forests

MDPI

Article

Taxonomy and Molecular Phylogeny of *Phellodon* (Thelephorales) with Descriptions of Four New Species from Southwest China

Chang-Ge Song [1], Xing Ji [1], Shun Liu [1], Xiao-Lan He [2] and Bao-Kai Cui [1,*]

[1] Institute of Microbiology, School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China; changgesong@126.com (C.-G.S.); jixingharper@163.com (X.J.); liushun2017@bjfu.edu.cn (S.L.)
[2] Soil and Fertilizer Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China; xiaolanhe1121@aliyun.com
* Correspondence: cuibaokai@yahoo.com; Tel./Fax: +86-10-6233-6309

Abstract: *Phellodon* is a genus of ectomycorrhizal fungi belonging to the group known as the stipitate hydnoids. It is associated with coniferous trees in forest ecosystems and is widely distributed in the northern hemisphere. *Phellodon*, together with *Hydnellum*, and *Sarcodon*, is classified in the Bankeraceae, members of which are generally considered as symbiotic fungi. Ectomycorrhizal fungi can help plant roots fix nitrogen and improve the absorption capacity of soil nutrients by trees, so they play an important role in ecosystem protection. Taxonomic and phylogenetic studies of Chinese *Phellodon* collections were carried out. Four new *Phellodon* species were discovered from southwestern China based on a combination of morphological characters and molecular data. *Phellodon atroardesiacus* is characterized by the blackish blue to dark grey pileus, dark grey to ash grey spines, and presence of clamp connections in spines. *Phellodon cinereofuscus* is distinguished by a cottony tomentose pileal margin, long spines which become clay-buff when dry, and echinulate basidiospores. *Phellodon stramineus* is characterized by a depressed and tomentose pileus, straw buff-colored pileal surface, and dark grey to ash grey spines. *Phellodon yunnanensis* is distinguished by a clay-pink to brown pileus, pale brown to white spines, and the presence of clamp connections in the outer layer of stipe. Detailed descriptions, illustrations, and ecological traits for the new taxa are provided. Phylogenetic analyses inferred from the internal transcribed spacer (ITS) regions confirmed that the four new species are distinct within *Phellodon*.

Keywords: ectomycorrhizal fungi; species identification; stipitate hydnoid fungi; taxonomy

Citation: Song, C.-G.; Ji, X.; Liu, S.; He, X.-L.; Cui, B.-K. Taxonomy and Molecular Phylogeny of *Phellodon* (Thelephorales) with Descriptions of Four New Species from Southwest China. *Forests* **2021**, *12*, 932. https://doi.org/10.3390/f12070932

Academic Editor: Artur Alves

Received: 10 June 2021
Accepted: 14 July 2021
Published: 16 July 2021

1. Introduction

Phellodon P. Karst. is a genus of ectomycorrhizal fungi associated with conifer trees in forest ecosystems. The genus *Phellodon*, together with *Hydnellum* P. Karst., and *Sarcodon* Quél. ex P. Karst., belongs to stipitate hydnoids, all of which are classified in the family Bankeraceae.

Ectomycorrhizal fungi are an important bridge between plant roots and soil and have ecological functions such as improving the absorption capacity of trees to soil nutrients [1]. Ectomycorrhizal fungal agents can also be widely used in seedling breeding of trees, exsitu protection of tree species, restoration and reconstruction of damaged ecosystems, and other processes [2]. Stipitate hydnoids are the emphasis of conservation in Europe because of their declining numbers [3]. Many British stipitate hydnoids species (14 species) were included in the UK Biodiversity Action Plan as priority species [4].

Phellodon was established by Petter Adolf Karsten and its type species is *P. niger* (Fr.) P. Karst [5]. According to the modern definition, species in *Phellodon* are characterized by the basidiomata consisting of a stipe and pileus with hydnoid hymenophores, uniform to

duplex context, hyaline and echinulate basidiospores [3]. Due to indeterminate growth, basidiomata of *Phellodon* are confluent and acquire irregular shape [6].

Around 18 species have been described in the genus according to He, et al. [7]. Most of these species were recorded from North America [6]. In the 20th century, species of *Phellodon* were described based only on morphological characteristics [8–15]. In recent years, molecular studies have been used to infer species limits in *Phellodon*. Parfitt et al. [3] combined morphological methods with DNA sequencing of the ITS1 region to clear the classification status of the known *Phellodon* species from the UK, which revealed more terminal clusters than conventionally recognized taxa. Baird et al. [7] conducted a study to reevaluate the species of stipitate hydnoid fungi from temperate southeastern United States; species of *Phellodon* were recorded and *Bankera fuligineoalba* (J.C. Schmidt) Pouzar was recombined in *Phellodon*. Then, they discovered a new species, *P. mississippiensis* R.E. Baird, L.E. Wallace & G. Baker, from the southern United States, which was observed to have rare clamp connections in the subhymenial hyphae [16]. The taxonomy and phylogeny of *Phellodon* are not well studied from China, and only one species, *P. subconfluens* H.S. Yuan & F. Wu in Liaoning Province, was recently described by Mu, et al. based on morphological characters and molecular data [17].

During investigations on macrofungi from Yunnan Province, southwestern China, some specimens with stipitate hydnoid basidiomata were collected. Morphological characters and phylogenetic analyses based on the internal transcribed spacer (ITS) regions indicated that these specimens represented four undescribed species of *Phellodon*. The aims of this study are to confirm the taxonomic affinities of the new species, explore the species diversity of *Phellodon* in southwestern China, and infer the evolutionary relationships among representative species of *Phellodon*.

2. Materials and Methods

2.1. Morphological Studies

The studied specimens were deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Macromorphological descriptions were based on field notes and herbarium specimens. Three specimens were examined of each of the 4 suspected new species, and 30 spores were counted per specimen. Microscopic characters, measurements, and drawings were made from slide preparations stained with Cotton Blue and Melzer's reagent and observed at magnifications up to ×1000 under a light microscope (Nikon Eclipse E 80i microscope, Nikon, Tokyo, Japan) following Sun, et al. [18] and Han, et al. [19]. In the text, the following abbreviations were used: IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, CB− = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W rationes between the specimens studied, and n = number of spores measured from given number of specimens. A field Emission Scanning Electron Microscope (FESEM) Hitachi SU-8010 (Hitachi, Ltd., Tokyo, Japan) was used to photograph the morphology of the basidiospores. Sections were studied at magnifications up to 1500× following Sun et al. [18].

2.2. Molecular Study and Phylogenetic Analysis

A CTAB rapid plant genome ex traction kit DN14 (Aidlab Biotechnologies, Beijing, China) was used to acquire total genomic DNA from dried specimens according to the manufacturer's instructions with some modifications [20,21]. The primer pairs ITS5/4 and MS1/MS2 were used to amplify ITS and the small subunit of mitochondrial rRNA gene (mtSSU) for one-way. The primer pairs LR0R/LR7, NS1/NS4, AF/Cr and 5F/7Cr were used to amplify the large subunit of nuclear ribosomal RNA gene (nLSU), the small subunit of nuclear ribosomal RNA gene (nSSU), DNA-directed RNA polymerase II subunit 1 (RPB1) and DNA-directed RNA polymerase II subunit 2 (RPB2) respectively [22] for two-way.

The PCR procedure for ITS and mtSSU was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 56 °C for 45 s and 72 °C for 1 min and a final extension of 72 °C for 10 min. The PCR procedure for nrLSU and nrSSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1.5 min and a final extension of 72 °C for 10 min. The PCR procedure for RPB1 and RPB2 was as follows: initial denaturation at 94 °C for 2 min, 9 cycles at 94 °C for 45 s, 60 °C for 45 s, followed by 36 cycles at 94 °C for 45 s, 53 °C for 1 min, 72 °C for 1.5 min and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced in Beijing Genomics Institute, China, with the same primers. All newly generated sequences were submitted to GenBank (Table 1).

Table 1. A list of species, specimens and GenBank accession numbers of sequences used in this study.

Species	Specimen No.	Locality	GenBank Accession No.					
			ITS	nrLSU	nuSSU	mtSSU	RPB1	RPB2
Phellodon alboniger	REB-70	USA	KC571749	-	-	-	-	-
P. alboniger	REB-57	USA	JN135206					
P. atratus	CL-72	Canada	MK281471	-	-	-	-	-
P. atratus	DAVFP28189	Canada	HQ650766	-	-	-	-	-
P. atroardesiacus	Cui 18449	China	**MZ221189**	**MZ225598**	**MZ225636 MZ225637**	-	-	-
P. atroardesiacus	Cui 18457	China	**MZ225577**	**MZ225599**	**MZ225637**	-	-	-
P. atroardesiacus	Cui 18458	China	**MZ225633**	**MZ225600**	**MZ225638**	-	-	-
P. atroardesiacus	Cui 18459	China	**MZ225634**	**MZ225601**	**MZ225639**	-	-	-
P. atroardesiacus	Cui 16951	China	**MZ225632**	**MZ225597**	**MZ225635**	-	**MZ343209**	**MZ343197**
P. brunneoolivaceus	REB-166	USA	KC571752	-	-	-	-	-
P. cinereofuscus	Cui 14231	China	**MZ225579**	-	-	-	-	-
P. cinereofuscus	Cui 16940	Australia	**MZ225580**	**MZ225602**	**MZ225640**	**MZ225623**	**MZ343210**	**MZ343198**
P. cinereofuscus	Cui 16944	China	**MZ225581**	**MZ225603**	**MZ225641**	**MZ225624**	**MZ343211**	**MZ343199**
P. cinereofuscus	Cui 16945	China	**MZ225582**	**MZ225604**	**MZ225642**	**MZ225625**	**-**	**-**
P. cinereofuscus	Cui 16962	China	**MZ225583**	**MZ225605**	**MZ225643**	**-**	**MZ352084**	**MZ343200**
P. cinereofuscus	Cui 16963	China	**MZ225584**	**MZ225606**	**MZ225644**	**MZ225627**	**MZ352085**	**MZ343201**
P. confluens	WAT28574	UK	EU622361	-	-	-	-	-
P. confluens	E00186901	UK	EU622362	-	-	-	-	-
P. ellisianus	REB-264	USA	KC571757	-	-	-	-	-
P. ellisianus	REB-407	USA	KC571759	-	-	-	-	-
P. fibulatus	REB-168	USA	JN135205	-	-	-	-	-
P. fibulatus	REB-34	USA	KC571761	-	-	-	-	-
P. fuligineoalbus	REB-271	USA	KC571760	-	-	-	-	-
P. fuligineoalbus	REB-285	USA	JN135196	-	-	-	-	-
P. fuligineoalbus	SL8	-	EU622316	-	-	-	-	-
P. melaleucus	LH4	UK	EU622368	-	-	-	-	-
P. melaleucus	E00219373	UK	EU622369	-	-	-	-	-
P. melaleucus	REB-408	USA	JN135197	-	-	-	-	-
P. mississippiensis	MS-1	USA	JN247563	-	-	-	-	-
P. mississippiensis	MS-3	USA	JN247564	-	-	-	-	-
P. niger	REB-46	USA	JN135202	-	-	-	-	-
P. niger	REB-282	USA	KC571766	-	-	-	-	-
P. cf. *nothofagi*	MES-175	Chile	MH930224	-	-	-	-	-
P. putidus	REB-8	USA	JN135200	-	-	-	-	-
P. secretus	0097	Russia	MG597404	-	-	-	-	-
P. sinclairii	PDD 89028	New Zealand	GU222291	-	-	-	-	-
P. stramineus	Cui 16942 16943	China	**MZ225585**	**MZ225607**	**MZ225645**	**-**	**MZ352086**	**-**
P. stramineus	Cui 16943	China	**MZ225586**	**MZ225608**	**MZ225646**	**-**	**MZ352087**	**MZ343202**
P. stramineus	Cui 16956 REB-57	China	**MZ225587**	**MZ225609**	**MZ225647**	**-**	**MZ352088**	**MZ343203**
P. stramineus	Cui 16959	China	**MZ225588**	**MZ225610**	**MZ225648**	**-**	**MZ352089**	**MZ343204**
P. stramineus	Cui 16961	China	**MZ225589**	**MZ225611**	**MZ225649**	**MZ225626**	**MZ352090**	**MZ343205**
P. stramineus	Cui 16964	China	**MZ225590**	**MZ225612**	**MZ225650**	**MZ225628**	**MZ352091**	**-**
P. subconfluens	Yuan11123	China	MK677464	-	-	-	-	-
P. subconfluens	Yuan11150	China	MK677465	-	-	-	-	-
Phellodon sp.1	REB-66	USA	KC571746	-	-	-	-	-
Phellodon sp.1	REB-83	USA	KC571747	-	-	-	-	-
Phellodon sp.1	REB-325	USA	KC571748	-	-	-	-	-

Table 1. *Cont.*

Species	Specimen No.	Locality	GenBank Accession No.					
			ITS	nrLSU	nuSSU	mtSSU	RPB1	RPB2
P. tomentosus	SL70	UK	EU622381	-	-	-	-	-
P. tomentosus	LH22	UK	EU622382		-	-	-	-
P. tomentosus	REB-274	USA	JN135203		-	-	-	-
P. violascens	2359-QFB-25626	-	KM406977	-	-	-	-	-
P. yunnanensis	Cui 14292	China	**MZ225591**	**-**	**-**	**-**	**-**	**-**
P. yunnanensis	Cui 14294	China	**MZ225592**	**-**	**-**	**-**	**-**	**-**
P. yunnanensis	Cui 17097	China	**MZ225593**	**MZ225613**	**MZ225651**	**MZ225629**	**-**	**MZ343206**
P. yunnanensis	Cui 17129	China	**MZ225594**	**MZ225614**	**MZ225652**	**MZ225630**	**-**	**MZ343207**
P. yunnanensis	Cui 17131	China	**MZ225595**	**MZ225615**	**MZ225653**	**MZ225631**	**-**	**MZ343208**
Sarcodon imbricatus	ELarsson384-10	Norway	MK602747	-	-	-	-	-
Sarcodon leucopus	OF296099		MK602755					

New sequences are shown in bold.

The sequences were aligned in MAFFT 6 [23] with the G-INS-i option provided by the CBRC (http://mafft.cbrc.jp/alignment/server/, accessed on 15 November 2020) and manually adjusted in BioEdit [24]. The sequences of *Sarcodon imbricatus* (L.) P. Karst. and *S. leucopus* (Pers.) Maas Geest. & Nannf. were used as outgroups. Phylogenetic analyses were performed as reported in Zhu, et al. [25] and Liu et al. [26]. The best-fit model of nucleotide evolution for the datasets was selected with AIC (Akaike Information Criterion) using MrModeltest 2.3 [27,28]. Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were performed based on ITS sequences.

The MP analysis was performed in PAUP* version 4.0b10 [29] with the heuristic search. All characters were equally weighted, and gaps were treated as missing data. Max-trees was set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BS) analysis with 1000 replicates [30]. Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Only the Maximum Parsimony best tree from all searches was kept.

The ITS region was divided into three partitions, ITS1, 5.8s, and ITS2, for the ML and Bayesian analyses [18]. ML searches were conducted with RA×ML-HPC2 under the GTRGAMMA model, with all model parameters estimated by the program. To assess branch support, 1000 rapid bootstrap replicates were run with the GTRCAT model.

BI was performed using MrBayes 3.2.6 on Abe through the Cipres Science Gateway (www.phylo.org, accessed on 18 November 2020) with 2 independent runs, each one beginning from random trees with 4 simultaneous independent Chains, performing 2 million replicates, sampling one tree every 100 generations. The first 25% of the sampled trees were discarded as burn-in. The remaining ones were used to construct a majority rule consensus and to calculate Bayesian posterior probabilities (BPP) of the clades.

Phylogenetic trees were constructed using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 19 November 2020). Branches that received bootstrap support for Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Posterior Probabilities (BPP) greater than or equal to 75% (MP and ML) and 0.95 (BPP) were considered as significantly supported, respectively.

3. Results

3.1. Phylogenetic Analyses

The ITS dataset included sequences from 59 fungal specimens representing 25 taxa. The dataset had an aligned length of 721 characters, of which 322 characters were constant, 43 were variable and parsimony-uninformative, and 356 were parsimony-informative. Maximum parsimony analysis yielded four equally parsimonious trees (TL = 1110, CI = 0.577, RI = 0.840, RC = 0.485, HI = 0.423). The best-fit model selected for these three partitions of ITS sequences was GTR+G for ITS1, JC for 5.8s, and HKY + G for ITS2. Bayesian and

MP analyses resulted in similar topologies as the ML analysis, with an average standard deviation of split frequencies of 0.006202. The ML topology is shown with MP (≥50%), ML (≥50%), and BPP (≥0.95) supported values at the nodes (Figure 1).

The phylogeny inferred from ITS sequences demonstrated that sampled specimens of the four new species: *Phellodon atroardesiacus*, *P. cinereofuscus P. stramineus*, and *Phellodon yunnanensis* formed distinct well-supported lineages (Figure 1).

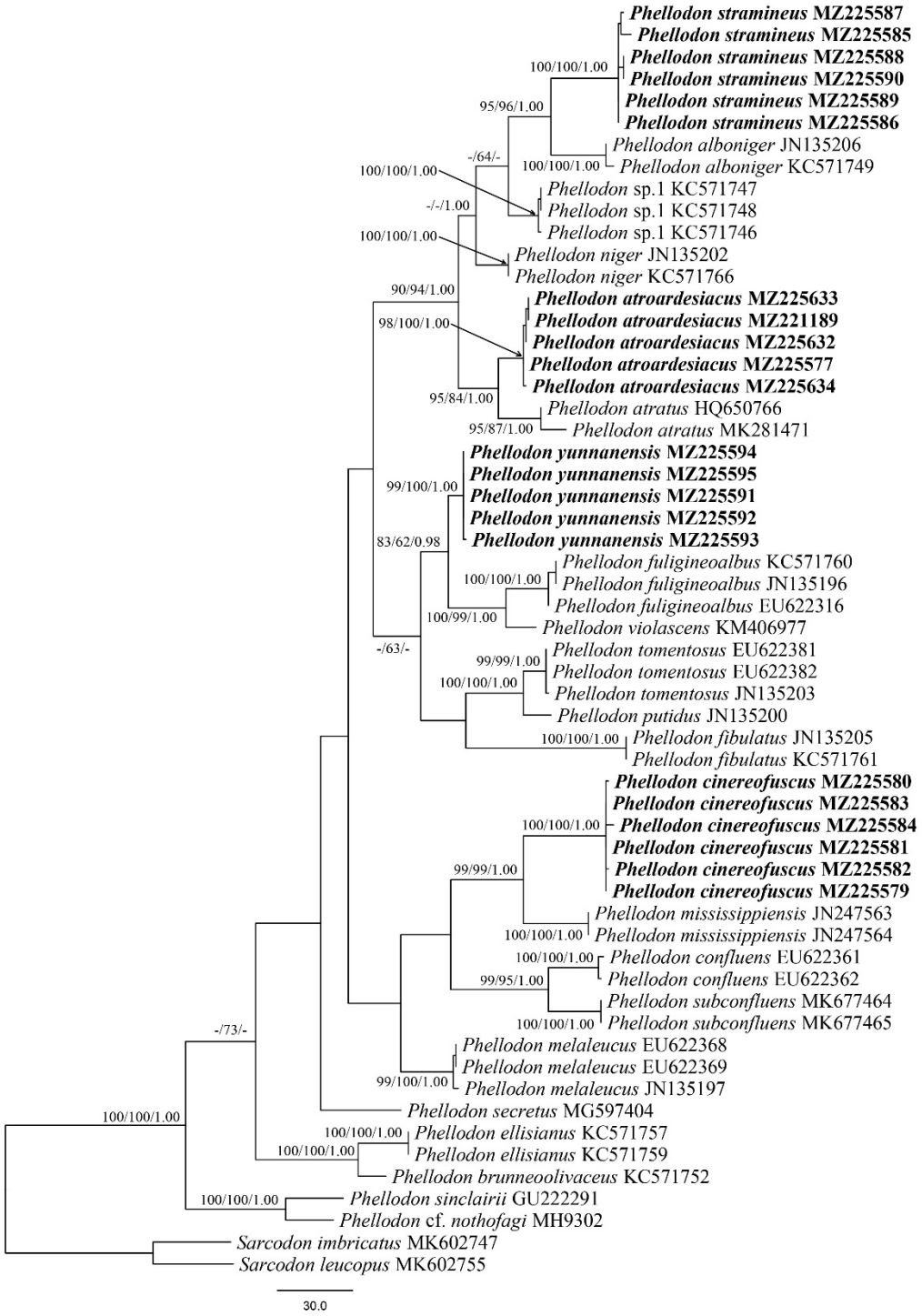

Figure 1. Maximum parsimony (MP) phylogram of the *Phellodon* species based on ITS sequences data. The supported branches are labeled with parsimony bootstrap proportions higher than 50%, maximum likelihood bootstraps higher than 50% and Bayesian posterior probabilities more than 0.95. Bold names = New species.

3.2. Taxonomy

Phellodon atroardesiacus B.K. Cui & C.G. Song, sp. nov., Figures 2a, 3a and 4.

MycoBank: 840306

Diagnosis—Differs from other *Phellodon* species by the blackish blue to dark grey pileus, scrobiculate at center, a mass of tomentum on pileus, short spines, and the presence of clamp connections in spines.

Etymology—*atroardesiacus* (Lat.): refers to the blackish blue basidiomata.

Holotype—**CHINA**. Xizang Autonomous Region, Chayu County, Cibagou Nature Reserve, on the ground of *Pinus densata* forest, approx. 97°04′ E 28°35′ N, elev. approx. 2900 m, 10 September 2020, Cui 18449 (BJFC 035310).

Fruitbody—Annual, centrally stipitate, single to concrescent, without odor or taste when fresh. Pileus orbicular to suborbicular, up to 5.5 cm in diam, 1.5 cm thick at the center. Pileal surface blackish blue to dark grey when fresh and becoming vinaceous brown upon drying, zonate, tomentose, scrobiculate at the center of the pileus; margin ash grey when fresh, becoming black after drying, up to 2 mm wide. Spines soft, dark greyish blue to ash grey when fresh, becoming fragile, pale mouse grey upon drying, up to 5 mm long. Context greyish blue, tough, azonate, up to 1 cm thick. Stipe cylindrical, glabrous, black, up to 5 cm long, 1 cm in diam.

Hyphal structure—Hyphal system monomitic; generative hyphae mostly with simple septa; all the hyphae IKI–, CB–; all the hyphae turned to olive green to black in KOH. Generative hyphae of pileal surface clay-buff, thick-walled, rarely branched, with simple septa, regularly arranged to parallel, 2.5–5 μm in diam. Generative hyphae in context clay-buff, slightly thick-walled, occasionally branched, with simple septa, parallel arranged, 3–6 μm in diam. Generative hyphae in spines vinaceous brown, thin-walled, occasionally branched, mostly with simple septa, occasionally with clamp connections, more or less parallel along the spines, 2–4 μm in diam. Generative hyphae in stipe clay-buff, thick-walled in the outer layer, rarely branched, mostly bearing simple septa, interwoven, 3–6 μm in diam; thick-walled in the inner layer, rarely branched, with simple septa, parallel along the stipe, 2.5–5 μm in diam.

Cystidia—Cystidia and other sterile hyphal elements absent.

Basidia—Clavate, bearing four sterigmata, 20–35 × 5–6 μm; sterigmata 2–4 μm long; basidioles similar to basidia in shape, but slightly smaller.

Spores—Basidiospores subglobose to globose, hyaline, thin-walled, echinulate, IKI–, CB–, 4–5 × (3–)3.5–4.5 μm, L = 4.45 μm, W = 3.78 μm, Q = 1–1.43 (n = 90/3, without the ornamentation).

Additional specimens (paratypes) examined—**CHINA**. Xizang Autonomous Region, Chayu County, Cibagou Nature Reserve, on ground of *Pinus densata* forest, alt. 2900 m, 10 September 2020, Cui 18457 (BJFC 035318) & Cui 18458 (BJFC 035319) & Cui 18459 (BJFC 035320).

Phellodon cinereofuscus B.K. Cui & C.G. Song, sp. nov., Figures 2b, 3b and 5.

MycoBank: 840307

Diagnosis—Differs from other *Phellodon* species by the cottony tomentose pileal margin, long spines which become clay-buff when dry, and echinulate basidiospores.

Etymology—*cinereofuscus* (Lat.): refers to the grey to pale brown spines.

Holotype—**CHINA**. Yunnan Province, Mouding County, Huafoshan Nature Reserve, on the ground of *Pinus* and *Fagaceae* forest, approx. 101°26′ E 25°19′ N, elev. approx. 2250 m, 13 September 2018, Cui 16962 (BJFC 030261).

Figure 2. Basidiomata of *Phellodon* species. (**a**,**b**). *P. atroardesiacus*, (**c**,**d**). *P. cinereofucus*, (**e**,**f**). *P. stramineus*, (**g**,**h**). *P. yunnanensis*. Scale bars: 2 cm.

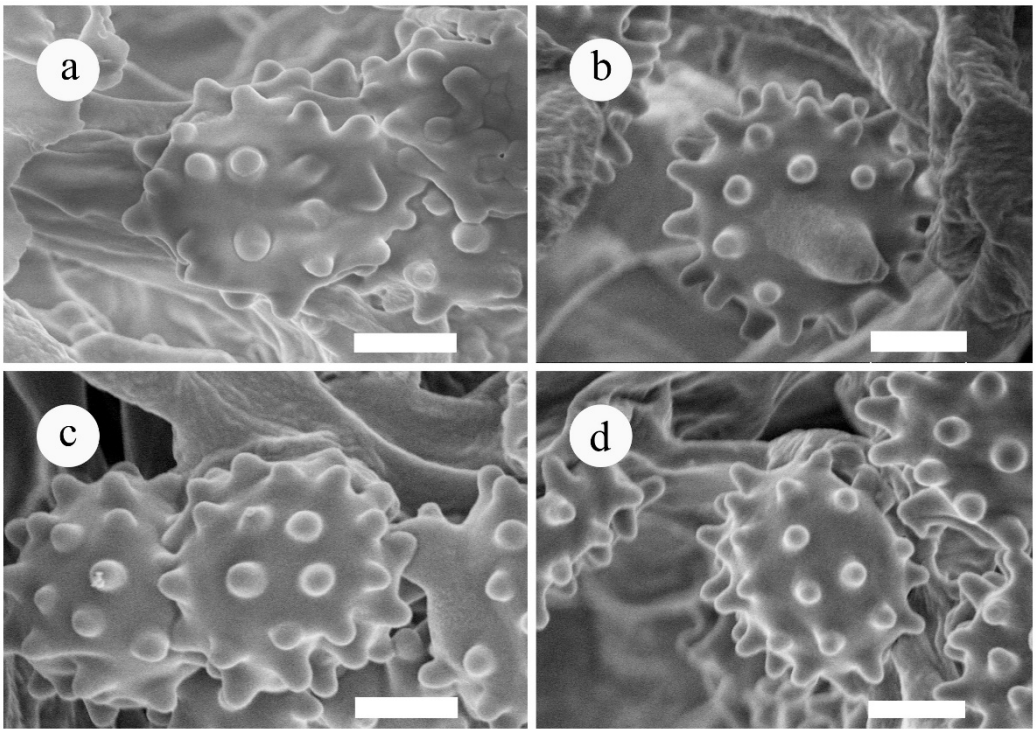

Figure 3. SEM of basidiospores of *Phellodon* species. (**a**). *P. atroardesiacus*, (**b**). *P. cinereofucus*, (**c**). *P. stramineus* (**d**). *P. yunnanensis*. Scale bars: 1.5 μm.

Fruitbody—Basidiomata annual, centrally or eccentrically stipitate, single to concrescent, with a fenugreek odor when fresh. Pileus irregularly shaped, infundibuliform, up to 11 cm in diam, 0.5 cm thick at the center. Pileal surface reddish brown to cinnamon brown when fresh and becoming greyish brown upon drying, color deeper at the center, zonate, glabrous, with radially aligned stripes at maturity; mature margin white when fresh and becoming cream to buff-yellow after drying, up to 1 cm wide. Spines soft, greyish brown to white when fresh, becoming fragile, buff to cinnamon-buff upon drying, up to 6 mm long. Context vinaceous buff, tough, azonate, up to 0.5 cm thick. Stipe cylindrical, glabrous, clay-buff to reddish buff, up to 4 cm long, 1.5 cm in diam.

Hyphal structure—Hyphal system monomitic; generative hyphae mostly with simple septa; all the hyphae IKI–, CB–; all tissues turning olive green to black in KOH. Generative hyphae in pileal surface hyaline to clay-buff, slightly thin-walled on the surface, rarely branched, with simple septa, regularly arranged to parallel, 3–6 μm in diam. Generative hyphae in context hyaline to clay-buff, thin-walled, occasionally branched, regularly arranged, with simple septa, 4–6.5 μm in diam. Generative hyphae in spines hyaline to clay-buff, thin-walled, mostly branched, with simple septa, more or less parallel along the spines, 2–4 μm in diam. Generative hyphae in stipe hyaline to clay-buff, thick-walled in the outer layer, rarely branched, bearing simple septa, interwoven, 3–7 μm in diam; thick-walled in the inner layer, with simple septa, parallel along the stipe, 3–6 μm in diam.

Cystidia—Cystidia and other sterile hyphal elements absent.

Basidia—Clavate, bearing four sterigmata and a basal simple septum, 17–34 × 5–7 μm; sterigmata 1–4 μm long; basidioles similar to basidia in shape, but slightly smaller.

Spores—Basidiospores subglobose to globose, hyaline, thin-walled, echinulate, IKI–, CB–, 4–5 × (3.5–)4–4.5 μm, L = 4.6 μm, W = 4.05 μm, Q = 1–1.25 (n = 90/3, without the ornamentation).

Additional specimens (paratypes) examined—CHINA. Yunnan Province, Nanhua County, Yulu Town, Sapiwu Village, on the ground of mixed forest dominated by trees of *Pinus* and *Quercus*, approx. 101°16′ E 25°11′ N elev. approx. 1800 m, 10 August 2016, Cui 14231 (BJFC 029099); Chuxiong, Zixishan Forest Park, on the ground of *Fagaceae* forest,

approx. 101°24′ E 25°1′ N elev. approx. 2100 m, 13 September 2018, Cui 16944 (BJFC 030243) & Cui 16945 (BJFC 030244); Mouding County, Huafoshan Nature Reserve, on the ground of *Pinus* and *Fagaceae* forest, approx. 101°26′ E 25°19′ N, elev. approx. 2250 m, 13 September 2018, Cui 16963 (BJFC 030262).

Phellodon stramineus B.K. Cui & C.G. Song, sp. nov., Figures 2c, 3c and 6.

MycoBank: 840308

Diagnosis—Differs from other *Phellodon* species by the greyish brown to olivaceous buff depressed and tomentose pileus, and long basidia with moderately long sterigmata.

Etymology—*stramineus* (Lat.), refers to the straw buff-colored pileal surface.

Holotype—**CHINA**. Yunnan Province, Mouding County, Huafoshan Nature Reserve, on the ground of forest dominated by *Pinus yunnanensis* and *Fagaceae*, approx. 101°26′ E 25°19′ N, elev. approx. 2250 m, 13 September 2018, Cui 16959 (BJFC 030258).

Fruitbody—Basidiomata annual, centrally or eccentrically stipitate, single to concrescent, with a fenugreek odor when dry. Pileus depressed or infundibuliform, up to 8 cm in diam, 5 cm thick at the center. Pileal surface straw buff when fresh and becoming buff upon drying, zonate, tomentose, with radially aligned stripes; margin dark grey to pale mouse grey when fresh, up to 3 mm wide. Spines soft, dark grey to ash grey when fresh, becoming fragile, pale mouse-grey to clay-buff upon drying, up to 3 mm long. Context tough, azonate, up to 3 mm thick. Stipe cylindrical, glabrous, olivaceous buff, up to 5.5 cm long, 0.8 cm in diam.

Hyphal structure—Hyphal system monomitic; generative hyphae mostly with simple septa; all the hyphae IKI–, CB–; tissues of pileus and stipe hyaline, while tissues of mature spines turned olive green in KOH. Generative hyphae hyaline and slightly thick-walled in pileus surface, rarely branched, with simple septa, regularly arranged to parallel, 4–6 μm in diam. Generative hyphae in context hyaline, thick-walled, occasionally branched, with simple septa, regularly arranged, 3–5 μm in diam. Generative hyphae in spines hyaline to clay-buff, thin-walled, occasionally branched, with simple septa, more or less parallel along the spines, 2–4 μm in diam. Generative hyphae in stipe hyaline to clay-buff, thick-walled, without branches, mostly bearing simple septa, subparallel along the stipe, 2–5 μm in diam.

Cystidia—Cystidia and other sterile hyphal elements absent.

Basidia—Clavate, bearing four sterigmata and a basal simple septum, 18–55 × 5–7 μm; sterigmata 1.5–5 μm long; basidioles similar to basidia in shape, but slightly smaller.

Spores—Basidiospores subglobose to globose, hyaline, thin-walled, echinulate, IKI–, CB–, 4–5.5(–6) × 4–5(–5.5) μm, L = 5.06 μm, W = 4.38 μm, Q = 1–1.5 (n = 90/3, without the ornamentation).

Additional specimens (paratypes) examined—**CHINA**. Yunnan Province, Chuxiong, Zixishan Forest Park, on the ground of *Fagaceae* forest, approx. 101°24′ E 25°1′ N elev. approx. 2250 m, 13 September 2018, Cui 16942 (BJFC 030241) & Cui 16943 (BJFC 030242); Mouding County, Huafoshan Nature Reserve, on the ground of forest dominated by *Pinus yunnanensis* and *Fagaceae*, approx. 101°26′ E 25°19′ N, elev. approx. 2250 m, 13 September 2018, Cui16956 (BJFC 030255) & Cui 16961 (BJFC 030260) & Cui 16964 (BJFC 030263).

Phellodon yunnanensis B.K. Cui & C.G. Song, sp. nov., Figures 2d, 3d and 7.

MycoBank: 840309

Diagnosis—Differs from other *Phellodon* species by a combination of glabrous pileus and stipe, moderately long spines, the presence of clamp connections in the outer layer of stipe, and tissues turning brown in KOH.

Etymology—*yunnanensis* (Lat.): referring to the holotype locality of the species in Yunnan Province.

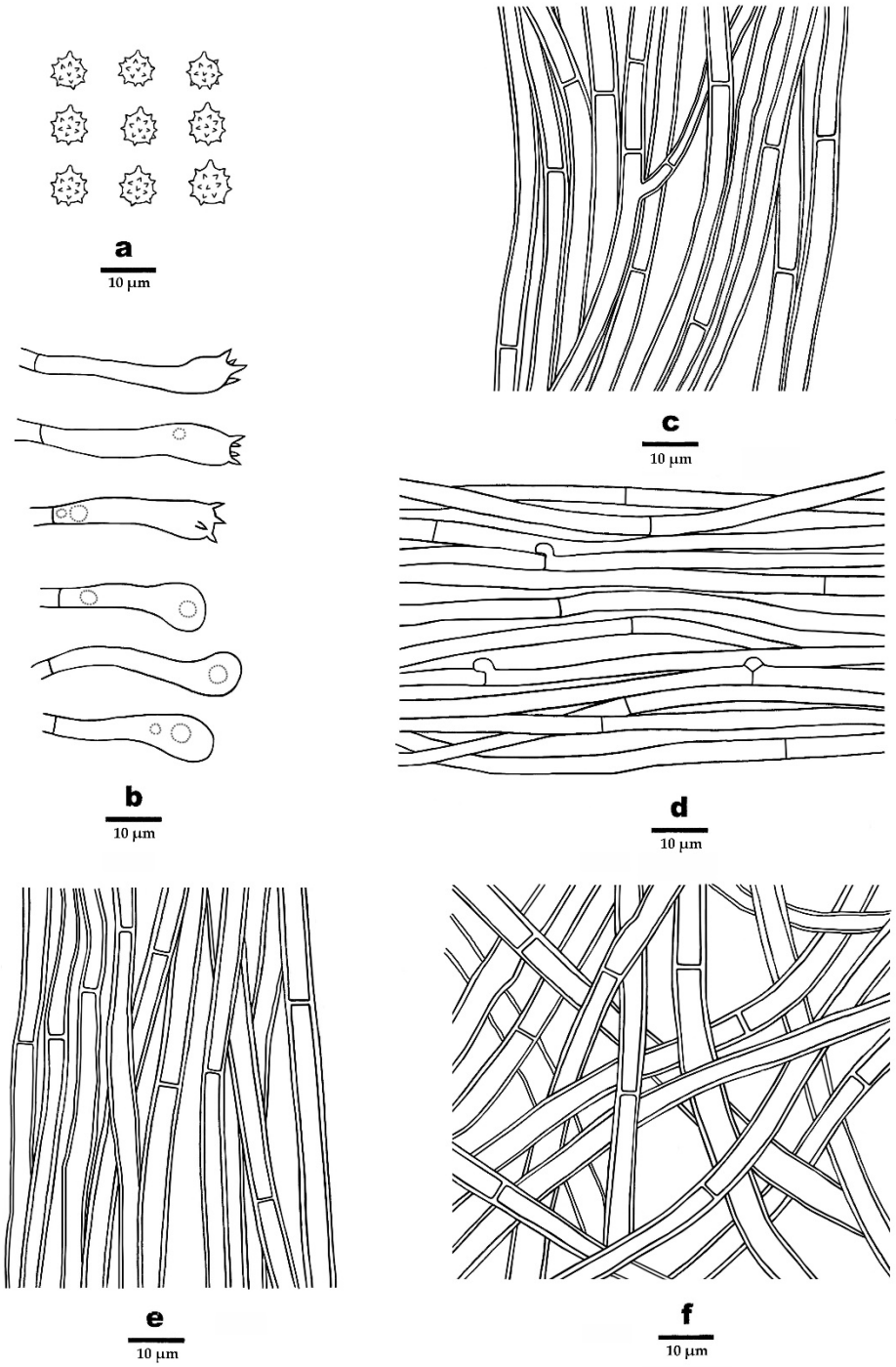

Figure 4. Microscopic structures of *P. atroardesiacus* (drawn from the holotype). (**a**). Basidiospores. (**b**). Basidia and basidioles. (**c**). Hyphae from spines. (**d**). Hyphae from context. (**e**). Hyphae from inner layer of stipe. (**f**). Hyphae from outer layer of stipe.

Holotype—**CHINA**. Yunnan Province, Lanping County, Tongdian Town, Jianganchang, on the ground of *Pinus armandii* and *Rhododendron* forest, approx. 99°32′ E 26°41′ N, elev. approx. 2600 m, 18 September 2018, Cui 17129 (BJFC 030429).

Fruitbody—Basidiomata annual, centrally or eccentrically stipitate, solitary or gregarious, with a fenugreek odor when fresh. Pileus irregularly shaped, depressed or infundibuliform, up to 8 cm in diam., 3 mm thick at the center. Pileal surface clay pink to brown when fresh and becoming greyish brown upon drying, zonate, glabrous, with radially aligned stripes; margin blunt or irregular, fawn to white when fresh, becoming

greyish brown with age, up to 3 mm wide. Spines soft, pale brown to white when fresh, becoming fragile, buff to cinereous upon drying, up to 5 mm long. Context tough, azonate, up to 3 mm thick. Stipe cylindrical, glabrous, basal tomentum absent, fawn, up to 3.5 cm long, 1.5 cm in diam.

Hyphal structure—Hyphal system monomitic; generative hyphae mostly with simple septa, occasionally with clamp connections; all the hyphae IKI–, CB–; tissues of pileus and stipe turning olive green to black, while tissues of mature spines turning brown in KOH. Generative hyphae in pileal surface hyaline, thin-walled to slightly thick-walled on the surface, rarely branched, with simple septa, regularly arranged to interwoven, 3–6.5 μm in diam. Generative hyphae in context hyaline, thin-walled, occasionally branched, with simple septa, regularly arranged, 2–6 μm in diam. Generative hyphae in spines hyaline, thin-walled, occasionally branched, with simple septa, more or less parallel along the spines, 2–4 μm in diam. Generative hyphae in stipe hyaline, slightly thick-walled in the outer layer, rarely branched, mostly bearing simple septa, occasionally with clamp connections, interwoven, 3–10 μm in diam., slightly thick-walled in the inner layer, rarely branched, with simple septa, subparallel along the stipe, 2–6 μm in diam.

Cystidia—Cystidia and other sterile hyphal elements absent.

Basidia—Clavate, bearing four sterigmata and a basal simple septum, 24–27 × 6–7 μm; sterigmata 1.5–5 μm long; basidioles similar to basidia in shape, but slightly smaller.

Spores—Basidiospores subglobose to globose, hyaline, thin-walled, echinulate, IKI–, CB–, 3.5–4.5(–5) × 3–4 (–4.5) μm, L = 3.99 μm, W = 3.64 μm, Q = 1.08–1.12 (n = 90/3, without the ornamentation).

Additional specimens (paratypes) examined—CHINA. Yunnan Province, Chuxiong, Zixishan Forest Park, on the ground of *Pinus* and *Fagaceae* forest, approx. 101°24′ E 25°1′ N elev. approx. 2300 m, 12 August 2016, Cui 14292 (BJFC 029160) & Cui 14294 (BJFC 029162); Xiangri-La, on the ground of *Pinus* forest, alt. 3200 m, 17 September 2018, Cui 17097 (BJFC 030397); Lanping County, Tongdian Town, Jianganchang, on the ground of *Pinus armandii* and *Rhododendron* forest, approx. 99°32′ E 26°41′ N, elev. approx. alt 2600 m, 18 September 2018, Cui 17131 (BJFC 030431).

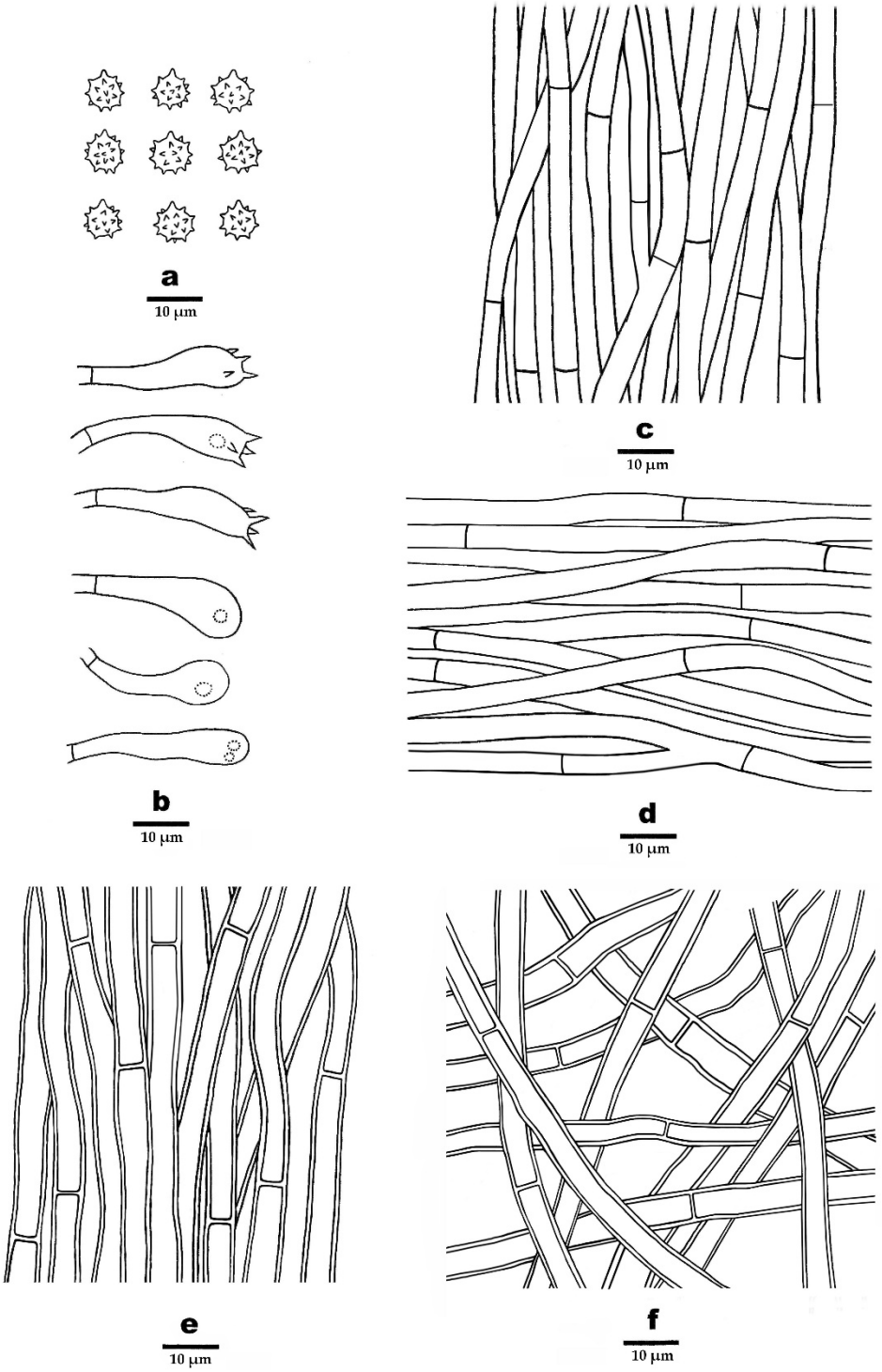

Figure 5. Microscopic structures of *P. cinereofucus* (drawn from the holotype). (**a**). Basidiospores. (**b**). Basidia and basidioles. (**c**). Hyphae from spines. (**d**). Hyphae from context. (**e**). Hyphae from inner layer of stipe. (**f**). Hyphae from outer layer of stipe.

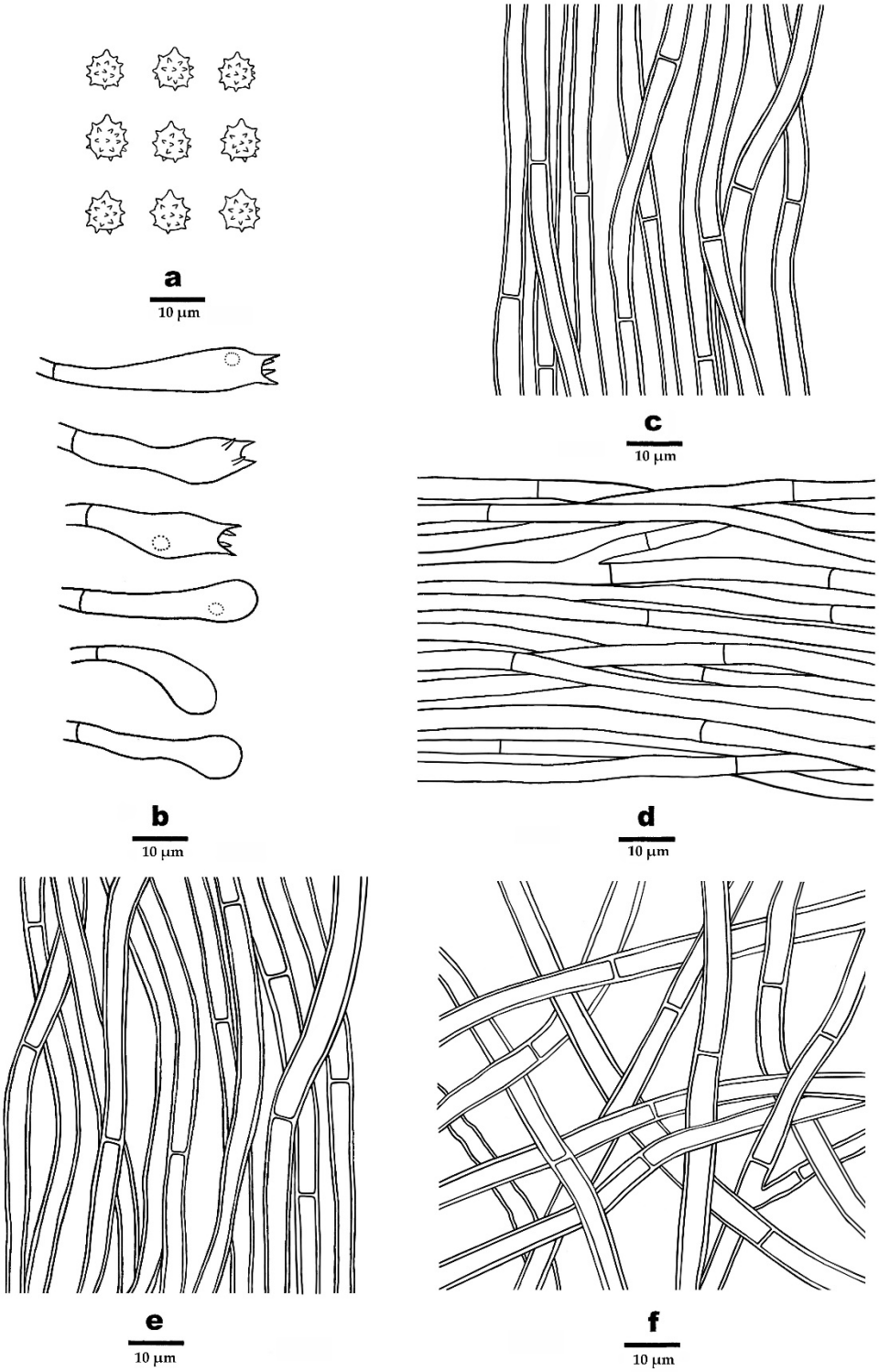

Figure 6. Microscopic structures of *P. stramineus* (drawn from the holotype). (**a**). Basidiospores. (**b**). Basidia and basidioles. (**c**). Hyphae from spines. (**d**). Hyphae from context. (**e**). Hyphae from inner layer of stipe. (**f**). Hyphae from outer layer of stipe.

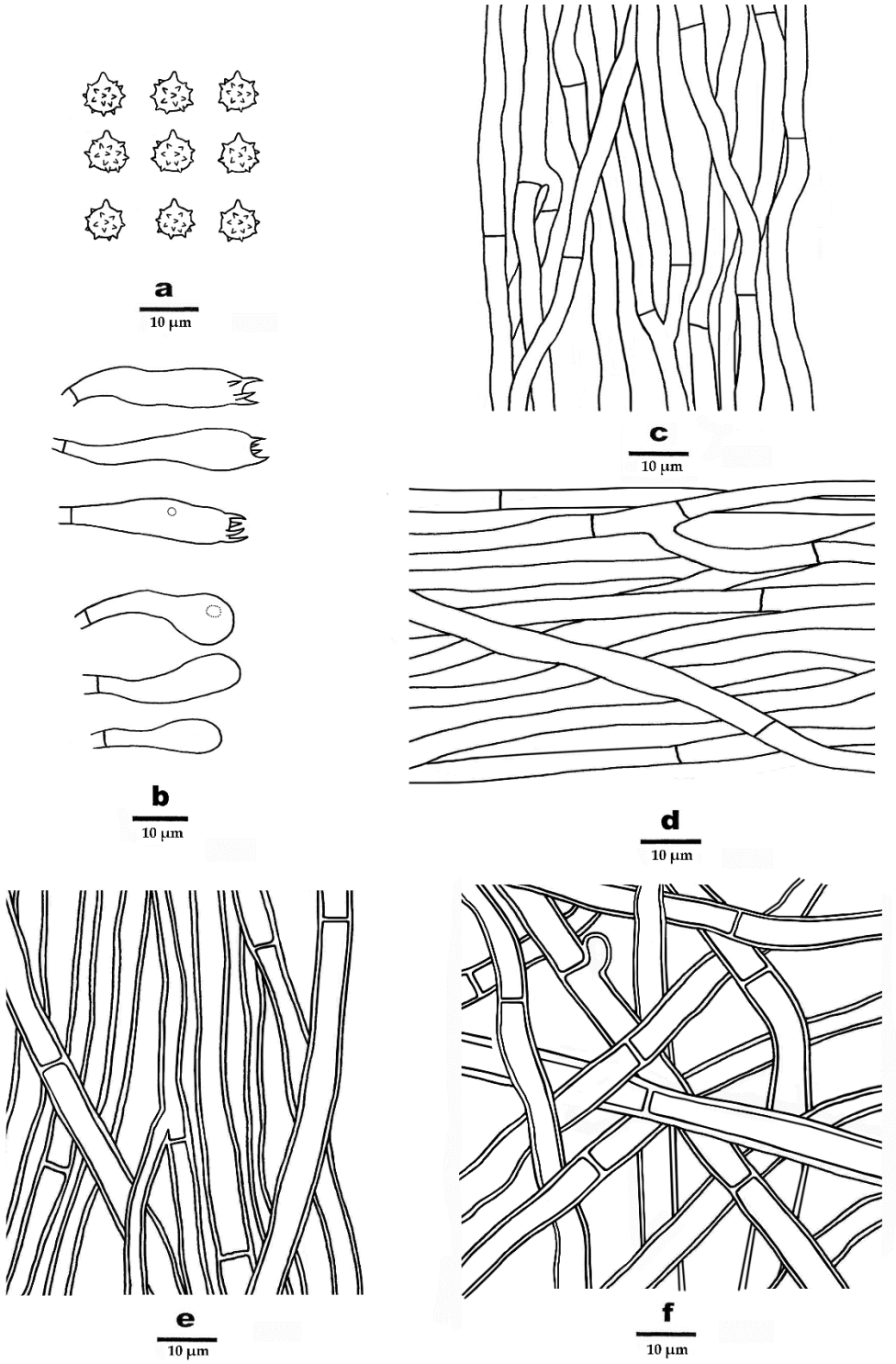

Figure 7. Microscopic structures of *P. yunnanensis* (drawn from the holotype). (**a**). Basidiospores. (**b**). Basidia and basidioles. (**c**). Hyphae from spines. (**d**). Hyphae from context. (**e**). Hyphae from inner layer of stipe. (**f**). Hyphae with clamps from outer layer of stipe.

4. Discussion

In the present study, four new species of *Phellodon* are described from southwestern China based on morphological characters and phylogenetic analyses of the ITS sequences. Due to the lack of multilocus sequences of other species in the genus, we are unable to construct a multilocus phylogenetic tree for the time being. We have sequenced the existing

specimens in our laboratory and provided the available sequences of nLSU, nSSU, mtSSU, RPB1 and RPB2 genes of the genus which might be useful for future studies.

Phellodon atroardesiacus is closely related to *P. atratus* K. Harrison in our phylogenetic analyses (Figure 1). Morphologically, *P. atratus* is similar to *P. atroardesiacus* in having a slate gray to black pileus and similar basidiospores (3.5–4.5 × 3.3–4.1 µm, [31]). However, *P. atratus* differs from *P. atroardesiacus* by having longer and narrower basidia (26–35 × 4.5–5.2 µm), and a lack of clamp connections [31]. These two species are then grouped together with *P. niger*. Macromorphologically, *P. niger* resembles *P. atroardesiacus* in having single to concrescent basidiomata, spongy tomentose pileus, and grey or bluish grey spines. Furthermore, *P. niger* can be distinguished from *P. atroardesiacus* by lacking clamp connections [5]. In addition, these three species are distinct from each other in the phylogenetic tree (Figure 1). *Phellodon fibulatus* K.A. Harrison resembles *P. atroardesiacus* in having single to gregarious basidiomata and abundant clamp connections in the spines. However, *P. fibulatus* is different from *P. atroardesiacus* by its greyish orange pileus and tissues becoming greyish in KOH [6].

Phellodon cinereofuscus is closely related to *P. mississippiensis* in the phylogenetic tree (Figure 1). Morphologically, *P. mississippiensis* is similar to *P. cinereofuscus* in having single to concrescent basidiomata, and white to pale orange, or light orange, spines. However, there are clamp connections in the subhymenial hyphae in *P. mississippiensis* while they are absent in *P. cinereofuscus*. Moreover, *P. mississippiensis* can be distinguished from *P. cinereofuscus* in having shorter basidia (16–22 × 5–6 µm, [6]). *Phellodon cinereofuscus* may be confused with *P. fibulatus* by having similar-colored pileal margin and similar-sized basidiospores and basidia. However, clamp connections are absent in *P. cinereofuscus* while present in *P. fibulatus*. Furthermore, *P. fibulatus* differs from *P. cinereofuscus* in having a longer stipe (3–7 cm, [12]). *Phellodon tomentosus* (L.) Banker is similar to *P. cinereofuscus* in having a brownish yellow to yellowish brown pileus which is cottony tomentose and often fibrillose at the margin. However, *P. tomentosus* differs from *P. cinereofuscus* in having thicker basidiomata (up to 2 mm, [17]) and clamp connections in stipe tissue. Moreover, our phylogenetic analyses showed that *P. tomentosus* is distinctly different from *P. cinereofuscus* (Figure 1).

Phellodon stramineus is closely related to *P. alboniger* (Fr.) Karsten in the phylogenetic tree (Figure 1). Morphologically, *P. alboniger* might be confused with *P. stramineus* by having single to concrescent basidiomata. However, *P. alboniger* is distinguished by its narrower basidiospores (3.8–5.2 × 3.6–4.4 µm) and shorter basidia (24–36 × 5–6.4 µm, [10]).

Phellodon yunnanensis is closely related to *P. violascens* (Alb. & Schwein.) A.M. Ainsw. and *P. fuligineoalbus* in our phylogenetic analyses (Figure 1). *Phellodon violascens* differs from *P. yunnanensis* by its white to flesh brown basidiomata, lack of clamp connections, and larger basidiospores (4.5–5.4 × 4.3–4.5 µm, [32]). *Phellodon fuligineoalbus* differs from *P. yunnanensis* in having yellow-white or light brown basidiomata, longer spines (up to 6 mm), lack of clamp connections, and larger basidiospores (4–6 × 4–5 µm, [16]). *Phellodon fibulatus* may be confused with *P. yunnanensis* by having clamp connections. However, clamp connections are abundant and occur in all parts in *P. fibulatus*, whereas in *P. yunnanensis* clamps are found only in the outer layer of stipe. Furthermore, it differs from *P. yunnanensis* in having tawny rhizomorphs and basal tomentum [6]. In addition, *P. fibulatus* is distinct from *P. yunnanensis* in the ITS-based phylogenetic analyses (Figure 1).

Stipitate hydnoid fungi, as critical functional components of forest ecosystems, are sensitive to nitrogen deposition. The diversity of stipitate hydnoid fungi can reflect the conservation state of forest ecosystems. Although approximately 16 stipitate hydnoid fungi have been recorded in China [7,33,34], species concepts for many of those fungi are still obscure. Therefore, comprehensive studies on the species diversity, taxonomy, and phylogeny of the hydnoid fungi are needed in the future.

5. Conclusions

In this study, four new species of ectomycorrhizal fungi belonging to *Phellodon* are described from southwestern China based on morphological characters, ecological distributions and ITS-based phylogeny.

Author Contributions: B.-K.C. designed the research; B.-K.C., X.-L.H., X.J., S.L. and C.-G.S. prepared the samples; C.-G.S., X.J. and S.L. conducted the molecular experiments and analyzed the data; C.-G.S., X.-L.H. and B.-K.C. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The research is supported by the National Natural Science Foundation of China (No. 31870008), Beijing Forestry University Outstanding Young Talent Cultivation Project (No. 2019JQ03016), and the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment, China (No. 2019HJ2096001006).

Data Availability Statement: The data and results of this study are available upon reasonable request. Please contact the main author of this publication.

Acknowledgments: We express our gratitude to Jia-Hui Xing (China), Yuan-Yuan Chen (China), Min Wang (China), Yi-Fei Sun (China), Yan Wang (China), Yu-Li Han (China) for help during field collections and molecular studies. Genevieve Gates (Australia) is thanked for improving the English of the text.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Erland, S.; Taylor, A.F.S. Resupinate ectomycorrhizal fungal genera. In *Ectomycorrhizal Fungi Key Genera in Profile*; Cairney, J.W.G., Chambers, S.M., Eds.; Springer: Berlin/Heidelberg, Germany, 1999; pp. 347–363. [CrossRef]
2. Yu, L.; Guo, L.D.; Ma, K.P. The role of mycorrhizal fungi in ecosystems. *Acta Phytoecol. Sin.* **2002**, *26*, 739–745.
3. Parfitt, D.; Ainsworth, A.M.; Simpson, D.; Rogers, H.J.; Boddy, L. Molecular and morphological discrimination of stipitate hydnoids in the genera *Hydnellum* and *Phellodon*. *Mycol. Res.* **2007**, *3*, 761–777. [CrossRef]
4. UK Biodiversity Group. Anon UK biodiversity group tranche 2 action plans. In *Plants and Fungi*; English Nature: Peterborough, ON, Canada, 1998.
5. Karsten, P.A. Enumeratio hydnearum Fr. fennicarum, systemate novo dispositarum. *Rev. Mycol. Toulouse* **1881**, *3*, 19–21.
6. Baird, R.; Wallace, L.E.; Baker, G.; Scruggs, M. Stipitate hydnoid fungi of the temperate southeastern United States. *Fungal Divers.* **2013**, *62*, 41–114. [CrossRef]
7. He, M.Q. Notes, outline and divergence times of Basidiomycota. *Fungal Divers.* **2019**, *99*, 105–367. [CrossRef]
8. Fries, E.M. *Observationes Mycologicae*; Gerh. Bonnier: Copenhagen, Denmark, 1815; p. 230. [CrossRef]
9. Banker, H.J. A contribution to a revision of the North American Hydnaceae. *Mem. Torrey Bot. Club* **1906**, *12*, 99–194. [CrossRef]
10. Coker, W.C.; Beers, A.H. *The Stipitate Hydnums of the Eastern United States*; University of North Carolina Press: Chapel Hill, NC, USA, 1951; p. 211.
11. Pouzar, Z. Sbírejte losákovité houby. *Ceská Mykol.* **1955**, *9*, 95–96.
12. Harrison, K.A. A new species of *Phellodon* possessing clamp connections. *Can. J. Bot.* **1972**, *50*, 1219–1221. [CrossRef]
13. Stalpers, J.A. The aphyllophoraceous fungi I. keys to the species of the Thelephorales. *Stud. Mycol.* **1993**, *35*, 1–168.
14. Pegler, D.N.; Roberst, P.J.; Spooner, B.M. *British Chanterelles and Tooth Fungi*; Kew: Royal Botanic Gardens, UK, 1997.
15. Karsten, P.A. Enumeratio boletinearum et polyporearum fennicarum, systemate novo dispositarum. *Rev. Mycol. Toulouse* **1881**, *3*, 16–19.
16. Baird, R.E.; Wallace, L.E.; Baker, G. Stipitate hydnums of the southern United States 1: *Phellodon mississippiensis* sp. nov. *Mycotaxon* **2013**, *123*, 183–191. [CrossRef]
17. Mu, Y.H.; Wu, F.; Yuan, H.S. Hydnaceous fungi of China 7. Morphological and molecular characterization of *Phellodon sub confluens* sp. nov. from temperate, deciduous forests. *Phytotaxa* **2019**, *414*, 280–288. [CrossRef]
18. Sun, Y.F.; Costa-Rezende, D.H.; Xing, J.H.; Zhou, J.L.; Zhang, B.; Gibertoni, T.B.; Gates, G.; Glen, M.; Dai, Y.C.; Cui, B.K. Multi-gene phylogeny and taxonomy of *Amauroderma* s. lat. (Ganodermataceae). *Persoonia* **2020**, *44*, 206–239. [CrossRef]
19. Han, M.L.; Chen, Y.Y.; Shen, L.L.; Song, J.; Vlasák, J.; Dai, Y.C.; Cui, B.K. Taxonomy and phylogeny of the brown-rot fungi: *Fomitopsis* and its related genera. *Fungal Divers.* **2016**, *80*, 343–373. [CrossRef]
20. Cui, B.K.; Li, H.J.; Ji, X.; Zhou, J.L.; Song, J.; Si, J.; Yang, Z.L.; Dai, Y.C. Species diversity, taxonomy and phylogeny of Polyporaceae (Basidiomycota) in China. *Fungal Divers.* **2019**, *97*, 137–392. [CrossRef]

21. Chen, J.J.; Cui, B.K.; Dai, Y.C. Global diversity and molecular systematics of *Wrightoporia* s.l. (Russulales, Basidiomycota). *Persoonia* **2016**, *37*, 21–36. [CrossRef]
22. Liu, S.; Shen, L.L.; Wang, Y.; Xu, T.M.; Gates, G.; Cui, B.K. Species diversity and molecular phylogeny of *Cyanosporus* (Polyporales, Basidiomycota). *Front. Microbiol.* **2021**, *12*, 631166. [CrossRef] [PubMed]
23. Katoh, K.; Toh, H. Recent developments in the MAFFT multiple sequence alignment program. *Brief. Bioinform.* **2008**, *9*, 286–298. [CrossRef]
24. Hall, T.A. Bioedit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
25. Zhu, L.; Song, J.; Zhou, J.L.; Si, J.; Cui, B.K. Species diversity, phylogeny, divergence time and biogeography of the genus *Sanghuangporus* (Basidiomycota). *Front. Microbiol.* **2019**, *10*, 812. [CrossRef]
26. Liu, S.; Han, M.L.; Xu, T.M.; Wang, Y.; Wu, D.M.; Cui, B.K. Taxonomy and phylogeny of the Fomitopsis pinicola complex with descriptions of six new species from east Asia. *Front. Microbiol* **2021**, *12*, 644979. [CrossRef] [PubMed]
27. Posada, D.; Crandall, K.A. Modeltest: Testing the model of DNA substitution. *Bioinform.* **1998**, *14*, 817–818. [CrossRef]
28. Nylander, J.A.A. *MrModeltest v2. Program. Distributed by the Author*; Evolutionary Biology Center: Uppsala University, Uppsala, Swden, 2004.
29. Swofford, D.L. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*; Version 4.0b10; Sinauer Associates: Sunderland, MA, USA, 2002.
30. Felsenstein, J. Confidence intervals on phylogenetics: An approach using bootstrap. *Evolution* **1985**, *39*, 783–791. [CrossRef]
31. Harrison, K.A. New or little known north American stipitate Hydnums. *Can. J. Bot.* **1964**, *42*, 1205–1233. [CrossRef]
32. Hrouda, P. Hydnaceous fungi of the Czech Republic and Slovakia. *Czech Mycol.* **1999**, *51*, 99–155. [CrossRef]
33. Dai, Y.C. A revised checklist of corticioid and hydnoid fungi in China for 2010. *Mycoscience* **2011**, *52*, 69–79. [CrossRef]
34. Mu, Y.H.; Hu, Y.P.; Wei, Y.L.; Yuan, H.S. Hydnaceous fungi of China 8. Morphological and molecular identification of three new species of *Sarcodon* and a new record from southwest China. *MycoKeys* **2020**, *66*, 83–103. [CrossRef] [PubMed]