Morpho-molecular characterisation of Arecophila, with A. australis and A. clypeata sp. nov. and A. miscanthi comb. nov.

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Abstract
Three arecophila-like fungal samples were collected on dead culms of gramineous plants in China. Morphological studies of our new collections and the herbarium specimen of Arecophila gulubiicola (generic type) were conducted and the morphological affinity of our new collections with Arecophila was confirmed. Maximum likelihood and Bayesian analyses using combined ITS, LSU, rpb2 and β-tubulin data from our collections revealed the phylogeny of Cainiaceae. The monospecific genus Alishanica (type species Al. miscanthi), which had been accepted in Cainiaceae, is revisited and synonymised under Arecophila. Based on morphology and phylogeny, Arecophila australis sp. nov. and A. clypeata sp. nov. are introduced as new species, while A. miscanthi is a new record for China. All the new collections are illustrated and described.

Keywords
Cainiaceae, gramineous plants, phylogeny, taxonomy

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Introduction

The current study is a part of a series of papers on Xylariales (Sordariomycetes) from China (Long et al. 2019; Xie et al. 2019, 2020; Pi et al. 2020). *Arecophila* K.D. Hyde, which is typified by *A. gulubiicola* K.D. Hyde, was introduced by Hyde (1996) with five species. *Arecophila* is characterised by immersed, subglose to lenticular ascomata, peridium with *textura angularis* cells, non- or poorly-developed clypeus, asci with a wedge-shaped, apical ring, J+ in Melzer’s reagent and 2-celled, brown ascospores with wall striations, surrounded by a mucilaginous sheath. Thanks to subsequently undertaken morphological studies of holotypes, several species have been transferred to *Arecophila* from genera such as *Amphisphaeria* Ces. & De Not., *Cainia* Arx & E. Müll., *Didymosphaeria* Fuckel and *Schizostoma* Ehrenb. ex Lév. (Hyde 1996; Umali et al. 1999; Wang et al. 2004).

Currently, there are 15 *Arecophila* epithets in Index Fungorum (http://www.indexfungorum.org/Names/Names.asp, May 2021), which have been introduced, based on morphology and lack sequence data (e.g. Hyde 1996; Umali et al. 1999; Wang et al. 2004). After searching for *Arecophila* in NCBI, there were only five hits of LSU, SSU and metagenomic sequences of *A. bambusae* and *Arecophila* sp. HKUCC 6487 in GenBank.

*Arecophila* was introduced as a genus of *Amphisphaeriaceae* (Hyde 1996), based on its unitunicate, cylindrical asci with a J+ apical ring and brown, 2-celled ascospores. Kang et al. (1999) reviewed the genus and accepted it in *Cainiaceae* and the occurrence on monocotyledons (palms and bamboo). The single and combined molecular analyses of LSU and SSU genes resulted in *Arecophila* grouping with *Cainia* in *Xylariales* (Smith et al. 2003). Based on analyses of partial LSU gene sequences, the generic placement of *Arecophila* within the *Cainiaceae* has been verified (Jeewon et al. 2003; Senanayake et al. 2015; Hyde et al. 2020; Wijayawardene et al. 2020). However, the available molecular data do not provide strong evidence of the phylogenetic affinity of *Arecophila* and related taxa.

During our continuous collecting of xylarialean taxa in China, we found some specimens that share a morphology resembling *Arecophila*. In this paper, two new species and a new record of *Arecophila* are provided with descriptions and illustrations. Furthermore, *Alishanica* is synonymised under *Arecophila*, based on morphology and phylogeny.

Materials and methods

Collection, isolation and morphology

Fresh samples were collected in Guizhou and Yunnan Provinces in China during the rainy season and taken to the laboratory in paper bags. Single-spore isolations were obtained following the method described in Chomnunti et al. (2014). The cultures on
Morphological studies of *Arecophila*

Potato dextrose agar (PDA) were transferred to 2 ml screw cap centrifuge tubes filled with 10% glycerol and sterile water to deposit at −20 °C and 4 °C, respectively. Herbarium materials were deposited at the Herbarium of Guizhou Agricultural College (GACP) and the Herbarium of Guizhou University (GZUH). Cultures were deposited at the Culture Collection of Guizhou University (GZUCC).

The morphological examination of fresh and herbarium specimens was carried out as described by Hyde (1996). Macro-morphological characters were examined and photographed using a digital camera (Canon 700D) fitted to the Olympus SZ61 stereomicroscope. Materials mounted in water, Melzer’s reagent and Indian ink were examined. At least 30 ascospores, 30 asci and 20 apical rings were measured for each taxa with Tarosoft (R) Image Frame Work (v. 0.9.0.7) and photographed using a digital camera (Nikon 700D) fitted to a light microscope (Nikon Ni).

**DNA extraction, polymerase chain reaction (PCR) amplification and sequencing**

Total genomic DNA was extracted from fresh mycelium scraped off from pure cultures with the BIOMIGA fungus genomic DNA extraction kit (GD2416) (Wijayawardene et al. 2013) following the manufacturer’s instructions. Primers, LR0R/LR5 (Vilgalys and Hester 1990), ITS4/ITS5 (White et al. 1990), RPB2-5F/RPB2-7cR (Liu et al. 1999), Bt2a/Bt2b and ACT-512F/ACT-783R (Hsieh et al. 2005) were used for amplifying partial large-subunit ribosomal RNA (LSU), internal transcribed spacer (ITS), partial second-largest subunit of the RNA polymerase II (*rpb2*), β-tubulin (*tub*) and α-actin gene (Hsieh et al. 2005). The amplification conditions were carried out according to Liu et al. (2011) and Hsieh et al. (2005). Amplified products were examined and sent to the sequencing company, Sangon Biotech, Shanghai, China. The obtained sequences were checked, assembled and uploaded to GenBank.

**Sequence alignment and phylogenetic analyses**

Following the NCBI BLAST results and literature (e.g. Jeewon et al. 2003; Senanayake et al. 2015), relevant sequences from all families of Xylariomycetidae were downloaded from GenBank for the phylogenetic analyses (Table 1). Sequences of each segment were aligned using MAFFT (http://mafft.cbrc.jp/alignment/server/index.html, Katoh and Standley 2019) and improved manually in BioEdit 7.2.3 (Hall 1999). The combined alignment of ITS, LSU, *rpb2* and β-tubulin was concatenated from individual datasets. Ambiguously aligned areas of each gene region were excluded and gaps were treated as missing data. The ALTER (http://sing.ei.uvigo.es/ALTER/) phylogeny website tool was used to obtain the phylip file for RAxML analysis and the nexus file for Bayesian analysis (Glez-Peña et al. 2010). Phylogenetic trees were visualised using FigTree v.1.4.0. and processed using Adobe Photoshop CS6 software (Adobe Systems, USA). The alignment for the tree in this paper was uploaded on the website (https://treebase.org/) with submission ID 26613.
Table 1. Sequences used for phylogenetic analyses in this study.

| Species                          | Strain number | Status | ITS         | LSU         | rpb2        | β-tubulin   | References                                      |
|----------------------------------|---------------|--------|-------------|-------------|-------------|-------------|-------------------------------------------------|
| Achaetomium macrosporum          | CBS 532.94    | –      | KX976574    | KX976699    | KX976797    | KX976915    | Wang et al. (2016)                               |
| Amphibambusa bambusicola         | MFLUCC 11-0617| HT     | KP744433    | KP744474    | N/A         | N/A         | Senanayake et al. (2015)                         |
| Amphiphaeria acericola           | MFLU 16-2479  | HT     | NR_171945   | MK640424    | N/A         | N/A         | Senanayake et al. (2019, submitted directly)     |
| Amphiphaeria thailandica         | MFLU 18-0794  | HT     | NR_168783   | NG_068588   | MK033640    | MK033639    | Samarakoong et al. (2019)                        |
| Amphiphaeria umbrina             | AFTOL-ID 1229 | AF009805| N/A         | FJ176863    | FJ238348    | N/A         | Schoch (2008, submitted directly)                |
| Apiospora bambucae               | ICMP 6889     | –      | N/A         | DQ368630    | DQ368649    | N/A         | Tang et al. (2007)                               |
| Apiospora hyphopodi              | MFLUCC 15-0003| HT     | KR069110    | KY356093    | N/A         | N/A         | Dai et al. (2016)                                |
| Apiospora setosa                 | ICMP 4207     | –      | N/A         | DQ368631    | DQ368650    | DQ368620    | Tang et al. (2007)                               |
| Apiospora yunnana                | MFLUCC 15-0002| HT     | KU940147    | NG_057104   | MK291950    | N/A         | Dai et al. (2017)                                |
| Arecophila australis             | GZUC0112      | HT     | MT742126    | MT742133    | N/A         | MT741734    | This study                                      |
| Arecophila australis             | GZUC0124      | –      | MT742125    | MT742132    | N/A         | N/A         | This study                                      |
| Arecophila bambucae              | HKUC 4794     | –      | N/A         | AF542038    | N/A         | N/A         | Kang et al. (1999)                               |
| Arecophila clypeata              | GZUC0110      | HT     | MT742129    | MT742136    | MT741732    | N/A         | This study                                      |
| Arecophila clypeata              | GZUC0127      | –      | MT742128    | MT742135    | N/A         | N/A         | This study                                      |
| Arecophila miscanthi             | GZUC0122      | –      | MT742127    | MT742134    | N/A         | N/A         | This study                                      |
| Arecophila miscanthi             | MFLU 19-2333  | HT     | NR_171235   | MK503827    | N/A         | N/A         | Hyde et al. (2020)                               |
| Arecophila sp.                   | HKUC 6487     | –      | N/A         | AF542039    | N/A         | N/A         | Jeewon et al. (2003)                             |
| Apiospora yunnana                | MFLUCC 15-0002| HT     | KU940147    | NG_057104   | KU940177    | MK291950    | Dai et al. (2017)                                |
| Artoropataspora partii           | MFLUCC 13-0444| HT     | KP352443    | N/A         | N/A         | N/A         | Thambugala et al. (2015)                         |
| Bagadiella lunata                | CBS 124762    | HT     | NR_132832   | NG_058637   | N/A         | N/A         | Cheewangkoon et al. (2009)                       |
| Barrmaelia nuppazii              | G2 = CBS 142771| HT     | MF488989    | MF488990    | MF488998    | MF489017    | Voglmayr et al. (2018)                           |
| Barrmaelia rhunicola             | BR = CBS 142772| HT     | MF488990    | MF488990    | MF488999    | MF489018    | Voglmayr et al. (2018)                           |
| Bartalinia pondensis             | CMW 31067     | –      | MH863602    | MH875078    | MH554904    | MH554663    | Vu et al. (2019)                                 |
| Beltnania pseudohormbica         | CBS 138003    | HT     | MH554124    | MH558667    | MH555032    | N/A         | Liu et al. (2019)                                |
| Beltnania rhombica               | CBS 125.58    | T      | MH857718    | MH868082    | MH554899    | MH704631    | Vu et al. (2019)                                 |
| Beltnaniopsis longiconidiophora  | MFLUCC 17-2139| HT     | NR_158353   | NG_066200   | N/A         | N/A         | Liu et al. (2017)                                |
| Biscogniauxia nummularia         | MUCL 51395    | ET     | KY610382    | KT281894    | KY624236    | KY271241    | Senanayake et al. (2015)                         |
| Cainia anthoxanthis              | MFLUCC 15-0539| HT     | NR_138407   | KR092777    | N/A         | N/A         | Senanayake et al. (2015)                         |
| Cainia graminis                  | CBS 136.62    | –      | MH858123    | AF431949    | N/A         | N/A         | Vu et al. (2019)                                 |
| Species                  | Strain number | Status | GenBank accession numbers | References                        |
|-------------------------|---------------|--------|---------------------------|-----------------------------------|
| Catania graminis        | MFLUCC 15-0540 | –      | KR092793, KR092781, N/A, N/A | Senanayake et al. (2015)          |
| Camillea obularia       | ATCC 28093    | –      | KY610384, KY610429, KY624238, KY271243 | Wendt et al. (2018)               |
| Castanediella acaciae   | CBS 139896    | HT     | NR_137985, NG_067293, N/A, N/A | Vu et al. (2019)                  |
| Castanediella couratarii| CBS 579.71    | HT     | KR145250, NG_066249, N/A, N/A | Crous et al. (2013)               |
| Castanediella eucalyptica| CPC 26539    | HT     | KX228266, KX228317, N/A, KX228382 | Wang et al. (2016)                |
| Chaetomium elongatum    | CBS 374.66    | –      | KC109758, KC109758, KF001820, KC109776 | Senanayake et al. (2015)          |
| Ciferriascossea fluctuatimura | MFLUCC 15-0541 | HT     | KR092789, KR092778, N/A, N/A | Senanayake et al. (2015)          |
| Ciferriascossea rectimura | MFLUCC 15-0542 | HT     | NR_153905, N/A, N/A, N/A | Senanayake et al. (2015)          |
| Clypeophysalospora latitans | CBS 141463  | ET     | KR153929, NG_058958, N/A, N/A | Giraldo et al. (2017)             |
| Coniocessia maxima      | CBS 593.74    | HT     | NR_137751, MH878275, N/A, N/A | Vu et al. (2019)                  |
| Coniocessia nodulosiporioides | CBS 281.77  | IT     | MH861061, AJ875224, N/A, N/A | Garcia et al. (2006)              |
| Creopharia sassafras    | STMA 14087    | –      | KY610411, KY610468, KY624265, KX271258 | Wendt et al. (2018)               |
| Cylindrium aeruginosum  | CBS 693.83    | –      | KM231854, KM231734, KM232430, KM232124 | Lombard et al (2014, submitted directly) |
| Cylindrium grande       | CBS 145655    | HT     | NR_165557, NG_068656, MK876481, MK876502 | Crous et al. (2019)               |
| Cylindrium purgamentum  | CPC 29580     | HT     | NR_155691, NG_067320, N/A, N/A | Koppel et al. (2017)              |
| Daldinia concentrica    | CBS 113277    | –      | AY616683, KT281895, KY624243, K877724 | Senanayake et al. (2015)          |
| Delonicicola siamensae  | MFLUCC 15-0670 | HT     | MF167586, NG_059172, MF158346, N/A | Peera et al. (2017)               |
| Diatrype palmicola      | MFLUCC 11-0018 | –      | KP744439, KP744481, N/A, N/A | Liu et al. (2015)                 |
| Diatrype whitmanensis   | ATCC MYA-4417 | –      | FJ74656, FJ430587, N/A, N/A | Igo et al. (2009, direct submission) |
| Entosordaria perfidiosa | EPE = CBS 142773 | ET    | MF488993, MF488993, MF489003, MF489021 | Voglmayr et al. (2018)            |
| Entosordaria quercina   | RQ = CBS 142774 | HT     | MF488994, MF488994, MF489004, MF489022 | Voglmayr et al. (2018)            |
| Eutypa flavovirens      | MFLUCC 13-0625 | –      | KR092798, KR092774, N/A, N/A | Senanayake et al. (2015)          |
| Eutypa laevata          | CBS 291.87    | –      | HM164737, N/A, HM164805, HM164771 | Trouillas and Guibler (2010)      |
| Eutypa lata             | CBS 208.87    | NT     | MH862066, MH187355, KF453595, DQ006969 | Vu et al. (2019)                  |
| Furfurella nigrescens   | CBS 143622    | HT     | MK527844, MK527844, MK523275, MK523332 | Daranagama et al. (2015)          |
| Furfurella stromatica   | CBS 144409    | HT     | NR_164062, MK527846, MK523277, MK523334 | Voglmayr et al. (2019)            |
| Graphostroma platystomum| AFTOL-ID 1249 | HT     | HG934115, DQ836906, DQ836893, HG934108 | Zhang et al. (2006)               |
| Hypocreaphylicola       | UME 31430     | –      | - , AY803834, N/A, N/A | Smith et al. (2002, submitted directly) |
| Hypocollaria fragiforme | MUCL51264     | ET     | KM186294, KM186295, KM186296, KM186293 | Marasinghe et al. (2019)          |
| Iodosphaeria honghensis | MFLU 19-0719  | HT     | MK737501, MK722172, MK791287, N/A | Li et al. (2015)                  |
| Iodosphaeria tongrenensis| MFLU 15-0393 | HT     | KR095282, KR095283, N/A, N/A | Senanayake et al. (2015)          |
| Species                                | Strain number | Status | GenBank accession numbers | References                      |
|----------------------------------------|---------------|--------|---------------------------|---------------------------------|
| Jackrogersella multiformis             | CBS 119016    | ET     | KC477234                  | Wendt et al. (2018)             |
| Kretzschmaria decusta                  | CBS 163.93    | –      | KC477237                  | Senanayake et al. (2015)        |
| Leptosyphilia fuchelii                 | CBS 140409    | NT     | NR_154123                 | Jaklitsch et al. (2016)         |
| Leptosilicia pistaciae                 | CBS 128196    | HT     | NR_160064                 | Voglmayr et al. (2019)          |
| Leptosilicia wienkampii                | CBS 143630    | ET     | NR_164067                 | Voglmayr et al. (2019)          |
| Longiapendispora chromolaenae         | MFLUCC 17-1485| HT     | NR_169723                 | Mapook et al. (2020)            |
| Lopadostoma americanum                | LG8           | HT     | KC774568                  | Jaklitsch et al. (2014)         |
| Lopadostoma dryophilum                | LG21          | ET     | KC774570                  | Jaklitsch et al. (2014)         |
| Lopadostoma fagi                       | LF1           | HT     | KC774575                  | Jaklitsch et al. (2014)         |
| Lopadostoma quercicola                | LG27          | HT     | KC774610                  | Jaklitsch et al. (2014)         |
| Lopadostoma turgidum                  | LT2           | ET     | KC774618                  | Jaklitsch and Voglmayr (2012)   |
| Melogramma campylosporum              | MBU           | –      | JF440978                  | Jaklitsch and Voglmayr (2012)   |
| Neophysalospora eucalypti             | CBS 111123    | –      | KP031107                  | Crous et al. (2014)             |
| Neophysalospora eucalypti             | CBS 138864    | –      | KP004462                  | Crous et al. (2014)             |
| Oxydothis metrysoylicola              | MFLUCC 15-0281| HT     | KY206774                  | Konta et al. (2016)             |
| Oxydothis pabnicola                   | MFLUCC 15-0806| HT     | KY206776                  | Konta et al. (2016)             |
| Oxydothis phoenicus                   | MFLUCC 18-0269| HT     | MK088065                  | Hyde et al. (2020)              |
| Phlogicylindrium uniforme             | CBS 131312    | HT     | JQ044426                  | Crous et al. (2011)             |
| Podosordaria tulamei                  | CBS 128.80    | –      | KT281902                  | Senanayake et al. (2015)        |
| Poronia punctata                      | CBS 056.78    | –      | KT281904                  | Wendt et al. (2018)             |
| Pseudomassaria chondropont            | MFLUCC 15-0545| –      | KR092790                  | Senanayake et al. (2015)        |
| Pseudomassaria sepincoliformis        | CBS 129022    | –      | JF440984                  | Jaklitsch and Voglmayr (2012)   |
| Pseudopodosporidium knawiae           | CBS 123529    | HT     | MH863299                  | Crous et al. (2017, submitted directly) |
| Pseudopodosporidium lambertii         | CBS 143169    | HT     | NR_156656                 | Crous et al. (2017)             |
| Pseudotruncatella anzozaensis         | MFLUCC 14-0988| HT     | NR_157489                 | Petra et al. (2018)             |
| Pseudotruncatella bolusanthi          | CBS 145532    | HT     | NR_165575                 | Crous et al. (2019)             |
| Robillarda roystonae                  | CBS 115445    | HT     | NR_154251                 | Liu et al. (2019)               |
| Sarosynson compunctum                 | CBS 359.61    | –      | MH858083                  | Wendt et al. (2018)             |
| Serifidium marginatum                 | CBS 140403    | ET     | NR_156602                 | Liu et al. (2019)               |
| Seynesia eurupiens                    | SMH 1291      | –      | N/A                       | Bhattacharya et al. (2000)      |
| Sordaria fimicola                     | CBS 723.96    | –      | MH862606                  | Vu et al. (2019)                |
| Species            | Strain number | Status | GenBank accession numbers | References                      |
|--------------------|---------------|--------|---------------------------|--------------------------------|
| *Sporocadus rotundatus* | CBS 616.83    | HT     | NR_161091 NG_069584 MH554974 MH554737 | Liu et al. (2019) |
| *Subsessila turbinata* | MFLUCC 15-0831 | HT     | NR_148122 NG_059724 N/A N/A | Lin et al. (2017) |
| *Vialaea insculpta*   | DAOM 240257   | –      | JX139726 JX139726 N/A N/A | Hambleton et al. (2010, submitted directly) |
| *Vialaea mangiferae*  | MFLUCC 12-0808 | HT     | NR_171903 NG_073594 N/A N/A | Senanayake et al. (2021, submitted directly) |
| *Vialaea minutella*   | BRIP 56959    | –      | KC181926 KC181924 N/A N/A | McTaggart et al. (2013) |
| *Xyladictyochaeta lusitanica* | CBS 143502 | – | MH107926 MH107972 N/A MH108053 | Crous et al. (2013) |
| *Xylaria hypoxylon*   | CBS 122620    | ET     | KY610407 KY610495 KY624231 KX271279 | Wendt et al. (2018) |
| *Xylaria obovata*     | MFLUCC 13-0115 | – | KR049088 KR049089 N/A N/A | Wendt et al. (2018) |
| *Xylaria polymorpha*  | MUCL 49884    | –      | KY610408 KT281899 KY624288 KX271280 | Wendt et al. (2018) |

Note. Type specimens are labelled with HT (holotype), ET (epitype) and IT (isotype), T (Type). N/A: not available.
Maximum likelihood (ML) analysis was performed on the CIPRES Science Gateway v.3.3 (http://www.phylo.org/portal2; Miller et al. 2010) using RAxML v.8.2.8 as part of the ‘RAxML-HPC BlackBox’ tool (Stamatakis et al. 2008). All free model parameters were estimated by RAxML with ML estimates of 25 per-site rate categories. GTRGAMMA + I model was chosen for RAxML, based on the result of MrModeltest 2.2. The best-scoring tree was selected with a final likelihood value of –10720.56919.

A Bayesian analysis (BY) was performed using MrBayes v.3.2.2 (Ronquist et al. 2012). The best-fit model was selected with MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala and Yang 1996) were determined by Markov Chain Monte Carlo sampling (MCMC) (Ronquist and Huelsenbeck 2003). Six simultaneous Markov chains were initially run for $30 \times 10^6$ generations and for every $1000^{th}$ generation, a tree was sampled (resulting in 30,000 total trees). The MCMC heated chain was set with a ‘temperature’ value of 0.15. All sampled topologies beneath the asymptote (20%) were discarded. The remaining 24,000 trees were used to calculate the posterior probability (PP) values in the majority rule consensus tree (Liu et al. 2011).

**Results**

**Phylogenetic analyses**

The resulted trees from ML and BY were similar in topology. *Cainiaceae* is a monophyletic group (Fig. 1) with 100%/1.00 (PP/BS) support. *Arecophila* species form two clades. Clade 1 consists of *A. miscanthi* (*≡ Alishanica miscanthi*), *A. clypeata* and *A. australis*, with high statistical support (100%/1.00 PP). In Clade 2, *A. bambusae* (HKUCC 4794) and *Arecophila* sp. (HKUCC 6487) display a close relationship with *Amphibambusa bambusicola*.

**Taxonomy**

*Arecophila* K.D. Hyde, Nova Hedwigia 63(1–2): 82 (1996)

MycoBank No: 27653

≡ *Alishanica* Karun., C.H. Kuo & K.D. Hyde, in Hyde et al., Mycosphere 11(1): 460 (2020)

**Sexual morph.** *Ascomata* immersed, raised, blackened areas on the host surface, a central erumpent, short, cone-shaped or umbilicate papilla, subglobose to lenticular in vertical section. *Clypeus* present or not, comprising host cells and intracellular brown hyphae. *Peridium* comprising several layers of angular cells. *Paraphyses* hypha-like, filamentous, septate, hyaline. *Asci* 8-spored, unitunicate, cylindrical, with an apical ring bluing in Melzer’s reagent or not. *Ascospores* ellipsoidal, 2-celled, constricted at the septum, brown, with longitudinal striations or a verrucose wall and surrounded by a wide mucilaginous sheath (Hyde 1996).

**Asexual morph.** Undetermined.
Figure 1. Phylogenetic tree, based on a combined ITS, LSU, rpb2 and β-tubulin gene dataset. Numbers close to each node represent Maximum Likelihood bootstrap values (≥ 75%) and Bayesian posterior probabilities (≥ 0.95). The hyphen (“–”) means a value lower than 75% (BS) or 0.95 (PP). New taxa are marked in red. Type materials are marked with T after the strains. The tree is rooted to Achaetomium macrosporum (CBS 532.94), Chaetomium elatum (CBS 374.66) and Sordaria fimicola (CBS 723.96).
Figure 1. Continued.
**Arecophila australis** Q.R. Li, J.C. Kang & K.D. Hyde, sp. nov.

Mycobank No: 836166

Fig. 2

**Diagnosis.** *Arecophila australis* differs from similar species by its dimension of ascospores (22.5–29 × 8–11 µm) covered by striations and ascomata with a disc area surrounding the ostioles.

**Holotype.** China, Guizhou Province, Guiyang City, Forest Park of Guiyang (26°32'55"N, 106°45'25"E), on dead culm of *Phragmites australis* (Cav.) Steud., 15 March 2014, Q.R. Li, GZ58 (GZUH0112, holotype, ex-type: GZUCC0112; GACP QR0152, isotype).

**Additional sequences.** ACT: MT741737

**Etymology.** In reference to the host, *Phragmites australis* (Cav.) Steud. *australis*

**Description.** Saprobic on dead culm of gramineous host. **Sexual morph:** Ascomata 420–560 × 290–380 µm (\(\bar{x}= 495 \times 325 \) µm, n = 10), immersed under a clypeus, solitary, slightly raised, blackened, dome-shaped areas, scattered or gregarious, globose to subglobose, with a central, erumpent, cone-shaped papilla in vertical section. Clypeus black, comprising host cells and intracellular brown hyphae. Ostioles papillate, black.

**Peridium** 15–25 µm (\(\bar{x}= 21 \) µm, n = 15) wide, comprising several layers, outer layer brown, thick-walled angular cells, inner layer hyaline. **Paraphyses** 3.3–5 µm (\(\bar{x}= 3.5 \) µm, n = 15) wide, hyaline, unbranched, septate. **Asci** 140–230 × 15.5–24 µm (\(\bar{x}= 183.5 \times 19 \) µm, n = 30), 8-spored, unitunicate, long-cylindrical, short-pedicellate, apically rounded, with a 4–5 × 2.5–3 µm (\(\bar{x}= 4.5 \times 2.7 \) µm, n = 20), trapezoidal, J+, apical ring. **Ascospores** 22.5–29 × 8–11 µm (\(\bar{x}= 25.5 \times 9 \) µm, n = 30), overlapping uniseriate, 2-celled, light brown to brown, equilateral ellipsoidal, constricted at the septum, longitudinal with sulcate striations, along the entire spore length, surrounded by a mucilaginous sheath, lacking germ slits and appendages. **Asexual morph:** undetermined.

**Culture characteristics.** Colonies on PDA, reached 3 cm diam. after one week at 25 °C, white, cottony, flat, low, dense, with slightly wavy margin.

**Known distribution.** China

**Additional material examined.** China, Guizhou Province, Guiyang City, Leigongshan National Nature Reserve (26°21'39"N, 108°9'59"E), on dead culm of an unidentified gramineous plant, 13 June 2015, Q.R. Li, GY67 (GACP QR0124, GZUH 0136; living cultures, GZUCC0124).

**Notes.** *Arecophila australis* resembles *A. serrulata* (Ellis & Martin) K.D. Hyde and *A. calamicola* K.D. Hyde (Hyde 1996). However, *A. serrulata* has white ring surrounding ostioles of ascomata, narrower ascospores (17–26 × 7–9.5 µm vs. 22.5–29 × 8–11 µm), smaller asci and apical ring (3.2 × 2.4 µm vs. 4.5 × 2.7 µm) compared to *A. australis* (Hyde 1996). *Arecophila calamicola* differs from *A. australis* in lacking clypeus, ascospores covered by verrucose ornamentation and surrounding by a mucilaginous sheath attached at the poles. Molecular phylogeny, based on combined ITS, LSU, rpb2 and β-tubulin sequences, shows that *A. australis* clusters as a distinctive clade in *Arecophila* (Clade 1). Based on its distinct morphology and
Figure 2. *Arecophila australis* (holotype) **A** material **B** ascoma on the surface of host **C** section of ascoma **D** peridium **E** paraphyses **F, G** ascus apex with a J+, apical ring (stained in Melzer’s reagent) **H–K** asci with ascospores **L–O** ascospores surrounded by a wide mucilaginous sheath (O stained in India ink). Scale bars: 300 µm (**B**); 50 µm (**C**); 5 µm (**D–O**).
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phylogeny, A. australis is introduced as a new species. Here, we need to explain the name of A. serrulata. Although Index Fungorum (02/07/2022) shows that the current name of A. serrulata is Roussoella serrulata (Ellis & G. Martin) K.D. Hyde & Aptroot, we have not found relevant literature. Hyde (1996) renamed Didymosphaeria serrulota Eltis & G. Martin and Roussoella serrulata as synonyms of A. serrulata (Ellis & G. Martin) K.D. Hyde. Arecophila serrulata was erected with the unitunicate asci with a blue-staining ring (Hyde 1996) which is clearly inconsistent with the morphological features of Roussoella Sacc. Therefore, we still compare with the original description of A. serrulata in this article.

**Arecophila clypeata** Q.R. Li, J.C. Kang & K.D. Hyde, sp. nov.
Mycobank No: 836167

**Fig. 3**

**Diagnosis.** Arecophila clypeata differs from similar species by its ascomata with clypeus and ascospores (18.5–22.5 × 6.5–9 µm).

**Holotype.** China, Yunnan Province, Kunming City, Kunming Botanical Garden (25°8'51"N, 102°44'57"E), on dead culm of gramineous plant, 20 March 2014, Q.R. Li, kib21 (holotype: GZUH0110; isotype: GACP QR0173; ex-type living cultures: GZUCC0110).

**Etymology.** In reference to the clypeus.

**Description.** Saprobic on dead stem of a gramineous. **Sexual morph:** Ascomata 367–448 × 278–363 µm (x̅ = 403 × 323 µm, n = 8), immersed under a black clypeus, solitary, slightly raised, dome-shaped areas, scattered or gregarious, subglobose to globose, with a central, erumpent, cone-shaped papilla, in vertical section. Ostioles papillate on the centre, black. **Peridium** 15–30 µm (x̅ = 25 µm, n = 10) wide, comprising several layers, outer layer brown, thick-walled angular cells, inner layer hyaline. **Paraphyses** 3–5 µm (x̅ = 4 µm, n = 15) wide, hyaline, unbranched, septate. **Asci** 180–245 × 10.5–14.5 µm (x̅ = 215.5 × 12 µm, n = 20), 8-spored, unitunicate, long-cylindrical, short-pedicellate, apically rounded, with a square-shaped, J+, apical ring, 3–4 × 3–4 µm. **Ascospores** 18.5–22.5 × 6.5–9 µm (x̅ = 20.5 × 7.5 µm, n = 30), overlapping uniseriate, 2-celled, light brown to brown, equilateral ellipsoidal, constricted at the septum, longitudinal, sulcate along the entire spore length, faint, surrounded by a mucilaginous sheath, lacking germ slits and appendages. **Asexual morph:** undetermined.

**Culture characteristics.** Colonies on PDA, reached 3 cm diam. after one week at 25 °C, white, cottony, flat, low, dense, with slightly wavy margin; fructifications were not observed in culture.

**Known distribution.** China

**Additional material examined.** China, Guizhou Province, Buyi and Miao Autonomous Prefecture in southern Guizhou Province, Maolan National Nature Reserve (25°17'17"N, 107°59'1"E), on dead culm of an unidentified gramineous plant, 12 June 2015, Q.R. Li, GZ120 (GACP QR0129; GZUH0127; living cultures, GZUCC0127).

**Additional sequences.** ACT: MT741737
Figure 3. *Arecophila clypeata* (holotype) A material B ascomata on the surface of host C, D section of ascomata E peridium F, G ascus apex with a J+, apical ring (stained in Melzer’s reagent) H–K asci with ascospores L–O ascospores. Scale bars: 500 µm (B, C); 100 µm (D); 10 µm (E, H–K); 5 µm (F, G, L–O).
Notes. *Arecophila clypeata* has long and weakly striate ascospores similar to *A. coronata* (Rehm) Umali & K.D. Hyde, *A. serrulata* (Ellis & G. Martin) K.D. Hyde and *A. bambusae* (Hyde 1996; Umali et al. 1999). However, *A. coronata* does not have a prominent clypeus and has longer and fusiform ascospores. *Arecophila clypeata* differs from *A. serrulata* by the ascomata without a central papilla surrounded by a circle of white tissue, further in having ascospores with wide sheaths (Hyde 1996). *Arecophila clypeata* is similar to *A. bambusae* which, however, has narrower ascospores (19–22.5 × 5.5–7 µm) covered by the strong striations and has ascomata without a central papilla surrounded by a black corolla protuberance (Umali et al. 1999).

*Arecophila gulubiicola* K.D. Hyde, Nova Hedwigia 63(1–2): 91 (1996)

*Mycobank No: 416041*

Fig. 4

Description. Saprobic on dead trunk of *Gulubia costata* (Becc.) Becc. Sexual morph: Ascomata 290–400 × 140–190 µm (x̄ = 336 × 167 µm, n = 8), immersed under a clypeus, solitary or clustered, in vertical section, lenticular, with a central ostiole. Clypeus raised, oval, blackened areas on the host surface, dome-shaped, well-developed and black. Peridium 25–35 µm wide, dense, compressed layers of brown-walled, angular cells, tightly adhered to the host tissues. Paraphyses 2–2.5 µm wide, filamentous, hyaline, septate, branched, tapering distally. Asci 107–145 × 11–13.5 µm (x̄ = 114.3 × 12.4 µm, n = 15), 8-spored, unitunicate, cylindrical, short-pedicellate, apically rounded, wedge-shaped, J+, subapical ring, 3–4 × 1–2 µm (x̄ = 3.5 × 1.5 µm, n = 15). Ascospores 14.5–18.5 × 6–9 µm (x̄ = 17.4 × 6.5 µm, n = 25), overlapping uniseriate, ellipsoidal, brown, 2-celled, septate at the centre, constricted at the septum, longitudinal, sulcate striations along the entire spore length, surrounded by a mucilaginous sheath. Asexual morph: Undetermined.

Material examined. Papua New Guinea, Central Province, 08°30’00"N, 147°24’35"E, on dead trunk of *G. costate* (Becc.) Becc. (*Arecaceae*), May 1992, K.D. Hyde, (BRIP 23002a, holotype).

Notes. *Arecophila gulubiicola* has deeply immersed, subglobose to lenticular ascomata with a small or lacking clypeus, cylindrical, short-pedicellate asci with a wedge-shaped, conical, apical ring and ellipsoidal, brown ascospores with wall striations and surrounded by a mucilaginous sheath (Hyde 1996). *Alishanica* has been introduced as a monospecific genus with the type species *Al. miscanthi* Karun. et al. on dead sheaths of *Miscanthus sinensis* (Poaceae) from Taiwan (Hyde et al. 2020). We re-examined both *A. gulubiicola* and *Al. miscanthi* herbarium specimens and observed that they are congeneric. *Alishanica miscanthi* has characters that immersed ascoma under a clypeus, unitunicate, cylindrical asci with a J+ apical ring and brown, 2-celled ascospores with longitudinal wall striations and a mucilaginous sheath which are consistent with the generic characteristics of *Arecophila*. The phylogeny of *Al. miscanthi* was mainly considered by the *A. bambusae* (HKUCC 4794) sequences (Hyde et al.
However, HKUCC 4794 is not the type material of *Arecophila* and cannot be used to represent *Arecophila*. In our phylogeny, HKUCC 4794 forms a distinct clade (Fig. 1; Clade 2) from the *Arecophila* representing the clade. Based on morphology and

**Figure 4.** *Arecophila gulubiicola* (BRIP 23002a, holotype) *A, B* herbarium material with label *C* ascomata on the host *D, E* sections of ascomata *F* paraphyses *G–J* asci *K* peridium *L, M* wedge-shaped, J+ apical ring bluing in Melzer’s reagent *N–Q* ascospores. Scale bars: 50 µm (*D, E*); 5 µm (*F–Q*).
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Phylogeny, we synonymise *Alishanica* under *Arecophila* and *Al. miscanthi* is accepted as an *Arecophila* species. Furthermore, *A. bambusae* needs to be recollected and provided with the phylogenetic affinity in future studies.

*Arecophila miscanthi* (Karun., C.H. Kuo & K.D. Hyde) Q.R Li & J.C. Kang, comb. nov.

MycoBank No: 839706

≡ *Alishanica miscanthi* Karun., C.H. Kuo & K.D. Hyde [as ‘miscanthii’], in Hyde et al., Mycosphere 11(1): 461 (2020)

**Description** (MFLU 19-2333). Saprobic on dead sheaths of *Miscanthus sinensis* (Poaceae). **Sexual morph:** Ascomata 272–277 × 283–296 µm (ˉx = 275 × 291.5 µm, n = 8), immersed beneath blackened aggregated clypeus of the surface of dead sheath, loosely aggregated or rarely solitary; dark brown to black, globose to subglobose, slightly depressed, uniloculate. Ostiole 92–110 µm long, 52–56 µm diameter (ˉx = 101 × 54 µm, n = 5), centrally erumpent, with periphyses, surrounded by distinct shiny black flanges, the tissue spreading down along the papilla. Peridium 51–60 µm wide, comprising 4–5 cell layers of thin-walled, brown cells of *textura angularis*, inwardly lighter. Paraphyses filamentous, distinctly septate, embedded in a hyaline gelatinous matrix. Asci 147–189 × 10–13 µm (ˉx = 167 × 11 µm, n = 30), 8-spored, unitunicate, cylindrical, short pedicellate, slightly truncate at the apex, with a wedge-shaped J+, subapical ring, 3.5–4 µm broad, 2–2.5 µm high. Ascospores 20–24 × 6–8 µm (ˉx = 22 × 7 µm, n = 40), overlapping, uniseriate, ellipsoid, slightly tapering at the ends, equally 2-celled and guttulate at both cells, constricted at the septum, brown with striations, surrounded by a thick, hyaline mucilaginous sheath, subglobose, parallel to the margin of the spore.

**Asexual morph:** Undetermined.

**Material examined.** China, Taiwan, Chiayi Province, Ali Mountain, Kwang Hwa, on dead sheaths of *Miscanthus sinensis* (Poaceae), 5 May 2018, A. Karunarathna, AKTW 44 (MFLU 19-2333, holotype)

**Additional material.** China, Yunnan Province, Kunming City, Kunming Botanical Garden (25°8’45”N, 102°44’59”E), on dead culm of monocotyledon, 20 March 2014, Q.R. Li, GZ43 (GZUH0122, GACP QR0201; living cultures, GZUCC0122).

**Note.** The characteristics of the holotype specimen *Arecophila miscanthi* (≡ *Alishanica miscanthi*) were revised, re-measured and described. *Alishanica miscanthi* is similar to *A. muroiana* and *A. serrulata* (Wang et al. 2004, Hyde et al. 2020). However, no clypeus was observed for *A. muroiana*. *Arecophila serrulata* has larger ascomata (480–560 × 280–320 µm) with a central papilla surrounded by a circle of white tissue (Hyde 1996) which differs from those of *A. miscanthi*. One new collection (GZUH0122, Fig. 5) shows the same traits of *A. miscanthi* (MFLU 19-2333) in having immersed ascomata with clypeus, a wedge-shaped J+, ascus subapical ring, same dimensions of ascospores and here we provide it as a new geographical record from China.
Figure 5. *Arecophila miscanthi* (GZUH0122) **A, B** ascomata on the surface of host **C** paraphyses and asci **D** section of ascoma **E** peridium **F, G** apical rings **H–K** asci with ascospores **M–P** ascospores. Scale bars: 50 µm (**C, D**); 10 µm (**E, F–K**); 5 µm (**L–P**).
Table 2. Synopsis of the species of *Arecophila*.

| Species          | Host                      | Clypeus   | Ascomata                        | Asci                        | Ascal ring                           | Ascospores                          | Distribution              |
|------------------|---------------------------|-----------|---------------------------------|-----------------------------|--------------------------------------|--------------------------------------|---------------------------|
| *A. australis*    | *Phragmites australis*    | Present   | 420–560 × 290–380 µm, globose to subglobose | 140–230 × 15.5–24 µm       | 4–5 × 2.5–3 µm, trapezoidal, J+      | 22.5–29 × 8–11 µm, wall striate, mucilaginous sheath | China (Guizhou)          |
| *A. bambusae*    | *Bambusa sp.*             | Absent    | 500–560 × 294–350 µm, globose to subglobose | 132.5–140 × 7.5–8 µm       | 2.5–3 µm in diam., ca. 2.5 µm high, wedge-shaped, J+ | 19–22.5 × 5.5–7 µm, slightly tapering at the ends, wall striate, mucilaginous sheath | Hong Kong               |
| *A. caamicola*   | *Calamus sp.*             | Absent    | 520 × 390 µm, subglobose        | 160–190 × 14–20 µm         | 4–4.8 µm diam., 3.2–4 µm high, wedge-shaped, J+ | 24–33 × 5.5–9 µm, wall striate, verrucose, mucilaginous sheath | Brunei, Indonesia        |
| *A. chamaeropis* | *Chamaerops humilis*      | Minute    | 400–700 × 300–400 µm, subglobose | 150–190 × 9–10 µm          | 3.5–4.5 diam., 1.5–2 µm high, wedge-shaped, J+ | 15–23 × 5.5–7 µm, wall striate, covered by pronounced verrucose ornamentation, mucilaginous sheath | Spain                     |
| *A. conranata*   | *Gigantochloa scribneriana, Bambusa sp.* | Present | 90–100 × 42–105 µm, subglobose or ellipsoidal | 132.5–157.5 × 7.5–9 µm      | 3.5–4 µm in diam., 2–2.5 µm high, wedge-shaped, J+, with a faint canal leading to the apex. | 29–31 × 5–5.5 µm, wall faint striate, mucilaginous sheath | Philippines, Hong Kong |
| *A. clypeata*    | A unknown graminous plant | Present   | 367–448 × 278–363 µm, subglobose to globose | 180–245 × 10.5–14.5 µm    | 3–4 × 3–4 µm, square-shaped, J+      | 18.5–22.5 × 6.5–9 µm, wall striate, mucilaginous sheath | China (Guizhou)          |
| *A. deutziae*    | *Deutzia stamineae*       | Absent    | 400–600 µm diam., globose       | 180–240 × 16–19 µm         | 3.5–4.5 µm diam., 1.5–2 µm high, wedge-shaped, J+ | 26–32 × 11–13 µm, wall striate | India                   |
| *A. eugeissonae* | *Eugeissona tristis*      | Absent    | 460–520 × 180–260 µm, Subglobose or ellipsoidal | 175–220 × 11–16.5 µm      | 3–4 µm diam., 1.5–2.0 µm high, discoid, J+ | 25–40 × 6.5–9 µm, wall weakly striate, verrucose, mucilaginous sheath | Malaysia                |
| *A. foveata*     | *Nolinae sp.*             | Present   | 300–400 × 400–500 µm, globose or ovoid | 130–150 × 14–15 µm         | 3–4 µm wide, 4–5 µm high, tubular, J+ | 16–20 × 8–10 µm, wall striate, foveate, surface aspect of numerous warts | USA                      |
| *A. gubucicola*  | *Gulubia costate*         | Present   | 290–400 × 140–190 µm, subglobose or lenticular | 107–145 × 11–13.5 µm      | 3.2–4 µm diam., 2.4–3.2 µm high, cylindrical, J+ | 14.5–18.5 × 5–6–9 µm with a minutely verrucose wall, mucilaginous sheath | Papua New Guinea        |
| *A. miscambhi*   | *Miscanthus sinensis*     | Present   | 283–296 × 272–277 µm, globose to subglobose | 147–189 × 10–13 µm        | 3.5–4 µm broad, 2–2.5 µm high, wedge-shaped, J+ | 20–24 × 6–8 µm, wall striate, mucilaginous sheath | China (Taiwan, Yunnan)   |
| *A. muroiana*    | *Phyllostachys bambusoides* | Absent | 350–460 × 320–400 µm, globose     | 125–165 × 10–12 µm        | 3.5–4 µm diam., 2–2.5 µm high, wedge-shaped, J+ | 20–25 × 6–7.5 µm, wall finely striate, mucilaginous sheath | Japan                    |
| Species          | Host                          | Clypeus | Ascomata                          | Asci                           | Ascal ring                          | Ascospores                      | Distribution                  |
|------------------|-------------------------------|---------|-----------------------------------|--------------------------------|-------------------------------------|---------------------------------|-------------------------------|
| A. notabilis     | Calamus, Bamboo               | Present | 400 × 360 µm, subglobose          | 180–220 × 11–14 µm             | 4–4.45 µm diam., 3–4.5 µm high, wedge-shaped, J+ | 20–26 × 6–8 µm, wall striate, finely verrucose, mucilaginous sheath | Brunei, Hong Kong, Indonesia   |
| A. nypae         | Nypa fruticans                | Absent  | 400–500 µm diam., subglobose      | 140–205 × 11–13 µm             | 4.5 µm diam., 2.5–4 µm high, wedge-shaped, J+ | 19–26 × 7–8 µm, wall striate, mucilaginous sheath | Malaysia                      |
| A. saccharicola  | Sacchari officinarum          | Absent  | 420–525 × 350–420 µm high         | 140–16 × 7–10 µm               | Not blued by Melzer's reagent       | 20–24 × 6–8 µm, wall smooth or striated | Jamaica                       |
| A. serrulata     | Korthalsia sp., Sabal sp., Serenoa sp. | Present | 480–560 × 280–320 µm, conical with flattened base | 110–112 × 10–12 µm, 3.2 µm diam., 2.4 µm high, wedge-shaped, J+ | 17–26 × 7–9.5 µm, wall striate, mucilaginous sheath | Brunei, USA, Florida            |
Discussion

Arecophila shares similar morphology to Atrotorquata, Cainia and Seynesia in having immersed ascomata and 2-celled ascospores (Hyde 1996). Arecophila, Atrotorquata, Cainia and Seynesia are accepted in Cainiaceae with newly-introduced genera, such as Amphibambusa and Longiappendispora (Mapook et al. 2020). Cainia has similar characteristics to Arecophila in its occurrence on monocotyledons, having asci with J+, apical rings and brown 2-celled ascospores (Kohlmeyer and Volkmann-Kohlmeyer 1993). The ascospores of Cainia are provided with several longitudinal germ slits and differ from those of Arecophila, where the ascospores are provided with ridges or a verrucose wall and lack germ slits. Seynesia produces ascospores that are smooth-walled and surrounded by mucilaginous sheaths that are drawn out at the poles with germ slits, which differ from Arecophila (Hyde 1995). The phylogenetic tree (Fig. 1) displays that Arecophila miscanthi (≡ Alishanica miscanthi) clusters in the Arecophila group with high support values (100%/1.00 PP).

Longiappendispora possesses ascospores with longitudinal striations and bristle-like polar appendages at both ends, without a gelatinous sheath, which differentiates it from other genera in Cainiaceae. Ascospores of Atrotorquata are provided with several longitudinal germ slits and differ from those of Arecophila (Kohlmeyer and Volkmann-Kohlmeyer 1993). At present, 16 Arecophila species have been described and a summary of each species is given in the Table 2.

The combined ITS, LSU, rpb2 and ß-tubulin phylogeny (Fig. 1) showed two clades of Arecophila as Clade 1 and Clade 2. The Arecophila differs from Amphibambusa and Cainia (see above). The sequence from the holotype of Atrotorquata spartii is noticeably clustered with Conioceia spp. in Conioceiae (Fig. 1). However, Atrotorquata spartii showed a close affinity with Cainia spp. in Cainiaceae, based on analysis of the combined LSU and ITS sequence alignment in Senanayake et al. (2015). Atrotorquata has similar characteristics to Arecophila and other genera of Cainiaceae (Hyde 1996). Hence, there should be more evidence to reassess Atrotorquata in the future. The unitunicate asci with a J+ apical ring in Melzer’s regent and brown ascospores covered with longitudinal wall striations, without germ slits can clearly distinguish Arecophila from its similar genera. In addition, a table including synopsis of the species of Arecophila is provided.

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