania Pratibha & Bhat
Phaeoappendicospora Senan. *et al.*
Phragmodiaporthe Wehm.
Plagiophiale Petr.
Plagiostigme Syd.
Prostratus Sivan. *et al.*
Pseudocryptosporella J. Reid & C. Booth
Pseudothis Theiss. & Syd.
Pseudovalsella Höhn.
Rabenhorstia Fr.
Savulescua Petr.
Skottsbergiella Petr.
Stioclettia Dennis
Trematovalsa Jacobesco
Uleoporthe Petr.
Vismaya V.V. Sarma & K.D. Hyde
Wehmeyera J. Reid & C. Booth
Wuestneia Auersw. ex Fuckel
Wuestneiopsis J. Reid & Dowsett

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Novel taxa of Diatrypaceae from Para rubber (*Hevea brasiliensis*) in northern Thailand; introducing a novel genus *Allocryptovalsa*

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Abstract

Species of Diatrypaceae are widespread on dead wood of plants worldwide. The delineation of this family is rather problematic because the characters of ascostromata are extremely variable and the names of taxa with sequence data are often misleading. In this paper, species of Diatrypaceae were collected from Para rubber in northern Thailand for examination and illustrations. Based on morphological characteristics and phylogenetic analyses, a new genus, *Allocryptovalsa*, is introduced to accommodate a new species *A. polyspora* and two species, *A. cryptovalsoidea* and *A. rabenhorstii* are transferred to the new genus. The new species, *Diatrypella heveae* and *Peroneutypa longiasca* are also introduced in this paper. Phylogenetic analyses of combined ITS and β-tubulin sequence data show their phylogenetic affinities in Diatrypaceae. Our study also shows that phylogenetic analyses of taxa of Diatrypaceae are highly confused as some genera are shown to be polyphyletic.

Key words – Phylogeny – Sordariomycetes – taxonomy – unitunicate fungi – Xylariales

Introduction

The family Diatrypaceae (Xylariales, Sordariomycetes) has a widespread distribution on variety of plants worldwide, comprising 16 genera and more than 1500 species (Trouillas et al. 2011, Maharachchikumbura et al. 2015, 2016, Mehrabi et al. 2015, Senanayake et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016, Shang et al. 2017). Species of this family are mostly saprobes inhibiting wood and bark of various angiosperms and some species (e.g. *Eutypa leptoplaca*, *Eutypella lata*, *E. microtheca*, *Cryptosphaeria pullmanensis*, *Cryptovalsa ampelina*, and *Diatrypella vulgaris*) have been reported as pathogens and/or endophytes (Acero et al. 2004, Trouillas et al. 2004, 2010, 2011, Trouillas & Gubler 2010, 2016, Grassi et al. 2014, Paolinelli-Alfonso et al. 2015, Mehrabi et al. 2016, Shang et al. 2017).

Taxa in Diatrypaceae are characterized by perithecial ascomata, with poor or well-developed ascostromata, immersed to erumpent in the host substrates, with ostiolate, and papillate ascomata,

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Shang et al. (2017) mentioned that the pedicellate character might be significant to determine the species in this genus. In this study, the absence of paraphyses and conspicuous sessile asci may not be significant characters to distinguish the genus from other genera in Diatrypaceae.

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Central America, the Caribbean, parts of North America, sub-Saharan Africa, southern and eastern Asia, Australia, New Zealand, and the Pacific Islands. This is the first report of *T. fuciformis* with the molecular phylogenetic confirmation from Sri Lanka.

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Phylogenetic revision of *Camarosporium* (*Pleosporineae*, *Dothideomycetes*) and allied genera

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Abstract: A concatenated dataset of LSU, SSU, ITS and *tef1* DNA sequence data was analysed to investigate the taxonomic position and phylogenetic relationships of the genus *Camarosporium* in *Pleosporineae* (*Dothideomycetes*). Newly generated sequences from camarosporium-like taxa collected from Europe (Italy) and Russia form a well-supported monophyletic clade within *Pleosporineae*. A new genus *Camarosporidiella* and a new family *Camarosporidiellaceae* are established to accommodate these taxa. Four new species, *Neocamarosporium korfii*, *N. lamiacearum*, *N. salicomiicola* and *N. salsolae*, constitute a strongly supported clade with several known taxa for which the new family, *Neocamarosporiaceae*, is introduced. The genus *Staurosphaeria* based on *S. lycii* is resurrected and epitypified, and shown to accommodate the recently introduced genus *Hazslinszkyomyces* in *Coniothyriaceae* with significant statistical support. *Camarosporium quaternatum*, the type species of *Camarosporium* and *Camarosporomyces flavigena* cluster together in a monophyletic clade with significant statistical support and sister to the *Leptosphaeriaceae*. To better resolve interfamilial/intergeneric level relationships and improve taxonomic understanding within *Pleosporineae*, we validate *Camarosporiaceae* to accommodate *Camarosporium* and *Camarosporomyces*. The latter taxa along with other species are described in this study.

Key words: Multigene phylogeny, Muriformly septate, Pleomorphism, *Pleosporales*, Taxonomy.

Taxonomic novelties: New families: *Camarosporiaceae* Wanas., K.D. Hyde & Crous, *Camarosporidiellaceae* Wanas., Wijayaw., Crous & K.D. Hyde, *Neocamarosporiaceae* Wanas., Wijayaw., Crous & K.D. Hyde; **New genus:** *Camarosporidiella* Wanas., Wijayaw. & K.D. Hyde; **New species:** *Camarosporidiella elaeagnicola* Wanas., Bulgakov & K.D. Hyde, *Ca. eufemiana* Wanas., Camporesi & K.D. Hyde, *Ca. halimodendri* Wanas., Bulgakov & K.D. Hyde, *Ca. italica* Wanas., Camporesi & K.D. Hyde, *Ca. mackenziei* Wanas., Bulgakov & K.D. Hyde, *Ca. melnikii* Wanas., Bulgakov & K.D. Hyde, *Ca. mirabellensis* Wanas., Camporesi & K.D. Hyde, *Ca. premilcurensis* Wanas., Camporesi & K.D. Hyde, *Ca. schulzeri* Wanas., Bulgakov & K.D. Hyde, *Staurosphaeria rhamnicola* Wanas., Yu. Sh. Gafforov & K.D. Hyde, *Neocamarosporium korfii* Wanas., E.B.G. Jones & K.D. Hyde, *N. lamiacearum* Dayar., E.B.G. Jones & K.D. Hyde, *N. salicorniicola* Dayarathne, E.B.G. Jones & K.D. Hyde, *N. salsolae* Wanas., Gafforov & K.D. Hyde; **New combinations:** *Camarosporidiella caraganicola* (Phukhams. *et al.*) Phukhams., Wanas. & K.D. Hyde, *Ca. aborescentis* (Phukhams. *et al.*) Phukhams., Wanas. & K.D. Hyde, *Ca. arezzoensis* (Tibpromma *et al.*) Wanas. & K.D. Hyde, *Ca. celtidis* (Shear) Thambugala, Wanas. & K.D. Hyde, *Ca. clematidis* (Wijayaw. *et al.*) Wijayaw., Wanas. & K.D. Hyde, *Ca. elongata* (Fr.) Wanas., Wijayaw. & K.D. Hyde, *Ca. laburni* (Pers.) Wanas., Bulgakov, Camporesi & K.D. Hyde, *Ca. laburnicola* (R.H. Perera *et al.*) Wanas. & K.D. Hyde, *Ca. moricola* (Chethana *et al.*) Wanas. & K.D. Hyde, *Ca. robiniicola* (Wijayaw. *et al.*) Wijayaw., Wanas. & K.D. Hyde, *Ca. spartii* (Trail) Wijayaw., Wanas. & K.D. Hyde, *Neocamarosporium chenopodii* (Ellis & Kellerm.) Wanas. & K.D. Hyde, *N. obiones* (Jaap) Wanas. & K.D. Hyde, *Staurosphaeria aloes* (Crous & M.J. Wingf.) Crous, Wanas. & K.D. Hyde; **New name:** *Staurosphaeria lyciicola* (Crous & R.K. Schumach.) Crous, Wanas. & K.D. Hyde; **Epitypification (basionym):** *Staurosphaeria lycii* Rabenh; **Generic abbreviations:** *Camarosporidiella*: *Ca.*, *Camarosporium*: *Cm.*, *Camarosporomyces*: *Cs.* & *Cucurbitaria*: *Cu.*

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INTRODUCTION

Morphological characteristics, cultural studies and host-fungal association have been considered as important aspects in the traditional taxonomy of coelomycetous fungi (Sutton 1980, Sivanesan 1984, Nag Raj 1993, Jeewon *et al.* 2002, 2003b, 2004, Wijayawardene *et al.* 2016). However, morphological plasticity of several coelomycetous genera led to poor generic and species delimitation, often resulting in incorrect taxonomic placement (Jeewon *et al.* 2003a, Shenoy *et al.* 2007, Wijayawardene *et al.* 2016). Proposing new genera (e.g. *Vermisporium*/*Seimatosporium*, *fide* Barber *et al.* 2011) and linking asexual genera with more than one sexual genus (e.g. *Phoma* and *Camarosporium*, *fide* Crous & Groenewald 2017) have resulted in taxonomic controversies among taxonomists and plant-pathologists (Wijayawardene *et al.* 2012a, b, Hyde *et al.*

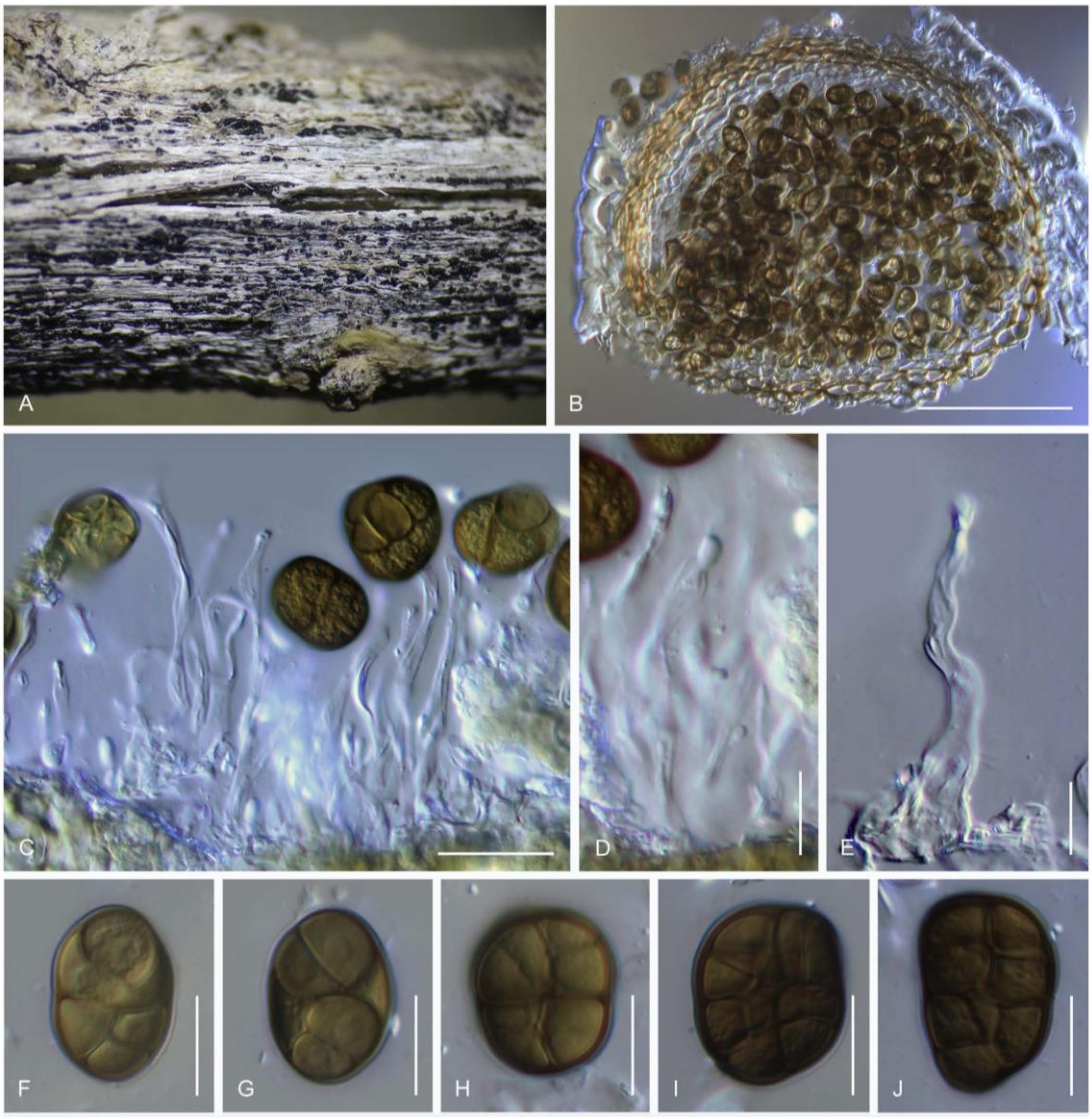

Fig. 26. *Neocamarosporium salsolae* (TASM 6100, holotype). **A.** Appearance of conidiomata on *Salsola* sp. **B.** Vertical section of conidioma. **C–E.** Conidiogenous cells and developing conidia. **F–J.** Conidia. Scale bars: B = 50 µm; C = 20 µm; D–J = 10 µm.

out DNA sequence-based studies. Wijayawardene *et al.* (2016) discussed the importance of re-collecting, and epitypifying of camarosporium-like taxa as currently it has more than 500 epithets in Index Fungorum (2017), and very few of these taxa are presently known from culture.

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ORIGINAL RESEARCH

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Abstract Knowledge of the relationships and thus the classification of fungi, has developed rapidly with increasingly widespread use of molecular techniques, over the past 10–15 years, and continues to accelerate. Several genera have been found to be polyphyletic, and their generic concepts have subsequently been emended. New names have thus been introduced for species which are phylogenetically distinct from the type species of particular genera. The ending of the separate naming of morphs of the same species in 2011, has also caused

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Species Fungorum 2017), *Xanthopyreniaceae*, *Collemopsidiales*, *Dothideomycetes*, 35 species, type: *Z. coepulonus* (Norman) Grube & R. Sant., asexual morph unknown, lichenicolous, terrestrial, worldwide, see Brackel (2008; new species), Diederich and Schultz (2009; new species), Halici and Candan (2009; new species), Roux (2009; new species), van den Boom (2010; new species), Lumbsch and Huhndorf (2010; outline, family placed as *Ascomycota* families *incertae sedis*), Kocakaya et al. (2011; new species), Pérez-Ortega et al. (2011a, 2016; new species, phylogeny), Kirk et al. (2013; genus accepted), Boom and Etayo (2014; new species), holotype of type: O-F72536, cultures available for the type: RO31.

Zygoascus M. T. Smith 1986, ?*Trichomonascaceae*, *Saccharomycetales*, *Saccharomycetes*, six species, type: *Z. hellenicus* M.T. Smith, asexual reproduction is by multilateral budding, saprophytic, but there are some clinical isolates, in soft drinks, fruit juices, insect tunnels, cactus fruits, soil, tanning fluids, clinical isolates, worldwide, see Lumbsch and Huhndorf (2010; outline), Kirk et al. (2013; genus accepted), cultures and sequences are available.

Zygopleurage Boedijn 1962, *Lasiosphaeriaceae*, *Sordariales*, *Sordariomycetes*, type: *Z. zygospora* (Speg.) Boedijn, saprobes, coprophilous, asexual morph phialophora-like, terrestrial, worldwide, see Richardson (2008, taxonomy), Chang et al. (2010, phylogeny), Mungai et al. (2011; taxonomy), Kruys et al. (2015, phylogeny).

Zygosaccharomyces B.T.P. Barker 1901 (= *Zygosaccharis* Clem. & Shear 1931; = *Zygosaccharomycodes* Nishiw. 1929 *fide* Species Fungorum 2017), *Saccharomycetaceae*, *Saccharomycetales*, *Saccharomycetes*, eleven species, type: *Z. rouxii* (Boutroux) Yarrow, asexual morph unknown, saprobes, terrestrial, worldwide, see Lumbsch and Huhndorf (2010; outline), Kirk et al. (2013; genus accepted), cultures and sequences are available.

Zygospermella Cain 1935 (= *Zygospermum* Cain 1934 *fide* Species Fungorum 2017), *Lasiosphaeriaceae*, *Sordariales*, *Sordariomycetes*, six species, type: *Z. setosa* (Cain) Cain, asexual morph unknown, saprobes, terrestrial, worldwide, see Lumbsch and Huhndorf (2010; outline), Kirk et al. (2013; genus accepted), Kruys et al. (2015; DNA, phylogeny), Maharachchikumbura et al. (2015b, 2016b; outline), cultures and sequences are available but lacking for the type.

Zygosporium Mont. 1842 (= *Pimina* Grove 1888; = *Urobasidium* Giesenh. 1893; = *Urophiala* Vuill. 1909 *fide* Species Fungorum 2017), *Ascomycota* genera *incertae sedis*, 16 species, type: *Z. oscheoides* Mont., hyphomycetous, saprobe, terrestrial, widespread, see Hyde et al. (2011; outline), Seifert et al. (2011; morphology), Kirk et al. (2013; genus accepted), Wijayawardene et al. (2012, 2017; outline), cultures and sequences are unavailable for type species.

Zygotorulaspora Kurtzman 2003, *Saccharomycetaceae*, *Saccharomycetales*, *Saccharomycetes*, two species, type: *Z. mrakii* (Capriotti) Kurtzman, asexual reproduction is by multilateral budding, saprophytic, fruit drinks, fruit flies, grape must, silage, worldwide, see Lumbsch and Huhndorf (2010; outline), cultures and sequences are available.

Zymoseptoria Quaedvl. & Crous 2011, *Mycosphaerellaceae*, *Capnodiales*, *Dothideomycetes*, seven species, type: *Z. tritici* (Desm.) Quaedvl. & Crous, coelomycetous, sexual morph mycosphaerella-like, plant pathogens on Poaceae, worldwide, see Quaedvlieg et al. (2011, 2014; morphology, phylogeny), epitype and ex-epitype culture of type: CBS H-20545, IPO 323 = CBS 115943.

Zythia Fr. 1825 (= *Zythia* Fr. 1849 fide Species Fungorum 2017), *Gnomoniaceae*, *Diaporthales*, *Sordariomycetes*, type: *Z. resinae* (Ehrenb.) P. Karst. 1890, coelomycetous, sexual morph unknown, endophytic, saprobes, widespread, see Ding et al. (2010; antimicrobial activity), Hyde et al. (2011; checklist), Wijayawardene et al. (2012, 2017; outline), Kirk et al. (2013; genus accepted), Maharachchikumbura et al. (2015b, 2016b; outline), cultures and sequences are unavailable.

Zyxiphora B. Sutton 1981, *Ascomycota* genera *incertae sedis*, one species, type: *Z. appendiculata* (B. Sutton) Nag Raj, hyphomycetous, sexual morph unknown, saprobes, terrestrial, Asia, see Seifert et al. (2011; morphology), Wijayawardene et al. (2012, 2017; outline), Kirk et al. (2013; genus accepted), cultures and sequences are unavailable, needs generic revision.

Doubtful genera

In Table 2, we listed the genera in *Ascomycota* which have been listed as nom. dub. in Kirk et al. 2008 and have not been included in recent literature.

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Novomicrothelia pandanicola sp. nov., a non-lichenized *Trypetheliaceae* species from *Pandanus*

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Abstract

A non-lichenized *Trypetheliaceae* species was collected and isolated from dead bark of *Pandanus tectorius* in Chanthaburi, Thailand. Morphological features of the fungus places it in the genus *Novomicrothelia*. Phylogenetic analyses of LSU sequence data showed that the fungus clustered together with *Novomicrothelia oleosa* and formed a well-supported basal clade in the family *Trypetheliaceae*. The fungus is phylogenetically distinct from the type species *N. oleosa,* and herein we introduce this taxon as *Novomicrothelia pandanicola* sp. nov. The new species shares similar ascomatal morphology and trabeculate pseudoparaphyses with *N. oleosa*, but differs in its muriform ascospores with a gelatinous sheath. *Novomicrothelia pandanicola* is described, illustrated and notes on its phylogenetic placement are provided.

Key words: Dothideomycetes, Phylogeny, Taxonomy, Trypetheliales

Introduction

Lichenization plays an important role in Ascomycota lifestyles (Lücking *et al.* 2017). Approximately 27% of the known Ascomycota are lichenized (Feuerer & Hawksworth 2007, Grube & Wedin 2016, Lücking *et al.* 2017). *Trypetheliaceae* Eschw. (Eschweiler 1824) is a dominant lichen-forming Dothideomycete family, and it is mostly corticolous or rarely saxicolous, accommodates primarily lichenized and less commonly non-lichenized taxa with a mainly tropical to subtropical and rarely temperate distribution (Harris 1984, Aptroot *et al.* 2008, Nelsen *et al.* 2009, 2011, 2014, Hyde *et al.* 2013, 2016).The family *Trypetheliaceae* is typified by the genus *Trypethelium* Spreng. and currently comprises 17 genera with approximately 421 species (Aptroot & Lücking 2016, Hyde *et al.* 2016, Lücking *et al.* 2017). Phylogenetic analysis places *Trypetheliaceae* together with a lichenicolous family *Polycoccaceae* Ertz, Hafellner & Diederich (Ertz *et al.* 2015) which are assigned to the order Trypetheliales Lücking, Aptroot & Sipman (Aptroot *et al.* 2008) within Dothideomycetes (Del Prado *et al.* 2006, Nelsen *et al.* 2009, 2011, 2014, Hyde *et al.* 2013, Lücking *et al.* 2016, Liu *et al.* 2017). Some taxa, such as *Arthopyrenia* A. Massal. and *Mycomicrothelia* Keissl. (Hawksworth 1985), species initially assigned to other groups were repositioned to this family based on phylogenetic studies (Nelsen *et al.* 2009, 2011, 2014, Hyde *et al.* 2016, Lücking *et al.* 2016).

The genus *Novomicrothelia* Aptroot, M.P. Nelsen & Lücking (Lücking *et al.* 2016) was introduced as a monotypic genus to accommodate a temperate, non-lichenized species, *N. oleosa* (Aptroot) Aptroot, M.P. Nelsen & Lücking. *Novomicrothelia oleosa* (≡ *Mycomicrothelia oleosa* Aptroot) was previously placed in *Mycomicrothelia* in a basal lineage of *Trypetheliaceae*. *Novomicrothelia* is characterized by an ecorticate, usually whitish thallus, solitary, roughly conical, erumpent to prominent, or exposed and black ascomata, apical ostioles, clavate asci, hyaline, clear or interspersed, filaments thin, anastomosing paraphysoids, as well as transversely 1-septate ascospores, with irregular

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Discussion

Novomicrothelia is monotypic with *N. oleosa* as the type, and has no distinct phenotypic difference from *Bogoriella*, but there is no sequence available for the type species *B. decipiens*, we are not able to clarify the phylogenetic relationship between these two genera, it needs further sequences data to solve this. The collection of *Novomicrothelia pandanicola* from Thailand has similar morphological features with *N. oleosa* in having an ecoricate thallus, solitary, erumpent black ascomata and anastomosing pseudoparaphyses, but it can differ from the latter in ascomatal shape, ascospores septation (muriform) and in having a gelatinous sheath.

The phylogenetic analysis showed that *Novomicrothelia pandanicola* and *N. oleosa* formed a monophyletic clade as the basal of *Trypetheliaceae* and is close to the genus *Bogoriella*. There are not many available data and evidences to solve their relationship, we consider that *Novomicrothelia* differs from *Bogoriella* in having muriform ascospores with wall invaginations at an early age, as well as thinner walls. The phylogenetic analyses showed that the two *Novomicrothelia* species are phylogenetically distinct species. Morphologically, the muriform ascospores with gelatinous sheaths of *N. pandanicola* distinguish it from *N. oleosa*, *B. collospora* and *B. decipiens* (Table 2). We presently maintain them as separate genera until additional evidence shows otherwise. Further studies are needed to elucidate the unique morphological characteristics of *Novomicrothelia*.

TABLE 2. Comparisons of *Novomicrothelia pandanicola* collection with similar *Novomicrothelia* and *Bogoriella* species.

		Ascomata		Asci size	Ascospores		Reference
Taxon	Thallus	morphology	size (µm)	(µm)	morphology	size (µm)	
Novomicrothelia pandanicola	ecorticate, non-lichenized, some part bordered by dark prothallus lines	solitary or irregulary confluent, immersed to erumpent, subglobose to flask-shaped, with black pseudoclypeus; apical or rarely eccentric ostioles	490–990 × 190–200	75–137 × 12–20	hyaline, becoming brown with age, ellipsoid to obovoid or fusiform, verruculose, muriform surrounded by irregular, gelatinous sheaths	16–32 × 5–10	This study
Novomicrothelia oleosa	ecorticate	single, roughly conical, erumpent to prominent, or exposed, not in pseudostromata; apical ostioles	/	/	brown, transversely 1-septate, with irregular endospore formation, becoming ornamented, elongated	length ≤28	Lücking *et al.* (2016)
Bogoriella collospora	dark olive-brown, nearly absent	2-10 together in dense groups, exposed and black, with lateral, fused ostioles	/	/	brown, ellipsoid to clavate, rounded ends, granularly, ornamented, 3-septate, surrounded by a hyaline gelatinous sheath	34–38 × 11–13	Aptroot & Lücking (2016)
Bogoriella decipiens	pale brownish to whitish, with dark prothallus lines	solitary, wide fringe, apical ostioles	700–900 × 200	100 × 28	grey (becoming brown with age), smooth, submuriform, 4–8 × (1–) 2–4 loculate, rounded ends	25–35 × 12–15	Aptroot & Lücking (2016)

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ORIGINAL ARTICLE

A novel marine genus, *Halobyssothecium* (Lentitheciaceae) and epitypification of *Halobyssothecium obiones* comb. nov.

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Abstract
A novel marine genus, *Halobyssothecium* (Lentitheciaceae), is introduced to accommodate *Byssothecium obiones* (= *Halobyssothecium obiones*) with evidence from phylogenetic analyses of concatenated LSU, SSU, ITS rDNA, and TEF1 sequence data. We could not locate any type material for this species and there is no molecular data available for the type of this species. Therefore, an epitype is designated for the precise delineation of this taxon. A detailed morphological description and DNA characterization based on LSU, SSU, ITS rDNA, and TEF1 sequence data are provided for the epitype, obtained from *Spartina* culms in Eastney, Langstone Harbour, Hampshire, UK.

Keywords New genus · Marine fungi · *Passeriniella* · *Leptosphaeria* · Phylogeny · *Spartina* · Taxonomy

Introduction

Byssothecium obiones (P. Crouan & H. Crouan) M.E. Barr was introduced by Crouan and Crouan (1867) as *Pleospora obiones* P. Crouan & H. Crouan from *Halimione portulacoides* (L.) Moq. However, the protologue lacks collection details and includes few morphological characters. The protologue of *Pleospora obiones* (Crouan and Crouan 1867) was as follows "*P. obionei* Crn. Mscr. Perithecium de 1 millim., naissant sous l'écorce, noir, conique, à ostiole très-court, thèques étroites subcylindriques à 8 spores jaunâtre, oblongues, subtoruleuses à 3 cloisons. Sur les tiges mortes d'Obione. Pr. r.". There are few other records of *Byssothecium obiones* from the original host *Halimione portulacoides* (Crouan and Crouan 1867; Grove 1933), while this species has been reported mostly from *Spartina* and/or *Phragmites* (Jones 1963; Hyde and Mouzouras 1988; Barr 2002). *Byssothecium obiones* occurring on decaying *Spartina* culm, has been assigned to various other genera viz. Didymosphaeria, *Heptameria*, *Pleospora*, *Leptosphaeria*, and *Passeriniella* (Jones 1962; Kohlmeyer and Kohlmeyer 1979; Hyde and Mouzouras 1988; Khashnobish and Shearer 1996a, 1996b; Barr 2002). Saccardo (1944) synonymized *Pleospora obiones* as *Leptosphaeria obiones* (Crouan & Crouan) Sacc. Subsequently, Apinis and Chester (Apinis and Chesters 1964) transferred it to the genus *Passeriniella*, named as *P. discors* (Sacc. & Ellis) Apinis & Chesters. Hyde and Mouzouras (1988) described ascospore morphologies of *L. obiones* as not typical of *Leptosphaeria* and hence referred it as *Passeriniella obiones*. Based on molecular analysis, Khashnobish and Shearer (1996a, 1996b) showed that *Byssothecium obiones* belonged to neither *Leptosphaeria* nor *Phaeosphaeria*. Barr (2002) assigned the species to *Byssothecium* in *Teichosporaceae* based on its versicolorous ascospores, two dark brown central cells and hyaline terminal

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the same clade as *B. circinans* (type) or within families Massarinaceae or Dacampiaceae but, grouped in Lentitheciaceae. Hence, we constructed a final analysis with Lentitheciaceae representatives and strains of *B. obiones* (08AV2569, 13AV2143, 20AV2566, 27AV2385 and MFLUCC) formed a well-separated (100% ML, 100% MP, 1.00 PP) lineage within Lentitheciaceae (Fig. 1).

Discussion

Halobyssothecium obiones is a widely occurring species in temperate climates (see Kohlmeyer and Kohlmeyer (1979) for geographical distribution) and growing on a wide range of salt marsh halophytes (*Agropyron junceiforme*, *Halimione portulacoides*, *Spartina* spp.) and on intertidal wood and test panels. Its occurrence on wood and bamboo requires further investigation. It is particularly common on *Spartina* species, both in the Atlantic and Pacific Oceans.

Halobyssothecium obiones is characterized by scattered, immersed to semi-immersed ascomata, pedicellate asci, and bi-seriate, pale brown to brown, septate ascospores, which are constricted at the septum (Crouan and Crouan 1867; Jones 1963; Apinis and Chesters 1964; Wagner 1965; Kohlmeyer and Kohlmeyer 1979; Hyde and Mouzouras 1988; Barr 2002; Barata 2002). It has been transferred to a number of different genera, as shown by the synonymies of this species since introduced in 1883 (Suetrong et al. 2009). Only ITS rDNA sequence data were available in GenBank prior to this study. Both the morphological and phylogenetic data available for this species were insufficient to classify the taxon in a stable position (Suetrong et al. 2009). Hence, we recollected this species from *Spartina* culms, on which this species has been abundantly reported and conducted a morphological and molecular study on this marine fungus. Although morphological features have referred it to many genera, such as *Didymosphaeria*, *Heptameria*, *Pleospora*, *Leptosphaeria*, and *Passeriniella*, a detailed phylogenetic study placed it in the Lentitheciaceae with strong statistical support. Within the Lentitheciaceae, *H. obiones* formed a new lineage in a sister group to *Lentithecium* species.

Lack of authentic specimens and molecular data for *Halobyssothecium obiones* has resulted in taxonomic confusion over the past 135 years (Hyde and Mouzouras 1988; Barr 2002; Suetrong et al. 2009). Even though there are several records of this species from different locations, few records of it from the original host *Halimione portulacoides* have been reported (Crouan and Crouan 1883; Grove 1933), while the majority of the collections have been made from different *Spartina* species: *S. alterniflora* (Jones 1963; Gessner and Goos 1973), *S. densiflora* (Peña and Arambarri 1998), and *S. maritima* (Barata 1997, 2002). A Halobyssothecium obiones-like species has been reported with much larger ascospore measurements than that of the type material, namely 38–56 × 16–22 μm (Jones 1963; Cavaliere 1968; Webber 1970), suggesting this may be another species. Likewise, reports of its occurrence from mangrove habitats with smaller ascospores may be due to misidentification (Cribb and Cribb 1960). Therefore, to avoid future taxonomic confusion, we designate our new collection as an epitype for the taxon with DNA characterization with four genes, i.e., LSU, SSU, ITS rDNA, and TEF1. However, Ariyawansa et al. (2014) mentioned that the epitype specimens' designation should probably be in accordance with the same host and origin to the initial material. We believe that it is reasonable to designate a type for this taxon from *Spartina* culms as several isolates of *Byssothecium obiones* from *Spartina* spp. conforms to the morphology originally described and it has been reported in many studies (Jones 1963; Apinis and Chesters 1964; Kohlmeyer and Kohlmeyer 1979; Hyde and Mouzouras 1988; Barr 2002) in accordance with suggestions by Dayarthne et al. (Dayarathne et al. 2016).

Spartina species are abundant grasses of the tidal salt marshes in coastal environments and contribute significantly to estuarine primary productivity (Gessner 1972, Gessner and Goos 1973). Energy stored by the plant is released through decomposition as detritus or decomposer biomass and *Halobyssothecium obiones* has been reported on pieces of wood, driftwood and dead plant stems (5.6% studied substrates) found in the intertidal zone of two sandy beaches on the Portuguese west coast (Gessner 1972; Gessner and Goos 1973; Figueira and Barata 2007).

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Abstract

This is the fifth in a series, *Mycosphere notes*, wherein 50 notes are provided on types of genera and other specimens with descriptions and illustrations. This includes one genus in *Arthoniomycetes*, one genus in *Eurotiomycetes*, 38 genera in *Dothideomycetes*, six genera in *Sordariomycetes*, two genera in *Ascomycota*, families *incertae sedis*, one genus in *Pezizomycotina*, and one taxon, *Angatia rondoniensis*, is treated as a doubtful species. *Pycnocarpon magnificum* is classified in *Asterinaceae*. We reinstate *Eopyrenula* in *Dacampiaceae* on the basis of its

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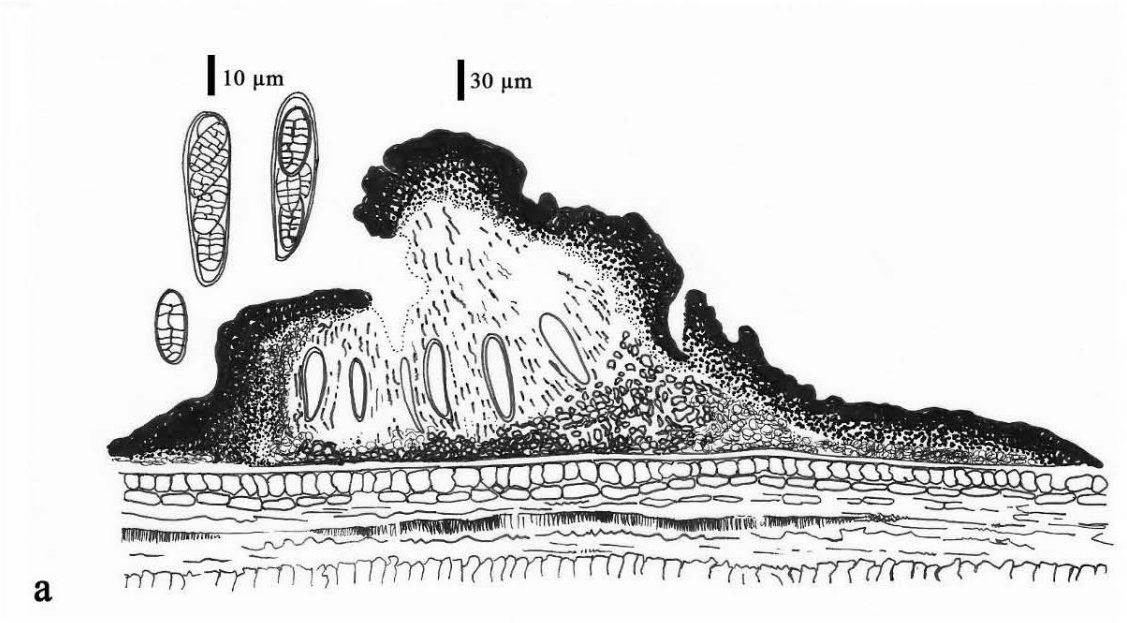

Figure 55 – *Angatia rondoniensis*. a Ascoma, asci and ascospores (redrawn from Batista et al. 1966; Page 79, Fig. 2). Scale bars: a = 30 µm for ascoma, 10 µm for asci and ascospores.

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Mycosphere notes 169–224

Hyde KD[1,2], Chaiwan N[2], Norphanphoun C[2,6], Boonmee S[2], Camporesi E[3,4], Chethana KWT[2,13], Dayarathne MC[1,2], de Silva NI[1,2,8], Dissanayake AJ[2], Ekanayaka AH[2], Hongsanan S[2], Huang SK[1,2,6], Jayasiri SC[1,2], Jayawardena RS[2], Jiang HB[1,2], Karunarathna A[1,2,12], Lin CG[2], Liu JK[7,16], Liu NG[2,15,16], Lu YZ[2,6], Luo ZL[2,11], Maharachchimbura SSN[14], Manawasinghe IS[2,13], Pem D[2], Perera RH[2,16], Phukhamsakda C[2], Samarakoon MC[2,8], Senwanna C[2,12], Shang QJ[2], Tennakoon DS[1,2,17], Thambugala KM[2], Tibpromma, S[2], Wanasinghe DN[1,2], Xiao YP[2,6], Yang J[2,16], Zeng XY[2,6], Zhang JF[2,15], Zhang SN[2,12,16], Bulgakov TS[18], Bhat DJ[20], Cheewangkoon R[12], Goh TK[17], Jones EBG[21], Kang JC[6], Jeewon R[19], Liu ZY[16], Lumyong S[8,9], Kuo CH[17], McKenzie EHC[10], Wen TC[6], Yan JY[13], Zhao Q[2]

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Bayesian posterior probability) (Fig. 93). The morphology of our isolates arein full agreement with the description for *T. angustata* (Sutton 1980). *Truncatella angustata* has a wide distribution and this is the first report of this species from *Alnus glutinosa* from Italy.

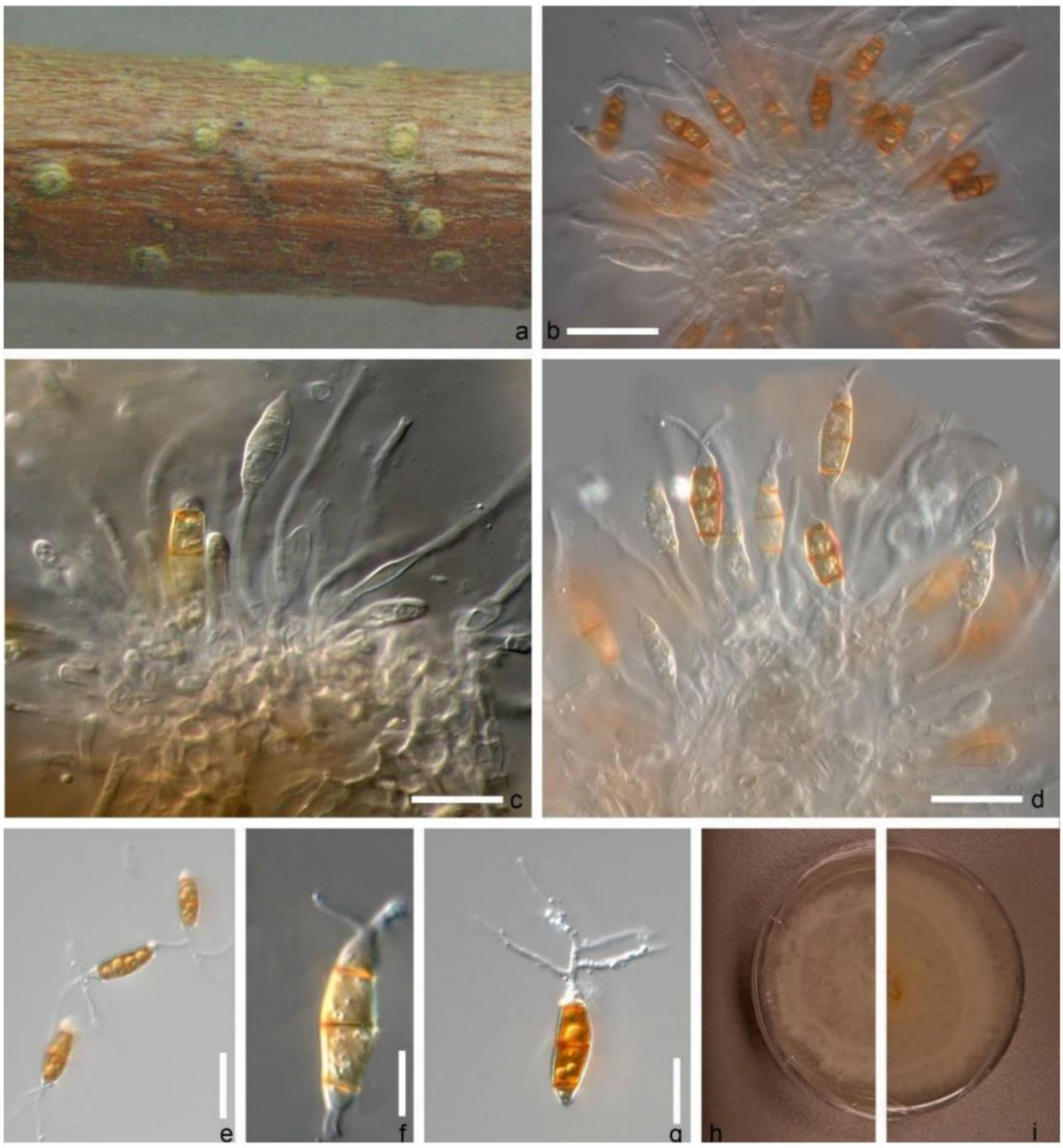

Figure 94 – Morphology of *Truncatella angustata*. a Conidiomata on host tissue. b–d Conidiophores and conidiogenous cells. e Conidia. f Immature conidia. g Branched apical appendages. h Above view of the culture after 7 days. i Reverse view of the culture after 7 days. Scale bars: b–d = 20 µm, e–g = 10 µm.

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Article
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Phylogenetic placement of *Akanthomyces muscarius*, a new endophyte record from *Nypa fruticans* in Thailand

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Abstract

A species of *Akanthomyces* (*Cordycipitaceae*, Hypocreales) was isolated as an endophyte from healthy leaves of *Nypa fruticans* collected in Krabi Province, Thailand. The species was identified as *Akanthomyces muscarius* based on phylogenetic analyses of the ITS gene region, as well as a combined LSU, SSU, TEF1 and RPB2 sequence dataset. Previously reported descriptions for *A. muscarius* are brief and based on few observations. In the present study, detailed descriptions of cultural and morphological characters of the new isolate are given. Phylogenies based on maximum likelihood and Bayesian analyses indicate that our new isolate clusters with extant strains of *A. muscarius* with good support and is sister to the genus *Lecanicillium*. Descriptions of the isolate match well with previously published data and our phylogeny supports the species identification. The asexual fungus *A. muscarius* is a new record for Thailand.

Key words – new record – *Akanthomyces* – *Cordycipitaceae* – mangrove

Introduction

Mangrove plants, especially leaves, are generally colonized by a variety of microfungi (Raghukumar et al. 1995, Swe et al. 2008, Sivakumar 2013, Doilom et al. 2017, Li et al. 2018, Devadatha et al. 2018). *Nypa fruticans* is a palm that develops in the upper regions of mangroves stretching from the brackish water zone at river mouths to almost inland freshwater (Rozainah & Aslezaeim 2010). Various terrestrial fungi have been recorded on the aerial parts of *N. fruticans*, e.g. *Fasciatispora petrakii, Astrosphaeriella nipicola, Oxydothis nypicola* (Hyde & Alias 1999, 2000, Poonyth et al. 2000), and some have been reported to be intertidal and endophytic (Hyde & Alias 1999).

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Table 2 Morphological comparison of *Akanthomyces muscarius* and closely related species.

Species	Strain no.	Distribution	Conidia (µm)	Phialides (µm)
Akanthomyces attenuatus	CBS 402.78	New Zealand, USA, Poland, Japan, India, Estonia	4.5–6.5 × 1.5–2.0	9–5.5 × 1–2
Lecanicillium aranearum	CBS 726.73a	Ghana, India	5-8 × 0.7–1.5	20–30 × 1.2–1·5
Akanthomyces aculeatus	**TS772** (Type)	USA, UK, Estonia, Ecuador	1-3 × 0.5-1 µm	5–8 × 2–3
Akanthomyces lecanii	CBS 101247	West Indies, Jamaica, Peru, UK, Italy, Finland, Turkey, Mexico, New Zealand, Sri Lanka	2.5–4.2 (3.5) × 1–1.5	11–30 (20) × 1.4–1.8
Akanthomyces muscarius	CBS 143.62	UK, Italy, Antarctica	(2–)2.5–5.5 (–6) × 1–1.7	15–35 (20) × 1–1.7
Akanthomyces muscarius	**MFLU 181145**	Thailand	2.5–6.8 (4.6) × 1.7–2.6 (1·8)	10.5–28.0 (15.0) × 1.5–2.8

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RESEARCH ARTICLE

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Morphological and phylogenetic characterisation of novel *Cytospora* species associated with mangroves

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Abstract

Mangroves are relatively unexplored habitats and have been shown to harbour a number of novel species of fungi. In this study, samples of microfungi were collected from symptomatic branches, stem and leaves of the mangrove species *Xylocarpus granatum*, *X. moluccensis* and *Lumnitzera racemosa* and examined morphologically. The phylogeny recovered supports our morphological data to introduce three new species, *Cytospora lumnitzericola*, *C. thailandica* and *C. xylocarpi*. In addition, a combined multi-gene DNA sequence dataset (ITS, LSU, ACT and RPB2) was analysed to investigate phylogenetic relationships of isolates and help in a more reliable species identification.

Keywords

3 new species, Cytosporaceae, *Lumnitzera racemosa*, Mangroves, Phylogeny, Taxonomy, *Xylocarpus granatum*, *Xylocarpus moluccensis*

conidia (Kohlmeyer and Kohlmeyer 1971). However, the phylogenies, generated herein, show that *C. xylocarpi* is distinct from *C. rhizophorae* (ATCC 38475), a strain from *Rhizophora mangle* that was identified by Kohlmeyer, the author of the species (Fig. 2). The two species also differ by 25 substitutions in ITS1+ITS2 and were collected from different hosts. Therefore, the collection in the present study is designated as a new species.

Our phylogeny also indicates a close relationship to unpublished sequences from GenBank (Figs 1, 2). Given that no morphological descriptions are available for these, the similarity in the ITS1 and ITS2 sequence between our strain and the sequences from GenBank (HAB16R13, M225, A761, MUCC302) are presented in Table 3. Those strains were collected from different hosts (Table 3) and, together with our strain, show substantial variation in ITS1 and ITS2 (Table 4). More collections are needed to further study morphological and genetic variation in this group.

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ORIGINAL ARTICLE

DGfM

Phylogenetic characterization of two novel *Kamalomyces* species in Tubeufiaceae (Tubeufiales)

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Abstract
Two novel species of bambusicolous fungi in the genus *Kamalomyces*, collected from northern Thailand, are described and illustrated herein. *Kamalomyces bambusicola* and *K. thailandicus* spp. nov. are typical of the genus *Kamalomyces* (Tubeufiaceae, Tubeufiales) and are morphologically distinct from known species with respect to their size of ascomata, asci and ascospores, ascospore septation and peridium structure, including the subiculum comprising hyphae on the host surface. Morphological examination reveals that the asexual morph of *K. bambusicola* is associated with its sexual morph in a subiculum forming dictyochlamydosporous conidia, which are similar to the asexual morph of *Chlamydotubeufia*. Phylogenetic analyses of combined LSU, ITS and TEF1-α sequence data also support these two species as distinct and confirm their phylogenetic affinities within the Tubeufiaceae. In particular, *Kamalomyces* shares a close phylogenetic relationship to *Helicoma*.

Keywords Bambusicolous fungi · Dothideomycetes · New species · Phylogeny · Taxonomy

Introduction

Tubeufiaceae is a family of Tubeufiales. The family was introduced to accommodate bitunicate ascomycetes occurring as saprobes on decaying wood (Barr 1979, 1980; Boonmee et al. 2011, 2014; Hyde et al. 2013). Taxa in Tubeufiaceae are characterized by superficial, soft and fleshy, pallid to bright, or black ascomata, bitunicate asci, and hyaline to pale brown, elongate, obovoid or oblong, septate ascospores, and hyphomycetous asexual morphs (Barr 1979, 1980; Boonmee et al. 2011, 2014; Hyde et al. 2013). Barr (1979) introduced Tubeufiaceae and designated *Tubeufia* Penz. & Sacc. as a generic type. The family was introduced to accommodate six genera viz. *Letendraea*, *Melioliphila*, *Podonectria*, *Rebentischia*, *Thaxteriella* and *Tubeufia* (Barr 1979, 1980; Kodsueb et al. 2006; Boonmee et al. 2011, 2014; Luo et al.

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Taxonomic circumscription of *Diaporthales* based on multigene phylogeny and morphology

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Abstract
Demarcation of family, genus and species boundaries in the *Diaporthales* has been tentative due to uninformative illustrations and descriptions, overlapping morphological characteristics, misplacement or poor condition of type specimens and shortage of molecular data from ex-type cultures. In this study, we obtained the type specimens or other authentic specimens of diaporthalean taxa from worldwide fungaria. We examined, described and illustrated them. This study is based on morphological characters from type or authentic specimens, details from protologue and original illustrations and molecular data obtained from GenBank. Combined analyses of nrITS, nrLSU, RPB2 and TEF1-α sequence data were used to construct the molecular phylogeny. Additionally, we provided separate phylogenetic trees for families when necessary to show the generic distribution within these families based on suitable gene markers. Based on morphology and phylogeny, we treat 17 genera previously assigned to *Diaporthales* genera *incertae sedis* within several families. For some genera we have designated new generic types as they are lacking type species or type species have affiliations with other families. We exclude *Anisomycopsis* from *Diaporthales* and place it in *Xylariomycetidae* genera *incertae sedis. Tirisporellaceae*, which was previously placed in *Tirisporellales* is placed in *Diaporthales* based on phylogeny and morphology. A new combination, *Dendrostoma leiphaemia* propose for *Amphiporthe leiphaemia* (Fr.) Butin. Based on the morphological characters and molecular data we accept 27 families and 138 genera within *Diaporthales*, 24 genera in *Diaporthales* genera *incertae sedis* and one genus in *Xylariomycetidae* genera *incertae sedis*. We provide notes for genera in *Diaporthales* genera *incertae sedis*, and excluded and doubtful genera are listed with notes on their taxonomy and phylogeny.

Keywords Authentic specimens · *Dendrostoma leiphaemia* · Incertae sedis · Classification · Taxonomy · Families · Accepted genera

Introduction

History

Fries (1823) accommodated the composite genus *Sphaeria*, which comprises most diaporthoid taxa, within *Sphaeriaceae*. Fuckel (1870) placed non-stromatic forms under subfamily *Ceratostomeae*, while stromatic forms were distributed among subfamilies *Melanconideae*, *Valseae*, *Dothideae* and *Melogrammeae*. Karsten (1873) revised *Sphaeriaceae* and divided it into four subfamilies as *Bertieae* (*Linospora*, *Ceuthocarpon*), *Diaportheae* (*Diaporthe*, *Gnomonia*, *Hypospila*), *Melanconideae* (*Pseudovalsa*, *Melanconis*, *Hercospora*, *Cytospora*) and *Valseae* (*Valsa*). Saccardo (1883) used ascospore septation and pigmentation to classify ascomycetes and diaporthoid taxa were distributed within various sections of his classification. He divided *Sphaeriaceae* into several subfamilies, those having stromatic tissues in *Ceratostomeae*, *Clypeosphaerieae*, *Valseae*, *Melanconideae*, *Melogrammeae* and non-stromatic taxa *Gnomonieae*. Lindau (1897) resurrected *Sphaeriales* to accommodate *Clypeosphaeriaceae*,

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similar characters to taxa in *Xylariomycetidae* and therefore we included this genus in *Xylariomycetidae* genera *incertae sedis*. *Tirisporellaceae* which was previously placed in *Tirisporellales* is revised here and it forms a clade within *Diaporthales*. Therefore, we included *Tirisporellaceae* as a family in *Diaporthales*. Based on morphological characters and molecular data we accepted 27 families, 138 genera within *Diaporthales*, 24 genera in *Diaporthales* genera *incertae sedis* and one genus in *Xylariomycetidae* genera *incertae sedis*.

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Fungal diversity notes 840–928: micro-fungi associated with Pandanaceae

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Abstract
This paper provides illustrated descriptions of micro-fungi newly found on Pandanaceae in China and Thailand. The fungi are accommodated in 31 families. New taxa described include a new family, seven new genera, 65 new species, 16 previously known species. A new family: Malaysiascaceae (Glomerellales). New genera are *Acremoniisimulans* (Plectosphaerellaceae), *Pandanaceomyces*, *Pseudoachroiostachy* (Nectriaceae), *Pseudohyaloseta* (Niessliaceae), *Pseudoornatispora* (Stachybotriaceae) and *Yunnanomyces* (Sympoventuriaceae). New species are *Acremoniisimulans thailandensis*, *Beltrania krabiensis*, *Beltraniella pandanicola*, *B. thailandicus*, *Canalisporium krabiense*, *C. thailandensis*, *Clonostachys krabiensis*, *Curvularia chonburiensis*, *C. pandanicola*, *C. thailandicum*, *C. xishuangbannaensis*, *Cylindrocladiella xishuangbannaensis*, *Dictyochaeta pandanicola*, *Dictyocheirospora nabanheensis*, *D. pandanicola*, *D. xishuangbannaensis*, *Dictyosporium appendiculatum*, *Di. guttulatum*, *Di. hongkongensis*, *Di. krabiense*, *Di. pandanicola*, *Distoseptispora thailandica*, *D. xishuangbannaensis*, *Helicoma freycinetiae*, *Hermatomyces biconisporus*, *Lasiodiplodia chonburiensis*, *L. pandanicola*, *Lasionectria krabiense*, *Menisporopsis pandanicola*, *Montagnula krabiensis*, *Musicillium pandanicola*, *Neofusicoccum pandanicola*, *Neohelicomyces pandanicola*, *Neooccultibambusa thailandensis*, *Neopestalotiopsis chiangmaiensis*, *N. pandanicola*, *N. phangngaensis*, *Pandanaceomyces krabiensis*, *Paracylindrocarpon nabanheensis*, *P. pandanicola*, *P. xishuangbannaensis*, *Parasarcopodium hongkongensis*, *Pestalotiopsis krabiensis*, *P. pandanicola*, *Polyplosphaeria nabanheensis*, *P. pandanicola*, *P. xishuangbannaensis*, *Pseudoachroiostachys krabiense*, *Pseudoberkleasmium pandanicola*, *Pseudochaetosphaeronema pandanicola*, *Pseudohyaloseta pandanicola*, *Pseudoornatispora krabiense*, *Pseudopithomyces pandanicola*, *Rostriconidium pandanicola*, *Sirastachys phangngaensis*, *Stictis pandanicola*, *Terriera pandanicola*, *Thozetella pandanicola*, *Tubeufia freycinetiae*, *T. parvispora*, *T. pandanicola*, *Vermiculariopsiella hongkongensis*, *Volutella krabiense*, *V. thailandensis* and *Yunnanomyces pandanicola*. Previous studies of micro-fungi on Pandanaceae have not included phylogenetic support. Inspiration for this study came from the book *Fungi Associated with Pandanaceae* by Whitton, McKenzie and Hyde in 2012. Both studies reveal that the micro-fungi on Pandanaceae is particularly rich in hyphomycetes. All data presented herein are based on morphological examination of specimens, coupled with phylogenetic sequence data to better integrate taxa into appropriate taxonomic ranks and infer their evolutionary relationships.

Keywords 65 new taxa · Ascomycota · Dothideomycetes · *Freycinetia* · Lecanoromycetes · Leotiomycetes · *Pandanus* · Saprobic · Sordariomycetes

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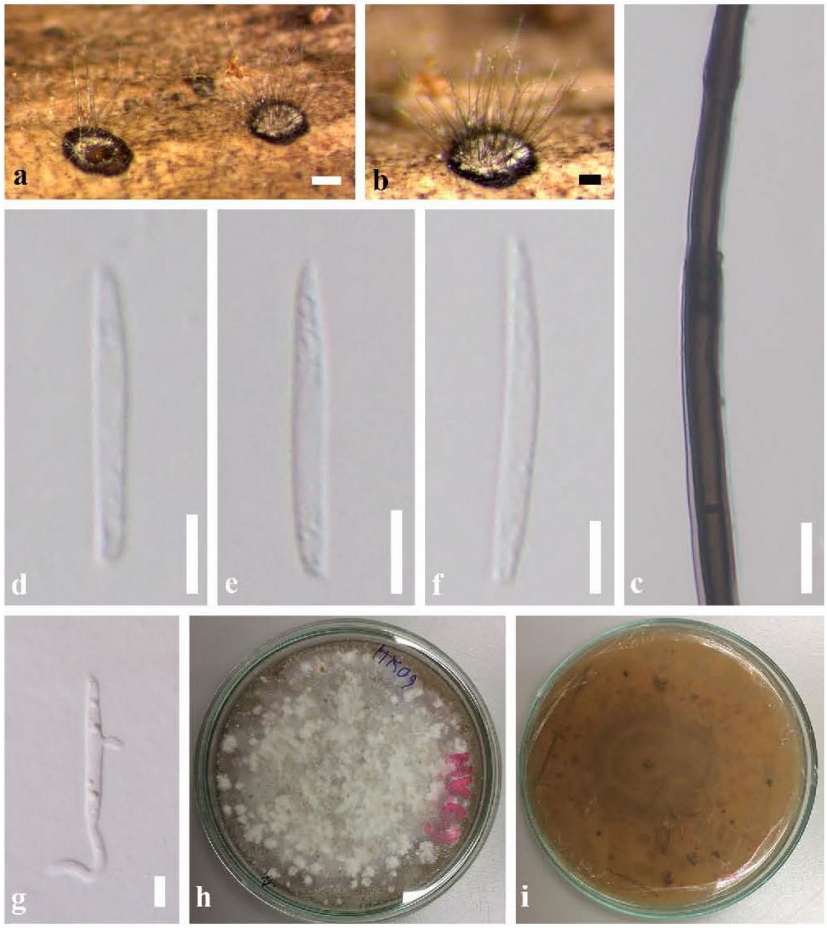

Fig. 112 ***Vermiculariopsiella hongkongensis*** (HKAS 100861, **holotype**). **a, b** Colonies on dead leaf of *Pandanus* sp. **c** Setae-walled. **d–f** Conidia. **g** Germinating conidium. **h, i** Colony on MEA from above and below. Scale bars: **a** = 200 μm, **b** = 100 μm, **b** = 10 μm, **c**, **g** = 5 μm, **d–f** = 2 μm

Notes: *Vermiculariopsiella* has never been reported from Pandanaceae. Based on phylogenetic analysis, *V. hongkongensis* is well-separated from other species of *Vermiculariopsiella* (0.99 in BYPP, Fig. 68). However, we compare the morphology *V. hongkongensis* with *V. acaciae* Crous & M.J. Wingf. and *V. immersa* (Desm.) Bender. but *V. acacia* has dimorphic conidia (Crous et al. 2016a), while *V. immerse* has fusoid to cylindrical conidia (Bender 1932). In a BLASTn search on NCBI GenBank, the closest matches of ITS sequence of KUMCC 17-0270 is *V. dichapetali culture* with 99% identity to the strain CBS:143440 (MH107924).

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Fungal diversity notes 709–839: taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on *Rosaceae*

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Abstract
This paper is the seventh in the Fungal Diversity Notes series, where 131 taxa accommodated in 28 families are mainly described from *Rosa* (*Rosaceae*) and a few other hosts. Novel fungal taxa are described in the present study, including 17 new genera, 93 new species, four combinations, a sexual record for a species and new host records for 16 species. *Bhatiellae*, *Cycasicola*, *Dactylidina*, *Embarria*, *Hawksworthiana*, *Italica*, *Melanocucurbitaria*, *Melanodiplodia*, *Monoseptella*, *Uzbekistanica*, *Neoconiothyrium*, *Neopaucispora*, *Pararoussoella*, *Paraxylaria*, *Marjia*, *Sporormurispora and Xenomassariosphaeria* are introduced as new ascomycete genera. We also introduce the new species *Absidia jindoensis*, *Alternaria doliconidium*, *A. hampshirensis*, *Angustimassarina rosarum*, *Astragalicola vasilyevae*, *Backusella locustae*, *Bartalinia rosicola*, *Bhatiellae rosae*, *Broomella rosae*, *Castanediella camelliae*, *Coelodictyosporium rosarum*, *Comoclathris rosae*, *C. rosarum*, *Comoclathris rosigena*, *Coniochaeta baysunika*, *C. rosae*, *Cycasicola goaensis*, *Dactylidina shoemakeri*, *Dematiopleospora donetzica*, *D. rosicola*, *D. salsolae*, *Diaporthe rosae*, *D. rosicola*, *Endoconidioma rosae-hissaricae*, *Epicoccum rosae*, *Hawksworthiana clematidicola*, *H. lonicerae*, *Italica achilleae*, *Keissleriella phragmiticola*, *K. rosacearum*, *K. rosae*, *K. rosarum*, *Lophiostoma rosae*, *Marjia tianschanica*, *M. uzbekistanica*, *Melanocucurbitaria uzbekistanica*, *Melanodiplodia tianschanica*, *Monoseptella rosae*, *Mucor fluvius*, *Muriformistrickeria rosae*, *Murilentithecium rosae*, *Neoascochyta rosicola*, *Neoconiothyrium rosae*, *Neopaucispora rosaecae*, *Neosetophoma rosarum*, *N. rosae*, *N. rosigena*, *Neostagonospora artemisiae*, *Ophiobolus artemisiicola*, *Paraconiothyrium rosae*, *Paraphaeosphaeria rosae*, *P. rosicola*, *Pararoussoella rosarum*, *Parathyridaria rosae*, *Paraxylaria rosacearum*, *Penicillium acidum*, *P. aquaticum*, *Phragmocamarosporium rosae*, *Pleospora rosae*, *P. rosae-caninae*, *Poaceicola agrostina*, *P. arundinicola*, *P. rosae*, *Populocrescentia ammophilae*, *P. rosae*, *Pseudocamarosporium pteleae*, *P. ulmi-minoris*, *Pseudocercospora rosae*, *Pseudopithomyces rosae*, *Pseudostrickeria rosae*, *Sclerostagonospora lathyri*, *S. rosae*, *S. rosicola*, *Seimatosporium rosigenum*, *S. rosicola*, *Seiridium rosarum*, *Setoseptoria arundelensis*, *S. englandensis*, *S. lulworthcovensis*, *Sigarispora agrostidis*, *S. caryophyllacearum*, *S. junci*, *S. medicaginicola*, *S. rosicola*, *S. scrophulariae*, *S. thymi*, *Sporormurispora atraphaxidis*, *S. pruni*, *Suttonomyces rosae*, *Umbelopsis sinsidoensis*, *Uzbekistanica rosae-hissaricae*, *U. yakutkhanika*, *Wojnowicia rosicola*, *Xenomassariosphaeria rosae*. New host records are provided for *Amandinea punctata*, *Angustimassarina quercicola*, *Diaporthe rhusicola*, *D. eres*, *D. foeniculina*, *D. rudis*, *Diplodia seriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *Lecidella elaeochroma*, *Muriformistrickeria rubi*, *Neofusicoccum australe*, *Paraphaeosphaeria michotii*, *Pleurophoma pleurospora*, *Sigarispora caulium* and *Teichospora rubriostiolata*. The new combinations are *Dactylidina dactylidis* (=*Allophaeosphaeria dactylidis*), *Embarria clematidis* (=*Allophaeosphaeria clematidis*), *Hawksworthiana alliariae* (=*Dematiopleospora alliariae*) and *Italica luzulae*

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Outline of *Ascomycota*: 2017

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Abstract
Taxonomic placement of genera have been changing rapidly as taxonomists widely use DNA sequence data in phylogenetic and evolutionary studies. It is essential to update existing databases/outlines based on recent studies, since these sources are widely used as a foundation for other research. In this outline, we merge both asexual and sexual genera into one outline. The phylum Ascomycota comprises of three subphyla *viz. Pezizomycotina* (including 13 classes, 124 orders and 507 families), *Saccharomycotina* (including one class, one order and 13 families) and *Taphrinomycotina* (five classes, five orders and six families). Approximately, 6600 genera have been listed under different taxonomic ranks including auxiliary (intermediate) taxonomic ranks.

Keywords Asexual genera · Classification · Sexual genera · Systematic · Taxonomic ranks

Introduction

The Kingdom *Fungi* contains a huge number of species, which continues to rise with more collections. Our current fungal classification system has been subjected to various updates over the years, following the discovery of many novel species and inclusion of DNA sequence data to provide a more natural system of classification that reflects evolutionary relationships. Lumbsch and Huhndorf (2010) provided an outline with three subphyla (*viz Pezizomycotina* with eleven classes; *Saccharomycotina* with one class and *Taphrinomycotina* with four classes). There have been major developments in fungal taxonomy over the last few years with many additions leading to subsequent classification changes (Liu et al. 2015; Senanayake et al. 2015; Li et al. 2016; Wijayawardene et al. 2016; Hyde et al. 2017a, b). Hence, it is essential to update our current classification to inadvertently avoid confusion and provide a platform where mycologists can use a standard system to assign taxa to appropriate ranks. There is also a need to integrate asexual species into our current classification. Kendrick (1979, 1989) advocated the incorporation of asexual genera in a natural classification system, but this was given little attention. However, with the concept of *One fungus- One name*, which deals with the inconsistencies of the dual nomenclatural system pertaining to pleomorphic fungi, this has changed (Hawksworth 2012). Hyde et al. (2011) compiled a number of asexual morph names into an outline with a view to providing a stable and reliable system to integrate asexual names in our classification and avoid nomenclatural bias. It is also very important to update our current classification system as several nomenclatural changes have been proposed since 2011 (Art. 59). DNA based sequence analyses have also

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Zygosporium Mont.

Li et al. (2017b) showed that this genus had distinct phylogenetic lineage in *Xylariales*, thus introduced *Zygosporiaceae*.

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ORIGINAL ARTICLE

DGfM

Acuminatispora palmarum gen. et sp. nov. from mangrove habitats

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Abstract
Fungi play a vital role as decomposers in mangrove ecosystems. A new ascomycete species, *Acuminatispora palmarum*, inhabiting decayed petioles and rachides of palms in mangrove habitats, is introduced in this paper based on morphological and phylogenetic evidence. Phylogenetic relationships of related taxa were inferred from combined LSU, SSU, *TEF1α*, and *RPB2* sequence data, and the analyses indicate that *A. palmarum* could be recognized as a distinct group in Pleosporales, but its familial placement needs to be further resolved. The morphological characters of this new taxon are also different from other members in Pleosporales by its deeply immersed ascomata, long pedicellate asci, and biseriate to triseriate, 1-(rarely 3) septate, brown, fusiform ascospores with acute or narrowly pointed ending cells. *Acuminatispora* gen. nov. (Pleosporales, *incertae sedis*) is therefore established to accommodate the new taxon *A. palmarum*. Furthermore, phylogenetic relationships of *Acrocordiopsis* and *Caryospora* are discussed with a consideration of morphological observations.

Keywords 2 new taxa · Dothideomycetes · Phylogeny · Sexual morph · Taxonomy

Introduction

Mangroves are distinctive coastal ecosystems comprising a diverse group of predominantly tropical trees and shrubs that are adapted to life in coastal intertidal marine locations (Tomlinson 1986). Breathing roots, salt-excreting leaves, and viviparous water-dispersed propagules are the three most important morphological and physiological traits (Duke 1992; Shi et al. 2005). Most studies on fungi colonizing mangroves are regarding to taxonomy (Cribb and Cribb 1955; Kohlmeyer and Kohlmeyer 1971, 1977; Kohlmeyer 1981, 1985; Kohlmeyer and Schatz 1985; Kohlmeyer and Volkmann-Kohlmeyer 1991), ecology and inventory (Kohlmeyer 1966, 1969; Hyde 1988; Hyde and Jones 1988; Hyde 1989a, b, c; Jones and Kuthubutheen 1989; Hyde et al. 1992; Hyde and Lee 1995; Alias et al. 2010; Pang et al. 2011), as well as a series of reviews (Jones 2000; Sarma and Hyde 2001; Jones 2011a, b; Jones et al. 2015; Sivakumar 2016), while other studies have considered the molecular phylogeny of mangrove fungi (Jones et al. 2009; Suetrong et al. 2009; Jones and Pang 2012). There are 74 mangrove species in 53 genera and 35 families that occurred along the protected shorelines of Thailand (Plathong and Plathong 2011), and 184 fungal species have been documented from Thai mangroves, of which approximately 85% are Ascomycota (Suetrong et al. 2017).

Pleosporales Luttr. ex M.E. Barr is the largest and most diverse group in Dothideomycetes (Ascomycota), including 75 families, 400 genera, and 52 genera *incertae sedis* (Schoch et al. 2006b, 2009; Hyde et al. 2013; Ariyawansa et al. 2015; Liu et al. 2017; Wijayawardene et al. 2018), and four new families were recently proposed in this order using multilocus phylogenetic evidence (Valenzuela-Lopez et al. 2018). Since molecular phylogeny has been used to rank marine fungi, most of the species found from intertidal mangrove wood, twigs, and leaves were identified as members of Pleosporales and are distributed in 12 accepted families: Aigialaceae,

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Table 2 Morphological comparison of *Acuminatispora palmarum*, *Coronopapilla mangrovei* and *Passeriniella savoryellopsis*

	Acuminatispora palmarum (this study)	*Coronopapilla mangrovei* (Hyde 1989c, Kohlmeyer and Volkmann-Kohlmeyer 1990, 1991)	*Passeriniella savoryellopsis* (Hyde and Mouzouras 1988)
Ascomata	380–610 μm high, 150–395 μm diam., subglobose, immersed with an erumpent short neck. Ostiole 72–85 μm diam., central, cylinder-like opening, periphysate	440–640 μm high, 360–480 μm diam., globose-pyriform, immersed becoming erumpent; Ostiolate with short necks, papillate	500–700 μm high, 800–1300 μm diam., globose to subglobose, immersed, ostiolate, periphyses, papillate
Peridium	10–20 μm thick, cells *textura angularis*	14–40 μm thick, at the side elongate cells, at the base angular cells	80–100 μm thick, cells *textura angularis*
Hamathecium	Trabeculate pseudoparaphyses up to 2.5 μm wide	Pseudoparaphyses very thin, less than 1.0 μm wide	Pseudoparaphyses 3.9–5.9 μm
Asci	93–125 × 13–22 μm, long pedicellate	215–260 × 28–34 μm	280–440 × 24–32 μm, 4-spored
Ascospores	24–30 × 7–10 μm, overlapping biseriate to triseriate, fusiform, with acute or narrowly pointed ending cells, 1-(rarely 3) septate, constricted at the central septa, the upper cell broader	36–60 × 16–24 μm, uniseriate, ellipsoidal, with tapering rounded tips, primary septum median, constricted, developing pseudosepta, thick-walled	64–88 × 24–28 μm, uniseriate, ellipsoidal, 3-septate, constricted at central septum, end cells small, conical and hyaline

change in a phylogenetic tree. Furthermore, the corresponding taxonomical classification may need to be modified.

Acrocordiopsis

The initial phylogenetic study of *Acrocordiopsis patilii* was conducted by Suetrong et al. (2009) who noted it grouped as a residual paraphyletic assemblage and was not assigned to any family. Subsequently, the genus was assigned to Salsugineaceae K.D. Hyde & S. Tibpromma (Hyde et al. 2013) based on the same sequence data as Suetrong et al. (2009), while Ariyawansa et al. (2015) placed *Acrocordiopsis* in the new family, Caryosporaceae with *Caryospora* and both of the above two treatments were based on phylogenetic analyses with few taxa representing the genera and families.

Wijayawardene et al. (2018) referred *Ac. patilii* in Salsugineaceae following Hyde et al. (2013). Jones and Pang (2012) also consider the phylogenetic placement of *Ac. patilii* unresolved. In our study, the phylogenetic position of the new taxon is not stable, as well as the taxa *Acrocordiopsis*, *Astrosphaeriella*, *Astrosphaeriellopsis*, and *Caryospora*. Currently, *Ac. patilii* and *Ac. sphaerica* are referred to the Salsugineaceae, although morphologically they have little in common with *Salsuginea ramicola* (website: marinefungi.org). Similarly, *Acrocordiopsis* is distinct from *Astrosphaeriellopsis bakeriana* both morphologically and phylogenetically. Further collections of *Ac. patilii*, *Ac. sphaerica*, and *Salsuginea ramicola* and new sequence data are required for all these species before their taxonomic positions are resolved.

Caryospora

The genus *Caryospora* and *Acuminatispora* formed an unsupported sister clade (ML/31, MP/17, and BYPP/0.55). *Caryospora* is an old genus, which currently includes approximately 12 species (*Caryospora aquatic* Huang Zhang, K.D. Hyde & Ariyaw., *C. australiensis* Abdel-Wahab & E.B.G. Jones, *C. callicarpa* (Curr.) Nitschke ex Fuckel, *C. coffeae* Pat. & Gaillard, *C. daweiensis* G.C. Zhao & R.L. Zhao, *C. langloisii* Ellis & Everh., *C. masonii* D. Hawksw., *C. minima* Jeffers, *C. obclavata* Raja & Shearer, *C. olearum* (Castagne) Sacc., *C. phyllostachydis* (Hara) I. Hino & Katum., *C. putaminum* (Schwein.) De Not.), and all have superficial or erumpent to nearly superficial ascomata, a carbonaceous peridium, and trabeculate pseudoparaphyses (Jeffers 1940; Barr 1979; Hawksworth 1982; Abdel-Wahab and Jones 2000; Raja and Shearer 2008; Hawksworth et al. 2010; Hu 2010; Ariyawansa et al. 2015). Only two species have been sequenced and further studies are required.

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Phylogenetic Revision of Savoryellaceae and Evidence for Its Ranking as a Subclass

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Morphology, phylogeny, and molecular clock analyses were carried out on Savoryellaceae in order to understand the placements of taxa in this family. *Ascotaiwania* and *Neoascotaiwania* formed a well-supported separate clade in the phylogeny of concatenated partial SSU, LSU, TEF, and RPB2 gene data. These two genera share similar morphological features, especially in their asexual morphs, indicating that they are congeneric. Hence, we synonymize *Neoascotaiwania* under *Ascotaiwania*. *Ascotaiwania hughesii* (and its asexual morph, *Helicoon farinosum*) and *Monotosporella setosa* grouped in a clade sister to Pleurotheciales and are excluded from *Ascotaiwania* which becomes monophyletic. A novel genus *Helicoascotaiwania* is introduced to accommodate *Ascotaiwania hughesii* and its asexual morph, *Helicoon farinosum*. A novel species, *Savoryella yunnanensis* is introduced from a freshwater habitat in Yunnan Province, China. Comprehensive descriptions and illustrations are provided for selected taxa in this family. In addition, we provide evolutionary divergence estimates for Savoryellomycetidae taxa and major marine based taxa to support our phylogenetic and morphological investigations. The taxonomic placement of these marine-based taxa is briefly discussed. Our results indicate that the most basal group of marine-based taxa are represented within Lulworthiales, which diverged from ancestral Sordariomycetes around 149 Mya (91–209) and Savoryellomycetidae around 213 Mya (198–303).

Keywords: freshwater, marine, morphology, phylogeny, Savoryellomycetidae, taxonomy

INTRODUCTION

The family Savoryellaceae (Savoryellales) was established by Jaklitsch and Réblová (2015) and is typified by the genus *Savoryella*. Boonyuen et al. (2011) had earlier introduced the order Savoryellales, but without designating a family. According to phylogenetic and molecular clock analyses (Hongsanan et al., 2017; Hyde et al., 2017), the orders Conioscyphales, Fuscosporellales,

The discovery of the extent new Caledonian species identified as *Monotosporella setosa* found developing on semi-solidified resin flows of *Agathis ovata* (Araucariaceae), is the first record of a *Monotosporella* species from modern resin substrates (Sadowski et al., 2012). Thus, the fossil age of this species could help to determine the exact phylogenetic placement of other *Monotosporella* species in the future. Most of the divergence time estimations have been conducted with mainly terrestrial representatives rather than those of marine origin and we recommend a thorough analysis of a wider range of marine fungal taxa.

AUTHOR CONTRIBUTIONS

AA-S, EJ, KH, KK, MD, RZ, SM, and VS planned the experiments. BD, WD, JY, MD, and WD conducted the experiments. AE, MD, and WD analyzed the data. BD, MD, SM, and EJ wrote the manuscript. All authors approved the manuscript.

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A stable phylogeny for Dactylosporaceae

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ABSTRACT
The apothecial ascomycete family Dactylosporaceae includes saprobes and lichenicolous fungi. In recent studies, the phylogenetic position of this family was unstable within the subphylum Pezizomycotina. The present study provides a stable phylogenetic placement for Dactylosporaceae within the class Eurotiomycetes and we introduce the new order: Dactylosporales Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, ord. nov. to accommodate this family. We also introduce two new species: *Dactylospora chiangraiensis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. and *Dactylospora fusiformis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. to this family and their relationships with other taxa are represented in a multigene phylogeny.

KEY WORDS
Apothecial ascomycetes, Eurotiomycetes, Pezizomycotina *incertae sedis*, polyphyletic, new order, new species.

RÉSUMÉ
Une phylogénie stable pour les Dactylosporaceae.
La famille des ascomycètes apothéciales, les Dactylosporaceae, comprend des saprobes et des champignons lichénicoles. Dans des études récentes, la position phylogénétique de cette famille était instable dans le sous-phylum Pezizomycotina. La présente étude fournit un placement phylogénétique stable pour les Dactylosporaceae dans la classe des Eurotiomycètes. Nous décrivons un nouvel ordre : les Dactylosporales Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, ord. nov. pour accueillir cette famille. Nous introduisons également deux nouvelles espèces: *Dactylospora chiangraiensis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. et *Dactylospora fusiformis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. dans cette famille, et leurs relations avec d'autres taxons sont représentées dans une phylogénie multigénique.

MOTS CLÉS
Ascomycètes apothéciaux, Eurotiomycètes, Pezizomycotina *incertae sedis*, polyphylétique, ordre nouveau, espèces nouvelles.

D. borealis was recorded near fresh water streams and coastal lowlands. According to the literature all those non-terrestrial species are morphologically similar by having ascospore wall ornamentations and/or ascospore appendages. *Dactylospora vrijmoediae* and *D. canariensis* have appendaged ascospores, *D. haliotrepha*, *D. mangrovei* and *D. borealis* have striate, verrucose and granulated ascospore walls, while other terrestrial *Dactylospora* have smooth ascospore walls (Kohlmeyer & Volkmann-Kohlmeyer 1998; Jones *et al.* 1999; Ihlen *et al.* 2004; Fryday & Coppins 2012; Pang *et al.* 2014). Many aquatic ascomycetes have ascospore walls with ornamentations or appendages and these are thought to be an adaptation for their aquatic life (Hyde & Jones 1989; Jones 1994, Shearer & Raja 2010). Moreover, according to the phylogenetic analyses II, three marine *Dactylospora* species form a monophyletic lineage, sister to the *Sclerococcum-D. parasitica* clade. Hence, we suggest these aquatic *Dactylospora* are a phylogenetic distinct group from the terrestrial *Dactylospora* species. Furthermore, they may have evolved from terrestrial *Dactylospora* ancestors and adapted to an aquatic life style (Shearer 1993; Jones 2006).

Currently there are two discomycete families within Eurotiomycetes viz. Mycocaliciaceae and Dactylosporaceae. Taxa of these two families are similar in having sessile to stipitate apothecia, sparingly branched paraphyses, cylindric clavate asci and ellipsoid to fusiform ascospores. However, Dactylosporaceae taxa differ by having amyloid asci (Schoch *et al.* 2009; Jaklitsch *et al.* 2016; Ekanayaka *et al.* 2017).

Dactylospora is a sexual genus and there are no available records of its asexual morph. The asexual genus *Sclerococcum* claded with *Dactylospora* but its phylogenetic limitations are still unclear. Moreover, the genera *Sclerococcum* and *Dactylospora* share similar habitats and ecological affinities (Schoch *et al.* 2009; Pang *et al.* 2014; Diederich 2015). By considering the phylogenetic and ecological similarities, we suggest that *Sclerococcum* could be the asexual morph of *Dactylospora*. However, further taxon sampling, culture studies and molecular data are required to resolve the generic relationship of these two genera.

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Fungal diversity notes 1036–1150: taxonomic and phylogenetic contributions on genera and species of fungal taxa

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Abstract

This article is the tenth series of the Fungal Diversity Notes, where 114 taxa distributed in three phyla, ten classes, 30 orders and 53 families are described and illustrated. Taxa described in the present study include one new family (viz. Pseudoberkleasmiaceae in Dothideomycetes), five new genera (*Caatingomyces*, *Cryptoschizotrema*, *Neoacladium*, *Paramassaria* and *Trochilispora*) and 71 new species, (viz. *Acrogenospora thailandica*, *Amniculicola aquatica*, *A. guttulata*, *Angustimassarina sylvatica*, *Blackwellomyces lateris*, *Boubovia gelatinosa*, *Buellia viridula*, *Caatingomyces brasiliensis*, *Calophoma humuli*, *Camarosporidiella mori*, *Canalisporium dehongense*, *Cantharellus brunneopallidus*, *C. griseotinctus*, *Castanediella meliponae*, *Coprinopsis psammophila*, *Cordyceps succavus*, *Cortinarius minusculus*, *C. subscotoides*, *Diaporthe italiana*, *D. rumicicola*, *Diatrypella delonicis*, *Dictyocheirospora aquadulcis*, *D. taiwanense*, *Digitodesmium chiangmaiense*, *Distoseptispora dehongensis*, *D. palmarum*, *Dothiorella styphnolobii*, *Ellisembia aurea*,

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isolated and is described here, based on morphological characteristics and phylogenetic analyses.

Rhizophydium koreanum Hyang B. Lee, S.J. Jeon, T.T. T. Nguyen, ***sp. nov.***

Index Fungorum number: IF554571; *Facesoffungi number*: FoF05792; Fig. 159

Etymology: *koreanum*, referring to the country from which the species was recorded.

Holotype: CNUFC-17CPW1-1

On PmTG (peptonized milk, tryptone and glucose) agar: *Zoosporangium* sphaerical, with many closely spaced and highly branched rhizoids, measured (100–)107.5–120.5(–131.5) µm diam. *Rhizoidal system* arising from a single point at the base of the sporangium. *Zoospores* abundantly produced, sphaerical, measured (3.0–)3.5(–4.0) µm diam., with a flagellum of (20–)23.5(–30) µm long. *Resting spore* sphaerical, measured (10–)12.5(–13.5) µm diam.

Culture characteristics: *Colonies* reaching 8.5 mm diam. on PmTG at 25 °C in 7 days, cream. Optimal growth was observed around 20 °C.

Material examined: REPUBLIC OF KOREA, from pond water collected at Chonnam National University Arboretum, Gwangju, Korea, 20 October 2017 (CNUFC-17CPW1-1, preserved as glycerol stock at − 80 °C in the Chonnam National University Fungal Collection; isotype in Culture Collection of Nakdonggang National Institute of Biological Resources [NNIBR], Sangju, Gyeongbuk Province).

Notes: Based on phylogenetic analyses and morphological comparison, our isolate belongs to *Rhizophydium*. *Rhizophydium koreanum* formed a distinct clade from other species in the phylogenetic tree (Fig. 160). It differs from the closely related species *R. globosum* and *R. brooksianum* by forming larger sporangia.

GenBank numbers: LSU: MH298649, MH298650.

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Karunarathna thanks CAS President's International Fellowship Initiative (PIFI) for funding his postdoctoral research (Grant No. 2018PC0006) and the National Science Foundation of China (NSFC, project code 31750110478). Department of Plant Medicine, National Chiayi University (NCYU) is thanked to provide facilities for DNA molecular experiment. Rajesh Jeewon thanks Mae Fah Luang University for the award of a Visiting Scholar. Walter Rossi and Marco Leonardi wish to thank the following entomologists who supplied them with the flies bearing Labouobeniales and/or identified the host insects: R. Andrade (Portugal), G. Cocks (Australia), J. Deeming (Great Britain), Martin J. Ebejer (Great Britain), Marina Krivosheina (Russia), Iain MacGowan (Great Britain), Thomas Pape (Denmark), Alfio Raspi (Italy), N. Vikhrev (Russia), M. von Tschirnhaus (Germany). H.B. 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We would like to thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) of the Parque Montanhas do Tumucumaque and the Instituto Brasileiro de Meio Ambiente (IBAMA) for support during the field trips; and Msc. RLM Alvarenga for the figures. We thank the Graduate Program for the Biodiversity and Biotechnology Network of the Legal Amazon (UFPA-MPEG, Brazil); the Museu Paraense Emílio Goeldi (MPEG), the Universidade do Estado do Amapa (UEAP) and the Universidade Federal de Pernambuco (UFPE) for the logistical support of its laboratories and herbaria. Further, we acknowledge the Conselho Nacional de Desenvolvimento Científico Programa de Capacitação for the scholarship to AMSS (Programa de Capacitação Institucional 303073/2018-7); CNPq (Sisbiota 563342/2010-2, PROTAX 562106/2010-3), and FACEPE (APQ 0788-2.03/12) for funding this research. T.G.L. Oliveira, C.M. Souza-Motta, J.D.P. Bezerra, and O.M.C. 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Barbosa acknowledgment scholarship and financial support from Conselho Nacional de Pesquisa e Desenvolvimento Cientifico (CNPq). We would like to acknowledge the URM Herbarium and URM Culture Collection staff and Professor Dr. Roger Fagner R. de Melo for taxonomy assistance. Guo-Jie Li and Rui-Lin Zhao would like to thank National Natural Science Foundation of China

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ORIGINAL ARTICLE

CrossMark

Taxonomic and phylogenetic characterizations reveal two new species and two new records of *Roussoella* (Roussoellaceae, Pleosporales) from Yunnan, China

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Abstract
Roussoella species mainly occur on monocotyledons, especially bamboo, and are characterized by raised, immersed ascostromata, cylindrical to clavate asci and ellipsoidal to fusiform, brown to dark brown, 1-septate, ornamented ascospores. They have cytospora-like asexual morphs with enteroblastic conidiogenous cells and hyaline to dark brown conidia, which are often minutely warty. In this article, two new species and two new records of *Roussoella* associated with dead bamboo, collected from Yunnan Province of China, are described and illustrated. These four taxa are similar in morphological characteristics, but can be distinguished by phylogenetic analyses of the concatenated ITS, LSU, TEF1-α, and RPB2 sequence data. Based on maximum likelihood and Bayesian inference analyses of the combined sequence dataset, we introduce *Roussoella kunmingensis* and *R. yunnanensis* spp. nov. *Roussoella mukdahanensis* is provided here as the first record on bamboo from China. The asexual morph of *R. pseudohysterioides* is illustrated and described on dead bamboo culms.

Keywords 2 new species · Bambusicolous fungi · Dothideomycetes · Morphology · Phylogeny

Introduction

Roussoellaceae J.K. Liu et al. is a well-resolved family in Pleosporales, introduced by Liu et al. (2014). According to Wijayawardene et al. (2018), the family accommodates seven genera viz. *Appendispora* K.D. Hyde (Hyde 1994; Ariyawansa et al. 2014), *Cytoplea* Bizz. & Sacc. (Bizzozero 1885; Wijayawardene et al. 2014), *Elongatopedicellata* J.F. Zhang et al. (Ariyawansa et al. 2015), *Immotthia* M.E. Barr (Barr 1987; Hyde et al. 2017; Doilom et al. 2018), *Neoroussoella* J.K. Liu, Phookamsak & K.D. Hyde (Liu et al. 2014), *Roussoella* Sacc. (Saccardo and Paoletti 1888; Liu et al. 2014), and *Roussoellopsis* I. Hino & Katum. (Hino and Katumoto 1965; Liu et al. 2014; Phookamsak et al. 2014). With the exception of *Appendispora*, *Cytoplea*, and *Immotthia*, all other genera in Roussoellaceae have molecular data in the GenBank database (https://www.ncbi.nlm.nih.gov/nuccore/; accessed 17 June 2018).

Roussoella, the type genus of Roussoellaceae, was formally introduced by Saccardo and Paoletti (1888) and is typified by *Roussoella nitidula* Sacc. & Paol. Until now, many new species of *Roussoella* have been discovered and mostly are found on bamboo and palms (Zhou et al. 2003; Ariyawansa et al. 2014; Liu et al. 2014; Dai et al. 2017; Hyde et al. 2018). *Roussoella* is characterized by raised, immersed to semi-immersed ascostromata containing long, cylindrical, thin-walled, bitunicate asci, trabeculate, anastomosed pseudoparaphyses (0.5–2 μm wide; Liew et al. 2000), and brown, two-celled, longitudinally striate or verrucose ascospores, sometimes

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Table 2 Synopsis of Roussoellaceae and Thyridariaceae discussed in this paper

Morphological characteristics	Roussoellaceae	Thyridariaceae
Sexual morph		
Ascostromata	Subconical to hemispherical or subglobose, uni- to multi-loculate immersed in a large, black clypeus to erumpent, with minute papillate.	Globose to subglobose, immersed to erumpent, forming valsoid groups in brown prosenchymatous tissue with yellowish or reddish pigments around the prominent ostiolar neck forming a disc.
Asci	Frequently cylindrical, subcylindric-clavate in *Roussoellopsis*.	Frequently cylindric-clavate to clavate.
Ascospores	Didymosporous, frequently overlapping 1-seriate, brown to dark brown, ellipsoidal to fusiform, 1-euseptate, rough-walled, with longitudinally striations or verrucose ornamentation, minutely warty in *Roussoellopsis*.	Phragmosporous or dictyosporous, frequently overlapping 1–2-seriate, pale brown to brown, or dark brown, ellipsoidal to subfusoid, 1–3-euseptate, or distoseptate, or muriform (in *Thyridariella*), smooth-walled.
Asexual morph	Cytoplea-like (*Roussoella*), melanconiopsis-like or neomelanconium-like (*Roussoellopsis*), neoconiothyrium-like (*Pseudoneoconiothyrium*), paraconiothyrium-like (*Neoroussoella*)	Cyclothyrium-like or Cytoplea-like (*Thyridaria*), phoma-like (*Cycasicola*), camarosporium-like (*Liua*)
References	Liu et al. (2014); Phookamsak et al. (2014); Wanasinghe et al. (2018)	Hyde et al. (2013); Crous et al. (2014); Jaklitsch and Voglmayr (2016); Wijayawardene et al. (2017); Wanasinghe et al. (2018)

R. siamensis which have few differences in morphological characteristics of ascomata, pseudoparaphyses, asci, and ascospores (Liu et al. 2014). They can be distinguished based on asci and ascospore dimension and the number of locules in the ascostromata. The most effective tool to identify species of *Roussoella* is combined morphology and multi-gene phylogenetic analyses. Phylogenetic relationships of the genera in Roussoellaceae were not well resolved in previous studies (Jaklitsch and Voglmayr 2016; Tibpromma et al. 2017; Wanasinghe et al. 2018). Phylogenetic status of *Roussoellopsis* and *Neoroussoella* could not be resolved based on multi-gene phylogenetic analyses. *Roussoellopsis* and *Neoroussoella* often cluster with *Roussoella* species but these genera have different morphological characters (Liu et al. 2014; Phookamsak et al. 2014). Further taxon sampling of *Roussoellopsis* and *Neoroussoella* as well as more informative genes is needed to confirm their phylogenetic affinities.

Jaklitsch and Voglmayr (2016) mentioned that Thyridariaceae should be recognized as containing *Neoroussoella*, *Parathyridaria*, *Roussoella*, *Roussoellopsis*, and *Thyridaria* (synonymized Roussoellaceae under Thyridariaceae) based on multi-gene phylogeny. However, based on phylogenetic analyses in Tibpromma et al. (2017) and this study, Roussoellaceae and Thyridariaceae are placed under separate clades. Besides, the two families have distinct morphological characters. Roussoellaceae can be distinguished from Thyridariaceae based on their both sexual and asexual morphs (see Table 2). We, therefore, conclude that these two families are distinct following Tibpromma et al. (2017), Wanasinghe et al. (2018) and Devadatha et al. (2018). However, further studies based on informative molecular data coupled with morphological distinctiveness are needed for the confirmation of their phylogenetic affinities.

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An online resource for marine fungi

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Abstract
Index Fungorum, Species Fungorum and MycoBank are the key fungal nomenclature and taxonomic databases that can be sourced to find taxonomic details concerning fungi, while DNA sequence data can be sourced from the NCBI, EBI and UNITE databases. Nomenclature and ecological data on freshwater fungi can be accessed on http://fungi.life.illinois.edu/, while http://www.marinespecies.org/provides a comprehensive list of names of marine organisms, including information on their synonymy. Previous websites however have little information on marine fungi and their ecology, beside articles that deal with marine fungi, especially those published in the nineteenth and early twentieth centuries may not be accessible to those working in third world countries. To address this problem, a new website www.marinefungi.org was set up and is introduced in this paper. This website provides a search facility to genera of marine fungi, full species descriptions, key to species and illustrations, an up to date classification of all recorded marine fungi which includes all fungal groups (Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Mucoromycota and fungus-like organisms e.g. Thraustochytriales), and listing recent publications. Currently, 1257 species are listed in the marine fungi website (www.marinefungi.org), in 539 genera, 74 orders, 168 families, 20 classes and five phyla, with new taxa continuing to be described. The website has curators with specialist mycological expertise who help to provide update data on the classification of marine fungi. This article also reviews knowledge of marine fungi covering a wide range of topics: their higher classification, ecology and world distribution, role in energy transfer in the oceans, origin and new chemical structures. An updated classification of marine fungi is also included. We would like to invite all mycologists to contribute to this innovative website.

Keywords Fungal classification · marine fungi website · High-throughput sequencing techniques · Fungal diversity · Origin of marine fungi

Introduction

Marine fungi have been studied since the first record of the species *Sphaeria posidoniae* (= *Halotthia posidoniae*) on the rhizome of the sea grass *Posidonia oceanica* in Algeria by Durieu and Montagne (in Montagne 1856), but as yet there has been no webpage to accommodate all of the information on these organisms. This review introduces the website, www.marinefungi.org which has been developed to provide an up-to-date compendium on marine fungi.

There have been various definitions as to what a marine fungus is, the generally quoted one is by Kohlmeyer and Kohlmeyer (1979): "obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat". Jones et al. (2015) broadened this as they were of the opinion it was too narrow and they included marine derived fungi, as many are taxa isolated during bioprospecting for new secondary metabolites (Fenical and Jensen 1993; Fenical et al. 1998). Marine derived fungi are

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the discovery of taxa whose morphology has yet to be established. Progress has been made in determining their ecological role in a number of habitats, their physiological requirements, and interactions in the colonization of substrates in the sea. Marine fungi have yielded an array of interesting secondary metabolies, some in advance stage of clearance. Some taxonomical groups require more intense study especially the Chytridiomycota and their role in the colonization of planktonic organisms. It is hoped that greater interaction between their study by traditional means and by high through put sequencing can be established to enable a better understanding of the global diversity of marine fungi.

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Appendix

List of marine fungi logged in the marine fungi website

Taxa with the prefix * are asexual morphs whose sexual stage is unknown; # indicates molecular data available for these fungi.
Taxa in underline text are new taxa in press.
Phylum: **BASIDIOMYCOTA**
Subphylum: **Ustilaginomycotina**
Class: **Ustilaginomycetes** R. Bauer, Oberw. & Vnky, Can. J. Bot. 75: 1311 (1997)
Subclass: **Ustilaginomycetidae** Jlich, Bibliotheca Mycologica 85: 54 (1981)
1. UROCYSTIDALES R. Bauer & Oberw., Can. J. Bot. 75 (8): 1311 (1997)
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Neopestalotiopsis alpapicalis sp. nov. a new endophyte from tropical mangrove trees in Krabi Province (Thailand)

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Abstract

Mangrove forests are dynamic systems primarily confined to tropical and subtropical coastal regions. Mangroves are highly complex habitats sustaining a diverse array of terrestrial and aquatic fungal species. Endophytic fungi are widely distributed in mangrove ecosystems and are integral contributors to global biodiversity. *Neopestalotiopsis* species occur as endophytes, saprobes and opportunistic pathogens of many plant hosts. Herein, a new species of *Neopestalotiopsis*, *N. alpapicalis*, was collected from the mangrove trees *Rhizophora apiculata* and *Rhizophora mucronata,* in Krabi, Thailand. Morphological features conform to those of *Neopestalotiopsis.* Number of apical appendages and size of apical cells of the newly described species differ from those of phylogenetically related species. A combined dataset of ITS, β-tub and TEF1 genes was used to infer the phylogenetic placement of the new species. The two strains of novel species, *N. alpapicalis* clustered together and have a close affinity to *N. rosicola.*

Key words: 1 new species, fungi, multigene phylogenetic analysis, opportunistic pathogen, *Sporocadaceae*, taxonomy

Introduction

Mangrove forests can be defined as woody trees and shrubs that grow in places, where river water mixes with seawater and are highly diverse habitats in tropical and subtropical coastal regions (Chaeprasert *et al.* 2010, Hamzah *et al.* 2018). Singh *et al.* (2014) estimated that 41.4% of global mangroves are found in South and Southeast Asia. In Thailand, mangrove forests populate the southern coastal region. *Rhizophoraceae* is the most abundant mangrove tree family and comprises four genera: *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora* (Polidoro *et al.* 2010). Of these, members of the genus *Rhizophora* have the widest distribution in the coastal areas of southern Thailand (Duke 2005, Pumijumnong 2014). *Rhizophora apiculata* and *R. mucronata* are the dominant plant species in mangrove forests of Thailand (Yan *et al.* 2016). Several studies have shown that *Rhizophora* spp. are hosts to fungal endophytes (Chaowalit 2009, Buatong 2010, Chaeprasert *et al.* 2010, Sakayaroj *et al.* 2010, Doilom *et al.* 2017). Endophytic fungi live within different tissues of the host plant without causing disease (Aly *et al.* 2011, Doilom *et al.* 2017, Kumar *et al.* 2018). It has been suggested that endophytes comprise a highly diverse group assisting plants to adapt to biotic and abiotic stress factors (Hartley *et al.* 2015, Amin 2016, Potshangbam *et al.* 2017). Common fungal genera found as endophytes from mangroves are *Colletotrichum*, *Diaporthe*, *Fusarium*, *Neopestalotiopsis*, *Phomopsis* and *Trichoderma* (Suryanarayanan *et al.* 2002, Cheng *et al.* 2009, Udayanga *et al.* 2011, de Souza Sebastianes *et al.* 2013). Nonetheless, only a few studies focus on identification of mangrove-derived endophytic fungi up to species level (Chaeprasert *et al.* 2010, Abraham *et al.* 2015, Thomas *et al.* 2016, Kumar *et al.* 2018). Zhou *et al.* (2018) reported that *Diaporthe* spp., *Neopestalotiopsis protearum*,

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Material examined: THAILAND, Krabi Province, fresh asymptomatic leaves of *Rhizophora mucronata* (Rhizophoraceae), 22 Sept. 2017, Vinit Kumar, RM5 (MFLU 19-0405), holotype, ex-type culture MFLUCC 17-2544), ibid. BBH isotype.

***Notes*:**—*Neopestalotiopsis alpapicalis* (MFLUCC 17-2544) falls within *Neopestalotiopsis* and forms a separate branch with a sister strain, MFLUCC 17-2545 (FIG. 1). *N. alpapicalis* is morphologically similar to *N. samarangensis*. However, *N. samarangensis* has 3 tubular apical appendages, which are long and thin, while *N. alpapicalis* has 1–4 short and thick apical appendages (Maharachchikumbura *et al.* 2013a). Along with this, *N. alpapicalis* has smaller apical cell as compared to other species within *Neopestalotiopsis* (Maharach. *et al.* 2014). When comparing the 472 TEF1 nucleotides of *N. alpapicalis* with other *N. rosicola* in the clade, there are only 6 bp differences, 5 bp differences in ITS, and 2 bp difference in β-tub. Morphological characters of the two strains of *N. alpapicalis* (MFLUCC 17-2544 and MFLUCC 17-2545), such as, the size of conidia, are often overlapping, but differ based on the size of apical cells, 0.75–2.3 ($\bar{x}$ = 1.25 µm) in MFLUCC 17-2544 and 2.0–4.0 ($\bar{x}$ = 2.75 µm). The sister taxon, *N. rosicola* (CFCC 51992, CFCC 51993), is different from *N. alpapicalis* in terms of conidial size and in the number and size of apical appendages (1–4 in *N. alpapicalis* and 2–4 in *N. rosicola*), while the size of apical appendages varies from 17–22.8 µm ($\bar{x}$ = 20.5 µm) in *N. rosicola* and 5.6–15 µm ($\bar{x}$ = 9.3 µm) in *N. alpapicalis* (Crous *et al.* 2012, Maharach. *et al.* 2014, Jiang *et al.* 2018).

Discussion

Herein, we introduce a new species *Neopestalotiopsis alpapicalis* (MFLUCC 17-2544 and MFLUCC 17-2545) within the genus *Neopestalotiopsis*. The genus *Neopestalotiopsis* (Maharachchikumbura *et al.* 2014) was separated from *Pestalotiopsis,* based on versicolor median cells of conidia and placed within *Amphisphaeriaceae* (Maharach. *et al.* 2014). Senanayake *et al.* (2015) introduced *Pestalotiopsidaceae* Maharach. (2015) as a separate family. Jaklitsch *et al.* (2016) synonymized *Pestalotiopsidaceae* to *Sporocadaceae.* Species of *Neopestalotiopsis* are characterized by their conidia with versicolor median cells and by indistinct conidiogenous cells (Maharach. *et al.* 2014). Furthermore, ITS sequences of *Neopestalotiopsis* have a distinct gap of ~20 bp located within the first 120 nucleotides. This gap is absent in *Pestalotiopsis* isolates and is useful for distinguishing *Neopestalotiopsis* species.

Both newly described strains have versicolorous conidia and median cells characteristic of *Neopestalotiopsis* (Maharach. *et al.* 2014). However, *N. alpapicalis* is distinguished from the other *Neopestalotiopsis* species based on number of conidial appendages and morphologically distinct apical cell (refer to the notes of the new species, FIG.2). The new isolates group together in the phylogenetic tree, but the relationship is not strongly supported, likely because of the short TEF1 sequence of *N. alpapicalis* (MFLUCC 17-2545) (FIG. 1). Along with this, we observed a 6 bp (1.27%) difference in the TEF1 alignment, 5bp in the ITS and 2 bp in β-tub when comparing *N. alpapicalis* with *N. rosicola* (details can be seen in the notes part). In general, the evolutionary relationships among species of *Neopestalotiopsis* are not robust and remain largely unresolved (Maharach. *et al.* 2014). This is evident herein and previous studies, whereby phylogenetic analyses resulted in trees with a variable number of polytomies and low statistical support at most nodes (Maharach. *et al.* 2014, Jayawardena *et al.* 2016, Jiang *et al.* 2018, Tibpromma *et al.* 2018). Nonetheless, *N. alpapicalis* along with *N. aotearoa, N. piceana, N. rosicola* and *N. samarangensis* consistently grouped together across all analyses (FIG.1). The latter four taxa also group together in previous studies (Tibpromma *et al.* 2018, Jiang *et al.* 2018).

Collectively, these results strongly suggest that the currently used genes might not be adequate to resolve relationships within the genus and that different and/or additional genes are necessary to properly delineate *Neopestalotiopsis* species.

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Rhytidhysteron mangrovei (*Hysteriaceae*), a new species from mangroves in Phetchaburi Province, Thailand

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Abstract

During an investigation of micro-fungi inhabiting mangrove forests, a new species of *Rhytidhysteron* was collected and isolated from dead twigs of a mangrove tree. *Rhytidhysteron mangrovei* sp. nov. is introduced, described, illustrated and compared with accepted species in the genus. Morphological comparison based on the size of exciple, the appearance of ascomata and ascospore septations and size as well as the multi-gene phylogenetic analyses based on LSU, ITS and TEF DNA sequences support its establishment in *Rhytidhysteron*. Placement of the genus in *Hysteriaceae* is also well-supported. In addition, phylogenetic analysis and DNA sequence data indicate that *Rhytidhysteron mangrovei* is closely related to *Rhytidhysteron thailandicum*. However, *R. mangrovei* is morphologically distinct from *R. thailandicum*, by having a relatively smaller size of exciple and perpendicularly rough-striate ascomata.

Keywords: 1 new species, Dothideomycetes, phylogenetics, saprobic, taxonomy

Introduction

The family *Hysteriaceae* was established by Chevallier (1826) as '*Hysterineae*'. Previously, the family has been classified under Pseudosphaeriales, Dothiorales, Dothideales, and Pleosporales, now within Hysteriales (Gäumann 1949, Müller & von Arx 1950, von Arx & Müller 1975, Barr 1987, Luttrell 1955, Kirk *et al.* 2001, 2008). *Hysteriaceae* is characterized by the presence of carbonaceous hysterothecium, navicular, characteristically dehiscing by an invaginated slit or sulcus, immersed to superficial, bitunicate asci and hyaline when immature to pigmented when mature, one to multi-septate, or muriform ascospores (Zogg 1962, Hyde *et al.* 2013, Ertz & Diederich 2014, Thambugala *et al.* 2016). Currently, there are 14 genera within this family (Wijayawardene *et al.* 2018). However, *Actidiographium*, *Gloniella*, *Hysterocarina,* and *Hysteropycnis* lack molecular data and hence their placement warrants further investigations (Boehm *et al.* 2009a, b, Jayasiri *et al.* 2018, Wijayawardene *et al.* 2018).

The genus, *Rhytidhysteron* was introduced by Spegazzini (1881) to accommodate *R. brasiliense* and *R. viride* and is typified by *R. brasiliense* (Spegazzini 1881, Silva-Hanlin & Hanlin 1999). The genus includes saprobic to weakly pathogenic fungi that grow on woody plants in terrestrial habitats (Yacharoen *et al.* 2015). Recently the genus was identified as a rare human pathogen, which causes subcutaneous mycoses in immunocompetent patients and is also associated with phaeohyphomycosis, a mycotic infection (Mishra *et al.* 2014, Chander *et al.* 2017). *Rhytidhysteron rufulum* has been reported as endophytes and is able to produce bioactive compounds, such as spirobisnaphthalenes

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Morphological and phylogenetic characterization of novel pestalotioid species associated with mangroves in Thailand

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Abstract

Pestalotioid fungi are associated with a wide variety of plants worldwide and are endophytes, pathogens and saprobes. The present study provides an updated phylogenetic placement of *Neopestalotiopsis*, *Pestalotiopsis* and *Pseudopestalotiopsis* using fresh collections from mangrove plants in Thailand. Twelve novel species are characterized based on combined sequence data analyses of internal transcribed spacer (ITS), beta tubulin (β-tubulin) and translation elongation factor 1-alpha (EF1α) coupled with morphological characters. The taxonomy and phylogenetic relationships of pestalotioid-fungi are reappraised with suggestions for future work.

Keywords – 12 new species – Asymptomatic leaves – *Acrostichum aureum* – *Avicenia marina* – Leaf spots – Mangroves – *Neopestalotiopsis* – *Pestalotiopsis* – pestalotiopsis-like – Phylogeny – *Pseudopestalotiopsis* – *Rhizophora* sp. – *Sonneronata alba* – Taxonomy

Introduction

Mangrove forests are one of the most productive ecosystems which play a major role in the ecological communities in coastal tropical and subtropical waters (Hyde & Lee 1995). They serve as hatcheries and nursery habitats for marine organisms and protect coastlines from catastrophic events such as storms and tidal surges (Hyde & Jones 1988, Fisher & Spalding 1993, Hyde & Lee 1995, Hyde et al. 1998). The greatest diversity of mangrove species occurs in Indonesia, Malaysia and Thailand, which also harbour a great diversity of micro-organisms especially fungi (Alias & Jones 2009, Alias et al. 2010). However, the phylogenetic and functional description of microbial diversity in mangrove ecosystems has not been addressed to the same extent as for other environments. Even though mangrove ecosystems are very rich in microbial diversity, less than 5% of species are believed to have been described (Thatoi et al. 2012). Recently developed technologies in molecular biology and genetics offer a great promise to explore the potential of microbial diversity. Hence, the present paper makes an effort to identify the species of fungi in

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Table 5 Comparison of conidia of *Pseudopestalotiopsis* species related to this study.

Species	Strain	Conidial size (µm)	Apical appendages (µm)		Basal appendage (µm)
			Number	Length	
Ps. ampullacea[b]	LC6618	21–31.5 × 6.5–9	2–3	17–25	3.5–7
Ps.avicenniae	MFLUCC 17-0434	(22–)22.5–26.5(–27) × (5–)5.5–6(–6.4)	1–3	(14–)15.5–28.5(–35.5)	(2–)3–4(–4.5)
Ps. chinensis[b]	LC3011	25.5–35.5 × 6–9	2–3	24–41	5–12
Ps.rhizophorae	MFLUCC 17-1560	(18.5–)22–25(–26.5) × (6.2–)6.5–7(–7.2)	1–2	(10.2–)20–29(–35)	(5.5–)9–12(–13.5)
Ps. dawaina[d]	MM14-F0015	22–31 × 8–9.5	3	20.5–33.5	2.5–6.5
Ps. elaeidis (=*Ps. myanmarina*)[c]	NBRC 112264	31–38.5 × 6.5–9	2–3	22.5–38.5	unbranched
Ps. jiangxiensis[b]	LC4479	22–29 × 6–9	2–4(3)	16.5–32	6.5–19.5
Ps. kawthaungina[d]	MM14-F0083	29.5–34.5 × 7–9	3	28–41	4.5–9
Ps. kubahensis[a]	UMAS KUB-P20	(26–)27–30(–33) × 5.6–7.3	2–4(3)	15.9–29.4	3.1–6.0
Ps.curvatispora	MFLUCC 17-1722/ MFLUCC 17-1723	(18.6–)19–26(–26.4) × (5.5–)6–7(–7.4)	2–3	(5.5–)6–24(–26.6)	(5.8–)5–11(–12.2)
Ps. simitheae[e]	MFLUCC 12-0121	22–30 × 5–6.5	2–4	14.5–26.5	4–6.5
Ps. taiwanensis[f]	NTUCC 17-002.1	21–26 × 6–7	2–5	16–25	3–7
Ps. thailandica	MFLUCC 17-1724/ MFLUCC 17-1725	(24–)24.5–30(–30.5) × (5–)5.5–6(–6.7)	1–3	(26.5–)28–36(–39.5)	(3.5–)4.5–5(–6.6)

Strain in this study are in bold.
[a]Lateef et al. (2015); [b]Liu et al. (2017); [c]Nozawa et al. (2017); [d]Nozawa et al. (2018); [e]Song et al. 2014; [f]Tsai et al. (2018).

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Families in *Botryosphaeriales*: a phylogenetic, morphological and evolutionary perspective

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Abstract

Botryosphaeriales was introduced in 2006 for a single family *Botryosphaeriaceae*. Since then the number of families has increased as a result of the transfer of one family (*Planistromellaceae*) into the order, re-instatement of another (*Phyllostictaceae*), while others resulted from raising genera to family status (*Aplosporellaceae*, *Endomelanconiopsisaceae*, *Melanopsaceae*, *Pseudofusicoccumaceae*, *Saccharataceae* and *Septorioideaceae*). All these decisions were based solely on phylogenetic analyses of several different loci. There has been no consensus on which loci are suitable markers at this taxonomic level and in some cases the datasets used to construct the phylogenies were incomplete. In this paper, the families of *Botryosphaeriales* were re-assessed in terms of morphology of the sexual morphs, phylogenetic relationships based on ITS and LSU sequence data, and evolutionary divergence times of lineages in relation to major events in the evolution of their hosts on a geological timescale. Six main lineages were resolved in the phylogenetic analyses and these correspond to six groups as defined on morphology of the sexual morphs. These lineages evolved during the Late epoch of the Cretaceous period and survived the catastrophic event that led to the mass extinction of non-avian dinosaurs and a great loss of plant diversity at the end of the Cretaceous period. They then diversified during the Paleocene and Eocene epochs of the Paleogene period. These six lineages are considered to represent families in *Botryosphaeriales*. Therefore, six families (*Aplosporellaceae*, *Botryosphaeriaceae*, *Melanopsaceae*, *Phyllostictaceae*, *Planistromellaceae* and *Saccharataceae*) are accepted in *Botryosphaeriales*, while three (*Endomelanconiopsisaceae*, *Pseudofusicoccumaceae* and *Septorioideaceae*) are reduced to synonymy under existing families.

Keywords Classification · Dothideomycetes · Evolutionary divergence times · Morphology

Introduction

The taxonomic history of *Botryosphaeriaceae*, introduced by Theisen and Sydow (1918) for *Botryosphaeria*, *Dibotryon* and *Phaeobotryon*, has been recalled in detail by Phillips et al. (2013). Briefly, the ordinal placement of this family has been the subject of much debate having been assigned to Pleosporales (Luttrell 1955; Barr 1979, 1987; Eriksson 1981) or Dothideales (Miller 1928; Barr 1972, 1976; von Arx and Müller 1975; Sivanesan 1984). It was only after the advent of the application of DNA sequence analysis to resolve phylogenetic relationships that the ordinal placement of the family was resolved.

In a multi-locus phylogeny for 96 taxa in the Dothideomycetes (Schoch et al. 2006) species of *Botryosphaeria* and *Guignardia* formed a clade that could not be associated with any known order. For that reason, Schoch

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underwent a remarkable adaptive radiation to occupy empty ecological niches. After the initial fern spike, which lasted for about 30,000 years, angiosperm abundance recovered to near Cretaceous levels (Vajda et al. 2001) accompanied by the appearance of newly evolved taxa that gave rise to modern day plants. It can be hypothesised that families in *Botryosphaeriales* that survived the mass extinction diversified on the Paleogene Angiosperms giving rise to what are now considered to be genera that adapted to the hosts that evolved especially during the Eocene epoch of the Paleogene period.

In this paper the families in *Botryosphaeriales* were re-evaluated in terms of morphology of their sexual states, phylogenetic relationships and evolutionary divergence times. The six main phylogenetic lineages resolved correlate with six groups defined on morphology of their sexual states. These six lineages survived the mass extinction event that occurred around 66 Mya and diversified on the surviving host plants, which are the ancestors of modern plants, and subsequently diverged into genera. We believe that these lineages represent families and for this reason we accept six families in *Botryosphaeriales* and reduce three others to synonymy. All six families are supported by morphology, phylogeny and evolutionary divergence times.

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Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi

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Abstract

This article is the ninth in the series of Fungal Diversity Notes, where 107 taxa distributed in three phyla, nine classes, 31 orders and 57 families are described and illustrated. Taxa described in the present study include 12 new genera, 74 new species, three new combinations, two reference specimens, a re-circumscription of the epitype, and 15 records of sexual-asexual morph connections, new hosts and new geographical distributions. Twelve new genera comprise *Brunneofusispora*, *Brunneomurispora*, *Liua*, *Lonicericola*, *Neoeutypella*, *Paratrimmatostroma*, *Parazalerion*, *Proliferophorum*, *Pseudoastrosphaeriellopsis*, *Septomelanconiella*, *Velebitea* and *Vicosamyces*. Seventy-four new species are *Agaricus memnonius*, *A. langensis*, *Aleurodiscus patagonicus*, *Amanita flavoalba*, *A. subtropicana*, *Amphisphaeria mangrovei*, *Baorangia major*, *Bartalinia kunmingensis*, *Brunneofusispora sinensis*, *Brunneomurispora lonicerae*, *Capronia camelliae-yunnanensis*, *Clavulina thindii*, *Coniochaeta simbalensis*, *Conlarium thailandense*, *Coprinus trigonosporus*, *Liua muriformis*, *Cyphellophora filicis*, *Cytospora ulmicola*, *Dacrymyces invisibilis*, *Dictyocheirospora metroxylonis*, *Distoseptispora thysanolaenae*, *Emericellopsis koreana*, *Galiicola baoshanensis*, *Hygrocybe lucida*, *Hypoxylon teeravasati*,

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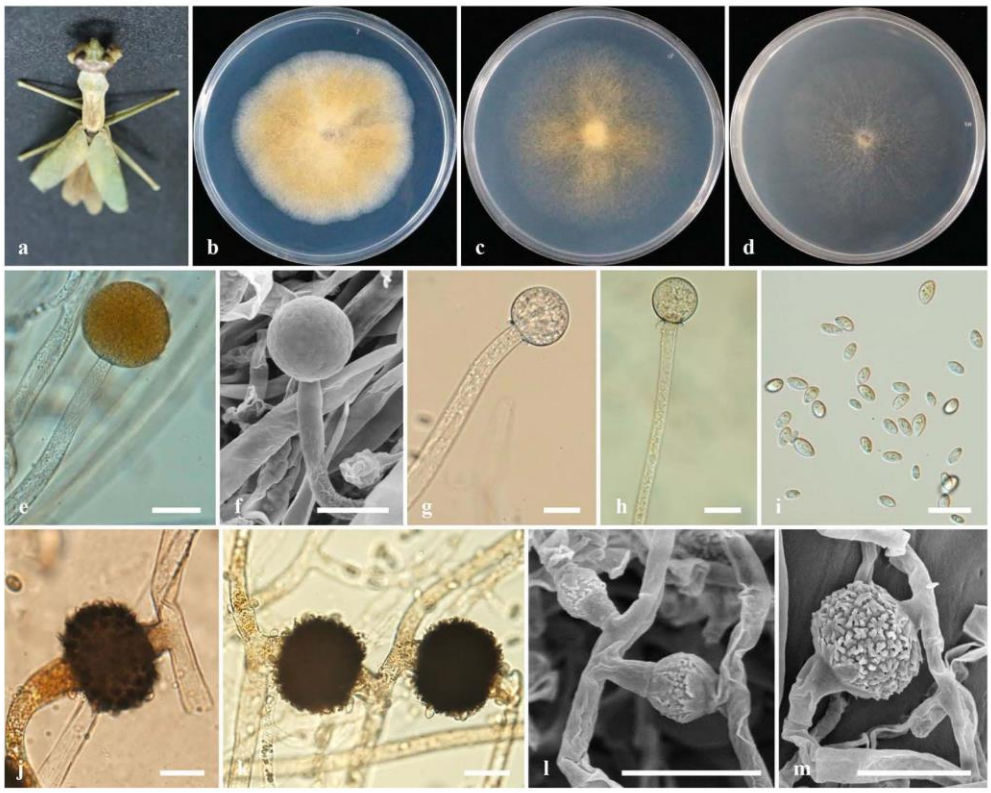

Fig. 178 ***Mucor orantomantidis*** (CNUFC-MID1-1, **holotype**). **a** A praying mantis. **b** Colony in potato dextrose agar. **c** Colony in synthetic mucor agar. **d** Colony in malt extract agar. **e, f** Sporangiophores and sporangia. **g, h** Columellae with collarette. **i** Sporangiospores. **j–m** Zygospores. (**e, g–k:** LM; **f, l, m:** SEM). *Scale bars* **e–k** = 20 μm, **l, m** = 50 μm

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RESEARCH ARTICLE

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Striatiguttulaceae, a new pleosporalean family to accommodate *Longicorpus* and *Striatiguttula* gen. nov. from palms

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Abstract

Palms represent the most morphological diverse monocotyledonous plants and support a vast array of fungi. Recent examinations of palmicolous fungi in Thailand led to the discovery of a group of morphologically similar and interesting taxa. A polyphasic approach based on morphology, multi-gene phylogenetic analyses and divergence time estimates supports the establishment of a novel pleosporalean family Striatiguttulaceae, which diversified approximately 39 (20–63) MYA (crown age) and 60 (35–91) MYA (stem age). Striatiguttulaceae is characterized by stromata or ascomata with a short to long neck, trabeculate pseudoparaphyses and fusiform to ellipsoidal, 1–3-septate ascospores, with longitudinal striations and paler end cells, surrounded by a mucilaginous sheath. Multi-gene phylogenetic analysis showed that taxa of Striatiguttulaceae form a well-supported and distinct monophyletic clade in Pleosporales, and related to Ligninsphaeriaceae and Pseudoastrosphaeriellaceae. However, these families can be morphologically demarcated by the slit-like ascomata and extremely large ascospores in Ligninsphaeriaceae and the rather narrow fusiform ascospores in Pseudoastrosphaeriellaceae. Eight strains of Striatiguttulaceae formed two monophyletic sub-clades, which can be recognized as *Longicorpus* **gen. nov.** and *Striatiguttula* **gen. nov.** Morphologically, the genus *Longicorpus* can be differentiated from *Striatiguttula* by its elongated immersed

The nature of the pseudoparaphyses (*sensu* Liew et al. 2000) is worth considering here and may provide evidence for separate lineages. The family Striatiguttulaceae, currently with three species, have trabeculate pseudoparaphyses, but also appearing septate. Phylogenetically closely related families of Ligninsphaeriaceae and Pseudoastrosphaeriellaceae are characterized by cellular pseudoparaphyses and trabeculate pseudoparaphyses respectively.

Considering the ecology of these Striatiguttulaceae species in relation to the mangrove ecosystem, salinity may be an important contributor to their presence. Loilong et al. (2012) have compared fungal community from *Nypa fruticans* at different salinities, and found freshwater species in lower salinity and marine species at higher salinity. Although no salinity was measured during our collections, *Longicorpus striataspora*, *Striatiguttula nypae* and *S. phoenicis* can be considered as manglicolous, because they are found from decayed rachides/petioles of palms, which are perennials submerged in soft mangrove mud and salty water, and well adapted to the varying salinity in mangroves by tidal water. On the other hand, their ascospores have mucilaginous sheaths and lack elaborate appendages, which are also typical characteristics of most mangrove fungi (Jones 2000).

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Biodiversity, phylogeny and role of fungal endophytes on above parts of *Rhizophora apiculata* and *Nypa fruticans*

By Kevin David Hyde

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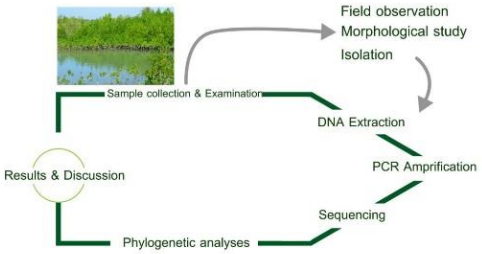
There are 1.5 million species of Fungi, with only 15% presently known. The fungi of aerial parts of mangroves are poorly known and in this study we investigate their biodiversity on two selected mangrove trees namely, *Rhizophora apiculata* and *Nypa fruticans*. Accurate identification of fungi can be done with the use of DNA sequence data up to genera and species level. We thoroughly assess the biodiversity, phylogeny and relationship of endophytes, saprobes and pathogens in the canopies of two mangrove hosts using morphology and molecular techniques. Fungal biodiversity and its role is poorly understood in these mangrove habitats. So, our study is the first comprehensive treatment of fungal biodiversity and its role. Our study will also reveal cryptic mangrove species in several pathogenic fungal groups and provide new phylogenetic data on higher fungal groups. Mangrove are the ideal habitat as they are less disturbed than other forest types and *Nypa* palm is an ancient plant.

OBJECTIVES

- To establish the biodiversity of fungi on the aerial parts of two mangrove species.
- To establish the percentage of new species as compared to known species and to describe all new species.
- To identify endophytic isolates and establish their phylogenetic relationships at different taxonomic levels based on DNA sequence data.
- To prepare herbarium material of all collections for future reference.
- To sequence appropriate genes of phylogenetically well-studied genera to identify cryptic species within species complex.
- To investigate evolutionary relationships of unidentified endophytes, poorly studied genera and new species based on a polyphasic approach.
- To establish if any species occupy more than one life.

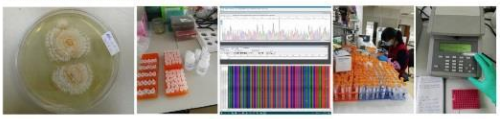
METHODS



COLLECTION TRIP & FIELD OBSERVATION

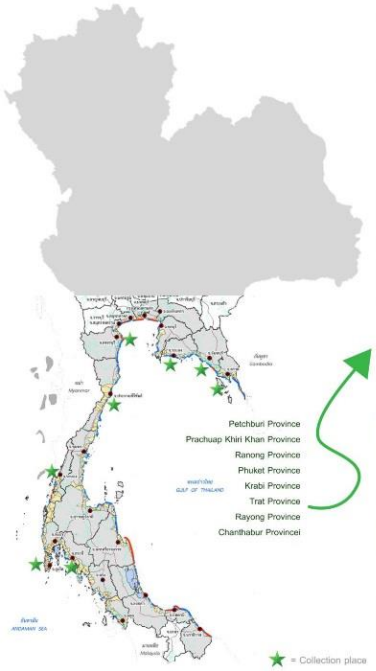
MORPHOLOGICAL STUDY

PHYLOGENETIC STUDY



PROGRESS & RESULTS

- More than 200 isolations, which includes endophytes, saprobes, pathogens.
- More than 150 strains have been identified and ITS gene region was considered as a prime identification gene.
- The taxa were identified as a members of these families (such as *Xylariaceae, Diatrypaceae, Halosphaeriaceae, Lulworthiaceae*) and genera (*Colletotrichum, Diaporthe, Hypoxylon, Neopestalotiopsis, Neodevriesia, Pestalotiopsis* and *Phyllosticta*).



- In the future more populations are yet to be discovered by employing Next gen sequencing method (NGS).

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ACKNOWLEDGEMENTS

We would like to thank the Thailand Research Fund (TRF) grant no RSA5980068 entitled "Biodiversity, phylogeny and role of fungal endophytes on above parts of *Rhizophora apiculata* and *Nypa fruticans*" for the financial support. We are appreciative the staff of Mangroves Forest Research Center at Ranong province, and thankful to the staff at Ao Kung Krabaen Bay Nature Center, The Sirindhorn International Environment Park, Sirinart Rajini Ecosystem Learning Center and Ngao Mangrove Forest Research Cente for giving us the knowledge, and granting us permission to study and collect mangrove specimens.

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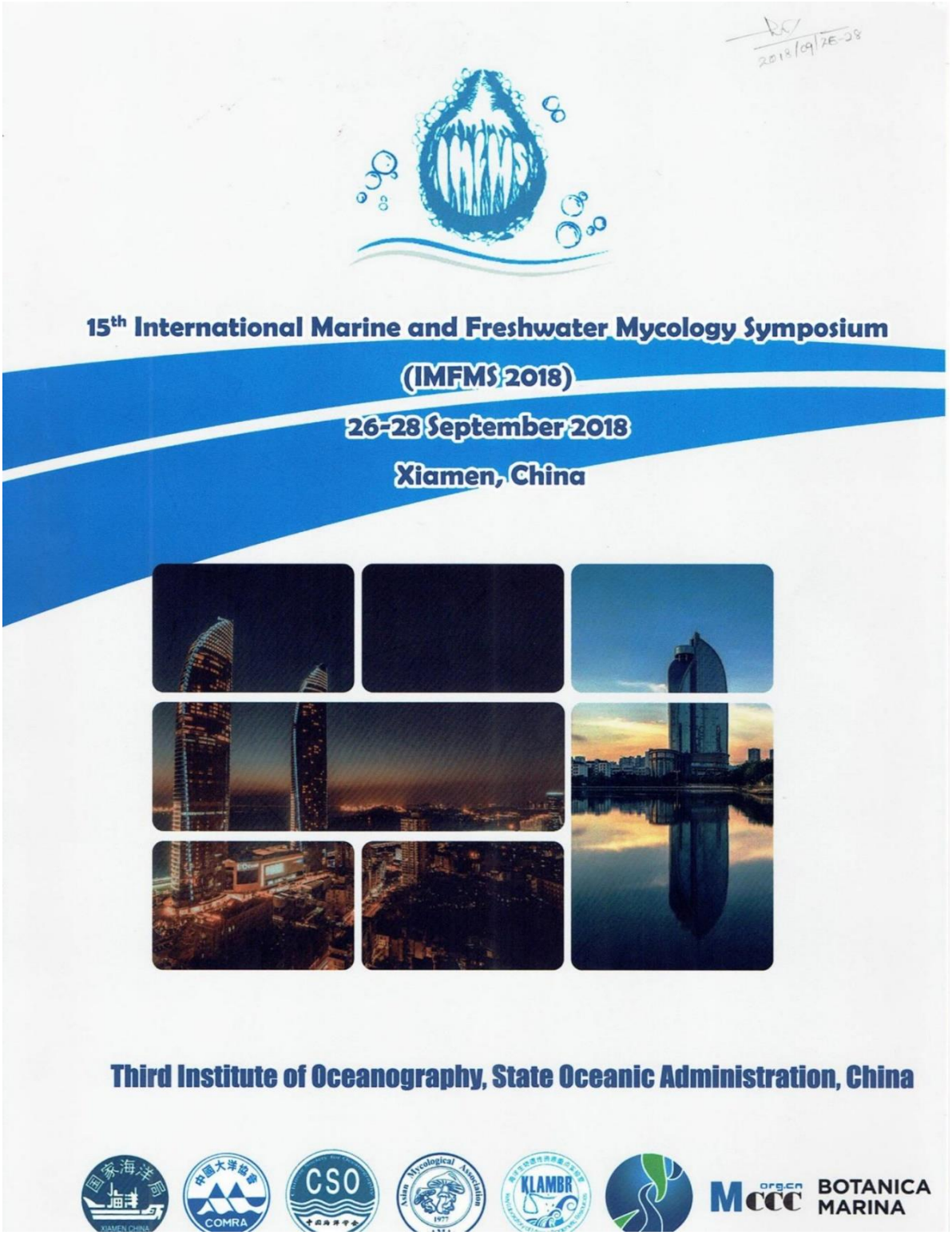
Conferences at 15th International Marine and Freshwater Mycology Symposium (IMFMS), Xiamen, China



As we embark on the 15th meeting of this conference series it is timely to provide a short introduction to this organisation. Willy Höhnk was responsible for this unique conference devoted to the study of marine fungi as the International Marine Mycology Symposium (IMMS) at the Instituts fűr Meeresforschung, Bremerhaven, Germany in October 1966. This was an imaginative concept and greatly furthered interest in marine fungi by bringing together mycologists from around the world. That it has continued for over 50 years shows the continued interest in the study of this ecological group of fungi. This initial meeting was followed by a well support meeting in September 1972 with many eminent mycologists attending: Fred Sparrow, Sam Meyers, Jan Kohlmeyer, Gill Hughes, Paul Kirk, E. Schnepe, Guy Willoughby and Alwin Gaertner (who organised the event). It also saw attendance by a number of young marine mycologists: David Porter, Frank Perkins and Gareth Jones. Over the next 20 years the conference attracted wider interest with venues in Morehead City, US (1979), Portsmouth, UK (1985), Vancouver, Canada (1989), Portsmouth, UK (1995) and Hong Kong, China (1999). At the meeting of Hong Kong, China, it was decided to include freshwater fungi and the meeting attract over 200 participants. In 2001 the meeting was hosted in Hurgada, Egypt and subsequent conferences were held in Asia: Chiang Mai, Thailand (2004), Penang, Malaysia (2007), when the group held a joint meeting with the Asian Mycological Association. The 11th IMFMS was held in Taichung (2009) and saw a revival with excellent attendance (over 200 participants). The next three meetings were held in Incheon, South Korea (2011), Beijing, PR China (2013), Goa, India (2015) with a meeting of the Marine Fungal Natural Products Consortium (MaFNaP) in Nantes, France (2015). Sadly, attendance has slipped and greater effort must be made to rejuvenate the conference. You may learn more about this conference at www.marinefungi.org.

E.B.Gareth Jones

This conference is organized by Third Institute of Oceanography, State Oceanic Administration, China.

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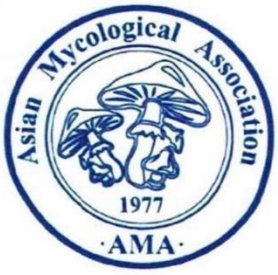

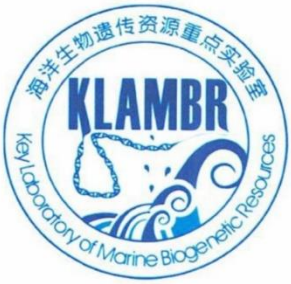

BOTANICA MARINA

Tuesday, 25th September

14:00-21:00 Registration

Wednesday, 26th September

08:00-09:00 Registration

09:00-09:30 Welcome remarks

09:30-10:10 Group photo & Tea break

Session 1: Diversity of aquatic fungi (Chairman: E.B. Gareth Jones and Guang-Yi Wang)

10:10-10:55 **Keynote lecture 1- E.B.Gareth Jones (KL1):** How many marine fungi: Biodiversity and Distribution

10:55-11:15 **Xuefeng Peng (SL1):** The ecology and size-fractioned diversity of fungi in the eastern tropical North Pacific oxygen minimum zone

11:15-11:35 **Hong-Mei Wang (SL2):** Investigation of fungal communities in a karst cave and the interaction between fungi and minerals

11:35-11:55 **Zong-Long Luo (SL3):** Lignicolous freshwater fungi of the Greater Mekong Subregion

12:00-14:00 Lunch break

14:00-14:45 **Keynote lecture 2- Guang-Yi Wang (KL2):** Impact of environmental factor on the dynamic and diversity of planktonic fungi in coastal environments

14:45-15:05 **Satinee Suetrong (SL4):** Community structure and distribution of Thai marine manglicolous fungi

15:05-15:25 **Sheng-Nan Zhang (SL5):** Investigation of palm fungi from marine (mangrove) ecosystems

15:25:-15:50 Tea break

Session 2: Taxonomy and Phylogeny of aquatic fungi (Chairman: Lei Cai and Zong-Hua Wang)

15:50-16:35 **Keynote lecture 3-Lei Cai (KL3):** Ocean currents shape the fungal distribution in East China Sea

16:35-16:55 **Monika Chandani Dayarathne (SL6):** Phylogenetic revision and evolution of Savoryellaceae and evidence for its ranking as a subclass

16:55-17:15 **Sung-Yuan Hsieh (SL7):** An ultrastructural study of sporangial development in Pythiogeton spp. in Taiwan

17:15-17:35 **Yong-Zhong Lu (SL8):** A taxonomic reassessment of helicosporous hyphomycetes from freshwater habitats

17:35-17:55 **Huang Zhang (SL9):** Towards a natural classification of *Annulatascaceae*-like taxa; introducing one new order and six new families.

18:30 Welcome Dinner

Thursday, 27th September

09:00-09:45 **Keynote lecture 4- Zong-Hua Wang (KL4):** Preliminary genomic study of marine fungi: A case study-using *Engyodontium ablum* as a

model

09:45-10:05 **Jing Yang (SL10):** Phylogenetic placement of *Cryptophiale, Cryptophialoidea, Nawawia* and *Phialosporostilbe* in Chaetosphaeriaceae

10:05-10:30 Tea break

Session 3: Community study of marine fungi (Chairman: Brandon Hassset)

10:30-11:15 **Keynote lecture 5-Brandon Hassett (KL5):** Marine Chytridiomycota: an Arctic case study

11:15-11:35 **Maxence Quemener (SL11):** Delving deeper into the diversity of fungal microorganisms in the uncharted sub-seafloor lower crust using different culturing techniques

11:35-11:55 **Kalyani Sen (SL12)**: Abundance and diversity of planktonic fungi in the waters of South China Sea

12:00-14:00 Lunch break

Session 4: Fungi of the extreme marine habitats (Chairman: Gaëtan Burgaud and Yuriko Nagano)

14:00-14:45 **Keynote lecture 6-Gaëtan Burgaud (KL6):** Studying (deep-sea) marine fungi through the meta-Omics prism to highlight their diversity, activity, ecological roles and biotechnological potential

14:45-15:05 **Ka-Lai Pang (SL13):** Diversity of fungi at the hydrothermal vent ecosystem at Gueishan Island, Taiwan

15:05-15:50 **Keynote lecture 7-Yuriko Nagano (KL7):** Fungal diversity in deep-sea environments

15:50-16:10 **Wei Xu (SL14):** Fungal diversity in the deep sea hadal sediments of the Yap Trench by cultivation and high throughput sequencing methods based on ITS rRNA gene

16:10-16:40 Tea break

Session 5: Natural substances of marine fungi (Chairman: Anake Kijjoa and Bin-Gui Wang)

16:40-17:25 **Keynote lecture 8-Bin-Gui Wang (KL8):** Chemical diversity and bioactivity of metabolites from marine derived fungi

17:25-17:45 **Panida Unagul (SL15):** Biotechnology production and application of the DHA-producing thraustochytrids for aquaculture

18:30 Dinner

Friday, 28th September

09:00-09:45 **Keynote lecture 9- Anake Kijjoa (KL9):** Bioactive secondary metabolites from marine-derived fungi collected in the Gulf of Thailand and the Andaman Sea (Anake Kijjoa)

09:45-10:05 **Bin Wu (SL16):** Structural characterization of two novel quinoline alkaloids with antibiotic activities from a hydrothermal vent sulfur-derived Fungus *Penicillium citrinum* Y43

10:05-10:25 **Lan Liu (SL17):** Marine fungal resources, from secondary metabolites to catalysts

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Abstracts
Keynote Lectures

KL 1: How many marine fungi: Biodiversity and Distribution

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The study of marine fungi has greatly expanded over the past 60 years as new techniques for their study have developed: microscopical, specialised isolation methods and high throughput sequencing studies. However, many authors using sequencing methods of sampling seawater and deep-sea sediments stress that marine fungi are poorly studied in comparison to the number of other marine microorganisms and that very little is known about their global distribution and diversity. Fungi require a substrate for growth so our studies of marine fungi in coastal waters e.g. mangroves, have yielded high numbers that are repeatedly collected. However, high throughput sequencing yields few taxa that match those found in coastal waters and often include terrestrial-like species. *Malassezia*-like organisms, generally associated with skin disease, such as dandruff and eczema, have been recovered from deep sea studies, but what is their role in the marine environment? Sequences of marine Chytridiomycota appear in high numbers from seawater samples, yet few have been described. These zoosporic fungi usually have specific hosts therefore a wider investigation of microalgal hosts is required.

Currently the number of marine fungi stands at 1,211 species (marinefungi.org) from diverse higher taxonomic groups with the Ascomycota dominant: genera in the Pleosporales and Halosphaeriaceae contributing high number of species. The most specious genera are *Aspergillus* and *Penicillium*, and generally isolated from seawater or as endophytes of various marine hosts. Marine fungi are world-wide in distribution, while some are restricted to the tropics, subtropics, temperate or cold-water habitats. They occur primarily as saprobes, while others are parasitic or endophytic or occur in special association with other organisms. Marine fungi have been recovered from coastal and oceanic sea waters, deep-sea sediments, hydrothermal vents, hypoxic and anoxic habitats, polar and hypersaline environments, all yield a wide range of taxa. Jones (2011) estimated the number of marine fungi as circa 12,000 species and identified areas for further investigation. While cellulosic materials have been widely explored for marine fungi, our knowledge of saprobic and parasitic fungi on seaweeds remains scant.

Marine mycology is a relative young science and many challenges remain for the curious and adventurous mycologist, new substrates and environments to sample as well as many geographical locations yet to be explored, in particular Africa.

Short CV

Gareth Jones is a renowned professor of marine mycology. He obtained his PhD from the University of Leeds, UK and was awarded a DSc from the University of Wales, UK. He has supervised over 100 PhD/MSc students and published over 500 research articles. He is now a visiting professor at Chiang Mai University, Thailand.

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Abstracts
Short Lectures

SL 3: Lignicolous freshwater fungi of the Greater Mekong Subregion

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Lignicolous freshwater fungi are those that grow on submerged woody debris in freshwater streams, ponds, lakes, tree hollows, peat swamps and dams. They play an important role in nutrient and carbon cycling, biological diversity and ecosystem functioning of freshwater ecosystems. The lignicolous freshwater fungi are highly diverse in the classes Dothideomycetes and Sordariomycetes. There are a few freshwater taxa belong to Eurotiomycetes, Orbiliomycetes and rarely Basidiomycetes collected from submerged wood in freshwater habitats. The diversity of lignicolous freshwater fungi of the Greater Mekong Subregion are currently being studied. In this study, 115 fresh collections are collected, five new genera, 44 new species are introduced based on their distinct morphology and evidence from molecular phylogeny.

Keywords: Asexual morphs, Freshwater fungi, Phylogenetics, Sexual morphs, Taxonomy

SL 5: Investigation of palm fungi from marine (mangrove) ecosystems

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Palms (*Arecaceae*) are widespread monocotyledonous plants and support a great biodiversity of fungi. Most studies of fungi associated with palms in mangroves are of a taxonomic nature, whereas few studies have incorporation with molecular phylogeny. In an ongoing study of taxonomy of palm fungi, we are employing molecular approaches and phylogenetic analyses to provide a backbone phylogeny for their taxonomy and nature classification, and further provide an updated checklist of palm fungi. Investigation of palm fungi from mangrove ecosystems herein, revealed two new taxa *Acuminatispora palmarum* gen. et sp. nov. (Pleosporales, Dothideomycetes) based on morphological comparison and phylogenetic analysis. Additionally, some genera are frequently encountered, such as *Anthostomella, Linocarpon*, *Oxydothis*. The typically marine taxa *Lignincola* and *Pileomyces*, as well as several interesting groups *Catabotrys*, *Distoseptispora*, *Fasciatispora, Kirschsteiniothelia*, *Tirisporella* and *Vaginatispora* have been collected and conducted phylogenetic analysis.

Keywords: *Acuminatispora palmarum*, phylogeny, palm fungi, taxonomy

SL 6: Phylogenetic revision and evolution of Savoryellaceae and evidence for its ranking as a subclass

Monika C. Dayarathne[1,2,3], Sajeewa S. N. Maharachchikumbura[5], E. B. Gareth Jones[4], Putarak Chomnunti[1], Dong Wei[1], Kevin D. Hyde[1,2,3,*]

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Taxonomic confusions of freshwater and marine taxa of Savoryellaceae are not fully resolved yet even it has been subjected to several morpho-molecular studies. Monophyly of the genera Savoryella and Canalisporium (and its sexual morph, Ascothailandia) was well established. Ascotaiwania and Neoascotaiwania formed a well-supported monophyletic clade in the phylogentic analyses of concatenated LSU, SSU, TEF1 and RPB2 data and they overlapped in their morphologies, especially in asexual morph characteristics indicating that these genera no longer to be two distinct genera. Hence, we synonymize Neoascotaiwania under Asotaiwania. Moreover, Ascotaiwania hughesii (and its asexual morph, Helicoön farinosum) and Monotosporella setosa grouped sister to Plurotheciales. Hence, those two taxa excluded from the genus and currently Ascotaiwania is no longer polyphyletic. A novel species, Savoryella yunnanensis introduce from a freshwater habitat in Yunnan province, China along with comprehensive descriptions and morphographs. Further, we provide evolutionary divergence estimates for the taxa of Savoryellomycetidae and major marine based taxa using Ophiocordyceps fossil evidence and secondary data using a combined LSU, SSU, TEF1 and RPB2 data set from 147 strains. The taxonomic placement of major marine based taxa is briefly discussed. Our results elaborated that the most basal group of marine based taxa are represented within Lulworthiales, which diverged from other Sordariomycetes around 149 MYA (91–209) and Savoryellomycetidae around 213 MYA (198–303).

SL 8: A taxonomic reassessment of helicosporous hyphomycetes from freshwater habitats

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This study deals with an extensive taxonomic reevaluation focusing on phylogenetic relationships and morphological characterization of helicosporous hyphomycetes in the order Tubeufiales. One hundred and forty new taxa are collected during an investigation of freshwater fungi along a north-south latitudinal gradient in the Asian region. Based on evidence from DNA sequence data and morphology, we introduce 12 new genera in the family Tubeufiaceae, *viz. Dematiohelicoma*, *Dematiohelicomyces*, *Dematiohelicosporum*, *Helicoarctatus*, *Helicohyalinum*, *Helicotruncatum*, *Helicotubeufia*, *Neochlamydotubeufia*, *Neohelicosporium*, *Pleurohelicosporium*, *Pseudohelicomyces* and *Pseudohelicoon*; transfer *Helicodochium* from Ascomycetes genera *incertae sedis* into Tubeufiaceae; introduced 65 new species, 43 new combinations and seven new records. The taxonomy of *Helicoma*, *Helicomyces*, *Helicosporium* and *Tubeufia* are revisited based on phylogenetic analyses and morphological evidence. A multi-gene phylogenetic tree based on maximum likelihood and Bayesian analyses of ITS, LSU, RPB2 and TEF1α sequence data of species of Tubeufiaceae is provided. Detailed descriptions and illustrations are provided, as well as the morphological comparison with similar taxa are explored.

Keywords: Asexual morphs, Phylogenetics, Taxonomy, Tubeufiaceae, Woody substrates

SL 9: Towards a natural classification of *Annulatascaceae*-like taxa; introducing one new order and six new families

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The family *Annulatascaceae* belongs to *Annulatascales*, *Sordariomycetes*, *Ascomycota*. Totally 25 genera have been put into *Annulatascaceae* based on morphological characters, especially the relatively massive, J-, refractive apical ring. However, with the development of phylogenetic studies, it showed that the number of heterogeneous species in *Annulatascaceae* was large as identified by nine excluded genera and only four reserved genera. The rest 12 genera were either reserved in *Annulatascaceae* or combined in other families by only morphological characters. Their affiliations are not convinced due to lack of molecular data. In this study, nine new *Annulatascaceae*-like taxa collected from Thailand were morphologically examined. Nine new strains and other known strains of Sordariomycetes species were used to establish a phylogenetic tree and evolution tree based on combined LSU, SSU, ITS and RPB2 sequence data. Phylogenetic analyses show that one new order *Atractosporales* and six new families are introduced to accommodate taxa excluded from *Annulatascaceae sensu stricto*, including *Atractosporaceae*, *Barbatosphaeriaceae*, *Conlariaceae*, *Lentomitellaceae*, *Pseudoproboscisporaceae* and *Woswasiaceae*. Two new species of *Fluminicola* are introduced. A new sexual morph, *Dictyosporella thailandensis,* is reported and *Dictyosporella* is excluded from *Annulatascaceae* and placed in Diaportheomycetidae, genera *incertae sedis*. The first sexual morph of *Sporidesmium*, *S. thailandense* is described. *Atractospora thailandensis*, *Diluviicola aquatica* and *Pseudoproboscispora thailandensis* are also introduced. *Platytrachelon* is added to *Papulosaceae*. *Aquaticola*, *Fusoidispora* and *Pseudoannulatascus* are excluded from *Annulatascaceae* and suggested place in Diaportheomycetidae, genera *incertae sedis*.

Keywords: Diaportheomycetidae, Phylogeny, Ring, Sexual states, Submerged wood

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SL 10: Phylogenetic placement of *Cryptophiale, Cryptophialoidea, Nawawia* and *Phialosporostilbe* in Chaetosphaeriaceae

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During a survey of freshwater fungi in China and Thailand, seven fresh collections representing four species in Chaetosphaeriaceae are described and illustrated based on morphological characters and phylogenetic analyses of combined LSU and ITS sequence data. *Cryptophiale udagawae*, *Cryptophialoidea fasciculata* and *Nawawia filiformis* were re-collected and are reported with the first sequence data for them. Two collections of *Phialosporostilbe* from China and Thailand are identified and introduced as a new species named *Phialosporostilbe scutiformis* with the first sequence data for the genus. A new combination is proposed for the genus *Phialosporostilbe*. Descriptions and illustrations of the new taxa and identified species are provided. Reference specimens of *Cryptophiale udagawae*, *Cryptophialoidea fasciculata* and *Nawawia filiformis* are designated in this study. Phylogenetic placements of the genera *Cryptophiale*, *Nawawia* and *Phialosporostilbe* are discussed.

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Abstracts Posters

PO 9: *Canalisporium dehongensis* sp. nov (*Savoryellaceae*) and *Distoseptispora dehongensis* sp. nov. (*Distoseptisporaceae*) from freshwater in Yunnan Province, China

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Canalisporium dehongensis sp. nov. and *Distoseptispora dehongensis* sp. nov. are described and illustrated from submerged wood collected from a freshwater habitat in Yunnan Province, China. Morphological examination and phylogenetic analyses support their classification in *Savoryellaceae* and *Distoseptisporaceae*. Their relationships with other morphologically similar taxa are discussed.

15th International Marine and Freshwater Mycology Symposium

Lignicolous freshwater fungi of China

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Abstract

Lignicolous freshwater fungi are those that grow on submerged woody substrates in freshwater habitats. We are carrying out a survey of the diversity of lignicolous freshwater fungi along a north-south gradient in the Asian region and the diversity of lignicolous freshwater fungi in China is a important part of this study. During our investigation on lignicolous freshwater fungi in China, 24 fresh specimens were collected from freshwater in Yunnan province. A new genus, eight new species were introduced based on based on morphological characters and phylogenetic analyses of combined ITS, LSU, SSU, RPB2 and TEF1α sequence data. A detailed description of *Kirschsteiniothelia rostrata* is also provided which is a new record for China.

Result

We are carrying out a survey on the diversity of lignicolous freshwater fungi along a north-south gradient in the Asian region. In our investigation on lignicolous freshwater fungi in China, 24 fresh specimens were collected from freshwater in Yunnan province. A new genus *Aquadictyospora*, eight new species viz *Aquadictyospora lignicola*, *Distoseptispora cangshanensis*, *D. obpyriformis*, *D. rostrata*, *D. submersa*, *Kirschsteiniothelia aquatica*, *K. cangshanensis*, *K. fluminicola* are introduced, and a detailed description of *Kirschsteiniothelia rostrata* is also provided,which is a new record for China.

Acknowledgments

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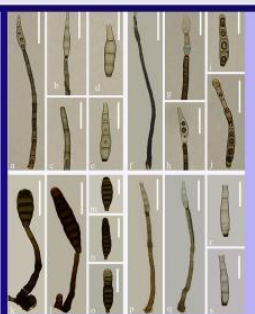
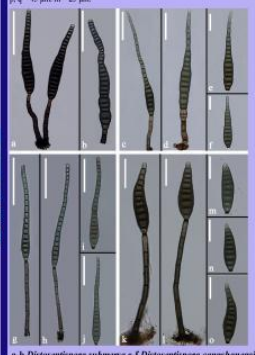


a-e *Kirschsteiniothelia cangshanensis* f-j *Kirschsteiniothelia fluminicola*
k-0 *Kirschsteiniothelia rostrata* p-s *Kirschsteiniothelia aquatica*
a = 35μm, b, g-j = 30 μm., c-e, r-s, i = 20 μm, f = 50 μm, k-l, n, o = 40 μm, p, q = 45 μm. m = 25 μm.



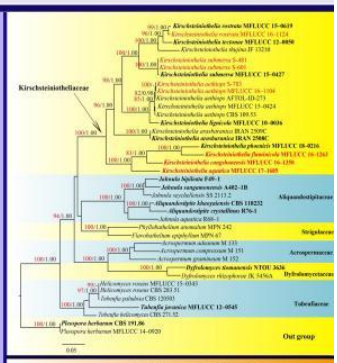
a-b *Distoseptispora submersa* c-f *Distoseptispora cangshanensis*
g-j *Distoseptispora rostrata* k-o *Distoseptispora obpyriformis*
a-b, i-j,k-l = 50 μm, c = 60 μm, d-f, m-o = 30 μm, g-h = 70 μm

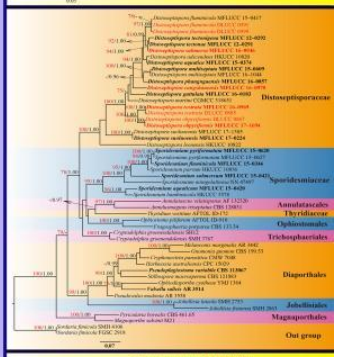
a-g *Pseudodictyosporium wuaense* h-n *Aquadictyospora lignicola*
a-g = 10 μm, h-n = 25 μm

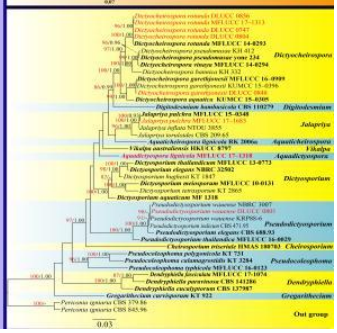
Kirschsteiniotheliaceae

Distoseptisporaceae

Dictyosporiaceae







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