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ORIGINAL ARTICLE

Two new species and a new record of *Nigrograna* (Nigrograna ceae, Pleosporales) from China and Thailand

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Abstract
Based on the morphological and phylogenetic analyses, four specimens, which were collected from China and Thailand respectively, are identified as two novel species *Nigrograna hydei* and *N. obtusispora* and a new record *N. magnoliae* in the present study. These share morphologic characters typical of *Nigrograna*, and cluster together with existing *Nigrograna* species in the phylogenetic analyses of a combined ITS-*TEF1-α* sequence dataset. Illustrations and comprehensive descriptions for these taxa are provided.

Keywords Biatriospora · Dothideomycetes · Multi-gene · Phylogeny · Taxonomy

Introduction

The genus *Nigrograna* was initially erected by De Gruyter et al. (2012) to accommodate an important human pathogenic species *Nigrograna mackinnonii* based on morphology and phylogeny. However, Ahmed et al. (2014) transferred *N. mackinnonii* to *Biatriospora* as it is phylogenetically close to the type species of *Biatriospora* (*B. marina*), and *Nigrograna* was therefore considered a synonym of *Biatriospora*. Jaklitsch and Voglmayr (2016) found that three newly collected taxa clustered with the above two *Biatriospora* species in their molecular study, but were morphologically and ecologically different from the type species of *Biatriospora*. Therefore, they established the family Nigrograna ceae to accommodate *Nigrograna*, with *N. mackinnonii* as the type species.

Members of *Nigrograna* are characterized by black ascomata, clavate, short pedicellate asci and pale to chocolate brown, asymmetric, fusoid to narrowly ellipsoid, septate ascospores. Currently, 16 species were reported according to Index Fungorum (September, 2020) and latest publication (Wanasinghe et al. 2020). Moreover, *Nigrograna*, the only genus in Nigrograna ceae, is also considered a geographically and ecologically diverse group as the species in this genus are reported as endophytes, saprobes, or pathogens, and widely distributed around the world (Jaklitsch and Voglmayr 2016; Kolařík et al. 2017; Tibpromma et al. 2017; Kolařík 2018; Zhao et al. 2018; Dayarathne et al. 2020; Mapook et al. 2020; Wanasinghe et al. 2020).

In this study, two novel species and a new record of *Nigrograna* were collected from China and Thailand. In order to explore their taxonomic placement and affinities with related species, the morphological characters along with genetic analyses based on combined ITS and *TEF1-α* sequences are provided.

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Material and methods

Collection, examination, and isolation

Samples were collected from Chiang Rai and Krabi Provinces, Thailand, and Guizhou Province, China respectively. Specimens were taken to the lab and then examined by using stereomicroscope (Motic SMZ 168) and compound microscopes (Nikon E100). Vertical sections of fruiting structures were made for microscope studies and photomicrography. Micro-morphological characters were observed using a Nikon ECLIPSE 80*i* compound microscope and captured by a Cannon EOS 70D digital camera. Measurements were processed in a program of Tarosoft (R) Image Frame Work v. 0.9.7 (Liu et al. 2010), and photographic plates were edited in Adobe Photoshop CS6 (Adobe Systems Inc., USA).

The fungi were isolated using single spore isolation as described in Chomnunti et al. (2014). The single germinated ascospores were picked up and transferred to potato dextrose agar (PDA; 39 g/l distilled water, Difco potato dextrose) for recording growth rates and culture characters. The holotypes are deposited at the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand, or Guizhou Academy of Agricultural Sciences (GZAAS) Guizhou, China. Ex-type living cultures are deposited in the Mae Fah Luang University Culture Collection (MFLUCC) Chiang Rai, Thailand, and Guizhou Culture Collection (GZCC), Guizhou, China. Facesoffungi and IndexFungorum numbers are provided as outlined in Jayasiri et al. (2015) and Index Fungorum (2020).

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from fresh fungal mycelium using an Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, Shanghai, People's Republic of China) following the manufacturer's instructions. The DNA amplification procedure was performed by Polymerase Chain Reaction (PCR) in a 25 μl reaction volume, which contained 9.5 μl distilled deionized water, 12.5 μl of 2 × Power Taq PCR Master Mix (TIANGEN Co., China), 1 μl DNA template, and 1 μl of each forward and reverse primers. Two partial gene regions were used in this study. The primers of ITS4 and ITS5 (White et al. 1990), EF1–983F and EF1–2218R (Rehner 2001) were used for amplifying the internal transcribed spacers (ITS) and translation elongation factor 1-alpha region (*TEF1*-α), respectively. The PCR thermal cycle programs for the above genes were as in the cited publications (White et al. 1990; Rehner 2001). The quality of PCR products was checked by 1.2% agarose gel electrophoresis stained with ethidium bromide and then sent for sequencing to Sango Biotechnology Co., Ltd. (Shanghai, People's Republic of China).

Phylogenetic analysis

Newly generated sequence data were used to carry out the BLASTn search in NCBI (https://www.ncbi.nlm.nih.gov) for a preliminary identification. Additional related sequences were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) based on the BLASTn results and recent publications (Dai et al. 2017; Jaklitsch and Voglmayr 2016; Hyde et al. 2017; Liu et al. 2017; Tibpromma et al. 2017; Kolařík 2018; Zhao et al. 2018; Dayarathne et al. 2020; Mapook et al. 2020; Wanasinghe et al. 2020). Single-gene sequence alignments were performed in MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) and edited manually where necessary in the program BioEdit v. 7.2 (Hall 1999). And the sequence dataset used to build the phylogenetic tree was uploaded as supplementary information 1 (Table 1).

Maximum-parsimony (MP) analyses were run using PAUP v. 4.0b10 (Swofford 2002) with 1000 replications and inferred using the heuristic search option with 1000 random taxa. All characters were unordered and of equal weight, and gaps were treated as missing data. Maxtrees was set as 5000, branches if zero length were collapsed, and all multiple equally parsimonious trees were saved. Stability of clade was accessed using a bootstrap (BT) analysis with 1000 replicates, each with ten replicates of random stepwise addition of taxa (Hillis and Bull 1993). Descriptive tree statistics for parsimony tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC), and homoplasy index (HI) were calculated.

Bayesian analyses were carried out by using MrBayes v. 3.2 (Ronquist et al. 2012). The best-fit models (SYM+I+G for ITS and GTR+I+G for *TEF1*-α) of evolution were estimated in MrModeltest 2.3 (Nylander 2008). Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo (MCMC) sampling in MrBayes v. 3.2. Six simultaneous Markov chains were run for 10,000,000 generations and trees were sampled every 100th generation. The temperature values were lowered to 0.15, burn-in was set to 0.25, and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01.

Trees were visualized with TreeView (Page 1996), and additionally layouts were done in the program of Adobe Illustrator CS v. 5 (Adobe Systems Inc., USA). Maximum-parsimony bootstrap (MPBP) values equal or greater than 50%, and Bayesian posterior probabilities (BYPP) equal or greater than 0.95 are given near the nodes.

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Table 1 Isolates and GenBank accession numbers of taxa used in the phylogenetic analyses. Newly generated sequences in this study are indicated in bold italics. The ex-type cultures were noted with T after specimen/strain numbers

Taxon	Specimen/strain no.	GenBank accession numbers		References
		ITS	*TEF1-α*	
Nigrograna antibiotica	CCF 4378^T	JX570932	JX570934	Kolařík et al. 2017; Kolařík 2018
Nigrograna antibiotica	CCF 1998	LT221894	-	Kolařík et al. 2017
Nigrograna cangshanensis	MFLUCC 15-0253^T	KY511063	KY511066	Tibpromma et al. 2017
Nigrograna carollii	CCF 4484^T	LN626657	LN626668	Kolařík et al. 2017; Kolařík 2018
Nigrograna chromolaenae	MFLUCC 17-1437^T	MT214379	MT235801	Mapook et al. 2020
Nigrograna fuscidula	CBS 141556^T	KX650550	KX650525	Jaklitsch and Voglmayr 2016
Nigrograna fuscidula	CBS 141476	KX650547	KX650522	Jaklitsch and Voglmayr 2016
Nigrograna fuscidula	MF1a	KX650548	KX650523	Jaklitsch and Voglmayr 2016
Nigrograna fuscidula	MF3	KX650549	KX650524	Jaklitsch and Voglmayr 201
Nigrograna hydei	***GZCC 19-0050^T***	***MN387227***	***MN389249***	***In this study***
Nigrograna impatientis	***GZCC 19-0042^T***	***MN387228***	***MN389250***	***In this study***
Nigrograna locuta-pollinis	CGMCC 3.18784^T	MF939601	MF939613	Zhao et al. 2018
Nigrograna locuta-pollinis	LC11690	MF939603	MF939614	Zhao et al. 2018
Nigrograna mackinnonii	CBS 674.75^T	NR_132037	KF407986	De Gruyter et al. 2012
Nigrograna mackinnonii	E5202H	JX264157	JX264154	Shaw et al. 2015
Nigrograna mackinnonii	E9303e	-	LN626673	Kolařík et al. 2017;
Nigrograna magnoliae	***GZCC 17-0057***	***MF399066***	***MF498583***	***In this study***
Nigrograna magnoliae	***MFLUCC 14-1187***	***MF399065***	***MF498582***	***In this study***
Nigrograna magnoliae	MFLUCC 20-0020^T	MT159628	MT159605	Wanasinghe et al. 2020
Nigrograna magnoliae	MFLUCC 20-0021	MT159629	MT159606	Wanasinghe et al. 2020
Nigrograna mycophila	CBS 141478^T	KX650553	KX650526	Jaklitsch and Voglmayr 2016
Nigrograna mycophila	CBS 141483	KX650555	KX650528	Jaklitsch and Voglmayr 2016
Nigrograna mycophila	MF6	KX650554	KX650527	Jaklitsch and Voglmayr 2016
Nigrograna norvegica	CBS 141485^T	KX650556	-	Jaklitsch and Voglmayr 2016
Nigrograna obliqua	CBS 141477^T	KX650560	KX650531	Jaklitsch and Voglmayr 2016
Nigrograna obliqua	CBS 141475	KX650558	KX650530	Jaklitsch and Voglmayr 2016
Nigrograna obliqua	MRP	KX650561	KX650532	Jaklitsch and Voglmayr 2016
Nigrograna peruviensis	CCF 4485^T	LN626658	LN626671	Kolařík et al. 2017; Kolařík 2018
Nigrograna rhizophorae	MFLUCC 18-0397^T	MN047085	MN077064	Dayarathne et al. 2020
Nigrograna samueliana	NFCCI-4383^T	MK358817	MK330937	Dayarathne et al. 2020
Nigrograna thymi	MFLUCC 14-1096^T	KY775576	KY775578	Hyde et al. 2017
Nigrograna yasuniana	YU.101026^T	HQ108005	LN626670	Kolařík et al. 2017; Kolařík 2018
Occultibambusa bambusae	MFLUCC 13-0855^T	KU940123	KU940193	Dai et al. 2017
Occultibambusa pustula	MFLUCC 11-0502^T	KU940126	-	Dai et al. 2017

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Results

Molecular analyses

Phylogenetic analyses of the combined ITS and *TEF1-α* matrix were used to determine the placement of newly collected taxa. The dataset consisted of 34 strains with *Occultibambusa bambusae* (MFLUCC 13-0855) and *O. pustula* (MFLUCC 11-0502) as the outgroup taxa. The dataset comprised 1402 characters (including gaps), of which 512 characters belonged to ITS (1–512), and 890 characters to *TEF1-α* (513–1402). Among these characters, of which 1052 were constant characters, 95 variable parsimony-uninformative characters, while 255 characters were parsimony-informative. The most parsimonious tree showed that TL = 698, CI = 0.622, RI = 0.788, RC = 0.490, and HI = 0.378. The MP tree revealed by the parsimony analysis was selected to represent the relationships among these taxa and is shown in Fig. 1. The trees generated from Bayesian analyses showed the similar topologies (not shown here). The new species *N. impatiensis* clusters with *N. chromolaenae*, where they are sister to the type species *N. mackinnonii*. *Nigrograna hydei* forms an independent clade, and it is phylogenetically related to the above three taxa, while our new strains GZCC 17-0057 and MFLLUCC 14-1178 group together with reported species *N. magnoliae* in a monophyletic clade.

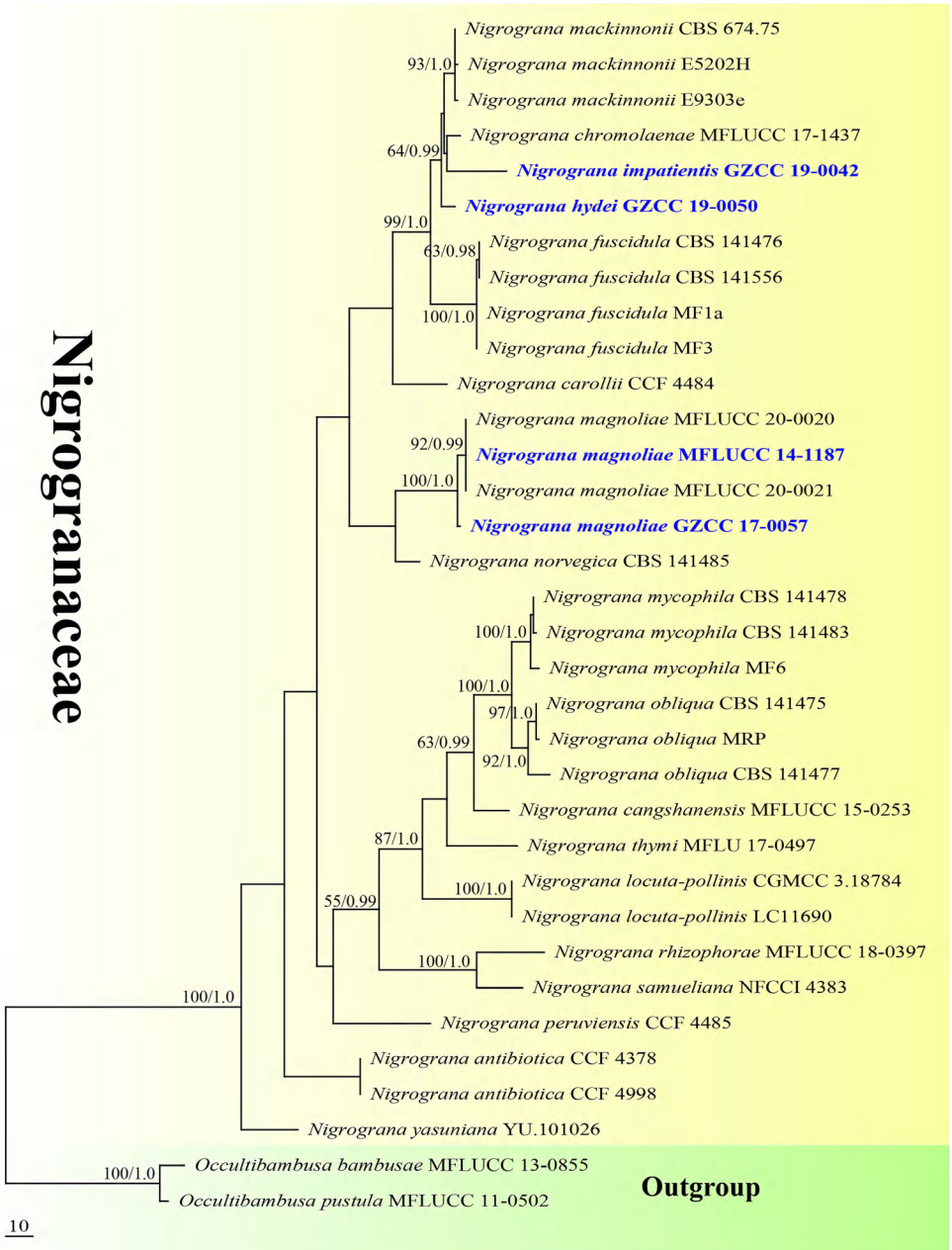

Fig. 1 Phylogram of the MP tree generated from combination of ITS and *TEF1-α* sequence dataset. MPBP ≥ 50% resulting from 1000 bootstrap replicates, and Bayesian posterior probabilities (BYPP) ≥ 0.95 are shown near the nodes. The original isolate numbers are noted after the species names. New strains are indicated in blue, bold letters. The tree is rooted to *Occultibambusa bambusae* (MFLUCC 13-0855) and *O. pustula* (MFLUCC 11-0502), and the scale bar shows 10 changes.

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Taxonomy

Nigrograna hydei J.F. Zhang, J.K. Liu & Z.Y. Liu, sp. nov. Fig. 2

Index Fungorum number: IF556749

Facesoffungi number: FoF 06294

Etymology: Named in honor of K.D. Hyde for his contributions in mycology.

Holotypus: MFLU 18-2073

Saprobic on decaying branches of unidentified host. Sexual morph: *Ascomata* immersed, with only ostiolar necks visible on the host surface or erumpent, normally solitary, scattered or aggregated in numbers of six, globose to subglobose, dark brown to black, surrounded by a 1.5–2.5 μm wide, brown to olivaceous, subicular hyphae. *Ostiole* central, periphysate. *Peridium* 15–28 μm thick, consisting of several layers of thick-walled, dark brown to lightly pigmented cells of *textura angularis*. *Hamathecium* comprising of numerous, filiform, 1 μm wide pseudoparaphyses, anastomosing above and between the asci. *Asci* 40–49(–59) × 7–8.5 μm ($\bar{x}$ = 48 × 8 μm, *n* = 20), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short

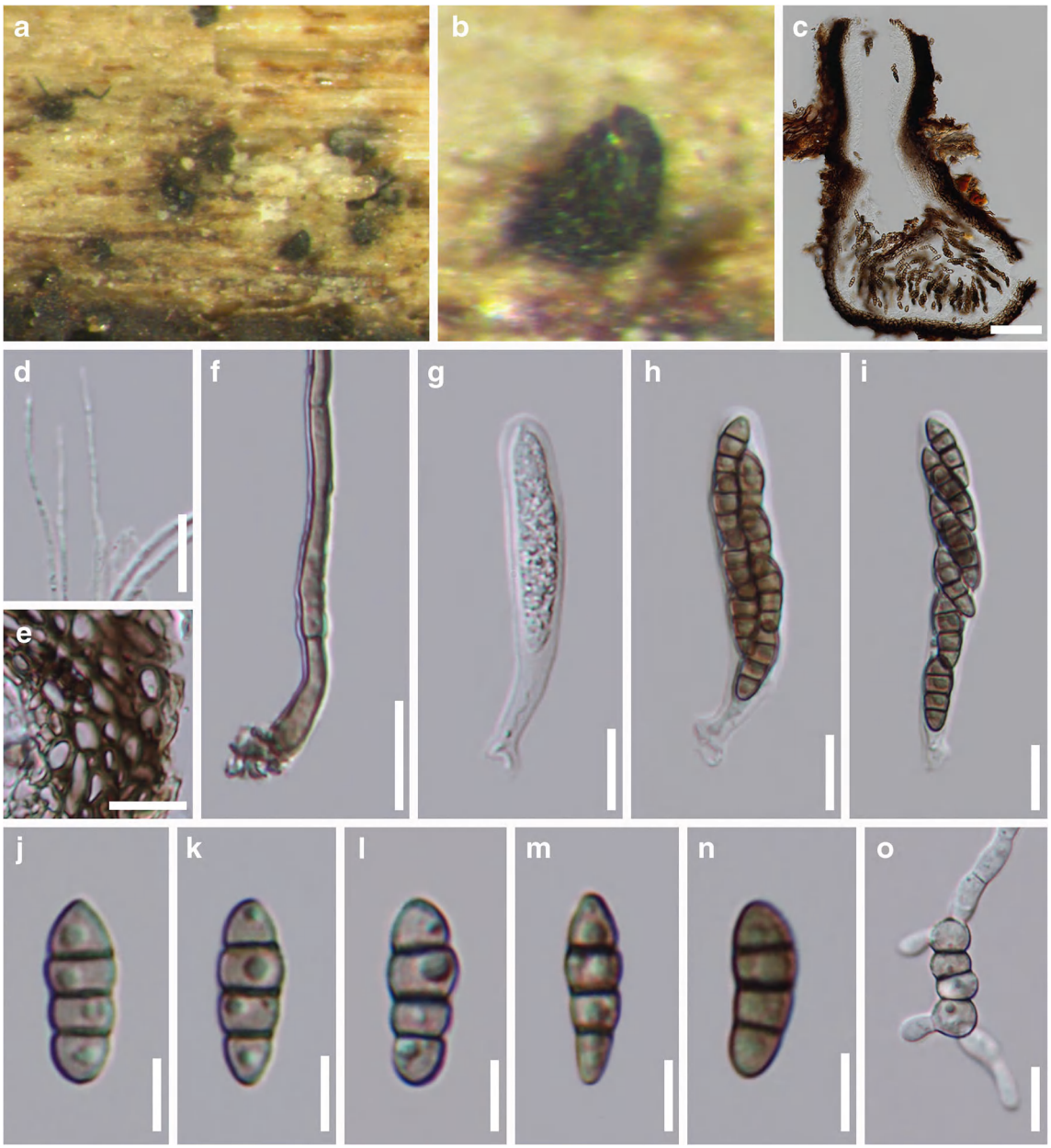

Fig. 2 *Nigrograna hydei* (MFLU 18-2073, holotype). **a**, **b** Ascomata immersed in the substrate with ostiolar necks erumpent throughout the host surface. **c** Vertical section through ascoma. **d** Pseudoparaphyses. **e** Section of peridium. **f** Subicular hypha. **g**–**i** Immature and mature asci. **j**–**n** Ascospores. **o** Germinating ascospore. Scale bars: **c** = 50 μm, **d**–**i**, **o** = 10 μm, **j**–**n** = 5 μm

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pedicellate with a swollen base, apically rounded with a developed ocular chamber. *Ascospores* 10–13 × 3.8–4.8 μm, ($\bar{x}$ = 12 × 4.3 μm, *n* = 30), uni- to bi-seriately arranged, fusoid to clavate, normally apical cell acute, and second cell slightly wider than others, straight or curved, 1-septate and pale brownish to yellow-brownish when young, becoming 3-septate, dark to chocolate-brownish at maturation, constricted at each septum. Asexual morph: Undetermined.

Culture characteristics Ascospores germinating on PDA within 24 h, colony reaching up to 33 mm on PDA after 35 days at 25 °C, irregular circular, entire edge, aerial mycelia dense, gray from above, brown to dark pigmented in reverse, and no asexual morph was observed.

Material examined Thailand, Krabi Province, on decaying branch of unidentified host, 22 December 2015, Kevin D. Hyde, MFL-06 (MFLU 18-2073, holotype; *Ibid.* GZAAS 18-0003, isotype); ex-type living culture (GZCC 19-0050).

Notes Our phylogenetic analyses showed that *N. hydei* forms an independent clade (MPBP 64% and BYPP 0.99), and is phylogenetically related to *N. chromolaenae*, *N. impatientis*, and *N. mackinnonii*. *Nigrograna hydei* shares the similar-sized asci and ascospores with *N. chromolaenae*, however, the former producing 3-septate ascospores, being constricted at each septum, while the ascospores in the latter are 3-euseptate, and only slightly constricted at the primary median septum (Mapnook et al. 2020). Moreover, the ascomata of *N. hydei* are surrounded by numerous, subicular hyphae, while these were not presented in *N. chromolaenae* (Mapnook et al. 2020). Both *N. impatitentis* and *N. hydei* share the similar-sized ascospores; however, the asci of *N. impatientis* are longer than N. *hydei* (55 × 8.5 μm vs. 48 × 8 μm), and the most remarkable difference between these two taxa is that *N. hydei* produces solitary ascomata, while the ascomata in *N. impatientis* are grouped and with convergent ostiolar necks penetrating together throughout the host surface. *Nigrograna hydei* and *N. mackinnonii* could not be compared directly in morphology as the latter was presented with asexual morph, while we did not observe the asexual morph in *N. hydei* (De Gruyter et al. 2012). Therefore, the recommendations of species delineation from Jeewon and Hyde (2016) were followed, and the comparison of each gene region between these taxa is processed and showed that there were 4 and 5 bp (base pair) differences in ITS and *TEF1-α* gene regions respectively. Even though this does not respond to their suggestions, *Nigrograna hydei* forms an independent clade in both MP and BY analyses with moderate support (MPBP 64% and BYPP 0.99). Therefore, we temporarily introduced *N. hydei* as new to science based on its phylogenetic placement, and more fresh specimens of this species are expected to be collected for consolidation of its availability in future study.

Nigrograna impatientis J.F. Zhang, J.K. Liu and Z.Y. Liu, sp. nov. Fig. 3

Index Fungorum number: IF556750

Facesoffungi number: FoF 06295

Etymology: Named after the host genus *Impatiens* from which the fungus was isolated.

Holotypus: MFLU 18-2072

Saprobic or *parasitic* on dead twigs of *Impatientis* sp. Sexual morph: *Ascomata* immersed in substrate, black, globose to subglobose, 127–153 μm wide, 227–330 μm high, normally 2–6 grouped together with convergent ostiolar necks penetrating together throughout the host surface, and stained the epidermis with a dark pigmented, often surrounded by a subiculum of brown to olivaceous, 1.5–2.5 μm wide hyphae. *Peridium* up to 13–30 μm thick, consisting of several layers of pseudoparenchymatous cells of *textura angularis*, becoming pale, thin-walled inside, and dark brown, thick-walled outside. *Hamathecium* comprising of numerous, 1–2 μm wide pseudoparaphyses, anastomosing above and between the asci. *Asci* (45–)50–58(–61) × 7–9.5 μm ($\bar{x}$ = 55 × 8.5 μm, *n* = 25), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, with a short stipe, apically rounded with a well-developed ocular chamber. *Ascospores* 12–14.5 × 4–5.5 μm, ($\bar{x}$ = 13 × 5 μm, *n* = 35) uni- to bi-seriate, fusoid to clavate, 3-euseptate, slightly constricted at the primary median septum, apical cell often acute, second cell usually slightly wider than others, straight or curved, verrucose, pale brownish to yellow-brown when young, turning dark to chocolate brown upon maturation, often guttulate. Asexual morph: Undetermined.

Culture characteristics Ascospores germinating on PDA within 24 h, colony reaching up to 18 mm on PDA after 14 days at 25 °C, circular, aerial mycelia dense, raised at the center, emerging several concentric rings in surface, white at the margin, becoming olivaceous grey towards the center, reverse white at the margin, brown towards inside, and dark pigmented at the center, and no asexual morph observed.

Specimen examined Thailand, Chiang Rai Province, on dead branch of *Impatiens* sp., 14 July 2017, J.F. Zhang, CR-01 (MFLU 18-2072, holotype; *Ibid.* GZAAS 18-0002, isotype); ex-type living culture (GZCC 19-0042).

Notes Present phylogenetic analyses (Fig. 1) showed that *N. impatientis* clusters together with *N. chromolaenae*, but without strong statistical bootstrap value support. *Nigrograna impatientis* is morphologically distinguished from *N. chromolaenae* as it produces longer asci (55 × 8.5 μm vs. 50 × 8.5 μm), and the ascomata in the former are grouped and with convergent ostiolar necks penetrating together throughout the host surface, while the asocomata in *N. chromolaenae* are solitary. Moreover, the ascomata of *N. impatientis* are often surrounded by brown subicular

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Fig. 3 *Nigrograna impatientis* (MFLU 18-2072, holotype). **a** Ascomata immersed in the substrate with convergent ostiolar necks penetrating together throughout the host surface. **b** Parallel section through ascomata. **c** Vertical section through ascomata. **d** Section of peridium. **e** Asci and pseudoparaphyses. **f**, **g** Asci. **h** Subicular hypha. **i**–**m** Ascospores. Scale bars: **c** = 200 μm, **d**, **i**–**m** = 5 μm, **e** = 20 μm, **f**–**h** = 10 μm

hyphae; however, this character was not found in *N. chromolaenae* (Mapook et al. 2020). In addition, the comparison of each gene region between these taxa was carried out according to the recommendations by Jeewon and Hyde (2016), and the results showed that there were 12 and 16 bp (base pair) differences across ITS and *TEF1-α* genes respectively. Besides, the comparison of each gene region between *N. impatientis* and the type species *N. mackinnonii* was also processed, and the results showed that there were 8 and 14 bp (base pair) differences in ITS and *TEF1-α* genes respectively.

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Therefore, *Nigrograna impatientis* is introduced herein as a new species based on the above information and recommendations from Jeewon and Hyde (2016).

Nigrograna magnoliae Wanas., PLoS One 15(7): e0235855 Fig. 4

Saprobic on decaying, submerged twigs in aquatic habitats, visible as black dots or masses of brown ascospores on the host surface. Sexual morph: *Ascomata* 200–300 μm wide, 100–150 μm high, solitary or aggregated in small groups, brown, immersed, appearing as slightly raised regions with black ostioles, ostioles lined with periphyses. *Peridium* 29.6–46.6 μm wide, comprising several fused layers of *textura angularis*, thin-walled and pale brown at the interior, becoming darker and thicker-walled to the outside. *Hamathecium* composed of hypha-like, 1.4–1.8 μm wide, slightly tapering pseudoparaphyses, anastomosing between and above the asci, embedded in a gelatinous matrix. *Asci* 50–85 × 6–10 μm ($\bar{x}$ = 70 × 8.5 μm, n = 25), 8-spored, bitunicate, fissitunicate, clavate to cylindrical, with a short stipe and knob-like base, apically rounded with a minute ocular chamber. *Ascospores* 12–16 × 4–6 μm ($\bar{x}$ = 4 × 5 μm, n = 40), uni- to bi-seriately arranged, fusoid to ellipsoid, with the second cell slightly enlarged, straight or slightly curved, with the obtuse to rounded ends, pale brown and one-septate when young, becoming chocolate brown or dark brown at maturation, 3-euseptate, and slightly constricted at the median septum. Asexual morph: Undetermined.

Culture characteristics Ascospores germinating on PDA within 12 h. Colonies reaching 21 mm diameter on PDA in 4 weeks at 25 °C, circular, raised and dense at the center, sparse and radiate at the margin, dark grey to pale olivaceous from above and dark olivaceous to black in reverse.

Specimen examined Thailand, Chiang Rai Province, Mae Fah Luang University, the drainage ditch on the campus, on submerged wood, 17 November 2014, Jing Yang, YJ-1 (MFLU 15-1167); living culture (MFLUCC 14-1187). China, Guizhou Province, Guiyang City, Huaxi District, Guizhou Academy of Agricultural Sciences, on decaying twigs of unidentified host, 17 July 2016, J.F. Zhang, GZ-03 (MFLU 18-2071; *Ibid.* GZAAS 18-0001); living culture (GZCC 17-0057).

Notes Phylogenetic analyses showed that our novel strains GZCC 17-0057 and MFLUCC 14-1187 and the existing species *N. magnoliae* group together in a monophyletic clade with strong statistical support (MPBP 100/100 and BYPP 1.0) (Fig. 1). Even though a short branch was presented between GZCC 17-0057 and another three strains (MFLUCC 14-1178, MFLUCC 20-0020, and MFLUCC 20-0021), there were only two and one bp (base pair) differences (not including the gaps) in the comparison of the 512 nucleotides of ITS regions and 890 nucleotides of *TEF1-α* regions respectively. Furthermore, morphological characteristics, i.e., ascomata, asci, and ascospores are not significantly different to each other between these specimens in shape and dimensions. Therefore, we identify these new collections as *N. magnoliae*. However, *Nigrograna magnoliae* was discovered in *Magnolia denudata* from China in the latest study (Wanasinghe et al. 2020), while the newly collected specimen MFLU 15-1167 was found on submerged twigs from Thailand. Therefore, we reported it herein as a new record from Thailand.

Discussion

Currently, 16 species were accepted in *Nigrograna*, but most of these taxa were discovered in America and Europe (Jaklitsch and Voglmayr 2016; Hyde et al. 2017; Kolařík et al. 2017; Tibpromma et al. 2017; Kolařík 2018; Zhao et al. 2018; Dayarathne et al. 2020; Wanasinghe et al. 2020). In this study, three newly collected taxa viz. *N. hydei*, *N. impatiensis*, and *N. magnoliae* were discovered from Thailand. And this is the third time to report *Nigrograna* species from Thailand as Dayarathne et al. (2020) and Mapook et al. (2020) described *N. rhizophorae* and *N. chromolaenae* respectively. In addition, *Nigrograna magnoliae* was also found in a different province from China in this study (Dayarathne et al. 2020). Therefore, *Nigrograna* is proposed to be a geographically diverse genus, and an ongoing investigation for *Nigrograna* species in China and Thailand is suggested to provide more fresh collections and explore the diversity of species in this genus.

Nigrograna is also an ecologically diverse genus as three taxa, i.e., *N. carollii*, *N. peruviensis*, and *N. yasuniana* have been reported as endophytes on various hosts (Kolařík et al. 2017), and around a quarter of existing species live as saprotrophs on the bark or corticated twigs of various hardwoods (Jaklitsch and Voglmayr 2016; Mapook et al. 2020; Wanasinghe et al. 2020), while even the species *N. mackinnonii* can cause mycetoma in human beings (De Gruyter et al. 2012). In this study, *Nigrograna hydei* and *N. magnoliae* were found to be saprobic on decaying branches of unidentified host, while another species *N. impatientis* was isolated from dead branches of *Impatiens* sp. However, pathology experiments were not carried out for *N. impatientis*, and we therefore cannot conclude whether it is a saprophyte or a pathogen.

In the present study, we also found that it is difficult to delimit the species in *Nigrograna* based only on their morphological characters, which agrees with the conclusion given by Jaklitsch and Voglmayr (2016) that molecular data are crucial for identification on interspecific level of this genus. Besides,

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Fig. 4 *Nigrograna magnoliae* (MFLU 15-1167). **a** Substrate. **b** Appearance of ostioles on surface of host, with masses of ascospores. **c** Mass of ascospores on host surface. **d** Vertical section of ascoma. **e** Section of peridium. **f** Psedoparaphyses. **g**–**l** Immature and mature asci. **m**–**v** Ascospores. **w** Germinating ascospore. **x**–**y** Colonies on PDA, **y** from bottom. Scale bars: **b** = 200 μm, **c**–**d** = 100 μm, **e**–**f** = 10 μm, **h**, **w** = 15 μm, **g**, **i**–**j** = 20 μm, **k** = 25 μm, **l** = 30 μm, **m**–**v** = 5 μm

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several *Nigrograna* species were established without strong bootstrap values support in recent studies and even in our study (Kolařík et al. 2017; Kolařík 2018; Mapook et al. 2020; Wanasinghe et al. 2020). Thus, more fresh *Nigrograna* collections and available sequence data are expected in future studies to resolve this issue.

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