Taxonomy, Phylogenetic and Ancestral Area Reconstruction in *Phyllachora*, with Four Novel Species from Northwestern China

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Abstract: The members of *Phyllachora* are biotrophic, obligate plant parasitic fungi featuring a high degree of host specificity. This genus also features a high degree of species richness and worldwide distribution. In this study, four species occurring on leaf and stem of two different species of grass were collected from Shanxi and Shaanxi Provinces, China. Based on morphological analysis, multigene (combined data set of LSU, SSU, and ITS) phylogenetic analyses (maximum likelihood and Bayesian analysis), and host relationship, we introduce herein four new taxa of *Phyllachora*. Ancestral area reconstruction analysis showed that the ancestral area of *Phyllachora* occurred in Latin America about 194 Mya. Novel taxa are compared with the related *Phyllachora* species. Detailed descriptions, illustrations, and notes are provided for each species.

Keywords: Ancestral reconstruction; Multigene phylogeny; Morphology; four new taxa; *Phyllachora*

1. Introduction

Phyllachorales is an ascomycetous order (in Sordariomycetes) introduced by Barr [1]. This order comprises biotrophic, obligate plant parasitic fungi that infect mostly plant leaves and stems [2–4]. Species of Phyllachorales are mainly distributed in tropical and subtropical regions [5–8]. Members of Phyllachorales present with shiny black stromata, leading to the common name ‘tropical tar spot fungi’. Most of the members of Phyllachorales are parasitic on angiosperms with a few notable exceptions including the lichenicolous *Lichenochora* species, the marine algicolous genus *Phycomelaina*, as well as ferns and gymnosperms [8–11].

Currently, Phyllachorales has four families, including Phaeochoraceae, Phaeochorel-laceae, Phyllachoraceae, and Telimenaceae [12,13]. They are morphologically characterized by black stromata of various shapes in the host plant; having paraphyses; unitunicate ascis cylindrical to clavate in shape, with an inconspicuous apical ring, usually 8-spored; and asceptate ascospores, which in most species are hyaline and 1-celled, appearing as brown in a few species (e.g., *Phyllachora stenostoma*) [1,4,6,8,14]. The asexual morph of Phyllachorales has been reported as a coelomycetous morph [15]. Large-scale phylogenetic studies comprising many representative species have confirmed the position of Phyllachorales in the subclass Sordariomycetidae with high support (100% MLBP) as well as the monophyly of...
of the order [4,8,16]. Mardones et al. [8] used three morphological characteristics and one ecological characteristic to reconstruct the ancestral state of genera in Phyllachorales based on the Likelihood Ancestral States method, reasoning that these characteristics had evolved independently numerous times. The ancestral state of members of Phyllachorales were monocotyledonous host plants with immersed perithecia, which was lost in the family Phaeochoraceae and evolved into erumpent or superficial perithecia in some species of Phyllachoraceae. The presence of clypeus as a morphological characteristic was lost only once in Phaeochoraceae. Therefore, it is thought that the presence of clypeus in these fungi is an evolutionarily stable characteristic. The ancestor of the Phyllachorales species had a black stroma, and the presence of bright black stromata may have evolved at least twice.

The family Phyllachoraceae was introduced by Theissen and Sydow [17] with *Phyllachora* as the type genus [3,18]. It is the largest family in Phyllachorales and currently comprises 54 genera [13]. Members of the family are characterized by forming leaf spots on the host that are abundant but scattered, raised, mostly rounded to oblong or elongated, sometimes parallel with leaf venation, surrounded by a light-brown necrotic region; lacking periphyses; having numerous paraphyses, branched or unbranched; 8-spored asci, persistent, cylindrical to fusiform, often present with an apical ring; ascospores fusiform to narrowly oval, hyaline, often with a mucilaginous sheath [4]. The type genus, *Phyllachora*, was introduced based on *P. agrostis*, which is a single species on the herbarium label in Fuckels exsiccate series ‘Fungi Rhenani’ [5]. Phyllachoraceae is similar to Phaeochoraceae, but Phaeochoraceae species are characterized by 6-8-spored asci, usually without apical structure, yellow to olivaceous ascospores or in various shades of brown, thick-walled; conversely, Phyllachoraceae species are characterized by 8-spored asci, an often-present an apical ring, usually hyaline ascospores, rarely pale brown, thin and smooth-walled [4,8]. These morphological characteristics can be used to distinguish the two families, and they form two independent branches in the phylogenetic tree [8].

*Phyllachora* is the type genus of Phyllachoraceae. Clements [19] designated the lectotype as *Phyllachora graminis*. Currently, *Phyllachora* is the largest genus within Phyllachoraceae, and about 1513 epithets are listed in the Index Fungorum (Index Fungorum 2022; accession date: 28.03.2022). Nevertheless, only 1382 species are accepted in the Species Fungorum (accession date: 28.03.2022). Species of the genus are morphologically characterized by clypeate pseudostroma in leaf tissues; generalized infection of the entire section of the mesophyll forming leaf spots on the host, mostly rounded to oblong or elongated, surrounded by a light-brown necrotic region; perithecium globose; numerous paraphyses, branched, slightly longer than asci; asci 8-spored, persistent, cylindrical to fusiform, short pedicellate, an apical ring often present; and ascospores 1–3 seriate, fusiform to narrowly oval, hyaline, sometimes with a gelatinous sheath [4,18,20]. Some members of the genus can inflict crop diseases, leading to yield loss. *Phyllachora maydis* is an example occurring in the United States, which can seriously impact quality and corn yield [21–24]. Owing to its biotrophic habit and high degree of host specificity, most *Phyllachora* species are given names based on host association and coevolution with the host [5,8,20,25]. *Phyllachora* species cannot grow on agar media since they are biotrophic [8]. *Phyllachora* species have been reported as pathogenic species on more than 1000 plant species (belonging to 121 families, including Cyperaceae, Fabaceae, Lauraceae, Moraceae, Myrtaceae, Poaceae, Proteaceae, and Rosaceae), and they are commonly found with Poaceae [20,26,27].

In this study, several specimens with tar spot diseases were collected. Based on polyphasic approaches (e.g., morphological analyses, information of host plant, and phylogenetic analyses), four novel species of *Phyllachora* are introduced herein. Based on paleontological evidence and paleoclimate records, we also reconstructed the ancestral area of *Phyllachora*. The analysis was restricted to members of *Phyllachora*, considering the history of their biogeographic diversity and dispersal route as well as estimating the divergence time and ancestral location of this genus.
2. Materials and Methods

2.1. Collecting, Morphological Study, and Depositing Specimens

Phyllachora-like fungi were collected from living leaves of *Cenchrus flaccidus* (Poaceae) and *Chloris virgata* (Poaceae) during field surveys in 2019 in Shanxi and Shaanxi Provinces, China. Specimens were taken to the laboratory in paper envelopes. Specimens were processed and examined with microscopes, and photos of ascomata and host were taken using a compound stereomicroscope (KEYENCE CORPORATION V.1.10 with camera VH-Z20R) following Wu et al. [28]. Hand sections were made under a stereomicroscope (OLYMPUS SZ61) and mounted in water and blue cotton, and photomicrographs of fungal structures were taken with a compound microscope (Nikon ECLIPSE 80i).

Images used for figures were processed with Adobe Photoshop CC v. 2015.5.0 software (Adobe Systems, San Jose, CA, USA).

Holotype collections were deposited at the herbarium of IFRD (International Fungal Research & Development Centre; Institute of Highland Forest Science, Chinese Academy of Forestry, Kunming, China). Registration numbers for new species were obtained in MycoBank Database (https://www.mycobank.org/, accession date: 10 April 2022).

For our specimens, no culture was obtained by multiple single-spore isolation or tissue isolation.

2.2. DNA Isolation, Amplification, and Sequencing

In accordance with the manufacturer’s instructions, genomic DNA was extracted from ascomata at room temperature using the Forensic DNA Kit (OMEGA, New York, NY, USA). The primers LR0R and LR5 were used to amplify the large subunit (LSU) rDNA [29]. The internal transcribed spacer (ITS) rDNA was amplified and sequenced with the primers ITS5 and ITS4 [30]. The partial small subunit (SSU) rDNA was amplified using primers NS1 and NS4 [30]. PCR reactions were in accordance with instructions from Golden Mix, Beijing TsingKe Biotech Co. Ltd, Beijing, China: initial denaturation at 98 °C for 2 min, followed by 30 cycles of 98 °C denaturation for 10 s, 56 °C annealing for 10 s and 72 °C extensions for 10 s (ITS and SSU) or 20 s (LSU), and a final extension at 72 °C for 1 min. All PCR products were sequenced by Biomed (Beijing Biomed Gen Technology Co., Ltd., Beijing, China). PCR products were sequenced by Biomed using the same primers as before.

2.3. Sequence Alignment and Phylogenetic Analyses

BioEdit version 7.0.5.3 [31] was used to re-assemble the sequences generated from forward and reverse primers for obtaining integrated sequences. Sequences of Phyllachoraceae species were downloaded from GenBank (Table 1) following the relevant publications [8,20,32,33]. All sequences were adjusted manually with Bioedit 7.0.5.3 [31] and aligned using the default setting of MAFFT version 7 online (https://mafft.cbrc.jp/alignment/server/, accession date: 10 April 2022).

Maximum Likelihood (ML) analysis using the aligned sequences as input was conducted with the help of RAxNLGUI v. 2.0 [35]. *Telimena bicincta* (MM-108) and *T. bicincta* (MM-133) were selected as an outgroup. One thousand nonparametric bootstrap iterations were employed with the “ML + rapid bootstrap” tools and “GTRGAMMA” arithmetic.

For Bayesian analysis, MrModeltest 2.3 [36] was used to estimate the best-fitting model for the combined LSU, SSU, and ITS loci, and model GTR+G was the best fit. In MrBayes v.3.2 [37], six simultaneous Markov chains were run for 2,000,000 generations; trees were sampled and printed every 100 generations. The first 5000 trees were submitted to the burn-in phase and discarded, while the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree [38–41].
Table 1. List of source and GenBank accession numbers used in this study. Sequences generated in this study are written in blue. Type collections are marked with "T".

| Species                  | Location                          | Source | Host Family | GenBank Accession Numbers | Reference |
|--------------------------|-----------------------------------|--------|-------------|---------------------------|-----------|
|                          |                                   |        |             | LSU | SSU  | ITS      |                             |
| **Ascovaginospora stellipala**<sup>T</sup> | North America (northern Wisconsin) | P5-13A  | Cyperaceae   | U85088 | U85087 | -        | [33]                          |
| **Camarotella costaricensis** | Latin America (Panama)          | MM-21  | Arecaceae    | KX430490 | KX451851 | KX451900 | [8]                           |
| **Camarotella costaricensis** | Latin America (Panama)          | MM-149  | Arecaceae    | KX430484 | KX451863 | KX451913 | [8]                           |
| **Camarotella sp.** | Latin America (Panama)          | MM-27  | Arecaceae    | KX430492 | KX451852 | KX451901 | [8]                           |
| **Coccodiella calathae**<sup>T</sup> | North America (Venezuela)  | CMU78543 | Melastomataceae | -        | U78543  | -        | [8]                           |
| **Coccodiella miconiicola** | Latin America (Panama)          | ppMP1342 | Melastomataceae | KX430506 | KX451871 | MF460365 | [8]                           |
| **Coccodiella miconiicola** | Latin America (Panama)          | TH-571  | Melastomataceae | KX430312 | KX451880 | -        | [8]                           |
| **Coccodiella miconiicola** | Latin America (Panama)          | CBMAP-H290A | Melastomataceae | MF460373 | MF460379 | MF460368 | [32]                          |
| **Coccodiella miconiicola** | Latin America (Ecuador)         | SO-15   | Melastomataceae | MF460374 | MF460380 | MF460369 | [32]                          |
| **Coccodiella toledoi** | Latin America (Ecuador)         | MM-165  | Melastomataceae | KX430488 | KX451865 | KX451917 | [8]                           |
| **Neophyllachora cerradensis** | Latin America (Brazil)     | UB21823 | Myrtaceae    | -        | -      | KC683470 | [18]                          |
| **Neophyllachora cerradensis**<sup>T</sup> | Latin America (Brazil)     | UB21900 | Myrtaceae    | -        | -      | KC683471 | [18]                          |
| **Neophyllachora myrciae** | Latin America (Brazil)          | UB21292 | Myrtaceae    | -        | -      | KC683463 | [18]                          |
| **Neophyllachora myrciae** | Latin America (Brazil)          | UB22192 | Myrtaceae    | -        | -      | KC683476 | [18]                          |
| **Neophyllachora myrciariae**<sup>T</sup> | Latin America (Brazil)         | UB21781 | Myrtaceae    | -        | -      | KC683469 | [18]                          |
| **Neophyllachora subcircinans** | Latin America (Brazil)     | UB09748 | Myrtaceae    | -        | -      | KC683441 | [18]                          |
| **Neophyllachora subcircinans** | Latin America (Brazil), South America (Paraguay) | UB21347 | Myrtaceae    | -        | -      | KC683466 | [18]                          |
| **Neophyllachora subcircinans** | Latin America (Brazil), South America (Paraguay) | UB21747 | Myrtaceae    | -        | KC902622 | KC683467 | [18]                          |
| **Neophyllachora truncatispora** | Latin America (Brazil)  | UB14083 | Myrtaceae    | -        | KC902614 | KC683448 | [18]                          |
| **Phyllachora arthraxonis** | East Asia (China)               | MHYAU:072 | Poaceae    | MG269803 | -      | MG269749 | [20]                          |
| **Phyllachora arundinellae** | East Asia (China)               | MHYAU:108 | Poaceae    | MG269815 | -      | MG269761 | [20]                          |
| **Phyllachora capillipediicola** | East Asia (China)               | MHYAU:20089 | Poaceae | MG356698 | -      | KY498084 | [32]                          |
| **Phyllachora capillipediicola** | East Asia (China)               | MHYAU:20090 | Poaceae | MG356699 | -      | KY498115 | [20]                          |
| **Phyllachora chloridis**<sup>T</sup> | Southeast Asia (Thailand)   | MFLU 15-0173 | Poaceae | MF197499 | MF197505 | KY94026 | [33]                          |
| **Phyllachora chloridis**<sup>T</sup> | Southeast Asia (Thailand)   | MFLU 16-2980 | Poaceae | MF197500 | MF197506 | KY94027 | [33]                          |
| **Phyllachora chloridis-virgatae** | East Asia (China)               | MHYAU:20136 | Poaceae | MG356685 | -      | KY498122 | [32]                          |
| **Phyllachora chloridis-virgatae** | East Asia (China)               | MHYAU:20058 | Poaceae | MG356683 | -      | KY498102 | [32]                          |
| Species | Location | Source | Host Family | GenBank Accession Numbers | Reference |
|---------|----------|--------|-------------|---------------------------|-----------|
| Phyllachora chloridis-virgatae | East Asia (China) | MHYAU 20137 | Poaceae | LSU: MG356686, SSU: - | KY498092 [32] |
| Phyllachora chrysopogonica | Southeast Asia (Thailand) | MFLU 16-2096 | Poaceae | LSU: MF372146, SSU: - | MF372145 [20] |
| Phyllachora cynodonticola | Southeast Asia (Thailand) | MFLU 16-2977 | Poaceae | LSU: MF197501, SSU: MF197507, ITS: KY594024 [33] |
| Phyllachora cynodonticola | Southeast Asia (Thailand) | MFLU 16-2978 | Poaceae | LSU: MF197502, SSU: MF197508, ITS: KY594025 [33] |
| Phyllachora cynodontis | East Asia (China) | MHYAU 20132 | Poaceae | LSU: KY498080, SSU: - | KY471328 [32] |
| Phyllachora cynodontis | East Asia (China) | MHYAU:20043 | Poaceae | LSU: KY498081, SSU: - | KY471329 [20] |
| Phyllachora cynodontis | East Asia (China) | MHYAU 20131 | Poaceae | LSU: KY498079, SSU: - | KY471327 [32] |
| Phyllachora cynodonticola | Southeast Asia (Thailand) | MFLU 16-2977 | Poaceae | LSU: MF197502, SSU: MF197508, ITS: KY594025 [33] |
| Phyllachora dendrocalami-hamiltoniicola | East Asia (China) | MHYAU 221 | Poaceae | LSU: MK614118, SSU: - | MK614102 [32] |
| Phyllachora dendrocalami-membranacei | East Asia (China) | MHYAU 222 | Poaceae | LSU: MK614119, SSU: - | MK614103 [32] |
| Phyllachora flaccidudis | East Asia (China) | IFRD9445 | Poaceae | LSU: ON072101, SSU: ON072097, ITS: ON075524 | This study |
| Phyllachora graminis | East Asia (China) | MHYAU 20137 | Poaceae | LSU: MG356686, SSU: - | KY498092 [32] |
| Phyllachora graminis | East Asia (China) | MHYAU:20043 | Poaceae | LSU: KY498081, SSU: - | KY471329 [20] |
| Phyllachora heterocladae | East Asia (China) | MFLU 18-1221 | Poaceae | LSU: MK296472, SSU: MK296468, ITS: MK305902 [20] |
| Phyllachora imperatae | East Asia (China) | MHYAU:014 | Poaceae | LSU: MG269800, SSU: - | MG269746 [20] |
| Phyllachora indosasae | East Asia (China) | MHYAU 125 | Poaceae | LSU: MG195662, SSU: - | MG195637 [32] |
| Phyllachora isachnicola | East Asia (China) | MHYAU:179 | Poaceae | LSU: MH018563, SSU: - | MH018561 [20] |
| Phyllachora jaensis | East Asia (China) | IFRD9448 | Poaceae | LSU: ON075440, SSU: ON072100, ITS: ON075527 | This study |
| Phyllachora keralensis | East Asia (China) | MHYAU 20082 | Poaceae | LSU: MG269792, SSU: - | KY498106 [20] |
| Phyllachora maqdis | North America (USA) | BPI 893231 | Poaceae | LSU: - , SSU: KU184459 | KU184459 [33] |
| Phyllachora maysis | North America (Wisconsin) | BPI 910560 | Poaceae | LSU: - , SSU: - | MG881846 [20] |
| Phyllachora miscanthi | East Asia (China) | MHYAU:167 | Poaceae | LSU: MG195669, SSU: - | MG195644 [20] |
| Phyllachora miscanthi | East Asia (China) | MHYAU:157 | Poaceae | LSU: MG195668, SSU: - | MG195643 [20] |
| Phyllachora panicicola | East Asia (China) | MFLU 16-2979 | Poaceae | LSU: MF197503, SSU: MF197504, ITS: KY594028 | [33] |
| Phyllachora pogonatheri | East Asia (China) | MHYAU:071 | Poaceae | LSU: MG269802, SSU: - | MG269748 [20] |
| Phyllachora pogonatheri | East Asia (China) | MHYAU:070 | Poaceae | LSU: MG269801, SSU: - | MG269747 [20] |
| Phyllachora pomigena | unknown | CBS 194.33 | Vochysiaceae | LSU: MH866861, SSU: - | MH855410 [20] |
| Phyllachora pomigena | unknown | CBS 193.33 | Vochysiaceae | LSU: MH866860, SSU: - | MH855409 [20] |
| Phyllachora quaecae | unknown | UB 21159 | Vochysiaceae | LSU: - , SSU: - | KL682781 [33] |
| Phyllachora quaecae | unknown | UB 21771 | Vochysiaceae | LSU: - , SSU: - | KL682780 [33] |
| Phyllachora sandiensis | East Asia (China) | IFRD9446 | Poaceae | LSU: ON075528, SSU: ON072098, ITS: ON075525 | This study |
| Phyllachora sinobambusae | East Asia (China) | MHYAU 085 | Poaceae | LSU: MG195655, SSU: - | MG195630 [32] |
| Phyllachora sphaerocaryi | East Asia (China) | MHYAU 178 | Poaceae | LSU: MK614114, SSU: - | MK614100 [32] |
| Phyllachora sphaerocaryi | East Asia (China) | MHYAU:178 | Poaceae | LSU: - , SSU: - | MH018560 [20] |
Table 1. Cont.

| Species                  | Location           | Source      | Host Family | GenBank Accession Numbers                  | Reference |
|--------------------------|--------------------|-------------|-------------|--------------------------------------------|-----------|
| *Phyllachora thysanolaenae* | Southeast Asia (Thailand) | MFLU 16-2071 | Poaceae     | LSU: - , SSU: MF372147 , ITS: -           | [20]      |
| *Phyllachora virgatae*   | East Asia (China)  | IFRD9447    | Poaceae     | LSU: ON075439 , SSU: ON072099 , ITS: ON075526 | This study|
| *Phyllachora yushaniae-falcataaurita* | East Asia (China) | MHYAU 123    | Poaceae     | LSU: MG195656 , SSU: - , ITS: MG195631    | [32]      |
| *Phyllachora yushaniae-polytrichae* | East Asia (China) | MHYAU 122    | Poaceae     | LSU: MG195657 , SSU: MH992455 , ITS: MG195632 | [32]      |
| *Phyllachora yushaniae-polytrichae* | East Asia (China) | MHYAU 158    | Poaceae     | LSU: MG195658 , SSU: - , ITS: MG195633    | [32]      |
| *Polystigma pusillum*    | Latin America (Costa Rica) | MM-113      | Fabaceae    | LSU: KX430474 , SSU: KX451858 , ITS: KX451907 | [8]       |
| *Polystigma pusillum*    | Latin America (Costa Rica) | MM-147      | Fabaceae    | LSU: KX430483 , SSU: KX451862 , ITS: -    | [8]       |
| *Polystigma sp.*        | Latin America (Panama) | MM-19       | Fabaceae    | LSU: KX430489 , SSU: KX451850 , ITS: KX451899 | [8]       |
| *Telimena bicincta*     | Latin America (Costa Rica) | MM-163      | Picramniaceae | LSU: KX430487 , SSU: KX451864 , ITS: KX451916 | [8]       |
| *Telimena bicincta*     | Latin America (Costa Rica) | MM-108      | Picramniaceae | LSU: KX430473 , SSU: KX451857 , ITS: KX451906 | [8]       |
|                         |                    | MM-133      | Picramniaceae | LSU: KX430478 , SSU: KX451861 , ITS: KX451910 | [8]       |

2.4. Reconstruction of Ancestral State

Members of *Phyllachora* were coded based on their collection locality according to field notes and references. Six areas were delimited based on the distribution data of *Phyllachora*: A = East Asia, B = Southeast Asia, C = North America, D = South America, E = Latin America, F = Central Europe, G = Unknown, using species from Asia, Europe, North America, South America, Latin America, and Central Europe. In MrBayes v.3.2, chains were run for 1 00000 generations; trees were sampled and printed every 100 generations. RASP 4.2 (Reconstruct Ancestral State in Phylogenies, http://mnh.scu.edu.cn/soft/blog/RASP, accession date: 10 April 2022) was used to reconstruct the ancestral state, and the most-optimal model was DEC [42].

2.5. Calibration Procedure

The second calibration time referenced the results of Dayarathne et al. [33] and Hongsanan et al. [43]. We followed the conclusion that the family Phyllachoraceae divergence time was about 217 Mya as a calibration point (root) for ancestral distribution reconstruction.

3. Results

3.1. Molecular Phylogenetic Results

We analyzed a three-loci (LSU, SSU, ITS) data set of *Phyllachora*. Based on the combined data of LSU, SSU, and ITS sequences. It was found that the two topological trees obtained by maximum likelihood (ML) and Bayesian were similar, and the best scoring RAxML tree was used as the representative tree (Figure 1). We generated a total of 161 sequences from 74 taxa of Phyllachorales, 57 sequences of LSU, 34 sequences of SSU, 70 sequences of ITS, and concatenated sequences of three genes, with 3341 characters including gaps. Bootstrap values of ML higher than 50% are shown on the phylogenetic tree, while values of Bayesian posterior probabilities above 0.5 are shown on the tree (Figure 1). Phylogenetic analysis showed that all four new taxa belonging to *Phyllachora* cluster together with *Phyllachora panicicola* with bootstrap values of 68% (in ML analysis) and Bayesian posterior probability of 0.92. *Phyllachora panicicola* and four new taxa form two clades independent from each other with bootstrap values of 100% and Bayesian posterior probabilities of 1.00.
Figure 1. Phylogenetic tree of maximum likelihood showing the relationships of Phyllachoraceae based on combined LSU, SSU, and ITS data set analysis. Bootstrap values of maximum likelihood higher than 50% are shown on the left, while values of Bayesian posterior probabilities above 0.5 are shown on the right. New species are given in bold, followed by the host of the species behind its strain number.

3.2. Ancestral Area Reconstruction Analysis for Phyllachora

Ancestral area reconstruction analysis revealed that Phyllachora species originated from Latin America about 194 Mya (Figure 2, node 145). Dispersal, vicariance, extinction, and other historical events affected the biogeographical distribution of the species. The evolutionary history of ancestors from the genus Phyllachora reveals that the species of this genus underwent 20 dispersals, 13 vicariances, and 1 extinction (Figure 2, blue coils represent dispersal, green coils represent vicariance, and orange coils represent extinction). Species of Phyllachora migrated from Latin America to Southeast Asia during the Jurassic period, with two dispersal events noted (Figure 2, node 127). In approximately 60–155 Mya,
there were frequent dispersal and vicariance events, and moreover, vicariances were always accompanied by dispersal events. There is only low support suggesting that species belonging to *Phyllachora* may have migrated from Latin America to Southeast Asia 119 Mya (Figure 2, node 108). About 100 Mya, species migrated from East Asia or North America to central Europe, with one dispersal and one vicariance (Figure 2, node 77).

Figure 2. Ancestral character state reconstruction based on the Bayesian tree. Each event is represented with a number at the nodes. Bayesian posterior probabilities are presented (≥0.5). The colored circle near the number at the nodes indicate that blue represents Dispersal, green represents Vicariance, orange represents Extinction. New species are given in bold.
3.3. Taxonomy of Fungi

*Phyllachora flaccidudis* H. X. Wu & J. C. Li. sp. nov. (Figures 3 and 4).

Figure 3. *Phyllachora flaccidudis* (IFRD9445, holotype). (a) Black spots on *Cenchrus flaccidus* (Poaceae); (b,c) Stromata; (d) Vertical section of ascomata in cotton blue; (e) Paraphyses; (f–i) Asci; (j–m) Ascospores. Scale bars, (b) 1 mm; (c) 0.5 mm; (d) 200 μm; (e) 50 μm; (f–i) 10 μm; (j–m) 5 μm. Microscopic techniques: DIC.
Figure 4. (a) P. flaccidudis ascospore in DIW (Deionized Water); (b,c) P. flaccidudis ascospores with gelatinous sheath in ink; (e) P. sandiensis ascospore in DIW; (f–h) P. sandiensis ascospores with gelatinous sheath in ink; (i) P. virgatae ascospore in DIW; (j,k) P. virgatae ascospores with gelatinous sheath in ink; (m) P. jiaensis ascospore in DIW; (n–p) P. jiaensis with gelatinous sheath in ink. Scale bars, (a–p) 5 μm. Microscopic techniques: DIC.

MycoBank: MB843395.

Etymology: Epithet derived from host species Cenchrus flaccidus.

Holotype: IFRD9445.

Parasitic on leaves and stems of Cenchrus flaccidus (Poaceae). Sexual morph: Stroma 1618–1900 × 641–764 μm (x = 1769 × 716 μm, n = 10) (Figure 3a–c), fusiform or cymbaeform, domed above the leaf surface, amphigenous, scattered, sometimes gregarious, like black nevus, no edge, black, carbonaceous. Section of stroma 307–379 μm high, multilocular, peridium 35–60 μm wide, composed of brown to dark brown cells of textura angularis (Figure 3d). Paraphyses 2–3 μm wide (x = 2.9 μm, n = 20), numerous, persistent, filiform, unbranched, aseptate, many guttules, slightly longer than asci (Figure 3e). Asci 82–110 × 7–10 μm (x = 91.6 × 9.0 μm, n = 20), thin-walled, 8-spored, persistent, cylindrical to clavate, apex obtuse, with pedicel (Figure 3f–i). Ascospores 11–13 × 4–7 μm (x = 11.4 × 5.9 μm, n = 20), 1-seriate, fusiform to oval, both ends obtuse, hyaline, aseptate, verrucose, with many guttules (Figure 3j–m) and a mucilaginous sheath (Figure 4a–d). Asexual morph: Not observed.

Material examined: China, Shanxi Province, Xinzhou, Wutai County, 38.71917699 N, 113.25921 E, on stems and leaves of Cenchrus flaccidus (Poaceae), 4 October 2019, Yuying Li IFRD9445, holotype. GenBank accession numbers: ITS: ON075524, LSU: ON072101, SSU: ON072097.

Notes: Phyllachora flaccidudis was collected from Cenchrus flaccidus (Poaceae) in Shanxi Province of China. According to the phylogenetic analysis, P. flaccidudis and P. sandiensis are closely related to P. panicicola (Figure 1). However, P. panicola was reported from Panicum
sp., and its asci were significantly longer and wider than in \textit{P. flaccidus} and \textit{P. sandiensis} (110–130 × 10–14 µm vs. 82–110 × 7–10 µm, 92–126 × 7–10 µm, respectively). Furthermore, the ascospores of \textit{P. panicola} are significantly larger than in \textit{P. flaccidus} and \textit{P. sandiensis} (14–16 × 6–8 µm vs. 11–13 × 4–7 µm, 10–14 × 6–7 µm, respectively). The ascospores of \textit{P. panicola} are rounded at both ends, with a central concave depression and lacking guttules different from \textit{P. flaccidus} and \textit{P. sandiensis}. Differences in morphological characteristics are also supported by the phylogenetic tree, as the two species cluster independently (100% bootstrap support) in a subclade of \textit{Phyllachora} (Figure 1).

The hosts of \textit{Phyllachora sphaerosperma} (= \textit{Phyllachora cenchricola}), \textit{P. flaccidus} and \textit{P. sandiensis} belong to species of \textit{Cenchrus}. However, the host of \textit{P. cenchricola} is \textit{Cenchrus echinatus}, which has been found in Brazil, the southern United States, South America, and the West Indies. The ascospores are nearly spherical, wider than both \textit{P. flaccidus} and \textit{P. sandiensis} (Table 2).

\textit{Phyllachora flaccidus} and \textit{P. chloridis} [33] have similar morphological characteristics, but their host plants are different. The host of \textit{P. flaccidus} and \textit{P. sandiensis} was reported from \textit{Cenchrus flaccidus} (Poaceae), while the host of \textit{P. chloridis} is \textit{Chloris} sp. Morphologically, the ascospores of \textit{P. flaccidus} and \textit{P. sandiensis} are significantly longer than those of \textit{P. chloridis} (Table 2) and feature pedicels, but they are absent in \textit{P. chloridis}. Ascospores of \textit{P. flaccidus} and \textit{P. sandiensis} have 1–2 or more guttules, while \textit{P. chloridis} has only one central guttule, which can serve as an important characteristic for species delimitation.

\textit{Phyllachora sandiensis} H. X. Wu & J. C. Li. sp. nov. (Figures 4 and 5).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{\textit{Phyllachora sandiensis} (IFRD9446, holotype). (a) Black spots on \textit{Cenchrus flaccidus} (Poaceae); (b,c) Stromata; (d) Vertical section of ascomata; (e) Paraphyses; (f) Ascus in cotton blue; (g–i) Asci; (j) Ascospore in cotton blue; (k–m) Ascospores. Scale bars, (c) 0.5 mm; (d) 100 µm; (e) 50 µm; (f–i) 10 µm; (j–m) 5 µm. Microscopic techniques: DIC.}
\end{figure}
### Table 2. Morphological comparison of four new species (in bold) and related species in *Phyllachora* reported from Poaceae.

| Fungal Taxa | Hosts | Color of the Stromata | Asci (μm) | Ascospores (μm) | Size | No. of Septa | Shape | References |
|-------------|-------|-----------------------|-----------|-----------------|------|-------------|-------|------------|
| *P. africana* (P. oblongospora) | Eremopezon delatoni, Lea clata, Chloris sp. | Black | 100–140 × 9–12.5 | 10-17 × 5–9 | Aseptate | ovoid | [44] |
| *P. sphaerosperma* (P. cenchricola) | Cenchrus echinatus | Black | 65–100 × 10–13 | 8–11 × 7–9 | Aseptate | nearly spherical | [14] |
| *P. centotheciaceae* | Centotheca lappacea | Bright | 46.3 × 9.0, pedicel 11.6 × 2.6 | 7.7–9.0 × 4.6–5.1 | Aseptate | oval | [45] |
| *P. coorgiana* | Chloris | Black | 51.2–54.0 × 11.6–14.9 | 15.7–19.1 × 6.3–8.2 | Aseptate | ovoid or ovate acuminately | [14,48] |
| *P. virgatae* | Chloris virgata | Black | 82–110 × 7–10 | 11–13 × 4–7 | Aseptate | drop shape, oval to ellipse, rounded at the ends | This study |
| *P. graminis* | Chloris sp., Elymus sp., Agropyron sp., Arrhenatherum sp., Asperula sp., Agrostis sp., Brachyelytvum sp., Bromus sp., Coix lachryma-jobi | Dark brown to black | 60–70 × 8–10 | 7–14 × 4–7 | Aseptate | oval to ovate or ovoid with obtuse end flattened or blunted | [14] |
| *P. graminis* var. cymodonticola | Cynodon dactylon | Bright | 82–87 × 7.7–8.1, with short peduncle 26–2.1 | 7.5–14 × 5.1–6.5 | Aseptate | usually oblique, rarely irregularly biseriate, ellipsoid or subglobose, rarely irregularly | [47] |
| *P. jiaensis* | Chloris virgata | Black | 77–114 × 8–12 | 9–17 × 8–9 | Aseptate | oval to ellipse, rounded at the ends | This study |
| *P. koondhookensis* | Chloris truncatae | Black | 75–87 × 12–16 | 14–16.5 × 5–5.5 | Aseptate | oval to ellipse, rounded at the ends | This study |
| *P. miniatissima* | Penniestium flaccidum, Chloris sp., Panicum sp., Pennisetum sp., Pennisetum glaucum sp., Setaria sp. | Black | 51.2–54.0 × 11.6–14.9 | 15.7–19.1 × 6.3–8.2 | Aseptate | ovate or ovate acuminately | [14,48] |
| *P. platyelliptica* | Themeda gigantea | Bright | 64.1–92.1 × 10.1–12.1 | 13.6–16.5 × 3.8–6.5 | Aseptate | narrow-ellipsoid | [48] |
| *P. paniculata* | Panicum sp. | Bright | 110–130 × 10–14 | 14–16 × 6–8 | Aseptate | ellipsoidal, rounded at the ends | [33] |
| *P. sandiensis* | Cenchrus flaccidus | Black | 92–126 × 7–10 | 10–14 × 6–7 | Aseptate | drop shape, oval to ellipse, rounded at the ends | This study |
| *P. virgatae* | Chloris virgata | Bright | 84–120 × 7–11 | 10–15 × 6–9 | Aseptate | oval to ellipse, rounded at the ends | This study |

MycoBank: MB 843396.

**Etymology.** Epithet derived from the type locality, a sandy forest park roadside (Shaanxi Province, Yulin City, Yuyang District) in China.

**Holotype:** IFRD9446.

Parasitic on leaves and stems of *Cenchrus flaccidus* (Poaceae). Sexual morph: Stroma 993–2742 × 371–438 μm diam. (x = 1378 × 415 μm, n = 10) (Figure 5a–c), domed above the leaf surface, amphiogenous, fusiform, cymbiform or irregular shape, like black nevus, scattered, sometimes gregarious, no edge, black, carbonaceous. Section of stroma 344–823 μm high, oval, multilocular, peridium 36–58 μm wide, composed of brown to dark-brown cells of textura angularis (Figure 5d). Paraphyses 2–4 μm wide (x = 3 μm, n = 20), numerous, persistent, filiform, unbranched, asperate, many guttules, slightly longer than asci (Figure 5e). Asci
Note: In the course of investigating the grass resources of northern China, two fungal species were collected from *Chloris virgata* (Poaceae). According to the phylogenetic analysis, *P. sandiensis* is closely related to *P. flaccidudis*, but the samples were collected from different locales. Morphologically, the size of asci and stromata of *P. flaccidudis* is significantly longer than in the case of *P. sandiensis* (Table 2). In addition, the ascospores of *P. sandiensis* are longer than those of *P. flaccidudis* (average 106.6 × 9.7 µm vs. 91.6 × 9.0 µm). According to sequence alignment results, LSU, SSU, and ITS sequences differed by 9 bases, 5 bases, and 13 bases between both taxa, respectively. Therefore, *P. sandiensis* is considered to be a new species of *Phyllachora*.

*Phyllachora virgata* H. X. Wu & J. C. Li. sp. nov. (Figures 4 and 6).

MycoBank: MB843397.

Etymology: Epithet derived from host species *Chloris virgata*.

Holotype: IFRD9447.

Parasitic on leaves and stems of *Chloris virgata* (Poaceae). Sexual morph: Stroma 738–2678 × 490–701 µm (τ = 1762 × 642 µm, n = 10) (Figure 6a–c), domed above the leaf surface, amphigenous, fusiform, cymbaeform or irregular shape, black spots, scattered, sometimes gregarious, no edge, shiny black, carbonaceous. Section of stroma 215–232 µm high, oval, multilocular, peridium 25–32 µm wide, composed of brown to dark-brown cells of textura angularis (Figure 6d). Paraphyses 2–3.5 µm wide (τ = 3.3 µm, n = 20), numerous, persistent, filiform, unbranched, asceptate, slightly longer than asci (Figure 6e). Asci 84–120 × 7–11 µm (τ = 102.6 × 9.1 µm, n = 20), thin-walled 8-spored, persistent, clavate, apex obtuse, with pedicel (Figure 6f–i). Ascospores 10–15 × 6–9 µm (τ = 12.9 × 6.9 µm, n = 20), 1-seriate, ovoid to oblong, acute at both ends, hyaline, asceptate, verrucose, with 1–2 or more guttules (Figure 6j–m) and a glutinous mucilaginous sheath (Figure 4e–h). Asexual morph: Not observed.

Material examined: China, Shanxi Province, Xinzhou, Dingxiang County, 38.49269099 N, 109.75387 E, on stems and leaves of *Chloris virgata* (Poaceae), 7 October 2019, Yuying Li, IFRD9446, holotype. GenBank accession numbers: LSU: ON075528, SSU: ON072098, ITS: ON075525.

Notes: *Phyllachora sandiensis* was collected from *Cenchrus flaccidus* in Shaanxi Province of China. According to the phylogenetic analysis, *P. sandiensis* is closely related to *P. flaccidudis*, but the samples were collected from different locales. Morphologically, the size of asci and stromata of *P. flaccidudis* is significantly longer than in the case of *P. sandiensis* (Table 2). In addition, the ascospores of *P. sandiensis* are longer than those of *P. flaccidudis* (average 106.6 × 9.7 µm vs. 91.6 × 9.0 µm). According to sequence alignment results, LSU, SSU, and ITS sequences differed by 9 bases, 5 bases, and 13 bases between both taxa, respectively. Therefore, *P. sandiensis* is considered to be a new species of *Phyllachora*.

*Phyllachora cynodontis* and *P. koondrookensis* have been reported from the same host (i.e., *Chloris*) [14,46]. However, *P. virgatae* and *P. jiaensis* are clearly distinguishable from the two species (Table 2). *Phyllachora chloridis-virgatae* (MHYAU 20136), *P. chloridis-virgatae* (MHYAU 20137), and *P. chloridis-virgatae* (MHYAU 20058) all have *Chloris virgata* as a host species, and there are no references about their morphological characteristics. However, they did not cluster with *P. virgatae* and *P. jiaensis* in the phylogenetic analysis.
Phyllachora virgatae (IFRD9447, holotype). (a) Black spots on Chloris virgata (Poaceae); (b,c) Stromata; (d) Vertical section of ascomata; (e) Paraphyses; (f) Ascus in cotton blue; (g–i) Asci; (j–m) Ascospores. Scale bars, (b) 1 mm, (c) 0.5 mm; (d) 100 μm; (e) 20 μm; (f–i) 10 μm; (j–m) 5 μm. Microscopic techniques: DIC.

We also searched for Phyllachora species reported from the same host genus in Farr et al. [49]. The results showed that Phyllachora africana (= P. oblongospora), P. graminis, and P. minutissima (=P. bonariensis) can also be parasitic on Chloris species. Phyllachora chloridis, P. graminis, P. africana, P. minutissima, P. virgatae, and P. jiaensis have been reported from the same host genus. However, morphologically they are easily distinguishable: the asci and ascospores of P. virgatae and P. jiaensis were significantly longer than in P. chloridis, P. graminis, and P. minutissima (Table 2). However, the asci of P. chloridis lack pedicels, and the asci of P. graminis have an ascus crown at the apex. Phyllachora minutissima has no paraphyses, while P. virgatae and P. jiaensis have pedicels and paraphyses and lack an ascus crown at the apex. The ascospores of P. virgatae and P. jiaensis have 1–2 or more guttules, but P. chloridis has only one central guttule. The asci of P. africana Parbery and P. minutissima were significantly longer than in P. virgatae and P. jiaensis. Hence, P. virgatae
is distinguishable by its different morphological characteristics, which qualify it as a new species of *Phyllachora*.

*Phyllachora jiaensis* H. X. Wu & J. C. Li. sp. nov. (Figures 4 and 7).

![Figure 7. Phyllachora jiaensis (IFRD9448, holotype). (a) Black spots on Chloris virgata (Poaceae); (b,c) Stromata; (d) Vertical section of ascomata; (e) Paraphyses; (f) Ascus in cotton blue; (g–i) Asci; (j–m) Ascospores. Scale bars, (c) 0.5 mm; (d) 100 μm; (e) 20 μm; (f–i) 10 μm; (j–m) 5 μm. Microscopic techniques: DIC.](image)

MycoBank: MB 843398.

Etymology: Epithet derived from the type locality, Jia County (Shaanxi Province, Yulin City) in China.

Holotype: IFRD9448.

Parasitic on leaves and stems of *Chloris virgata* (Poaceae). Sexual morph: Stroma 825–2321 × 347–640 μm diam. (τ = 1372 × 501 μm, n = 10) (Figure 7a–c), domed above the leaf surface, amphigenous, fusiform, cymbaeform or of irregular shape, black spots, scattered, sometimes gregarious, without an edge, shiny black, carbonaceous. Section of stroma 143–170 μm high, oval, multilocular, peridium 18–27 μm wide, composed of brown to dark-brown cells of textura angularis (Figure 7d). Paraphyses 3–4 μm wide (τ = 3.1 μm, n = 20), numerous, persistent, filiform, unbranched, aseptate, slightly longer than asci.
Phyllachora jiaensis was collected from Shaanxi Province. According to phylogenetic analysis, *P. virgatae* and *P. jiaensis* are closely related; however, they were collected from different locales. Morphologically, the stromata color of *P. virgatae* is bright black, and in *P. jiaensis* it is black. In addition, the asci of *P. virgatae* were longer than in *P. jiaensis* (102.6 × 9.1 µm vs. 93.4 × 9.4 µm). The ascospores of *P. jiaensis* were also longer than in *P. virgatae* (13.4 × 6.5 µm vs. 12.9 × 6.9 µm) (Table 2).

Phylogenetically, *P. virgatae* and *P. jiaensis* clustered together with high bootstrap support and probability value (100/1.0), with *P. jiaensis* forming a long branch. The LSU, SSU, and ITS loci differ by 8 bases, 109 bases, and 3 bases, respectively. Phylogenetically, *P. virgatae* grouped with *P. jiaensis* to form one clade, and *P. flaccidudis* with *P. sandiensis* to form another clade, with high bootstrap and probability values (100/1.0), but they occur on different hosts. *P. virgatae* and *P. jiaensis* both occur on *Chloris virgata*, and the host of *P. flaccidudis* and *P. sandiensis* is *Cenchrus flaccidus*. The ascospores of *P. flaccidudis* and *P. sandiensis* are acute at one end and blunt at the opposite end, while the ascospores of *P. virgatae* and *P. jiaensis* are blunt at both ends.

The four new species described herein have ascospores with gelatinous sheaths that differ in black ink (Figure 4). The gelatinous sheaths of *P. flaccidudis* and *P. sandiensis* are larger than in *P. virgatae* and *P. jiaensis*. Hence, based on both morphological and phylogenetic evidence, we introduce the novel species, *P. jiaensis*.

### 4. Discussion

In this study, we introduced four new taxa of *Phyllachora* (*P. flaccidudis*, *P. sandiensis*, *P. virgatae*, and *P. jiaensis*) that have morphological characteristics typical of *Phyllachora*: black leaf spots, peridium clypeate, multicellular, asci cylindrical, an unobvious apical ring, shortly pedicellate, numerous paraphyses and slightly longer than asci, and asceptate ascospores with guttules [4,33,50]. All novel taxa were introduced based on morphological characteristics and novel phylogenetic lineages in *Phyllachora* (Figure 1). We compared the morphological characteristics of the four new species and similar *Phyllachora* taxa (Table 2).

In recent years, several *Phyllachora* species have been introduced in many places of China, such as *Phyllachora heterocladae* (Sichuan, China), *P. panicicola* (Yunnan, China), *P. eriochloae* var. *colombiensis* (Yunnan, China), *P. graminis* var. *cynodonticola* Speg. (Yunnan, China), and *P. eriochloae* Speg. var. *eriochloae* (Yunnan, China) [20,33,43–48]. However, relevant molecular data only exists for a few of these species (e.g., *P. heterocladae* and *P. panicicola*). The majority of these species were reported from Yunnan Province (e.g., *P. panicicola*). Host specificity plays an important role when introducing novel *Phyllachora* species [3,4]. Yang et al. [20] proposed that phyllachora-like species that are parasitic on Poaceae should be treated as *Phyllachora*, and our study also provides strong evidence that supports this hypothesis.

Yang et al. [20] introduced *Phyllachora heterocladae* from Sichuan Province, and the phylogenetic tree was artificially divided into five lineages based on the host plants. Most species of *Phyllachora* that cluster within lineage I are graminicolous (Poaceae), but *P. quaeeae* grows on *Qualea multiflora* (Vochysiaceae). They formed a distinct subclade with *P. arundinellae* (MHYAU:108), *P. cynodontis* (MHYAU:20043), and *P. imperatae* (MHYAU:014). Species within lineage II and lineage IV are bambusicolous fungi. Lineage III is solely composed of *P. thyssanolaenae* (MFLU 16-2071), which is an unstable species in the phylogeny.
Lineage V contains only P. pomigena, associated with an unknown host plant. Li et al. [32] introduced two new species, P. dendrocalamii-membranacei and P. dendrocalamii-hamiltonii, and phylogenetic analysis was generated four main clades. Lineage I consisted of all Phyllachora species obtained from the subfamily Agrostidoideae of the Poaceae, except for Polystigma pusillum (MM-19), which was found growing on Fabaceae. Neophyllachora species occurred in the family Myrtaceae within Lineage II. Lineage III and Lineage IV are Phyllachora species collected from the subfamily Bambusoideae of the Poaceae. However, it is important to note that Yang et al. [20] did not include all Phyllachora species in their analysis.

In this study, the generated phylogenetic tree comprises 74 species belonging to six genera (viz., Ascovaginospora, Camarotella, Coccodiella, Neophyllachora, Phyllachora, and Polystigma). We found that the Phyllachora genus is paraphyletic. Because the host of P. pomigena remains unknown, the species Phyllachora pomigena formed a single clade [20,51]. In the phylogenetic analysis, the new species described herein are included within the Phyllachora genus and separated from other taxa with a single subclade. Their hosts are Cenchrus flaccidus and Chloris virgata, both belonging to Poaceae (graminicolous).

The study revealed that the ancestor of Phyllachora species originated from Latin America. Phyllachora species ancestors initially spread from Latin America to North America, East Asia, South America, and eventually to Central Europe. The characteristic of Phyllachora species in Latin America are consistent with the ancestral characteristics of Phyllachora genus found in Mardones et al. [8]. For example, existing species P. maydis and P. graminis still retain ancestral characteristics, such as growing on monocotyledonous hosts, immersed perithecia, black stromata, and the presence of clypeus [8]. Reconstruction analysis of ancestral location indicates that a vicariance event (i.e., the splitting of the range of a taxon or biota into two or more geographical subdivisions by the formation of natural barriers, for example, mountain building, glaciation, plate tectonics or climate change) affected speciation allowing some species to retain ancestral morphological characteristics [52]. The appearance of Polystigma could have resulted from the extinction event (Figure 2 node 143). The extinction event may have resulted in the host of Polystigma species shifting from monocotyledons to dicotyledons (Fabaceae).

During the Cretaceous geological upheaval, orogeny, continental drift as well as the emergence of the Atlantic and the Indian Ocean led to dramatic terrestrial climate changes across the earth’s surface [53]. These led to the mass extinction of the dominant Mesozoic gymnosperm and ferns in the tropics, subtropical plains, and low mountains areas, which were replaced by angiosperms (the origin of Poaceae) that flourished in the Paleogene [54]. The emergence of angiosperms may have triggered the evolution and migration of the ancestors of the Phyllachora fungi.

There are few studies examining the co-evolution and ancestral state reconstruction of Phyllachora species; this is because of the scarcity of existing species with high-quality molecular data, which adds uncertainty to the process of ancestral state reconstruction. Extensive sampling and high-quality molecular data will reveal more accurate changes in the ancestral status of species in this group. Ancestor state reconstruction currently requires inferring phenotypes of ancestral species using observations from present-day species [35,56]. As new classical and molecular methods for identifying fungi continue to develop [57], ancestor state reconstruction analysis of fungal taxonomy is at the forefront of a new trend [8,58–60]. Future studies on species diversity and evolution of Phyllachora species require more extensive sampling and high-quality molecular data.

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