

Description of three new *Clavulina* species from Baiyun Mountain, Guangzhou, China

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Research Article

Keywords: 3 New taxa, Morphological feature, Phylogenetic analysis, Taxonomy

Posted Date: May 23rd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2919422/v1>

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Version of Record: A version of this preprint was published at Mycological Progress on November 24th, 2023. See the published version at <https://doi.org/10.1007/s11557-023-01929-x>.

Abstract

Three new *Clavulina* species, *C. baiyunensis*, *C. minor* and *C. lilaceorosea* were described from specimens collected from Baiyun Mountain, Guangzhou, China based on detail morphological studies and phylogenetic analyses of three gene fragments ITS, LSU and *RPB2*. *C. baiyunensis* is characterized by light brown to brown basidiomata with hyaline tramal hyphae. *C. minor* has diminutive, dull white to pale brown basidiomata with hyaline tramal hyphae. *C. lilaceorosea* has pale brown to ochre brown basidiomata with palmate branches, tramal hyphae walls light brown. Both three new species have abundant clamp connections, basidia with two cornuted sterigmata, postpartal septa common, and cystidia absent. A key of known *Clavulina* species original from China is presence.

Introduction

The genus *Clavulina* J. Schröt. (Clavulinaceae, Cantharellales) is characterized by clavarioid to coralloid, simple or branched basidiomata with colors ranging from white, to grey or purple; smooth, globose to subglobose basidiospores with one or several translucent guttules; and cylindrical to sub-clavate basidia usually with two cornuted sterigmata and often developing a transversal septum after the release of the basidiospores (Corner 1950; Petersen 1988; Henkel 2005; Olariaga et al. 2009).

Clavulina, considered to be ectomycorrhizal (ECM) fungi depending on morphological (Tedersoo et al. 2003; Wazny 2014) and molecular evidences (Tedersoo et al. 2010; Smith et al. 2011), is widely distributed in temperate and tropical ecosystems. Currently there are approximately 89 recognized species of *Clavulina* (Wu et al. 2019; Yuan et al. 2020), with 20 of them being described since 2010, including 8 from Guyana (Henkel et al. 2011; Uehling et al. 2012a, b), 4 from Brazil (Wartchow 2012; Tibpromma et al. 2017), 3 each from Mexico (Eduardo et al. 2019; Yuan et al. 2020) and China (He et al. 2016; Wu et al. 2019), 1 each from India (Phookamsak et al. 2019) and Cyprus (Crous et al. 2019), showing particularly diverse in the tropics.

Baiyun Mountain (113°16'51"–113°19'40" E, 23°9'39"–23°13'32" N) is located in Guangdong Province, southern China, covering an area of 28 square kilometers. The subtropical monsoon climate and high average annual precipitation (1,689–1,877 mm) favors the establishment of a diverse macrofungal community. In this study, three new *Clavulina* species, named as *C. baiyunensis*, *C. minor* and *C. lilaceorosea*, were described from specimens collected in the area by the authors during a macrofungal survey using both phylogenetic analyses and detail morphological examinations.

Materials and methods

Sampling and morphology

Specimens used in this study were collected from Baiyun Mountain, Guangzhou, China from May 2020 to August 2022 by the authors during a macrofungal survey. They were dehydrated in a specimen drier at 40–45°C and then deposited in the Herbarium of Microbiology Institute of Guangdong (GDGM). Fresh

fruitbodies were photographed under daylight and their macroscopic characteristics were briefly recorded in the field and detailed morphological data were obtained in the laboratory.

Microscopic characters were observed under a Nikon Eclipse Ni-U microscope from dried materials. Basidiospores from dry materials were observed and measured in Melzer's reagent. Other micromorphological structures (basidia, tramal hyphae) were recorded from dry specimens rehydrated in 5% KOH for 1 min and then observed in 1% aqueous Congo Red reagent. Color designation refers to HTML Color Codes (<https://htmlcolorcodes.com>). Scanning electron images of basidiospores were captured with a Field Emission Scanning Electron Microscope (ZEISS Gemini500). One hundred basidiospores were measured from each species and [n/m/p] means n basidiospores from m basidiomata of p specimens. The measurements are given as (a)b–c–d(e), in which a is the lowest value, c is the mean value, b–d covers a minimum of 90% of the values and e is the biggest value. Q stands for the ratio of length and width of a spore, and Q_m for the average and the standard deviation of Q for all basidiospores sampled.

DNA extraction, amplification and sequencing

The genomic DNA was extracted from fresh basidiomata using a modified CTAB procedure (Doyle and Doyle 1987). The internal transcribed spacer (ITS), a fragment of large subunit ribosomal (LSU) region and the second subunit of RNA polymerase (*RPB2*) were amplified using primer sets ITS1/ITS4 (White et al. 1990), LROR/LR5 (Vilgalys and Hester 1990) and RPB2-6F/fRPB2-7cR (Liu et al. 1999), respectively. The PCR amplifications were performed in a total volume of 25 μ L of PCR mixture containing 0.5 μ L TransTaq HiFi DNA polymerase, 2.5 μ L of 10 \times HiFi buffer, 2.5 μ L of dNTPs, 2 μ L of genomic DNA, 1 μ L of each primer and 16 μ L of deionized water. PCR reactions were conducted using the following profiles: pre-degeneration at 95°C or 94°C (ITS), followed by 35 cycles of denaturation at 95°C for 60 s or 94°C for 30 s (ITS), annealing at 55°C for 120 s or 52°C for 30 s (ITS), elongation at 72°C for 90 s or 60 s (ITS), and a final elongation at 72°C for 10 min. PCR products were purified using E.Z.N.A Gel Extraction Kit (OMEGA) and sequenced on an ABI3730 DNA Analyzer (RuiBiotech, Guangzhou, China) using the same primers used for PCR amplification.

Phylogenetic analyses

ITS, LSU and *RPB2* sequences of *Clavulina* from Wu et al. (2019), Phookamsak et al. (2019) and He et al. (2016), were downloaded from GenBank for phylogenetic analysis. *Hydnum repandum* L. and *Hydnum rufescens* Pers. were used as outgroups following He et al. (2016) (Table 1). The datasets were aligned with MAFFT v7.520 (Kato and Standley 2013), then manually adjusted with BioEdit v7.0.5.3 (Hall 1999), including trimming of ambiguous sites at the beginning and end of the datasets. Both Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms were used for phylogenetic analyses. ML analysis was executed on RAxML 8.2.10 (Stamatakis 2014) using ML search and rapid bootstrapping for 1000 replicates with GTRGAMMAI model. All parameters in RAxML analysis were kept at default. BI analysis was performed with MrBayes 3.2 (Ronquist et al. 2012) through the Markov Chain Monte Carlo (MCMC) method under the model selected using ModelFinder (Kalyaanamoorthy et al. 2017). Two independent runs with 4 chains were made; each was run for 10 million generations, and trees were sampled every 1000

generations, until the average standard deviation of split frequencies was below 0.01 at the end of the algorithm. The phylogenetic trees were visualised using MEGA 7.0.26 (Kumar et al. 2016).

Table 1

Information on the specimens and sequences used in this study. The new species are in bold. Voucher no. with T represents holotype.

	Location	Voucher	ITS	LSU	<i>RPB2</i>
<i>C. amazonensis</i>	Guyana	TH8742	—	HQ680361	JN228249
<i>C. amazonensis</i>	Colombia	AMV1847	KT724111	KT724124	—
<i>C. amazonensis</i>	Guyana	TH9191	HQ680356	—	—
<i>C. baiyunensis</i>	China, Guangzhou	B21051526	OP738990	OP737357	OP745528
<i>C. baiyunensis</i>	China, Guangzhou	B21062712 (T)	OP738991	OP737359	OP745529
<i>C. baiyunensis</i>	China, Guangzhou	B21062720	OP738992	OP737358	—
<i>C. caespitosa</i>	Guyana	TH8709 (T)	DQ056371	DQ056370	JN228234
<i>C. castaneipes</i>	Costa Rica	TENN056432	JX287357	—	—
<i>C. castaneipes</i>	USA	OSC 108705	EU669209	EU669261	—
<i>C. cerebriformis</i>	Guyana	MCA4022 (T)	JN168690	JN228222	JN228233
<i>C. cf. amethystina</i>	Norway	PRM 896664	EU862203	—	—
<i>C. cf. amethystina</i>	Norway	O 62152	EU862204	—	—
<i>C. cf. amythystina</i>	USA	SE-2015	KT275670	—	—
<i>C. cf. cinerea</i>	—	MES427	—	JN228226	JN228239
<i>C. cf. cinerea</i>	Canada	UBC:F29630	MZ868607	—	—
<i>C. cf. cinerea</i>	Canada	UBC:F29600	MZ868605	—	—
<i>C. cf. cinerea</i>	—	BIO 10304	EU862226	—	—
<i>C. cf. cinerea</i>	—	BIO 10294	EU862225	—	—
<i>C. cinerea</i>	USA	ECV4030	MG663298	MF797670	—
<i>C. cinerea</i>	USA	JKU9	JN228228	—	JN228242
<i>C. cinerea</i>	—	H 6002012	EU862209	—	—
<i>C. cinerea</i>	India	CONTIG_SL47	—	OL688379	—
<i>C. cinereoglebosa</i>	Guyana	TH8561 (T)	NR_119975	JN228232	JN228246
<i>C. cirrhata</i>	Guyana	TH8940	JQ677059	—	JQ677046
<i>C. cirrhata</i>	Guyana	TH9266	JQ677061	—	—

	Location	Voucher	ITS	LSU	RPB2
<i>C. cirrhata</i>	Guyana	TH9207	JQ677062	—	—
<i>C. coralloides</i>	—	HBAU15770	MW850398	—	—
<i>C. coralloides</i>	—	110116MFBPL0405	MW554410	—	—
<i>C. coralloides</i>	China: Yunnan	HKAS122411	ON794279	—	—
<i>C. craterelloides</i>	Colombia	AMV1401	—	KT724127	—
<i>C. craterelloides</i>	Guyana	TH8234 (T)	JQ911749	—	—
<i>C. cristata</i>	USA	DUKE9312	—	JN228215	JN228250
<i>C. cristata</i>	Spain	229	MN649216	—	—
<i>C. cristata</i>	USA	JKU8	JN228227	—	JN228241
<i>C. cristata</i>	Spain	BIO 9641	EU862228	—	—
<i>C. cristata</i>	Spain	BIO 10291	EU862223	—	—
<i>C. dicymbetorum</i>	Guyana	TH8730	DQ056364	DQ056369	—
<i>C. effusa</i>	Guyana	TH9193 (T)	—	JN228230	JN228245
<i>C. effusa</i>	Colombia	AMV1837	KT724116	KT724129	—
<i>C. effusa</i>	Guyana	TH8511	JN228231	—	—
<i>C. flava</i>	China, Yunnan	MHHNU9825 (T)	MK564139	MK564129	MK564149
<i>C. grisea</i>	Brazil	AMO357	KX811200	KX811195	—
<i>C. guyanensis</i>	Guyana	TH9257	JQ677057	—	—
<i>C. guyanensis</i>	Guyana	TH9245 (T)	JQ677056	—	JQ677049
<i>C. humicola</i>	Guyana	TH8737 (T)	DQ056368	DQ056367	JN228244
<i>C. iris</i>	Cyprus	LIP 0401586 (T)	MN028412	MN028396	—
<i>C. kunmudlutsa</i>	Guyana	MCA3117	—	HQ680362	—
<i>C. kunmudlutsa</i>	Guyana	TH9206	HQ680358	—	—
<i>C. kunmudlutsa</i>	Guyana	TH8932 (T)	HQ680359	—	—
<i>C. lilaceorosea</i>	China, Guangzhou	B22052918 (T)	OP738996	OP737363	OP745532
<i>C. lilaceorosea</i>	China, Guangzhou	B21082106	OP738995	OP737362	OP745533

	Location	Voucher	ITS	LSU	RPB2
<i>C. livida</i>	China: Guizhou	MCCNNU00959 (T)	KU219603	—	—
<i>C. mahiscolorata</i>	Mexico	FCME 27662 (T)	MH542554	MN049496	MN053719
<i>C. minor</i>	China, Guangzhou	B21081646 (T)	OP738993	OP737360	OP745530
<i>C. minor</i>	China, Guangzhou	B22082717	OP738994	OP737361	OP745531
<i>C. monodiminutiva</i>	Guyana	TH8738	DQ056365	DQ056372	JN228237
<i>C. nigricans</i>	Guyana	TH8284	JN228224	—	JN228238
<i>C. nigricans</i>	Guyana	G200	KJ786649	KJ786553	—
<i>C. ornatipes</i>	USA	TH9598	—	JN228229	JN228243
<i>C. pakaraimensis</i>	Guyana	TH9194 (T)	NR_121533	—	JQ677047
<i>C. pakaraimensis</i>	Guyana	TH9212	JQ677053	—	—
<i>C. pakaraimensis</i>	Guyana	TH9244	JQ677054	—	—
<i>C. parvispora</i>	Mexico	FCME 27650 (T)	MH542550	MN049492	MN053718
<i>C. purpurascens</i>	China, Yunnan	MHHNU9848 (T)	MK564137	MK564127	MK564147
<i>C. reae</i>	Mexico	FCME 27623	MH542526	MN049487	MN053717
<i>C. reae</i>	Mexico	FCME 27629	MH542525	MN049488	—
<i>C. rosiramea</i>	Guyana	TH8954 (T)	JQ677064	JQ677044	JQ677048
<i>C. rugosa</i>	—	QHU20421	OM970961	OM942743	—
<i>C. rugosa</i>	Tunisia	H21587	KU973837	—	—
<i>C. rugosa</i>	—	BIO 10293	EU862220	—	—
<i>C. rugosa</i>	—	BIO 10300	EU862217	—	—
<i>C. samuelsii</i>	—	TENN065723	JQ638712	—	—
<i>C. samuelsii</i>	New Zealand	PDD:89881	GU222317	—	—
<i>C. sphaeropedunculata</i>	Mexico	FCME 27663 (T)	MH542557	MK253717	—
<i>C. sprucei</i>	Guyana	TH9122	HQ680355	JN228223	JN228236
<i>C. sprucei</i>	Guyana	TH9120	HQ680353	—	—

	Location	Voucher	ITS	LSU	<i>RPB2</i>
<i>C. subrugosa</i>	–	TENN043395	JQ638711	–	–
<i>C. subrugosa</i>	New Zealand	TN43395	JN228221	–	–
<i>C. tepurumenga</i>	Guyana	MCA3116	–	HQ680363	JN228248
<i>C. tepurumenga</i>	Guyana	TH8217 (T)	HQ680357	–	–
<i>C. thindii</i>	India	US_1428 (T)	MG892054	–	–
<i>Hydnum repandum</i>	Slovenia	LJU GIS 1337	AJ547871	–	–
<i>Hydnum rufescens</i>	Slovenia	LJU GIS 1332	AJ547868	–	–

Results

Phylogeny

A total of 20 sequences generated in this study were deposited in GenBank (ITS: OP738990–OP738996, LSU: OP737357–OP737363, *RPB2*: OP745528–OP745533) and were analyzed by Blast in NCBI database. OP738991, OP738993 and OP738996, the ITS sequence of the type specimen of *C. baiyunensis*, *C. minor* and *C. lilaceorosea*, has the highest similarity of 93.6%, 94.7% and 90.0% with the validly described species of *C. cinerea* (MG663298), *C. subrugosa* (JN228221) and *C. castaneipes* (JX287357), respectively. There was no sequence of uncultured or unnamed specimens in GenBank which has a similarity higher than 97% with OP738991, OP738993 and OP738996, except for the sequence OL998855 from a specimens collected from Fuzhou, Fujian Province, China which has a similarity of 99.7% with OP738996, indicating that *C. lilaceorosea* may present Fujian Province as well.

The final dataset used for phylogenetic analysis consisted of 144 sequences (75 ITS, 39 LSU and 30 *RPB2*), representing 53 *Clavulina* taxa and two outgroups *Hydnum repandum* L. and *Hydnum rufescens* Pers. (Table 1). The final alignment of the ITS and the 3-locus concatenated dataset was 916 bp and 2581 bp (916 bp for ITS, 880 bp for LSU and 785 bp for *RPB2*), respectively. BI phylogenies were inferred under GTR + I + G + F model for both ITS and LSU datasets with an average standard deviation of split frequencies equal to 0.002652 and 0.005212, in which the initial 25% of sampled data were discarded as burn-in. Tree topologies derived by ML and BI analyses were almost identical, therefore, only the ML tree was showed with the bootstrap value (BS) and posterior probability (PP) when they were greater than or equal to 70% and 0.90, respectively (Figs. 1 and 2).

Phylogenetic analysis based on the ITS (Fig. 1) and 3-locus (Fig. 2) dataset showed consistently that all members of genus *Clavulina* in the datasets form a single clade and new species *Clavulina baiyunensis*, *Clavulina minor* and *Clavulina lilaceorosea* formed three independent clades within *Clavulina* which clearly separated from all described species examined with strong support (BS = 100, PP = 1). The 3-locus phylogenetic tree (Fig. 2) showed there were three major clades in the genus *Clavulina* which was partly

supported by the main distinguishing morphological features of the genus, such as presence or absence of clamp or cystidia, hypha wall character etc.. *C. baiyunensis* clustered together with *C. flava* P. Zhang which was a sister to a major cluster including *C. minor*, *C. cinerea* (Bull.) J. Schröt., *C. samuelsii* R.H. Petersen and *C. subrugosa* in the clade III. *C. lilaceorosea* was located within a cluster containing *C. castaneipes* (G.F. Atk.) Corner, *C. ornatipes* (Peck) Corner and *C. purpurascens* P. Zhang in the clade I.

Taxonomy

Clavulina baiyunensis X.X. Huang & L.H. Qiu, sp. nov. (Figs. 3 and 4)

MycoBank: MB847699

Etymology: the epithet '*baiyunensis*' refers to the type location, Baiyun Mountain.

Type: China, Guangdong Province, Baiyun Mountain, elevation, 238 m, N 23° 11' 20.71", E 113° 18' 21.03", 27 Jun 2021, L.H. Qiu (B21062712, GDGM 89546). GenBank ITS: OP738991, LSU: OP737359, *RPB2*: OP745529.

Basidiomata coralloid, gregarious in caespitose clusters; clusters 20–70 mm tall, 20–50 mm wide across branches. Individual basidiomata 20–70 mm tall, 10–30 mm, wide across branches, forming 3–5 ranks in multiple planes, branching pattern polychotomous to dichotomous-ascending; branch tips sharply acuminate when young becoming blunt with age; initially white, maturing brown (#CD853F) from the base with white apices. Stipes 5–30 × 3–8 mm, subclavate, white when immature to light brown (#D2B48C) or brown (#CD853F). Hymenium amphigenous, white to dull-white (#FAEBD7). Odor unapparent.

Basidiospores [100/5/3] (4.7)5.1–5.8–6.5(6.6) × (4.4)4.8–5.4–6.0(6.1) μm, Q = 1.00–1.09–1.20(1.22), Q = 1.09 ± 0.07, globose to subglobose, rarely broadly ellipsoid, with a hilar appendix 0.5–0.9 μm long, with one or several translucent guttules, thin walled, inamyloid, hyaline in H₂O and KOH. Basidia 18–29 μm long, 4–7 μm wide, subcylindrical to clavate, tapering from apex to base, with numerous granular contents, clamps occasionally present; postpartal septa observed in some basidia, occurring 6–11 μm below basidia tips; two sterigmata per basidium, 2.3–4.6 μm long, cornute. Basidioles numerous, subcylindrical to clavate, basally clamped. Cystidia absent. Tramal hyphae smooth, thin walled, hyaline in H₂O and KOH, 2.1–7.3 μm wide, clamp connections abundant.

Habit, habitat, and distribution: Gregarious to caespitose in humus layers on soils under evergreen coniferous and broad-leaved mixed forest. Basidiomata occur generally from May to July and only known specimens are from Guangdong Province of China so far.

Specimens examined: China, Guangdong Province, Baiyun Mountain, elevation, 238 m, N 23° 11' 35.24", E 113° 18' 53.30", 27 Jun 2021, L.H. Qiu (B21062720, GDGM 89547); same location, elevation, 238 m, N 23° 10' 42.68", E 113° 18' 6.31", 15 May 2021, L.H. Qiu (B21051526, GDGM 89545).

Commentary: *Clavulina baiyunensis* is characterized by its light brown to brown basidiomata with micro-morphological features include the presence of clamp connections, postpartal septa, and two cornute

sterigmata per basidium. *Clavulina baiyunensis* is phylogenetically close to *C. flava* in our phylogenetic analyses (Figs. 1 and 2), but the latter has prominently yellow basidiomata and tramal hyphae shows pale yellow in water (Wu et al. 2019). *Clavulina cinerea* (Bull.) J. Schröt. and *C. incrustata* Wartchow are macromorphologically similar to *C. baiyunensis*, but *C. cinerea* can be distinguished by its lilac-gray to gray basidiomata, larger basidiospores ($7.5\text{--}9.2 \times 7.2\text{--}8.7 \mu\text{m}$) and basidia ($38\text{--}65 \mu\text{m}$ long, up to $7 \mu\text{m}$ wide) (Burt 1922); *C. incrustata*, first reported in Brazil, has pale vinaceous hymenial surface and very rare clamp connections that only observed in the context (Wartchow 2012).

Clavulina minor X.X. Huang & L.H. Qiu, sp. nov. (Figs. 3 and 5)

MycoBank: MB847700

Etymology: the epithet '*minor*' refers to the small basidiomata.

Type: China, Guangdong Province, Baiyun Mountain, elevation, 238 m, N $23^{\circ} 11' 28.85''$, E $113^{\circ} 18' 5.39''$, 16 Aug 2021, L.H. Qiu (B21081646, GDGM 89548). GenBank ITS: OP738993, LSU: OP737360, *RPB2*: OP745530.

Basidiomata coralloid, solitary or scattered. Individual basidiomata 15–20 mm tall, 2–4 mm wide, simple or sparsely branched one to two times, monopodial or irregularly dichotomous towards branch apices and polychotomous at the base, branches clavate to subterete; branch tips rounded acuminate when young becoming blunt with age; dull white (#FFFFFF0) when young, turning khaki (#F0E68C) to pale brown (#F4A460) from above the base when mature. Stipes 5–10 × 2–4 mm, subclavate to subterete, concolorous with branches. Hymenium amphigenous, smooth, ivory (#FFFFFF0) to dull white (#FFFFFF0), light brown (#CD853F) when mature. Odor unapparent.

Basidiospores [100/3/2] (5.8)6.0–6.5–7.3(7.9) × (5.1)5.5–6.0–6.7(7.3) μm , $Q = (1.00)1.01\text{--}1.08\text{--}1.19(1.25)$, $Q = 1.08 \pm 0.06$, globose to subglobose, rarely broadly ellipsoid, smooth, inamyloid, hyaline in H_2O and KOH, with one or several translucent guttules, apicules 0.4–0.6 μm long. Basidia 29–45 μm long, 4–7 μm wide, subcylindrical to subclavate or clavate, with numerous granular contents, postpartial septa observed on some basidia, occurring 6–12 μm below basidia tips, clamps occasionally present, two sterigmata per basidium, 3.1–6.5 μm long, cornute. Basidioles numerous, subcylindrical to clavate, basally clamped. Cystidia absent. Tramal hyphae smooth, thin walled, hyaline, 2.7–5.7 μm wide, clamp connections abundant.

Habit, habitat, and distribution: Solitary to scattered or caespitose in mossy soils under evergreen coniferous and broad-leaved mixed forest. Basidiomata occur in the summer, usually in August; and only known specimens are from Guangdong Province of China so far.

Specimens examined: China, Guangdong Province, Baiyun Mountain, elevation, 238 m, N $23^{\circ} 11' 20.23''$, E $113^{\circ} 18' 21.56''$, 16 Aug 2022, L.H. Qiu (B22082717, GDGM 89549).

Commentary: *Clavulina minor* is characterized by its diminutive, dull white to pale brown basidiomata, globose to subglobose basidiospores, basidium with postpartial septa and two cornute sterigmata per

basidium, clamp connections abundant in tramal hyphae. In the phylogenetic analyses (Figs. 1 and 2), *C. minor* is phylogenetically close to *C. cinerea*, *C. samuelsii* R.H. Petersen and *C. subrugosa* (Cleland) Corner. *C. cinerea* differs by its numerous compact branches, lilac-gray to gray basidiomata and larger basidiospores ($7.5\text{--}9.2 \times 7.2\text{--}8.7 \mu\text{m}$) (Burt 1922). *C. samuelsii* differs from *C. minor* in having tiller buff basidiomata and presence of cystidia (Petersen 1988). *C. subrugosa* has similar simple or filiform basidiomata with *C. minor*, but the former differs from *C. minor* in having white to pale greyish brown basidiomata and subglobose to broadly ellipsoid basidiospores ($Q = 1.11\text{--}1.32$) (Corner 1950; Petersen 1988). *C. rugosa* (Bull.) J. Schröt is macromorphologically similar to *C. minor*, but the former can be distinguished by its white basidiomata with noncristate and obtuse apices and sometimes a longitudinally wrinkled surface, relatively larger basidiospores ($9\text{--}14 \times 8\text{--}12 \mu\text{m}$) (Corner 1950; Olariaga et al. 2009).

Clavulina lilaceorosea X.X. Huang & L.H. Qiu, sp. nov. (Figs. 3 and 6)

MycoBank: MB847701

Etymology: the epithet '*lilaceorosea*' refers to the pale brown to ochre brown basidiomata.

Type: China, Guangdong Province, Baiyun Mountain, elevation, 230 m, N 23° 11' 53.84", E 113° 19' 11.75", 29 May 2022, L.H. Qiu (B22052918, GDGM 89550). GenBank ITS: OP738996, LSU: OP737363, *RPB2*: OP745532.

Basidiomata solitary or coralloid, gregarious in caespitose clusters; clusters 20–50 mm tall, 20–35 mm wide across branches. Individual basidiomata 20–50 mm tall, 4–15 mm wide across branches, branching pattern polychotomous to dichotomous-ascending, forming 3–5 ranks in multiple planes, branches subterete to flattened, palmate; branch tips rounded acuminate to irregularly coronate when young becoming blunt with age; context subsolid to hollow, concolorous with branches. Stipes 5–20 × 3–8 mm, subclavate or flattened, pale brown (#F4A460) to ochre brown (#A7621F). Hymenium amphigenous, sienna (#A0522D) to dark orange (#FF8C00). Odor unapparent.

Basidiospores [$100/4/2$] (4.9)5.2–5.9–6.8(7.2) × (4.4)4.8–5.5–6.4(6.9) μm , $Q = (1.00)1.01\text{--}1.07\text{--}1.16(1.20)$, $Q = 1.07 \pm 0.05$, globose to subglobose, rarely broadly ellipsoid, smooth, inamyloid, hyaline in H_2O and KOH, with one or several translucent guttules, apicules 0.5–0.9 μm long. Basidia 20–32 μm long, 4–7 μm wide, subcylindrical to subclavate or clavate, with numerous granular contents, postpartial septa observed on some basidia, occurring 7–11 μm below basidia tips, clamps occasionally present, two sterigmata per basidium, 2.3–5.9 μm long, cornute. Basidioles numerous, subcylindrical to clavate, basally clamped. Cystidia absent. Tramal hyphae smooth, thin, light brown walls in KOH, 2.8–6.3 μm wide, clamp connections abundant.

Habit, habitat, and distribution: Gregarious to caespitose or solitary in humus layers on soils under trees of Fagaceae. Basidiomata occur generally from May to August; the only known specimens are from Guangdong Province of China so far, but it may present in Fujian Province of China as well according to the sequence available in GenBank.

Specimens examined: China, Guangdong Province, Baiyun Mountain, elevation, 238 m, N 23° 11' 20.23", E 113° 18' 21.56", 21 Aug 2021, L.H. Qiu (B21082106, GDGM 89551).

Commentary: *Clavulina lilaceorosea* is characterized by its pale brown to ochre brown basidiomata with palmate branches, globose to subglobose basidiospores, the presence of postpartal septa and clamp connections. The phylogenetic tree (Figs. 1 and 2) shows that *C. lilaceorosea* forms a monophyletic clade in *Clavulina* and closely related to *C. castaneipes* (G.F. Atk.) Corner. (= *C. ornatipes* (Peck) Corner) (Petersen 1964), and *C. purpurascens* P. Zhang. *C. castaneipes* resemble *C. lilaceorosea* in appearance of palmate basidiomata, but the former has strigose, hispid fibrils on its stem and clamp connections absent (Corner 1950; Petersen 1964). *C. purpurascens* differs from *C. lilaceorosea* by its pale purple basidiomata, simple or sparsely branched one to two times and lager basidiospores of 8.6–12.7 × 7.7–10.6 μm (Wu et al. 2019).

Discussion

In this study, three new species of *Clavulina* from Baiyun Mountain, Guangdong, China are identified and described. China is rich in macrofungal diversity through the subtropical monsoon climate and high vegetation coverage, but knowledge of *Clavulina* species is relatively restricted. By far, only following eight *Clavulina* species were described from specimens collected in China: *C. bessonii* (Pat.) Corne, *C. castaneipes* (= *C. ornatipes*), *C. coralloides* (L.) J. Schröt. (= *C. cristata* (Holmsk.) J. Schröt.), *C. cinerea* and *C. rugosa* (Teng 1963; Zang et al. 1996; Li et al. 2015), *C. livida* (He et al. 2016), *C. flava* and *C. purpurascens* (Wu et al. 2019). The description of three new *Clavulina* species in this study increase the our understanding to the biodiversity of the genus and the macrofungal community of Baiyun Mountain in Guangzhou, China.

Currently no molecular-based subgenus classification system of *Clavulina* was proposed. Corner (1950) divided the genus *Clavulina* into two subgenera based mainly on the micromorphological characteristics which have been used ever since: subg. *Fusco-Clavulina* Corner containing species with brown wall tramal hyphae while subg. *En-Clavulina* Corner including those with colorless wall. And the latter was further divided into three groups: 1. hyphae without clamps, 2. hyphae with clamps and presence of cystidia, and 3. hyphae with clamps but absence of cystidia. The phylogenetic tree based on 3-locus dataset (Fig. 2) showed that the color of hyphae wall could be a good character to distinguish the taxa at subgenus levels, as all species in the clade I have brown hyphae wall, those in clade III have colorless while those in the clade II have mix color hyphae walls, indicating an evolution history from brown to colorless hyphae walls. However, the presence or absence of clamps and cystidia seem not a good character for the demarcation of subgroups in subg. *En-Clavulina*. A worldwide sampling and applying more gene loci for phylogenetic analysis are needed to establish a reliable taxonomic system for the genus *Clavulina*.

Declarations

Acknowledgments

We are very grateful to the Herbarium of Microbiology Institute of Guangdong (HMIGD) for accepting our collections. The reviewers' comments and suggestions for improving the manuscript are highly appreciated.

Data availability

Publicly available datasets were analyzed in this study which can be found here:
<https://www.ncbi.nlm.nih.gov/>; <https://indexfungorum.org/>; <https://www.mycobank.org/>.

Funding

This research is supported partly by the Guangdong Province Science and Technology Innovation Strategy Special Fund (Grant No. 2018B020205003).

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Contributions

Material preparation, data collection, and analysis were performed by Xiao-Xia Huang, Yi-Xin Chen, Zhi-Jun Lin, Ying-Shan Liang and Li-Hong Qiu. The draft of the manuscript and hand-sketched graphics were written and drew by Xiao-Xia Huang. All authors commented on previous versions and confirmed the final manuscript.

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Figures

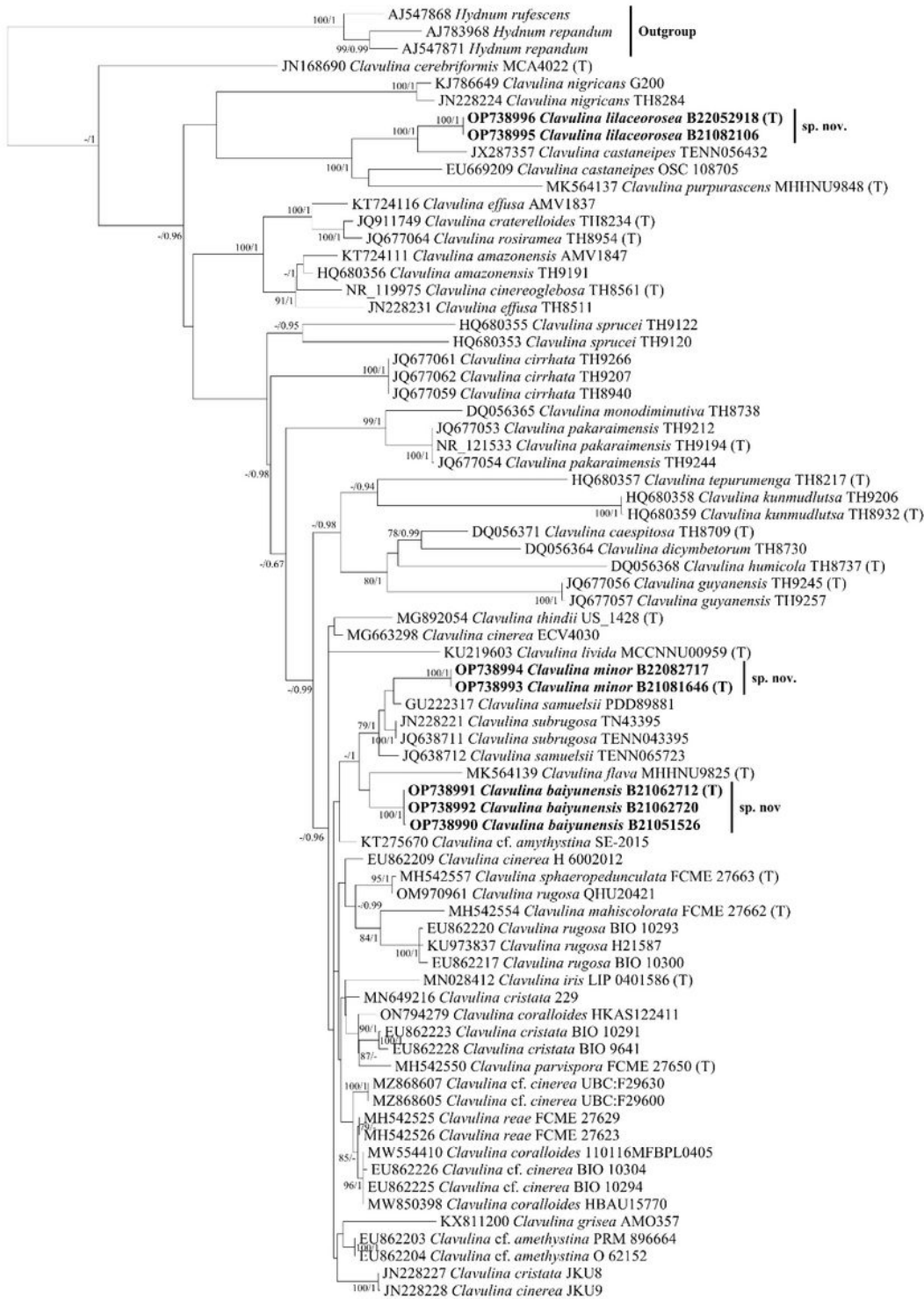


Figure 1

Phylogeny of *Clavulina* inferred from the ITS dataset rooted with *Hydnum repandum* and *Hydnum rufescens* using ML analysis. Bootstrap value (BS) and posterior probability (PP) higher than 70 and 0.90, respectively, are displayed around nodes. Scale bar: 0.02 substitutions. The new species are in bold.

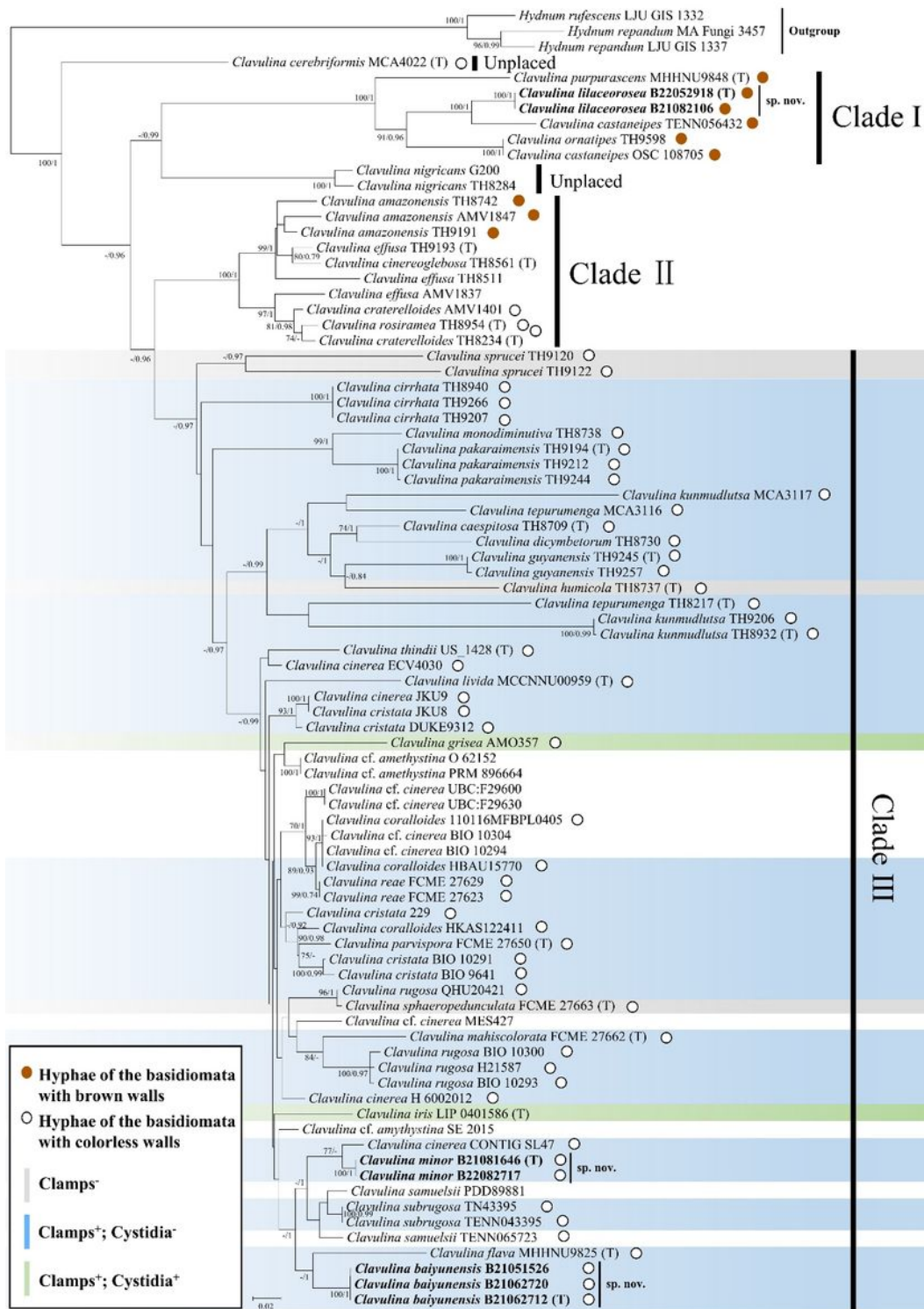


Figure 2

Phylogeny of *Clavulina* inferred from III dataset rooted with *Hydnum repandum* and *Hydnum rufescens* using ML analysis. Bootstrap value (BS) and posterior probability (PP) higher than 70 and 0.90, respectively, are displayed around nodes. Scale bar: 0.02 substitutions. The new species are in bold. The absence of any marks indicates that the corresponding information is not available.

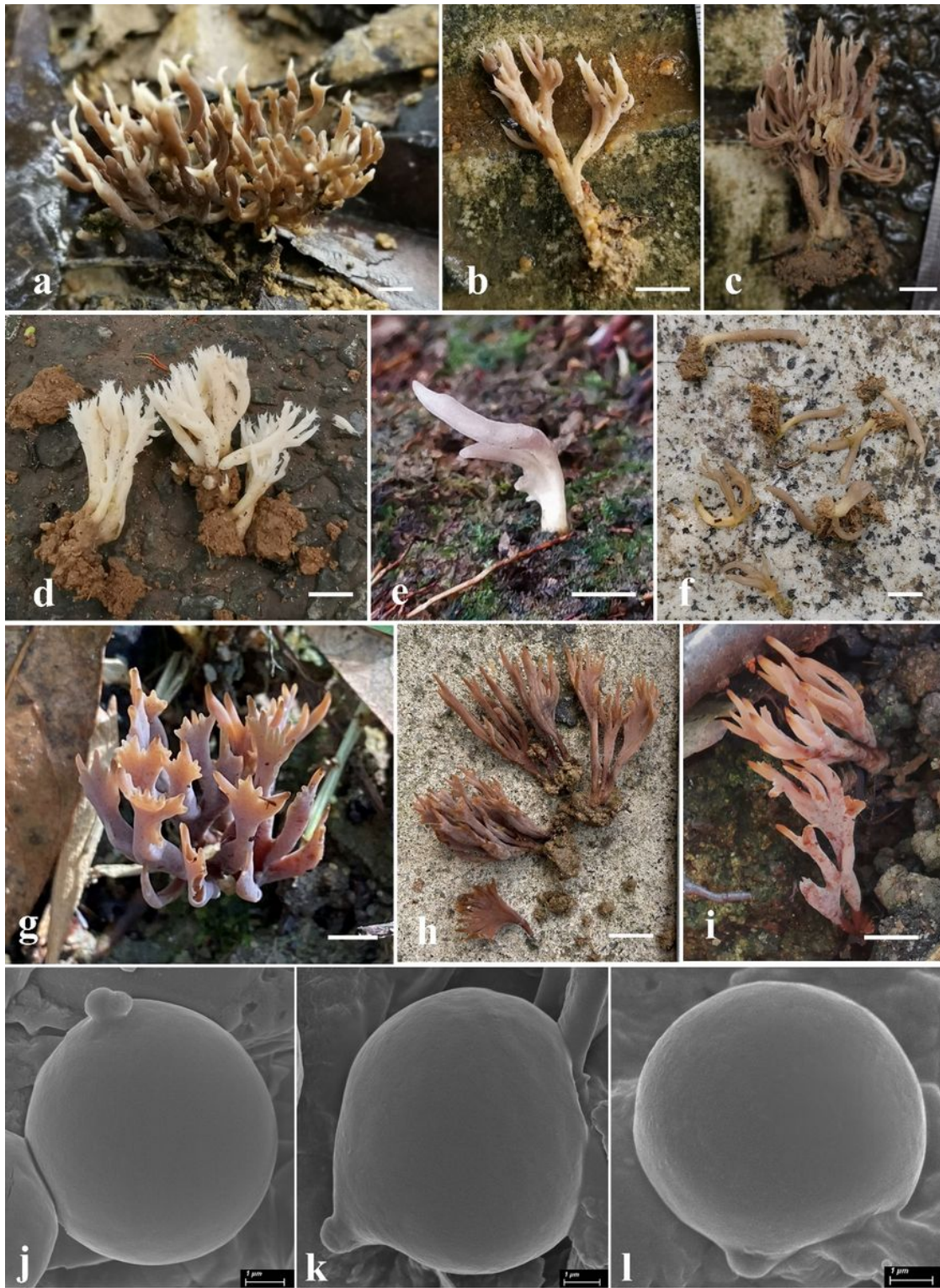


Figure 3

Basidiomata of three new species: a–d *Clavulina baiyunensis* (a, b B21062712 holotype; c B21062720; d B21051526); e, f *Clavulina minor* (e B21081646 holotype; f B21081646); g–i *Clavulina lilaceorosea* (g, h B21081646 holotype; i B21082106); J–I Basidiospores of *C. baiyunensis*, *C. minor* and *C. lilaceorosea*, respectively. Scale bars a–i 10 mm; J–I 1 μm .

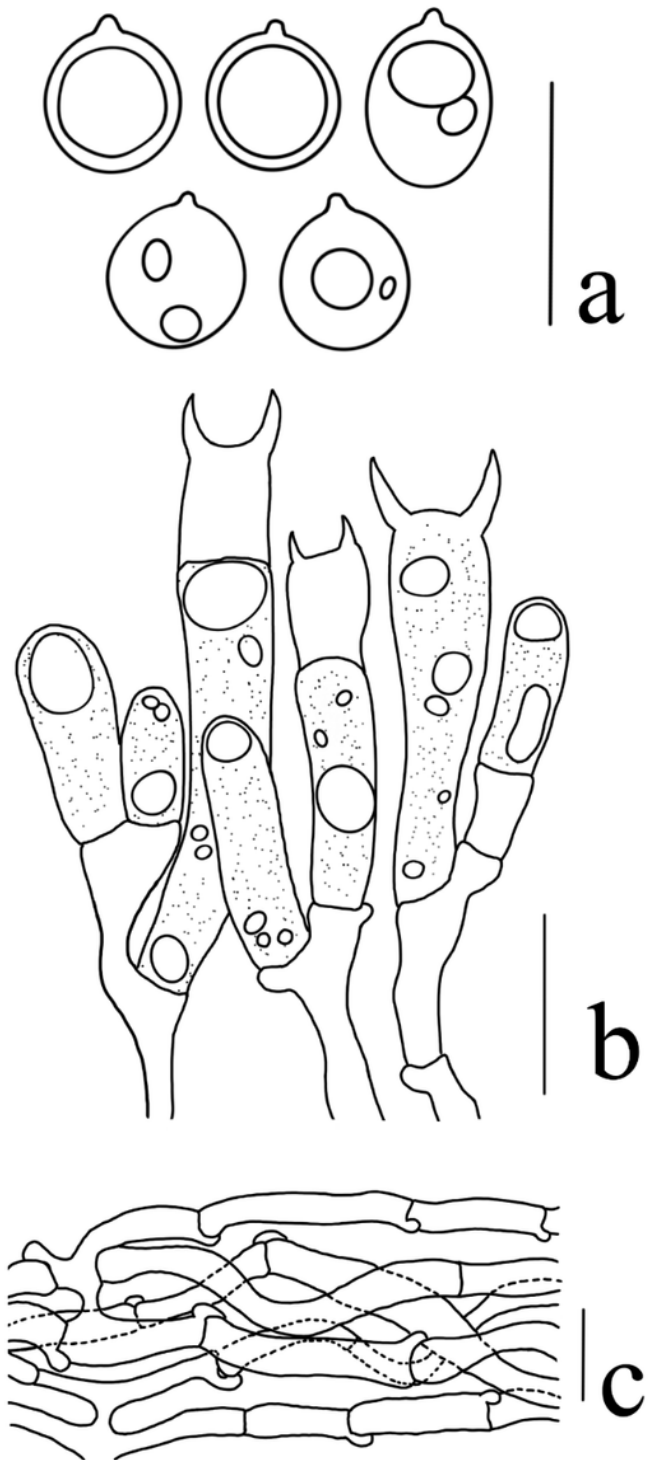


Figure 4

Microscopic characteristics of *Clavulina baiyunensis*: a basidiospores; b basidia; c tramal hyphae. Scale bars = 10 μm .

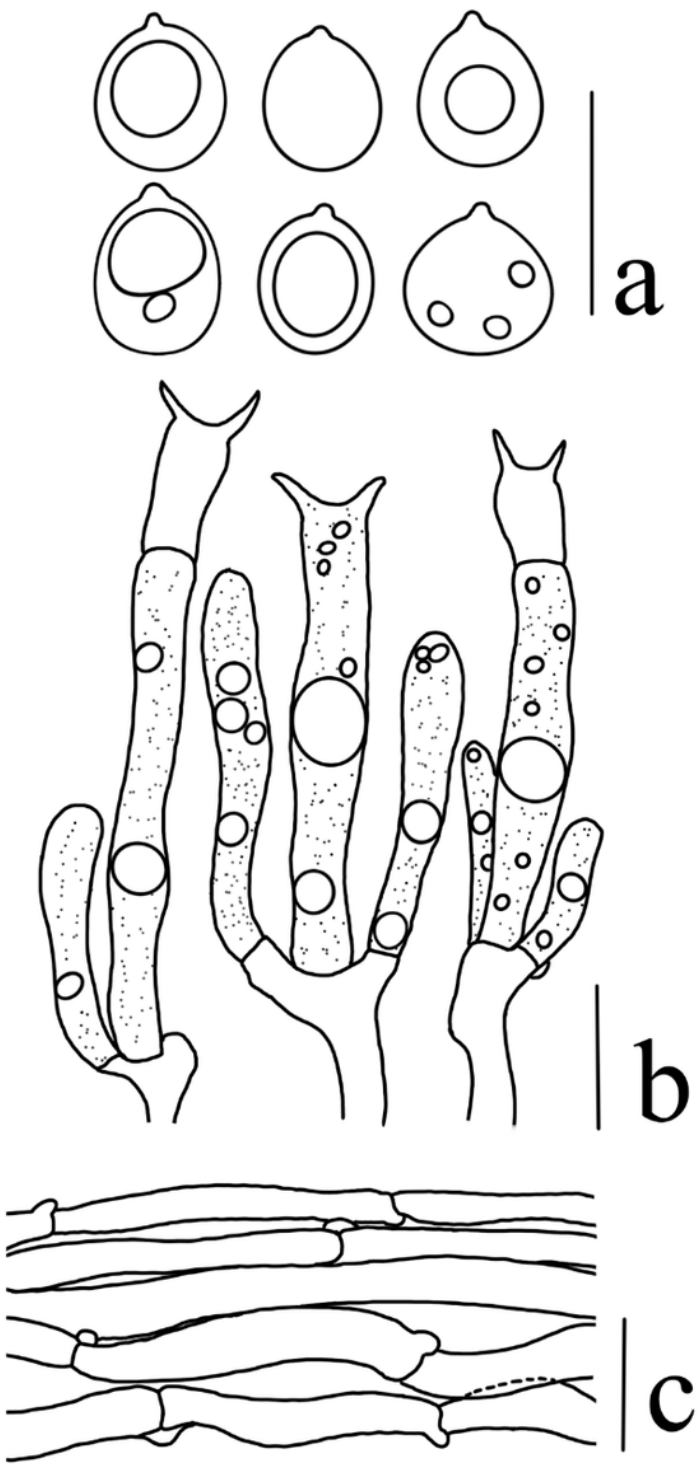


Figure 5

Microscopic characteristics of *Clavulina minor*. a basidiospores; b basidia; c tramal hyphae. Scale bars = 10 μm .

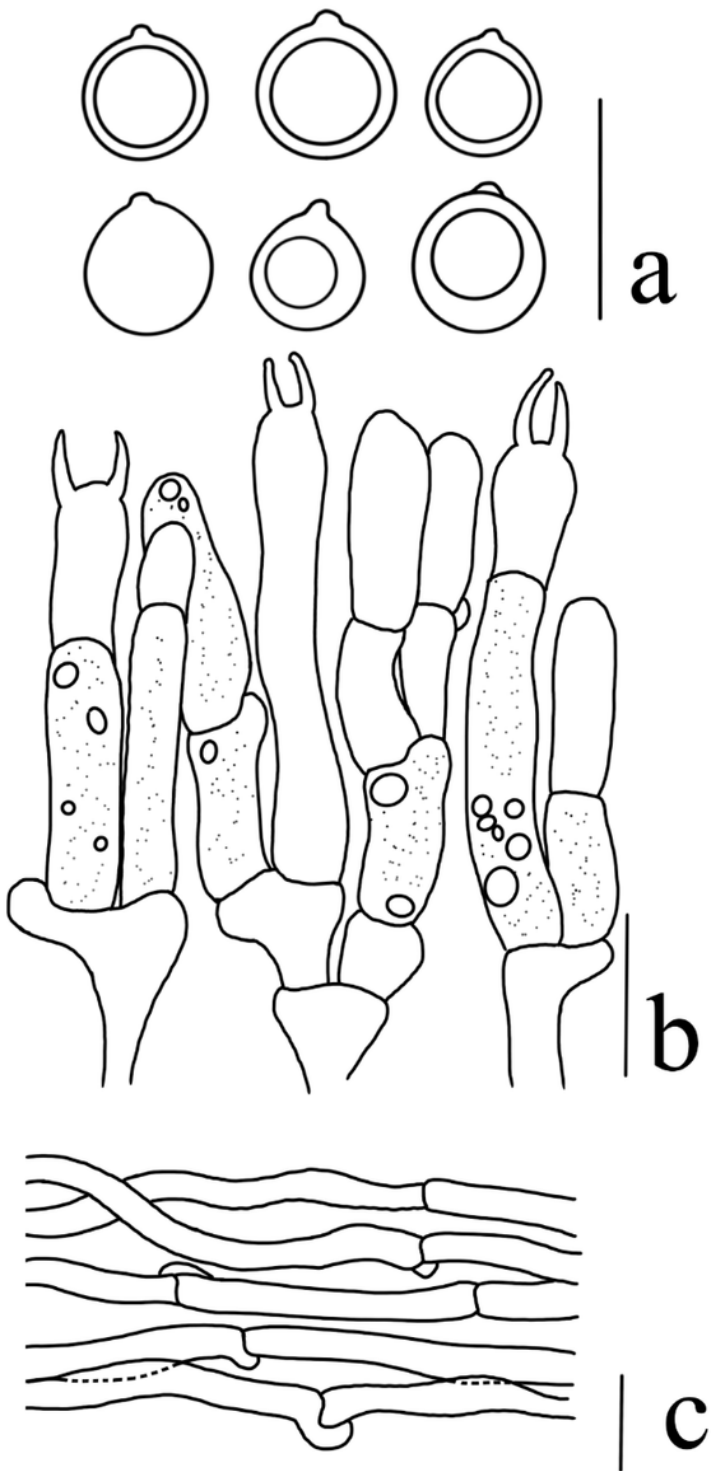


Figure 6

Microscopic characteristics of *Clavulina lilaceorosea*: a basidiospores; b basidia; c tramal hyphae. Scale bars = 10 μm .

Supplementary Files

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