The numbers of fungi: is the descriptive curve flattening?

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Abstract

The recent realistic estimate of fungal numbers which used various algorithms was between 2.2 and 3.8 million. There are nearly 100,000 accepted species of Fungi and fungus-like taxa, which is between 2.6 and 4.5% of the estimated species. Several forums such as Botanica Marina series, Fungal Diversity notes, Fungal Biodiversity Profiles, Fungal Systematics and Evolution—New and Interesting Fungi, Mycosphere notes and Fungal Planet have enhanced the introduction of new taxa and nearly 2000 species have been introduced in these publications in the last decade. The need to define a fungal species more accurately has been recognized, but there is much research needed before this can be better clarified. We address the evidence that is needed to estimate the numbers of fungi and address the various advances that have been made towards its understanding. Some genera are barely known, whereas some plant pathogens comprise numerous species complexes and numbers are steadily increasing. In this paper, we examine ten genera as case studies to establish trends in fungal description and introduce new species in each genus. The genera are the ascomycetes *Colletotrichum* and *Pestalotiopsis* (with many species or complexes), Atrocalyx, Dothiora, Lignosphaeria, Okeanomyces, Rhamphoriopsis, Thozetella, Thyrostroma (relatively poorly studied genera) and the basidiomycete genus Lepiota. We provide examples where knowledge is incomplete or lacking and suggest areas needing further research. These include (1) the need to establish what is a species, (2) the need to establish how host-specific fungi are, not in highly disturbed urban areas, but in pristine or relatively undisturbed forests, and (3) the need to establish if species in different continents, islands, countries or regions are different, or if the same fungi occur worldwide? Finally, we conclude whether we are anywhere near to flattening the curve in new species description.

Keywords 11 new taxa · Atrocalyx · Colletotrichum · Dothiora · fungal numbers · Host-specificity · Lepiota · Lignosphaeria · Okeanomyces · Pestalotiopsis · Rhamphoriopsis · Thozetella · Thyrostroma

Introduction

The estimated numbers of fungi have always been a compelling topic with numerous discussion papers over time. Mycologists have always pondered over how many fungal species there are, as this has important implications for conservation practices. Fungi, including the seen mushrooms and unseen microorganisms are diverse and form an integral component of life's genetic diversity, but their actual

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numbers are poorly understood and the estimates available so far are debatable. Hawksworth (1991) provide a comprehensive account and argument for the numbers being 1.5 million. This was based on, amongst other metrics, there being circa six taxa as unique to each plant species. Since this critical paper, there have been several revisions (Table 1) with estimates ranging from 0.5 to 13.2 million, and the latest estimate being 11.7–13.2 million (Wu et al. 2019). Hawksworth and Lücking (2017) based their estimates (2.2–3.8 M), taking into account cryptic species, the rates and patterns at which new species are being described, unexplored niches and DNA based species from environmental DNA. New generation sequencing has also provided



Extended author information available on the last page of the article

Table 1Published estimates offungal species

References	Estimated species (mil- lions)	Notes
Hawksworth (1991)	1.5	
Hammond (1992)	1	
Smith and Waller (1992)	1	Only tropical plants
Hywel-Jones (1993)	1.5	Only insect fungi
Rossman (1994)	1	
Dreyfuss and Chapela (1994)	1.3	Only endophytes
Hammond (1995)	1.5	
Cannon (1997)	9.9	
Shivas and Hyde (1997)	0.27	Only plant pathogens
Fröhlich and Hyde (1999)	> 1.5	Considered very conservative
May (2000)	0.5	
Arnold et al. (2000)	> 1.5	
Aptroot (2001)	0.04-0.07	Only ascomycetes
Hawksworth (2001)	0.5–9.9	
De Meeûs and Renaud (2002)	0.06	Only ascomycota
De Meeûs and Renaud (2002)	0.025	Only basidiomycota
O'Brien et al. (2005)	3.5–5.1	
Crous et al. (2006)	0.17	Only South Africa
Schmit and Mueller (2007)	0.72	
Dai and Zhuang (2010)	0.18	Only China
Blackwell (2011)	5.1	
Mora et al. (2011)	0.61	Only land
Mora et al. (2011)	0.005	Only oceans
Hawksworth (2012)	1.5–3	
Hawksworth and Lücking (2017)	2.2–3.8	
Wu et al. (2019)	11.7–13.2	

new information on fungal numbers, but the methodology needs to be much improved before we can use this data (Hongsanan et al. 2018; Thines et al. 2018). Tedersoo et al. (2014) obtained over 80,000 fungal OTUs from 14,600 soil samples taken worldwide. They reported that tropical rainforests harbour the highest fungal diversity, but the number of fungal species in the world may have been greatly overestimated. The main problem with all of these estimates is that the authors were dealing with a limited amount of data from which to extrapolate. For example, is the ratio of six species of fungi to one host species realistic? Some genera such as Aspergillus, Fusarium and Penicillium are far more speciose than other genera (Seifert et al. 2007; Samson et al. 2014; Al-Hatmi et al. 2016; Hubka et al. 2018). Are the large numbers of species in these genera because they are better studied, or they are commonly found in urban environments? This paper examines the various issues relating to estimates of fungal species. We use ten genera as case studies and conclude by addressing some of the issues that require further research with more data, better resolution or a better understanding, before we can move forward to accurately realize exactly how many fungi there are.

Problems in estimating species numbers

What is a species?

One of the most important aspects in estimating fungal species numbers is accurately defining a species. There have been numerous debates with regards to what comprises a species and this has recently been summarized in Steenkamp et al. (2018). Most mycologists have relied heavily on morphological attributes and DNA sequence data to identity and distinguish species (Liu et al. 2015; Wu et al. 2016; Thambugala et al. 2017b; Tibpromma et al. 2017; Hyde et al. 2019, 2020a; Phookamsak et al. 2019). Simply, we mostly rely on phenotypic and phylogenetic aspects and consider a species as a group of individuals sharing similar phenotypes and sufficient DNA similarities (Pausas and Verdú 2010; Steenkamp et al. 2018; Moore et al. 2020). However, to be a distinct species they must be sufficiently distinct from another sister group / species and have ample differences in DNA, supported with phylogeny or other analyses (Jeewon and Hyde 2016; Bhunjun

et al. 2020). It is presently impossible to derive a standard system for differentiating species for all groups of fungi (Lücking 2020).

Kurtzman and Robnett (1998) proposed that new yeasts should be characterized by divergence in the variable D1/D2 domain of the large subunit (26S) ribosomal DNA and ITS region and this has been followed widely by yeast mycologists (Wang et al. 2015). The topic of defining a species is presently receiving much interest and the outcomes will heavily affect fungal numbers. For example, many cryptic species of plant pathogens are currently being recognized and numbers continue to increase (Cai et al. 2011). Cryptic species have similar phenotypes, but different phylotypes and this has resulted in highly speciose genera, such as in *Aspergillus, Colletotrichum* and *Fusarium* (Samson et al. 2014; Al-Hatmi et al. 2016; Hubka et al. 2018; Jayawardena et al. in press). Whether such phenomena are prevalent in the lesser studied fungal genera, has yet to be addressed.

Cryptic species/species complexes

Evolution has not only shaped life on earth, but has also influenced species diversity. Speciation has resulted in the formation of new and distinct species in the course of evolution due to separation of populations (Giraud et al. 2008), but this has also led to contention concerning the existence of species complexes and cryptic species. Mycologists are increasingly discovering novel cryptic species and this will have an impact on fungal numbers as they constitute important units of biodiversity. These cryptic species are not only important for taxonomic purposes and estimating biodiversity, but also for understanding community dynamics, especially with plant pathogens, and to gain insights into ecological and evolutionary processes that drive speciation.

DNA sequence data has revealed that in many cases, phenotypically identical or similar species actually represent different species and these might be more diverse than previously anticipated. This is evident in speciose genera such as Colletotrichum (Jayawardena et al. 2020), Diaporthe (Udayanga et al. 2014; Dissanayake et al. 2017), Pestalotiopsis (Jeewon et al. 2003; Maharachchikumbura et al. 2013a, 2014; Solarte et al. 2018) and Fusarium (O'Donnell et al. 2008), but may also be true of poorly studied genera. Genealogical concordance phylogenetic species recognition analysis (GCPSR) analyzed by the pairwise homoplasy index (PHI) test was used to determine the recombination level within closely related species (Bruen et al. 2006). The relationships between closely related taxa are visualized by constructing splits graphs from concatenated datasets, using the LogDet transformation and splits decomposition options. If the PHI results value is lower than 0.05 ($\Phi w < 0.05$), it indicates that significant recombination is present in the

dataset. This may be an important method to provide further evidence to justify a species.

Unstudied habitats, regions and continents

Fungi are ubiquitous and have been recovered from numerous diverse habitats. For example, they are pathogens of economically important crops (Jayawardena et al. 2019a, b), live inside plant tissues as endophytes (Tibpromma et al. 2018; Rashmi et al. 2019), act as decomposers and are important in nutrient cycling (Hyde et al. 2005; Tang et al. 2005; Kuehn et al. 2011; Saikkonen et al. 2015; Juan-Ovejero et al. 2020; Op De Beeck et al. 2020) or associated with roots as arbuscular mycorrhizal fungi (Kivlin et al. 2011; Cao et al. 2018; Powell and Rillig 2018; Wang et al. 2019). Some fungal communities have been well-studied because of human importance, while others have barely been studied, and therefore fungal diversity might be underestimated (Hawksworth 2001). There are numerous understudied habitats which harbour numerous species, and if they are studied diverse, new species may be discovered (Hyde et al. 2018b). For example, less research has focused on agroforestry systems despite the fact that fungi play vital roles in forestry health (Udawatta et al. 2019). Entomopathogenic fungi on insects are less-well studied and can also be commercially exploited (Butt et al. 2016; Hyde et al. 2019). Similarly, fungi in ant domains have only recently been discovered and are likely to comprise numerous new species (Mueller 2002; Schlick-Steiner et al. 2008; Luiso et al. 2020). Marine habitats have been well-studied for fungi with numerous new species being described with emphasis on bioprospecting (Hyde et al. 2000; Kohlmeyer and Kohlmeyer 2013; Lozada and Dionisi 2015; Gladfelter et al. 2019; Jeewon et al. 2019). The ocean is however, vast and fungi from deep sea oceans, endophytes of seaweeds and microsporidia causing diseases of marine animals need to be investigated (Sweet and Bateman 2015; Gnavi et al. 2017; Xu et al. 2019). Other less researched habitats where fungi could be abundant and reveal numerous novel species discoveries are Karst fungi, caves, forests (especially pristine rainforests), extreme environments, volcanoes, mountains, deserts, freshwater aquatic systems, lakes, grasslands, indoor environment and many others (Connell et al. 2009; Shapiro and Pringle 2010; Sterflinger et al. 2012; Hagen et al. 2013; Ariyawansa et al. 2016a, b, c; Woudenberg et al. 2017; Bensch et al. 2018). Thus, research of understudied habitats is likely to result in unexpectedly large numbers of new fungi.

The study of fungi has been extremely disparate. The fungi of Australia, most of Europe, Japan, North America, New Zealand and South Africa have been relatively well-studied (Chethana et al. 2020), but in most other parts of the world, further studies are warranted. The fungi in large areas of Africa (e.g. Malawi, Nigeria, Zambia), Asia (e.g.

Cambodia, Indonesia, Myanmar), Europe (e.g. Bulgaria, Greece), Central Asia (e.g. Uzbekistan), the Pacific Islands (e.g. Fiji) and South America (Peru, Venezuela) have hardly been studied. If we study these regions will we find large numbers of new species or will they be the same as other parts of the world? It has been suggested that a large proportion of new species awaits discovery and possibly lie in tropical regions such as Thailand (Hyde et al. 2018b).

Ratio of hosts to fungal species

The numbers and ratio of fungal species to plant or animal hosts have barely been investigated. Although, Ellis and Ellis (1985) provided a significant text of the fungi on land plants, this was a general text that only dealt with the UK where habitats are highly disturbed and plant diversity relatively low. Very few studies have addressed the diversity of fungi in pristine rainforests. Are fungal species ubiquitous on most plant hosts in such habitats or are they specific to certain host species, genera or families? In a landmark study, Fröhlich and Hyde (1999) reported the fungal communities on two palm species (Licuala sp. and Licuala ramsavi) growing in north Queensland, Australia and an area of pristine rainforest in Brunei Darussalam. They identified 242 taxa based on morphology and remarkably only 30 taxa overlapped between *Licuala* sp. and *Licuala ramsayi*. With such low overlap between two palm species, but in different countries, what is the situation with other plant hosts? Studies on Heliconia, teak, bamboo, Pandanaceae, Quercus and Clematis also indicate that the fungi on these hosts barely overlap, thus we are far from understanding the relationships between fungal species and their hosts (Dai et al. 2017; Doilom et al. 2017; Tibpromma et al. 2018; Phukhamsakda et al. 2020).

Case studies

In this section we introduce new species in ten genera and discuss the likelihood of further new species being discovered. We follow the recent outlines in He et al. (2019), Hyde et al. (2020b) and Hongsanan et al. (2020) for the arrangement of taxa.

Ascomycota Dothideomycetes

Dothideales

For the latest treatments of Dothideales we follow Hongsanan et al. (2020).

Dothioraceae Chevall.

Dothideales is typified by Dothioraceae, a family introduced by Chevallier (1826). Members of Dothideaceae are cosmopolitan in their distribution and associated with woody plants (Hyde et al. 2013). Eighteen genera are presently accommodated in the family (Hongsanan et al. 2020).

Dothiora Fr.

Dothiora is typified by D. pyrenophora which was collected from dead branches of Sorbus aucuparia (Rosaceae) in Switzerland (Thambugala et al. 2014). Eighty-four epithets are listed under Dothiora in Index Fungorum (2020) and most of these species are recorded as saprobes or pathogens on plants in terrestrial habitats (Thambugala et al. 2014). The realistic number of extant *Dothiora* is likely much less than 84. The genus has not been monographed recently and many species are synonymized and others belong to genera such as Botryosphaeria, Diplodia, and Myriangium. In this paper, Dothiora omaniana is described from Punica granatum (pomegranate) from a commercial orchard in Oman. The species is conspicuous as it causes leaf spot disease. However, are we likely to find many more new species in this genus? Twenty-two Dothiora species have sequence data in GenBank and are confirmed as distinct species. The taxa are from 23 different hosts and 17 plant families indicating that the genus has a wide host range and that species are possibly specific to families or genera (Table 2). Species have been collected mainly in Europe, and thus other continents and numerous regions have never been studied for the genus (Table 2). Thus, we believe that comprehensive studies on this genus are likely to result in numerous new species.

Dothiora omaniana Maharachch. & Al-Sadi, sp. nov.

MycoBank number: MB834630; Facesoffungi number: FoF 07704; Fig. 1

Etymology: Named after the country Oman, where the fungus was collected.

Holotype: SQU H-111

Associated with leaf spot on leaves of Punica granatum. Asexual morph: Conidiomata pycnidial, brown, immersed to erumpent through host tissue, solitary, globose, to 250 µm diam; wall of 2–6 layers of brown *textura angularis*. Conidiophores reduced to conidiogenous cells lining the inner cavity, hyaline, smooth, aseptate, ampulliform $4–10\times3-4$ µm, with central phialidic locus. Conidia solitary, hyaline, smooth, subcylindrical to oblong, guttulate, apex obtuse, aseptate, (4–)6–8(–9)×2–4 µm. Hyphae brown, verruculose, and constricted at septa.

Culture characteristics: Colonies on potato dextrose agar (PDA) flat, spreading, with sparse aerial mycelium, margins feathery, reaching 5 cm diam after 1 wk.

Species	Host/family	Origin	Life mode	References
Dothiora agapanthi	Agapanthus sp. (Amaryllidaceae)	South Africa	_	Crous and Groenewald (2016)
Dothiora bupleuricola	Bupleurum fruticosum (Apiaceae)	France	Pathogen	Crous and Groenewald (2016)
Dothiora buxi	Buxus sempervirens (Buxaceae)	Italy	Saprobe	Hyde et al. (2016)
Dothiora cactacearum	Cactaceae	USA	Saprobe	Crous and Groenewald (2017)
Dothiora cannabinae	Daphne cannabina (Thymelaeaceae)	India	Saprobe	Froidevaux (1972)
Dothiora ceratoniae	Nerium oleander (Apocynaceae), Arbutus unedo (Ericaceae), Ceratonia siliqua (Fabaceae)	Italy, Spain	Saprobe	Crous and Groenewald (2016)
Dothiora corymbiae	Corymbia citriodora (Myrtaceae)	Australia	-	Crous et al. (2018b)
Dothiora cytisi	Cytisus scoparius (Fabaceae)	Italy	Saprobe	Li et al. (2016a)
Dothiora elliptica	Vaccinium uliginosum (Ericaceae)	Switzerland	-	Fuckel (1873)
Dothiora europaea	Salix helvetica (Salicaceae)	Switzerland	-	Froidevaux (1972)
Dothiora infuscans	Wall surface	Spain	Saprobe	Crous et al. (2018a)
Dothiora laureolae	Daphne laureola (Thymelaeaceae)	Italy	Saprobe	Froidevaux (1972)
Dothiora maculans	Populus tremuloides (Salicaceae), Acer pseudoplatanus (Aceraceae)	Canada, Netherlands	Saprobe	Crous and Groenewald (2016)
Dothiora mahoniae	Mahonia repens (Berberidaceae)	USA	Pathogen	Crous et al. (2018b)
Dothiora oleae	Olea europaea (Oleaceae)	Turkey, Italy, Spain, Greece	Saprobe	Crous and Groenewald (2016)
Dothiora omaniana	Punica granatum (Lythraceae)	Oman	Pathogen	Present study
Dothiora phaeosperma	Lonicera coerulea (Caprifoliaceae)	Switzerland	Saprobe	Froidevaux (1972)
Dothiora pyrenophora	Sorbus aucuparia (Rosaceae)	Germany, Sweden	Saprobe	Fries (1849)
Dothiora prunorum	Prunus domestica (Rosaceae)	UK	-	Crous and Groenewald (2016)
Dothiora rhamni-alpinae	Rhamnus alpina (Rhamnaceae)	Italy	Saprobe	Froidevaux (1972)
Dothiora sorbi	Sorbus aria (Rosaceae)	Switzerland, France	-	Fuckel (1870)
Dothiora viburnicola	Viburnum tinus (Adoxaceae)	Italy	Saprobe	Crous and Groenewald (2016)

Table 2	Hosts and	distribution	of Dothiord	<i>i</i> species that	have molec	ular data
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Material examined: OMAN, Al Jabal al-Akhdar (Green Mountain), from leaves of *Punica granatum* (Lythraceae), July 2016, SSN Maharachchikumbura OM39 (SQU H-111, **holotype**); ex-type culture = SQUCC 13293.

GenBank numbers: LSU: MT077209, ITS: MT077213, TEF: MT081204, TUB: MT081205.

Notes—Among *Dothiora species*, the new taxon *D. omaniana* formed a robust monophyletic lineage with high statistical support (ML: 93%: Fig. 2) sister to *D. agapanthi* which was isolated from leaves of *Agapanthus* sp. in South Africa (Crous and Groenewald 2016). *Dothiora omaniana* can be distinguished from *D. agapanthi* (conidia = (8-)10-12(-13)×3(-3.5) µm) by its smaller conidia ($(4-)6-8(-9) \times 2-4$ µm) as well as shape of the conidiogenous cells.

Pleosporales

For the latest treatments of Pleosporales we follow Hongsanan et al. (2020).

Dothidotthiaceae Crous & A.J.L. Phillips

Species of Dothidotthiaceae are parasitic on living hosts and saprobic on wood and branches in terrestrial habitats (Hyde et al. 2013; Pem et al. 2019a; Senwanna et al. 2019). Currently, seven genera are recognized in Dothidotthiaceae.

Thyrostroma Höhn.

Senwanna et al. (2019) and Pem et al. (2019b) provided the most recent treatments of *Thyrostroma*. In this study, a new species *Thyrostroma alhagi* collected from *Alhagi kirghisorum* (Fabaceae) in Uzbekistan is introduced. The new species is compared to similar species in the genus and is established based on phylogenetic analyses (Fig. 3).

There are 33 epithets under the genus Thyrostroma in Index Fungorum (2020) but the actual number of extant species may be less as most of them have been synonymized and transferred to other genera such as Botryosphaeria and Melanconis. Only 13 Thyrostroma species are confirmed with molecular data although there are more than 200 sequences of Thyrostroma in GenBank. Some of those sequences are unidentified species such as Thyrostroma sp. isolate TX11 and other undescribed isolates. It seems that we are likely to discover many more new species of Thyrostroma. The taxa confirmed with molecular data are from 11 different hosts which belong to ten plant families. The genus appears to have a wide host range and species are possibly specific to families or genera (Table 3). Thyrostroma species have only been collected in Korea, Russia, Ukraine, the USA and Uzbekistan from a limited number of host genera and families. Many other regions have never been studied for the



Fig. 1 Dothiora omaniana (SQU H-111, holotype) **a**, **b** Conidiomata on host surface. **c**, **d** Vertical sections of conidioma. **e**, **f** Conidiomata on PDA. **g** Hyphae giving rise to conidiogenous cells. **h**, **i** Conidiogenous cells. **j**, **k** Conidia. Scale bars: **c**, **d** = 50 μ m, **g**-**k** = 10 μ m

genus (Table 3). Thus, we believe that comprehensive studies are likely to result in numerous new species.

Thyrostroma alhagi D. Pem, Gafforov & K.D. Hyde, *sp. nov*.

Index Fungorum number: IF 557028; Facesoffungi number: FoF 07080; Fig. 4

Etymology: The epithet is derived from the host genus. Holotype: TASM 6137

Saprobic on dead stem of Alhagi kirghisorum. Sexual morph: Undetermined. Asexual morph: (Pseudo)sporodochia 660-750 µm high, 850-1150 µm diam., solitary or in groups, semi-immersed or immersed to erumpent, sporodochial, convex, dark brown or black, rough. Conidiophores $14-28 \times 9.6-9.9 \ \mu m \ (\bar{x} = 22.5 \times 9.8 \ \mu m, n = 10)$, hyaline to pale brown, finely roughened, cylindrical to subcylindrical. Conidiogenous cells 5–6×7–8 μ m (\bar{x} = 6×8.5 μ m, n = 10), holoblastic, hyaline to pale brown, subcylindrical, finely roughened, integrated, terminal. Conidia 45-65 × 23-26 µm $(\bar{x} = 51.7 \times 25.2 \,\mu\text{m}, n = 10)$ acrogenous, straight or curved, variable in shape, generally clavate to obpyriform, dark brown, rough-walled, with 1-3 transverse septa, and 0-4 oblique or longitudinal septa, strongly constricted at the septa, rounded at apex, truncate at base with of 9.6-9.8 µm diam.

Culture characteristics: Conidia germinating on malt extract agar (MEA) within 48 h. Colonies growing on MEA, reaching 3 cm diam. in 1 week at 16 °C. Mycelium dense, circular, flat, surface smooth, edge slightly fimbriate, thinly hairy, radially striated, above and reverse dark-grey.

Material examined: UZBEKISTAN, Tashkent Province, Bostanliq District, Xojikent Village, Ugam Range of Western Tien Shan Mountains, on dead stem of *Alhagi kirghisorum* (Fabaceae), 10 April 2016, Yusufjon Gafforov (TASM 6137, **holotype**; MFLU 17–0060, **isotype**); ex-type living culture, MFLUCC 17–1949.

GenBank numbers: LSU: MN846098, SSU: MN846097, ITS: MN846099.

Notes—*Thyrostroma alhagi* forms a lineage close to *T. ephedricola. Thyrostroma alhagi* however, differs from *T. ephedricola* in having larger sporodochia (660–750 µm high, 850–1150 µm diam., vs. 90–166 µm high, 70–150 µm diam.) and conidia (45–65 × 23–26 µm vs. 25–34 × 14–22 µm). *Thyrostroma alhagi* differs from *T. robiniae* in having shorter and wider conidiophores (14–28 × 9.6–9.9 µm vs. 23–35 × 4–9 µm) and larger conidia (45–65 × 23–26 µm vs. 38–50 × 13–20 µm). *Thyrostroma alhagi*, also differs from *T. compactum* (type species) in having narrower conidiophores (14–28 × 9.6–9.9 µm vs. 20–60 × 5–12 µm) and truncate

Dothiora infuscans FMR 16326
50 Dothiora laureolae CBS 744.71 57
Dothiora cannabinae CBS 737.71
Dothiora cactacearum CBS 142492
Dothiora buxi MFLU 15-3404
Dothiora corymbiae CBS 145060
Dothiora europaea CBS 739.71
Dothiora viburnicola CBS 274.72
Dothiora bupleuricola CBS 112.75
Dothiora pyrenophora CPC 30634
- Dothiora sorbi CBS 742.71
Dothiora rhamni-alpinae CBS 745.71
Dothiora prunorum CBS 933.72
Dothiora phaeosperma CBS 870.71
Dothiora cytisi MFLUCC 14-0970
L Dothiora elliptica CBS 736.71
Dothiora ceratoniae CBS 477.69
Dothiora oleae CBS 152.71
Dothiora agapanthi CPC 20600
Dothiora maculans CBS 299.76
Dothiora matoniae CBS 299.76
Dothidea ribesia CPC 30638

0.05

Fig. 2 Phylogram generated from maximum likelihood analysis based on combined LSU+ITS+TEF+TUB sequence data of species in *Dothiora*. Bootstrap support values greater than 50% are given above the nodes. The new isolate is in blue and the tree is rooted with *Dothidea ribesia* (CPC 30638)

bases (9.6–9.8 μ m diam. vs. 6–11 μ m). A comparison of the 479 ITS (+ 5.8S) nucleotides (without gaps) of *T. alhagi* with that of *T. ephedricola* and *T. compactum* reveals 8 (1.7%) and 16 (3.4%) nucleotide differences respectively. Thus, the new species is justified based on molecular evidence and morphological differences. Recent research on the ascomycetous microfungi have yielded some species previously unknown in Uzbekistan, as well as several new to science which include nine new genera and more than 30 species (Gafforov 2017; Gafforov and Rakhimov 2017; Wanasinghe et al. 2017, 2018a, b; Pem et al. 2018, 2019d, e; Samarakoon et al. 2018; Gafforov et al. 2019; Hyde et al. 2019). The Central Asia region characterised by arid and semi-arid and xerophytic plants provides an excellent opportunity to unravel the species diversity with the possibility of discovering additional novel species from *Thyrostroma*.

Lophiotremataceae K. Hiray. & Kaz. Tanaka

Lophiotremataceae species are saprobic on herbaceous and woody plants in terrestrial habitats (Hyde et al. 2013; Hashimoto et al. 2017). Currently, six genera are placed in Lophiotremataceae (Wijayawardene et al. 2018, 2020; Hongsanan et al. 2020).

Atrocalyx A. Hashim. & Kaz. Tanaka

Atrocalyx species are found on twigs or bark of woody plants or seeds and have been collected in Belgium, China, Japan, Spain and Thailand (Hashimoto et al. 2017; Tibpromma et al. 2017; de Silva et al. 2018; Jaklitsch et al. 2018; Jayasiri et al. 2019). The morphology of this genus is similar to Lophiotrema (Hashimoto et al. 2017). There are eight Atrocalyx epithets in Index Fungorum (2020), all confirmed with molecular data. The members of Atrocalyx are saprobes on various hosts. The known taxa of Atrocalyx are from five different hosts distributed in four plant families indicating that the genus has a wide host range and species are possibly specific to families or genera (Table 4). All known Atrocalyx taxa have been collected from Asia and Europe, while numerous regions have never been studied for this genus (Table 4). Thus, we believe that comprehensive studies on this genus are likely to result in numerous new species.

Atrocalyx quercus Tibpromma & K.D. Hyde, sp. nov.

Index Fungorum Number: IF557254; Facesoffungi number: FoF 08145; Fig. 5

Etymology: The specific epithet "quercus" refers to the host genus.

Holotype: HKAS107388

Saprobic on twig of Quercus variabilis. Sexual morph: Undetermined. Asexual morph: Coelomycetous. Conidiomata 140–300 µm diam., 120–200 µm high ($\bar{x} = 185.85 \times 158.68$ µm, n = 5), pycnidial, globose to subglobose, unilocular, immersed, black, without ostiolar neck. Pycnidial wall 5–25 µm wide, multilayered, outer layer with red-brown walled cells, inner wall with hyaline to pale brown walled cells of *textura angularis*. Conidiophores arising from the basal cavity around conidiomata. Conidiogenous cells holoblastic, determinate, ampulliform to cylindrical, short, hyaline, smooth-walled. Conidia 2–3.5 × 2.5–4 µm ($\bar{x} = 2.53 \times 3.27$ µm, n = 40), oval to ellipsoid, hyaline when young, becoming brown at maturity, 1-celled, smooth-walled.

Culture characteristics: Colonies on PDA irregular in shape, endulate in edge, white-grey at the margin with black at the center, wrinkled, zonate with different sector colony

Fig. 3 Phylogram generated from maximum likelihood analysis based on combined LSU, SSU, and ITS sequence data representing Dothidotthiaceae and the outgroup taxa. Related sequences are taken from Senwanna et al. (2019) Sixty strains are included in the combined analyses which comprise (728 characters for LSU. 760 characters for SSU, 479 characters for ITS) after alignment. Didymella exigua (CBS 183.55) and Phoma herbarum (CBS 615.75) in Didvmellaceae (Pleosporales) are used as the outgroup taxa. Single gene analyses were also performed to compare the topology and clade stability with combined gene analyses (data not shown). Tree topology of the maximum likelihood analysis is similar to the Bayesian analysis. The best RaxML tree with a final likelihood values of - 4234.299902 is presented. The matrix had 196 distinct alignment patterns, with 19.70% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.249839, C = 0.215788, G = 0.276425,T = 0.257948; substitution rates AC=2.531606, AG=9.487087, AT = 5.410984, CG = 1.628327, CT=19.366870, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.784351$. Bootstrap values for maximum likelihood (ML) equal to or greater than 50% and clade credibility values greater than 0.90 (the rounding of values to 2 decimal proportions) from Bayesian-inference analysis labeled on the nodes. Ex-type strains are in bold and black, the new isolate is indicated in bold and blue



in the upper part; reverse irregular in shape, black and not producing pigments on agar.

Material examined: CHINA, Yunnan Province, Kunming, on dead twig of *Quercus variabilis* (Fagaceae), 10 June 2019, S. Tibpromma, QS05 (HKAS107388, **holotype**); ex-type living culture KUMCC 20-0032.

GenBank numbers: LSU: MT274527, ITS: MT274562, SSU: MT274563, TEF1: MT307302.

Notes: The asexual morph of *Atrocalyx quercus* shares similar morphology with *A. acutisporus* and *A. krabiensis* in having ellipsoidal and aseptate conidia (Hashimoto et al. 2017; Jayasiri et al. 2019). Our new species has hyaline conidia, differing from *A. acutisporus* and *A. krabiensis* which have brown conidia (Hashimoto et al. 2017; Jayasiri et al. 2019) and they are also phylogenetically different (Fig. 6).

Species	Host/family	Origin	Life mode	References
T. alhagii	Alhagi kirghisorum (Fabaceae)	Uzbekistan	Saprobic	Present study
T. celtidis	Celtis occidentalis (Cannabaceae)	Russia	Pathogenic	Senwanna et al. (2019)
T. cornicola	Cornus officinalis (Cornaceae)	Korea	Saprobic or pathogenic	Crous et al. (2016)
T. ephedricola	Ephedra equisetina (Ephedraceae)	Uzbekistan	Saprobic	Pem et al. (2019c)
T. franseriae	Franseria sp. (Asteraceae)	USA	Saprobic or pathogenic	Marin-Felix et al. (2017)
T. jaczewskii	Elaeagnus angustifolia (Elaeagnaceae)	Ukraine	Saprobic or pathogenic	Pem et al. (2019c)
T. lycii	Lycium barbarum (Solanaceae)	Russia	Pathogenic	Senwanna et al. (2019)
T. moricola	Morus alba (Moraceae)	Russia	Saprobic or pathogenic	Senwanna et al. (2019)
T. robiniae	Robinia pseudoacacia (Fabaceae)	Russia	Saprobic or pathogenic	Senwanna et al. (2019)
T. styphnolobii	Styphnolobium japonicum (Ulmaceae)	Russia	Saprobic or pathogenic	Senwanna et al. (2019)
T. tiliae	Tilia cordata (Malvaceae)	Russia	Saprobic or pathogenic	Senwanna et al. (2019)
T. ulmicola	Ulmus pumila (Ulmaceae)	Russia	Saprobic or pathogenic	Senwanna et al. (2019)
T. ulmigenum	Ulmus pumila (Ulmaceae)	Russia	Saprobic or pathogenic	Senwanna et al. (2019)

Table 3 Hosts and distribution of Thyrostroma species that have molecular data

In the phylogenic analysis, our new species clusters with *A. bambusae* (MFLUCC 10–0558) with weak support (Fig. 6). Therefore, we compared nucleotide base pairs of *A. quercus* with *A. bambusae* to justify our new species based on the guidelines of Jeewon and Hyde (2016). Five bp differences in LSU, 23 bp differences in ITS, 12 bp differences in SSU and 50 bp differences in TEF1 were observed (gaps were excluded). Phylogenetic relationships of our new species are shown in Fig. 6 and the GCPSR results ($\Phi w = 0.9853$, Fig. 7) also support that our species is distinct. This is the first record of *Atrocalyx* on *Quercus*.

Phaeoseptaceae Boonmee, Thambug. & K.D. Hyde

Phaeoseptaceae was established to accommodate lignicolous fungal lineages on wood. There are currently five genera in this family: *Decaisnella, Lignosphaeria, Phaeoseptum* (generic type), *Pleopunctum* and putative strains of *Thyridaria macrostomoides* (Abdel-Wahab and Jones 2003; Zhang et al. 2012a; Ariyawansa et al. 2015; Thambugala et al. 2015; Hyde et al. 2018b; Liu et al. 2019b; Phukhamsakda et al. 2019).

Lignosphaeria Boonmee, Thambug. & K. D. Hyde

There are currently two epithets under *Lignosphaeria* in Index Fungorum (2020), but the realistic number of species is likely to be higher, as both species have been collected as saprobes on dead plant material in Chiang Mai Province, Thailand (Table 5). In this study we introduce a new species, *Lignosphaeria diospyrosa* on *Diospyros malabarica* from Krabi Province, Thailand. Morphological and phylogenetic analyses provide strong evidence that our collection is a new species (Figs. 8, 9). The two other species have been collected from an undetermined host, so based on current data we are unable to speculate if the species in this genus are host-specific, but all the species have been found on woody bark. We believe that extensive sampling of unstudied hosts and regions is likely to result in numerous new species.

Lignosphaeria diospyrosa Bhunjun, Phukhams. & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF557335; Facesoffungi number: FoF 07751; Fig. 9

Etymology: The epithet reflects the genus of host plant, *Diospyros malabarica*.

Holotype: MFLU 17–1543

Saprobic on dried bark of Diospyros malabarica. Sexual morph: Ascomata 315-350 × 240-330 µm, gregarious, scattered, solitary, immersed, with only black shiny ostioles visible, globose to compressed, carbonaceous to sub-carbonaceous, black, with a well-developed pseudoclypeus, ostiolate. Ostioles 130-200×145-165 µm, central, long, elongate, oblong, carbonaceous, filled with hyaline periphyses. Peridium 10–30 µm wide ($\bar{x} = 20 \mu m, n=20$), uniform, up to 45 μ m at apex, composed of 4(-5) layers of thick-walled cells of textura angularis, cells towards the inside lighter, with a thin, hyaline, gelatinous inner layer. Hamathecium of dense, 0.8-1.6 µm wide, filamentous, branched, septate, anastomosing pseudoparaphyses, situated between and above the asci, embedded in a gelatinous matrix. Asci 48–75 \times 5–10 µm diam. ($\bar{x} = 65 \times 7$ µm, n = 50), 8-spored, bitunicate, fissitunicate, oblong to clavate, with long, pedicel furcate, apically rounded, with an ocular chamber. Ascospores $10-15 \times 2.5-5 \ \mu m \ (\bar{x} = 14 \times 4 \ \mu m, n = 50)$, bi-seriate or partially overlapping, broad fusiform, tapering towards the ends, acute ends, hyaline, 3-septate, strongly constricted at median septum, slightly swollen near the Fig. 4 Thyrostroma alhagi (TASM 6137, holotype). a, b Appearance of conidiomata on host surface. c Vertical section through the conidioma. d-k Conidia. l Germinating conidium on MEA after 48 hours. Scale bars: a=2 mm, b, $c=500 \mu m$, $d=40 \mu m$, $e=50 \mu m$, $f-l=20 \mu m$



Table 4Hosts and distributionof Atrocalyx species that havemolecular data

Species	Host/family	Origin	Life mode	References
A. acervatus	Acer sp. (Sapindaceae)	China	Saprobic	de Silva et al. (2018)
A. acutisporus	Unidentified woody plant	Japan	Saprobic	Hashimoto et al. (2017)
A. asturiensis	Cytisus sp. (Fabaceae)	Spain	Saprobic	Jaklitsch et al. (2018)
A. bambusae	Bamboo (Poaceae)	Thailand	Saprobic	Hyde et al. (2016)
A. guttulata	Unidentified plant	Thailand	Saprobic	Tibpromma et al. (2017)
A. krabiensis	Acacia sp. (Fabaceae)	Thailand	Saprobic	Jayasiri et al. (2019)
A. lignicola	Populus sp. (Salicaceae)	Belgium	Saprobic	Hashimoto et al. (2017)
A. quercus	Quercus variabilis (Fagaceae)	China	Saprobic	Present study



Fig. 5 Atrocalyx quercus (HKAS107388, holotype). a, b Conidiomata on the substrate. c, d Sections of pycnidium. e Pycnidium wall. f, g Developing conidia attached to conidiogenous cells. h–l Conidia.

median septum, with guttule in each cell, smooth-walled. Asexual morph: Undetermined.

Culture characters: Colonies on MEA reaching 30 mm diam. after 4 weeks of incubation at 25 °C. Culture above dark brown to black, with dense mycelia, circular, umbonate, rough surface, dull, covered with yellow aerial mycelium, oil **m** Germinating conidium. **n**, **o** Top and reverse view of colonies on PDA media. Scale bars: **c**, $d=40 \ \mu m$, **e**, $h=10 \ \mu m$, **f**, **g**, $m=5 \ \mu m$, $i-l=2 \ \mu m$

droplets formed in the culture; reverse black, orange pigment diffusing in the agar.

Material examined: THAILAND, Krabi Province, dried bark of *Diospyros malabarica*, 17 December 2015, C. Phukhamsakda, Kr018 (MFLU 17–1543, **holotype**); ex-type living culture, MFLUCC 16–0426.



Fig. 6 Phylogram generated from maximum likelihood analysis based on combined LSU, SSU, RPB2, TEF1 and ITS sequence data. Related sequences are taken from Hashimoto et al. (2017) and de Silva et al. (2018). Thirty-tree strains are included in the combined analyses which comprise 4111 characters (884 characters for LSU, 1026 characters for SSU, 728 characters for RPB2, 938 characters for TEF1, 535 characters for ITS) after alignment. *Hermatomyces thailandicus* (MFLUCC 14-1143) and *Hermatomyces tectonae* (MFLUCC 14-1140) in Hermatomycetaceae (Pleosporales) are used as the outgroup taxa. Single gene analyses were also performed to compare the topology and clade stability with combined gene analyses. Tree topology of the maximum likelihood analysis is similar

to the Bayesian analysis. The best RAxML tree with a final likelihood values of -23101.855495 is presented. The matrix had 1245 distinct alignment patterns, with 11.01% undetermined characters or gaps. Estimated base frequencies were as follows: A=0.247175, C=0.254162, G=0.268145, T=0.230519; substitution rates AC=1.719468, AG=4.211003, AT=1.547790, CG=1.204277, CT=9.847753, GT=1.000000; gamma distribution shape parameter α =0.168107. Bootstrap values for maximum likelihood (ML) equal to or greater than 70% and clade credibility values greater than 0.95 (the rounding of values to 2 decimal proportions) from Bayesian-inference analysis labeled on the nodes. Ex-type strains are in bold black, the new isolate is indicated in bold blue



Fig.7 Split graphs showing the results of the pairwise homoplasy index (PHI) tests of closely related taxa using LogDet transformation and splits decomposition. PHI test results (Φ w) \leq 0.05 indicate significant recombination within the dataset

Table 5 Hosts and distributionof Lignosphaeria species that	Species	Host/family	Origin	Life mode	References
have molecular data	Lignosphaeria thailandica Lignosphaeria fusispora	Unknown Unknown	Thailand Thailand	Saprobe Saprobe	Thambugala et al. (2015) Thambugala et al. (2015)
	Lignosphaeria diospyrosa	Diospyros malabar- ica (Ebenaceae)	Thailand	Saprobe	Present study

GenBank numbers: LSU: MT221674, SSU: MT221675, ITS: MT199809, TEF1: MT221676, RPB2: MT221677.

Notes: Lignosphaeria diospyrosa is introduced as a new sexual morph species. This species has overlapping asci size (48–75×5–10 µm vs. 63–68×7–8.5 µm in L. thailandica vs. 47.5–74×8–13 µm in L. fusispora), but smaller ascospores (10–15×2.5–5 µm vs. 15–19×5–6 µm in L. thailandica vs. 15–19×3–5 µm in L. fusispora). Lignosphaeria thailandica can be distinguished from L. fusispora based on its coriaceous to carbonaceous ascomata with rounded ostioles and 2–3-seriate, narrowly fusiform ascospores with acute ends.

Lignosphaeria diospyrosa forms a sister clade to L. thailandica and L. fusispora with strong statistical support (Fig. 8). There is no base pair difference in the LSU sequences between Lignosphaeria diospyrosa and the other species. Interestingly, there is no base pair difference in the ITS and LSU gene between Lignosphaeria thailandica and L. fusispora. The TEF1, RPB2 and SSU gene regions are not available for Lignosphaeria thailandica and L. fusispora. Lignosphaeria thailandica and L. fusispora. Lignosphaeria thailandica and L. fusispora could represent the same species based on available sequence data and extensive sampling is needed to differentiate between the distinct phenotypes. Lignosphaeria diospyrosa differs from L. thailandica and L. fusispora by 18 base pairs (4.3%) in the partial ITS sequence.

Sordariomycetes

The latest treatment of this class is Hyde et al. (2020b).

Amphisphaeriales D. Hawksw. & O.E. Erikss.

Amphisphaeriales was introduced by Eriksson and Hawksworth (1986) in the subclass Xylariomycetidae. Seventeen families are accepted by Hyde et al. (2020b) based on their placement and divergence time estimates, i.e., Amphisphaeriaceae, Apiosporaceae, Beltraniaceae, Castanediellaceae, Clypeophysalosporaceae, Cylindriaceae, Hyponectriaceae, Iodosphaeriaceae, Melogrammataceae, Oxydothidaceae, Phlogicylindriaceae, Pseudomassariaceae, Pseudosporidesmiaceae, Pseudotruncatellaceae, Sporocadaceae, Vialaeaceae and Xyladictyochaetaceae.

Sporocadaceae Corda

Sporocadaceae represents endophytic, pathogenic and saprobic fungi, with a wide host range. Jaklitsch et al (2016) re-validated Sporocadaceae and accepted 22 genera. Liu et al. (2019b) revised this family, based on morphological characters and phylogenetic analysis of LSU, ITS, ef1 α , tub2 and rpb2. Hyde et al. (2020b) accepted 32 genera in this family based on published data and provided notes on each genus.

Pestalotiopsis Steyaert

Species of *Pestalotiopsis* are common phytopathogens that cause a variety of diseases or saprobes or endophytes on a wide variety of plants (Maharachchikumbura et al. 2011, 2014). They are widely distributed in tropical and temperate regions, and produce wide range of chemically novel metabolites (Guba 1961; Barr 1975; Uecker and Raj 1994;



Fig. 8 Bayesian 50% majority-rule consensus phylogram based on combined LSU, SSU, ITS, TEF1 and RPB2 sequence data. Related sequences were retrieved from GenBank. Fifteen strains were included in the analysis of the combined loci and comprises 4081 characters after alignment (810 characters for LSU, 1017 characters for SSU, 484 characters for ITS, 895 characters for TEF1, 875 characters for RPB2, including gaps). The tree is rooted with *Lophiostoma arundinis* (CBS 621.86) and *L. crenatum* (CBS 629.86) in Lophiostomataceae. Maximum parsimony analysis of 705 parsimony informative characters resulted in a most parsimonious tree (CI=0.812, RI=0.769, RC=0.625, HI=0.188). The best scoring RAxML tree received a final likelihood value of -13097.434362. The matrix had 809 distinct alignment patterns, with 36.94% undetermined characters.

Xu et al. 2010; Maharachchikumbura et al. 2012, 2014; Debbab et al. 2013; Norphanphoun et al. 2019). In this entry, we introduce a new species *Pestalotiopsis kandelicola* based on morphology and phylogeny. The fungus was isolated from the asymptomatic leaves of *Kandelia candel* in Taiwan.

Although there are 366 *Pestalotiopsis* epithets in Index Fungorum (2020), the number of known *Pestalotiopsis* is likely to be much less, as many species are synonyms and others belong to other genera such as *Monochaetia*, *Neopestalotiopsis*, *Truncatella* and *Pseudopestalotiopsis*

acters and gaps. Estimated base frequencies were: A=0.241927, C=0.259640, G=0.276058, T=0.222376; substitution rates AC=1.377930, AG=3.094856, AT=1.588916, CG=1.478544, CT=8.506796, GT=1.000000; gamma distribution shape parameter $\alpha=0.703865$. In our analysis, GTR+I+G model was used for each partition in Bayesian posterior analysis. Bootstrap values (BS) from maximum parsimony (MP, left), maximum likelihood (ML, right) higher than 50% BS and Bayesian posterior probabilities (BYPP, below) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 50% BS/0.90 BYPP. Thick branches represent significant support values from all analyses (BS $\geq 75\%$ /BYPP ≥ 0.95). The ex-type strains are in bold and black. The newly generated sequence is in bold and blue

(Maharachchikumbura et al. 2011, 2014). Thus 285 species are presently listed in *Pestalotiopsis* in Species Fungorum (2020). However, are we likely to find many more new species in the genus? Eighty *Pestalotiopsis* species have sequence data in GenBank and are confirmed as distinct species. The taxa are from 56 different hosts and 31 plant families indicating that the genus has a wide host range and that species are possibly specific to families or genera (Table 6). Species have been collected from Africa, Asia, Australia, European, North America, South America but, numerous



Fig. 9 Lignosphaeria diospyrosa (MFLU 17-1543, holotype). a–c Appearance of ascomata on natural substrate. d Vertical section through ascoma. e Ostiole canal. f Partial peridium layer. g Pseudoparaphyses. h, i Asci (h, i Asci stained with Congo red solution). j

regions have never been studied for the genus (Table 6). Thus, we believe that comprehensive studies on this genus are likely to result in numerous new species. Ocular chamber. **k**–**n** Ascospores. **o** Germinated ascospore. **p**, **q** Culture characteristics on MEA. Scale bars: $c = 200 \ \mu\text{m}$, $d = 100 \ \mu\text{m}$, **e**, $g-i=50 \ \mu\text{m}$, $f=20 \ \mu\text{m}$, $j-n=10 \ \mu\text{m}$, $p, q=30 \ \text{mm}$

Pestalotiopsis kandelicola Norph., C.H. Kuo & K.D. Hyde, sp. nov.

Index Fungorum number: IF557755; Facesoffungi number: FoF 08934, Fig. 10

Species	Host/family	Origin	Life mode	References
Pestalotiopsis aggestorum	Camellia sinensis (Theaceae)	China	Pathogen	Liu et al. (2017)
P. anacardiacearum	Mangifera indica (Anacardi- aceae)	China	Pathogen	Maharachchikumbura et al. (2013b)
P. arceuthobii	Arceuthobium campylopodum (Santalaceae)	USA	N/A	Maharachchikumbura et al. (2014)
P. arengae	Arenga undulatifolia (Are- caceae)	Singapore	Saprobe	Maharachchikumbura et al. (2014)
P. australasiae	Knightia sp. (Proteaceae)	New Zealand	N/A	Maharachchikumbura et al. (2014)
P. australis	<i>Grevillea</i> sp. (Proteaceae), <i>Protea neriifolia × susannae</i> (Proteaceae)	Australia, South Africa	N/A	Maharachchikumbura et al. (2014)
P. biciliata	Paeonia sp. (Paeoniaceae), Platanus × hispanica (Pla- tanaceae), Taxus baccata (Taxaceae)	Slovakia, Italy, Netherlands	Saprobe	Maharachchikumbura et al. (2014)
P. brachiata	Camellia sp. (Theaceae)	China	N/A	Liu et al. (2017)
P. brassicae	Brassica napus (Brassicaceae)	New Zealand	N/A	Maharachchikumbura et al. (2014)
P. bulbophylli	Bulbophyllum thouars (Orchi- daceae)	China	Saprobe	Wang et al. (2017)
P. camelliae	Camellia japonica (Theaceae)	China	Pathogen	Zhang et al. (2012c)
P. chamaeropis	<i>Chamaerops humilis</i> (Are- caceae)	Italy	N/A	Maharachchikumbura et al. (2014)
P. chinensis	Taxus sp. (Taxaceae)	China	Endophyte	Maharachchikumbura et al. (2012)
P. clavata	Buxus sp. (Buxaceae)	China	Endophyte	Maharachchikumbura et al. (2012)
P. coffeae-arabicae	Coffea arabica (Rubiaceae)	China	N/A	Song et al. (2013)
P. colombiensis	Eucalyptus eurograndis (Myrtaceae)	Colombia	N/A	Maharachchikumbura et al. (2014)
P. dianellae	Dianella sp. (Asphodelaceae)	Australia	N/A	Crous et al. (2017)
P. digitalis	<i>Digitalis purpurea</i> (Plantagi- naceae)	New Zealand	Pathogen	Liu et al. (2015)
P. diploclisiae	Diploclisia glaucescens (Men- ispermaceae)	Hong Kong	N/A	Maharachchikumbura et al. (2014)
P. distincta	Camellia sinensis (Theaceae)	China	N/A	Crous et al. (2017)
P. diversiseta	Rhododendron sp. (Ericaceae)	China	Endophyte	Maharachchikumbura et al. (2012)
P. doitungensis	Dendrobium sp. (Orchidaceae)	Thailand	Endophyte	Ma et al. (2019)
P. dracontomelon	Dracontomelon dao (Anacar- diaceae)	Thailand	Pathogen	Liu et al. (2015)
P. ericacearum	Rhododendron delavayi (Eri- caceae)	China	Pathogen	Zhang et al. (2013)
P. formosana	On dead grass	Taiwan	Saprobe	Ariyawansa and Hyde (2018)
P. furcata	Camellia sinensis (Theaceae)	Thailand	N/A	Maharachchikumbura et al. (2013a)
P. gaultheria	Gaultheria forrestii (Eri- caceae)	China	Pathogen	Zhang et al. (2013)
P. gibbosa	Gaultheria shallon (Ericaceae)	Canada	N/A	Watanabe et al. (2018)
P. grevilleae	Grevillea sp. (Proteaceae)	Australia	N/A	Maharachchikumbura et al. (2014)

 Table 6
 Hosts and distribution of Pestalotiopsis species that have molecular data

Species	Host/family	Origin	Life mode	References
P. hainanensis	Camellia sinensis (Theaceae), Podocarpus spp. (Podocar- paceae), Tamarindus indica (Fabaceae)	China	Endophyte	Liu et al. (2007)
P. hawaiiensis	<i>Leucospermum</i> sp. (Pro-teaceae)	USA	N/A	Maharachchikumbura et al. (2014)
P. hispanica	Proteaceae	Spain	N/A	Liu et al. (2019b)
P. hollandica	Sciadopitys verticillata (Scia- dopityaceae)	The Netherlands	N/A	Maharachchikumbura et al. (2014)
P. humicola	Acacia mangun (Fabaceae)	Malaysia	N/A	Liu et al. (2019b)
P. humus	Soil	Papua New Guinea	Saprobe	Maharachchikumbura et al. (2014)
P. inflexa	Dead plant material	China	Saprobe	Maharachchikumbura et al. (2012)
P. intermedia	Dead plant material	China	Saprobe	Maharachchikumbura et al. (2012)
P. italiana	Cupressus glabra (Cupressaceae)	Italy	Saprobe	Liu et al. (2015)
P. ixorae	Ixora sp. (Rubiaceae)	Japan	N/A	Watanabe et al. (2012)
P. jesteri	Fagraea bodenii (Gentian- aceae)	Papua New Guinea	N/A	Maharachchikumbura et al. (2014)
P. jiangxiensis	Camellia sp. (Theaceae)	China	N/A	Liu et al. (2017)
P. jinchanghensis	Camellia sinensis (Theaceae)	China	N/A	Liu et al. (2017)
P. kandelicola	Kandelia candel (Rhiz- ophoraceae)	Taiwan	Endophyte	Present study
P. kenyana	Coffea sp. (Rubiaceae)	Kenya	N/A	Maharachchikumbura et al. (2014)
P. knightiae	Knightia sp. (Proteaceae)	New Zealand	N/A	Maharachchikumbura et al. (2014)
P. krabiensis	Pandanaceae	Thailand	Endophyte	Tibpromma et al. (2018)
P. kunmingensis	Podocarpus macrophyllus (Podocarpaceae)	China	Endophyte	Wei and Xu (2004)
P. leucadendri	Leucadendron sp. (Proteaceae)	South Africa	Saprobic	Liu et al. (2019b)
P. lijiangensis	Castanopsis carlesii (Fagaceae)	China	Endophyte	Zhou et al. (2018)
P. longiappendiculata	Camellia sinensis (Theaceae)	China	N/A	Liu et al. (2017)
P. lushanensis	Camellia sp. (Theaceae)	China	N/A	Liu et al. (2017)
P. macadamiae	Macadamia sp. (Proteaceae)	Australia	Pathogen	Akinsanmi et al. (2017)
P. malayana	Macaranga triloba (Euphor- biaceae)	Malaysia	N/A	Maharachchikumbura et al. (2014)
P. monochaeta	Quercus robur (Fagaceae)	Netherlands	N/A	Maharachchikumbura et al. (2014)
P. monochaetioides	N/A	Netherlands	N/A	Vu et al. (2019)
P. montellica	Dead plant material	China	Saprobe	Maharachchikumbura et al. (2012)
P. neolitseae	Neolitsea villosa (Lauraceae)	Taiwan	Endophyte	Ariyawansa and Hyde (2018)
P. novae-hollandiae	Banksia grandis (Proteaceae)	Australia	N/A	Maharachchikumbura et al. (2014)
P. oryzae	Oryza sativa (Oryzeae), Telo- pea sp. (Proteaceae)	Denmark, Italy, USA	N/A	Maharachchikumbura et al. (2014)
P. pandanicola	Pandanaceae	Thailand	Saprobe	Tibpromma et al. (2018)
P. papuana	Coastal soil	Papua New Guinea	N/A	Maharachchikumbura et al. (2014)

Fungal Diversity

Table 6 (continued)
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Species	Host/family	Origin	Life mode	References
P. parva	Delonix regia (Fabaceae)		N/A	Maharachchikumbura et al. (2014)
P. photinicola	Photinia serrulata (Rosaceae)	China	Pathogen	Chen et al. (2017)
P. pinicola	Pinus armandii (Pinaceae)	China	Saprobe, endophyte	Tibpromma et al. (2019)
P. portugalica	N/A	Portugal		Maharachchikumbura et al. (2014)
P. rhizophorae	Rhizophora apiculata (Rhiz- ophoraceae)	Thailand	Pathogen	Norphanphoun et al. (2019)
P. rhododendri	Antidesma ghaesembilla (Phyl- lanthaceae), Rhododendron sinogrande (Ericaceae)	China	Saprobe	Maharachchikumbura et al. (2012)
P. rhodomyrtus	Rhodomyrtus tomentosa (Myrtaceae)	China	N/A	Song et al. (2013)
P. rosea	Pinus sp. (Pinaceae)	China	Endophyte	Maharachchikumbura et al. (2012)
P. scoparia	Chamaecyparis sp. (Cupres- saceae)		Saprobe	Maharachchikumbura et al. (2014)
P. sequoiae	Cupressaceae sempervirens (Cupressaceae)	Italy	Endophyte, pathogen	Hyde et al. (2016)
P. shorea	Shorea obtusa (Dipterocar- paceae)	Thailand	Saprobe	Song et al. (2014)
P. spathulata	Gevuina avellana (Proteaceae)	Chile	Pathogen	Maharachchikumbura et al. (2014)
P. spathuliappendiculata	Phoenix canariensis (Are- caceae)	Australia	N/A	Liu et al. (2019b)
P. telopeae	Telopea sp. (Proteaceae)	Australia	Pathogen	Maharachchikumbura et al. (2014)
P. terricola	Soil	Pacific Island	N/A	Liu et al. (2019b)
P. thailandica	Rhizophora apiculata (Rhiz- ophoraceae)	Thailand	Endophyte	Norphanphoun et al. (2019)
P. trachicarpicola	Chrysophullum sp. (Sapota- ceae), Podocarpus macro- phyllus (Podocarpaceae), Schima sp. (Theaceae), Trachycarpus fortunei (Are- caceae)	China	Endophyte, pathogen	Zhang et al. (2012b)
P. unicolor	Rhododendron sp. (Ericaceae)	China	Endophyte	Maharachchikumbura et al. (2012)
P. verruculosa	Rhododendron sp. (Ericaceae)	China	Endophytes	Maharachchikumbura et al. (2012)
P. yanglingensis	Camellia sinensis (Theaceae)	China	N/A	Liu et al. (2017)
P. yunnanensis	Podocarpus macrophyllus (Podocarpaceae)	China	Endophyte	Wei et al. (2013)

Etymology: name reflects the host genus *Kandelia*. Holotype: NCYU 19-0355 (dried ex-culture)

Isolated from asymptomatic leaf of Kandelia candel. Sexual morph: Undetermined. 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, smoothand thin-walled, simple, collarette present and not flared, $10-25 \times 2-5 \mu m$. Conidia $20-23.5 \times 4-6 \mu m$ ($\bar{x}=22 \times 5 \mu m$), 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, $(3-)3.5-4.5(-5) \mu m$ long; three median cells $(12-)13-14(-15) \mu m$ long, brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, $(3-)3.5-4(-5) \mu m$ long; third cell brown, $(3-)4-5(-6) \mu m$ long; fourth cell brown, $(3-)4-5(-6) \mu m$ long; apical cell $(3-)3.5-4(-4.5) \mu m$ long, hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci

Fig. 10 Pestalotiopsis kandelicola (NCYUCC 19-0355, ex-type). a Habitat. b, c Kandelia candel. d 7 days of culture on PDA (leaf-above, right-reverse). e-g Colony sporulating on PDA. h Conidiogenous cells giving rise to conidia. i-m Conidia. Scale bars: e, g=1 mm, f=500 μ m, h-n=10 μ m



in a crest at the apex of the apical cell, branched, flexuous, $(11-)13-14(-15) \ \mu m \ long \ (\bar{x} \pm SD = 13 \pm 1.5 \ \mu m)$; single basal appendage, tubular, unbranched, centric, $(2-)2.5-3(-3.5) \ \mu m \ long \ (\bar{x} \pm SD = 2 \pm 0.5 \ \mu m)$.

Culture characteristics: Colonies on PDA reaching 6–7 cm diam. after 14 d at room temperature (± 25 °C), under light 12 h/dark 12 h, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, fluffy, white from above and reverse.

Material examined: TAIWAN, New Taipei, tissue isolation from asymptomatic leave of *Kandelia candel*, 15 July 2018, Chada Norphanphoun TPE1P-2A (NCYU, holotype; MFLU, isotype); NCYUCC 19-0355, ex-living cultures; TPE1P-2C, NCYUCC 19-0354, living cultures.

GenBank Number: NCYUCC 19-0355: ITS: MT560722, LSU: MT560710, EF1α: MT563101, TUB2: MT563099;

NCYUCC 19-0354: ITS: MT560723, LSU: MT560711, EF1α: MT563102, TUB2: MT563100.

Notes: Two strains of *Pestalotiopsis kandelicola* form a distinct subclade, sister to *P. parva* with high bootstrap support (96% MP, 99% ML, 1.00 PP, Fig. 11). *Pestalotiopsis kandelicola* differs from *P. parva* in having longer and narrower conidia (*P. kandelicola*: $(20-)21-22(-23.5)\times(4-)4.5-5(-6) \mu m vs.$ *P. parva* $: <math>(16-)16.5-20(-21)\times5-7(-7.5) \mu m$); longer apical appendages (*P. kandelicola*: $(11-)13-14(-15) \mu m vs.$ *P. parva* $: <math>(6-)6.5-12(-13) \mu m$) and shorter basal appendages (*P. kandelicola*: $(2-)2.5-3(-3.5) \mu m vs.$ *P. parva* $: <math>2-4 \mu m$) (Maharachchikumbura et al. 2014).

Chaetosphaeriales

We follow (Hyde et al. 2020b) for treatment of this order.

Fig. 11 One of the 1000 most parsimonious trees obtained from a heuristic search of combined ITS, β-tubulin and EF1a sequence data for Pestalotiopsis. The tree is rooted to Neopestalotiopsis saprophytica (MFLUCC 12-0282). Maximum parsimony and maximum likelihood bootstrap values \geq 50%, Bayesian posterior probabilities \geq 0.95 (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 hold



Chaetosphaeriaceae Réblová, M.E. Barr & Samuels

The asexual morphs in *Chaetosphaeriaceae* have been reported as both coelomycetes and hyphomycetes (Réblová et al. 1999; Shenoy et al. 2007; Lu et al. 2016; Wei et al. 2018; Lin et al. 2019). There are currently 44 genera accommodated in the family (Wijayawardene et al. 2020). However, some genera are polyphyletic and phylogenetic

relationships are still unresolved (Tibpromma et al. 2018; Wei et al. 2018; Lin et al. 2019; Phookamsak et al. 2019). Further taxon sampling with molecular data is required to resolve these ambiguous genera (Jeewon et al. 2009; Perera et al. 2016; Tibpromma et al. 2018; Wei et al. 2018; Lin et al. 2019).

Thozetella Kuntze

Thozetella was introduced by Kuntze (1891) and is typified by T. nivea. The genus is characterized by sporodochial or synnematous conidiomata, with terminated, phialidic conidiogenous cells and hyaline, naviculate to fusiform or ellipsoid, aseptate conidia, with unbranched setula at each end, and forming sterile microawns (Sutton and Cole 1983; Perera et al. 2016; Tibpromma et al. 2018). Most Thozetella species have similar conidial characters, however, these species can be distinguished by their sporodochial formations and microawns coupled with molecular analysis (Paulus et al. 2004; Jeewon et al. 2009; Da Silva and Grandi 2011; Perera et al. 2016; Tibpromma et al. 2018; Phookamsak et al. 2019). Thozetella species have been reported as saprobes in soil and on decaying plants in terrestrial and freshwater habitats from temperate and tropical regions and there is no reported sexual morph (Morris 1956; Agnihothrudu 1958; Waipara et al. 1996; Sivichai et al. 2002; Delgado-Rodríguez et al. 2004; Paulus et al. 2004; Jeewon et al. 2009; Barbosa et al. 2011; Da Silva and Grandi 2011, 2013; Perera et al. 2016; Crous et al. 2018a, 2019; Tibpromma et al. 2018; Phookamsak et al. 2019). There are 26 species accommodated in the genus (Index Fungorum 2020). We follow the latest treatment and updated accounts of Thozetella in Perera et al. (2016), Tibpromma et al. (2018) and Phookamsak et al. (2019). There are 114 sequences of 16 *Thozetella* species in GenBank, however, these taxa only have ITS and LSU sequence data, and no DNA sequence data from protein coding gene.

A new species of *Thozetella*, *T. bambusicola*, from dead bamboo branches is introduced here. It is the first time to discover sporodochia surrounded by black, hairy setae in this genus. The species is conspicuous as it has short sporodochial or synnematous conidioma. We are likely to find more new species as taxa in this genus can be found from bamboo, leaf litter, palm parts, seed pods, shrubs and debris in at least 14 plant families indicating that the genus has a wide host range and that *Thozetella* are possibly specific to host families or genera (Table 7). Most species have been collected in America, Asia and Oceania, and numerous regions have never been studied for the genus (Table 7). Thus, we believe that comprehensive studies on this genus are likely to result in numerous new species.

Thozetella bambusicola H.B. Jiang, Phookamsak & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF557717; Facesoffungi number: FoF 08140; Fig 12

Etymology: Refers to the fungal bamboo host. Holotype: KUN-HKAS 101776

Saprobic on dead bamboo branches. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. Sporodochia solitary, scattered, superficial, yellowish to orange, cylindrical or subulate, surrounded by black, hairy setae growing from the base. *Microawns* 70–100 µm long, 3.5–5 µm wide, aseptate, smooth-walled, thick-walled, straight or curved, sometimes L-shaped. *Conidiophores* $37-60 \times 2-2.5$ µm, macronematous, synnematous, abundant, brown, elongated, cylindrical, septate, branched, densely compacted along the synnemata axis, smoothwalled. *Conidiogenous cells* 1–2.2 µm wide, phialidic, hyaline, subcylindrical, smooth-walled. *Conidia* $13.7-15.5 \times 2.1-2.7$ µm (\bar{x} = 14.6 × 2.4 µm, n=20), naviculate to fusiform or ellipsoid, with rounded ends, hyaline, smooth-walled, with a single filiform setula at each end, 4.8–5.7 µm long.

Material examined: CHINA, Sichuan Province, Yibin City, Changning County, Shunan Bamboo Forest, on dead bamboo branches, 23 July 2019, H.B. Jiang and R. Phookamsak, SC025 (KUN-HKAS 101776, holotype).

GenBank numbers: LSU: MT102918, SSU: MT102919, ITS: MT102917.

Notes—*Thozetella bambusicola* is unique and can be distinguished from other *Thozetella* species in having sporodochia surrounded by black, hairy setae. *Thozetella bambusicola* forms an independent lineage distinct from other *Thozetella* taxa based on phylogenetic analyses of a combined ITS, LSU and TUB2 sequence dataset (Fig. 13). Through the NCBI BLASTn search of ITS region, *T. bambusicola* is most similar to *Thozetella* sp. (JMGB06_7A1) with 94.4% similarity, however, they differ in 27/481 bp (5.6%).

Glomerellales

Glomerellales was invalidly introduced by Chadefaud (1960) and was validated by Réblová et al. (2011). This order currently includes five families *viz*. Australiascaceae, Glomerellaceae, Malaysiascaceae, Plectosphaerellaceae and Reticulascaceae (Maharachchikumbura et al. 2016; Tibpromma et al. 2018; Hyde et al. 2020b; Wijayawardene et al. 2020).

Glomerellaceae

Glomerellaceae was invalidly published by Locquin (1984) and was validated by Zhang et al. (2006) with a Latin description placing this family within the subclass Hypocreomycetidae. Maharachchikumbura et al. (2016) provided evidence for the phylogenetic position of Glomerellaceae within Glomerellales. This family is monotypic with *Colletotrichum*.

Colletotrichum Corda

Colletotrichum is typified by *C. lineola* which was collected from on a dead stem of an undetermined host of Apiaceae in the Czech Republic (Corda 1831). Nine-hundred and two epithets are listed under *Colletotrichum* in Index Fungorum (2020) and most these species are recorded as

Table 7 Hosts and	Table 7 Hosts and distribution of Thozetella species that have molecular data	data		
Species	Host/family	Origin	Life mode	Life mode References
Thozetella acerosa	Cryptocarya mackinnoniana (Lauraceae)	Australia	Saprobe	Paulus et al. (2004)
T. boonjiensis	Cryptocarya mackinnoniana (Lauraceae) and Opisthiolepis heterophylla (Proteaceae)	Australia	Saprobe	Paulus et al. (2004)
T. bambusicola	Dead bamboo	China	Saprobe	Present study
T. cristata	 Alchornea triplinervia (Euphorbiaceae), Andira fraxinifolia (Fabaceae), Caesalpinia echinata (Fabaceae), Cedrela fissilis (Meliaceae), Chamae- crista desvauxii (Fabaceae), Clusia melchiorii (Clusiaceae), Clusia nemorosa (Clusiaceae), Euterpe edulis (Arecaceae), Persea barbonia (Lauraceae) 	Australia, Brazil, Cuba, the USA, Italy, Japan, Mexico, Venezuela	Saprobe	Pirozynski and Hodges Jr. (1973), Lunghini and Quadraccia (1990), Grandi (1998), Heredia (1999), Castañeda-Ruiz et al. (2003a, b), Paulus et al. (2004), da Silva and Grandi (2008), Barbosa et al. (2009), Dos Santos Santa Izabel et al. (2011), Da Silva and Grandi (2013)
T. fabacearum	Fabaceae sp.	Thailand	Saprobe	Perera et al. (2016)
T. falcata	Cryptocarya mackinnoniana (Lauraceae), Ficus pleurocarpa (Moraceae)	Australia, Brazil	Saprobe	Paulus et al. (2004), Da Silva and Grandi (2013)
T. gigantea	Caesalpinia echinate (Fabaceae), Cryptocarya mackinnoniana (Lauraceae)	Australia, Brazil	Saprobe	Paulus et al. (2004), Barbosa et al. (2011)
T. havanensis	Calophyllum Antillanum (Calophyllaceae)	Brazil, Cuba, Nigeria	Saprobe	Castañeda-Ruiz (1984), Mercado-Sierra et al. (1997), Calduch et al. (2002), da Silva and Grandi (2013)
T. lithocarpi	Lithocarpus sp. (Fabaceae)	Thailand	Saprobe	Phookamsak et al. (2019)
T. neonivea	Archontophoenix cunninghamiana (Arecaceae)	New Zealand	Saprobe	Crous et al. (2019)
T. nivea	Dead wood of unidentified plant	Australia, the USA	Saprobe	Kuntze (1891), Pirozynski and Hodges Jr. (1973)
T. pandanicola	Pandanus sp. (Pandanaceae)	Thailand	Saprobe	Tibpromma et al. (2018)
T. pindobacuensis	Decaying leaves of unidentified plant	Brazil	Saprobe	Crous et al. (2018a)
T. pinicola	Pinus elliotti (Pinaceae)	China	Saprobe	Jeewon et al. (2009)
T. queenslandica	Caesalpinia echinate (Fabaceae), Cryptocarya mackinnoniana (Lauraceae)	Australia, Brazil	Saprobe	Paulus et al. (2004), Gusmão et al. (2006), Dos Santos Santa Izabel et al. (2011)
T. tocklaiensis	Camellia sinensis (Theaceae), Eucalyptus sp. (Myrtaceae), debris	Argentina, Australia, Brazil, India, New Zealand, Papua New Guinea, South Africa	Saprobe	Agnihothrudu (1958), Maia (1983, 1998), Waipara et al. (1996), Maia et al. (2002), Piontelli and Giusiano (2004)



Fig. 12 *Thozetella bambusicola* (KUN-HKAS 101776, holotype). a Sporodochia on a dead bamboo branch. b, g Conidiogenous cells. c, d Conidiophores. e, f Microawns. h–j Conidia. Scale bars: b–d, g–j=10 μ m, e=15 μ m, f=20 μ m

pathogens, endophytes or saprobes from terrestrial habitats (Cannon et al. 2012; Jayawardena et al. 2016a, b). In this paper, we introduce a new species of *Colletotrichum*, which was collected from leaves of *Citrus medica* in China.

Although there are a large number of Colletotrichum epithets, the number of known Colletotrichum species is likely to be much less. Thus 493 species are presently listed in Colletotrichum in Species Fungorum (2020). There are 253 Colletotrichum species in GenBank confirmed with molecular data, however only 248 species are accepted in Jayawardena et al. (2020). In this paper, we describe a new species, Colletotrichum citrus-medicae, from Citrus medica (citron) from a commercial orchard in China (Figs. 14, 15). The species is conspicuous as it causes leaf spot disease. However, are we likely to find many more new species in the genus? The majority of species belong to 14 species complexes while a few species as singletons (Damm et al. 2019). All species are from different hosts and plant families with a wide geographic distribution, indicating that the genus has a wide host range and we may find many new species with further sampling. Several species are host-specific (Jayawardena et al. 2016a), especially species in the caudatum and graminicola species complexes from Poaceae. However, it is still unclear whether this is due to inadequate sampling or whether these species are truly host-specific. The number of species accepted by (Jayawardena et al. 2016a) is 190, and during the past four years 63 species from different hosts and different countries have been reported (Bhunjun et al. 2019; Damm et al. 2019; Fu et al. 2019). There are many studies on *Colletotrichum*, but not all countries or host families have been investigated (Cannon et al. 2012; Jayawardena et al. 2016a) and most of these studies focus on economically important crops. Thus, we believe that comprehensive studies on this genus are likely to result in numerous new species.

Colletotrichum citrus-medicae Qian Zhang, Yong Wang bis, Jayawardena & K.D. Hyde, *sp. nov*.

Index Fungorum number: IF 557772; Facesoffungi number: FoF 08677, Fig. 15

Etymology: *citrus-medicae*, in reference to the host *Citrus medica*.

Holotype: HGUP 1554

Pathogenic on leaves of Citrus medica. Sexual morph: Undetermined. Asexual morph: Sclerotia abundant, black, globose to irregular. Aerial mycelium in small tufts,



Fig. 13 Phylogram generated from maximum likelihood analysis based on combined ITS, LSU and TUB2 sequence dataset representing *Chaetosphaeriaceae* and the outgroup taxa. Related sequences are derived from Phookamsak et al. (2019). Fifty-seven strains are included in the combined analyses which comprise 2586 characters (601 characters for ITS, 935 characters for LSU, 1050 characters for TUB) after alignment. *Sordaria fimicola* (CBS 508.50) and *Gelasinospora tetrasperma* (AFTOL-ID 1287) are used as the outgroup taxa. Single gene analyses were also performed to compare the topology of the maximum likelihood analysis is similar to the Bayesian inference analysis. The best RAXML tree with a final likelihood values

of -15195.632060 is presented. The matrix had 901 distinct alignment patterns, with 55.21% undetermined characters or gaps. Estimated base frequencies were as follows: A=0.219814, C=0.276912, G=0.298070, T=0.205204; substitution rates AC=1.072117, AG=1.547562, AT=1.767556, CG=0.918998, CT=7.063468, GT=1.000000; gamma distribution shape parameter α =0.481499. Bootstrap values for maximum likelihood (ML) equal to or greater than 70% and clade credibility values greater than 0.90 (the rounding of values to 2 decimal proportions) from Bayesian-inference analysis labeled on the nodes as ML/PP. Ex-type strains are in bold and black, the new isolate is indicated in bold and blue

white, sparse, with orange to dark orange conidial masses. Acervuli absent in culture. Setae absent. Conidiophores hyaline, smooth-walled, simple, $13.5-56.5 \times 3.5-9.5 \ \mu m$ ($\bar{x} = 32 \times 4.9 \ \mu m$, n = 20). Conidiogenous cells $4-18.5 \times 3.5-6.5 \ \mu m$ ($\bar{x} = 7.5 \times 4.9 \ \mu m$, n = 20), hyaline, smooth-walled, cylindrical to slightly inflated. Conidia 13.5–17 × 5.5–9 µm ($\bar{x} = 15 \times 7.5$ µm, n = 50), hyaline, smooth, one-celled, cylindrical with obtuse ends (oblong), occasionally slightly narrowing at the center, guttulate. *Appressoria* 6–9.5×5.5–8.5 µm ($\bar{x} = 7.5 \times 7$ µm, n = 30), solitary to aggregated, medium to dark brown, smooth-walled, round or oval or irregular.



Fig. 14 The MP consensus tree inferred from the combined ITS, GAPDH, CHS, ACT, TUB and HIS sequence alignments of the *Collectotrichum* dataset. Parsimony bootstrap support values above

Culture characteristics—Colonies on PDA reaching 45 mm in 7 days at 28 °C, at first white and becoming pale brownish to pinkish, reverse pale yellowish to pinkish.

Material examined—CHINA, Kunming Botanical Gardens, Kunming, Yunnan Province, on diseased leaves of *Citrus medica*, 15 January 2018, Q. Zhang (HGUP 1554, **holotype**); ex-type living culture GUCC 1554 = MFLUCC 19–0173, *ibid*. (GUCC 1555 and GUCC 1556 **ex-paratypes**).

GenBank numbers: GUCC 1554: ITS: MN959910, GAPDH: MT006331, CHS: MT006328, ACT: MT006325, TUB2: MT006337, HIS-MT006334; GUCC 1555: ITS: MN959911, GAPDH: MT006332, CHS: MT006329, ACT: MT006326, TUB2: MT006338, HIS: MT006335; GUCC 1556: ITS: MN959912, GAPDH: MT006333, CHS: MT006330, ACT: MT006327, TUB2: MT006339, HIS: MT006336.

Notes: We selected five gene fragments for MP and BYPP phylogenetic analyses. Based on DNA sequences, *C. citrus-medicae* does not belong to any known *Colletotrichum* species complex; the closest matches in blastn searches of the ex-holotype strain in GenBank with 50%, and Bayesian posterior probability values above 0.90 are shown at the nodes. *Monilochaetes infuscans* (CBS 869.96) was set as outgroup

sequences of the different loci resulted in sequences of strains from different species complexes. Strains (HGUP 1554, HGUP 1555 and HGUP 1556) formed an independent branch with high MP bootstrap and BYPP support (100/1.00) (Fig. 14). Colletotrichum citrus-medicae is a singleton species closely related to C. sydowii. The newly described species shows 51 bp differences in ITS, 59 bp in CHS and more 50 bp differences in GAPDH, ACT and Tub2 with C. sydowii. Colletotrichum citrus-medicae differs from C. sydowii by relatively shorter conidia (13.5-17 vs 18-21) and smaller simple, un-lobed appressoria (6-9.5 vs 8-18) (Weir et al. 2012). Thirty-two species of Colletotrichum have been reported from Citrus sp. worldwide and 15 species have been reported from China (Farr and Rossman 2020). Among them C. boninense, C. brevisporum, C. gloeosporioides, C. novae-zelandiae and C. truncatum are known from Citrus medicae.

Microascales Luttr. ex Benny & Kimbr.

Hyde et al. (2020b) provided the latest treatment of Microascales. See also Réblová et al. (2011),



Fig. 15 *Colletotrichum citrus-medicae* (HGUP 1554, holotype). a, b Upper (a) and reverse (b) sides of cultures on PDA 7 days after inoculation. d-g Conidiophores, conidiogenous cells and conidia. h-m Appressoria. n Conidia. Scale bars: $d-g=20 \mu m$, $h-m=5 \mu m$, $m=10 \mu m$

Maharachchikumbura et al. (2016) and Wijayawardene et al. (2018, 2020) for additional information about this order.

Halosphaeriaceae E. Müll. & Arx ex Kohlm

Halosphaeriaceae, typified by *Halosphaeria*, is one of the seven currently accepted families in Microascales, viz Ceratocystidaceae, Chadefaudiellaceae, Gondwanamycetaceae, Graphiaceae, Halosphaeriaceae, Microascaceae, Triadelphiaceae (Hyde et al. 2020b). It includes 163 species in 64 genera (Pang 2002; Jones et al. 2009, 2015, 2017, 2019; Maharachchikumbura et al. 2015; Wijayawardene et al. 2017, 2018, 2020; Hyde et al. 2020b). Halosphaeriaceous species are cosmopolitan in distribution and are primarily found in marine environments thriving on different substrates (e.g. driftwood, mangrove wood, seagrasses, marine algae) (Jones 2011; Jones and Pang 2012; Jones et al. 2013), and some are freshwater species (Pang and Jheng 2012; Cai et al. 2014). Around 75% of halosphaeriaceous species have been sequenced (Jones et al. 2017). The type species for the family is *Halosphaeria* and type species *H. appendiculata*. Kohlmeyer (1972) assigned 12 species to the genus, but the genus was subsequently split into a number of different genera based on the ultrastructure and ontogeny of the ascospore appendages (Jones et al. 1983, 1984). *Halosphaeria appendiculata* and *H. cucullata* were retained in the genus (Jones 1995).

Okeanomyces (Kohlm.) K.L. Pang & E.B.G. Jones

Okeanomyces was introduced by Pang et al. (2004) to accommodate *Halosphaeria cucullata*. This species was originally described as *Remispora cucullata* by Kohlmeyer (1986) and subsequently transferred to *Halosphaeria* by Kohlmeyer (1972), however, it differs from *H. appendiculata* in the morphology of the ascospores and its appendages. Halosphaeria cucullata has a single polar cap-like or globose gelatinous deciduous appendage on the ascospores, while H. appendiculata has polar and equatorial spoonshaped appendages. Furthermore, ascospores of the former are more cylindrical as compared to the latter. In addition to the differences in morphology, molecular data supports the separation of this species from Halosphaeria and Pang et al. (2004) introduced Okeanomyces. In this paper, with support of molecular data we describe the second Okeanomyces species, isolated from an intertidal rocky shore in Thailand. Periconia prolifica is the asexual morph of Okeanomyces cuc*ullatus* initially established in culture by Kohlmeyer (1969) and subsequently confirmed by sequence data (Pang et al. 2004). Other marine Periconia species include P. abyssa and P. salina (Kohlmeyer 1977; Dayarathne et al. 2020). Periconia abyssa was recovered at depths of 3975 m and 5315 m in the Gulf of Angola and Iberian Sea, on wood. It has also been documented many times from mangrove habitats, but whether this is the same taxon remains to be determined. Periconia salina was recently described by Dayarathne et al. (2020) from buried bark in a sand dune in Wales. Periconia is highly polyphyletic with circa 200 epithets listed in Index Fungorum (2020) with species referred to Pleosporales (e.g. Apiosporaceae, Davidiellaceae, Herpotrichiellaceae and others), Microascaceae, Ophiostomataceae and Halosphaeriaceae. Periconia species have also been described from freshwater habitats: P. aquatica (Hyde et al. 2017), P. byssoides (Shearer 1972; Luo et al. 2004), P. cookei (Hyde et al. 2018a), *P. digitata* (Luo et al. 2004), *P. homothallica* (Tanaka et al. 2015), *P. laminella* (Abdel-Aziz 2016), *P. lichenoides* (Borse et al. 2016), *P. minutissima* (Hyde et al. 2017), *P. prolifica* (Shearer 1972; Abdel-Aziz 2016) *P. pseudobyssoides* (Hyde et al. 2018a), *P. pseudodigitata* (Tanaka et al. 2015) and *P. saraswatipurensis* (Borse et al. 2016), *P. submersa* (Hyde et al. 2017).

Although a single Okeanomyces species is presently known and 200 Periconia epithets are listed in Index Fungorum, it is unlikely that many more Okeanomyces species remain to be discovered; more likely the asexual morph Periconia may yield further marine taxa as in the recently described P. salina. In this paper, we describe a new species Okeanomyces marinus from submerged decaying wood collected at Nai Yang Beach, Thailand. However, are we likely to find many more new species in the genus? Two Okeanomyces species with sequence data in GenBank are confirmed as distinct species. Okeanomyces cucullatus is widely distributed in tropical countries, primarily as the asexual morph (Australia, Bahamas, Belize, Brazil, Brunei, Great Abaco, Guatemala, Hawaii, India, Japan, Malaysia, Mauritius, Mexico, South Africa, Thailand, USA). However, numerous regions have never been studied for the genus e.g. the African and South American tropical locations.

New marine fungi continue to be described, especially from mangrove habitats and it is likely that this will continue to be the case. Jones et al. (2019) listed 1257 species in 539 genera while currently 1692, species in 685, are listed in



Fig. 16 Number of novel marine fungi discovered from 2010–2019

www.marinefungi.org. The highest number of marine fungi described was in the decade 1990-1999 with 150 taxa (Jones and Pang 2012). In the decade 2000 to 2009 this dropped to 42. Recent numbers of marine fungi introduced are presented in Figure 16, with 132 new marine fungi introduced in the decade 2010–2019. Jones (2011) considered the question "Are there more marine fungi waiting to be described?" and estimated that the number of marine fungi may be 10,000 to 12,500 species based on the substrates and geographical locations to be sampled. Kis-Papo (2005) postulated there are 10,029 marine fungi based on the assumption that only circa 5% of all fungi have been described. Potential sources of more marine species include marine derived fungi isolated from algae, soils, sand, and water, planktonic fungi, deep-sea fungi, unculturable fungi, and cryptic species (Jones 2011). We can attribute the increase in the number of marine fungi to those described from mangroves substrates, isolated from seaweeds, deep sea fungi and many new marine yeasts (Limtong and Yongmanitchai 2010; Statzell-Tallman et al. 2010; Fell et al. 2011; Gnavi et al. 2017; Devadatha and Sarma 2018; Xu et al. 2018; Jones et al. 2019; Zhang et al. 2019). The continued discovery of new marine fungi can also be attributed to a wider selection of marine substrates investigated for fungi e.g. algae (Cheng et al. 2015; Garzoli et al. 2018), sponges (Gao et al. 2008; Bovio et al. 2018), sampling of deep sea environments (Xu et al. 2018, 2019) and new host mangrove substrates e.g. decaying petiole of the palm Phoenix paludosa from intertidal zone (Jones et al. 2019), and on decaying wood of the maritime salt marsh plant Suaeda monoica (Devadatha and Sarma 2018).

The significant increase in the number of new marine taxa introduced in the decade 2010-2019 supports the notion that many more marine fungi can be expected to be documented in the years to come and supports the estimates of numbers of new fungi proposed by Jones (2011) (Fig. 16). One other source of marine fungi that has been neglected is plankton and zoosporic animals (Hassett and Gradinger 2016; Hassett et al. 2017, 2019). Comeau et al. (2016) noted that novel chytrid lineages dominate fungal sequences in diverse marine habitats. Frenken et al. (2017) noted that a survey of key databases for fungal taxonomic assignment reveals that Chytridiomycota represent between 0.1 and 4% of the fungal sequences, while the number of parasitic species may be fewer than a few dozen. Greater numbers of marine fungi can be expected by surveying marine algae as some 9200–12,500 are described and they cover vast areas of the oceans yielding huge biomass annually, also microsporidial diseases of marine animals is another source of new taxa (Pang personal communication).

Spatafora and Blackwell (1994) were the first to undertake the sequencing of a marine ascomycete namely, *Halosphaeriopsis medsiosetigera* and resulted in a significant advancement in the study of the phylogeny of marine fungi, especially their higher order classification (Kong et al. 2000; Abdel-Wahab et al. 2001). Such studies enabled the identification of polyphyletic marine genera, e.g. *Lignincola* and *Nais* (Pang et al. 2003a) and *Halosarpheia* (Pang et al. 2003b), supporting data for the description of new genera (Pang et al. 2003b) and the systematic reassessment of selected genera *Torpedospora* and *Swampomyces* (Sakayaroj et al. 2005). It also enabled the taxonomic assignment of known asexual marine fungi e.g. *Cirrenalia, Cumulospora* and *Orbimyces* in the Lulworthiales (Abdel-Wahab et al. 2010). It has also helped to clarify many novel species of marine fungi and the trend is expected to continue.

Okeanomyces marinus Calabon, E.B.G. Jones, Boonmee & K.D. Hyde, *sp. nov*.

Index Fungorum number: IF 557251, Facesoffungi number: FoF 08424, Fig. 17

Etymology: In reference to the marine habitat.

Holotype: MFLU 20-0202

Saprobic on decaying wood submerged in intertidal rocky shore. Sexual morph: Undetermined. Asexual morph: Colonies on the substratum superficial, dark brown to black. Conidiophores reduced to conidiogenous cells. Conidiogenous cells erect, aggregated in clusters on hyphae, hyaline to brown, smooth, spathulate to ampulliform. Conidia 7–15×7–11 µm (\bar{x} = 9.45×8.52 µm, n=30), globose, subglobose, irregular, hyaline to brown, thick and smooth-walled.

Culture characteristics: Conidia germinating on MEA within 48 h. Colonies growing on MEA, slow-growing, reaching 5–7 mm diam. in 30 days at 25 °C. Mycelium dense, irregular, raised to convex, surface rough, opaque, above and reverse dark-grey to black.

Material examined: THAILAND, Phuket Province, Thalang District, saprobic on submerged decaying wood, 5 May 2018, M.S. Calabon, 21NYHY1 (MFLU 20–0202, **holotype**), ex-type living culture MFLUCC 20–0123.

GenBank numbers: LSU: MT068207, SSU: MT509714

Notes—*Okeanomyces marinus* has a similar conidial morphology (globose to subglobose, smooth) to *Periconia prolifica*, the asexual morph of *Okeanomyces cucullatus*, and *Periconia abyssa*. The conidiophore sets the difference between *Okeanomyces marinus* and *Periconia prolifica* wherein the former has conidiophores that is reduced to conidiogenous cells while the latter has hyaline erect conidiophores (5–200×2.5 µm) (Kohlmeyer and Kohlmeyer 1979). Both *Okeanomyces marinus* and *Periconia abyssa* have reduced conidiogenous cells but differ in the size of conidia (7–13 µm vs 16–20 µm). Phylogenetic analysis shows that they are different species in *Okeanomyces* (Fig. 18). Fig. 17 Okeanomyces marinus (MFLUCC 20–0123, holotype). a Host material. b–e Appearance of colonies on host surface. f–j Conidiogenous cells and conidia. k–s Conidia. t Germinated conidium in MEA. u Culture on MEA (obverse, reverse), 45 days. Scale bars: b, c=500 μ m, d=200 μ m, e=100 μ m, f=10 μ m, g–j=20 μ m, k–s=10 μ m, t=20 μ m



Diaporthomycetidae families incertae sedis

Rhamphoriaceae Réblová

For the latest treatments of Rhamphoriaceae see Réblová and Štěpánek (2018) and Hyde et al. (2020b).

Rhamphoriopsis Réblová & Gardiennet

Réblová and Štěpánek (2018) introduced this genus with a single species. In this study, a new species, *Rhamphoriopsis sympodialis*, is established based on multigene analyses (Fig. 19). *Rhamphoriopsis sympodialis* is from unidentified decaying wood in China and is the first report of this genus in China, or even in Asia. However, are we likely to find many more new species in the future? The taxon is from one host and on one plant family (*Buxus sempervivens*, Buxaceae). Species have now been collected in Europe and China, and thus numerous regions have never been studied for the genus. Therefore, we believe that comprehensive studies are likely to lead to the discovery of many new species.



Fig. 18 Phylogram generated from maximum likelihood analysis based on large subunit (LSU) ribosomal RNA and small subunit ribosomal RNA (SSU) gene sequence data for species of Halosphaeriaceae. Two species from Microascaceae, *Petriella setifera* (AFTOL-ID 956) and *Microascus trigonosporus* (AFTOL-ID 914), serve as the outgroup taxa. A dataset for the phylogenetic analysis comprising 60 species was taken from Jones et al. (2017). Tree topology of the maximum likelihood analysis is similar to the Bayesian analysis. The best scoring RAxML tree with a final likelihood value of - 15977.663202 is presented. The matrix with 810 distinct alignment patterns and 21.06% proportion of gaps and completely undetermined characters, 1422 constant, 268 parsimony uninformative analysis.

resulted a single most parsimonious tree (TL=2556, CI=0.405, RI=0.590, RC=0.238, HI=0.595). Estimated base frequencies were as follows: A=0.256051, C=0.220023, G=0.284681, T=0.239244; substitution rates AC=1.004308, AG=2.550493, AT=1.322458, CG=0.907161, CT=7.373340, GT=1.000000; gamma distribution shape parameter α =0.230695. Bayesian analysis resulted in 10,001 trees after 10,000,000 generations. MP and ML bootstrap support values equal to or greater than 50% are given while Bayesian posterior probability equal to or higher than 0.90 are specified (ML/MP/BYPP). Hyphen ('-') indicates a value lower than 50% for both RAxML and parsimony, and Bayesian posterior probability lower than 0.90. Newly generated sequences are in blue



Fig. 19 Phylogram generated from maximum likelihood analysis based on combined LSU, and ITS sequence data representing Rhamphoriaceae and the outgroup taxon *Brachysporum nigrum*. Related sequences are taken from Réblová and Štěpánek (2018). Fifteen strains are included in the combined analyses which comprise 1371 characters (825 characters for LSU, 546 characters for ITS) after alignment including the gaps. Single gene analyses were also performed to compare the topology and clade stability with combined gene analyses. Tree topology of the maximum likelihood analysis is similar to the maximum parsimony and Bayesian analysis. The best RAxML tree with a final likelihood values of -4521.041838 is pre-

Rhamphoriopsis sympodialis C.G. Lin, K.D. Hyde & Jian K. Liu, *sp. nov*.

Index Fungorum number: IF557278, Facesoffungi number: FoF07570; Fig. 20

Etymology: In reference to the sympodial conidiophores. Holotype: HKAS 105172

Saprobic on decaying wood. Sexual morph undetermined. Asexual morph hyphomycetous. Colonies effuse, pale white, hairy. Mycelium partly superficial, partly immersed. Conidiophores $14-45 \times 1.9-3.3 \mu m$ ($\bar{x} = 26 \times 2.7 \mu m$, n = 17), macronematous, mononematous, scattered or in small groups, unbranched, straight or flexuous, smooth-walled, thin-walled, cylindrical, subulate, 0–1-septate, subhyaline to pale brown at the base, sented. The matrix had 299 distinct alignment patterns, with 11.76% undetermined characters or gaps. Estimated base frequencies were as follows: A=0.249421, C=0.243580, G=0.289761, T=0.217238; substitution rates AC=1.616849, AG=2.214612, AT=2.109943, CG=0.754591, CT=8.574084, GT=1.000000; gamma distribution shape parameter α =0.683528. Bootstrap values for maximum likelihood (ML) and maximum parsimony (MP) equal to or greater than 60% and clade credibility values greater than 0.80 (the rounding of values to 2 decimal proportions) from Bayesian-inference analysis labeled on the nodes. Ex-type strains are in bold and black, the new isolate is indicated in bold and blue

hyaline at the apex, mostly reduced to conidiogenous cells. *Conidiogenous cells* 11–42×1.8–3.2 µm ($\bar{x} = 22 \times 2.4$ µm, n = 17), polyblastic, integrated, terminal becoming intercalary, cylindrical, tapering apically, sympodial, with numerous indistinctive denticles. *Conidia* 1.9–4×1.4–2.2 µm ($\bar{x} = 3.2 \times 1.9$ µm, n = 21), solitary, acropleurogenous, simple, dry, smooth, thin-walled, aseptate, hyaline, ellipsoidal to obovoid.

Culture characteristics: Conidia germinating on WA within 48 h. Colonies on PDA white-brown, reaching a diam. of 0.5–0.7 cm in 10 days at 28 °C.

Material examined: CHINA, Guizhou Province, Qiannan Buyi Miao Autonomous Prefecture, Dushan County, Guizhou Zilinshan National Forest Park (Shengou Fig. 20 *Rhamphoriopsis sympodialis* (HKAS 105172, holotype). **a** Host material. **b** Conidiophores on the host surface. **c**, **d** Conidiophores. **e** Conidiogenous cells. **f**-**k** Conidia. Scale bars: **c**, **d**=20 μ m, **e**-**k**=5 μ m



District), unnamed road, on decaying wood, 6 July 2018, Chuan-Gen Lin, DS 2-38 (HKAS 105172, **holotype**), extype living culture GZCC 18-0095.

GenBank numbers: LSU: MT079191, ITS: MT079187

Notes: *Rhamphoriopsis sympodialis* differs from *Rhamphoriopsis muriformis* by its mononematous, short, paler, sharper and sympodial conidiophores reduced to conidiogenous cells. All the taxa of *Rhamphoriopsis* formed a monotypic lineage and the isolate of *Rhamphoriopsis sympodialis* was in a distinct lineage within *Rhamphoriopsis* clade. *Rhamphoriopsis sympodialis* is a phylogenetically distinct species from *Rhamphoriopsis muriformis* (Fig. 19).

Basidiomycota

We follow He et al. (2019) and Wijayawardene et al. (2020) for the latest treatment of Basidiomycota.

Agaricomycetes Doweld

The class Agaricomycetes was established by Doweld (2001). Twenty-two orders were arranged in this class, and details are provided in He et al. (2019).

Agaricales Underw.

Agaricales is the largest group of gilled mushrooms in the class Agaricomycetes consisting of 38 families, 508 genera and 17291 species (Underwood 1899; Kirk et al. 2008; He et al. 2019).

Agaricaceae Chevall.

The modern taxonomies of the family based on evidence of molecular data were investigated by Vellinga (2004), Vellinga et al. (2011) and Brandon Matheny et al. (2007). Recently, 54 genera were included in Agaricaceae (He et al. 2019).

Lepiota (Pers.) Gray

Lepiota, are commonly known as white-spored mushrooms, species are diverse worldwide and there are six sections in the genus (Vellinga 2001). There are 1515 *Lepiota* epithets in Index Fungorum and 1179 accepted epithets in Species Fungorum (2020). There are however, many synonyms and other species in related genera in lepiotaceous fungi. He et al. (2019) accepted 450 species in *Lepiota*. There are 1225 sequences of *Lepiota* in GenBank, but species number is not confirmed, and some species names are not correct. *Lepiota* has a saprotrophic lifestyle, they are distributed in both temperate and tropical regions, and fruit on humus soil, mull soil, decaying leaves and pant material, dung and other substrates (Vellinga 2004). Phylogenetic studies of *Lepiota* were carried out by Vellinga (2003) and Liang et al. (2011). Sysouphanthong et al. (2011, 2012, 2013, 2016) and Tibpromma et al. (2017) studied the diversity, taxonomy and phylogeny of *Lepiota* in Northern Thailand, and they described eight new species in the region. We surveyed and collected six specimens of *Lepiota* from Northern Thailand (Table 8) and describe two new species in section *Lilaceae*.

Lepiota chiangraiensis Sysouph. Thongkl. & K.D. Hyde, sp. nov.

MycoBank number: MB 834495; Facesoffungi number: FoF 07516, Figs. 21 and 22

Etymology: the name '*chiangraiensis*' is derived from location where species is distributed.

Holotype: MFLU 20-0197

 Table 8 New collections and sequences of Lepiota in this study

Species	Strain no.	Substrate	Origin	GenBank accesion no.	
				ITS	LSU
L. chiangraiensis	MFLU 20-0197	Humus soil mixed with leave litter	Thailand	MT020094	MT020099
L. chiangraiensis	MFLU 20-0198	Humus soil mixed with leave litter	Thailand	MT020095	MT020100
L. chiangraiensis	MFLU 20-0199	Humus soil mixed with leave litter	Thailand	MT020096	MT020101
L. chiangraiensis	MFLU 20-0200	Humus soil mixed with leave litter	Thailand	_	_
L. pleurocystidiata	MFLU 20-0196	Humus soil mixed with leave litter	Thailand	MT020093	MT020098
L. pleurocystidiata	MFLU 09-0056	Humus soil mixed with leave litter	Thailand	MT020097	MT020102

Fig. 21 Lepiota chiangraiensis. **a** MFLU 20–0197 (**holotype**). **b** MFLU 20–0199. **c** MFLU 20–0201



Fig. 22 Lepiota chiangraiensis (MFLU 20–0197, holotype). a Pileus covering. b Basidiospores. c Basidia. d Cheilocystidia



Pileus 30–75 mm, when young parabolic, expanding to convex or umbonate, soon campanulate with broad umbo, applanate to plano-concave, with straight margin; when young, completely brownish orange (7C7-8) to reddish brown (8E7-8) at umbo, glabrous, smooth, with concentrically crowded squamules around umbo toward margin, brownish-orange (7E6), when fully mature glabrous to squamose at umbo, splitting up into concentrically brownish orange (7E6) squamules toward margin, on white to light brown (7D5) fibrillose background; marginal zone with squamules to fibrillose squamules, with white to light brown (7D5) fibrillose, sometime surface peeling and leaving white sulcate to fringed edge. Lamellae free, subventricose, 7-8 mm wide, white to pale yellow (4A3) with age, crowded, with eroded edge. Stipe $50-85 \times 6-10$ mm, cylindrical, slightly tapering to apex; white fibrillose at apex to middle and orange-white to pale orange (5A2-3) background, darker toward base and with brown (6E7-8) fibrils and minute squamules attached around base zone. Annulus not persistent, made up of partial veil, brownish orange (7C7-8) to reddish brown (8E7-8) squamules on white to pale yellow (4A3), sometimes disappeared in mature stage. Context white to pale yellow (4A3) in pileus, 4.5-6 mm wide; concolorous with surface in stipe, hollow. Taste not observed. Smells like *Termitomyces* spp. Spore print white. Basidiospores [75,3,3] 8.5–10.2×5–6.2 µm, \bar{x} = 9.6×5.7 µm, Q = 1.5–1.8, Qav = 1.7, in side-view ellipsoid to oblong ovoid, in frontal view ellipsoid ovoid, thick-walled, hyaline, non-dextrinoid, congophilous, cyanophilous, non-metachromatic. Basidia 24.5–30 × 11.5–13.5 µm, clavate, 4-spored, colourless. Lamella edge crowded with cheilocystidia. Cheilocystidia 22.5–35 × 7.5–13.5 µm, clavate, utriform, narrowly clavate, hyaline, slightly thick-walled. Pleurocystidia absent. Pileus covering a hymeniderm made up of clavate, narrowly clavate, cylindrical elements, $18.5-65 \times 10-20$ µm, slightly thick-walled, with pale brown intracellular pigment, with an under layer of hyaline to pale brown incrusted hyphae, 3–4.5 µm wide. Stipe covering a cutis made up of hyaline, cylindrical elements, 3–5 µm in diameter; in squamules a hymeniderm same as those on pileus. Clamp-connections present in all tissues.

Habit and distribution: growing in small to large groups, rarely solitary; on rich humus soil with dead leaves and wood under shade trees of *Samanea saman* or beside grassland; commonly found on Mae Fah Luang University campus.

Material examined: THAILAND, Chiang Rai Province, Muang District, Campus of Mae Fah Luang University, N 18° 05' 59.1", E 102° 40' 22.9", alt. 488 m., 28 June 2018, P. Sysouphanthong, PS2018-35 (MFLU 20–0197, **holotype**); Chiang Rai Province, Muang District, Campus of Mae Fah Luang University, N 18° 05' 59.1", E 102° 40' 22.9", alt. 488 m., 2 August 2018, P. Sysouphanthong, PS2018-59 (MFLU 20–00198); 6 August 2019, P. Sysouphanthong, PS2019-57 (MFLU 20–0200); 13 August 2019, P. Sysouphanthong, PS2019-73 (MFLU 20–0201); Pa Daed District, forest of Pa Ngae Village, N 19° 34' 57", E 100° 00' 51", alt. 510–540 m, 29 August 2018, P. Sysouphanthong, PS2018-139 (MFLU 20–0199). GenBank numbers: MFLU 20–0197: ITS: MT020094, LSU: MT020099; MFLU 20–0198: ITS: MT020095, LSU: MT020100; MFLU 20–0199: ITS: MT020096, LSU: MT020101.

Notes: Lepiota chiangraiensis has a hymenodermal structured pileus covering and ellipsoid to oblong ovoid basidiospores and is placed in Lepiota sect. Lilaceae (Vellinga 2001). A related species L. bengalensis from Bangladesh is similar to L. chiangraiensis in macro characters, but differs in its smaller basidiospores, $(5.6)6-6.6(7) \times 3-3.6(4) \mu m$ and smaller basidia, $18-22(25) \times 6-7(8) \mu m$ (Hosen et al. 2016). A second species, L. ochraceofulva, a rare species from Europe, is also similar to L. chiangraiensis in macro characters, but differs in smaller basidiospores, $5.5-7 \times 3.5-4.5 \,\mu m$ (Candusso and Lanzoni 1990; Vellinga 2001). A related species with brown to dark brown pilei from the same region is L. aureofulvella, but L. aureofulvella has smaller basidiomata, a cutis structure of the pileus covering and spurred basidiospores (Sysouphanthong et al. 2011). Lepiota sect. Lilaceae with a hymenoderm structure of the pileus covering is not monophyletic (Vellinga 2003, 2010). In phylogenetic analysis based on ITS and LSU (Fig. 23a, b) L. chiangraiensis is sister to L. bengalensis and L. ochraceofulva.

Lepiota pleurocystidiata Sysouph. Thongkl. & K.D. Hyde, sp. nov.

MycoBank number: MB 834496; Facesoffungi number: FoF 07515, Figs. 24 and 25

Etymology: the name '*pleurocystidiata*' is from the presence of pleurocystidia.

Holotype: MFLU 20-0196

Pileus 10-15 mm, campanulate to umbonate, with wide umbo, with straight margin, with crowded brown (6E5) to dark brown (6F7-8) squamules at umbo toward margin, on white fibrillose background; margin squamulose or floccose, with white fibrils and brown (6E5) squamules. Lamellae free, crowded, broadly ventricose, white, 1.5-2 mm wide, with eroded edge. Stipe $28-35 \times 2.5-3$ mm, cylindrical or tapering to apex, white fibrillose at upper part, squamulose from annular zone downwards base, with brown (6E5) to dark brown (6F7-8) squamules, hollow. Annulus a white fibrillose annular zone; underside with brown (6E5) to dark brown (6F7-8) squamules. Context white and dull, 1-1.3 mm wide in pileus; white in stipe. Smell and taste not observed. Spore print white. Basidiospores [50,2,2] $5-6 \times 3-3.8 \mu m$, $avl \times avw = 5.5 \times 3.3 \mu m$, Q = 1.5–1.8, Qav = 1.6, in side-view oblong to ellipsoidal ovoid, in frontal view oblong ovoid, hyaline, slightly thick-walled, dextrinoid, congophilous, cyanophilous, not metachromatic. Basidia $15-20 \times 6-7 \mu m$, clavate, hyaline, thin-walled, 4-spored. Cheilocystidia not seen. Pleurocystidia 18-26×5.5-8 µm, lageniform or clavate with short to long appendiculate (5-8 µm). Pileus covering a hymeniderm made up of clavate to irregularly clavate elements, thin-walled, with brown parietal and intracellular pigment, $22.5-47.5(-50) \times 8-20 \mu m$. Stipe covering in squamules similar to those on pileus. Clamp-connections present in all tissues.

Habitat and distribution: grow solitary to a small group; on soil with thick humus, in deciduous mixed rain forest dominated by *Castanopsis armata*, *Lithocarpus* spp.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng District, Pha Deng Village, N 19' 07' 13.7", E 98'43' 52,9", alt. 905 m, 13 August 2007, P. Sysouphanthong, PNG14 (MFLU 09-0056, **holotype**).

GenBank numbers: MFLU 20–0196: ITS: MT020093, LSU: MT020098; MFLU 09–0056: ITS: MT020097, LSU: MT020102.

Notes: This species is recognized by its brown to dark brown squamules on the pileus and lower half of stipe, ellipsoidal ovoid basidiospores, absence of cheilocystidia, and pleurocystidia. According to its main character, the species belongs to Lepiota sect. Lilaceae (Vellinga 2001). The absence of cheilocystidia was found in some species in Sect. Lilaceae e.g. Lepiota hymenoderma, but it differs in larger basidiomata and absence of pleurocystidia; and L. apatelia differs in a paler pileus with orange brown to yellow brownish squamules, absence of squamules on stipe, and absence of pleurocystidia (Vellinga and Huijser 1998; Vellinga 2001). Other species without cheilocystidia are L. cristatoides and L. cystophoroides, but both species lack pleurocystidia (Vellinga and Huijser 1998; Vellinga 2001). In a phylogenetic analysis based on ITS (Fig. 23a) L. pleurocystidiata is sister to L. farinolens, L. sosuensis and L. lahorensis with high bootstrap support; and a phylogenetic analysis based on LSU (Fig. 23b) shows that L. pleurocystidia is related to L. subincarnata with low bootstrap support.

Discussion

Fungal numbers have been estimated in between 2.2-3.8 million species (Hawksworth and Lücking 2017) and yet, even considering this large range, it is clear we are far from providing a reliable estimate. Wijayawardene et al. (2020) provided an outline of all Fungi and fungus-like taxa with an estimated number of extant species for each genus. Thus, there are approximately 100,000 extant Fungi and funguslike taxa, which is between 2.6-4.5% of the 2.2-3.8 million estimated species. There are however, numerous black holes that impede estimates of fungal diversity that need research answers. For example, we are still unclear as to what exactly is a species, and have no real idea of the ratio of fungi to each host. Hawksworth (1991) used amongst other metrics, a ratio of about six fungal taxa to each plant, to estimate that there were 1.5 million fungi. Recent estimates of flowering plants range from 220,000 (Mora et al.



Fig. 23 Maximum likelihood phylogenetic tree of *Lepiota* based on sequences of ITS (a) and LSU (b). Tree was performed in RAxML7.2.6 (Stamatakis et al. 2008). Bootstrap support values \geq 70% are shown above the branches. New sequences are in blue.

The GenBank accession number is indicated after species name. L = Lepiota, M = Macrolepiota. The tree is rooted with Macrolepiota procera (Scop.) Singer
Fig. 24 *Lepiota pleurocystidiata.* **a–c** MFLU 20-0196 (**holotype**). **d–e** MFLU 09–0056



2011) to 420,000 (Cheek et al. 2020) and following a ratio of circa 6:1 there would be roughly 2.5 million fungi. However, almost 30 years on, we are nowhere nearer to establishing how accurate the ratio of 6:1 is. Hyde (2001) asked the question, where are the missing fungi, as at that time the estimated number of extant fungi was about 5% of the estimated 1.5 million species (Hawksworth 1991). Hyde (2001) suggested that poorly studied countries and regions would reveal many of the missing fungi and this has been true of studies in northern Thailand (Hyde et al. 2018b).

In this paper, we provide ten case studies of genera, with new species introduced in each. In the speciose, mainly pathogenic genus *Colletotrichum*, which comprises 14 species complexes and 247 species (Jayawardena et al. 2020), it is apparent than many more species will be revealed from unstudied hosts. Similarly, in *Pestalotiopsis*, where species boundaries may be better defined, we expect many more species to be discovered in understudied regions and hosts (Maharachchikumbura et al. 2014; Song et al. 2014). Several poorly studied genera (*Atrocalyx, Lignosphaeria*, Okeanomyces, Rhamphoriopsis, Thyrostroma, Thozetella) also reveal that many taxa are likely to be discovered once we look for these genera in unstudied countries and hosts. Even in the prominent mushrooms, such as Lepiota, we are discovering new species. Extensive studies of Lepiota in the last 15 years in northern Thailand, has revealed nine new and 34 extant species (Sysouphanthong et al. 2011, 2012, 2013, 2016; Tibpromma et al. 2017; Hyde et al. 2020b). Thus, 26% of Lepiota species collected in northern Thailand were new to science. This is lower than the estimated numbers of new Amanita (83%) and Agaricus (93%) discovered, but still high (Hyde et al. 2018b). Collections in other, less seasonal parts of Thailand, where it is rarely cool and much wetter, and surrounding countries and other continents would surely reveal numerous new taxa. The case studies illustrate we are far from establishing whether taxa are host-specific, ubiquitous, or even common. However, each study provides a small advance and new discoveries are plentiful. The above case studies, however, serve to illustrate that we are nowhere near levelling off the curve in new species discovery.

Fig. 25 Lepiota pleurocystidiata (MFLU 20–0196, holotype). a Pileus covering. b Basidia. c Basidiospores. d Pleurocystidia



One of the most important criterion in predicting fungal numbers is whether a species is host-specific (Zhou and Hyde 2001). Cursory evidence however, would suggest that this is not the case, as ubiquitous fungi are often reported in publications (Shin et al. 2004; Ortiz-Bermúdez et al. 2007; Rosling et al. 2011). Most studies are however, from highly disturbed areas (e.g. managed forests, grasslands, urban parks), with very few studies in pristine forests in tropical regions (Gilbert et al. 2002; Liu et al. 2019a). It is therefore likely, that the fungi discovered are weedy, ubiquitous, often visible taxa, which have adapted to these highly disturbed habitats (Lodge 1997). One remarkable study, was that of Fröhlich and Hyde (1999) which compared fungal communities on three individual Licuala ramsayi palms in northern Queensland, Australia and a different Licuala species (again three trees) in a pristine tropical rainforest in Brunei Darussalam. One-hundred fungal taxa were identified from the palm trees in Australia and 172 taxa from the palm trees in Brunei Darussalam. There were 30 overlapping species, but these identifications may have been clumped as it was not possible to resolve taxa using molecular data. If fungal species were not mainly host or genus-specific, we question how is it that almost totally different communities occurred on these palm species?

There have been relatively few studies that have seriously addressed whether fungi are host-specific or generalists in pristine or relatively undisturbed forests, where most undiscovered species are thought to occur (Bills and Polishook 1994; Hyde 2001). Most studies have been the result of short surveys or visits to tropical forests by experts concentrating on specific groups (Hawksworth 2001, 2012). We are aware of no long-term studies of all fungi in a specific forest. There are several methods by which we can establish whether fungi are host-specific. This includes the study of fungi on specific hosts (Promputtha et al. 2002, 2004, 2017; Wang et al. 2008; Doilom et al. 2017; Hyde et al. 2017), comparisons of fungi on different hosts in the same forest (Parungao et al. 2002; Paulus et al. 2006), or to establish how saprobes could be host-specific (Chethana et al. pers. comm.). It is impossible to address all of these topics in this paper in detail and therefore we address them briefly below.

Parungao et al. (2002) took a unique approach, examining the fungal species on ten leaves from 13 different tree types, at two small plots in pristine tropical forests in northern Queensland. Of the 57 microfungi identified, 36 taxa were found only on one leaf type, indicating possible hostspecificities. In another landmark study, Paulus et al. (2006) studied the diversity of fungi on six tree species in pristine Australian tropical rainforests. Using direct observation of fruiting bodies and particle filtrations a high level of diversity was discovered with 185 species from leaves and 419 morphotypes discovered from particle filtation, respectively. The microfungal assemblages on leaves of the tree species were relatively distinct, with 60% of the taxa being recorded on a single host, and only ca 3% of taxa occurring on all tree species. Evidence suggested that the fungal communities on the tree species in the same family, were more similar than those in different families. Further studies of this type, are needed with better methodology, i.e., determining species based on molecular data, identification of trees, more leaves and leaves at various stages of decay, as we suspect that an even higher diversity and less overlap would be revealed.

Another way to look at species diversity is to examine the fungi on a single host species, genus or family. The examples compared below include similar methodology and identifications based on morphology and some with molecular data. At the species level, Bills and Polishook (1994) reported the fungi on Heliconia mariae, Promputha et al. (2005) on Magnolia, Doilom et al. (2017) on teak (Tectona grandis), and Thambugala et al. (2017a) on Tamarix (Table 9). At the genus or subfamily level, relatively detailed studies are those of Dai et al. (2017) on fungi of bamboo (Bambusoideae, Gramineae), Phukhamsakda et al. (2020) on *Clematis* (Ranunculaceae), Wanasinghe et al. (2018b) on Rosaceae, Tibpromma et al. (2018) mostly on Pandanus (Pandanaceae), and Bills and Polishook (1991) on Carpinus caroliniana and results with species overlap are shown in Table 9. The overlapping taxa on each host is low and illustrates that the fungi on the different hosts appear to be host, genus or family specific.

Perhaps the only feasible explanation for the difference in fungal communities found in the two Licuala palms by Fröhlich and Hyde (1999) or different tree species by Parungao et al. (2002) and Paulus et al. (2006) is that endophytes within a host become saprobes (Promputtha et al. 2007, 2010), or less commonly pathogens (Photita et al. 2004). The ability for an endophyte to colonize a host may be dependent on it overcoming the hosts defenses (Chethana et al. pers. comm.), and thus the endophytes are likely to be hostspecific to some extent. Several studies have investigated endophytes becoming saprobes and it appears that leaves are colonized by endophytes which become saprobes when leaves senesce (Promputtha et al. 2007). This would account for the differences in fungal communities on different hosts and also account for host-specificity of saprobes. In succession studies the primary and middle colonizers appear to be endophytes. Only the late colonizers appear to have been derived from the environment.

In the real world, the estimates of fungal numbers are based on taxonomically accepted species, and future predicted novelty (Hawksworth 1991; Lücking and Hawksworth 2018). However, a new era of environmental metabarcoding have added more diversity (in terms of short sequences) to the fungi, than since when fungi were first formally studied until now (Lücking et al. 2020). Estimates of fungal communities in sediments of subtropical Chinese seas have been proposed based on DNA metabarcoding data (Li et al. 2016b). These unnamed sequences, known as OTU's or dark taxa (8,608 of species hypotheses (at 1.5% threshold) in UNITE (https://unite.ut.ee/; Nilsson et al. 2019)) are voucher less taxa and are presently not accepted in the International Code of Nomenclature for Algae, Fungi, and Plants (Turland et al. 2018) which require a type. Much discussion on the future and perils of describing taxa based on sequences have been published (Hongsanan et al. 2018; Thines et al. 2018). However, the diversity revealed through metabarcoding does have some significance to fungal estimates, but at present many of the OTU's or dark taxa are inadequate or erroneous sequences. Thus, the methodology must be improved and become more accurate and this is quickly happening. Thus, back in the real world, we must base fungal number estimates on reliable data, until metabarcoding reaches acceptable standards.

The above discussion serves to illustrate how little we know, rather than what we know and leads to several recommendations for future research. (1) There is a need to accurately establish what a species is, (2) there is a need to establish how host-specific fungi are, not in highly disturbed urban areas, but in pristine or relatively undisturbed forests. The fact that Fröhlich and Hyde (1999), Parungao et al. (2002) and Paulus et al. (2006) found such differences in communities on host plants, may be because the forests were undisturbed. We need to establish if a fungal species is specific to a plant species, genus or family or if species are mostly ubiquitous. We have very little idea at this time and therefore studies need to be targeted to answer this question, and (3) there is a need to establish if species in different continents, islands, countries or regions are different, or do the same taxa occur worldwide?

Because data on plant/fungi relationships and global distributions are inadequate, we are far from being able to accurately estimate fungal species numbers. Recent studies have shown that freshwater genera in different stream systems comprise different species. Originally clumped as *Acrogenospora sphaerotheca* and *Cancellidium applanatum* in streams worldwide (Bao et al. 2020; Hyde pers. comm.), these genera have been shown to comprise several species in a small region of the world studied with morphological and molecular evidence. Thus, the diversity of lignicolous freshwater fungi may be much higher than originally thought, but can only be established by detailed studies. Other groups of fungi are likely to reveal similar trends, once they are thoroughly researched. Besides, the estimates of the insect-associated fungi were uncertain due to the high level of uncertainty of

ts and host families
various hosts and hos
Overlapping taxa on various host
Table 9

Host	Tamarix (24)	Tamarix (24) Tectona grandis (188)		Bambu- <i>Clematis</i> (88) soideae (44)	Rosaceae (114)	Pandanus (81)	Carpinus caroliniana References (155)	References
Magnolia (66)	1	Lasiodiplodia theo- bromae, Lasiodip- lodia pseudothe- obromae	I	Diaporthe ravennica Lasiodiplodia pseu- dotheobromae	Lasiodiplodia pseu- dotheobromae	1	1	Promputtha et al. (2005)
Tamarix (24)	I	1	I	I	I	I	1	Thambugala et al. (2017a)
Tectona grandis (188)	1	1	I	1	Lasiodiplodia pseu- dotheobromae	Chaetomium globo- sum, Lasiodiplodia pseudotheobromae, Pseudofusicoccum adansoniae	Chaetomium globo- sum	Doilom et al. (2016, 2017)
Bambusoideae (44)	I	1	I	I	I	I	I	Dai et al. (2017)
Clematis (88)	I	1	I	1	Angustimassarina rosarum, Diaporthe rudis	Dictyocheirospora xishuangbannaen- sis, Torula chromo- laenae	1	Phukhamsakda et al. (2020)
Rosaceae (114)	I	I	I	I	I	I	I	Wanasinghe et al. (2018b)
Pandanus (81)	I	1	I	I	I	I	Chaetomium globo- sum	Tibpromma et al. (2018)
Carpinus caroliniana (155)	I	I	I	I	I	I	I	Bills and Polishook (1991)

The number in brackets is the total number of fungi obtained from each host Overlapping between all hosts = 9 species

insect diversity (Ødegaard et al. 2000). However, the diversity of insects and insect-associated fungi could be indirectly affected by plant diversity (Hawksworth 1998). Hence, plant diversity may help to estimate insect-associated fungal diversity in the future (Schmit and Mueller 2007).

Returning to the title of the paper, is the species description curve flattening? The answer appears to be a resounding No!

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