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Morphological and Phylogenetic Analyses Reveal Dictyostelids (Cellular Slime Molds) Colonizing the Ascocarp of *Morchella*

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Abstract: *Morchella* spp. (true morels) are precious edible mushrooms consumed around the world, with a delicious taste, rich nutritional value, and unique healthcare effects. Various fungi and bacteria have been reported to colonize the ascocarps of *Morchella*, damaging their fruiting bodies and leading to serious economic losses in cultivation. The species identification of these colonizing organisms is crucial for understanding their colonization mechanisms on morels. Slime molds, which have characteristics of both "fungi" and "animals", can occasionally colonize crops and edible fungi. However, there have been no reports of dictyostelid cellular slime molds (dictyostelids) colonizing plants and fungi to date. In this study, we discovered that dictyostelids colonized the surface of one wild ascoma of *Morchella* in the forest of Chongqing, China, with the tissues being black and rotten. Macro- and micro-morphological observations, along with molecular phylogenetic analyses, identified the specimens investigated in this study as *Dictyostelium implicatum* and *Morchella* sp. *Mel*-21. The results provide new knowledge of dictyostelid colonization on organisms and contribute to the diversity of species colonizing true morels. Moreover, this is also the first report of dictyostelids distributed in Chongqing, China. This study enhances our insights into the life history and potential ecological significance of dictyostelids and updates their distribution area in China. Further research will be conducted to uncover the mechanisms behind the colonization observed in this study.

Keywords: *Dictyostelium*; 18S rRNA; true morel; multi-gene; distribution; sorocarp

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1. Introduction

True morels (*Morchella* spp., phylum Ascomycota), a group of the world's most prized edible and medicinal mushrooms, are of very important economic and scientific value [1]. They are rich in protein, carbohydrate compounds, vitamins, minerals, and other nutrients [2], which have many health benefits, and abundant microorganisms are present on the fruiting bodies [3,4]. Due to the high demand for true morels and their increasing economic importance, morel cultivation has been a global research focus for more than 100 years [5,6]. In recent years, the outdoor cultivation of morels has been successful and greatly expanded in China [6,7]. However, the occurrence of fungi and bacteria colonizing the fruiting bodies of *Morchella* at cultivation sites has been increasingly and commonly reported [8–15] and causes the development of white plaques, dark-black lesions, wrinkled and rotten apothecia, and even perforation symptoms [16–21], resulting in decreased harvest yields, declined commodity quality, and reduced final profits [6,22–25].

Among these harmful organisms colonizing the ascomata of *Morchella*, *Pseudodiploospora longispora* (Matsush.) Jing Z. Sun, X.Z. Liu & H.W. Liu [17,18,26,27] can colonize both the caps and stipes of true morels and are recognized as serious pathogens, which produce numerous conidia spreading rapidly around the cultivation areas, resulting in up to 80%

of morel yield losses every year [12]. The *Fusarium incarnatum–F. equiseti* species complex [16] is a group of fungal pathogens distributed worldwide that mainly colonize the stipes of *Morchella importuna* M. Kuo, O'Donnell & T.J. Volk and develop spindle, dark brown, sunken patches with sparse white hyphae on their surfaces. Similar symptoms have also been reported in *Morchella sextelata* M. Kuo due to the colonization of *Clonostachys solani* (Harting) Schroers & W. Gams [21]. Additionally, *Alternaria alternata* (Fr.) Keissl., an opportunistic pathogen noted in economically important fruit crops [9], was found to invade the hymenia of *M. importuna*, resulting in halted fruiting body growth and abnormal morphology [13]. Furthermore, *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson has been observed to colonize the ascocarps of *Morchella rufobrunnea* Guzmán & F. Tapia [10], while *Trichoderma atroviride* P. Karst [8]., *Pseudomonas chlororaphis* subsp. *aureofaciens* Peix and *Bacillus subtilis* (Ehrenberg) Cohn [15], and *Penicillium raperi* G. Sm. [14] have been documented as pathogens colonizing *M. sextelata.* Unstable environmental conditions provide opportunities for bacteria [15] and fungi [28] to proliferate and colonize morels, damaging their fruiting bodies and causing various diseases [6,21,29]. However, there have been no reports that protists can colonize *Morchella*.

Slime molds, characterized by features of "fungi" and "animals" during their life cycle [30], include endoparasitic slime molds (Phytomyxea), acrasid cellular slime molds (acrasids), dictyostelid cellular slime molds (dictyostelids), plasmodial slime molds (Myxogastrea), and other heterotypic slime molds [31–35]. Most slime molds are saprophytic without significant economic value, and only Myxogastrea can colonize crops and edible fungi in the form of plasmodia or sporangia, affecting the growth of crops and fungi and even causing severe decay and death [36–39]. For example, *Polymyxa graminis* Ledingham, as a lower eukaryote, obligatorily colonizes plant roots and transmits nine kinds of wheat viruses, specifically *Bymovirus* sp. and *Furovirus* sp. [40]. *Plasmodiophora brassicae* Woronin can colonize plants and damage the roots of most cruciferous plants [41]. In addition, *Stemonitis splendens* Rostaf [42]., *Physarella oblonga* (Berk. & M.A. Curtis) Morgan [43], and *Stemonaria longa* (Peck) Nann.-Bremek., Y. Yamam. & R. Sharma [44] have also been reported to colonize mushrooms. Dictyostelid cellular slime molds (dictyostelids) predominantly inhabit the soil and leaf litter layer, along with animal dung, where they feed mostly on bacteria [45–48]. Because of their crucial evolutionary status, unique life cycle, and significant interactions with the environment and human health, dictyostelids have become model organisms with significant research value in biological characters, genetics, and applications [49,50]. However, to our knowledge, there have been no reports of dictyostelids colonizing plants or fungi.

In this study, we found that dictyostelids colonized the surface of one wild *Morchella* ascoma in a forest in Chongqing, China, with the tissue observed to be black and rotten. The *Morchella* sample was identified to be *Morchella* sp. *Mel*-21, and the dictyostelids were recognized as *Dictyostelium implicatum* H. Hagiw. based on morphological and molecular phylogenetic studies. Our results contribute to expanding the knowledge about species colonizing *Morchella*, especially wild ascomata, and offer novel perspectives on the potential ecological significance of dictyostelids as well as their distribution in China.

2. Materials and Methods

2.1. Specimens

The specimens of *Morchella* and dictyostelids were collected from Chongqing, China, in March 2023, then dried with silica gel, and finally deposited in Chongqing Normal University, Chongqing, China. The strain numbers of *Morchella* and dictyostelids are FCNU1120 and H1054, respectively.

2.2. Morphological Study of Dictyostelids

2.2.1. Macroscopic Morphological Observation

The macroscopic morphological characteristics of the dictyostelids were observed under a stereo microscope (Leica S9 Series, Shanghai, China), including the size and

morphology of aggregations and pseudoplasmodia, the length of sorocarps, the color and branching pattern of sorophores, and the color of sori.

2.2.2. Microscopic Morphological Observation

Several intact and complete dictyostelids were selected and placed on a microscope slide. Before the morphological observation, the specimens were stained with 1% aqueous Congo red solution. Microscopic features were observed using an Optec BK-FL light microscope (Optec, Chongqing, China), including the spore shape and size, the presence of polar granules, macrocysts and microcysts, the width of the sorophores, the number of stalk cell columns, and the characteristics of the top and base of the sorophores. Then, images were captured with an Optec CCD TP510 digital camera (Optec, Chongqing, China) and processed using Adobe Photoshop CC 2019 v.20.0.4.

2.3. DNA Extraction, Sequencing, and Phylogenetic Analyses

Under the stereo microscope, ten sorocarps of dictyostelids growing on the cap surface of a single *Morchella* ascoma were randomly selected and transferred to a clean centrifuge tube, and a few tissues from the stipe of *Morchella* where no dictyostelids were observed to colonize were placed in another centrifuge tube. Methods for genomic DNA extraction and Sanger sequencing followed Du et al. [51]. The 18S ribosomal RNA (18S rRNA) [52] gene for dictyostelids [53] and translation elongation factor 1-a (*EF1-a*) [51,54,55], internal transcribed spacers 1 and 2 within 5.8S rDNA (ITS) [52,56], RNA polymerase II largest subunit (*RPB1*) [51], and RNA polymerase II second largest subunit (*RPB2*) [51] genes for *Morchella* [57] were selected. The primers used for the PCR amplification and sequencing of the five genes are given in Table 1. Each PCR reaction contained 22 µL of T3 Super PCR Mix (Beijing Tsingke Biotech Co., Ltd., Beijing, China), 1 µL of each primer (Sangon Co., Ltd., Shanghai, China), and 1 µL of template DNA; the final volume was 25 µL. PCRs were conducted in a T1000 Thermal Cycle (Bio-Rad, Singapore) using the cycling parameters shown in Table 2. Amplicons were electrophoresed in 1.5% agarose (Sangon Co., Ltd., Shanghai, China) in 1× TAE, stained with Gold View™ (Chongqing Siding Biotech Ltd., Chongqing, China), and then photographed over an ultraviolet transilluminator (Beijing Labgic Technology Co., Ltd., Beijing, China). Then, the PCR products were sequenced with an ABI 3730 capillary sequencer (Sangon Co., Ltd., Shanghai, China). Newly generated sequences were assembled and edited using SeqMan v.7.1.0 (DNA STAR package; DNAStar Inc., Madison, WI, United States). In addition, 54 sequences of *EF1-a*, ITS, *RPB1*, and *RPB2* genes from 28 species previously reported in *Morchella* [57] and 61 sequences of 18S rRNA from 57 species of dictyostelids [53] were retrieved from GenBank and included in the following phylogenetic analysis. Their accession numbers are, respectively, given in Tables 3 and 4.

Table 1. Detailed information on PCR and sequencing primers.

Locus	Primer	Sequence (5′-3′)	Taxon	Reference
18S rRNA	NS1	GTAGTCATATGCTTGTCTC	*Dictyostelium*	[52]
	NS2	GGCTGCTGGCACCAGACTTGC		
EF1-a	EF-595F	CGTGACTTCATCAAGAACATG	*Morchella*	[54]
	EF-1R	GGARGGAAYCATCTTGACGA		[51]
ITS rDNA	ITS1F	CTTGGTCATTTAGAGGAAGTAA	*Morchella*	[56]
	ITS4	TCCTCCGCTTATTGATATGC		[52]
RPB1	RPB1B-F	AACCGGTATATCACGTYGGTAT	*Morchella*	[51]
	RPB1B-R	GCCTCRAATTCGTTGACRACGT		
RPB2	RPB2B-F	TAGGTAGGTCCCAAGAACACC	*Morchella*	[51]
	RPB2B-R	GATACCATGGCGAACATTCTG		

Table 2. PCR programs used for amplification of 18S rRNA, *EF1-a*, ITS, *RPB1*, and *RPB2* in this study.

Gene	PCR Program
18S rRNA	2′ −98 °C, 35× (10″ −98 °C, 10″ −45 °C, 20″ −72 °C), 10′ −72 °C
EF1-a	2′ −98 °C, 35× (10″ −98 °C, 10″ −50 °C, 90″ −72 °C), 10′ −72 °C
ITS	2′ −98 °C, 35× (10″ −98 °C, 10″ −50 °C, 20″ −72 °C), 10′ −72 °C
RPB1	2′ −98 °C, 35× (10″ −98 °C, 10″ −50 °C, 90″ −72 °C), 10′ −72 °C
RPB2	2′ −98 °C, 35× (10″ −98 °C, 10″ −50 °C, 90″ −72 °C), 10′ −72 °C

Table 3. Detailed information on the retrieved sequences of dictyostelids used in this study. Newly generated sequence information indicated in bold.

Species	Voucher	Locality	GenBank Accession Number
			18S rRNA
Acytostelium anastomosans	PP1	America	AM168115
A. amazonicum	HN1B1	Honduras	HQ141511
A. digitatum	OH517	America	AM168114
A. leptosomum	212rjb	Portugal	HQ141512
A. longisorophorum	DB10A	America	AM168109
A. magnisorum	08A	America	HQ141513
A. serpentarium	SAB3A	America	AM168113
A. singulare	FDIB	America	HQ141514
A. subglobosum	LB1	America	AM168110
Dictyostelium aureum	SL1	America	AM168028
D. australe	NZ80B	New Zealand	AM168029
D. bifurcatum	UK5	America	AM168084
D. brefeldianum	TNS-C-115	Japan	AM168030
D. brunneum	WS700	America	AM168031
D. capitatum	91HO-50	Japan	AM168032
D. caveatum	WS695	America	AM168077
D. coeruleo-stipes	CRLC53B	America	AM168036
D. crassicaule	93HO-33	Japan	AM168037
D. delicatum	TNS-C-226	Japan	AM168093
D. deminutivum	MexM19A	America	AM168092
D. discoideum	V34	America	AM168039
	M1A	Costa Rica	KJ394476
D. exiguum	TNS-C-199	Japan	AM168085
D. gloeosporum	TCK52	Japan	AM168074
D. gracile	TNS-C-183	Japan	AM168078

Table 3. *Cont.*

Species	Voucher	Locality	GenBank Accession Number
			18S rRNA
D. implicatum	93HO-1	Japan	AM168043
	H1054	**China**	**PP658424**
D. lacteum	/ [1]	France	AM168045
D. laterosorum	AE4	America	AM168046
D. longosporum	TNS-C-109	Japan	AM168048
D. macrocephalum	B33	Japan	AM168049
D. medium	TNS-C-205	Japan	AM168050
D. medusoides	OH592	America	AM168088
D. microsporum	TNS-C-38	Japan	AM168090
D. minutum	Boots_07_A1	America	JN590753
	Boots_07_B1	America	JN590758
D. monochasioides	HAG653	Japan	AM168052
D. mucoroides	Ice211A1	Sweden	KC865597
D. polycarpum	OhioWILDS	America	AM168058
D. polycephalum	AP	India	GU562439
D. potamoides	FP1A	America	AM168069
D. pseudobrefeldianum	91HO-8	Japan	AM168059
D. purpureum	cavender	America	HQ141481
D. rhizopodium	AusKY-4	Japan	AM168063
D. rosarium	M45	America	AM168065
D. septentrionalis	AK2	America	AM168067
D.sphaerocephalum	Ice241A1	America	KC865595
	Boots_14_A2	America	JN590756
	Boots_07_A2	America	JN590754
Lamproderma puncticulatum	162	Switzerland	HQ687202
Polysphondylium anisocaule	NZ47B	New Zealand	AM168096
P. asymetricum	HN20C	Honduras	HQ141503
P. australicum	NB1AP	Australia	HQ141508
P. colligatum	HN13C1	Honduras	HQ141505
P. equisetoides	B7JB	America	AM168099
P. filamentosum	SU-1	America	AM168100
P. luridum	LR-2	America	AM168101
P. multicystogenum	AS2	Africa	HQ141506
P. patagonicum	/[1]	Argentina	GQ496156
P. pseudocandidum	TNS-C-91	America	AM168107
P. stolonicoideum	K12A	Australia	HQ141507
P. tikalense	HN1C1	Honduras	HQ141509
P. tikaliensis	OH595	America	AM168106

[1] The voucher information of this sample unavailable.

Table 4. Detailed information on the retrieved sequences of *Morchella* used in this study. Newly generated sequences information indicated in bold.

Species	Voucher	Locality	GenBank Accession Number			
			ITS	*EF1-a*	*RPB1*	*RPB2*
Morchella angusticeps	M304 M65	America America	JQ723055 GU551433	GU551560 GU551396	GU551658 GU551470	GU551707 GU551516
M.arbutiphila	HT193	Turkey	JN085141	JN085085	JN085201	JN085257
M. australiana	M338 T35077	Australia Australia	KC753472 KC753470	KC753468 KC753466	KC753475 KC753477	KC753480 KC753478
M.brunnea	M35 M431	Canada America	GU551415 GU551414	GU551378 GU551377	GU551452 GU551451	GU551492 GU551491
M. confericola	HT106 HT479	Turkey Turkey	JN085140 JN085127	JN085084 JN085071	JN085200 JN085187	JN085256 JN085243
M. confusa	FCNU1027 FCNU1028	China China	MK321848 MK321849	MK321866 MK321867	MK321854 MK321855	MK321860 MK321861
M. eohespera	M215 HKAS62873 HKAS62875	Sweden China China	GU551404 JQ321878 JQ321890	GU551367 JQ321846 JQ321858	GU551441 JQ321942 JQ321954	GU551478 JQ321974 JQ321986
M. eximioides	HKAS62883 HKAS62884 M231	China China Sweden	JQ321898 JQ321899 GU551428	JQ321866 JQ321867 GU551391	JQ321962 JQ321963 GU551465	JQ321994 JQ321995 GU551508
M.fekeensis	HT401 HT510	Turkey Turkey	JN085114 JN085133	JN085058 JN085077	JN085174 JN085193	JN085230 JN085249
M. hispaniolensis	M374	Dominican Republic	MH014725	GU551554	GU551652	GU551484
M. importuna	HKAS62868 HKAS62871	China Germany	JQ321874 JQ321903	JQ321842 JQ321871	JQ321938 JQ321967	JQ321970 JQ321999
M. kaibabensis	TAC-1376 TAC-1708	America America	MH014727 MH014728	MH014721 MH014722	MH014732 MH014733	MH014737 MH014738
M. laurentiana	10.05.19AV02 13.05.14AV01	Canada Canada	KT819376 KT819374	KT819387 KT819385	KT819353 KT819351	KT819364 KT819362
M.magnispora	HT470 HT471	Turkey Turkey	JN085122 JN085123	JN085066 JN085067	JN085182 JN085183	JN085238 JN085239
M.mediterraneensis	HT448 HT520	Turkey Turkey	JN085118 JN085135	JN085062 JN085079	JN085178 JN085195	JN085234 JN085251
M.pulchella	HT472	Turkey	JN08512	JN085068	JN085184	JN085240
M.purpurascens	HKAS62876 HT297 M214 M476	China Turkey Sweden China	JQ321895 JN085111 GU551406 GU551426	JQ321863 JN085055 GU551369 GU551389	JQ321959 JN085171 GU551443 GU551463	JQ321991 JN085227 GU551480 GU551505
M. septentrionalis	M9	America	JQ723064	GU551556	GU551654	GU551703
M. synderi	M299 M433	America America	GU551413 GU551425	GU551376 GU551388	GU551450 GU551462	GU551490 GU551503
Morchella sp. *Mel*-13	HKAS62889 HKAS62893 M424	China China India	JQ321884 JQ321888 GU551429	JQ321852 JQ321856 GU551392	JQ321948 JQ321952 GU551466	JQ321980 JQ321984 GU551511
Morchella sp. *Mel*-14	HKAS62885 HKAS62886	China China	JQ321887 JQ321891	JQ321855 JQ321859	JQ321951 JQ321955	JQ321983 JQ321987
Morchella sp. *Mel*-17	M315	Bulgaria	JQ723057	GU551561	GU551659	GU551708
Morchella sp. *Mel*-21	HKAS62878 HKAS62880 M225 **FCNU1120**	China China Japan **China**	JQ321894 JQ321882 JN085156 **PP658423**	JQ321862. JQ321850 GU551559 **PP695543**	JQ321958 JQ321946 GU551657 **PP693901**	JQ321990 JQ321978 GU551507 **PP693900**

Table 4. *Cont.*

Species	Voucher	Locality	GenBank Accession Number			
			ITS	***EF1-a***	***RPB1***	***RPB2***
Morchella sp. *Mel*-23	M495 M542	Norway Denmark	JN085153 JQ723063	GU551381 GU551562	JN085212 GU551660	GU551495 GU551709
Morchella sp. *Mel*-26	HT508	Turkey	JN085131	JN085075	JN085191	JN085247
Morchella sp. *Mel*-34	HKAS62877	China	JQ321896	JQ321864	JQ321960	JQ321992
Morchella sp. *Mel*-37	CIEFAP5 CIEFAP71 CIEFAP74	Argentina Argentina Argentina	KJ439678 KJ439673 KJ439674	KJ569626 KJ569630 KJ569631	KJ569594 KJ569596 KJ569598	KJ569620 KJ569624 KJ569625
Morchella sp. *Mel*-38	ALV3206	Cyprus	KU865009	KU865050	KU865040	KU865042

Newly generated sequences of dictyostelids and *Morchella* were separately combined in an alignment with downloaded sequences from each genus. In addition, *Lamproderma puncticulatum* and *M. importuna* were chosen, respectively, as the outgroups of dictyostelids and *Morchella*. Sequence alignments were performed separately for each gene dataset with MAFFT v.7.475 using the E-INS-i strategy [58] and then manually checked with BioEdit v.7.0.9 [59]. Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses were conducted for the combined four-gene dataset (ITS-*EF1a*-*RPB1*-*RPB2*) and the 18S rRNA dataset using RAxML v.8.2.12 [60] and MrBayes v.3.2.7a [61], respectively. Rapid bootstrapping with 1000 replicates was executed for ML analysis with the GTR + GAMMA + I model chosen by ModelTest v.3.8 [62]. The BI analysis was run for one million generations, sampling the trees every 100 generations, and used four Markov Chain Monte Carlo (MCMC) chains. When the mean standard deviation of split frequencies was below 0.01, the runs were terminated. The burn-in summary of the top 25% of samples was performed using the "sumt" and "sump" commands to obtain posterior possibilities.

3. Results

The substantial proliferation of white and transparent dictyostelids colonizing the cap surface of one *Morchella* ascoma from a forest habitat was found in Chongqing, with the colonized area observed to be blackened and decayed (Figure 1). After a thorough inspection of the surrounding area, only one ascoma with dictyostelids growing on the surface was identified. The specimen of *Morchella* was identified to be *Morchella* sp. *Mel*-21 based on multi-gene phylogenetic analyses. Based on morphological observations, the slime molds were first considered to belong to the genus *Dictyostelium* and were further inferred to be *D. implicatum* by molecular phylogenetic analysis.

Figure 1. Slime molds colonizing the ascoma of *Morchella* in the field. (**A**) Distant view; (**B**,**C**) close-up view. Slime molds indicated by white arrows.

3.1. Molecular Phylogenetic Analysis of the Morchella Specimen

In this study, four sequences of the *Morchella* specimen were obtained through PCR amplification targeting the ITS, *EF1-a*, *RPB1*, and *RPB2* genes with accession numbers PP658423, PP695543, PP693901, and PP693900. The alignments of sequences, which included those newly generated in this study and the 54 retrieved sequences from GenBank (Table 3) for ITS, *EF1-a*, *RPB1*, and *RPB2* datasets, respectively, were 646, 777, 692, and 680 bp. The final aligned multi-gene sequence matrix contained 28 species and a total of 55 sequences with 3287 bp. The phylogenetic trees were inferred from the combined four-gene dataset based on ML and BI analyses. No significant topological differences were detected between the two analyses, and the ML phylogenetic tree is shown in Figure 2. The phylogenetic analyses strongly supported the studied specimen being *Morchella* sp. *Mel*-21 (Figure 2) since it clustered together with HKAS62878 and HKAS62880 from China and M225 from Japan with high support (100%/1); these were previously identified as *Morchella* sp. *Mel*-21 [51,63]. Therefore, based on molecular phylogenetic analyses, the species identity of the *Morchella* specimen used in this study was recognized as *Morchella* sp. *Mel*-21.

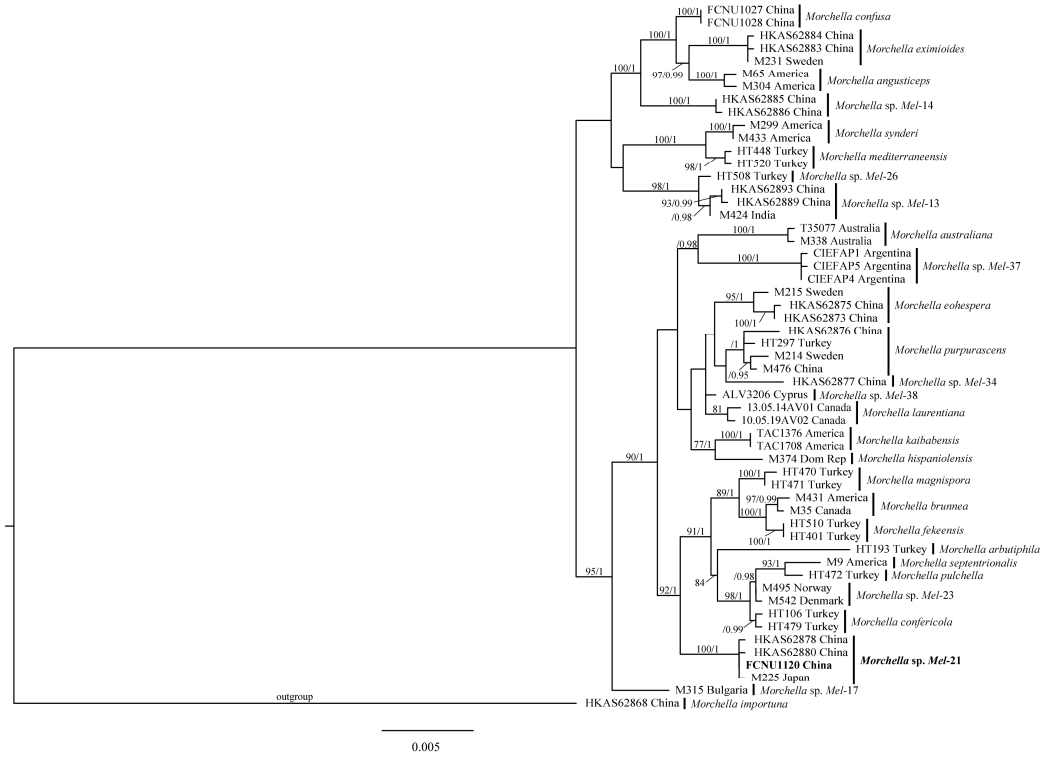

Figure 2. The phylogenetic tree of 28 *Morchella* species inferred from ML analyses based on the concatenated dataset (ITS, *EF1-a*, *RPB1*, and *RPB2*). Bootstrap values over 75% and Bayesian posterior probabilities over 0.95 shown on the branches. The new specimen of *Morchella* used in this study indicated in bold.

3.2. Morphological Observation of Dictyostelids

***Dictyostelium* sp.**

Cell aggregations (Figure 3A) with ample radiate streams. Pseudoplasmodia (Figure 3B) often migrating without sorophore formation. Mexican-hat-like protrusion (Figure 3C–E) and the sorocarp formation period with mastoid structure (Figure 3F–H) observed. Sorocarps (Figure 3I) solitary and unbranched, some erect while others prostrate. Sori white or milk-white, globose. Spores (Figure 4A) hyaline, elliptical, mostly 5.77–7.97 × 3.63–4.85 µm, without polar granules. Spore germination observed (Figure 4B). Microcyst (Figure 4C,D) globose.

Sorophores generally stout, tapering from bases to tips, consisting of several tiers of cells, bases (Figure 4E) clavate, tips (Figure 4F) acuminate.

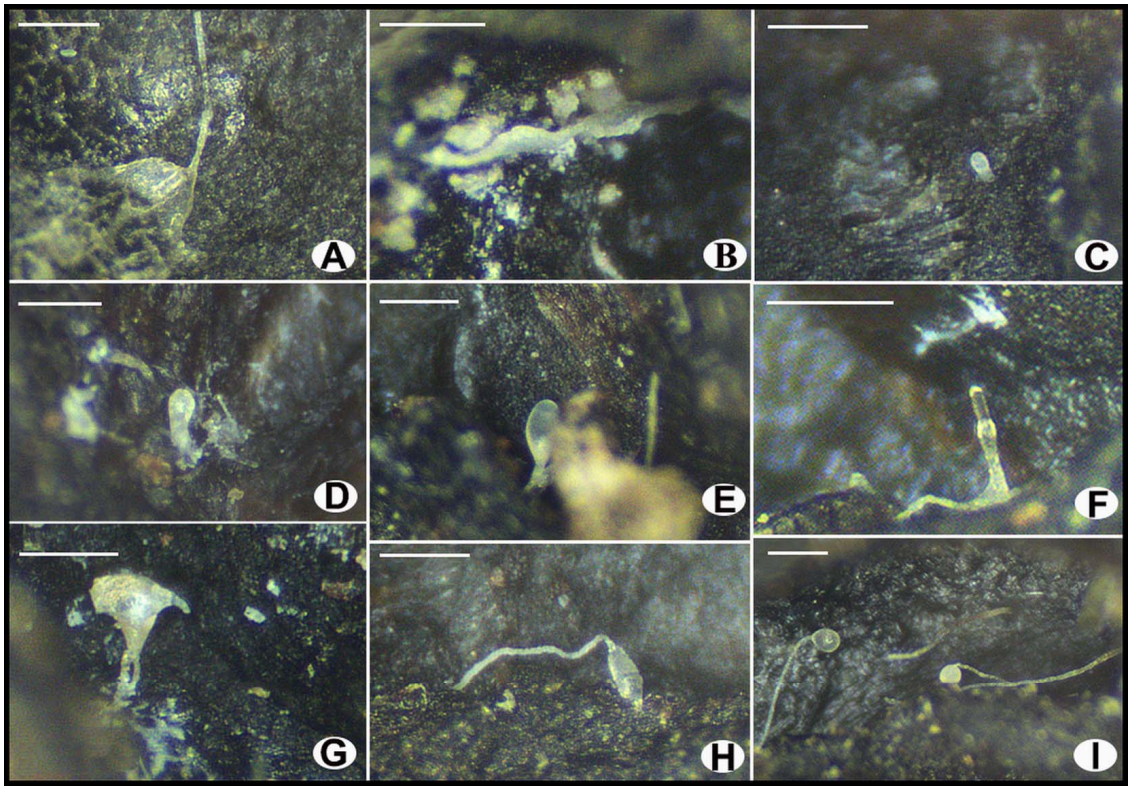

Figure 3. Morphological characteristics of *Dictyostelium* sp. investigated in this study under a stereo microscope. (**A**) Cell aggregation; (**B**) pseudoplasmodium; (**C**–**E**) mexican-hat-like protrusion; (**F**–**H**) the sorocarp formation period with mastoid structure; (**I**) sorocarps. Scale bars = 200 μm.

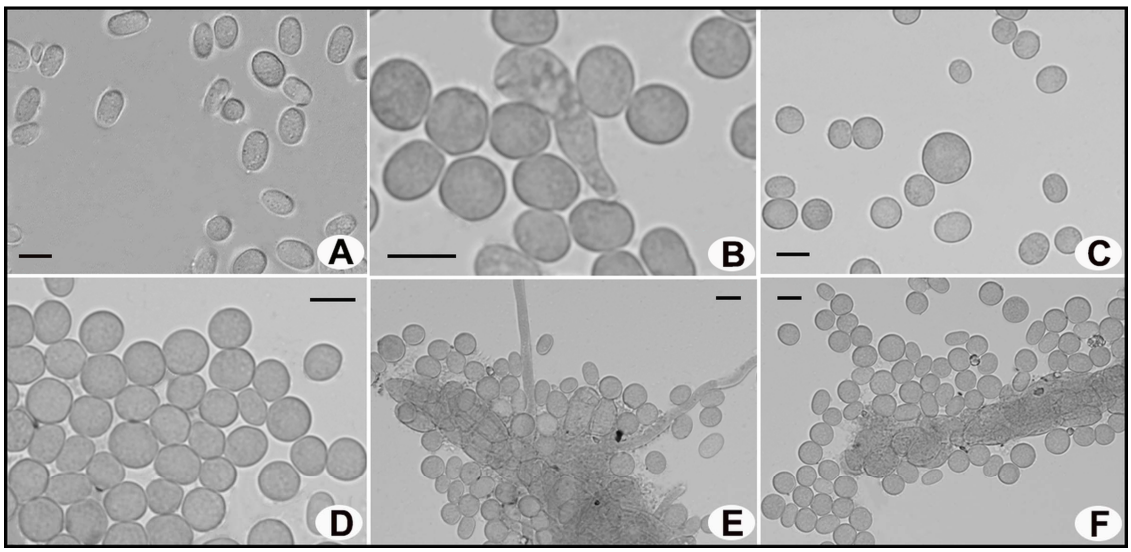

Figure 4. Microscopic morphological characteristics of *Dictyostelium* sp. observed under a light microscope. (**A**) Spores; (**B**) spore germination; (**C**,**D**) microcysts; (**E**) base of sorophores; (**F**) tip of sorophores. Scale bars = 5 μm.

Specimens examined. H1054. Isolated from the surface of one wild ascoma of *Morchella* in 2023 from Chongqing, China.

Known distribution. China, America, Germany, Korea, Japan, India, Pakistan, Ukraine, Thailand.

Commentary. The morphological observation was performed after the samples were dried, and the length and diameter of the fresh dictyostelids' sorocarps and sori could not be measured. Consequently, the dictyostelids were initially identified as belonging to *Dictyostelium* sp. based solely on morphological features.

3.3. Molecular Phylogenetic Analysis of Dictyostelium Implicatum Specimens

The newly generated 18S rRNA sequence in this study was 531 bp with accession number PP658424 and aligned with the 61 sequences retrieved from GenBank (Table 4). The final alignment matrix contained 1259 bp with 62 sequences and a total of 57 species. The phylogenetic trees were obtained based on the ML and BI analyses, and the ML tree was presented in Figure 5. The dictyostelids in this study clustered together with AM168043 from Japan with high support (92%/1), which was previously identified as *Dictyostelium implicatum* [64]. Therefore, based on molecular phylogenetic analysis, dictyostelids that colonized the cap surface of *Morchella* ascoma found in this study were identified as *D. implicatum*.

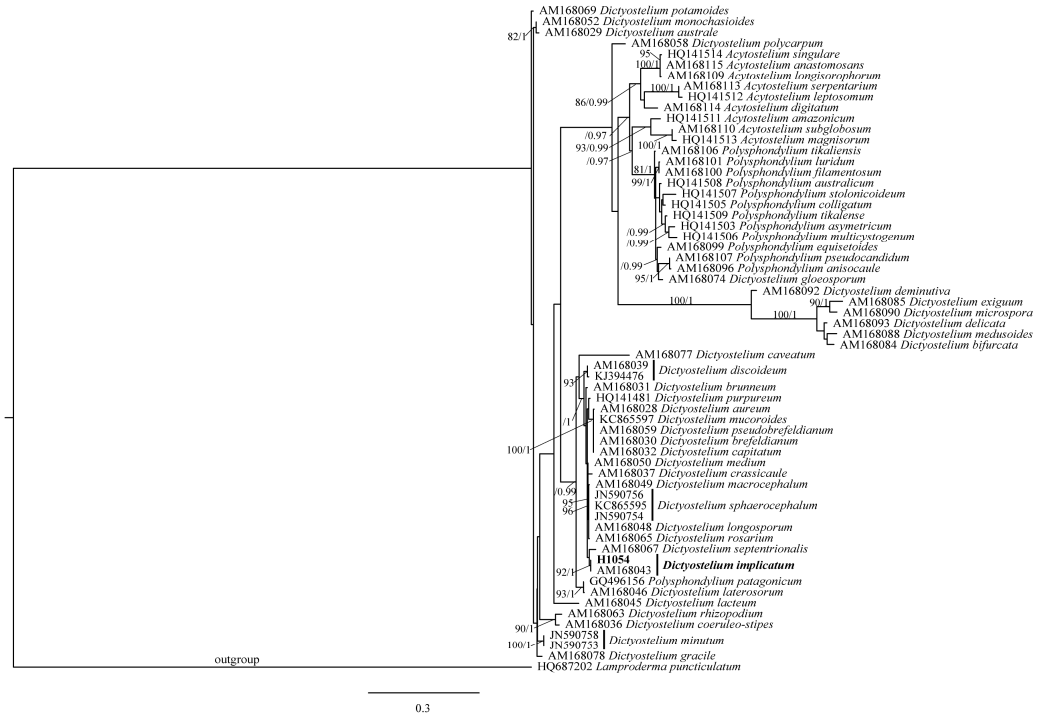

Figure 5. The phylogenetic tree of 57 species of dictyostelids inferred from ML analyses based on 18S rRNA. Bootstrap values over 75% and Bayesian posterior probabilities over 0.95 reported on the branches. The new collection of *Dictyostelium* used in this study indicated in bold.

4. Discussion

The large-scale commercial cultivation of morels has become a part of an emerging industry for edible fungi in China and globally, showcasing their significant economic and scientific value [6,7]. The colonization of bacteria and fungi is one of the key factors affecting the artificial cultivation of morels and causing serious economic losses [6,21–25]. Increasing numbers of bacteria and fungi, such as *Ps. longispora* [17,18,26,27], *F. incarnatum-equiseti* [16], *C. solani* [21], *Pu. lilacinum* [10], *T. atroviride* [8], *Ps. chlororaphis* subsp. *aureofaciens* and *B. subtilis* [15], *A. alternata* [13], *Pe. raperi* [14], and so on, have been discovered to colonize *Morchella* species and harm their fruiting bodies. Investigating the species diversity of these colonizing organisms is the premise for further revealing their colonization mechanisms

and is also crucial for drawing the attention of planters and researchers to them during morel cultivation and in the field.

Dictyostelids, well known as dictyostelid cellular slime molds that feed on bacteria and other microbes [50], have never been reported to act as pathogens of any organisms before [36–39]. Though tiny and difficult to find in nature with the naked eye [65], dictyostelids have been documented worldwide [46], such as in China, America, Germany, Korea, Japan, India, Pakistan, Ukraine, Thailand, etc. [45–47,66–77]. In China, they have been previously reported in Beijing, Jilin, Shanxi, Heilongjiang, Liaoning, Hunan, Henan, Xizang, Yunnan, Sichuan, Guizhou, Hainan, Guangxi, Guangdong, Taiwan, and so on [46,47,76–83]. Based on the phylogenetic analysis of ITS, 18S rRNA, 5.8S rRNA, α-tubulin, and β-tubulin genes [64,84–87], dictyostelids have been reported to include 11 genera [88], of which *Dictyostelium*, *Polysphondylium*, and *Acytostelium* are the most common [89].

In this study, we found that dictyostelids had colonized the surface of one wild ascoma of *Morchella*, with the tissue being black and rotten. The wild ascoma was identified as *Morchella* sp. *Mel*-21 through molecular phylogenetic studies. Interestingly, this species was previously reported to be successfully cultivated in China, albeit with low and unstable yields [90,91]. Though the length and size of fresh sorocarps and sori are crucial for dictyostelids species identification [78], due to the availability of only dried specimens for the microscopic morphological observation of dictyostelids in this study, we were unable to obtain data on their length and size. Consequently, these dictyostelids were initially identified as belonging to the genus *Dictyostelium* based on morphology. Further molecular phylogenetic analysis was conducted to uncover the species identity of the dictyostelids using the 18S rRNA and ITS genes, which are widely accepted for species identification in the genus *Dictyostelium* [89,92–94]. However, only a clear 18S rRNA sequence was obtained with clean peaks, while the ITS sequences were always messy after multiple attempts and were then discarded. Based on the phylogenetic tree of the 18S rRNA dataset, the dictyostelids in this study were identified to be *D. implicatum*, with support values being 92%/1, slightly lower than 97%/1, probably due to the newly generated sequence (531 bp) being much shorter than the referenced ones (mainly around 670 bp), chosen according to An and Li [89], but no better sequences of 18S rRNA could be obtained from the dictyostelids after multiple attempts. Notably, to the best of our knowledge, the dictyostelids found in this study are reported for the first time from Chongqing, located in southwestern China, further broadening their distribution record in China.

Considering the lack of previous reports of dictyostelids acting as pathogens of any organisms [36–39], with the aim of conducting inoculation experiments to determine whether dictyostelids could be pathogens of *Morchella* based on our discovery, we tried to isolate the dictyostelids and hoped to reveal their colonization mechanisms. However, despite multiple attempts across different laboratories, we were unable to successfully isolate them. Given that abundant microbial communities have been reported on the ascomata of *Morchella* [3,4], based on the available literature and our data, we currently hypothesize that the colonization mechanism of dictyostelids discovered in this study is likely consuming the microorganisms present on the surface of the ascoma, and the activity of these microorganisms may, in turn, contribute indirectly to the blackening and decay of the *Morchella* ascoma. Subsequent studies will be conducted to continue trying to resolve this problem and provide a more comprehensive understanding.

This study represents the first report of dictyostelids (*D. implicatum*) colonizing the fruiting body of *Morchella* and introduces novel research advancements for exploring the life history and potential ecological significance of dictyostelids. While organism colonization has previously only been documented in cultivated morels, this study observes it for the first time on wild ascomata. The new finding of dictyostelids colonizing *Morchella* sp. *Mel*-21 could serve as a valuable reference and attract attention for artificial cultivation in the future.

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References

1. Du, X.H.; Zhao, Q.; Yang, Z.L. A review on research advances, issues, and perspectives of morels. *Mycology* **2015**, *6*, 78–85. [CrossRef] [PubMed]
2. Tian, J.F.; Shang, Y.; Xiao, Z.N. Research progress on nutrient composition, function and processing of morels. *Sci. Technol. Food Ind.* **2023**, *45*, 419–428.
3. Li, Y.T.; Chen, H.Y.; Zhang, X. Cultivation, nutritional value, bioactive compounds of morels, and their health benefits: A systematic review. *Front. Nutr.* **2023**, *10*, 1159029. [CrossRef]
4. Jiang, K.; Li, L.; Yang, Z.; Chen, H.; Qin, Y.; Brennan, C. Variable characteristics of microbial communities and volatile organic compounds during post-harvest storage of wild morel mushrooms. *Postharvest Biol. Technol.* **2023**, *203*, 112401. [CrossRef]
5. Liu, W.; Chen, L.F.; Cai, Y.L.; Zhang, Q.Q.; Bian, Y.B. Opposite polarity monospore genome de novo sequencing and comparative analysis reveal the possible heterothallic life cycle of *Morchella importuna*. *Int. J. Mol. Sci.* **2018**, *19*, 2525. [CrossRef] [PubMed]
6. Xu, Y.Y.; Tang, J.; Wang, Y.; He, X.L.; Tan, H.; Yu, Y.; Chen, Y.; Peng, W.H. Large scale commercial cultivation of morels: Current state and perspectives. *Appl. Microbiol. Biot.* **2022**, *106*, 4401–4412. [CrossRef]
7. Du, X.H.; Yang, Z.L. Mating systems in true morels (*Morchella*). *Microbiol. Mol. Biol. Rev.* **2021**, *85*, e0022020. [CrossRef]
8. Fu, Y.W.; Wu, H.H.; Wang, S.; Yu, Q.; Tian, D.S.; Xu, X. First report of *Trichoderma atroviride* causing rot of *Morchella sextelata* in Anhui Province, China. *Crop Prot.* **2023**, *168*, 106206. [CrossRef]
9. Gupta, S.; Saxena, S. Endophytes: Saviour of apples from post-harvest fungal pathogens. *Biol. Control* **2023**, *182*, 105234. [CrossRef]
10. Masaphy, S. First report on *Purpureocillium lilacinum* infection of indoor-cultivated morel primordia. *Agriculture* **2022**, *12*, 695. [CrossRef]
11. Shi, X.F.; Liu, D.; He, X.H.; Liu, W.; Yu, F.Q. Epidemic identification of fungal diseases in *Morchella* cultivation across China. *J. Fungi* **2022**, *8*, 1107. [CrossRef] [PubMed]
12. Wang, X.X.; Peng, J.Y.; Sun, L.; Bonito, G.; Guo, Y.X.; Li, Y.; Fu, Y.P. Genome sequencing of *Paecilomyces Penicillatus* provides insights into its phylogenetic placement and mycoparasitism mechanisms on morel mushrooms. *Pathogens* **2020**, *9*, 834. [CrossRef] [PubMed]
13. Wei, Y.F.; Fu, M.H.; Li, W.L.; Luo, Y.; Zhang, Y.T.; Sun, W.L.; Zou, J. First report on apothecium deformity of *Morchella importuna* caused by *Alternaria alternata* in China. *Plant Dis.* **2024**, *108*, 1398. [CrossRef] [PubMed]
14. Xu, X.; Yang, C.B.; Wang, S.; Jiao, C.Y.; Sun, J.Z.; Fan, X.Y.; Wang, X.J.; Xiang, W.S.; Zhao, J.W. *Penicillium raperi* causes rot disease on *Morchella sextelata* in Heilongjiang Province, China. *Crop Prot.* **2024**, *175*, 106479. [CrossRef]
15. Zhu, X.T.; Ma, K.L.; Sun, M.Y.; Zhang, J.M.; Liu, L.J.; Niu, S.Q. Isolation and identification of pathogens of *Morchella sextelata* bacterial disease. *Front. Microbiol.* **2023**, *14*, 1231353. [CrossRef]
16. Guo, M.P.; Chen, K.; Wang, G.Z.; Bian, Y.B. First report of stipe rot disease on *Morchella importuna* caused by *Fusarium incarnatum–F. equiseti* species complex in China. *Plant Dis.* **2016**, *100*, 2530. [CrossRef]
17. He, P.X.; Li, C.C.; Cai, Y.L.; Ya, Z.; Bian, Y.B.; Liu, W. First report of pileus rot disease on cultivated *Morchella importuna* caused by *Diploöspora longispora* in China. *J. Gen. Plant Pathol.* **2018**, *84*, 65–69. [CrossRef]

18. Hyde, K.D.; Maharachchikumbura SS, N.; Hongsanan, S.; Samarakoon, M.C.; Lücking, R.; Pem, D.; Harishchandra, D.; Jeewon, R.; Zhao, R.L.; Xu, J.C.; et al. The ranking of fungi: A tribute to David L. Hawksworth on his 70th birthday. *Fungal Divers.* **2017**, *84*, 1–23. [CrossRef]
19. Liu, J.M.; Feng, J.C.; Wu, Z.T.; Yang, X.Z. Study on pathogen identification and biological morel of *Morchella* white mold disease in Hexi area. *North. Hortic.* **2023**, 116–123.
20. Tu, S.; Zhang, Y.; Chen, X.; Song, L.; Lv, B.; Chen, Y. First report of *Aspergillus niger* causing rot of *Morchella sextelata* in China. *Plant Dis.* **2024**, *108*, 804. [CrossRef]
21. Yu, F.M.; Jayawardena, R.S.; Luangharn, T.; Zeng, X.Y.; Li, C.J.Y.; Bao, S.X.; Ba, H.; Zhou, D.Q.; Tang, S.M.; Hyde, K.D.; et al. Species diversity of fungal pathogens on cultivated mushrooms: A case study on morels (*Morchella*, Pezizales). *Fungal Divers.* **2024**, *125*, 157–220. [CrossRef]
22. Liu, W.; Cai, Y.L.; He, P.X.; Ma, X.L.; Bian, Y.B. Occurrence and control of pests and diseases in field cultivation of *Morchella* mushrooms. *Acta Edulis Fungi* **2019**, *26*, 128–134.
23. Lv, B.; Yu, S.; Chen, Y.; Yu, H.; Mo, Q. First report of *Lecanicillium aphanocladii* causing rot of *Morchella sextelata* in China. *Plant Dis.* **2022**, *106*, 3202. [CrossRef] [PubMed]
24. Peng, B.; Bian, Y.B.; Gong, Y.H.; Xiao, Y. Diseases of *Morchella* and their comprehensive prevention and control techniques. *Edible Med. Mushrooms* **2024**, *32*, 129–135.
25. Zhao, Q.; Lü, M.L.; Li, L.; Huang, W.; Zhang, Y.F.; Hao, Z. Temptation and trap of morel industry in China. *J. Fungal Res.* **2021**, *19*, 1232–1237.
26. Sun, J.Z.; Yu, S.; Lu, Y.Z.; Liu, H.W.; Liu, X.Z. Proposal of a new family *Pseudodiploösporeaceae* fam. nov. (*Hypocreales*) based on phylogeny of *Diploöspora longispora* and *Paecilomyces penicillatus*. *Mycology* **2022**, *14*, 60–73. [CrossRef]
27. Liu, Q.Z.; Ma, H.S.; Zhang, Y.; Dong, C.H. Artificial cultivation of true morels: Current state, issues and perspectives. *Crit. Rev. Biotechnol.* **2018**, *38*, 259–271. [CrossRef]
28. Liu, Z.H.; Cong, Y.L.; Sossah, F.L.; Lu, Y.Z.; Kang, J.C.; Li, Y. Characterization and genome analysis of *Cladobotryum mycophilum*, the causal agent of cobweb disease of *Morchella sextelata* in China. *J. Fungi* **2023**, *9*, 411. [CrossRef]
29. He, X.S.; Wang, J.; Wang, Y.P.; Li, X.Y.; Wang, Y.Z.; Zhu, X.Q.; Zhou, L.; Zhao, M.; Zhang, N.; Xie, J.Y. The high-yield operating procedures of morels cultivation in southwest China. *Edible Med. Mushrooms* **2020**, *28*, 454–460.
30. Li, S.; Dou, W.J.; Peng, X.Y.; Wang, Q.; Li, Y. Research progress of Myxomycetes resources in China. *J. Fungal Res.* **2023**, *21*, 93–102.
31. Adl, S.M.; Leander, B.S.; Simpson AG, B.; Archibald, J.M.; Anderson, O.R.; Bass, D.; Bowser, S.S.; Brugerolle, G.; Farmer, M.A.; Karpov, S.; et al. Diversity, nomenclature, and taxonomy of protists. *Syst. Biol.* **2007**, *56*, 684–689. [CrossRef] [PubMed]
32. Cavalier-Smith, T. Protist phylogeny and the high-level classification of Protozoa. *Eur. J. Protistol.* **2003**, *39*, 338–348. [CrossRef]
33. Cavalier-Smith, T.; Chao, E.E.Y. Phylogeny and megasystematics of phagotrophic heterokonts (kingdom Chromista). *J. Mol. Evol.* **2006**, *62*, 388–420. [CrossRef]
34. Chen, S.L. The taxonomic history of Myxomycetes (Myxogastrea). *Mycosystema* **2023**, *42*, 38–49.
35. Ruggiero, M.A.; Gordon, D.P.; Orrell, T.M.; Bailly, N.; Bourgoin, T.; Brusca, R.C.; Cavalier-Smith, T.; Guiry, M.D.; Kirk, P.M. A higher level classification of all living organisms. *PLoS ONE* **2015**, *10*, e0119248.
36. Howard, F.L.; Currie, M.E. Parasitism of myxomycete plasmodia on fungous mycelia. *J. Arnold Arbor.* **1932**, *13*, 438–447. [CrossRef]
37. Michalczyk-Wetula, D.; Jakubowska, M.; Felska, M.; Skarżyński, D.; Mąkol, J.; Płonka, P.M. *Tyrophagus putrescentiae* (Sarcoptiformes: Acaridae) in the in vitro cultures of slime molds (Mycetozoa): Accident, contamination, or interaction? *Exp. Appl. Acarol.* **2021**, *84*, 445–458. [CrossRef] [PubMed]
38. Stephenson, S.L.; Feest, A. Ecology of soil eumycetozoans. *Acta Protozool.* **2012**, *51*, 201–208.
39. Zhang, Z.J.; Zhai, C.; Li, Y.; Stephenson, S.L.; Liu, P. Slime molds (Myxomycetes) causing a "disease" in crop plants and cultivated mushrooms. *Front. Plant Sci.* **2024**, *15*, 1411231. [CrossRef]
40. Cubeta, M.A.; Cody, B.; Williams, P.H. First report of *Plasmodiophora brassicae* on cabbage in eastern North Carolina. *Plant Dis.* **1998**, *82*, 129. [CrossRef]
41. Ledingham, G.A. Studies on *Polymyxa graminis*, n. gen.; n. sp.; a plasmodiophoraceous root parasite of wheat. *Can. J. Res.* **1939**, *17*, 38–51. [CrossRef]
42. Lee, J.H.; Kim, D.R.; Kwak, Y.S. First report of *Stemonitis splendens Rostaf* causing bark decay of oak logs used for shiitake cultivation in Korea. *Mycobiology* **2014**, *42*, 279–281. [CrossRef]
43. Li, Y.S.; Zhang, B.; Jiang, S.C.; Hsiang, T.; Li, Y.; Wang, X.L. First report of *Physarella oblonga* on *Lentinula edodes* in China. *Plant Dis.* **2017**, *101*, 2146. [CrossRef]
44. Zhang, B.; Li, S.; Li, H.M.; Li, Y. First report of a new myxogastria (*Stemonaria longa*) causing rot disease on shiitake logs (*Lentinula edodes*) in China. *Plant Dis.* **2017**, *102*, 1032. [CrossRef]
45. Liu, P.; Zou, Y.; Hou, J.; Stephenson, S.L.; Li, Y. *Dictyostelium purpureum* var. *pseudosessile*, a new variant of dictyostelid from tropical China. *BMC Evol. Biol.* **2019**, *19*, 78.
46. Liu, P.; Zhang, S.; Zou, Y.; Li, Z.; Stephenson, S.L.; Li, Y. Distribution and ecology of dictyostelids in China. *Fungal Biol. Rev.* **2020**, *34*, 170–177. [CrossRef]
47. Zhang, Z.; Yang, Y.; Zhao, J.; Li, Y.; Stephenson, S.L.; Qiu, J.; Liu, P. Environmental factors influencing the diversity and distribution of dictyostelid cellular slime molds in forest and farmland soils of western China. *Microbio. Spectr.* **2023**, *11*, e0173223. [CrossRef]
48. Zou, Y.; Liu, P. Research progress on ecology of dictyostelid cellular slime molds. *Mycosystema* **2023**, *42*, 160–169.

49. Li, Y.; Wang, Q.; Li, S. Development of myxomycetology. *Mycosystema* **2021**, *40*, 261–269.
50. Zhou, Y. Biodiversity of Dictyostelid Cellular Slime Molds in Different Habitat Gradients of Changbai Mountain. Master's Dissertation, Jilin Agricultural University, Jilin, China, 1 May 2019.
51. Du, X.H.; Zhao, Q.; O'Donnell, K.; Rooney, A.P.; Yang, Z.L. Multigene molecular phylogenetics reveals true morels (*Morchella*) are especially species-rich in China. *Fungal Genet. Biol.* **2012**, *49*, 455–469. [CrossRef]
52. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, H.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: San Diego, CA, USA, 1990; Volume 8, pp. 315–322.
53. An, Y.; Li, Y. Molecular phylogeny of dictyostelid cellular slime molds from China. *J. Jilin Agric. Univ.* **2019**, *41*, 294–302.
54. Kauserud, H.; Schumacher, T. Outcrossing or inbreeding: DNA markers provide evidence for type of reproductive mode in *Phellinus nigrolimitatus* (Basidiomycota). *Mycol. Res.* **2001**, *105*, 676–683. [CrossRef]
55. Rehner, S.A.; Buckley, E. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **2005**, *97*, 84–98. [CrossRef] [PubMed]
56. Gardes, M.; Bruns, T.D. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **1993**, *2*, 113–118. [CrossRef]
57. Du, X.H.; Wu, D.M.; He, G.Q.; Wei, W.; Xu, N.; Li, T.L. Six new species and two new records of *Morchella* in China using phylogenetic and morphological analyses. *Mycologia* **2019**, *111*, 857–870. [CrossRef]
58. Katoh, K.; Misawa, K.; Kuma, K.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef]
59. 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.
60. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef] [PubMed]
61. Ronquist, F.; Teslenko, M.; Van Der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef]
62. Posada, D. ModelTest server: A web-based tool for the statistical selection of models of nucleotide substitution online. *Nucleic Acids Res.* **2006**, *34*, W700–W703. [CrossRef]
63. O'Donnell, K.; Rooney, A.P.; Mills, G.L.; Kuo, M.; Weber, N.S.; Rehner, S.A. Phylogeny and historical biogeography of true morels (*Morchella*) reveals an early Cretaceous origin and high continental endemism and provincialism in the Holarctic. *Fungal Genet. Biol.* **2011**, *48*, 252–265. [CrossRef] [PubMed]
64. Schaap, P.; Winckler, T.; Nelson, M.; Alvarez-Curto, E.; Elgie, B.; Hagiwara, H.; Cavender, J.; Milano-Curto, A.; Rozen, D.E.; Dingermann, T.; et al. Molecular phylogeny and evolution of morphology in the social amoebas. *Science* **2006**, *314*, 661–663. [CrossRef] [PubMed]
65. Sun, J.Y. Studies on the Life Cycle of Important Taxa in Dictyosteliaceae. Master's Dissertation, Jilin Agricultural University, Jilin, China, 1 June 2011.
66. Cavender, J.C. *Dictyostelium dimigraformum*, *Dictyostelium laterosorum* and *Acytostelium ellipticum*: New Acrasieae from the American tropics. *J. Gen. Microbiol.* **1970**, *62*, 113–123. [CrossRef]
67. Cavender, J.C.; Lakhanpal, T.N. Distribution of dictyostelid cellular slime molds in forest soils of India. *Mycologia* **1986**, *78*, 56–65. [CrossRef]
68. Cavender, J.C.; Cavender-Bares, J.; Hohl, H.R. Ecological distribution of cellular slime molds in forest soils of Germany. *Bot. Helv.* **1995**, *105*, 199–219.
69. Cavender, J.C.; Vadell, E.M.; Perrigo, A.L.; Landolt, J.C.; Stephenson, S.L.; Liu, P. Four new species of dictyostelids from soil systems in northern Thailand. *J. Fungi* **2022**, *8*, 593. [CrossRef]
70. Hagiwara, H. *The Taxonomic Study of Japanese Dictyostelid Cellular Slime Molds*; National Science Museum Monographs: Tokyo, Japan, 1989; pp. 1–128.
71. Hagiwara, H. Dictyostelid cellular some molds of Pakistan. I. Distribution and occurrence in soils of forests, cultivated fields and alpine pastures. In *Cryptogamic Flora of Pakistan*; Nakaike, T., Malik, S., Eds.; Bulletin of the National Science Museum: Tokyo, Japan, 1992; Volume 1, pp. 87–98.
72. Liu, P.; Li, Y. New species and new records of dictyostelids from Ukraine. *Mycologia* **2011**, *103*, 641–645. [CrossRef]
73. Liu, P.; Zou, Y.; Li, S.; Stephenson, S.L.; Wang, Q.; Li, Y. Two new species of dictyostelid cellular slime molds in high-elevation habitats on the Qinghai-Tibet Plateau, China. *Sci. Rep.* **2019**, *9*, 5. [CrossRef]
74. Liu, P.; Zou, Y.; Li, W.; Li, Y.; Li, X.; Che, S.; Stephenson, S.L. Dictyostelid cellular slime molds from Christmas Island, Indian Ocean. *Msphere* **2019**, *4*, 10–1128. [CrossRef]
75. Hong, J.S.; Chang, N.K. A new species of cellular slime molds from Korea, *Dictyostelium flavidum* sp. nov. *J. Plant Biol.* **1992**, *35*, 393–401.
76. Zhao, M.J. Studies on Taxonomy of Dictyostelid Cellular Slime Molds of Eastern China. Master's Dissertation, Jilin Agricultural University, Jilin, China, 1 May 2014.

77. Zou, Y.; Hou, J.; Guo, S.; Li, C.; Li, Z.; Stephenson, S.L.; Pavlov, I.N.; Liu, P.; Li, Y. Diversity of dictyostelid cellular slime molds, including two species new to science, in forest soils of Changbai Mountain, China. *Microbiol. Spectr.* **2022**, *10*, e0240222. [CrossRef] [PubMed]
78. He, X.L. Preliminary Taxonomy Studies on Dictyosteliaceae in China. Master's Dissertation, Jilin Agricultural University, Jilin, China, 1 June 2008.
79. Bai, R.L. Preliminary study of Acrasiomycetes. *Mycosystema* **1983**, *2*, 173–178.
80. Hagiwara, H.; Yeh, Z.Y.; Chien, C.Y. *Dictyostelium macrocephalum*, a new dictyostelid cellular slime mold from Taiwan. *Bull. Natl. Sci. Mus. Ser. B* **1985**, *11*, 103–108.
81. Li, C. Studies on Taxonomy of Dictyostelid Cellular Slime Molds in Henan and Hunan Provinces. Master's Dissertation, Jilin Agricultural University, Jilin, China, 29 May 2013.
82. Ren, Y.Z. Studies on Distribution of Dictyostelid Cellular Slime Molds in the Tropic and Frigid Zone of China. Master's Dissertation, Jilin Agricultural University, Jilin, China, 31 May 2013.
83. Yuan, H.Y. Studies on Taxonomy of Dictyostelid Cellular Slime Molds in the Southwest of China. Master's Dissertation, Jilin Agricultural University, Jilin, China, 28 May 2013.
84. McCarroll, R.; Olsen, G.J.; Stahl, Y.D.; Woese, C.R.; Sogin, M.L. Nucleotide sequence of the *Dictyostelium discoideum* small-subunit ribosomal ribonucleic acid inferred from the gene sequence: Evolutionary implications. *Biochemistry* **1983**, *22*, 5858–5868. [CrossRef]
85. Triviños-Lagos, L.; Ohmachi, T.; Albrightson, C.; Burns, R.G.; Ennis, H.L.; Chisholm, R.L. The highly divergent α- and β-tubulins from *Dictyostelium discoideum* are encoded by single genes. *J. Cell Sci.* **1993**, *105*, 903–912. [CrossRef]
86. Romeralo, M.; Spiegel, F.W.; Baldauf, S.L. A fully resolved phylogeny of the social amoebas (Dictyostelia) based on combined SSU and ITS rDNA sequences. *Protist* **2010**, *161*, 539–548. [CrossRef] [PubMed]
87. Romeralo, M.; Cavender, J.C.; Landolt, J.C.; Stephenson, S.L.; Baldauf, S.L. An expanded phylogeny of social amoebas (Dictyostelia) shows increasing diversity and new morphological patterns. *BMC Evol. Biol.* **2011**, *11*, 1–10. [CrossRef] [PubMed]
88. NCBI Taxonomy: A Comprehensive Update on Curation, Resources and Tools. *Database* **2020**, *2020*, baaa062. [CrossRef]
89. An, Y. Studies on Multigene and Fatty-Acids Biomarker in Representative Genera Taxonomy of Dictyostelid Cellular Slime Molds. Ph.D. Dissertation, Jilin Agricultural University, Jilin, China, 2 December 2015.
90. Du, X.H. Review on species resources, reproductive modes and genetic diversity of black morels. *J. Fungal Res.* **2019**, *17*, 240–251.
91. Cai, Y.L.; Ma, X.L.; Lu, D.X.; Zhang, Y.; Liu, W. Phylogenetic analysis and domestication of wild morel *Mel*-21. *Acta Edulis Fungi* **2020**, *27*, 23–29.
92. Romeralo, M.; Fiz-Palacios, O.; Lado, C.; Cavender, J.C. A new concept for *Dictyostelium sphaerocephalum* based on morphology and phylogenetic analysis of nuclear ribosomal internal transcribed spacer region sequences. *J. Can. Bot.* **2007**, *85*, 104–110. [CrossRef]
93. Romeralo, M.A.; Escalante, R.; Sastre, L.; Lado, C. Molecular systematics of dictyostelids: 5.8S ribosomal DNA and internal transcribed spacer region analyses. *Eukaryot. Cell* **2007**, *6*, 110–116. [CrossRef] [PubMed]
94. Sheikh, S.; Thulin, M.; Cavender, J.C.; Escalante, R.; Kawakami, S.I.; Lado, C.; Landolt, J.C.; Nanjundiah, V.; Queller, D.C.; Strassmann, J.E.; et al. A new classification of the dictyostelids. *Protist* **2018**, *169*, 1–28. [CrossRef] [PubMed]