RESEARCH ARTICLE

A new study of *Nagrajomyces*: with two new species proposed and taxonomic status inferred by phylogenetic methods

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Abstract

Nagrajomyces (*incertae sedis*, *Ascomycota*) is a monotypic genus with a previously unknown systematic position. In this report, two new species are proposed, *Nagrajomyces fusiformis* and *Nagrajomyces laojunshanensis*. These new taxa are proposed based on morphological characteristics evident via light microscopy and molecular data. Multi-locus phylogenetic analyses (ITS rDNA, nrLSU rDNA, *RPB2*, and *TEF1-a*) show that specimens recently collected in Yunnan Province, China are closely related to *Gnomoniaceae*. Both new species and known species were discovered repeatedly in their asexual developmental form exclusively on twigs of *Rhododendron* spp. (*Ericaceae*). This indicates a host specificity of *Nagrajomyces* spp. for species of *Rhododendron*.

Keywords

host specificity, *Nagrajomyces*, new taxa, phylogeny

Introduction

Gnomoniaceae is a distinct family of *Diaporthales*, established by Winter (1886). The traditional classification of species in *Gnomoniaceae* was mainly based on morphological features, such as the morphology of ascomata and ascospores as well as the position of necks (Barr 1978; Monod 1983). Sogonov et al. (2008) used phylogenetic analyses of molecular sequence data of several genes (*TEF1-a*, nrLSU, and *RPB*2) to revise the concepts of leaf-inhabiting genera, and discovered that several traditional genera in *Gnomoniaceae* are polyphyletic. Phylogenetic analyses indicate that host specificity can be used to circumscribe genera and species of *Gnomoniaceae*. Based on phylogenetic analyses and morphological characteristics, Senanayake et al. (2017) described new taxa and excluded some genera from *Gnomoniaceae*. Since then, additional genera have been introduced for species observed in sexual developmental stages, as well as those that have only been observed as pycnidial asexual morphs, and rarely for species known in both sexual and asexual forms (Senanayake et al. 2018; Crous et al. 2019; Jiang et al. 2019; Minoshima et al. 2019; Yang et al. 2020).

Many species of *Gnomoniaceae* are important plant pathogens, such as *Apiognomonia errabunda* (Roberge ex Desm.) Höhn, which causes oak anthracnose (Sogonov et al. 2007), *Gnomoniopsis fructicola* (G. Arnaud) Sogonov, which causes strawberry stem rot (Maas 1998), and *Ophiognomonia leptostyla* (Fr.) Sogonov, which causes walnut anthracnose (Neely and Black 1976). Species of *Gnomoniaceae* can also have wide host ranges, with species on *Fagaceae*, *Onagraceae*, and *Rosaceae* being frequently infected. Species of *Rhododendron* (*Ericaceae*), which is the largest genus of woody plants in the northern hemisphere, are also known hosts of species of *Gnomoniaceae* (Monod 1983).

Nagrajomyces (*incertae sedis*, *Ascomycota*) is a monotypic genus based on *N. dictyosporus* Mel'nik (Mel'nik 1984; Nag Raj 1993). It was discovered in Russia, where it grows on twigs of *Rhododendron aureum* and develops stalked, unilocular, or plurilocular conidiomata as well as muriform conidia, and each conidium bears an apical appendage that is single, unbranched, attenuated, flexuous and can be more than 100 μm long (Mel'nik 1984; Nag Raj 1993).

In the present study, two new species were discovered on twigs of *Rhododendron* spp. in Yunnan and assigned to the genus *Nagrajomyces* based on morphological characteristics, habitat, and host. Phylogenetic analysis revealed that the proposed *Nagrajomyces* species belong to *Gnomoniaceae*.

Materials and methods

Specimen collections and isolation

Fieldwork for the discovery of fungi was conducted during June 2021 in Yunnan Province, China. Fresh pycnidia were repeatedly discovered and collected on twigs of *Rhododendron* spp. Twigs with conidiomata were packed in paper bags and transported to the laboratory

for morphological tests. Conidiomata were cut off in the laboratory using a razor blade, wrapped in paper packets, disinfected with 75% ethanol for 10 s, then 10% sodium hypochlorite for 2 min 30 s, and rinsed with distilled water three times. After absorbing the water with sterile filter paper, the conidiomata were transferred to potato dextrose agar (PDA) plates (Jiang et al. 2021) then incubated at 25 °C to obtain cultures. Dry specimens were deposited at the China Forest Biodiversity Museum of the Chinese Academy of Forestry (CAF; http://museum.caf.ac.cn) and the Herbarium of the College of Life Science, Capital Normal University (BJTC; http://smkxxy.cnu.edu.cn). Ex-type living cultures were deposited at the China Forestry Culture Collection Center (CFCC; http://cfcc.caf.ac.cn/).

Morphological analysis

Conidiomata were photographed and cut by hand using a razor blade under a Nikon SMZ-1000 stereomicroscope (Japan). Morphological characteristics of conidiomata, conidiophores, and conidia were photographed and measured with an Olympus EX-51 upright microscope (Japan), and for each structure at least 20 measurements were made. Color values were taken from ColorHexa (https://www.colorhexa.com/).

DNA extraction, polymerase chain reaction amplification, and phylogeny

Genomic DNA was extracted from specimens and cultures via the M5 Plant Genomic DNA Kit (Mei5 Biotechnology Co., Ltd., China) in accordance with the manufacturer's instructions. Table 1 summarizes the primers used to obtain sequence data for ITS rDNA, nrLSU rDNA, *RPB2*, and *TEF1-a*, and the polymerase chain reaction (PCR) amplification protocols. PCR products were analyzed in 1% electrophoretic agarose gel with a 200-bp DNA ladder, purified, and sequenced by Beijing Zhongke Xilin Biotechnology Co., Ltd. (Beijing, China). SeqMan was used to align the sequences obtained by forward and reverse primers to obtain a consensus sequence. A partition homogeneity test was performed to determine the congruence of the four datasets (Farris et al. 1994). Sequences for phylogenetic analyses were selected based on Yang et al. (2020), supplemented by sequences of *Apiosporopsis carpinea* (Fr.) Mariani, *Apiosporopsis* sp., *Juglanconis juglandina* (Kunze) Voglmayr & Jaklitsch, *Juglanconis oblonga* (Berk.) Voglmayr & Jaklitsch, and *Melanconis marginalis* (Peck) Wehm. from Senanayake et al. (2018) used as outgroup taxa. All sequences used in this study are listed in Table 2. Subsequent alignments were generated with online MAFFT tools

Table 1. Primer information and PCR amplification protocols.

Gene	Primer pairs	Reference	Amplification conditions
ITS rDNA	ITS1F/ITS4	White et al. (1990); Gardes and Bruns (1993)	Phillips et al. (2008)
LSU rDNA	LR0R/LR5	Vilgalys and Hester (1990); Rehner and Samuels (1994)	Phillips et al. (2008)
TEF1-a	EF1-728F/EF1-986R	Carbone and Kohn (1999)	Glass and Donaldson (1995)
RPB2	fRPB2-5F/fRPB2-7cR	Liu et al. (1999)	Liu et al. (1999)

Table 2. Sequences used in phylogenetic analyses. References to sequences generated in the present study are emphasized in bold.

Taxa	Voucher	ITS rDNA	LSU rDNA	*RPB2*	*TEF1-α*	References
Alnecium auctum	CBS 124263	KF570154	KF570154	KF570170	KF570200	Voglmayr and Jaklitsch (2014)
Ambarignomonia petiolorum	CBS 116866	EU199193	AY818963	EU199151	–	Mejía et al. (2008)
Ambarignomonia petiolorum	CBS 121227	EU254748	EU255070	EU219307	EU221898	Mejía et al. (2008)
Amphiporthe tiliae	CBS 119289	EU199178	EU199122	EU199137	–	Mejía et al. (2008)
Apiognomonia errabunda	AR 2813	DQ313525	–	DQ862014	DQ313565	Sogonov et al. (2007)
Apiognomonia veneta	MFLUCC 16-1193	MF190114	MF190056	–	–	Senanayake et al. (2017)
Apioplagiostoma populi	858501	KP637024	–	–	–	Wijekoon et al. (2021)
Apiosporopsis carpinea	CBS 771.79	–	AF277130	–	–	Zhang and Blackwell (2001)
Apiosporopsis sp.	Masuya 11Af2-1	–	AB669034	–	–	Osono and Masuya (2012)
Asteroma alneum	CBS 109840	EU167609	EU167609	–	–	Simon et al. (2009)
Asteroma sp.	Masuya 8Ah9-1	–	AB669035	–	–	Osono and Masuya (2012)
Cryptodiaporthe acerina	AR 3822	EU254755	EU255075	EU219253	EU221879	Sogonov et al. (2008)
Cryptodiaporthe aubertii	CBS 114196	KX929767	KX929803	KX929838	KX929732	Meyer et al. (2017)
Cryptosporella hypodermia	CBS 116866	EU199181	AF408346	EU199140	–	Mejía et al. (2008)
Ditopella biseptata	MFLU 15-2661	MF190147	MF190091	MF377616	–	Senanayake et al. (2017)
Ditopella ditopa	CBS 109748	DQ323526	EU199126	EU199145	–	Mejía et al. (2008)
Ditopellopsis sp.	CBS 121471	EU254763	EU255088	EU219254	EU221936	Sogonov et al. (2008)
Flavignomonia rhoigena	CFCC 53118	MK432674	MK429917	MK578102	–	Jiang et al. (2019)
Flavignomonia rhoigena	CFCC 53119	MK432675	MK429918	MK578103	–	Jiang et al. (2019)
Gnomonia gnomon	CBS 199.53	DQ491518	AF408361	EU219295	EU221885	Sogonov et al. (2008)
Gnomonia gnomon	CBS 829.79	AY818957	AY818964	–	EU221905	Sogonov et al. (2005)
Gnomoniella microspora	BPI 877571	EU254765	–	–	–	Sogonov et al. (2008)
Gnomoniopsis alderdunensis	CBS 125680	GU320825	–	–	–	Walker et al. (2010)
Gnomoniopsis chamaemori	CBS 803.79	EU254808	EU255107	–	–	Sogonov et al. (2008)
Gnomoniopsis racemula	AR 3892	EU254841	EU255122	EU219241	EU221889	Sogonov et al. (2008)
Juglanconis juglandina	WU 35960	KY427145	KY427145	KY427195	KY427214	Voglmayr et al. (2017)
Juglanconis oblonga	TFM FPH 2623	KY427153	KY427153	KY427203	KY427222	Voglmayr et al. (2017)

| Taxa	Voucher	ITS rDNA	LSU rDNA	*RPB2*	*TEF1-α*	References
Mamianiella coryli	BPI 877578	EU254862	–	–	–	Sogonov et al. (2008)
Marsupiomyces epidermoidea	MFLU 15-2921	–	MF190058	–	–	Senanayake et al. (2017)
Marsupiomyces quercina	MFLUCC 13-0664	MF190116	MF190061	–	–	Senanayake et al. (2017)
Melanconis marginalis	BPI 748234	–	–	EU219299	EU221886	Sogonov et al. (2008)
Melanconis marginalis	BPI 748446	EU199197	AF408373	EU219301	EU221991	Sogonov et al. (2008)
Neognomoniopsis quercina	CBS 145575	MK876399	MK876440	–	–	Crous et al. (2019)
Nagrajomyces fusiformis	**CAF 800050**	**OP473599**	**OP473595**	**OP484756**	**OP484760**	**This study**
Nagrajomyces fusiformis	**BJTC 1773**	**OP473602**	**OP473598**	**–**	**OP484763**	**This study**
Nagrajomyces laojunshanensis	**CFCC 58177**	**OP456161**	**OP473594**	**OP484755**	**OP484759**	**This study**
Nagrajomyces laojunshanensis	**CAF 800049**	**OP473600**	**OP473596**	**OP484757**	**OP484761**	**This study**
Nagrajomyces laojunshanensis	**BJTC 1849**	**OP473601**	**OP473597**	**OP484758**	**OP484762**	**This study**
Occultocarpon ailaoshanense	LCM 524.01	JF779849	JF779853	JF779856	–	Mejía et al. (2011)
Occultocarpon ailaoshanense	LCM 522.01	JF779848	JF779852	JF779857	JF779862	Mejía et al. (2011)
Ophiognomonia melanostyla	LCM 389.01	JF779850	JF779854	JF779858	–	Mejía et al. (2011)
Ophiognomonia vasiljevae	AR 4298	EU254977	EU255162	EU219331	EU221999	Sogonov et al. (2008)
Phragmoporthe conformis	AR 3632	–	AF408377	–	–	Castlebury et al. (2002)
Plagiostoma aesculi	AR 3640	EU254994	EU255164	EU219269	–	Sogonov et al. (2008)
Plagiostoma rhododendri	CBS 847.79	EU255044	EU255187	EU219272	–	Sogonov et al. (2008)
Pleuroceras oregonense	AR 4333	EU255060	EU255196	EU219313	EU221931	Sogonov et al. (2008)
Pleuroceras pleurostylum	CBS 906.79	EU255061	EU255197	EU219311	EU221962	Sogonov et al. (2008)
Sirococcus conigenus	BPI 871248	EU199201	EU199134	EU199157	–	Mejía et al. (2008)
Sirococcus piceicola	BPI 871166	EU199202	EU199135	EU199158	–	Mejía et al. (2008)
Sirococcus tsugae	BPI 871167	EU199203	EU199136	EU199159	–	Mejía et al. (2008)
Sirococcus tsugae	AR 4010	EF512478	EU255207	EU219289	EU221928	Sogonov et al. (2008)
Tenuignomonia styracis	BPI 892786	–	LC379289	LC379295	LC379283	Minoshima et al. (2019)
Tenuignomonia styracis	BPI 892785	–	LC379288	LC379294	LC379282	Minoshima et al. (2019)
Valsalnicola oxystoma	AR 5137	JX519561	–	–	–	Crous et al. (2012)
Valsalnicola oxystoma	AR 4833	JX519559	JX519563	–	–	Crous et al. (2012)
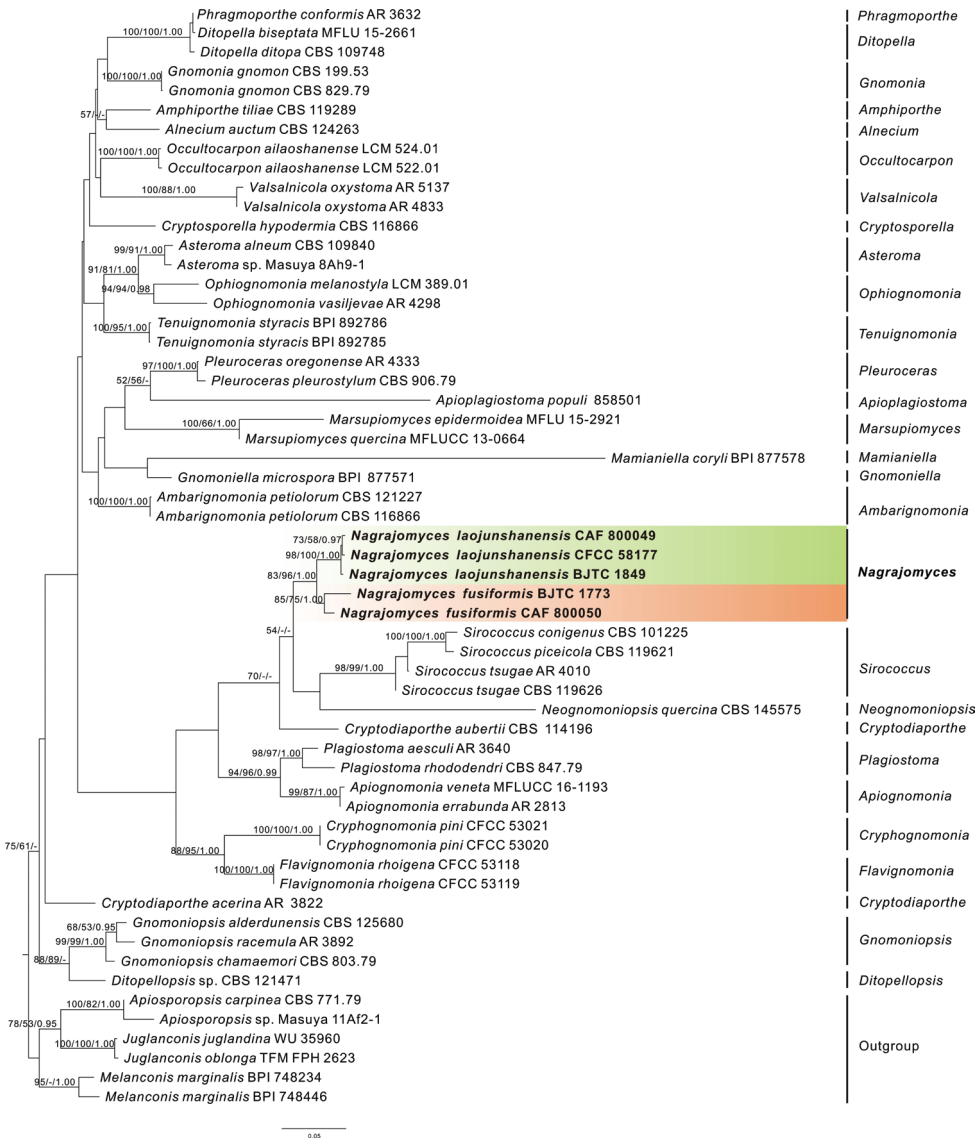
Figure 1. Phylogenetic tree based on an ML analysis of combined ITS rDNA, nrLSU rDNA, *RPB2*, and *TEF1-a* sequences of species of Gnomoniaceae. Bootstrap support values for RAxML and maximum parsimony above 50% and Bayesian posterior probability values above 0.95 are shown at the nodes. The tree is rooted with sequences of *Apiosporopsis carpinea*, *Apiosporopsis* sp., *Juglanconis juglandina*, *J. oblonga*, and *Melanconis marginalis*. References to new sequences are in bold, and the names of the two new species are highlighted by colors.

(https://www.ebi.ac.uk/Tools/msa/mafft/) and edited with Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The maximum likelihood (ML) tree was constructed using RAxML version 8.2.12 (Stamatakis et al. 2005;

Stamatakis 2006; Stamatakis 2014) with GTRGAMMA model and 1000 bootstrap iterations. The multi-locus Bayesian Inference (BI) tree was built by MrBayes version 3.2.6 (Ronquist and Huelsenbeck 2003). Models of nucleotide substitution for each gene used in the Bayesian analysis were determined by MrModeltest v.2.3 (Nylander 2004). Analyses of four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 100,000,000 generations, and other operational methods were applied used as described by Guo et al. (2021). The maximum parsimony (MP) tree was constructed using PAUP version 4.0 beta 10 (Swofford 2003) with 1000 random sequence additions, 1000 maxtrees were obtained, and bootstrap analysis was conducted based on 1000 replicates, with 10 replicates of random stepwise additions of taxa. For further details see Guo et al. (2021). Trees were viewed via Treeview (Page 1996).

Results

Phylogenetic analysis

Multi-locus phylogenetic analyses of species of *Gnomoniaceae* (*Diaporthales*) include sequences of 51 ingroup taxa and sequences of an outgroup formed by *Apiosporopsis carpinea*, *Apiosporopsis* sp., *Juglanconis juglandina*, *J. oblonga*, and *Melanconis marginalis* (Fig. 1). The multi-locus dataset (ITS rDNA, LSU rDNA, *RPB2* and *TEF1-a*) comprises 2875 characters, of which 945 are parsimony-informative, 200 are parsimony-uninformative and 1730 are constant. Maximum parsimony analysis of sequences resulted in one most parsimonious tree with a length (TL) of 3730 steps, a consistency index (CI) of 0.463, a retention index (RI) of 0.690, and a homoplasy index (HI) of 0.537. Bayesian and maximum likelihood trees exhibited topologies similar to this parsimony tree.

The topology of the phylogenetic tree obtained in the current study was similar to the topology presented by Yang et al. (2020). Nineteen sequences of five specimens recently collected on *Rhododendron* spp. in China form a clade with high support values. This clade is sister to sequences of species of *Siroccocus* and *Neognomoniopsis*, but with poor support values. The newly discovered clade is divided into two small subclades labeled *Nagrajomyces fusiformis* and *N. laojunshanensis*.

Taxonomy

Nagrajomyces fusiformis C. L. Hou & L. Zhuo, sp. nov.
MycoBank No: 845666
Figs 2, 3

Etymology. The epithet *fusiformis* refers to fusoid conidia.
Type. China, Yunnan province, Lijiang, Yulong, 26°40'55"N, 99°54'01"E, alt. 2762 m, on dying twigs of *Rhododendron vellereum* Hutch. ex Tagg., 20 June 2021, coll. C.L. Hou, M.J. Guo, H. Zhou (holotype CAF 800050).
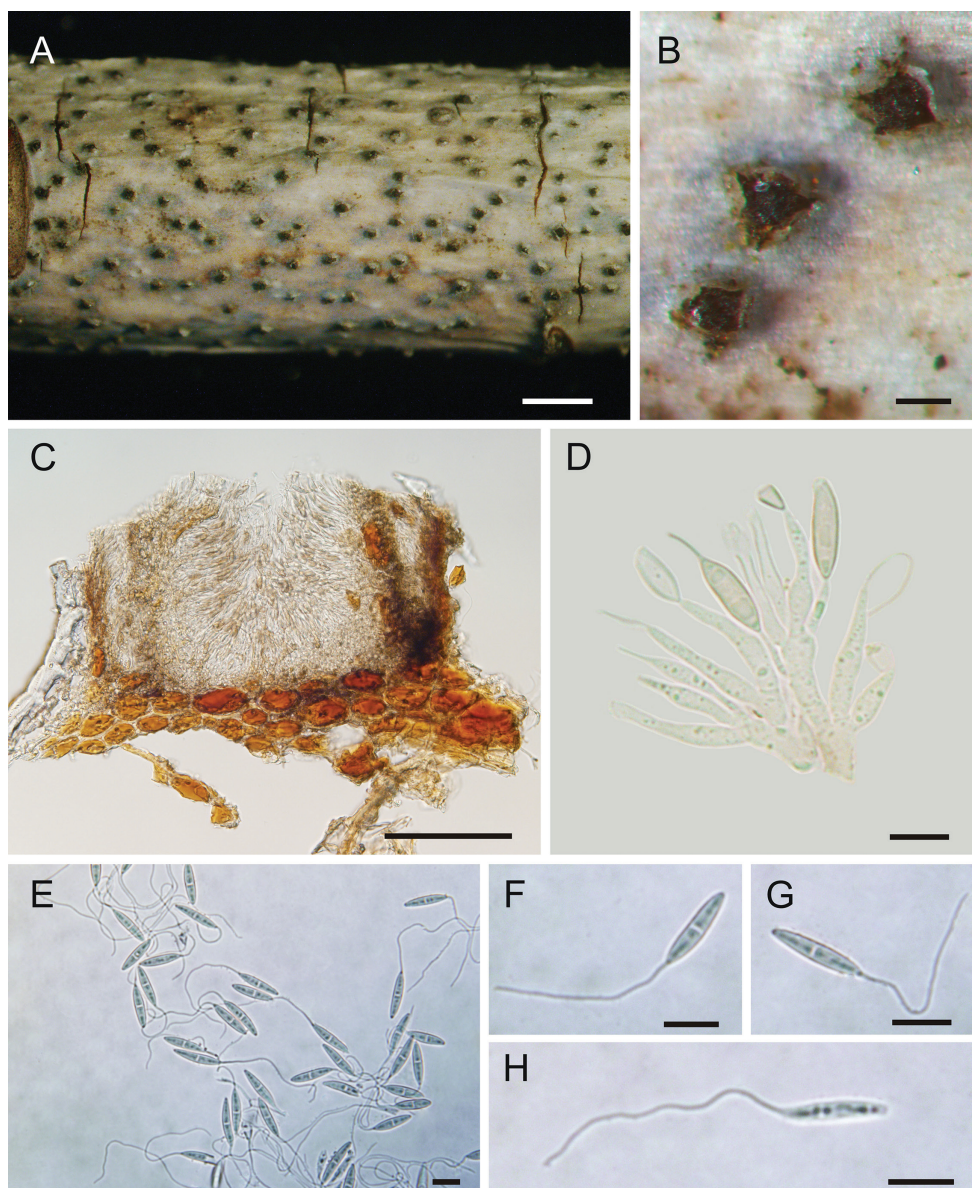
Figure 2. Micrographs of *Nagrajomyces fusiformis* (holotype CAF 800050) on twigs of *Rhododendron vellereum* **A, B** conidiomata on a dying twig **C** vertical section of a conidioma **D** conidiophores and conidia at diverse developmental stages **E–H** conidia with appendages. Scale bars: 2 mm (**A**); 200 μm (**B**); 100 μm (**C**); 10 μm (**D–H**).

Diagnosis. This new species differs from *N. dictyosporus* and *N. laojunshanensis* by fusoid to elongate-fusoid conidia with pointed ends, usually 1-septate and smaller.

Description. *Conidiomata* solitary, pycnidial, irregularly plurilocular, subepidermal in origin, immersed at first, then becoming erumpent through the periderm of the host, 545–554 μm diameter, 520–546 μm high, peridium
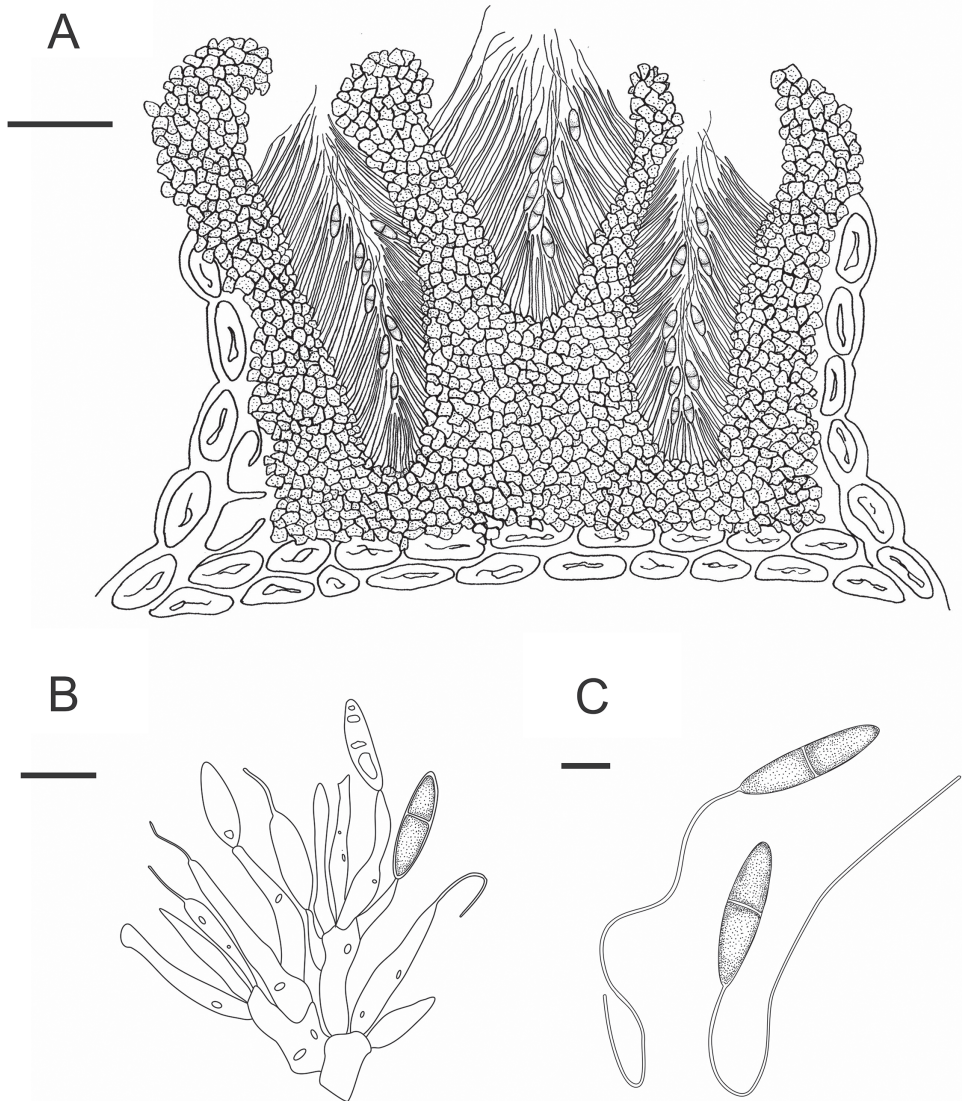
Figure 3. *Nagrajomyces fusiformis* (holotype CAF 800050) **A** vertical section of a conidioma **B** conidiophores and conidia **C** conidia with appendages. Scale bars: 100 μm (**A**); 10 μm (**B**); 5 μm (**C**).

dark brown, 47.0–67.5 μm thick. ***Conidiophores*** ampulliform, smooth, hyaline, multiguttulate, 12–29 × 2.0–3.5 μm ($\overline{x}$ = 19 × 3 μm, *n* = 20). ***Conidia*** fusoid to elongate-fusoid, 1-septate, cells equal, smooth, hyaline to pale brown, 13.5–19.0 × 3–4 μm ($\overline{x}$ = 16.5 × 3.5 μm, *n* = 20), with a whip-like appendage at the tip of each conidium, 30–77 μm ($\overline{x}$ = 51 μm, *n* = 20) in length (Fig. 4). ***Sexual morph*** not observed.

Additional specimen examined. CHINA, Yunnan Province, Lijiang, Laojunshan, 26°37'56"N, 99°43'30"E, alt. 3873 m, on dying twigs of *Rhododendron vellereum*, 20 June 2021, coll. C.L. Hou, M.J. Guo, H. Zhou (BJTC 1773).

Notes. *Nagragomyces fusiformis* differs from other species of *Nagrajomyces* by narrower and 1-septate conidia.

Nagrajomyces laojunshanensis C. L. Hou & L. Zhuo, sp. nov.
MycoBank No: 845665
Figs 4, 5

Etymology. The epithet *laojunshanensis* refers to the location where the type specimen was collected.

Type. China, Yunnan Province, Lijiang, Laojunshan, 26°39'44"N, 99°46'58"E, alt. 2910 m, on living twigs of *Rhododendron cinnabarinum* Hook. f., 20 June 2021, coll. C.L. Hou, M.J. Guo, H. Zhou (holotype CAF 800049). Ex-type culture CFCC 58177.

Diagnosis. This new species differs from *N. fusiformis* by conidia that are elongate-elliptical, blunter at both ends, and usually 3-septate and larger. *Nagrajomyces laojunshanensis* differs from *N. dictyosporus* by conidiomata that are unilocular and without stalks.

Description. *Conidiomata* solitary, pycnidial, unilocular, subglobose to ellipsoidal, subepidermal in origin, immersed at first, then becoming erumpent, 218–406 μm wide, 188–275 μm high, peridia black, 37–43 μm thick, opening irregularly in the upper part, with faint yellow content. ***Conidiophores*** ampulliform, smooth, hyaline, multiguttulate, 16.0–25.5 × 2–4 μm ($\overline{x}$ = 21 × 3 μm, *n* = 20). ***Conidia*** elongate-elliptical, 1–3-septate, mostly 3-septate, smooth, hyaline, 18–23 × 5.5–7.0 μm ($\overline{x}$ = 19.5 × 6.5 μm, *n* = 20), with a long, whip-like appendage at the tip of each conidium, 70–200 μm ($\overline{x}$ = 143.5 μm, *n* = 20) in length. ***Sexual morph*** not observed.

Culture characteristics. *Cultures* (ex-type CFCC 58177) on PDA 8 cm diameter after 1 month, with irregular margins, sparse aerial mycelium, colonies with whitish margins, with center turning black olive (#3b3c36) with increasing age. On MEA, 5.7 cm diameter after 1 month, with irregular margins, colonies with beaver (#9f8170) -colored margins, with center turning black olive (#3b3c36) with increasing age. ***Conidia*** not observed.

Additional specimen examined. China, Yunnan province, Kunming, Luquan, Jiaozixueshan, 26°05'04"N, 102°50'54"E, alt. 3823 m, on living twigs of *Rhododendron cinnabarinum* Hook. f., 23 June 2021, coll. C.L. Hou, M.J. Guo, H. Zhou, (BJTC 1849).

Notes. *Nagrajomyces laojunshanensis* differs from *N. dictyosporus* by conidia that are colorless and conidiomata that are without stalks. *Nagrajomyces laojunshanensis* differs from *N. fusiformis* by elongate-elliptical conidia with blunter ends, which are longer (18–23 μm vs. 13–19 μm) and wider (5.7–7.0 μm vs. 2.8–3.7 μm). Conidia of *N. laojunshanensis* are mostly 3-septate, whereas those of *N. fusiformis* are 1-septate. Molecular sequence data confirm the presence of two distinct species.
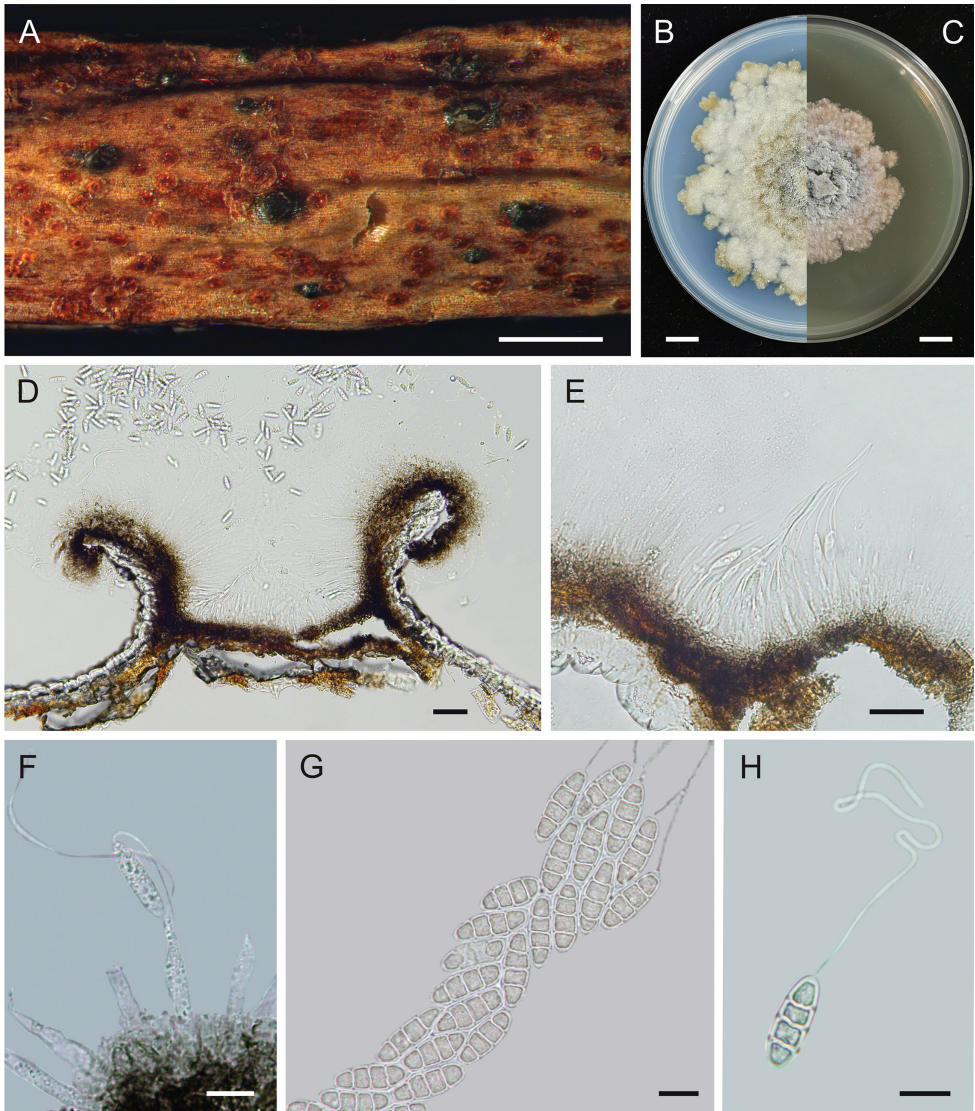
Figure 4. Micrographs of *Nagrajomyces laojunshanensis* on *Rhododendron cinnabarinum* (holotype CAF 800049) **A** conidiomata on a living twig **B** ex-type culture (CFCC 58177) on PDA after 30 days, seen from above **C** ex-type culture (CFCC 58177) on MEA after 30 days, seen from above **D** vertical section of a conidioma **E, F** conidiophores and conidia **G** conidia forming a cirrus **H** conidium with appendage. Scale bars: 1 mm (**A**); 1 cm (**B, C**); 100 μm (**D, E**); 10 μm (**F–H**).

Discussion

Morphologically, the most distinctive features of the new species of *Nagrajomyces* are septate conidia with long, single, apical appendages. The presence of this structure distinguishes them from all anamorphic genera known to belong to *Gnomoniaceae*.
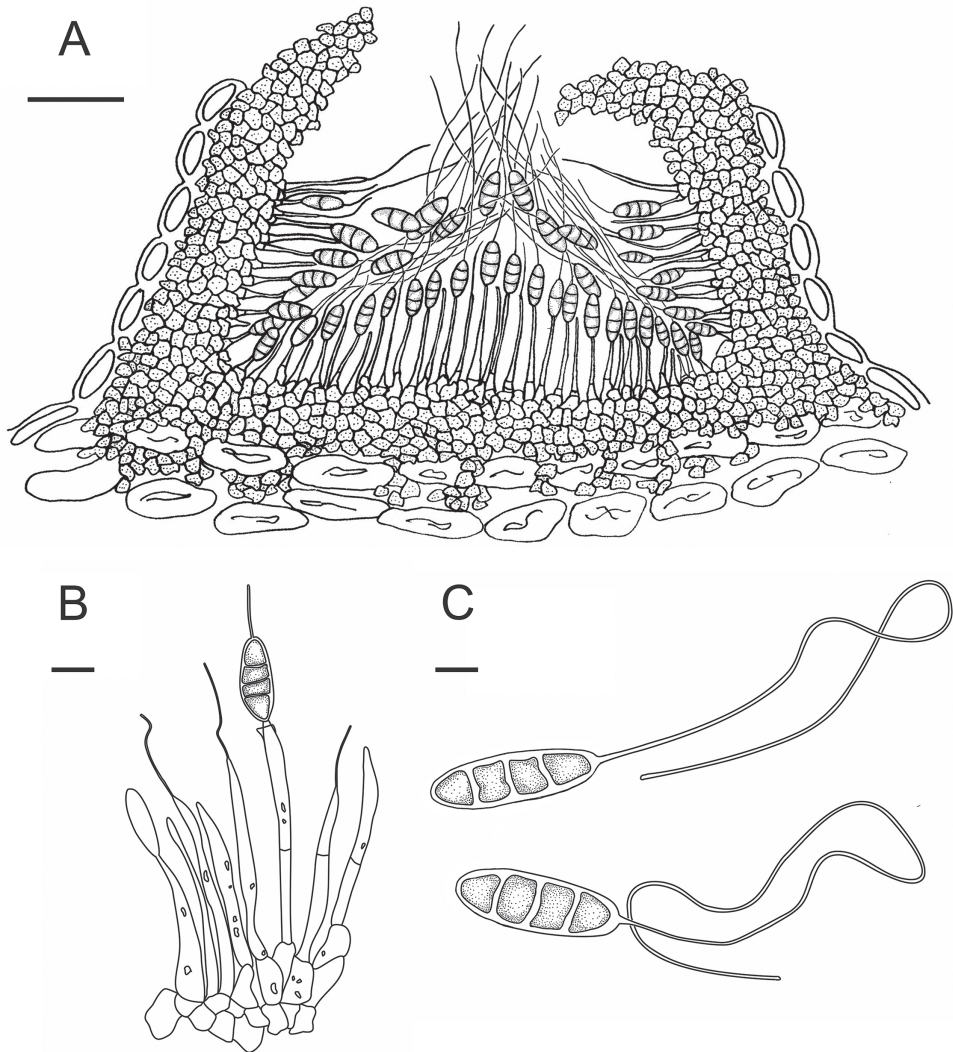
Figure 5. *Nagrajomyces laojunshanensis* (holotype CAF 800049) **A** vertical section of a conidioma **B** conidiophores and conidia **C** conidia with appendages. Scale bars: 50 μm (**A**); 10 μm (**B**); 5 μm (**C**).

Both new species proposed in the present study and the known species *N. dictyosporus* inhabit twigs of *Rhododendron*. In spite of the absence of molecular data for the type species of *Nagrajomyces*, these two new species are accommodated in *Nagrajomyces* based on significant morphological features (distinctive conidia) and identical ecology.

Many coelomycetous genera have conidia with appendages (Nag Raj 1993), and some of them share morphological characteristics with the new species proposed in this study. For example, species of *Uniseta* and *Urohendersonia* have septate conidia and a long apical appendage attached to each conidium. *Uniseta* is a monotypic genus typified by *U. flagellifera* (Ellis & Everh.) Ciccar. (Ciccarone 1947). Nag Raj (1974, 1993)

mentioned that *U. flagellifera* has a sexual morph called *Cryptodiaporthe comptoniae* (Schwein.) Barr (Barr 1991, syn. *C. aubertii* var. *comptoniae* (Schwein.) Wehm.) that is considered a synonym of *Cryptodiaporthe aubertii* (Westend.) Wehm. (Wehmeyer 1933). Conidia of this species are two-celled, hyaline, relatively inequilateral or curved, and bear a long flagellate appendage at one end (Wehmeyer 1933). *Cryptodiaporthe acerina* J. Reid & Cain and *C. aubertii* are included in the phylogenetic tree (Fig. 1) and located distant from the new species proposed herein. Furthermore, *U. flagellifera* differs from the new species proposed here by an asexual morph growing on branches of *Comptonia asplenifolia* damaged by fire (Ellis and Everhart 1889), while *Nagrajomyces* spp. develop on twigs of *Rhododendron*. Because of these differences, we consider the genus *Uniseta* to be separate from the genus *Nagrajomyces*.

Spegazzini (1902) introduced *Urohendersonia* Speg. with *Ur. platensis* Speg. as the type species. Nag Raj (1993) listed only five species in this genus, including the type species. *Urohendersonia* spp. differ from *Nagrajomyces* spp. by having globose to subglobose conidiomata immersed in host tissues, and yellowish brown to brown conidia each with an extracellular gelatinous appendage, and their host species (Nag Raj 1993). *Urohendersonia* spp. occur on diverse host species and various substrates of host, such as on leaves of *Erythrina* sp., *Manihot carthagenensis*, *Pongamia pinnnata*, *Stipa spartea*, or in the rhizospheres of *Acerva persica* and *Dactyloctenium aegyptium* (Nag Raj 1993; Wijayawardene et al. 2016). Unfortunately, there are no molecular sequence data available for any species within those genera.

In the phylogenetic analysis presented herein, the two new species, *N. fusiformis* and *N. laojunshanensis* form a clade with high support values, which is separate from other species of *Gnomoniaceae* represented by sequence data in GenBank. These two new species described in this study fill gaps in the molecular data of *Nagrajomyces* and also enable the taxonomic status of the new species to be determined.

A total of 38 genera are currently included in the family *Gnomoniaceae* based on morphological and molecular analyses (Senanayake et al. 2018; Crous et al. 2019; Jiang et al. 2019; Minoshima et al. 2019; Yang et al. 2020). Sexual morphs have been described for all but four; *Asteroma*, *Flavignomonia*, *Millerburtonia*, and *Sirococcus*. *Sirococcus* spp. are closely related to the new species described herein, whereas phylogenetic data indicate that the other three genera are distantly related to *Nagrajomyces* spp. *Asteroma* spp. have cylindrical to fusiform, acicular or broadly fusiform conidia (Senanayake et al. 2018). Conidia of *Flavignomonia* are cylindrical to oblong (Jiang et al. 2019), and conidia of *Millerburtonia* are filiform and aciculate (Ciferri 1951).

In addition to morphological characteristics and molecular sequence data, host ranges are often useful to delineate genera and species of *Gnomoniaceae* (Sogonov et al. 2008). Species of *Gnomonia*, for example, are generally associated with host plants in the *Betulaceae* family, mostly belonging to the subfamily *Coryloideae* (Sogonov et al. 2008). The two new species identified in the present study, and the known species, all develop on twigs of *Rhododendron* spp. indicating that they are specialized with respect to this host. The differences in conidiomatal structure could be explained by differences in host epidermal features or maturity. Two species of *Gnomoniaceae* are known

to inhabit *Rhododendron* spp. *Plagiostoma rhododendri* (Auersw.) Sogonov was reported on dry twigs and inflorescences of *Rhododendron hirsutum* L., and occasionally on dead leaves of *R. ferrugineum* L. (Monod, 1983). Only the sexual form of this species has been described, and phylogenetic analysis places it somewhat distant to species of *Nagrajomyces* (Fig. 1). The second species is *Gnomonia* sp., reported on rotten leaves of *R. ferrugineum* (Rehm 1906). This species lacks a specific morphological description.

Rhododendron is the largest genus of woody plants in the northern hemisphere, and its species diversity is highest in the Himalaya-Hengduan Mountains and Southeast Asia (Chamberlain et al. 1996; Shrestha et al. 2018). Considering the host preference of *Gnomoniaceae* species and the biodiversity of *Rhododendron* worldwide, additional *Gnomoniaceae* species are expected to exist on these plants.

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References

Barr ME (1978) The Diaporthales in North America with emphasis on *Gnomonia* and its segregates. Mycologia Memoir 7: 1–232. https://doi.org/10.1002/fedr.19800910313

Barr ME (1991) Revisions and additions to the Diaporthales. Mycotaxon 41(1): 287–305.

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553–556. https://doi.org/10.1080/00275514.1999.12061051

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002) A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences. Mycologia 94(6): 1017–1031. https://doi.org/10.1080/15572536.2003.11833157

Chamberlain D, Hyam R, Argent G, Fairweather G, Walter KS (1996) The genus *Rhododendron*: its classification and synonymy. Royal Botanic Garden Edinburgh, Edinburgh, 181 pp.

Ciccarone A (1947) Morfologia di Nobarclaya natalensis Syd. Nuovo Giornale botanico italiano 54(3): 1–15. https://doi.org/10.1080/11263504709440463

Ciferri R (1951) Schedae Mycologicae I–XI. Mycopathologia et mycologia applicata 6(1): 19–27. https://doi.org/10.1007/BF02115730

Crous PW, Carnegie AJ, Wingfield MJ, Sharma R, Mughini G, Noordeloos ME, Santini A, Shouche YS, Bezerra JDP, Dima B, Guarnaccia V, Imrefi I, Jurjević Ž, Knapp DG, Kovács GM, Magistà D, Perrone G, Rämä T, Rebriev YA, Shivas RG, Singh SM, Souza-Motta CM, Thangavel R, Adhapure NN, Alexandrova AV, Alfenas AC, Alfenas RF, Alvarado P, Alves AL, Andrade DA, Andrade JP, Barbosa RN, Barili A, Barnes CW, Baseia IG, Bellanger JM, Berlanas C, Bessette AE, Bessette AR, Biketova AY, Bomfim FS, Brandrud TE, Bransgrove

K, Brito ACQ, Cano-Lira JF, Cantillo T, Cavalcanti AD, Cheewangkoon R, Chikowski RS, Conforto C, Cordeiro TRL, Craine JD, Cruz R, Damm U, de Oliveira RJV, de Souza JT, de Souza HG, Dearnaley JDW, Dimitrov RA, Dovana F, Erhard A, Esteve-Raventós F, Félix CR, Ferisin G, Fernandes RA, Ferreira RJ, Ferro LO, Figueiredo CN, Frank JL, Freire KTLS, García D, Gené J, Gêsiorska A, Gibertoni TB, Gondra RAG, Gouliamova DE, Gramaje D, Guard F, Gusmão LFP, Haitook S, Hirooka Y, Houbraken J, Hubka V, Inamdar A, Iturriaga T, Iturrieta-González I, Jadan M, Jiang N, Justo A, Kachalkin AV, Kapitonov VI, Karadelev M, Karakehian J, Kasuya T, Kautmanová I, Kruse J, Kušan I, Kuznetsova TA, Landell MF, Larsson KH, Lee HB, Lima DX, Lira CRS, Machado AR, Madrid H, Magalhães OMC, Majerova H, Malysheva EF, Mapperson RR, Marbach PAS, Martín MP, Martín-Sanz A, Matočec N, McTaggart AR, Mello JF, Melo RFR, Mešić A, Michereff SJ, Miller AN, Minoshima A, Molinero-Ruiz L, Morozova OV, Mosoh D, Nabe M, Naik R, Nara K, Nascimento SS, Neves RP, Olariaga I, Oliveira RL, Oliveira TGL, Ono T, Ordoñez ME, Ottoni AM, Paiva LM, Pancorbo F, Pant B, Pawłowska J, Peterson SW, Raudabaugh DB, Rodríguez-Andrade E, Rubio E, Rusevska K, Santiago ALCMA, Santos ACS, Santos C, Sazanova NA, Shah S, Sharma J, Silva BDB, Siquier JL, Sonawane MS, Stchigel AM, Svetasheva T, Tamakeaw N, Telleria MT, Tiago PV, Tian CM, Tkalčec Z, Tomashevskaya MA, Truong HH, Vecherskii MV, Visagie CM, Vizzini A, Yilmaz N, Zmitrovich IV, Zvyagina EA, Boekhout T, Kehlet T, Læssøe T, Groenewald JZ (2019) Fungal Planet description sheets: 868–950. Persoonia 42: 291–473. https://doi.org/10.3767/persoonia.2019.42.11

Crous PW, Shivas RG, Wingfield MJ, Summerell BA, Rossman AY, Alves JL, Adams GC, Barreto RW, Bell A, Coutinho ML, Flory SL, Gates G, Grice KR, Hardy GE, Kleczewski NM, Lombard L, Longa CM, Louis-Seize G, Macedo F, Mahoney DP, Maresi G, Martin-Sanchez PM, Marvanová L, Minnis AM, Morgado LN, Noordeloos ME, Phillips AJ, Quaedvlieg W, Ryan PG, Saiz-Jimenez C, Seifert KA, Swart WJ, Tan YP, Tanney JB, Thu PQ, Videira SI, Walker DM, Groenewald JZ (2012) Fungal Planet description sheets: 128–153. Persoonia 29: 146–201. https://doi.org/10.3767/003158512X661589

Ellis JB, Everhart BM (1889) New and rare species of North American fungi. Journal of Mycology 5: 145–157. https://doi.org/10.2307/3752949

Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics 10(3): 315–319. https://doi.org/10.1111/j.1096-0031.1994.tb00181.x

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61(4): 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Guo MJ, Wang QT, Cheng YH, Hou CL (2021) Identification of *Aspergillus tubingensis* causing pomegranate fruit rot in China. Australasian Plant Pathology 50: 233–240. https://doi.org/10.1007/s13313-020-00769-7

Jiang N, Voglmayr H, Bian DR, Piao CG, Wang SK, Li Y (2021) Morphology and Phylogeny of *Gnomoniopsis* (Gnomoniaceae, Diaporthales) from *Fagaceae* Leaves in China. Journal of Fungi 7(10): 792. https://doi.org/10.3390/jof7100792

Jiang N, Yang Q, Liang YM, Tian CM (2019) Taxonomy of two synnematal fungal species from *Rhus chinensis*, with *Flavignomonia* gen. nov. described. MycoKeys 60: 17–29. https://doi.org/10.3897/mycokeys.60.46395

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16(12): 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Maas JL (1998) Compendium of strawberry diseases, 2nd Edn. American Phytopathology Society Press, Minnesota, 38–39. https://doi.org/10.1094/9780890546178

Mejía LC, Castlebury LA, Rossman AY, Sogonov MV, White JF (2008) Phylogenetic placement and taxonomic review of the genus *Cryptosporella* and its synonyms *Ophiovalsa* and *Winterella* (*Gnomoniaceae*, *Diaporthales*). Mycological Research 112(1): 23–35. https://doi.org/10.1016/j.mycres.2007.03.021

Mejía LC, Rossman AY, Castlebury LA, Yang ZL, White Jr JF (2011) *Occultocarpon*, a new monotypic genus of Gnomoniaceae on *Alnus nepalensis* from China. Fungal Diversity 52: 99–105. https://doi.org/10.1007/s13225-011-0108-y

Mel'nik VA (1984) Coelomycetes of the SSSR. II. *Nagrajomyces dictyosoporus* Mel'nik gen. et sp. nov. and *Phragmotrichum vassiljevae* Mel'nik sp. nov. mikologiya i fitopatologiya 18: 9–14.

Meyer JB, Trapiello E, Senn-Irlet B, Sieber TN, Cornejo C, Aghayeva D, González AJ, Prospero S (2017) Phylogenetic and phenotypic characterisation of *Sirococcus castaneae* comb. nov. (synonym *Diplodina castaneae*), a fungal endophyte of European chestnut. Fungal Biology 121(8): 625–637. https://doi.org/10.1016/j.funbio.2017.04.001

Minoshima A, Walker DM, Takemoto S, Hosoya T, Walker AK, Ishikawa S, Hirooka Y (2019) Pathogenicity and taxonomy of *Tenuignomonia styracis* gen. et sp. nov., a new monotypic genus of *Gnomoniaceae* on *Styrax obassia* in Japan. Mycoscience 60(1): 31–39. https://doi.org/10.1016/j.myc.2018.08.001

Monod M (1983) Monographie taxonomique des Gnomoniaceae. Beihefte zur Sydowia 9: 1–315.

Nag Raj TR (1974) Icones generum coelomycetum VI. University of Waterloo Biology Series 13: 1–41.

Nag Raj TR (1993) Coelomycetous anamorphs with appendage-bearing conidia. Mycologue Publications, Waterloo.

Neely D, Black WM (1976) Anthracnose of black walnuts in the Midwest. Plant Disease Reporter 60(6): 519–521.

Nylander JAA (2004) MrModeltest v2, program distributed by the author. Uppsala University, Uppsala.

Osono T, Masuya H (2012) Endophytic fungi associated with leaves of Betulaceae in Japan. Canadian Journal of Microbiology 58(4): 507–515. https://doi.org/10.1139/w2012-018

Page RD (1996) TreeView: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12(4): 357–358. https://doi.org/10.1093/bioinformatics/12.4.357

Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21: 29–55. https://doi.org/10.3767/003158508X340742

Rehm H (1906) Beiträge zur Ascomycetenflora der Voralpen und Alpen. Oesterreichische botanische Zeitschrift 56: 291–348. https://doi.org/10.1007/BF01672393

Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98(6): 625–634. https://doi.org/10.1016/S0953-7562(09)80409-7

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Senanayake IC, Crous PW, Groenewald JZ, Maharachchikumbura SSN, Jeewon R, Phillips AJL, Bhat JD, Perera RH, Li QR, Li WJ, Tangthirasunun N, Norphanphoun C, Karunarathna SC, Camporesi E, Manawasighe IS, Al-Sadi AM, Hyde KD (2017) Families of *Diaporthales* based on morphological and phylogenetic evidence. Studies in Mycology 86: 217–296. https://doi.org/10.1016/j.simyco.2017.07.003

Senanayake IC, Jeewon R, Chomnunti P, Wanasinghe DN, Norphanphoun C, Karunarathna A, Pem D, Perera RH, Camporesi E, McKenzie EHC, Hyde KD, Karunarathna SC (2018) Taxonomic circumscription of *Diaporthales* based on multigene phylogeny and morphology. Fungal Diversity 93: 241–443. https://doi.org/10.1007/s13225-018-0410-z

Shrestha N, Wang ZH, Su XY, Xu XT, Lyu LS, Liu YP, Dimitrov D, Kennedy J, Wang QG, Tang ZY, Feng XJ (2018) Global patterns of *Rhododendron* diversity: The role of evolutionary time and diversification rates. Global Ecology and Biogeography 27(8): 913–924. https://doi.org/10.1111/geb.12750

Simon UK, Groenewald JZ, Crous PW (2009) *Cymadothea trifolii*, an obligate biotrophic leaf parasite of *Trifolium*, belongs to Mycosphaerellaceae as shown by nuclear ribosomal DNA analyses. Persoonia 22: 49–55. https://doi.org/10.3767/003158509X425350

Sogonov MV, Castlebury LA, Rossman AY, Farr DF, White JF (2005) The type species of genus *Gnomonia*, *G. gnomon*, and the closely related *G. setacea*. Sydowia 57(1): 102–119.

Sogonov MV, Castlebury LA, Rossman AY, Mejía LC, White JF (2008) Leaf-inhabiting genera of the *Gnomoniaceae*, *Diaporthales*. Studies in Mycology 62(1): 1–77. https://doi.org/10.3114/sim.2008.62.01

Sogonov MV, Castlebury LA, Rossman AY, White JF (2007) The type species of *Apiognomonia*, *A. veneta*, with its *Discula anamorph* is distinct from *A. errabunda*. Mycological Research 111(6): 693–709. https://doi.org/10.1016/j.mycres.2007.03.013

Spegazzini CL (1902) Mycetes argentinenses (Series II). Anales del Museo Nacional de Buenos Aires 8: 49–89.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21): 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stamatakis A, Ludwig T, Meier H (2005) RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 21(4): 456–463. https://doi.org/10.1093/bioinformatics/bti191

Swofford DL (2003) PAUP*. Phylogenetic Analysis using Parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Massachusetts.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Voglmayr H, Castlebury LA, Jaklitsch WM (2017) *Juglanconis* gen. nov. on *Juglandaceae*, and the new family Juglanconidaceae (Diaporthales). Persoonia 38: 136–155. https://doi.org/10.3767/003158517X694768

Voglmayr H, Jaklitsch WM (2014) Stilbosporaceae resurrected: generic reclassification and speciation. Persoonia 33: 61–82. https://doi.org/10.3767/003158514x684212

Walker DM, Castlebury LA, Rossman AY, Sogonov MV, White Jr JF (2010) Systematics of genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three gene phylogeny, host associations, and morphology. Mycologia 102(6): 1479–1496. https://doi.org/10.3852/10-002

Wehmeyer LE (1933) The genus *Diaporthe* Nitschke and its segregates. University of Michigan studies. Scientific series 9: 1–349.

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wijayawardene NN, Hyde KD, Wanasinghe DN, Papizadeh M, Goonasekara ID, Camporesi E, Bhat DJ, McKenzie EHC, Phillips AJ, Diederich P, Tanaka K, Li W, Tangthirasunun N, Phookamsak R, Dai D, Dissanayake AJ, Weerakoon G, Maharachchikumbura SS, Hashimoto A, Matsumura M, Bahkali AH, Wang Y (2016) Taxonomy and phylogeny of dematiaceous coelomycetes. Fungal Diversity 77: 1–316. https://doi.org/10.1007/s13225-016-0360-2

Wijekoon CP, Kalischuk ML, Brunelle P, Howard RJ, Kawchuk LM (2021) Characterization of bronze leaf disease in western Canadian aspen and poplar trees. Canadian Journal of Plant Science 102(1): 11–19. https://doi.org/10.1139/CJPS-2021-0104

Winter G (1886) Fungi Australienses. Revue mycologique 8: 207–213.

Yang Q, Jiang N, Tian CM (2020) Tree inhabiting gnomoniaceous species from China, with *Cryphogonomonia* gen. nov. proposed. MycoKeys 69: 71–89. https://doi.org/10.3897/mycokeys.69.54012

Zhang N, Blackwell M (2001) Molecular phylogeny of dogwood anthracnose fungus (*Discula destructiva*) and the Diaporthales. Mycologia 93(2): 355–365. https://doi.org/10.2307/3761657