Additions to Phaeosphaeriaceae (Pleosporales): Elongaticollum gen. nov., Ophiosphaerella taiwanensis sp. nov., Phaeosphaeriopsis beaucarneae sp. nov. and a new host record of Neosetophoma poaceicola from Musaceae

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Abstract

A novel ascomycetous genus, Elongaticollum, occurring on leaf litter of Hedychium coronarium (Zingiberaceae) in Taiwan, is described and illustrated. Elongaticollum is characterized by dark brown to black, superficial, obpyriform, pycnidial conidiomata with a distinct elongate neck, and oval to oblong, hyaline, asceptate conidia. Phylogenetic analyses (maximum likelihood, maximum parsimony and Bayesian) of combined ITS, LSU, SSU and tef1-α sequence data revealed Elongaticollum as a distinct genus within the family Phaeosphaeriaceae with high statistical support. In addition, Ophiosphaerella taiwanensis and Phaeosphaeriopsis beaucarneae are described as new species from dead leaves of Agave tequilana and Beaucarnea recurvata (Asparagaceae), respectively. Neosetophoma poaceicola is reported as a new host record from dead leaves of Musa acuminata (Musaceae). Newly described taxa are compared with other similar species and comprehensive descriptions and micrographs are provided.
Keywords
Asparagaceae, Dothideomycetes, leaf litter, new taxa, Zingiberaceae

Introduction

Plant litter is considered as one of the main contributors to net above-ground primary productivity of terrestrial ecosystems (Swift et al. 1979; Berg and McLaugherty 2008; Krishna and Mohan 2017). Since plant litter is returned back to the soil, it represents a major source of organic carbon in forest soils (Berg 2003). Plant litter can be defined as a collection of fallen leaves, twigs, seeds and other woody debris that accumulate on the ground as a natural part of the forest ecosystem (Johnson and Catley 2002; Berg and McLaugherty 2008). In particular, leaf litter is the main source of organic matter and nutrients of the soil, compared to other litter types (Robertson and Paul 1999; Berg and McLaugherty 2008; Krishna and Mohan 2017). Leaf litter decomposition is a key process contributing to biogeochemical cycles in any forest ecosystem. Microorganisms are the primary agents in this process (Purahong et al. 2016; Mlambo et al. 2019). Fungi are considered as the “key players” in leaf litter decomposition, because of their ability to produce a wide range of extracellular enzymes (Pointing et al. 2005; Berg and McLaugherty 2008; Bani et al. 2018). Many researchers have been carrying out studies of fungal species inhabiting leaf litter and have described numerous new species in Dothideomycetes (Hyde et al. 2019; Phookamsak et al. 2019; Tennakoon et al. 2019).

The family Phaeosphaeriaceae is considered to be one of the most species-rich families in Dothideomycetes and includes species that inhabit a wide range of ecosystems (i.e., marine, terrestrial, and mangroves) (Phookamsak et al. 2014, 2017; Bakhshi et al. 2019; Jones et al. 2019; Luo et al. 2019; Tennakoon et al. 2019). Phaeosphaeriaceae was established by Barr (1979), who designated Phaeosphaeria I. Miyake as the generic type of the family. Phaeosphaeriaceae species have immersed to superficial, globose to subglobose ascomata, short papilla, bitunicate asci and hyaline to pigmented, fusiform to ellipsoidal, filiform, or muriform ascospores (Bakhshi et al. 2019; Chaivan et al. 2019; Maharachchikumbura et al. 2019; Yang et al. 2019). Members of Phaeosphaeriaceae are cosmopolitan, since they exhibit diverse lifestyles as saprobes, endophytes and pathogens of economically important plants (Barr 1992; Phookamsak et al. 2014, 2017; Yang et al. 2016; Hyde et al. 2020; Mapook et al. 2020). Apart from being cosmopolitan in nature, it appears that this family is phylogenetically highly diverse. Thus, recent studies have revealed a large number of new genera in this family. For instance, in the space of two years, eleven genera have been introduced, viz. Bhagirathimyces S.M. Singh & S.K. Singh (Hyde et al. 2020), Hydemyces Maharachchikumbura et al. (Maharachchikumbura et al. 2019), Hydeopsis J.F. Zhang et al. (Zhang et al. 2019), Neostagonosporella C.L. Yang, et al. (Yang et al. 2019), Parastagonosporella M. Bakhshi, Arzanlou & Crous (Bakhshi et al. 2019), Pseudoophiosphaerella J.F. Zhang...
et al. (Zhang et al. 2019), *Murichromolaenicola* Mapook & K.D. Hyde (Mapook et al. 2020), *Neoophiobolus* Mapook & K.D. Hyde (Mapook et al. 2020), *Paraleptospora* Mapook & K.D. Hyde (Mapook et al. 2020), *Pseudostaurosphaeria* Mapook & K.D. Hyde (Mapook et al. 2020) and *Vittaliana* Devadatha et al. (Devadatha et al. 2019). Currently, more than 70 genera are accommodated in this family (Wanasinghe et al. 2018; Bakhshi et al. 2019; Maharachchikumbura et al. 2019; Phookamsak et al. 2019; Hongsanan et al. 2020; Hyde et al. 2020).

We are investigating the diversity of microfungi on leaf litter in the tropics with the aim of clarifying their taxonomy based on morphology coupled with multi-gene phylogeny. As a part of this study, we have collected and isolated four taxa from Taiwan, which belong to the family Phaeosphaeriaceae. We present herein comprehensive morphological descriptions and an in-depth phylogenetic investigation of the newly introduced species.

**Materials and methods**

**Sample collection, morphological studies and isolation**

Decaying leaf litter samples of *Agave tequilana* F.A.C. Weber (Asparagaceae), *Beaucarnea recurvata* Lem. (Asparagaceae), *Hedychium coronarium* J.Koenig (Zingiberaceae), and *Musa acuminata* Colla (Musaceae) were collected from Dahu Forest Area in Chiayi, Taiwan and taken to the laboratory in Zip lock plastic bags. Specimens were examined with a LEICA EZ4 stereomicroscope. Micro-morphological characters were determined using AXIOSKOP 2 PLUS compound microscope and images were captured with a Zeiss AXIOCAM 506 COLOR digital camera. Observations and photomicrographs were made from materials mounted in water. Permanent slides were preserved in lactoglycerol, sealed by applying nail-polish around the margins of cover slip. All measurements were made with ZEN2 (blue edition) and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Single ascospore and conidial isolation was carried out following the method described in Phookamsak et al. (2014). The single germinated spore was picked up and transferred to potato dextrose agar (PDA) and incubated at 25 °C in natural light. Subsequent sub-culturing was done carefully to obtain pure culture and ensure absence of contaminants. Culture characteristics were observed after three weeks. Colonies were photographed and colonial characters were noted and described. Type specimens of new taxa were deposited at the herbarium of Mae Fah Luang University (MFLU) and National Chiayi University Herbarium (NCYU). Living cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and National Chiayi University Culture Collection (NCYUCC). Faces of Fungi and Index Fungorum numbers were provided as in Jayasiri et al. (2015) and Index Fungorum (2020).
DNA extraction and PCR amplification

Total genomic DNA was extracted from scraped fresh fungal mycelium using the DNA extraction kit E.Z.N.A Fungal DNA Mini Kit (D3390-02, Omega Bio-Tek) following the manufacturer’s protocol. The DNA product was kept at 4 °C for DNA amplification and maintained at -20 °C for long term storage. DNA was amplified by polymerase chain reaction (PCR) for four genes, the large subunit (28S, LSU), small subunit (18S, SSU), internal transcribed spacers including the 5.8s rDNA (ITS1-5.8S-ITS2) and translation elongation factor 1 alpha (tef1-α). The partial LSU gene was amplified by using the primer combination LR0R and LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1994); partial SSU was amplified with NS1 and NS4 (White et al. 1990), nuclear ITS was amplified with primers ITS5 and ITS4 (White et al. 1990), and tef1-α gene was amplified using the primers EF1-983F and EF1-2218R (Rehner et al. 2001). Amplification reactions were performed in 25 µl of total reaction that contained 9.5 µl of sterilized water, 12.5 µl of 2×Power Taq PCR MasterMix (Tri-I Biotech, Taipei, Taiwan), 1 µl of each forward and reverse primers and 1 µl of DNA template. The PCR thermal cycle program of ITS, LSU, SSU and tef1-α gene was processed initially at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 50 seconds, elongation at 72 °C for 1 minute and a final extension at 72 °C for 10 minutes and a holding temperature of 4 °C. The PCR products were analyzed by 1.5% agarose gels containing the Safeview DNA stain (GeneMark, Taipei, Taiwan) to confirm their expected molecular weight. PCR products were purified and sequenced with primers mentioned above by Tri-I Biotech, Taipei, Taiwan. Nucleotide sequences were deposited in GenBank (Table 1).

Phylogenetic analysis

Phylogenetic analyses were performed using a combined LSU, SSU, ITS and tef1-α sequence dataset. Newly generated sequence data were initially subjected to blast search in NCBI to obtain the closest matches in GenBank. Sequences generated from this study were analyzed with related taxa in the family Phaeosphaeriaceae, which were obtained from GenBank and from recently published data (Bakhshi et al. 2019; Hyde et al. 2017; Maharachchikumbura et al. 2019; Yang et al. 2019; Mapook et al. 2020) (Table 1). The combined dataset consisted of 168 sequences including our newly generated sequences. Multiple alignments were automatically made with MAFFT v. 7 at the web server (http://mafft.cbrc.jp/alignment/server), using default settings (Katoh and Standley 2013). The alignment was refined manually with BioEdit v. 7.0.5.2 (Hall 1999), where necessary.

Evolutionary models for phylogenetic analyses were selected independently for each locus using MrModeltest v. 3.7 (Posada and Crandall 1998) under the Akaike Information Criterion (AIC). Phylogenetic trees were obtained from Randomized Accelerated Maximum Likelihood (RAxML), maximum parsimony analysis (MP) and
| Species                              | Strain/Voucher no. | GenBank accession no. | ref/t– |
|--------------------------------------|--------------------|-----------------------|--------|
| *Acericola italica*                  | MFLUCC 13-0609     | MF167429 MF167430 MF167428 | –      |
| *Allophaeosphaeria muriformia*       | MFLUCC 13-0277     | KX910089 KX950400 KX926415 | –      |
| *Alloennoticia thailandica*          | MFLUCC 15-0576     | – – – – | –      |
| *Amarengraphium ammophilicola*       | MFLU 17-2571       | MN017847 MN017913 MN047087 MN077065 | –      |
| *Amarenyomyces daecylidis*           | KUMCC 18-0154      | MK356345 MK356539 MK356371 | –      |
| *Arezzomyces cytisi*                 | MFLUCC 15-0649     | KT306950 KT306954 KT306947 | –      |
| *Banksiophoma australiensis*         | CBS 142163         | KY979794 – KY979739 KY979889 | –      |
| *Bhagirathimyc es himalayensis*      | AMH 10127          | MK386020 MN121697 MK386021 | –      |
| *Bhatiellae rosae*                  | MFLUCC 17-0664     | MG829898 MG829101 MG829887 | –      |
| *Brunneomurispora lonicerae*         | KUMCC 18-0157      | MK386346 MK386360 MK386373 MK386965 | –      |
| *Camarosporioides phragmitis*        | MFLUCC 13-0365     | KX572345 KX572350 KX572340 KX572354 | –      |
| *Chaetosphaeronema achilleae*        | MFLUCC 16-0476     | KX765266 – KX765265 | –      |
| *C. hispidulum*                      | CBS 216.75         | KF251652 EU754045 KF251148 KF253108 | –      |
| *Dematiopleospora cirsii*            | MFLUCC 13-0615     | KX274250 – KX274243 KX284708 | –      |
| *D. mariae*                          | MFLUCC 13-0612     | KJ749653 KJ749652 KJ742423 KX284708 | –      |
| *Didymocyrtis xanthomendozae*        | CBS 129666         | – – KIP10651 KIP10677 | –      |
| *Diederichomyces ficuzzae*           | CBS 128019         | JQ238616 – KIP10674 KIP10673 | –      |
| *Dhawkworthia scutaticola*           | MFLUCC 17-0693     | MG829038 MG829144 MG828929 | –      |
| *D. lonicerae*                       | MFLUCC 14-0955     | MG829012 MG829121 MG828902 MG829203 | –      |
| *Edenia gomezpompae*                 | JLCC 34533         | – – KC193601 | –      |
| *Embarria clematidis*                | CBS 128019         | JQ238616 – KIP10674 KIP10673 | –      |
| *Equiseticola fusispora*             | MFLUCC 14-0522     | KU987669 KU987670 KU987668 MG528095 | –      |
| *Gallicola baobabianensis*           | HKAS 102234        | MK356348 MK356362 MK356374 MK359906 | –      |
| *G. pseudophaeosphaeria*             | MFLUCC 17-0693     | MG829038 MG829144 MG828929 | –      |
| *Hydeomyces desertipleosporoides*    | SQUCC 15259        | MK290839 MK290843 MK290841 MK290848 | –      |
| *Hydeopsis verrucipora*              | SD 2016-5          | MK522498 MK522504 MK522508 MK523388 | –      |
| *Italica acchilae*                   | MFLUCC 14-0955     | MG829012 MG829121 MG828902 MG829203 | –      |
| *I. luzulae*                         | MFLUCC 14-0932     | KT306951 – – – | –      |
| *Jeromyomyces labinae*               | CBS 144617         | MK442529 – MK442589 MK442695 | –      |
| *Junaceicola italica*                | MFLUCC 13-0750     | – – KX500110 MG528097 | –      |
| *J. luzulae*                         | MFLUCC 13-0780     | KX449530 KX449531 KX449529 | –      |
| *Kwanghwaensis miscanthi*            | MFLUCC 14-0522     | KU987669 KU987670 KU987668 MG528095 | –      |
| *Leptosphaeria doliolum*             | CBS 505.75         | GU301827 GU296159 JF740205 GU349069 | –      |
| *Leptospora rubella*                 | CPC 11006          | DQ195792 DQ195803 DK195780 | –      |
| *L. thailandica*                     | MFLUCC 16-0385     | KX655549 KX655554 KX655559 KX655564 | –      |
| *Longispora clematidis*              | MFLUCC 13-0750     | – – KX500110 MG528097 | –      |
| *Loratospora aestuarii*              | CBS 117592         | – – MH863024 | –      |
| *Magninella scutetae*                | CBS 239.58         | MH863023 – MH857770 | –      |
| *Melinicta anthoxanthi*              | MFLUCC 14-1011     | KU848204 KU848205 – – | –      |
| *Murichromaena chiangraiensis*       | MFLUCC 17-1488     | MN994559 MN994606 MN994582 MN998163 | –      |
| *M. chromolaenae*                    | MFLUCC 17-1489     | MN994560 MN994606 MN994583 MN998164 | –      |
| *Muriophaeosphaeria galatellae*       | MFLUCC 14-0614     | KT438329 KT438331 KT438333 MG520900 | –      |
| *Muriophaeosphaeria galatellae*       | MFLUCC 14-0614     | KT438329 KT438331 KT438333 MG520900 | –      |
| *Neoophiobolus chromolaenae*         | MFLUCC 17-1467     | MN994562 MN994606 MN994583 MN998164 | –      |
| Species                        | Strain/Voucher no. | GenBank accession no. | LSU | SSU | ITS | tef-2 |
|-------------------------------|-------------------|-----------------------|-----|-----|-----|-------|
| *N. chromolaenae*             | MFLUCC 17-1449    | MN994561 MN994607 MN994584 MN998165 |
| *Neosetophoma sp.*            | MFLUCC 17-0844    | MG829035 MG829141 MG828926 MG829219 |
| *N. aepatica*                 | CBS 145363        | MK540024 – MK539953 – |
| *N. camprodii*                | MFLUCC 15-0682    | KU302778 – KU302779 – |
| *N. clematidis*               | MFLUCC 13-0734    | KP684153 KP684154 KP744450 – |
| *N. gareyi*                   | MFLUCC 14-0528    | – KY501126 – KY514402 – |
| *N. geyangensis*              | GZ13              | MH018132 MH018136 MH018134 MH051889 |
| *N. italica*                  | MFLU 14-0809      | KP711361 KP711366 KP711356 – |
| *N. lonicerae*                | KUMCC 18-0155     | MK356349 MK356363 MK356375 MK359067 |
| *N. lunariae*                 | CPC 26671         | KX306789 – KX306763 – |
| *N. micanihi*                 | MFLU 18-2675      | MK503826 MK503832 MK503820 – |
| *N. phragmitis*               | CBS 145364        | MK540025 – MK539954 MK540148 |
| *N. poaceicola*               | MFLUCC 16-0886    | KY50382 KY50383 KY568986 – |
| *N. rosea*                    | MFLUCC 18-1632 MT321809 MT321802 MT321795 – |
| *N. rosea*                    | MFLUCC 17-0844    | MG829035 MG829141 MG828926 MG829219 |
| *N. rosana*                   | MFLUCC 17-0768    | MG829037 MG829143 MG828928 – |
| *N. rosarium*                 | MFLUCC 17-0308    | MG829036 MG829142 MG828927 – |
| *N. salis*                    | MFLU 17-0118      | MK608026 – MK608025 – |
| *N. samarororum*              | CBS 138.96        | KF251666 GQ387517 MH862569 KF253119 |
| *N. sambuci*                  | CBS 145365        | MK540026 – MK539955 MK540149 |
| *N. shoemakeri*               | MFLU 16-1606      | MG602199 MG602201 MG602203 MG844352 |
| *N. tienshanensis*            | MFLUCC 17-0844 MT321809 MT321802 MT321795 – |
| *N. xingreens*                | GZAAAS18 0100     | MH018133 – MH018135 – |
| *Neosphaerellopsis thailandica* | CPC 21659        | KP170721 – KP170652 KP170678 |
| *Neostagonospora caricis*      | CBS 135992        | KF251667 – KF251163 – |
| *N. phragmitis*               | MFLUCC 16-0493    | KX910090 KX950401 KX926416 MG520902 |
| *Neostagonospora sichuanensis* | MFLUCC 18-1228    | – – – – – – |
| *N. aquatica*                 | MFLUCC 18-1231    | – – – – – – |
| *N. korrae*                   | ATCC 56289        | – – KC848509 KC848515 |
| *N. narmari*                  | ATCC 64688        | – – KC848510 KC848516 |
| *O. taiwanica*                | NTUCC 14-0033     | KX767089 KX767090 KX767088 MG520911 |
| *O. herpotricha*              | k28               | – – KP690992 KP691016 |
| *O. korrae*                   | ATCC 56289        | – – KC848509 KC848515 |
| *O. narmari*                  | ATCC 64688        | – – KC848510 KC848516 |
| *O. taiwanensis*              | MFLU 18-2534 MT321815 MT321808 MT321801 MT328758 |
| *O. taiwanica*                | NTUCC 17-024      | MN082419 – MN082417 – |
| *Paraleptosphaeria dryadis*   | CBS 643.86        | GU301828 KC584632 JF740213 GU349009 |
| *Paraleptospora chromolaenae* | MFLUCC 17-1481    | MN994563 MN994609 MN994587 MN998167 |
| Species                        | Strain/Voucher no. | GenBank accession no. |
|-------------------------------|--------------------|-----------------------|
|                               |                    | LSU          | SSU          | ITS          | rtf<sup>–2</sup> |
| **Phaeosphaeriaceae**         |                    |              |              |              |                |
| P. chromolaenica              | MFLUCC 17-1450     | MN994564     | MN994961     | MN994588     | MN998168      |
| Paraphiobolus arundinis       | MFLUCC 17-1789     | MG520965     | MG520984     | MG520945     | MG520912      |
| P. plantaginis                | MFLUCC 17-0245     | KY815010     | KY815012     | KY977641     | MG520913      |
| Paraloratospora camporesii    | MFLU 18-0915       | MN756637     | MN756635     | MN756639     | –              |
| Paraphoma chrysanthemiocola   | CBS 522.66         | KF251670     | GQ387521     | KF251166     | KF253124      |
| P. radicina                   | CBS 111.79         | KF251676     | EU754092     | KF251172     | KF253130      |
| Parastagonospora dactylidis   | MFLUCC 13-0375     | KY824767     | KY824769     | KY824766     | –              |
| Parastagonospora falliparia   | CBS 135981         | MH460545     | –            | MH460543     | MH460549      |
| P. falliparia                 | CCTU 1151-1        | MH460546     | –            | MH460544     | MH460550      |
| Pheoascopia muriformis        | MFLUCC 17-0372     | MF611638     | MF611639     | MF611637     | –              |
| P. festucae                   | MFLUCC 17-0056     | KY442547     | –            | KY442611     | KY442702      |
| Pheaeoseptoria zeae           | CBS 144614         | KM434277     | KM434287     | KM434267     | KM434296      |
| Pheaeosphaeria musae          | MFLUCC 11-0133     | –            | –            | –            | –              |
| P. oryzae                     | CBS 110110         | KF251689     | GQ387530     | KF251186     | –              |
| P. papayae                    | CBS 135416         | KF251676     | EU754092     | KF251172     | KF253130      |
| Pheaeosphaeriopsis agapanthi  | CPC 26303          | KX228311     | –            | KX228260     | –              |
| P. agarensis                  | CPC 29122          | KY173520     | –            | KY173430     | –              |
| P. aloes                      | CBS 145367         | MK540030     | –            | MK539959     | MK540153      |
| P. aloicola                   | CBS 145368         | MK540031     | –            | MK539960     | MK540154      |
| P. amblyospora                | CBS 110131         | –            | –            | MH862851     | –              |
| **Phaeosphaeriopsis**         | MFLU 18-2586       | MT321813     | MT321806     | MT321799     | MT328756      |
| **Phaeosphaeriopsis**         | MFLU 18-2587       | MT321814     | MT321807     | MT321800     | MT328757      |
| P. dracaenica                 | MFLUCC 11-0157     | KM434283     | KM434292     | KM434273     | KM434301      |
| P. glaucopunctata             | MFLUCC 13-0265     | KJ522477     | KJ522481     | KJ522473     | MG520918      |
| P. gregilae                   | CBS 145369         | MK540032     | –            | MK539961     | MK540155      |
| P. nolinae                    | CBS 102205         | KY090667     | KY090693     | KY090635     | –              |
| P. obtusispora                | CBS 246.64         | JX681119     | –            | KY090644     | –              |
| P. omaniana                   | SQUCC:14333        | MT075849     | –            | MT075840     | –              |
| P. phacidiomorpha             | CBS 198.35         | AF275496     | AF275515     | FJ462742     | –              |
| P. pseudoagavacearum          | CBS 145370         | MK540033     | –            | MK539962     | –              |
| P. trisepata                  | MFLU 17-1800A      | MN750592     | MN750607     | MN750613     | MN756837      |
| P. yuccae                     | MFLUCC 16-0558     | KJ522479     | KJ522484     | KJ522475     | MG520919      |
| Pseudophaeosphaeria huishuiensis | HS13             | MK522499     | MK522505     | MK522509     | MK523389      |
| Pseudophiobolus rubi           | MFLUCC 17-1490     | MN994570     | MN994616     | MN994593     | MN998175      |
| Pseudostauroporpha chromolaena | MFLUCC 17-1491   | MN994571     | MN994617     | MN994594     | MN998175      |
| P. chromolaenica              | MFLUCC 17-0128     | MG829060     | MG829165     | –            | MG829232      |
| P. rosacea                    | MFLUCC 17-0125     | MG520966     | –            | MG520946     | –              |
| Pseudophiobolus achilleae      | MFLUCC 17-2257     | MG520967     | MG520989     | MG520947     | MG520926      |
| P. galii                      | MFLUCC 17-0925     | MG520966     | –            | MG520946     | –              |
| Pseudophiobolus buishuiensis   | HS13              | MK522499     | MK522505     | MK522509     | MK523389      |
| Pseudophiobolus rubi           | MFLUCC 14-0259     | KX765299     | KX765300     | KX765298     | MG520934      |
| Pseudostauroporpha chromolaena | MFLUCC 17-1490   | MN994570     | MN994616     | MN994593     | MN998175      |
| P. amblyospora                | MFLUCC 17-0128     | MG829060     | MG829165     | –            | MG829232      |
| P. trisepata                  | MFLUCC 17-0125     | MG520966     | –            | MG520946     | –              |
| Sclerostagonospora rosicola    | MFLUCC 15-0129     | MG829068     | MG829172     | MG828957     | MG829229      |
| Scoleciopsis minutiscissiulus  | MFLUCC 12-0089     | KF366382     | KF366383     | –            | –              |
| Septoria phragmitis           | CPC 24118          | KR873279     | –            | KR873251     | –              |
| S. pseudophragmitis           | CBS 145417         | –            | –            | MK601616     | MK559452      |
| Setomelanomma bolmii           | CBS 110217         | GU301871     | GU296196     | KT389542     | GU349028      |
| Setophoma antiqua              | CBS 145369         | MK11947     | –            | MK11909      | MK250709      |
| S. chromolaenae               | CBS 135105         | KF251747     | –            | KF251244     | KF253195      |
| S. endophytica                | LC3163            | MK511956     | –            | MK511931     | MK525092      |
| Species                  | Strain/Voucher no. | GenBank accession no. |
|-------------------------|-------------------|-----------------------|
|                         |                  | LSU | SSU | ITS | tef1–α |
| *S. longinqua*           | LC6593           | MK511946 – MK511908 | MK525069 |
| *S. pseudosacchari*      | CBS 145373       | MK540039 – MK539969 |
| *S. sacchari*            | MFLUCC 11-0154   | KJ476146 – KJ476144 | KJ461319 |
|                         | MFLUCC 12-0241   | KJ476147 – KJ476149 | KJ461318 |
| *S. terrestris*          | CBS 335.29       | KF251749 – GQ87526  | KF251246 – KF253196 |
| *S. vernoniae*           | CBS 137988       | KJ869198 – KJ869141 | MK540162 |
| *S. yingyishenii*        | LC12696          | MK511950 – MK511914 | MK525075 |
| *S. yunnanensis*         | LC6532           | MK511945 – MK511907 | MK525068 |
| *Stagonospora folicola*  | CBS 110111       | KF251759 – EU754118 | KF251256 – KF253206 |
| *Sulcispora sp.*         | MFLUCC 14-0995   | KP271444 – KP271445 | KP271444 – MH665366 |
| *Sulcispora pleurospora* | CBS 460.84       | –   –   –   –   –   – |
| *Tinteltobia destructans* | CBS 127737      | KY090664 – KY090698 | KY090652 – |
| *T. apantiae*            | CBS 376.91       | GU238123 – GU238226 | KY090651 – |
| *Vagicolia vagans*       | CBS 604.86       | KU058727 – KF251193 | KF253149 |
| *Vitalliana mangrovei*   | NFCCI 4251       | MG767312 – MG767313 | MG767311 – MG767314 |
| *Vrystatia aloeicola*    | CBS 135107       | KF251781 – KF251278 – |
| *Wingfeldomyces cyperi*  | CBS 141450       | KX228337 – KX228286 | MK540163 |
| *Wojnowiciella eucalypti*| CPC 25024        | KR477674 – KR477674 | LT990617 |
| *W. kunmingensis*        | KUMCC 18-0159    | MK356354 – MK356368 | MK356380 – MK359071 |
| *Xenophoma punctelliae*  | CBS 128022       | JQ238619 – – – – – | KP170686 |
| *Xeneopteria neoaccardi* | CBS 120.43       | KF251783 – KF251280 | KF253227 |
| CBS 128665              | KF251784 – KF251281 | KF253228 |
| *Yunnanensis chromolaenae* | MFLUCC 17-1486  | MN994573 – MN994596 | MN998177 |
|                         | MFLUCC 17-1487   | MN994574 – MN994597 | MN998178 |
| *Yunnanensis phagnitis*  | MFLUCC 17-0315   | MF684863 – MF684876 | MF684862 – MF683624 |
|                         | MFLUCC 17-1361   | MF684865 – MF684871 | MF684869 – |

Bayesian inference analyses (BI). ML trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008; Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. The MP analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002), with parameters as described in Tennakoon et al. (2019). Descriptive tree statistics for parsimony, such as Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC) and Homoplasy Index (HI) were calculated.

The BI analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) to evaluate posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo sampling (MCMC). Six MCMC chains were run simultaneously, starting from random trees for 3,000,000 generations. Trees were sampled every 100th generation for a total of 30,000 trees. The first 6,000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 24,000 trees. Phylograms were visualized with FigTree v1.4.0 (Rambaut 2012) and annotated in Microsoft Power Point (2010). Sequences of the new strains generated in this study are deposited in GenBank. The final alignment and trees were deposited in TreeBASE, submission ID: 26088.
Results

Phylogenetic analysis

The combined dataset of ITS, LSU, SSU and tef1-α sequences comprised 3423 characters, of which 2418 characters are constant, 697 characters are parsimony-in-
formative, while 308 variable characters are parsimony-uninformative in the maximum parsimony (MP) analysis (TL = 6364, CI = 0.250, RI = 0.657, RC = 0.164, HI = 0.750). The RAxML analysis of the combined dataset yielded a best scoring tree (Figure 1) with a final ML optimization likelihood value of \(-34492.801018\). The matrix had 1331 distinct alignment patterns, with 37.25% of undetermined characters or gaps. Estimated base frequencies are; A = 0.247120, C = 0.228182, G = 0.268238, T = 0.256459; substitution rates AC = 1.250439, AG = 3.526348, AT = 2.517351, CG = 0.798250, CT = 6.907432, GT = 1.000; proportion of in-
variable sites $I = 0.596400$; gamma distribution shape parameter $\alpha = 0.492378$. All analyses (ML, MP and BI) gave similar results and are in agreement with previous studies based on multi-gene analyses (Hyde et al. 2019, 2020; Phookamsak et al. 2019). Phylogenetic analyses of the combined data matrix resulted in well-resolved clades, many of which had considerably high statistical support (Figure 1). Bootstrap support values for maximum likelihood, maximum parsimony $\geq 70\%$, and Bayesian posterior probabilities (BYPP) $\geq 0.95$ are given above each branch in that order (Figure 1). Phylogenetic position and statistical support are noted in the taxonomy section.

**Figure 1.** Continued.
Taxonomy

**Elongaticollum Tennakoon, C.H. Kuo & K.D. Hyde, gen. nov.**

Index Fungorum number: IF 557486
Facesoffungi number: FoF07849

**Etymology.** Refers to the fact that the pycnidia have elongated necks.

**Diagnosis.** Saprobic on dead leaves of *Hedychium coronarium* J. Koenig. **Sexual morph:** Undetermined. **Asexual morph:** Coelomycetous. *Conidiomata* pycnidial, solitary, superficial, dark brown to black, obpyriform, papillate. **Neck** elongate, dark brown, usually straight, but sometimes slightly curved. *Conidiomatal wall* composed of 4–5 layers of light brown cells, arranged in **textura angularis. Conidiophores** reduced to **conidiogenous cells**. **Conidiogenous cells** hyaline, aseptate, smooth, ampulliform, arising from the inner cell wall of the apex. **Conidia** oval to oblong, smooth and thin-walled, hyaline, aseptate, with 1–2-minute guttules.

**Type species.** *Elongaticollum hedychii* Tennakoon, C.H. Kuo & K.D. Hyde.

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**Elongaticollum hedychii** Tennakoon, C.H. Kuo & K.D. Hyde, sp. nov.

Index Fungorum number: IF 557487
Facesoffungi number: FoF07850

**Figure 2**

**Etymology.** Name reflects the host *Hedychium coronarium* J. Koenig, from which the holotype was collected.
Holotype. MFLU 18-2542.

Diagnosis. Saprobic on dead leaves of *Hedychium coronarium* J. Koenig. Sexual morph: Undetermined. Asexual morph: Coelomycetous. **Conidiomata** 120–140 µm high, 60–70 µm diam., pycnidial, solitary, scattered, superficial, visible as small black spots on host surface, dark brown to black, obpyriform, papillate. **Neck** up to 80–100 µm long, 20–30 µm diam., elongated, dark brown, usually straight, but sometimes slightly curved. **Conidiomatal wall** up to 80–100 µm wide, composed of 4–5 layers of light brown, thick-walled cells, arranged in texture angularis. **Conidiophores** reduced to **conidiogenous cells**. **Conidiogenous cells** 3–4 × 3–3.5 µm (\( \bar{x} = 3.6 \times 3.2 \) µm, \( n = 10 \)), arising from the inner cell wall of the apex, hyaline, aseptate, smooth, ampulliform. **Conidia** 4–5 × 1.8–2.2 µm (\( \bar{x} = 4.6 \times 2.1 \) µm, \( n = 30 \)), oval to oblong, smooth, thin-walled, hyaline, aseptate, with 1–2-minute guttules.

Culture characteristics. Colonies on PDA reaching 30 mm diameter after 3 weeks at 20–25 °C, colonies medium sparse, circular, raised, surface slightly rough with entire edge, margin entire, colony from above: light brown to grey at the margin, dark brown at middle, dark brown to black at the center; reverse, light brown to yellowish at the margin, brown at middle, dark brown to black at the center; mycelium light brown to grey with tufts; not producing pigments in PDA.

Material examined. Taiwan, Chiayi, Fanlu Township area, Dahu Forest, dead leaves of *Hedychium coronarium* J. Koenig (Zingiberaceae), 15 August 2018 (23°27.514’N, 120°36.302’E), D.S. Tennakoon, TLF031-A (MFLU 18-2542, holotype), ex-type living culture (MFLUCC 18-1638 = NCYUCC 19-0163); ibid. 20 August 2018 (23°27.530’N, 120°36.314’E), TLF031-B (NCYU19-0139, paratype), living culture (NCYUCC19-0286); ibid. 25 August 2018 (23°27.512’N, 120°36.301’E), TLF031-C (NCYU19-0140, paratype), living culture (NCYUCC 19-0287).

Notes. The genus *Elongaticollum* differs from other asexual morphs in Phaeosphaeriaceae in dark brown to black, superficial, obpyriform, pycnidial conidiomata with distinct elongate necks (80–100 µm) and a globose base and oval to oblong, hyaline, aseptate conidia (Figure 2). Multi-gene phylogenetic analyses (LSU, SSU, ITS, tef1-α), show *Elongaticollum* strains constitute a highly supported independent lineage nested between *Setophoma sensu lato* and *Neostagonosporella* (97% ML, 80% MP , 1.00 BYPP, Figure 1). However, the asexual morph of *Setophoma* can be distinguished from *Elongaticollum* in having setose conidiomata without elongate necks and oblong to ellipsoidal conidia, whereas, *Elongaticollum* have conidiomata with distinct elongate necks and lacking setae and oval to oblong conidia (De Gruyter et al. 2010; Phookamsak et al. 2014). Despite some *Setophoma* species not having setae (i.e. *S. antiqua*, *S. endophytica*, and *S. yunnanensis*) (Liu et al. 2019), *Elongaticollum* species can be distinguished by its superficial conidiomata with elongate necks.

The asexual morph of *Neostagonosporella* differs from *Elongaticollum* in having multiloculate conidiomata without distinct elongate necks and two types of conidia (macroconidia: subcylindrical to cylindrical, transversely multi-septate, hyaline and microconidia oval, ellipsoidal or long ellipsoidal, aseptate, hyaline), whereas *Elongaticollum* has uni-loculate conidiomata with distinct elongate necks and oval to oblong conidia (Figure 2, Yang et al. 2019).
Phylogenetic investigations herein provide insights into the taxonomy of *Setophoma* as well (Figure 1). Two major clades of *Setophoma* are recovered (*Setophoma sensu stricto* and *Setophoma sensu lato*). The *Setophoma sensu stricto* clade includes *S. brachypodii*, *S. poaceicola* and *S. terrestris* (type species). *Setophoma sensu lato* comprises *S. antiqua*, *S. chromolaenae*, *S. endophytica*, *S. pseudosacchari*, *S. sacchari*, *S. vernoniae*, *S. yingyisheniae* and *S. yunnanensis* (Figure 1). *Elongaticollum*, differs from *Setophoma sensu lato* in having distinct superficial, obpyriform, pycnidial conidiomata with a globose base and distinct elongated necks (Figure 2, Liu et al. 2019). Further work is needed to resolve relationships between *Setophoma sensu stricto* and *Setophoma sensu lato*.

*Ophiosphaerella* Speg., Anal. Mus. nac. B. Aires, Ser. 3 12: 401 (1909)

**Notes.** *Ophiosphaerella* was introduced by Spegazzini (1909) to accommodate *O. graminicola* Speg. as the type species. The species of this genus are characterized by papillate ascomata bearing fissitunicate, cylindrical asci frequently narrower near the
base, with a short furcate pedicel and filamentous, pale brown, multi-septate ascospores without swollen cells or separating into part spores. Barr (1987) placed *Ophiophysaerella* in Phaeosphaeriaceae and this was confirmed by Zhang et al. (2009, 2012) and Hyde et al. (2013) based on molecular phylogeny. Most *Ophiophysaerella* species are often found as pathogens or saprobes worldwide on Poaceae and Cyperaceae (Câmara et al. 2000). Currently, twelve *Ophiophysaerella* species are listed in Index Fungorum (2020). In this study, we introduce *Ophiophysaerella taiwanensis* from *Agave tequilana* F.A.C. Weber (Asparagaceae) as a new species.

**Ophiophysaerella taiwanensis** Tennakoon, C.H. Kuo & K.D. Hyde, sp. nov.
Index Fungorum number: IF 557488
Facesoffungi number: FoF07851
Figure 3

**Etymology.** Named after Taiwan, where this fungus was collected.

**Holotype.** MFLU 18-2534.

**Diagnosis.** Saprobic on dead leaf of *Agave tequilana* F.A.C. Weber (Asparagaceae).

**Sexual morph:** *Ascomata* 270–310 µm high, 220–260 µm diam., solitary, scattered, immersed to slightly erumpent through host tissue with papilla, visible as raised, small black dots in host surface, globose to subglobose, uniloculate, glabrous, dark brown to black, ostiole central, periphysate. *Peridium* 20–25 µm wide, thick-walled, of equal thickness, composed of 6–7 layers of small, flattened, brown to dark brown pseudo-parenchymatous cells, hyaline towards the inside, arranged in a textura angularis, fusing and indistinguishable from the host tissues. *HAMATHECIUM* of 1.5–2.5 µm wide, cellular, septate, rarely branching, pseudoparaphyses, anastomosing mostly above the asci and embedded in a mucilaginous matrix. *Asci* 115–140 × 8.5–10 µm (x̄ = 121.6 × 9.2 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded, with a well-developed ocular chamber. *Ascospores* 110–132 × 2.2–2.7 µm (x̄ = 117.2 × 2.4 µm, n = 20), fasciculate, parallel, scolecosporous, filiform, 12–13-septate, narrowing towards ends, pale brown to brown, smooth-walled.

**Asexual morph:** Undetermined.

**Culture characteristics.** Colonies on PDA reaching 25 mm diameter after 3 weeks at 20–25 °C, colonies medium sparse, circular, raised, surface slightly rough with entire edge, margin well-defined, colony from above: gray to light brown at the margin, gray to cream at the center; reverse, gray to light brown at the margin, dark brown to black at the center; mycelium whitish gray with tufting; not producing pigments in PDA.

**Material examined.** Taiwan, Chiayi, Fanlu Township area, Dahu Forest, dead leaf of *Agave tequilana* F.A.C. Weber (Asparagaceae), 15 August 2018 (23°27.520′N, 120°36.310′E), D.S. Tennakoon, TLF016 (MFLU 18-2534, holotype; *ibid.* (NCYU19-0131, isotype), ex-type living culture, NCYUCC 19-0152.

**Notes.** The scolecosporous specimen was collected from dead leaves of *Agave tequilana* (Asparagaceae) in Taiwan. The multi-gene phylogenetic analysis (Figure 1)
Figure 3. Ophiosphaerella taiwanensis (MFLU 18-2534, holotype) a, b appearance of ascomata on host c close-up of ascomata d vertical section through ascoma e apex of ascoma f peridium g pseudoparaphyses h–j asci k, l ascospores m germinated ascospore in PDA n colony from above o colony from below. Scale bars: 100 µm (d, e), 15 µm (f), 50 µm (g–m).

shows our strain (Ophiosphaerella taiwanensis, NCYUCC 19-0152), cluster with other Ophiosphaerella species, in particular with close affinity to Ophiosphaerella agrostidis with high bootstrap support (88% ML, 70% MP, 0.99 BYPP, Figure 1). Morphological characters of our collection (NCYUCC 19-0152) differ from Ophiosphaerella agrostidis in having periphyses in the ostiole, 12–13 septate ascospores and host occurrence (Asparagaceae). Ophiosphaerella agrostidis was introduced by Câmara et al. (2000) on Agrostis palustris (Poaceae), and is lacking periphyses, comprises 15-septate ascospores (Phookamsak et al. 2014). A comparison of the 619 nucleotides across the tef1-α gene region of Ophiosphaerella taiwanensis and O. agrostidis (MFLUCC 11-0152) reveals 17 base pair differences (2.74%).

Phaeosphaeriopsis M.P.S. Câmara, M.E. Palm & A.W. Ramaley, Mycol. Res. 107(5): 519 (2003)

Notes. The genus Phaeosphaeriopsis was introduced by Câmara et al. (2003) to accommodate Paraphaeosphaeria-like taxa, viz. P. agavensis A.W. Ramaley, M.E. Palm &
Additions to Phaeosphaeriaceae (Pleosporales)

M.E. Barr, *P. glaucopunctata* (Grev.) Shoemaker & C.E. Babc., *P. nolinae* A.W. Ramaley, *P. obtusispora* (Speg.) O.E. Erikss, *Phaeosphaeriopsis amblyspora* A. W. Ramaley and *Phaeosphaeriopsis amblyspora* A. W. Ramaley. The genus is typified by *P. glaucopunctata* and characterized by having immersed, sub-epidermal, globose to subglobose to pyriform ascomata, cylindric asci and septate, punctate or verrucose ascospores (Câmara et al. 2003; Phookamsak et al. 2014; Thambugala et al. 2014; Tibpromma et al. 2017). Currently, 17 *Phaeosphaeriopsis* species are accepted in Index Fungorum (2020). In this paper, *Phaeosphaeriopsis beaucarneae* is introduced from *Beaucarnea recurvata* (Asparagaceae) as a new species and the sexual/asexual morph connection between strains isolated from the natural habitat was established based on molecular sequence data.

*Phaeosphaeriopsis beaucarneae* Tennakoon, C.H. Kuo & K.D. Hyde, sp. nov.

Index Fungorum number: IF 557489
Facesoffungi number: FoF07852
Figures 4, 5

**Etymology.** Name reflects the host *Beaucarnea recurvata* Lem., from which the holotype was collected.

**Holotype.** MFLU 18-2586.

**Diagnosis.** Saprobic on dead leaf of *Beaucarnea recurvata* Lem. (Asparagaceae).

**Sexual morph:** *Ascomata* 160–200 µm high, 220–250 µm diam., scattered, solitary, gregarious, coriaceous, immersed to semi-immersed, slightly raised, erumpent, visible as black spots on host surface, uniloculate, dark brown to black, globose to subglobose, ostiolate. *Ostiole* central, papillate. *Peridium* 20–30 µm wide, thick-walled, of equal thickness, composed of 4–5 layers of dark brown to brown, thick-walled, pseudoparenchymatous cells of textura angularis. *Hamathecium* of 1.5–2.5 µm wide, cellular, septate, rarely branching, pseudoparaphyses, anastomosing mostly above the asci and embedded in a mucilaginous matrix. *Asci* 80–90 × 9–10 µm (\(\bar{x} = 86.5 \times 9.6 \) µm, \(n = 25\)), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded, with a well-developed ocular chamber. *Ascospores* 20–25 × 5.5–7 µm (\(\bar{x} = 22.6 \times 6.2 \) µm, \(n = 20\)), overlapping 1–2-seriate, oblong to cylindrical, yellowish to light brown, slightly narrowing towards the end cells, mostly 5-septate, constricted at the septa, enlarged at the 4th cell from above, verruculose, straight to curved, lacking a mucilaginous sheath. **Asexual morph:** *Conidiomata* 180–200 µm high, 140–160 µm diam., pycnidial, solitary, immersed to erumpent, small black spots on host surface, globose to subglobose with centrally placed ostiole. *Conidiomatal wall* 28–34 µm wide, composed of 6–7 layers of dark brown cells, arranged in textura angularis. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–4 × 2.6–3.1 µm, holoblastic, phialidic, single, discrete, sometimes integrated, ampulliform or cylindric-clavate, hyaline, arising from basal stratum. *Conidia* 6.8–7.4 × 3–4 µm (\(\bar{x} = 7.1 \times 3.4 \) µm, \(n = 30\)), 1-celled, globose to subglobose, initially hyaline, becoming brown to dark brown, aseptate, rough-walled.
**Figure 4.** Phaeosphaeriopsis beaucarneae (MFLU 18-2586, holotype) **a** appearance of ascomata on host **b** close up of ascoma **c** vertical section through ascoma **d** peridium **e** pseudoparaphyses **f-i** asci **j-n** ascospores **o** germinated ascospore in PDA **p** colony from above **q** colony from below. Scale bars: 100 µm (**c**), 15 µm (**d**), 50 µm (**e–i**), 10 µm (**j–o**).

**Culture characteristics.** Colonies on PDA reaching 27 mm diameter after 3 weeks at 20–25 °C, colonies medium sparse, circular, raised, surface slightly rough with entire edge, margin irregular, colony from above: light brown at the margin, white to cream at the center; reverse, yellow to light brown at the margin, light brown to brown at the center; mycelium white to cream with tufting; not producing pigments in PDA.

**Material examined.** Taiwan, Chiayi, Fanlu Township area, Dahu Forest, dead leaf of Beaucarnea recurvata Lem. (Asparagaceae), 21 July 2018 (23°27.514’N, 120°36.302’E), D.S. Tennakoon, SV027 (MFLU 18-2586, holotype); *ibid.* (NCYU19-0184, isotype), ex-type living culture, NCYUCC 19-0106; *ibid.*, Dahu forest, dead leaf of Beaucarnea recurvata Lem. (Asparagaceae), 25 July 2018 (23°26.534’N, 120°36.220’E), D.S. Tennakoon, SV028 (MFLU 18-2587, paratype); living culture, NCYUCC 19-0107.

**Notes.** Phaeosphaeriopsis beaucarneae is similar to other Phaeosphaeriopsis species in having scattered, semi-immersed to erumpent, globose to subglobose, ostiolate ascomata and cylindrical to clavate asci and light brown, verrucose ascospores (Phookamsak et al. 2014; Thambugala et al. 2014; Hyde et al. 2020). According to
Figure 5. *Phaeosphaeriopsis beaucarneae* (MFLU 18-2586, paratype) **a** appearance of conidiomata on host **b** close up of conidiomata **c** vertical section through conidioma **d** conidiomatal wall **e, f** conidiogenous cells and developing conidia **g–i** conidia **j** germinated conidium in PDA **k** colony from above **l** colony from below. Scale bars: 100 µm (**c**), 20 µm (**d**), 3 µm (**e, f**), 5 µm (**g–j**).

the present multi-gene phylogenetic analyses (Figure 1), *Phaeosphaeriopsis beaucarneae* is grouped with other *Phaeosphaeriopsis* species, in particularly closely to *P. grevilleae* (CBS 145369) with high statistical support (70% ML, 75% MP, 0.99 BYPP, Figure 1). The asexual morph of *P. grevilleae* was isolated from leaves of *Grevillea* sp. (Proteaceae) and introduced by Marin-Felix et al. (2019). *Phaeosphaeriopsis beaucarneae* differs from *P. grevilleae* in having larger conidia (6.8–7.4 × 3–4 µm), whereas *P. grevilleae* has comparatively smaller conidia (5 × 3.5 µm). A comparison of the 516 nucleotides across the ITS (+5.8S rDNA) gene region of *Phaeosphaeriopsis beaucarneae* and *P. grevilleae* (CBS 145369) revealed 16 base pair differences (3.10%). In addition, we compared our new taxon with *P. grevilleae* based on base pair differences in the tefl-α gene region. We found a total of 19 base pair differences (3.06%) across 619 nucleotides.

Recent studies have revealed that *Phaeosphaeriopsis* is a species rich genus and numerous *Phaeosphaeriopsis* species have been described during the last few years (Thambugala et al. 2014; Tibpromma et al. 2017; Marin-Felix et al. 2019; Al-Jaradi et al. 2020; Hyde et al. 2020). With this study, the number of *Phaeosphaeriopsis* species increases to 18.
**Neosetophoma** Gruyter, Aveskamp & Verkley, *Mycologia* 102(5): 1075 (2010)

**Notes.** *Neosetophoma* was introduced by de Gruyter et al. (2010), typified by *N. samararum* (Desm.) Gruyter, Aveskamp. & Verkley. Species of *Neosetophoma* are characterized by globose to irregular conidiomata, with papillate ostioles, and yellowish conidia that are attenuate at one end (De Gruyter et al. 2010; Liu et al. 2015). Tibpromma et al. (2017) introduced *Neosetophoma garethjonesii* Tibpromma, E.B.G. Jones & K.D. Hyde as the first report of the sexual morph of *Neosetophoma*. *Neosetophoma* species have a diverse distribution as saprobes, endophytes, plant pathogens and soil fungi (Phookamsak et al. 2014; Hernandez-Restrepo et al. 2016; Karunarathna et al. 2017; Tibpromma et al. 2017; Wanasinghe et al. 2018). Currently, 19 *Neosetophoma* species are accepted in Index Fungorum (2020). In this study, we found *Neosetophoma poaceicola* Goonas., Thambug. & K.D. Hyde from dead leaves of *Musa acuminata* Colla in Taiwan. This is the first *Neosetophoma* species recorded from the plant family Musaceae.

*Neosetophoma poaceicola* Goonas., Thambug. & K.D. Hyde. *Mycosphere* 8: 742 (2017)
Index Fungorum number: IF552974
Facesoffungi number: FoF00262
Figure 6

**Diagnosis.** Saprobic on dead leaf petioles of *Musa acuminata* Colla (Musaceae). **Sexual morph:** Ascomata 70–100 µm high, 90–130 µm diam., solitary, gregarious, coriaceous, immersed to semi-immersed, slightly raised, visible as black spots on host surface, uni-loculate, dark brown to black, globose to ovoid. Peridium 15–20 µm wide, thick-walled, of equal thickness, composed of several layers of dark brown to brown, pseudoparenchymatous cells of textura angularis. Hamathecium of 1–2 µm wide, cellular, rarely branching, pseudoparaphyses, anastomosing mostly above the asci and embedded in a mucilaginous matrix. Asci 60–80 × 7–8 µm (x = 70.6 × 7.6 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindric-clavate with a short, rounded pedicel, apically rounded. Ascospores 20–30 × 3–4 µm (x = 25.5 × 3.7 µm, n = 40), overlapping 1–2-seriate, hyaline, fusiform, with acute ends, 1-septate, 3–4 eu-septate, cell near the septum slightly larger, slightly constricted at the septum, straight to curved, smooth-walled, guttulate. **Asexual morph:** Undetermined.

**Culture characteristics.** Colonies on PDA reaching 30 mm diameter after 3 weeks at 20–25 °C, colonies medium sparse, circular, flat, surface slightly rough with entire edge, margin well-defined, colony from above: yellow to light brown at the margin, brown at the center; reverse: yellow to light brown at the margin, dark brown at the center; mycelium light brown to whitish grey with tufting; not producing pigments in PDA.

**Material examined.** Taiwan, Chiayi, Fanlu Township area, Dahu Forest, dead leaf petiole of *Musa acuminata* Colla (Musaceae), 21 July 2018 (23°27.530’N, 120°36.340’E), D.S. Tennakoon, SV049 (MFLU 18-2597, new host record), living culture, MFLUCC 18-1632,NCYUCC 19-0119.
Additions to Phaeosphaeriaceae (Pleosporales)

Notes. As morphological characters (immersed to semi-immersed ascomata, cylindrical-clavate, apically rounded asci with short rounded pedicel and hyaline, fusiform, 1-septate ascospores) largely overlap with those of *Neosetophoma poaceicola* (MFLUCC 16–0886), we report our collection (MFLUCC 18-1632) as a new host record of *N. poaceicola* from dead leaves of *Musa acuminata* (Musaceae) in Taiwan. Combined multi-gene (LSU, SSU, ITS and *tefl-α*) based phylogenies also showed that our collection clustered with *Neosetophoma poaceicola* (MFLUCC 16-0886), with high bootstrap support (100% ML, 100% MP, 1.00 BYPP, Figure 1). *Neosetophoma poaceicola* was introduced by Thambugala et al. (2017) from dead leaves of grass species in Thailand. However, our collection slightly differs from *Neosetophoma poaceicola* (MFLUCC 16-0886) in having comparatively slightly larger ascospores (20–30 × 3–4 µm, versus 18.5–22.5 × 3.5–5 µm).

*Neosetophoma* species have been recorded from various host families, viz. Brassicaceae, Caprifoliaceae, Iridaceae, Malvaceae, Ranunculaceae, Salicaceae, but most are reported from Poaceae (Phookamsak et al. 2014; Karunarathna et al. 2017; Tiptromma et al. 2017, Wanasinghe et al. 2018; Marin-Felix et al. 2019). Interestingly, this is the first *Neosetophoma* species record (MFLU 18-2597) from the plant family Musaceae.

**Figure 6.** *Neosetophoma poaceicola* (MFLU 18–2597, new host record) a appearance of ascomata on host b close up of ascomata c vertical section through ascoma d peridium e pseudoparaphyses f–h asci i–k ascospores l germinated ascospore in PDA m colony from above n colony from below. Scale bars: 50 µm (c), 20 µm (d), 30 µm (e–h), 15 µm (i–l).
Discussion

The taxonomy of Phaeosphaeriaceae has been subjected to several changes in recent years. Traditionally, morphology-based identification was the main means for identifying Phaeosphaeriaceae species (Barr 1979, 1992; Tomilin 1993). However, species identification has been revolutionized by the application of molecular based approaches incorporating DNA sequence data in Phaeosphaeriaceae (Phookamsak et al. 2014, 2017; Tennakoon et al. 2016; Wanasinghe et al. 2018; Bakhshi et al. 2019; Chethana et al. 2020; Hyde et al. 2020). Phaeosphaeriaceae species are adapted to a wide range of ecological environments and are present in soils, fresh and marine habitats and cause infections in humans (Yuan 1994; Phookamsak et al. 2014, 2017; Ahmed et al. 2017; Maharachchikumbura et al. 2019; Valenzuela-Lopez et al. 2019). Members of the Phaeosphaeriaceae have also been recorded from both temperate and tropical countries (i.e. Austria, Belgium, Bulgaria, Canada, China, Germany, Italy, Japan, Norway, Poland, Thailand, Sweden, Switzerland) and from different host families (i.e. Acoraceae, Arecales, Cyperaceae, Asparagaceae, Brassicaceae, Fabaceae, Poaceae, Marantaceae) (Shoemaker and Babcock 1989; Phookamsak et al. 2014, 2019; Wanasinghe et al. 2018; Maharachchikumbura et al. 2019; Farr and Rossman 2020). Due to their cosmopolitan distribution, in the last few years, many researchers have paid significant attention to the Phaeosphaeriaceae (Phookamsak et al. 2014, 2019; Tennakoon et al. 2016; Wanasinghe et al. 2018; Bakhshi et al. 2019; Hyde et al. 2020).

The fungi that decay leaf litter are highly diverse and may be host-specific (Parungao et al. 2002). Several studies have examined the succession of leaf degrading communities and found unique sets of species on different types of litter (Promputtha et al. 2002, 2017; Duong et al. 2008). Additional ecological studies are therefore needed to establish whether these fungi are generalists or specialists. This study provides evidence to indicate the fungal diversity in leaf litter, even within a single family, Phaeosphaeriaceae. Additional work is necessary to identify if the newly described species are host specific.

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