Diversity of Ascomycota in Jilin: Introducing Novel Woody Litter Taxa in Cucurbitariaceae

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Abstract: Cucurbitariaceae has a high biodiversity worldwide on various hosts and is distributed in tropical and temperate regions. Woody litters collected in Changchun, Jilin Province, China, revealed a distinct collection of fungi in the family Cucurbitariaceae based on morphological and molecular data. Phylogenetic analyses of the concatenated matrix of the internal transcribed spacer (ITS) region, the large subunit (LSU) of ribosomal DNA, the RNA polymerase II subunit (rpb2), the translation elongation factor 1-alpha (tef1-α) and β-tubulin (β-tub) genes indicated that the isolates represent Allocucurbitaria and Parafenestella species based on maximum likelihood (ML), maximum parsimony (MP) and Bayesian analysis (BPP). We report four novel species: Allocucurbitaria mori, Parafenestella changchunensis, P. ulmi and P. ulnicola. The importance of five DNA markers for species-level identification in Cucurbitariaceae was determined by Assemble Species by Automatic Partitioning (ASAP) analyses. The protein-coding gene β-tub is determined to be the best marker for species level identification in Cucurbitariaceae.

Keywords: ASAP; fungal barcode; multi-loci phylogeny; northeast China; Pleosporales; taxonomy

1. Introduction

Fungi are known to have a high diversity; however, the number of named and classified fungi is still lower than the estimated number of species [1–4]. This could be because several regions are yet to be explored. China is the third largest country in the world by area, with several different climatic conditions [5–8]. Jilin is a province located in northeast (NE) China where the temperature is hot and dry in summers and has a harsh winter with temperatures down to −20 °C [9]. The vegetation in the eastern mountains includes tree genera such as the Betula, Fraxinus, Juglans, Larix, Pinus, Quercus, Salix, Sorbus and Ulmus [10]. These trees are common in the northern hemisphere and in temperate climates [11].

The family Cucurbitariaceae was established by Winter [12], and it is characterized by clustered ascomata and scattered, black, and shiny ostioles, surrounded with olivaceous-to-brown hyphae and having yellow-to-dark olivaceous, brown and muriform ascospores [13–15]. Asexual morphs are known to occur as pycnidia with hyaline conidia [14]. Cucurbitariaceae has received much attention in recent years, and it includes 13 genera: Allocucurbitaria Valenz.-Lopez, Stchigel, Guarro & Cano, Astragalicola Jaklitsch & Voglmayr, Cucitella Jaklitsch & Voglmayr, Cucurbitaria Gray (=Pleurostromella Petr.), Fenestella Tul. & Tul C., Neocucurbitaria Wan., E.B.G. Jones & K.D. Hyde, Paracucurbitaria Valenz.-Lopez Stchigel, Guarro & Cano, Parafenestella Jaklitsch & Voglmayr, Protopenestella Jaklitsch & Voglmayr, Rhytidella Zalasky, Seltsamia Jaklitsch & Voglmayr, Syncarpella Theiss. & Syd. and Syngenestella Jaklitsch & Voglmayr [13]. Jaklitsch et al. [15] provided a comprehensive study of fenestelloid
clades of Cucurbitariaceae using fresh collections. Various type specimens were verified, and all the genera of Cucurbitariaceae formed a well-supported clade in a multi-locus phylogeny [15]. However, the phylogenetic placement of Rhytidiella and Syncarpella remain to be confirmed as they lack molecular data [15]. Fenestella, Neocucurbitaria and Parafenestella have a wide distribution mainly in temperate regions and can be found on various hosts [14,16–19]. For example, Parafenestella salicum was found on the twigs of Salix alba and Fenestella parafenestrata on the branches of Quercus robur in Austria, while Neocucurbitaria subcaespitosa was isolated from the twigs of Sorbus aria in Switzerland [14,15].

This study mainly focuses on ascomycetous fungi from the northern part of China. The novel taxa are introduced based on morphology and molecular data. In this study, Allocucurbitaria was used to demonstrate important characteristics for distinguishing the asexual morph at the generic level. This study also determines the best barcode out of five DNA markers for species delineation in Cucurbitariaceae by applying assemble species by automatic partitioning (ASAP) analyses.

2. Materials and Methods

2.1. Collection and Isolation

Dried branches of Morus alba, Populus species and Ulmus pumila were collected from Jilin Agricultural University in Changchun, Jilin Province, China (longitude: 125.410385; latitude: 43.810433). Specimens were kept in sealed paper bags indicating the location, time and host details. The specimens were processed following Senanayake et al. [20] for isolation. Single-spore isolation was performed using potato dextrose agar (PDA) and incubated at 25 °C in the dark [16]. Germinated ascospores were transferred aseptically to PDA and grown at 25 °C for 2 weeks. Pure cultures were deposited at the Engineering Research Center of the Chinese Ministry of Education for Edible and Medicinal Fungi at the Jilin Agricultural University (CCMJ), Changchun, China, and type specimens were deposited in the Herbarium of Mycology, Jilin Agricultural University (HMJAU). The new taxa were registered with Mycobank [17,18].

2.2. Morphological Observation

The specimens were examined using a Zeiss Stemi 2000C stereomicroscope equipped with a Leica DFC450C (Leica, Heidelberg, Germany) digital camera. A thin section of partial ascoma was prepared and placed on glass slides with a drop of sterile water. The structure and size of microcharacters were observed and photographed using a digital Axiocam 506 color camera equipped with Zeiss Image A2 (Zeiss, Oberkochen, Germany). Fructification of asexual morph in the sterile culture was observed after four weeks of incubation in the dark.

2.3. DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was extracted using NuClean PlantGen DNA Kit (CWBio, Taizhou, China) according to the manufacturer’s protocol. The internal transcribed spacer region of ribosomal DNA (ITS) [21], the large subunit (LSU) of ribosomal DNA [22], the RNA polymerase II second-largest subunit (rpb2) [23], the translation elongation factor 1-alpha (tef1-α) and beta-tubulin (β-tub) were amplified as described in Table 1. The amplification reactions were performed using 20 µL PCR mixtures containing 9 µL of ddH2O, 10 µL of 2× EsTag MasterMix (Dye), 0.4 µL of DNA template and 2 µL of 2 µmol/µL of each forward and reverse primer. All PCR products were visualized with electrophoresis using a 1% agarose gel. The PCR products were sequenced by Sangon Biotech (Shanghai) Co., Ltd., China.
Table 1. The PCR primers and amplifying conditions used in this study.

| Amplification Loci (Primer Pair Forward/Reverse) | PCR Conditions                                                                 | References            |
|-------------------------------------------------|-------------------------------------------------------------------------------|-----------------------|
| ITS (ITS5/ITS4)                                 | An initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C and 90 s at 72 °C, and a final extension step of 10 min at 72 °C, and 10 °C for holding temperature | White et al. [21]      |
| rpb2 (fRPB2-5F/fRPB2-7cR)                       |                                                                                 | Vilgalys et al. [23]  |
| tef1-a (2218F/983R)                             |                                                                                 | Carbone and Kohn [24] |
|                                                 |                                                                                 | Rehner and Buckley [25]|
| LSU (LROR/LR5)                                 | An initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 45 s at 53 °C and 90 s at 72 °C, and a final extension step of 10 min at 72 °C, and 10 °C for holding temperature | Vilgalys and Hester [22]|
| B-tub (T1/Bt2b)                                |                                                                                 | O'Donnell and Cigelnik [26]|

2.4. Phylogenetic Analysis

The sequence data were assembled using Geneious Prime 2021 (Biomatters Ltd., Auckland, New Zealand). The closest matches for the new strains were obtained using BLASTn searches (http://www.blast.ncbi.nlm.nih.gov/, accessed on 17 December 2021), and reference sequence data were downloaded from recent publications [14,15]. The sequences were aligned with MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/, accessed on 8 July 2022) [27], and ambiguous nucleotides were manually adjusted following visual examination in AliView version 1.26 [28]. Leading or trailing gaps exceeding the primer binding site were trimmed from the alignments, and the alignment gaps were treated as missing data. The concatenation of the multilocus data was created using Sequence Matrix version 1.8 [29].

Phylogenetic analyses were conducted using maximum likelihood, maximum parsimony and Bayesian inference methods. Maximum likelihood analysis was performed using RAxML-HPC2 on XSEDE on the CIPRES web portal (http://www.phylo.org/portal2/, accessed on 8 July 2022) [30–32]. The GTR+I+G model of nucleotide evolution was used for the datasets, and RAxML rapid bootstrapping of 1000 pseudo-replicates was performed [33]. The best-fit evolutionary models for individual and combined datasets were estimated under the Akaike information criterion (AIC) using jModeltest 2.1.10 on the CIPRES web portal for posterior probability [34]. The GTR+I+G model was the best model for the datasets. Maximum parsimony analysis of the combined matrices was performed using a parsimony ratchet approach. Descriptive tree statistics for parsimony (Consistency Index [CI], Homoplasy Index [HI] Tree Length [TL], Retention Index [RI] and Relative Consistency Index [RC]) were calculated for the trees generated under the different optimality criteria. The resulting best trees were then analyzed using PAUP and subjected to a heuristic search with TBR branch swapping (MulTrees option in effect, steepest descent option not in effect) [35]. Bayesian inference analyses were conducted using MrBayes v. 3.2.6 on the CIPRES web portal. Simultaneous Markov chains were run for seven million generations, and trees were sampled every 100th generation [36]. The phylogenetic trees were visualized in FigTree 1.4.3 [37] and edited in Adobe Illustrator CS v. 6 (Adobe, San Jose, CA, USA).

2.5. Analysis of Matrix Partitions by Assemble Species by Automatic Partitioning

Puillandre et al. [38] introduced the assemble species by automatic partitioning (ASAP) method to build species partitions. The ASAP method circumscribes species partitions using an implementation of a hierarchal clustering algorithm based on pairwise genetic distances (Kimura 2-Parameter). The pairwise genetic distances are used to build a list of partitions ranked by a score that is computed using the probabilities of groups to define panmictic species. The ASAP delimitations were run on the online version (https://bioinfo.mnhn.fr/abi/public/asap/ (accessed on 13 January 2022)) using single-locus datasets that included 107 strains of Cucurbitariaceae. The partition with the lowest ASAP score is known to represent the best partitions [38,39], and thus partitions with the lowest ASAP score were considered for each dataset [39,40].
3. Results
3.1. Phylogenetic Analyses

The final concatenated dataset comprised 110 ingroup taxa and two outgroup taxa, with 4607 characters including gaps (651 bases for ITS, 911 bases for LSU, 1063 bases for \texttt{rpb2}, 1281 bases for \texttt{tef1-\alpha}, and 701 bases for \texttt{\beta-tub}). The RAxML analysis yielded a best-scoring tree with a final ML optimization likelihood value of $-39123.587750$. The matrix consisted of 1740 distinct alignment patterns, with 25.90% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.234707, C = 0.269983, G = 0.265086, T = 0.230223; substitution rates AC = 1.287870, AG = 4.563896, AT = 1.434736, CG = 1.144629, CT = 6.919700, GT = 1.000000; proportion of invariable sites I = 0.606319; gamma distribution shape parameter $\alpha$ = 0.967784. The maximum parsimony dataset consisted of 1230 parsimony-informative characters and 246 variable characters. The parsimony analysis yielded 256 most parsimonious trees out of 1000 (TL = 6467, CI = 0.368, RI = 0.806, RC = 0.296, HI = 0.632). In the BPP analysis, 2437 trees were sampled after the 20% burn-in with a stop value of 0.009904. The maximum parsimony dataset consisted of 3132 parsimony-informative characters and 241 variable characters. The parsimony analysis yielded 512 most parsimonious trees out of 1000 (TL = 6468, CI = 0.368, RI = 0.806, RC = 0.297, HI = 0.632). In the BPP analysis, 1461 trees were sampled after the 20% burn-in with a stop value of 0.009955. The phylogenetic trees generated from the ML, MP and BPP had similar topologies (Figures S6 and S7).

In the ML analysis of the ITS region, \textit{Parafenestella ulmi} (CCMJ 5001 and CCMJ 5002) and \textit{P. ulmicola} (CCMJ 5003 and CCMJ 5004) clustered together with high support (ML = 95%), while \textit{P. changchunensis} (CCMJ 5007) formed a clade with \textit{P. vindobonensis} (CBS 145256) with relatively low support (ML = 63%) in \textit{Parafenestella} (Figure S1). \textit{Parafenestella ostryae} (MFLU 16-0184) and \textit{P. pittospori} (CPC 34462) resided in the \textit{Neocucurbitaria} clade (Figure 1) similar to the combined dataset. \textit{Allocucurbitaria mori} (CCMJ 5005 and CCMJ 5006) formed a clade with \textit{A. botulispora} (CBS 142452), \textit{Seltsamia galinsogisoli} (CBS 140956), \textit{S. ulmi} (CBS 143002) and two unidentified \textit{Seltsamia} species (EAB-67-11b and SGSF207) (ML = 100%). The LSU locus could not accurately distinguish taxa at the genus and species level in \textit{Cucurbitariaceae} (Figure S2). In the ML analysis of \texttt{\beta-tub} gene, \textit{Parafenestella ulmi} (CCMJ 5001 and CCMJ 5002) and \textit{P. ulmicola} (CCMJ 5003 and CCMJ 5004) formed a clade with high support (ML = 94%), while \textit{P. changchunensis} (CCMJ 5007) clustered with \textit{P. pseudosalicis} (CBS 145264) with moderate support (ML = 71%). \textit{Allocucurbitaria mori} (CCMJ 5005 and CCMJ 5006) and \textit{A. botulispora} (CBS 142452) formed a clade with moderate support (ML = 54%, Figure S5). In the \texttt{tef1-\alpha} analysis, \textit{Parafenestella ulmi} (CCMJ 5001 and CCMJ 5002) and \textit{P. ulmicola} (CCMJ 5003 and CCMJ 5004) formed a clade with relatively high support (ML = 89%) (Figure S4). \textit{Parafenestella changchunensis} (HMJAU 60182) formed a clade with \textit{P. salicis} (CBS 145270 and C303), \textit{P. pseudosalicis} (CBS 145264), \textit{P. vindobonensis} (CBS 145265) and \textit{P. alpina} (CBS 145263 and C249) with relatively high support (ML = 79%). \textit{Allocucurbitaria mori} (CCMJ 5005 and CCMJ 5006) clustered with \textit{Synfenestella pyri} (CBS 144855) with low support (ML = 41%).

In the multi-locus phylogenetic analysis, \textit{Parafenestella ulmi} (CCMJ 5001 and CCMJ 5002) and \textit{P. ulmicola} (CCMJ 5003 and CCMJ 5004) formed a clade with high support (ML = 100%; MP = 100%; BPP = 1.00). \textit{Parafenestella changchunensis} (CCMJ 5007) clustered with \textit{P. pseudosalicis} (CBS 145264) and \textit{P. salicis} (CBS 145270 and C303) with high support (ML = 99%; MP = 96%; BPP = 1.00). \textit{Parafenestella changchunensis} (CCMJ 5007) is closely related to \textit{P. pseudosalicis} (ML = 75%; MP = 96%). The fresh collections from \textit{Morus alba} revealed a new species \textit{Allocucurbitaria mori} (CCMJ 5005 and CCMJ 5006). The two isolates (CCMJ 5005 and CCMJ 5006) formed a close relationship to an unidentified \textit{Seltsamia} species (SGSF207) with strong statistical support (ML = 100%; MP = 100%; BPP = 1.00).
Figure 1. The Bayesian 50% majority-rule consensus phylogram based on a concatenated ITS, LSU, rpb2, tef1-α and β-tub dataset of Cucurbitariaceae. The tree is rooted with *Pyrenochaetopsis americana* (UTHSC DI16225) and *P. confluens* (CBS 142459). Bootstrap support values for maximum likelihood and maximum parsimony analysis greater than 70% (ML = left; MP = middle) and Bayesian posterior probabilities ≥ 0.90 (BPP, right) are shown at the nodes. The new species are indicated in blue. The type-derived strains are indicated in bold and marked with T.
3.2. ASAP: Assemble Species by Automatic Partitioning

Five single-locus datasets were used that comprised 110 sequences of ITS, 109 sequences of LSU, 101 sequences of rpb2, 96 sequences of β-tub and 88 sequences of tef1-a. The ASAP analysis of the ITS region assigned all members of Cucurbitariaceae into 45 groups (Figure 2); β-tub gene into 65 groups (Figure 2); LSU into 43 groups (Figure S8); rpb2 gene into 65 groups (Figure S9); tef1-a gene into 45 groups (Figure S10).

**Figure 2.** Dendrogram from ASAP analysis based on two datasets (ITS and β-tub markers). The results of species delimitation are indicated by red bars. Sequences generated in this study are in blue.

The ASAP analysis recovered *P. ulmi* (CCMJ 5001 and CCMJ 5002), *P. ulmicola* (CCMJ 5003 and CCMJ 5004) and twelve other strains including *P. pseudoplataini* (CBS 142392), *P. austriaca* (CBS 145262), *P. rosaccarum* (C203, FM1, C269, C283, CBS 145268, C315, CBS145272, C320), *P. germanica* (CBS 145267) and *P. tetratrupha* (CBS 145266) as one group in the LSU data. *Parafenestella changchunensis* (CCMJ 5007) and *P. pseudosalici* (CBS 145264) were recovered as one group in the LSU data. The ASAP analysis of the ITS region recovered *P. ulmi* and *P. ulmicola* as one group (Figure 2). The ASAP result of the β-tub gene was similar to the combined dataset (Figure 2). *Parafenestella ulmi* and *P. ulmicola* were not delineated by the tef1-a and rpb2 genes (Figures S9 and S10). *Parafenestella changchunensis*, *P. pseudosalici* (CBS 145264) and *P. salici* (CBS 145270 and C303) were recovered as one group in the tef1-a data. *Allocucurbitaria mori* (CCMJ 5005 and CCMJ 5006) grouped with *Synfenestella pyri* (CBS 144855) in the ASAP analysis of the tef1-a gene, but both were recovered as individual groups in the ITS, LSU, rpb2, and β-tub datasets.

In the ASAP analysis, the β-tub gene was the best marker for identifying *Parafenestella* and *Allocucurbitaria* taxa. *Parafenestella ulmi* and *P. ulmicola* were recovered as a group in ASAP analysis of the ITS and other markers but were recovered as separate groups in the β-tub dataset (similar to the combined dataset). *Parafenestella changchunensis* and *P.
vindobonensis (CBS 145265) were recovered as a group in the ITS region but were recovered as distinct species in the \( \beta \)-tub dataset. Alloccurbitaria mori was recovered as an individual group in all single-marker analyses (except \( tef1-\alpha \) gene). Based on the current results, the \( \beta \)-tub gene is the best marker for the identification of Cucurbitariaceae taxa at the species level.

### 3.3. Taxonomy

**Allocurbitaria mori** W.X. Su, Phukhams. & Y. Li, *sp. nov.* (Figure 3).

**MycoBank Number:** MB844413.

**Etymology:** Named after the host genus *Morus*.

**Holotype:** HMJAU 60183.

**Description:** Saprobic on dead twigs of *Morus alba*.

**Sexual morph:** Undetermined.

**Asexual morph:** Stromata poorly developed, multiloculate, with 5–8 locules forming groups in stromata, immersed. Conidiomata 108–180 × 103–201 \( \mu m \) (\( \overline{\mu} = 142 \times 143 \mu m, n = 6 \)), pycnidia, solitary or aggregated, sometimes confluent, semi-immersed, visible as black protrusions, globose to ellipsoid, coriaceous, black, without distinguishable ostioles. Pycnidial wall 5–9 \( \mu m \) wide, thick-walled, composed of 7–10 layers of thin-walled cells of textura angularis, dark brown on the outside to gradually lighter on the inside, inner layer subhyaline, lining layer bearing conidiogenous cells. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 6–14 × 1–5 \( \mu m \) (\( \overline{\mu} = 10 \times 2 \mu m, n = 30 \)), enteroblastic, solitary, long cylindrical, arising from the inner layer of conidioma, smooth-walled, hyaline. Conidia 3–5 × 1–2 \( \mu m \) (\( \overline{\mu} = 4 \times 1.5 \mu m, n = 50 \)), oblong, hyaline, aseptate, with a minute guttule, smooth.

**Cultural characters:** Colonies on MEA reaching 32–38 mm diam after 4 weeks at 25 \( ^\circ \)C. Cultures from above, gray at the center, dense in the middle, sparse at the edge, circular, papillate, black lumps produced on the surface of cultures, white at the edge.

**Material examined:** CHINA, Jilin Province, Changchun, Jilin Agricultural University, from *Morus alba* (Moraceae) twigs, 20 May 2021, Wenxin Su and C. Phukhamsakda, S057 (HMJAU 60183, holotype); ex-type living culture, CCMJ5005; isotype = HMJAU 60184; ex-isotype living culture, CCMJ5006.

**GenBank accession numbers:** CCMJ5005: LSU = OL897171, ITS = OL996120, \( tef1-\alpha \) = OL944601, \( rpb2 \) = OL944505, and \( \beta \)-tub = OL898725. CCMJ5006: LSU = OL897172, ITS = OL996121, \( tef1-\alpha \) = OL944602, \( rpb2 \) = OL944506 and \( \beta \)-tub = OL898720.

**Notes:** Alloccurbitaria mori (CCMJ5005 and CCMJ5006) formed a separate clade in Allocurbitaria/Seltsamia with high support (ML = 98%; MP = 97%; BPP = 1.00). Morphologically, *A. mori* (HMJAU 60183) is similar to *A. botulispora* (CBS 142452) and *S. galinsogisoli* (CBS 140956) in having cylindrical, enteroblastic, solitary conidiogenous cells and aseptate conidia [41,42] (Figure 4). However, *S. galinsogisoli* (CBS 140956) has longer conidia, while *A. botulispora* (CBS 142452) has distinct guttulate at the conidia ends [41,42].

A BLASTn search of the ITS region of *A. mori* strain CCMJ 5005 showed a high query cover and similarity (99.80%) to an unidentified Seltsamia sp. (SGSF207) from soil. However, there are no other loci available in public databases for comparison. Hence, we introduce *Allocurbitaria mori* as a novel species, and this is the first report of *Allocurbitaria* on *Morus* tree [41–43].
ever, there are no other loci available in public databases for comparison. Hence, we introduce *Allocucurbitaria* *mori* as a novel species, and this is the first report of *Allocucurbitaria* on *Morus* tree [41–43].

**Figure 3.** *Allocucurbitaria* *mori* (HMJAU 60183, holotype) The red arrow indicates the conidiomata in face view. (a,b) Appearance of conidiomata on host substrate. (c,d) Vertical section of partial conidiomata. (e) Section of partial conidioma wall. (f–h) Conidiogenous cells and conidia. (i) Conidia. (j) Culture characteristics on PDA. Scale bars: (a) = 500 μm; (b) = 200 μm; (c,f) = 100 μm; (d) = 50; (e,g,h) = 10 μm; (i) = 5 μm.
**Parafenestella changchunensis** W. X. Su, Phukhams. & Y. Li, sp. nov. (Figure 5).

**MycoBank Number:** MB844412.

**Etymology:** referring to Changchun City where the sample was collected.

**Holotype:** HMJAU 60182.

**Description:** Saprobic on dead stems of Populus L.

**Sexual morph:** Ascomata 174–416 × 226–486 μm (x = 280 × 353 μm, n = 5), single or gregarious, scattered, globose to depressed globose, submersed, visible as black dots and protruding host surface, solitary or aggregated. Ostioles 61 × 100 μm, center, protruding filled with periphyses. Peridium 12–27 μm wide, thick-walled, composed of 6–10 wall layers, outer part comprising dark brown cells of textura angularis, inner layer thin-walled, dark brown from the outside radiating light brown cells to hyaline towards the inside. Hamathecium of dense, 1.6–2.0 μm (x = 1.7 μm, n = 10) wide, filamentous, septate, cellular pseudoparaphyses surrounding asci. Asci 95–138 × 16–21 μm (x = 121 × 18 μm, n = 10), 6–8 ascospores, bitunicate, fissitunicate, broad cylindrical, some curved, short-pedicellate, apically rounded with an ocular chamber. Ascospores 18–25 × 8–13 μm (x = 21 × 10 μm, n = 30), uniseriate, partially overlapping, fusiform to oval, slightly asymmetrical, the
middle of ascospores is slightly contracted, with 4–6 transverse septa, 2–3 vertical septa, the upper part is slightly larger than the lower part, light yellow to dark brown.

**Asexual morph:** *Pyenia* produced in PDA after 2 weeks of incubation in the dark, mycelium white. **Conidiomata** confluent or scattered, superficial, covered with dense vegetative hyphae, with turbid whitish drops, globose, black. **Conidia** 5–8 × 2.5–4.5 µm (X = 6.5 × 3.7 µm, n = 30), oblong to allantoid, hyaline, aseptate, with 1–2 guttules.

**Culture characteristics:** Colonies on PDA, reaching 26–31 mm diam after 2 weeks at 25 °C. Culture from above, mycelium dense and producing hyphal coil structures; from the center to the outer edge, the color changes from grey to greyish-green to white, with obvious concentric wheel patterns, a clear radiation pattern at the back, round.

**Material examined:** CHINA, Jilin Province, Changchun, Jilin Agricultural University, from dead stems of *Populus L. (Salicaceae)*, 18 April 2021, Wenxin Su, S12-16 (HMJAU 60182, holotype); ex-type living culture, CCMJ5007.

**GenBank accession numbers:** CCMJ5007: LSU = OL897170, SSU = OL891808, ITS = OL996119, tef-α = OL944600, and β-tub = OL898719.

**Notes:** In our phylogenetic analysis, *P. changchunensis* (CCMJ5007) is closely related to *P. pseudosalicis* (CBS 145264) with moderate support (ML = 75%; MP = 96 %; Figure 1). *Parafenestella changchunensis* is morphologically similar to *P. pseudosalicis* in having immersed, concave apex ascomata, with the upper part of young ascospores often wider, ends concolorous and smooth walled [14]. The immature spores of *P. changchunensis* have four horizontal septa and form 2–3 vertical septa during the maturation process. However, the immature spores of *P. pseudosalicis* have 2 transverse septa turning into 2–4 longitudinal septa during the maturation process [15]. *Parafenestella changchunensis* mycelium nodules gradually form fruiting bodies on the medium, while there are no reports of the asexual morph of *P. pseudosalicis* [15].

A BLASTn search of the ITS region of *P. changchunensis* (CCMJ 5007) showed a high similarity and query cover (98.81%) to *P. vindobonensis* (CBS 145265). The β-tub sequence of *P. changchunensis* (CCMJ 5007) showed a high query cover and similarity (96.82%) to *P. pseudosalicis* (C301). There were 0.96% (6/627 bases), 0.34% (3/885), 1.78% (13/730) and 7.99% (43/538 bases) base differences in the ITS, LSU, tef-α and β-tub genes between *P. changchunensis* (CCMJ 5007) and *P. vindobonensis* (CBS 145265), excluding gaps. There were 1.75% (11/627 bases), 0.11% (1/885), 1.10% (8/730) and 3.16% (17/538 bases) base differences in the ITS, LSU, tef-α and β-tub genes of *P. changchunensis* (CCMJ 5007) and *P. pseudosalicis* strain C301, excluding gaps. Therefore, we introduce *P. changchunensis* as a novel species, and this is the first report of *Parafenestella* on the *Populus* tree [14,15].

**Parafenestella ulmi** W.X. Su, Phukhams., & Y. Li, sp. nov. (Figure 6).

**Mycobank Number.** MB844410.

**Etyymology:** Named after the host genus *Ulmus*.

**Holotype:** HMJAU 60178.

**Description:** Saprobic on dead stems of *Ulmus pumila*.

**Sexual morph:** Ascomata 170–225 × 194–260 µm (X = 201 × 229 µm, n = 5), immersed, visible as black spots or having a convex surface, solitary, scattered, globose to ellipsoid, flat at the base, coriaceous, black. **Peridium** 19–39 µm wide, composed of 6–10 layers, outer part comprising dark brown cells of *textura angularis*, inner layer comprising thin-walled, light brown cells of *textura angularis*. **Hamathecium** of dense, 1.5–4.5 µm wide (X = 2.2 µm, n = 20), filamentous, septate, pseudoparaphyses surrounding asci. **Asci** 115–181 × 11–15 µm (X = 132 × 13 µm, n = 20), 8 ascospores, bitunicate, cylindrical, mostly curved, short-pedicellate, apically rounded with an oscular chamber, clearly visible when immature. **Ascospores** 18–24 × 8–12 µm (X = 22 × 10 µm, n = 30), uniseriate to partially overlapping, broadly ellipsoid, slightly pointed at both ends, 5–8 transversely septate, 1–2 vertically septate, mature spores constricted at the middle septum, slightly curved, initially hyaline, becoming yellowish to brown at maturity, the cell above median septum slightly wider, smooth-walled.
Figure 5. Parafenestella changchunensis (HMJAU 60182, holotype). (a) Ascomata on host surface. (b) Vertical section through partial ascoma. (c) Ostioles. (d) Partial peridium. (e) Pseudoparaphyses. (f–i) Asci. (j–v) Development stages of ascospores. (w) Germinating ascospore (x) Culture characteristics on PDA. (y) Pycnidia. (z) Hyphal coil structures formed by mycelia. (a1) Conidia. Scale bars: (a) = 500 µm; (b,c) = 100 µm; (d,e) = 20 µm; (f–i) = 50 µm; (j–v,a1) = 10 µm.

Asexual morph: Pycnidia produced in PDA after 2 weeks of incubation in the dark, mycelium greenish, 1–3 µm (μ = 2.2 µm, n = 20), uniloculate, confluent or scattered, superficial, covered with dense vegetative hyphae, globose, dark brown to black. Conidiogenous cells 18–24 × 8–12 µm (μ = 22 × 10 µm, n = 30), enteroblastic, phialidic, determinate,
conidia 3–5 × 1–2 µm (± 4.3 × 1.5 µm, n = 30), long ellipsoid to cylindrical, aseptate, with two small guttulate at the polar ends, hyaline, smooth-walled.

**Culture characteristics:** Colonies on PDA, reaching 45–48 mm diam after two weeks at 25 °C. Culture from above the center to the outer edge, the color radiating from black to dark green to yellow and white edges, with obvious concentric wheel patterns, dense intermediate hyphae and sparse white mycelium at the outer circle; reverse greenish-black, round.

**Material examined:** CHINA, Jilin Province, Changchun, Jilin Agricultural University, from Ulmus pumila (Ulmaceae) stem litter, 15 March 2021, Wexin Su and C. Phukhamsakda, S12 (HMJAU 60178, holotype); ex-type living culture, CCMJ 5001, isotype = HMJAU 60179; ex-isotype living culture, CCMJ 5002.

**GenBank accession numbers:** CCMJ5001: LSU = OL897166, SSU = OL891806, ITS = OL996115, tef1-α = OL944596, rpb2 = OL944501, and β-tub = OL898723. CCMJ5002: LSU = OL897167, ITS = OL996116, tef1-α = OL944597, rpb2 = OL944502, and β-tub = OL898717.

**Notes:** In our phylogenetic analysis, P. ulmi (CCMJ 5001 and CCMJ 5002) and P. ulmicola were found on dead branches of Ulmus pumila in Jilin Province, China, which lies in the temperate zone. Parafenestella taxa are mainly recorded in Austria, followed by England, Germany and Ukraine, which are all temperate countries [15]. Morphologically, the ascomata of P. ulmi and P. ulmicola are semi-immersed, visible as black spots or convex surfaces. The asci of P. ulmi are longer than P. ulmicola but similar in width (132 × 13 vs. 119 × 13 µm). The immature ascospores of P. ulmi present 2–3 transverse septa without longitudinal septate, but the spores have 4–8 transverse septa with 1–3 longitudinal septate at mature stages. The ascospores of P. ulmicola showed indentation when immature that disappeared during maturation. The ascospores of P. ulmicola showed 5–8 transverse septa and 1–2 vertically septate after maturity with less constriction at the septum. The ascospores of P. ulmi are yellowish to brown, while P. ulmicola have dark brown ascospores at maturity. In PDA, the colonies of P. ulmicola have wavy and aggregated colony edges. The colonies of P. ulmi are blue-black (reverse view) with back-green edges, while P. ulmicola is gray-brown with white edges.

A BLASTn search of the ITS region of P. ulmi strain CCMJ 5001 showed a high query cover and similarity (96.45%) to P. tetratrupha (CBS 145266) while the β-tub sequence of P. ulmi strain CCMJ 5001 showed a high similarity and query cover (97.07%) to P. germanica strain C307. Therefore, we introduce P. ulmi as a novel species.

**Parafenestella ulmicola** W.X. Su, Phukhams., & Y. Li, sp. nov. (Figure 7).

**Mycobank Number:** MB844411.

**Etymology:** Named after the host genus Ulmus.

**Holotype:** HMJAU 60180.

**Description:** Saprobic on twigs debris of Ulmus pumila L.

**Sexual morph:** Ascomata 242–434 × 310–462 µm (± 306 × 359 µm, n = 5) µm wide, semi-immersed, visible as a convex hemisphere, globose to subglobose, solitary or mostly aggregated, scattered, coarse-walled, coriaceous, black, with a papilla. Ostiole 21–24 µm, centrally located. Peridium 21–68 µm wide, composed of 11–20 wall layers, with dark brown cells of textura angularis. Asci 105–153 × 11–14 µm (± 119 × 13 µm, n = 20), 8 ascospores, bitunicate, fissitunicate, broadly cylindrical, apically rounded, some curved, short-pedicellate, ocular chamber is not visible at maturity. Ascospores 17–22 × 8–12 µm (± 19 × 9 µm, n = 30), uniseriate, rarely overlapping, broadly oval, blunt at both ends, narrow towards the ends, with 4–8 transversely separte, 1–3 vertically separte, constricted at the middle septum, initially hyaline, becoming yellowish to brown at maturity, smooth-walled.
Figure 6. Parafenestella ulmi (HMJAU 60178, holotype). (a) Ascomata on host surface. (b) Vertical section through ascoma. (c) Partial peridium in vertical section. (d,e) Asci arrangement along with pseudoparaphyses. (f–h) Development stages of asci. (i–s) Development stages of ascospores. (t) Germinating ascospore. (u) Four-week-old culture characteristics on PDA. (v) Pycnidia formed in sterile culture after two weeks of incubation on PDA. (w,x) Conidiogenous cells and conidia. (y) Conidia. Scale bars: (a) = 500 μm; (b) = 100 μm; (c–h) = 50 μm; (i–s) = 20 μm; (v) = 200 μm; (w–y) = 5 μm.
Asexual morph: Pycnidia produced in cultures on PDA after four weeks of incubation in the dark, mycelium greenish, 41–158 μm diam, covered with white mycelium, ellipsoid, semi-immersed, scattered or aggregated, black, ostiole central. Peridium with brown cells of textura angularis. Conidia 1.4–2.5 × 0.6–0.9 μm (x = 1.9 × 0.7 μm, n = 30), cylindrical to allantoid, hyaline, smooth, aseptate, with a minute guttulate.

Culture characteristics: Colonies on PDA reaching 35–41 mm diam after 2 weeks at 25 °C. Culture from above the center to the outer edge, the color changes from grey to white, with obvious concentric wheel patterns; a few weeks later, the outer circle hyphae grow into round dark green hyphae with a thin surface.

Material examined: CHINA, Jilin Province, Changchun, Jilin Agricultural University, from Ulmus pumila (Ulmaceae) twigs debris, 15 March 2021, Wexin Su and C. Phukham-sakda, S16 (HMJAU 60180, holotype); ex-type living culture, CCMJ 5003, isotype = HMJAU 60181; ex-isotype living culture, CCMJ 5004.

GenBank accession numbers: CCMJ5003: LSU = OL897168, SSU = OL891807, ITS = OL946117, tef1-α = OL944598, rpb2 = OL944503 and β-tub = OL898724. CCMJ5004: LSU = OL897169, ITS = OL996118, tef1-α = OL944599, rpb2 = OL944504 and β-tub = OL898719.

Notes: Sixteen Parafenestella species are listed in Species Fungorum [44], of which six species were reported on Rosaceae, four on Salicaceae and three on Betulaceae, while one species was reported on Pittosporaceae, Salicaceae and Sapindaceae [14,15,45,46]. Parafenestella ulmicola (CCMJ 5003 and CCMJ 5004) is closely related to P. ulmi (CCMJ 5001 and CCMJ 5002) within Parafenestella (ML = 100%; MP = 100%; BPP = 1.00, Figure 1). There were 2.31% (12/518) base differences in the β-tub, 0.14% (1/733) base differences in the tef1-α and 0.27% (2/736) base differences in the rpb2 gene between P. ulmicola (CCMJ 5003 and CCMJ 5004) and P. ulmi (CCMJ 5001 and CCMJ 5002), excluding gaps. There were no base differences in the ITS and LSU sequences.

Parafenestella ulmi and P. ulmicola are phylogenetically close to P. tetraprupha but differ from P. tetraprupha by having a less longitudinal septa being visible at the surface [20]. Parafenestella tetraprupha ascospores are ellipsoid, yellow-brown to reddish-brown to dark brown, with 1–3 main septa, 8–17 distinct transverse and 2–4 longitudinal septa; they are darker and longer than P. ulmi and P. ulmicola (26.5–33.5 × 13–16.5 vs. 18–24 × 8–12 vs. 17–22 × 8–12 μm) and have more transverse septa than P. ulmi and P. ulmicola (Table 2). In the multi-locus phylogenetic analysis, although P. rosacearum was divided into six groups (Figure 1), it was still identified as one species because the tef1-α sequences of C203, C283 and C309 are almost the same. The rpb2 sequences of strains C203, C315, FM1 and FP11 are identical, while C269 and C283 differ from C203, C283 and C309 by 20 nucleotides [15]. In the phylogenetic analysis, P. germanica and P. pseudoplatani clustered in the same clade as P. parasalicum and P. salicium. These strains were identified as different species due to morphological distinctiveness [15]. The ascospores of P. germanica were larger than P. pseudoplatani (29–39.5 × 13–16.5 vs. 25–29 × 12–14 μm). The ascospores of P. parasalicum were larger than P. salicium (36–44 × 15.8–19.3 vs. 27–33 × 12.5–16 μm) (Table 3). There were 0.40% (2/494) base differences in the ITS, 2.28% (16/701) base differences in β-tub, 0.51% (4/789) base differences in tef1-α and 1.41% (15/1063) base differences in rpb2 between P. germanica and P. pseudoplatani. There were 3.42% (24/701) base differences in β-tub, 1.90% (15/789) base differences in tef1-α and 1.32% (14/1063) base differences in rpb2 between P. parasalicum and P. salicium. Thus, the species boundaries of P. ulmi and P. ulmicola were justified based on their distinct morphological traits and nucleotides differences. Therefore, we introduce P. ulmicola as a novel species, and this is first report of Parafenestella on Ulmus trees.
Figure 7. *Parafenestella ulmicola* (HMJAU 60180, holotype). (a) Ascomata on host surface. (b) Vertical section through ascoma. (c) Ostiole. (d) Partial peridium wall. (e) Pseudoparaphyses. (f–h) Asci. (i–s) Developmental stages of ascospores. (t) Germinating ascospore. (u) Pycnidia produced in four weeks old cultures on PDA. (v) Conidiomata. (w) Conidia. (x) Four weeks old culture on PDA. Scale bars: (b) = 100 μm; (c) = 50 μm; (d,e) = 20 μm; (f–h) = 50 μm; (i–s) = 10 μm; (u) = 200 μm; (v) = 100 μm; (w) = 5 μm.
### Table 2. The dataset used for phylogenetic analysis. The type-derived sequences are in bold.

| Taxon                        | Strain          | Host/Substrate                  | Typification Status | GenBank Accession Numbers | GenBank Accession Numbers | GenBank Accession Numbers | GenBank Accession Numbers | GenBank Accession Numbers | GenBank Accession Numbers |
|------------------------------|-----------------|---------------------------------|--------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| *Allocucurbitaria botulispora* | CBS 142452      | human scab on leg               | Holotype           | LT592932                  | LN907416                  | LT593070                  | –                         | LT593001                  |                           |
| *Allocucurbitaria mori*      | HMJAU 60183      | *Morus alba*                    | Holotype           | OL996120                  | OL897171                  | OL944505                  | OL944601                  | OL898725                  |                           |
| *Allocucurbitaria mori*      | HMJAU 60184      | *Morus alba*                    | Isotype            | OL996121                  | OL897172                  | OL944506                  | OL944602                  | OL898720                  |                           |
| *Astragalicola amorpha*       | CBS 142999      | *Astragalus angustifolius*      | Holotype           | MF795753                  | MF795753                  | MF795795                  | MF795842                  | MF795883                  |                           |
| *Cucitella opali*            | CBS 142405      | *Acer opalus*                   | Holotype           | MF795754                  | MF795754                  | MF795796                  | MF795843                  | MF795884                  |                           |
| *Cucurbitaria berberidis*    | C39             | *Berberis vulgaris*             | –                  | MF795755                  | MF795755                  | MF795797                  | MF795844                  | MF795885                  |                           |
| *Cucurbitaria berberidis*    | CB              | *Berberis vulgaris*             | –                  | MF795757                  | MF795757                  | MF795799                  | MF795846                  | MF795887                  |                           |
| *Cucurbitaria berberidis*    | CBS 130007 = CB1 | *Berberis vulgaris*            | Epitype            | MF795758                  | MF795758                  | MF795800                  | –                         | –                         |                           |
| *Cucurbitaria oromediterranea* | CBS 142401 = C241 | *Berberis sp.*                | –                  | MF795756                  | MF795756                  | MF795798                  | MF795845                  | MF795886                  |                           |
| *Cucurbitaria oromediterranea* | C265            | *Berberis aetnensis*           | –                  | MF795762                  | MF795762                  | MF795804                  | MF795850                  | MF795891                  |                           |
| *Cucurbitaria oromediterranea* | C29             | *Berberis hispanica*           | –                  | MF795759                  | MF795759                  | MF795801                  | MF795847                  | MF795888                  |                           |
| *Cucurbitaria oromediterranea* | C86             | *Berberis hispanica*           | –                  | MF795760                  | MF795760                  | MF795802                  | MF795848                  | MF795889                  |                           |
| *Cucurbitaria oromediterranea* | CB2             | *Berberis cretica*             | –                  | MF795763                  | MF795763                  | MF795805                  | MF795851                  | MF795892                  |                           |
| *Cucurbitaria oromediterranea* | CB3             | *Berberis hispanica*           | –                  | MF795764                  | MF795764                  | MF795806                  | MF795852                  | –                         |                           |
| *Cucurbitaria oromediterranea* | CBS 142399 = C229 | *Berberis cretica*            | Holotype           | MF795761                  | MF795761                  | MF795803                  | MF795849                  | MF795890                  |                           |
| *Fenestella crataegi*        | C287            | *Crataegus monogyna*           | –                  | MK356281                  | MK356281                  | MK357554                  | –                         | MK357598                  |                           |
| *Fenestella crataegi*        | CBS 144857 = C314 | *Crataegus monogyna*          | Epitype            | MK356282                  | MK356282                  | MK357512                  | MK357555                  | MK357599                  |                           |
| Taxon                    | Strain        | Host/Substrate | Typification Status | GenBank Accession Numbers |
|-------------------------|---------------|----------------|---------------------|--------------------------|
|                         |               |                |                     | ITS          | LSU          | rpb2        | tef1-α      | β-tub        |
| *Fenestella fenestrata* | CBS 143001 = FP9 | *Alnus glutinosa* | Epitype             | MF795765   | MF795765   | MF795807   | MF795853   | MF795893   |
| *Fenestella gardienneti* | CBS 144859 = FM | *Acer saccharum* | Holotype            | MK356283   | MK356283   | MK357513   | MK357556   | MK357600   |
| *Fenestella granatensis* | CBS 144854 = C279 | *Acer granatense* | Holotype            | MK356284   | MK356284   | MK357514   | MK357557   | MK357601   |
| *Fenestella media*      | CBS 144860 = FP | *Corylus avellana* | Epitype             | MK356285   | MK356285   | MK357515   | MK357558   | MK357602   |
| *Fenestella media*      | FCO           | *Carpinus orientalis* | –                   | MK356286   | MK356286   | MK357516   | MK357559   | –           |
| *Fenestella media*      | FP1           | *Corylus avellana* | –                   | MK356287   | MK356287   | MK357517   | MK357560   | MK357603   |
| *Fenestella media*      | FP3           | *Acer pseudoplatanus* | –                   | MK356288   | MK356288   | MK357518   | MK357561   | MK357604   |
| *Fenestella media*      | FP7           | *Castanea sativa* | –                   | MK356289   | MK356289   | MK357519   | MK357562   | MK357605   |
| *Fenestella media*      | FP10          | *Tilia cordata* | –                   | MK356290   | MK356290   | MK357520   | MK357563   | MK357606   |
| *Fenestella parafenestrata* | CBS 144856 = C306 | *Quercus robur* | Holotype            | MK356291   | MK356291   | MK357521   | MK357564   | MK357607   |
| *Fenestella parafenestrata* | C317           | *Salix sp.* | –                   | MK356292   | MK356292   | MK357522   | MK357565   | MK357608   |
| *Fenestella subsymmetrica* | CBS 144861 = FP6 | *Acer campestre* | Holotype            | MK356297   | MK356297   | MK357525   | MK357569   | MK357610   |
| *Fenestella subsymmetrica* | C285           | *Juglans regia* | –                   | MK356293   | MK356293   | MK357523   | MK357566   | –           |
| *Fenestella subsymmetrica* | C286           | *Juglans regia* | –                   | MK356294   | MK356294   | –           | MK357567   | –           |
| *Fenestella subsymmetrica* | C286x          | *Juglans regia* | –                   | MK356295   | MK356295   | –           | –           | –           |
| *Fenestella subsymmetrica* | FP4            | *Corylus avellana* | –                   | MK356296   | MK356296   | MK357524   | MK357568   | MK357609   |
| *Fenestella subsymmetrica* | FP8            | *Salix caprea* | –                   | MK356298   | MK356298   | MK357526   | MK357570   | MK357611   |
| *Fenestella viburni*    | CBS 144863 = FVL | *Viburnum lantana* | Holotype            | MK356300   | MK356300   | MK357528   | MK357572   | MK357613   |
Table 2. Cont.

| Taxon                  | Strain     | Host/Substrate                  | Typification Status | GenBank Accession Numbers |
|------------------------|------------|---------------------------------|---------------------|---------------------------|
|                        |            |                                 |                     | ITS | LSU | rpb2 | tef1-α | β-tub   |
| *Fenestella viburni*   | FP2        | *Viburnum lantana*              | –                   | MK356299 | MK356299 | MK357527 | MK357571 | MK357612 |
| *Neocucurbitaria*      | CBS 142398 = C225 | *Genista acanthoclada*               | Holotype            | MF795766 | MF795766 | MF795808 | MF795854 | MF795894 |
| *acanthocladae*        |            |                                 |                     |     |     |     |     |       |
| *Neocucurbitaria*      | C26a       | *Acer pseudoplatanus*            | –                   | MF795767 | MF795767 | MF795809 | MF795855 | MF795895 |
| *acerina*              | CBS 142403 = C255 | *Acer pseudoplatanus*                 | –                   | MF795768 | MF795768 | MF795810 | MF795856 | MF795896 |
| *Neocucurbitaria*      | CBS 142404 = C261 | *Genista aetnensis*               | Holotype            | MF795769 | MF795769 | MF795811 | MF795857 | MF795897 |
| *aetnensis*            | C270       | *Genista aetnensis*              | –                   | MF795770 | MF795770 | MF795812 | MF795858 | MF795898 |
| *Neocucurbitaria*      | CBS 297.74 | Sea water                       | Holotype            | LT623221 | EU754177 | LT623278 | –       | LT623238 |
| *aquatica*             |            |                                 |                     |     |     |     |     |       |
| *Neocucurbitaria*      | CBS 115979 | –                               | –                   |–     |–     |–    |–     |–       |
| *cava*                 | CBS 257.68 | Wheat-field soil                 | Epitype             | JF740260 | EU754199 | LT717681 | –       | KT389844 |
| *Neocucurbitaria*      | CBS 142406 = KU9 | *Genista cinerea*               | Holotype            | MF795771 | MF795771 | MF795813 | MF795859 | MF795899 |
| *cinerea*              | CBS 142402 = C244 | *Cistus monspeliensis*            | Holotype            | MF795772 | MF795772 | MF795814 | MF795860 | MF795900 |
| *Neocucurbitaria*      | CBS 142109 = CPC 28920 | *Hakea*                       | Holotype            | KY173436 | KY173526 | KY173593 | –       | KY173613 |
| *hakeae*               |            |                                 |                     |     |     |     |     |       |
| *Neocucurbitaria*      | CBS 142791 | Subcutaneous tissue from injured human arm | Holotype            | LT592916 | LN907372 | LT593054 | –       | LT592985 |
| *irregularis*          | C316       | *Quercus rubra*                  | –                   | MK356301 | MK356301 | MK357529 | MK357573 | MK357614 |
| *Neocucurbitaria*      | CBS 142390 = BW6 | *Juglans regia*                  | Holotype            | MF795773 | MF795773 | MF795815 | MF795861 | MF795901 |
| *juglandicola*         |            |                                 |                     |     |     |     |     |       |
| *Neocucurbitaria*      | CBS 121759 | From human corneal scrapings (keratitis) | Holotype            | EU885415 | LT623215 | LT623275 | –       | LT623236 |
| *keratinophila*        |            |                                 |                     |     |     |     |     |       |
Table 2. Cont.

| Taxon                      | Strain          | Host/Substrate       | Typification Status | GenBank Accession Numbers |
|----------------------------|-----------------|----------------------|---------------------|---------------------------|
| Neocucurbitaria populi     | CBS 142393 = C28| Populus sp.          | Holotype            | MF795774                  |
|                            |                 |                      |                     | MF795774                  |
| Neocucurbitaria prunicola  | CBS 145033      | Prunus padus         | –                   | MK442594                  |
|                            |                 |                      |                     | MK442594                  |
| Neocucurbitaria quercina   | CBS 115095      | Quercus robur        | Neotype             | LT623220                  |
|                            |                 |                      |                     | GQ387619                  |
| Neocucurbitaria rhamni     | CBS 142391 = C1 | Rhamnus frangula     | Epitype             | MF795775                  |
|                            |                 |                      |                     | MF795775                  |
| Neocucurbitaria rhamni     | C112            | Rhamnus frangula     | –                   | MF795776                  |
|                            |                 |                      |                     | MF795776                  |
| Neocucurbitaria rhamni     | C133            | Rhamnus frangula     | –                   | MF795777                  |
|                            |                 |                      |                     | MF795777                  |
| Neocucurbitaria rhamni     | C190            | Rhamnus frangula     | –                   | MF795778                  |
|                            |                 |                      |                     | MF795778                  |
| Neocucurbitaria rhamni     | C277            | Rhamnus saxatilis    | –                   | MF795779                  |
|                            |                 |                      |                     | MF795779                  |
| Neocucurbitaria rhamnicola| CBS 142396 = C185| Rhamnus lycioides   | Holotype            | MF795780                  |
|                            |                 |                      |                     | MF795780                  |
| Neocucurbitaria rhamnicola| KRx             | Rhamnus alaternus    | –                   | MF795781                  |
|                            |                 |                      |                     | MF795781                  |
| Neocucurbitaria rhamnioides| C222           | Rhamnus saxatilis subsp. prunifolius | – | MF795783 |
|                            |                 |                      |                     | MF795783                  |
| Neocucurbitaria rhamnioides| C223           | Rhamnus saxatilis subsp. prunifolius | – | MF795784 |
|                            |                 |                      |                     | MF795784                  |
| Neocucurbitaria rhamnioides| CBS 142395 = C118| Rhamnus myrtifolius | Holotype            | MF795782                  |
|                            |                 |                      |                     | MF795782                  |
| Neocucurbitaria ribicola   | CBS 142394 = C55| Ribes rubrum         | Holotype            | MF795785                  |
|                            |                 |                      |                     | MF795785                  |
| Neocucurbitaria ribicola   | C155            | Ribes rubrum         | –                   | MF795786                  |
|                            |                 |                      |                     | MF795786                  |
| Neocucurbitaria unguis-hominis| CBS 111112   | Agapornis sp.        | –                   | LT623222                  |
|                            |                 |                      |                     | GQ387623                  |

Note: The table continues with additional entries for GenBank Accession Numbers.
Table 2. Cont.

| Taxon                     | Strain               | Host/Substrate                     | Typification Status | GenBank Accession Numbers |
|--------------------------|----------------------|------------------------------------|---------------------|---------------------------|
|                          |                      |                                    |                     | ITS          | LSU          | rpb2        | tef1-α       | β-tub       |
| **Neocucurbitaria vachelliae** | CBS 142397 = C192   | Vachellia gummitfera               | Holotype            | MF795787    | MF795787    | MF795829    | MF795875    | MF795913    |
| **Paracucurbitaria italica** | CBS 234.92          | Olea europaea                      | Holotype            | LT623219    | EU754176    | LT623274    | –            | LT623235    |
| **Paracucurbitaria riggenbachii** | CBS 248.79          | Fraxinus excelsior with bacterial canker | Holotype            | LT903672    | GQ387608    | LT903673    | –            | LT900365    |
| **Parafenestella alpina** | CBS 145263 = C198   | Cotoneaster integerrimus           | Holotype            | MK356302    | MK356302    | MK357530    | MK357574    | MK357615    |
| **Parafenestella alpina** | C249                 | Salix appendiculata                | –                   | MK356303    | MK356303    | MK357531    | MK357575    | MK357616    |
| **Parafenestella austriaca** | CBS 145262 = C152   | Rosa canina                        | Holotype            | MK356304    | MK356304    | MK357532    | MK357576    | MK357617    |
| **Parafenestella changchunensis** | HMJAU 60182        | Populus L.                         | Holotype            | OL996119    | OL897170    | –            | OL944600    | OL898719    |
| **Parafenestella faberi** | MFLUCC 16-1451      | Rosa canina                        | Holotype            | KY563071    | KY563074    | –            | –            | –            |
| **Parafenestella germanica** | CBS 145267 = C307   | Corylus avellana                   | Holotype            | MK356305    | MK356305    | MK357533    | MK357577    | MK357618    |
| **Parafenestella ostryae** | MFLU 16-0184        | Ostrya carpinifolia                | –                   | KY563072    | KY563075    | –            | –            | –            |
| **Parafenestella pittospori** | CPC 34462           | Pittosporum tenuifolium            | Holotype            | MN562098    | MN567606    | –            | –            | –            |
| **Parafenestella pseudoplatani** | CBS 142392 = C26   | Acer pseudoplatanus                | Holotype            | MF795788    | MF795788    | MF795830    | MF795876    | MF795914    |
| **Parafenestella pseudosalicis** | CBS 145264 = C301  | Salixcf. alba                      | Holotype            | MK356307    | MK356307    | MK357535    | MK357579    | MK357620    |
| **Parafenestella rosacearum** | CBS 145268 = C309   | Pyracantha coccinea                | Holotype            | MK356311    | MK356311    | MK357539    | MK357583    | MK357624    |
| **Parafenestella rosacearum** | C203                | Pyrus communis                     | –                   | MK356308    | MK356308    | MK357536    | MK357580    | MK357621    |
Table 2. Cont.

| Taxon                     | Strain | Host/Substrate       | Typification Status | GenBank Accession Numbers |
|---------------------------|--------|----------------------|---------------------|---------------------------|
|                           |        |                      | ITS LSU rpb2 tef1-α β-tub |
| Parafenestella rosacearum| C269   | Crataegus monogyna   | –                   | MK356309 MK356309 MK357537 MK357581 MK357622 |
| Parafenestella rosacearum| C283   | Pyrus communis       | –                   | MK356310 MK356310 MK357538 MK357582 MK357623 |
| Parafenestella rosacearum| C315   | Rosa canina          | –                   | MK356312 MK356312 MK357540 MK357584 MK357625 |
| Parafenestella rosacearum| C320   | Sorbus aria          | –                   | MK356315 MK356315 MK357543 MK357587 – |
| Parafenestella rosacearum| CBS 145272 = FP11 | Prunus domestica | –                   | MK356314 MK356314 MK357542 MK357586 MK357627 |
| Parafenestella rosacearum| FM1    | Rosa canina          | –                   | MK356313 MK356313 MK357541 MK357585 MK357626 |
| Parafenestella salicis    | CBS 145270 = C313 | Salix alba         | Neotype             | MK356317 MK356317 MK357545 MK357589 MK357629 |
| Parafenestella salicis    | C303   | Salix alba           | –                   | MK356316 MK356316 MK357544 MK357588 MK357628 |
| Parafenestella salicic    | CBS 145269 = C311 | Salix alba         | Holotype            | MK356318 MK356318 MK357546 MK357590 MK357630 |
| Parafenestella tetratrupha| CBS 145266 = C304 | Alnus glutinosa     | Epitype             | MK356319 MK356319 MK357547 MK357591 MK357631 |
| Parafenestella ulmi       | HMJAU 60178 | Ulmus pumilaL.   | Holotype            | OL996115 OL97166 OL944501 OL944596 OL898723 |
| Parafenestella ulmi       | HMJAU 60179 | Ulmus pumilaL.   | Isotype             | OL996116 OL97167 OL944502 OL944597 OL898717 |
| Parafenestella ulmicola   | HMJAU 60180 | Ulmus pumilaL.   | Holotype            | OL996117 OL97168 OL944503 OL944598 OL898724 |
| Parafenestella ulmicola   | HMJAU 60181 | Ulmus pumilaL.   | Isotype             | OL996118 OL97169 OL944504 OL944599 OL898718 |
| Parafenestella vindobonensis | CBS 145265 = C302 | Salix babylonica   | Holotype            | MK356320 MK356320 MK357548 MK357592 MK357632 |
| Protofenestella ulmi      | CBS 143000 = FP5 | Ulmus minor    | Holotype            | MF795791 MF795791 MF795833 MF795879 MF795915 |
Table 2. Cont.

| Taxon                      | Strain                  | Host/Substrate                              | Typification Status | GenBank Accession Numbers |
|----------------------------|-------------------------|---------------------------------------------|---------------------|---------------------------|
|                            |                         |                                             |                     | ITS | LSU | rpb2 | tef1-α | β-tub |
| Pyrenochaeta nobilis       | CBS 407.76 = AFTOL-ID 1856 | Laurus nobilis leaves                       | Neotype             | MF795792 | MF795792 | MF795834 | MF795880 | MF795916 |
| Pyrenochaetopsis americana | UTHSC DI16-225          |                                             | Holotype            | LT592912 | LN907368 | LT593050 | –        | LT592981 |
| Pyrenochaetopsis confluens | CBS 142459              | Deep tissue/ fluids from human blood sample | Holotype            | LT592950 | LN907446 | LT593089 | –        | LT593019 |
| Seltsamia galinosogisoli   | CBS 140956 = CGMCC 3.17981 = SYPF 7336 | Soil of a Galinsoga parvisflora | Epitype             | KU759584 | KU759581 | –        | –        | –        |
| Seltsamia sp.              | EAB-67-11b              | Emerald ash borer                           | –                   | MT777389 | –       | –        | –        | –        |
| Seltsamia sp.              | SGSF207                 |                                             | –                   | MK192899 | –       | –        | –        | –        |
| Seltsamia ulmi             | CBS 143002 = L150      | Ulmus glabra                                | Holotype            | MF795794 | MF795794 | MF795836 | MF795882 | MF795918 |
| Synfenestella pyri         | CBS 144855 = C297     | Pyrus communis                              | Holotype            | MK356321 | MK356321 | MK357549 | MK357593 | MK357633 |
| Synfenestella sorbi        | C298                    | Sorbus aucuparia                            | –                   | MK356325 | MK356325 | MK357553 | MK357597 | MK357636 |
| Synfenestella sorbi        | CBS 144858 = C196     | Sorbus aucuparia                            | Holotype            | MK356324 | MK356324 | MK357552 | MK357596 | MK357635 |
| Synfenestella sorbi        | CBS 144862 = FR        | Sorbus aucuparia                            | Epitype             | MK356322 | MK356322 | MK357550 | MK357594 | MK357634 |
| Synfenestella sorbi        | FRa                     | Sorbus aucuparia                            | –                   | MK356323 | MK356323 | MK357551 | MK357595 | –        |
| Taxon      | Ascomata                                                                 | Sexual Morph                                                                 | Ascospores                                                                 |
|-----------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------|
|           |                                                                           | Asci                                                                       | 24–30.5 × 12–14 µm, typically ellipsoid to fusoid often inequilateral, pale or yellowish-brown, eventually dark brown, with 7–15 transverse and 2–4 longitudinal septa. |
| P. alpina | 240–375 µm diam, globose, subglobose or pyriform, usually tightly aggregated in bark on a perithecial host fungus in small numbers, with brown to black, subicular hyphae. | 170–208 × 18.5–21.5 µm, cylindrical to oblong, a short stipe and simple or knob-like base, containing 6–8 ascospores in uniseriate arrangement. | 27–32.5 × 13–15 µm, broadly ellipsoid, symmetric, dark brown or dark reddish-brown, with 9–14 distinctly spaced transverse and 3–5 longitudinal septa. |
| P. austriaca | 283–431 µm diam, subglobose to pyriform, scattered or aggregated, basally and laterally surrounded by subhyaline to dark brown subicular hyphae. | 159–205 × 16–19.5 µm, cylindrical, with a short stipe and simple or knob-like base, containing 4–8 ascospores in uniseriate arrangement. | 18–25 × 8–13 µm, fusiform to oval, light yellow to dark brown, developing 2 main septa, 4–6 transverse septa, 2–3 longitudinal septa. |
| P. changchunensis | 280 × 353 µm, globose to depressed globose, solitary or aggregated forming visible black bumps submerged under bark. | 95–138 × 16–21 µm, broad cylindrical, short-pedicellate, curved, some curved, 6–8 spores ocular chamber is not visible at maturity, uniseriate arrangement. | 28.5–36 × 12.5–16 µm, variable in shape, pale or yellowish-brown to dark brown, with 1–4 main septa, 7–14 transverse and 1–5 longitudinal septa. |
| P. faberi | 300–500 µm diam, tightly or loosely aggregated in small numbers, with ostiolar, partly erumpent through bark fissures, maxing with Cytospora species. | 135–180 × 18.5–23.5 µm, cylindrical to oblong or narrowly clavate, a short stipe and simple or knob-like base, 4–8 ascospores in uniseriate to partly biseriate arrangement. | 29–39.5 × 13–16.5 µm, ellipsoid to broadly fusoid, turning yellow to yellow-brown to dark brown, with 1–3 main septa, 8–15 transverse and 3–6 longitudinal septa. |
| P. germanica | 230–450 µm diam, black, solitary or in small groups on inner bark or on the ostiolar level of old Diaporthe decedens. | 140–173 × 17.5–22 µm, cylindrical to oblong, with a short stipe and simple or knob-like base, containing 2–8 ascospores (obliquely or overlapping), uniseriate arrangement. | 36–44 × 15.8–19.3 µm, fusoid or ellipsoid, yellow-brown to dark brown, with 2 main septa, 11–16 distinct transverse septa and 3–5 longitudinal septa. |
| P. parasalicum | 270–400 µm diam, immersed in bark, globose, subglobose or pyriform, forming groups, maxing with Cytospora species. | 185–219 × 22–27 µm, cylindrical to oblong, with a short stipe and simple or knob-like base, containing 4–8 ascospores (overlapping, obliquely), uniseriate to partly biseriate arrangement. | 25–29 × 12–14 µm, ellipsoid, yellow-brown to dark brown, with 1–3 main septa, 7–11 transverse and 2–4 longitudinal septa, with minute guttules. |
| P. pseudosalicis | 300–400 diam, subglobose to subpyriform, immersed in bark or on ascomata of an effete perithecial fungus, often with concave apex, covered with subicular hyphae. | 186–215 × 17.5–19 µm, cylindrical to oblong, with a short stipe and simple or knob-like base, containing 4–8 ascospores in uniseriate arrangement. | 25–29 × 12–14 µm, ellipsoid, yellow-brown to dark brown, with 1–3 main septa, 7–11 transverse and 2–4 longitudinal septa, with minute guttules. |
Table 3. Cont.

| Taxon             | Ascomata                                                                                                                                  | Sexual Morph                                                                                       | Ascospores                                                                                       |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| *P. rosacearum*   | 285–432 µm diam, globose, subglobose to subpyriform, immersed on often blackened inner bark, scattered or in small groups, erumpent through bark fissures. | 181–240 × 19–22 µm, cylindrical to oblong, with a short-contorted stipe and simple or knob-like base, containing 2–8 ascospores in uniseriate, rarely partly biseriate arrangement. | 28–35 × 13.5–16.5 µm, ellipsoid, symmetric to inequilateral, yellow-brown to dark brown, with 1–3 main septa, 7–15 transverse and 2–5 longitudinal septa. |
| *P. salicis*      | 275–442 µm diam, globose, subglobose to pyriform or subconical, immersed below the epidermis on inner bark, partly erumpent through bark fissures. | 141–188 × 16–19 µm, cylindrical to oblong, with a short stipe and simple or knob-like base, containing 1–8 ascospores in (obliquely) uniseriate to partly biseriate arrangement. | 23–29 × 11–13.5 µm, ellipsoid to fusoid, symmetric, golden yellow-brown (when fresh) to dark brown, with 1–3 main septa, 5–11 transverse and 1–3 longitudinal septa. |
| *P. salicum*      | 270–420 diam, globose, subglobose or pyriform, immersed in bark, the inner bark layers connected to the host, scattered or aggregate, cover with subicular hyphae. | 181–228 × 19.5–24 µm, cylindrical, with a short stipe and simple or knob-like base, containing 6–8 ascospores in (overlapping) uniseriate arrangement. | 27–33 × 12.5–16 µm, broadly ellipsoid to broadly fusoid, first 2-celled and hyaline, turning golden yellow to dark brown or dark reddish-brown, with 9–14 transverse and 3–4 longitudinal septa. |
| *P. tetratrupha*  | 300–500 µm diam, globose, subglobose or pyriform, immersed, tightly or loosely aggregated in whitish to dark brown subiculum, erumpent through fissures. | 154–229 × 18.5–22.2 µm, cylindrical, with a short stipe and simple or knob-like base, containing 2–8 ascospores in uniseriate arrangement. | 26.5–33.5 × 13–16.5 µm, ellipsoid, yellow-brown to reddish-brown to dark brown, with 1–3 main septa, 8–17 distinct transverse and 2–4 longitudinal septa. |
| *P. ulmi*         | 170–225 × 194–260 µm, globose to ellipsoid, immersed under the host epidermis, visible as black spots or having a convex surface. | 115–181 × 11–15 µm, cylindrical, mostly curved, short-pedicellate, containing 8 ascospores, uniseriate to partially overlapping. | 18–24 × 8–12 µm, broadly ellipsoid, yellowish to brown, with 5–8 transversely septate, 1–2 longitudinal septa. |
| *P. ulmicola*     | 242–434 × 310–462 µm, globose to subglobose, on the surface, semi-immersed, visible as a convex hemisphere, with a papilla. | 105–153 × 11–14 µm, broad cylindrical, some curved, short-pedicellate, containing 8 ascospores, short-pedicellate, uniseriate, rarely overlapping. | 17–22 × 8–12 µm, broadly oval, yellowish to brown, with 4–8 transversely septate and 1–3 vertical septate. |
| *P. vindobonensis*| 308–425 µm diam, globose, subglobose or pyriform, immersed in bark, partially erumpent, tightly aggregated in small groups on inner bark mixing with pseudostromata of a *Cytospora* sp. | 179–214 × 13.5–15.5 µm, cylindrical, with a short stipe and simple or knob-like base, containing 4–8 ascospores in uniseriate arrangement. | 24.5–30.5 × 9.5–11 µm, oblong, fusoid or narrowly ellipsoid, turning yellowish to medium brown, 1–6 main septa, when mature with 7–11 thick transverse and 1–3 septa, containing minute droplets. |
4. Discussion

The family 

\[ Cucurbitariaceae \]

was introduced by Winter [12] and typified by 

\[ Cucurbitaria berberidis \] (Pers.) Gray [46]. Members of this family occur worldwide and are commonly recorded in Austria, Germany, England and Ukraine as saprobic or necrotrophic on various substrates including plant debris, soil and wood [14,15,47]. Although ribosomal markers and the ITS region are important for phylogenetic analyses, other loci are often needed for better resolution at the species level [48–51]. The ITS region can have low support values on key evolutionary nodes and cannot be used to accurately classify species in most genera [52,53]. Housekeeping genes and protein-coding genes such as \( \alpha\text{-tub} \), \( \beta\text{-tub} \), \( \text{cal} \), \( \text{gapdh} \), \( \text{rpb}2 \) and \( \text{tef}1\text{-}\alpha \) are thus usually recommended for a stable and reliable topology in phylogenetic analyses [54–56].

In this study, ASAP [38] was used to determine the most informative loci for 

\[ Parafenestella \]. The \( \beta\text{-tub} \) gene provided the best species level identification of 

\[ Parafenestella \], followed by \( \text{rpb}2 \), \( \text{tef}1\text{-}\alpha \), ITS and LSU based on ASAP analyses (Figure 2, Figures S8–S11). ASAP analyses based on the \( \beta\text{-tub} \) gene provided the best resolution of 

\[ P. ulmi \] and 

\[ P. ulnicola \], in addition to 

\[ P. changchunensis \], 

\[ P. pseudosalis \] and 

\[ P. salis \] (Figure 2). The ITS region is an important marker; however, it could not delineate between 

\[ P. pseudoplantani \] (CBS 142392), 

\[ P. parasallicum \] (CBS 145271), 

\[ P. salicum \] (CBS 145269), 

\[ P. australica \] (CBS 145262), 

\[ P. germanicola \] (CBS 145267) and 

\[ P. rosacearum \] (C203, C269, C283, C315, C320, CBS 145272, CBS 145268, FM1) as they were recovered as a group in ASAP analysis. In the ASAP analysis of the \( \beta\text{-tub} \) gene, this clade was divided into seven groups: (1) 

\[ P. australica \] (CBS 145262), 

(2) 

\[ P. germanicola \] (CBS 145267), 

(3) 

\[ P. rosacearum \] (C269, C283, C315, FM1), (4) 

\[ P. rosacearum \] (CBS 145272, CBS 145268) and 

\[ P. rosacearum \] (C203), (5) 

\[ P. pseudoplantani \] (CBS 142392), (6) 

\[ P. parasalicum \] (CBS 145271) and (7) 

\[ P. salicum \] (CBS 145269) (Figure 2). The \( \beta\text{-tub} \) gene exists in all eukaryotes and is involved in the formation of the spindle during cell division [57]. \( \beta\text{-tubulin} \) plays an important role in defining the characteristics of species [58]. The ASAP analysis of the \( \beta\text{-tub} \) gene likely reflects the interspecific relationship within 

\[ Parafenestella \]. Thus, we encourage the inclusion of \( \beta\text{-tub} \) in the phylogenetic studies of 

\[ Parafenestella \] species. This result is also supported by the phylogeny of single genes, two loci datasets (ITS + \( \beta\text{-tub} \), Figure S14); ITS + \( \text{rpb}2 \), Figure S12); ITS + \( \text{tef}1\text{-}\alpha \), Figure S13) and multi-loci dataset (Figures S7 and S11).

Valenzuela-Lopez et al. [58] established 

\[ Allocurbitaria \] in 

\[ Cucurbitariaceae \] based on morphological and phylogenetic analysis. 

\[ Allocurbitaria botulispora \] (CBS 142452) was classified as 

\[ Pyrenochaeta \] species [43]. Valenzuela-Lopez et al. [41] examined the morphology of 

\[ Pyrenochaeta \] and suggested that 

\[ A. botulispora \] was more similar to phoma-like taxa. As it clustered in 

\[ Cucurbitariaceae \], the authors classified the species under the genus 

\[ Allocurbitaria \] within 

\[ Cucurbitariaceae \] [41]. 

\[ Seltsamia \] was introduced with the unique characteristics of pleomassaria-like fungus [14]. There is no confirmed report of the holomorph character of the type species (\( S. ulmi \)), and thus the generic status is constrained. Three species of 

\[ Allocurbitaria \] are listed in Species Fungorum [44], with one species reported on 

\[ Ulmus glabra \] in Norway, one species from soil in China and one species reported from diseased human scab in the USA [41,59]. Notably, the 

\[ Allocurbitaria \] strains can be saprophyte and can harbor soil and/or opportunistic fungal disease in humans [41–43]. We provide the first report of 

\[ Allocurbitaria \] on dead twigs of 

\[ Populus morus \].

\[ Parafenestella \] is the fourth most speciose genera in 

\[ Cucurbitariaceae \] (\( Cucurbitaria \) 94 species; 

\[ Fenestella \] 28 species; 

\[ Neocucurbitaria \] 21 species; 

\[ Parafenestella \] 14 species; 

\[ Synccarpella \] 7 species; 

\[ Rhytidella \] 4 species; 

\[ Allocurbitaria \] 2 species; 

\[ Astragalicola \] 2 species; 

\[ Paracucurbitaria \] 2 species; 

\[ Synfenestella \] 2 species; 

\[ Cucitella \] 1 species; 

\[ Protofenestella \] 1 species; 

\[ Seltsamia \] 1 species) [44]. 

\[ Parafenestella \] species are commonly distributed over temperate areas including northeast China but are rarely found in the tropical regions [11,13]. All three novel species in this study were collected during early spring in Changchun, Jilin Province, China. Jilin Province (40°52′–46°18′ N) belongs to a temperate continental climate, and the study of similar vegetation from similar climates is likely to result in many 

\[ Parafenestella \] taxa [60]. We speculate that extensive investigations in the temperate regions would result
in numerous Parafenestella members. Climate conditions also affect the infection degree of Cucurbitariaceae fungi to hosts, as temperatures below 0 °C may stop fungal development [15]. The age of the host including branch size and thickness may also affect the development of Cucurbitariaceae [15].

Parafenestella is characterized by immersed to erumpent and aggregated or clusters of ascomata [15]. The number of ascomata in Parafenestella (as a cluster) is often less than 10, which is higher than in Fenestella and Synfenestella [14,15]. Parafenestella does not form distinct pseudostromata, while Fenestella forms a pustular pseudostroma appearing as bumps, and Synfenestella forms conspicuous pseudostromatic pustules on pseudostromata [15]. The ascospores of Parafenestella are irregularly arranged and partially overlapping, while the ascospores of Fenestella and Synfenestella are borne in a uniseriate arrangement [14,15]. The sexual morph of Cucurbitariaceae is usually found on the wood and bark of trees and shrubs (Corylus avellana, Prunus domestica, Rosa canina, Sorbus aucuparia) [15]. The asexual morph of Parafenestella has not been reported from the natural host and is successfully produced only in culture [14,15]. However, pycnidia in artificial culture often lack conidiophores, which could be due to environmental conditions [61].

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jof8090905/s1, Figure S1: The best-scoring RAxML tree based on a concatenated ITS dataset. Figure S2: The best-scoring RAxML tree based on a concatenated LSU dataset. Figure S3: The best-scoring RAxML tree based on a concatenated rpb2 dataset. Figure S4: The best-scoring RAxML tree based on a concatenated tef1-a dataset. Figure S5: The best-scoring RAxML tree based on a concatenated tub2 dataset. Figure S6: The best-scoring RAxML tree based on a concatenated ITS, LSU, rpb2, tef1-a and tub2 dataset. Figure S7: Phylogram generated from maximum parsimony analysis based on combined ITS, LSU, rpb2, tef1-a and tub2 dataset. Figure S8: Phylogram generated from ASAP analysis using LSU sequence data. Figure S9: Phylogram generated from ASAP analysis using rpb2 sequence data. Figure S10: Phylogram generated from ASAP analysis using tef1-a sequence data. Figure S11: Phylogram generated from ASAP analysis using ITS, LSU, rpb2, tef1-a and tub2 dataset. Figure S12: The best-scoring RAxML tree based on a concatenated ITS + rpb2 dataset. Figure S13: The best-scoring RAxML tree based on a concatenated ITS + tef1-a dataset. Figure S14: The best-scoring RAxML tree based on a concatenated ITS + tub2 dataset.

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References

1. Hawksworth, D.L. The magnitude of fungal diversity: The 1.5 million species estimate revisited. *Mycolological* 2001, 105, 1422–1432. [CrossRef]

2. Bhunjun, C.S.; Niskanen, T.; Suwannarach, N.; Wannathes, N.; Chen, Y.J.; McKenzie, E.H.; Maharachchikumbura, S.S.; Buyck, B.; Zhao, C.L.; Fan, Y.G.; et al. The numbers of fungi: Are the most speciose genera truly diverse? *Fungal Divers.* 2022, 27, 387–462. [CrossRef]

3. Phukhamsakda, C.; Nilsson, R.H.; Bhunjun, C.S.; de Farias, A.R.; Sun, Y.R.; Wijesinghe, S.N.; Raza, M.; Bao, D.F.; Lu, L.; Tiplompra, S.; et al. The numbers of fungi: Contributions from traditional taxonomic studies and challenges of metabarcoding. *Fungal Divers.* 2022, 28, 327–386. [CrossRef]

4. Wu, B.; Hussain, M.; Zhang, W.; Stadler, M.; Liu, X.; Xiang, M. Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. *Mycolology* 2019, 10, 127–140. [CrossRef]

5. Liu, J.; Diamond, J. China’s environment in a globalizing world. *Nature* 2005, 435, 1179–1186. [CrossRef][PubMed]

6. Luo, Z.L.; Hyde, K.D.; Liu, J.K.; Bhat, D.J.; Bao, D.F.; Li, W.L.; Su, H.Y. Lignicolous freshwater fungi from China II: Novel *Distoseptispora* (*Distoseptisporaceae*) species from northwestern Yunnan Province and a suggested unified method for studying lignicolous freshwater fungi. *Mycosphere* 2018, 9, 444–461. [CrossRef]

7. Zheng, H.; Wan, Y.K.; Li, J.; Rafael, F.C.R.; Yu, Z.F. *Phialolunuluspora ternisspora* (*Chaetosphaeriaceae, Sordariomycetes*), a novel asexual genus and species from freshwater in southern China. *Mycoses* 2020, 76, 17. [CrossRef]

8. Zhang, Z.K.; Wang, X.C.; Zhuang, W.Y.; Cheng, X.H.; Zhao, P. New species of *Talaromyces* (Fungi) isolated from soil in Southwestern China. *Biologia* 2021, 70, 415. [CrossRef]

9. Zheng, P. *China’s Geography*. China Intercontinental Press: Beijing, China, 2006.

10. Zhang, X.; Wang, W.C.; Fang, X.Q.; Ye, Y. Vegetation of Northeast China during the late seventeenth to early twentieth century as revealed by historical documents. *Reg. Environ. Change* 2011, 11, 869–882. [CrossRef]

11. Yuan, D.Y.; Zhu, L.J.; Cherubini, P.; Li, Z.S.; Zhang, Y.D.; Wang, X.C. Species-specific indication of 13 tree species growth on climate warming in temperate forest community of northeast China. *Ecol. Indic.* 2021, 133, 108389. [CrossRef]

12. Winter, H.G. Pilze—Ascomyceten. In *GL Rabenhorst’s Kryptogrammen-Flora von Deutschland, Oesterreich und der Schweiz*; Verlag von Eduard Kummer: Leipzig, Germany, 1885; Volume 1, pp. 65–528.

13. Wijayawardene, N.N.; Hyde, K.D.; Al-Ani, L.K.T.; Tedersoo, L; Haelewaters, D.; Rajeshkumar, K.C.; Zhao, R.L.; Apte, S.; Leonтьev, D.V.; Saxena, R.K.; et al. Outline of fungi and fungus-like taxa. *Mycosphere* 2020, 11, 1060–1456. [CrossRef]

14. Jaklitsch, W.M.; Checa, J.; Blanco, M.N.; Olariaga, I.; Tello, S.; Voglmayr, H. A preliminary account of the *Cucurbitariaceae*. *Mycologia* 2019, 105, 30, 71–118. [CrossRef][PubMed]

15. Jaklitsch, W.M.; Voglmayr, H. Fenestelloid clades of the *Cucurbitariaceae*. *Persoonia* 2020, 44, 1–40. [CrossRef][PubMed]

16. Monka, J.; Tiplompra, S.; Manowong, A.; Mapook, A.; Norphanphoun, C.; Hyde, K.D.; Promputtha, I. Discovery of three novel *Cytopsora* species in Thailand and their antagonistic potential. *Diversity* 2021, 13, 488. [CrossRef]

17. Index Fungorum. 2022. Available online: http://www.indexfungorum.org/names/names.asp (accessed on 11 April 2022).

18. Jayasiri, S.C.; Hyde, K.D.; Ariyawansa, H.; Bhat, D.J.; Buyck, B.; Cai, L.; Dai, Y.C.; Abd-Elsalam, K.A.; Ertz, D.; Hidayat, I.; et al. The faces of fungi database: Fungal names linked with morphology, phylogeny and human impacts. *Fungal Divers.* 2015, 74, 3–18. [CrossRef]

19. De Hoog, G.S.; Gerrits van den Ende, A.H.G. Molecular diagnostics of clinical strains of filamentous basidiomycetes. *Mycoses* 1998, 41, 183–189. [CrossRef]

20. Senanayake, I.C.; Rathnayaka, A.R.; Marasinghe, D.S.; Calabon, M.S.; Gentekaki, E.; Lee, H.B.; Hurdeal, V.G.; Pem, D.; Dissanayake, L.S.; Wijesinghe, S.N.; et al. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere* 2020, 11, 2678–2754. [CrossRef]

21. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic, *PCR Protoc. Guid. Methods Appl.* 1990, 172, 4238–4246. [CrossRef]

22. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *J. Bacteriol.* 1990, 172, 4328–4329. [CrossRef]

23. Voglmayr, H.; Akulov, O.Y.; Jaklitsch, W.M. Reassessment of *Allantonectria*, phylogenetic position of *Thyronectria*, and *Thyronectria caraganae* sp. nov. *Mycol. Prog.* 2016, 15, 921. [CrossRef][PubMed]

24. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 1999, 91, 553–556. [CrossRef]

25. Rehner, S.A.; Buckley, E. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 2005, 97, 84–98. [CrossRef]

26. O’Donnell, K.; Cigelnik, E.; Weber, N.S.; James, M.T. Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. *J. Mol. Evol.* 1997, 48, 48–65. [CrossRef]

27. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* 2019, 20, 1160–1166. [CrossRef]

28. Larsson, A. AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 2014, 30, 3276–3278. [CrossRef]
57. Chen, H.R. Structural and Functional Relationship between JWA and α-Tubulin. Master’s Thesis, Nanjing Medical University, Nanjing, China, 2004.

58. Samson, R.A.; Seifert, K.A.; Kuijpers, A.F.A.; Houbraken, J.A.M.P.; Frisvad, J.C. Phylogenetic analysis of Penicillium subgenus Penicillium using partial β-tubulin sequences. Stud Mycol. 2004, 49, 175–200.

59. Magaña-Dueñas, V.; Stchigel, A.M.; Cano-Lira, J.F. New Coelomycetous fungi from freshwater in Spain. J. Fungi 2021, 7, 368. [CrossRef] [PubMed]

60. Chen, Y.L.; Xu, T.L.; Veresoglou, S.D.; Hu, H.W.; Hao, Z.P.; Hu, Y.J.; Liu, L.; Deng, Y.; Rillig, M.C.; Chen, B.D. Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. Soil Biol. Biochem. 2017, 110, 12–21. [CrossRef]

61. De Gruyter, J.; Woudenberg, J.H.C.; Aveskamp, M.M.; Verkley, G.J.M.; Groenewald, J.Z.; Crous, P.W. Systematic reappraisal of species in Phoma section Paraphoma, Pyrenochaeta and Pleurophoma. Mycologia 2010, 102, 1066–1081. [CrossRef] [PubMed]