Updated taxonomy of Chinese Clavaria subg. Syncoryne (Clavariaceae, Agaricales): description of two new species and one newly recorded species

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Abstract
Species of Clavaria (Clavariaceae, Agaricales) collected from China were studied using morphological and molecular methods. Two species, C. aspersa and C. hupingshanensis, are here described as new to science; C. aspersa possesses simple, white basidiomata in gregarious or scattered fascicles, whereas C. hupingshanensis possesses simple, rose-white to seashell-pink basidiomata in gregarious to caespitose clusters. In addition, C. amoenoides is described as a newly recorded species for China; this species is characterized by simple, very pale orange-yellow to picric-yellow basidiomata. A phylogenetic analysis was conducted based on a combined dataset of internal transcribed spacer, nuclear ribosomal RNA large subunit, and the RNA polymerase II second largest subunit sequences. The phylogenetic reconstruction resolved accessions of the three species into three independent lineages within the Clavaria. The morphology of the three species is described in detail and is illustrated with line drawings and photographs. Holotypes of the new species are deposited in the Mycological Herbarium of Hunan Normal University. The sequences newly generated in this study have been deposited in GenBank. An updated key to the known Clavaria species in China is provided.

Keywords Clavariaceae · Phylogeny · Morphology · Taxonomy

Introduction

The genus Clavaria Vaill. ex L. is a member of the family Clavariaceae Chevallier (1826). The name “Clavaria” was first proposed by Vaillant (1727) and was subsequently used by Linnaeus (1753) for all species of fungi with an erect, club-shaped, or branched fruit body. As now circumscribed, the genus is characterized by its simple clavarioid or branched basidiomata and the absence of clamps in contextual hyphae. The species are classified into two subgenera: Syncoryne Fr. lacks clamps at the basidium base, and Holocoryne (Fr.) Quél. has a broad, loop-like clamp at the base of the basidium (Coker 1923; Corner 1950, 1970; Petersen 1978, 1988).

The color of the basidiomata is an important character for classification in Clavaria, which comprises species that produce basidiomata with a high diversity of colors, including white, pink, and purple (Burt 1922; Corner 1950). In recent years, many studies on the color of basidiomata in Clavaria have been undertaken; for example, Roberts (2007) studied black and brown Clavaria species in the British Isles, Kautmanová et al. (2012) focused on European species of Clavaria with dark basidiomata, and Olariaga et al. (2015) studied yellow Clavaria species with a loop-like clamp at the base of the basidium.

Before the present study, eight species of Clavaria were recorded in China and approximately 35 species were recognized worldwide (Tai 1979; Olariaga et al. 2015; Chen & Zhang 2019; Yan et al. 2020). In China, only Clavaria acuta Sowerby and Clavaria gibbsiae Ramsb. have a clamp at the base of the basidium; the other species native to China are classified in subg. Syncoryne. Since 2010, we have collected a number of Clavaria specimens with clampless basidia in several provinces of China. Morphological and molecular studies showed that these specimens belong to two species that are new to science, and to one species not previously recorded in China. Descriptions and illustrations of the three species are presented herein.
Materials and methods

Sample sources

Specimens of the two new species, *C. aspersa* and *C. hupingshanensis*, were collected by the authors in Hunan Province or Anhui Province, Central China, between 2010 and 2020. Material of the newly recorded species in China, *C. amoenoides*, was collected in Jilin Province between 2019 and 2020. The dried vouchers are housed in the Mycological Herbarium of Hunan Normal University (MHHNU), Changsha, China. We extracted DNA from the dried specimens, and amplified and sequenced three DNA regions: the internal transcribed spacer (ITS), the nuclear ribosomal RNA large subunit (nrLSU), and the RNA polymerase II second largest subunit gene (*RPB2*). A dataset comprising the concatenated 27 ITS, 26 nrLSU, and 22 *RPB2* sequences, combined with selected sequences downloaded from GenBank, were used for phylogenetic analyses. Voucher information, GenBank accession numbers, and other relevant information are listed in Table 1.

Morphological descriptions

The macromorphological data were based on field notes and habitat photographs. Micromorphological characters were recorded from microscopic observation. The color of basidiomata was described using color codes (Komerup & Wanscher 1978) and color terms (Ridgway 1912). Dried material was sectioned, rehydrated with 5% KOH solution, and stained with 1% Congo red solution. The stained basidiospores, basidia, and hyphae were observed in 5% KOH solution with a light microscope. The notation \[ n/m/p \] indicates that *n* spores were measured from *m* basidiomata of *p* specimens. The basidiospore dimensions are described using the notation \( a \)–\( b \)–\( c \)–\( d \), where the range \( b \)–\( c \) includes a minimum of 90% of the measured values, and extreme values (i.e., \( a \) or \( d \)) are provided in parentheses. \( Q \) is the length/width ratio of a basidiospore in lateral view, \( Q \) is the average \( Q \) of all basidiospores ± sample standard deviation.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from dried specimens using the modified cetyltrimethylammonium bromide method of Doyle and Doyle (1987). The following primer pairs were used to amplify the selected DNA regions: ITS4 and ITS5 (Vilgalys & Hester 1990; White et al. 1990; Gardes & Bruns 1993) for ITS region; LR0R and LR5 (Vilgalys & Hester 1990) for nrLSU region; and rpB2-5F, rpB2-6F, and rpB2-7.1R (Liu et al. 1999; Matheny et al., 2007) for *RPB2* gene. Each PCR amplification was conducted using an Eppendorf Mastercycler thermal cycler (Eppendorf Inc., Germany) in a 25-μL reaction volume. The thermal-cycling protocol was as follows: initial denaturation at 94 °C for 4 min; denaturation for 34 cycles of 94 °C for 40 s, annealing at an appropriate temperature (55 °C for 40 s for ITS and nrLSU; 50 °C for 1 min for *RPB2*), and extension at 72 °C for 1 min; and a final extension at 72 °C for 8 min (Liu et al. 2017; Wu et al. 2019). The PCR products were separated by 1% agarose gel electrophoresis (Songang Inc., China). The purified PCR products were sequenced using an ABI 3730 DNA Analyzer (PerkinElmer Inc., USA). The same primers used for PCR amplification were used for sequencing reactions. Newly generated sequences were deposited in GenBank (accession numbers are listed in Table 1).

Alignment and phylogenetic analyses

A multiple sequence alignment for each DNA region (comprising 27 ITS, 26 nrLSU, and 22 *RPB2* sequences) was generated using default settings for gap openings and gap extension penalties with MUSCLE (Edgar 2004). The alignment was manually edited as necessary. A concatenated sequence dataset was then assembled with PAUP 4.0 (Swofford 2002) for subsequent phylogenetic analyses. Phylogenetic analyses were conducted using the maximum likelihood (ML) method with RAxML 7.2.6 (Stamatakis et al., 2005, 2006). The GTR+Gamma evolutionary model was used (Stamatakis et al., 2008). A ML bootstrap analysis with 1000 replicates was performed to assess topological support. Bayesian inference (BI) was performed using MrBayes 3.1 (Ronquist & Huelsenbeck 2003). The BI analyses were run for 1,000,000 generations using four Metropolis-coupled Monte Carlo Markov chains to calculate posterior probabilities. The tree files were visualized with FigTree 1.4.2 (Rambaut 2012) and edited using Adobe Photoshop CS6 (Adobe Systems Inc., USA) and Illustrator CS5 (Adobe Systems Inc., USA).

Results

Taxonomy

**Clavaria amoenoides** Corner, K. S. Thind & Anand, Trans. Brit. Mycol. Soc. 39 (4): 483, 1956. Figures 1 and 2

Diagnosis: 1. basidiomata simple, pale orange-yellow to picric yellow, gregarious or caespitose; 2. basidiospores ellipsoid, smooth, 4–6 × 2.5–4 μm; 3. basidia clavate to subcylindrical, clampless, 4 sterigmata.

Basidiomata (Fig. 1a, b) gregarious to caespitose, 30–100 mm tall, 2–4 mm wide, simple, apex rounded. Fertile part cylindrical in outline, smooth, sometimes slightly sinuous, occasionally longitudinal depressions or grooves with age, yellow to very pale orange-yellow [1A2–3, Matius Yellow, Picric Yellow, Pale Orange-Yellow]. Apex paler,
### Table 1  Voucher information and GenBank accession of taxa used in this study

| Identification   | Specimen no. | GenBank no. (ITS) | GenBank no. (28S) | GenBank no. (RPB2) | Location | References                          |
|------------------|--------------|-------------------|-------------------|-------------------|----------|-------------------------------------|
| *C. amoenoides*  | Lueck4       | KP965768          | KP965786          |                   | Germany  | Karich et al. (2015)               |
| *C. amoenoides*  | MHHNU10306   | ON228386          | ON231688          | ON246172          | China    | Present study                      |
| *C. amoenoides*  | MHHNU10522   | ON228387          | ON231689          | ON246173          | China    | Present study                      |
| *C. amoenoides*  | MHHNU10525   | ON228388          | ON231690          | ON246174          | China    | Present study                      |
| *C. amoenoides*  | MHHNU10551   | ON228389          | ON231691          | ON246175          | China    | Present study                      |
| *C. aspersa*     | MHHNU32157   | ON228390          | ON231692          | ON246176          | China    | Present study                      |
| *C. aspersa*     | MHHNU32397   | ON228391          | ON231693          | ON246177          | China    | Present study                      |
| *C. aspersa*     | MHHNU32698   | ON228392          | ON231694          | ON246178          | China    | Present study                      |
| *C. aspersa*     | MHHNU32750   | ON228393          | ON231695          | ON382047          | China    | Present study                      |
| *C. fragilis*    | MHHNU10527   | ON228394          | ON231696          | ON246179          | China    | Present study                      |
| *C. fragilis*    | MHHNU32418   | ON228395          | ON231697          | ON246180          | China    | Present study                      |
| *C. fragilis*    | TENN033244   | KP257121          | KP257195          |                   | USA      | Birkebak et al. (2016)             |
| *C. aff. fragilis* | JMB08171003 | KP257124          | HQ877689          | KP257252          | USA      | Birkebak et al. (2013, 2016)       |
| *C. fumosa*      | MR00170      | JN214482          | HQ877696          |                   | USA      | Birkebak et al. (2013, 2016)       |
| *C. fumosa*      | TENN060724   | KP257126          | KP257199          |                   | Russia   | Birkebak et al. (2016)             |
| *C. griseollacina* | MHHNU9722  | MT028142          | ON231725          | ON246185          | China    | Yan et al. (2020); present study   |
| *C. griseollacina* | MHHNU10149 | MT028141          | ON231726          | ON246186          | China    | Yan et al. (2020); present study   |
| *C. hupingshanensis* | MHHNU7362 | ON228396          | ON231698          | ON246181          | China    | Present study                      |
| *C. rosea*       | TENN063100   | KP257133          | KP257205          | KP257256          | USA      | Birkebak et al. (2016)             |
| *C. rosea*       | TENN065117   | KP257134          | KP257206          | KP257257          | USA      | Birkebak et al. (2016)             |
| *C. sinensis*    | MHHNU8198    | MT028140          | ON231727          | ON246187          | China    | Yan et al. (2020); present study   |
| *C. zollingeri*  | MHHNU10528   | ON228397          | ON231699          | ON246182          | China    | Present study                      |
| *C. zollingeri*  | MHHNU10548   | ON228398          | ON231700          | ON246183          | China    | Present study                      |
| *C. zollingeri*  | MHHNU10550   | ON228399          | ON231701          | ON246184          | China    | Present study                      |
| *C. zollingeri*  | TENN064095   | KP257141          | HQ877700          | KP257263          | USA      | Birkebak et al. (2013, 2016)       |
| *C. zollingeri*  | TENN58652    | AY854071          | AY639882          | AY480940          | USA      | Birkebak et al. (2013)             |
| *Mucronella flava* | IO.16.84 | MT232354          | MT232307          |                   | Sweden   | Olariaga et al. (2020)             |
| *Mucronella sp.* | PDD95742     | HQ533013          |                   |                   | New Zealand | Unpublished                       |

New sequences are shown in bold

Fig. 1  Basidiomata of *Clavaria amoenoides* (a MHHNU10525; b MHHNU10551). Bars = 2 cm
Concolorous. Sterile part indistinct, without tomentum at base and mycelial patch indistinct. Context frail, hymenium concolorous but slightly paler. Taste and odor, and macrochemical reactions not recorded.

Basidiospores (Fig. 2a) [60/5/4] 4.0–6.0(7.0) × (2.0)2.5–4.0 μm [\(Q = 1.50–2.08(2.50), Q = 1.94 \pm 0.27\)], narrowly ellipsoid or subcylindrical, thin-walled, hyaline, smooth, hilar appendage present (3.5–6.0 μm in length), with granular contents, nonamyloid. Basidia (Fig. 2b) (30)35–50 × (4)5–8 μm, clavate to subcylindrical, clampless, hyaline, multiguttulate, sterigmata four, tapered; incrustations or crystals absent; subhymenium clearly delimited from the context, composed of densely interwoven hyphae. Hyphae of the context cylindrical to inflated, thin-walled, parallel, without secondary septa, lacking clamp connections, hyaline. Hyphae near subhymenium 3–8 μm wide; hyphae distant from subhymenium 9–22 μm wide.

Habitat: Gregarious to caespitose in humus layers of soil in broad-leaved forest or on soil in pine-oak forest. Basidiomata produced in summer or autumn, usually throughout August to September.

Distribution: India (Corner et al., 1956), Germany (Karich et al., 2015), China.

Specimens examined: China. Jilin Province: Jiaohe City, Hongye Valley, 43°44′07″ N, 126°05′14″ E, alt. 740 m, 28th Aug. 2020, P. Zhang (MHHNU10522; MHHNU10525; MHHNU10551).

Fig. 2  Microscopic features of *Clavaria amoenoides* (MHHNU10525). a Basidiospores; b Basidia

Comments: *Clavaria amoenoides* is primarily characterized by its yellow to very pale orange-yellow basidiomata and ellipsoidal spores. In the genus *Clavaria*, a number of species have yellow basidiomata. Most of these species, such as *Clavaria argillacea* Pers., *Clavaria flavipes* Pers., *Clavaria flavostellifera* Oariaga et al., and *Clavaria sphagnicola* Boud., are classified in subg. *Holocoryne*. However, *C. amoenoides* lacks clamped basidia and thus belongs in subg. *Syncoryne*. On the basis of this character, *Clavaria straminea* Cotton is similar to *C. amoenoides*. In comparison, *C. straminea* usually has a very distinct, cinnamon-yellow stem, and has globose basidiospores, 5–7 μm diam. (Cotton 1910).

In the genus *Clavulinopsis* Overeem, *Clavulinopsis amoena* (Zoll. & Moritzi) Corner is the most difficult to distinguish from *C. amoenoides* in the field, but the hyphae and basidia of the former have obvious clamp connections.

Based on macro- and micromorphological examination of four specimens collected in China, we determined that *C. amoenoides* occurs naturally in China.

**Clavaria aspersa** P. Zhang & Ju. Yan, sp. nov.

**MycoBank**: 844449

Diagnosis: 1. basidiomata simple, white, scattered to gregarious or weakly fascicled; 2. basidiospores ellipsoid, hyaline, smooth, 4–5 × 2.5–4 μm; 3. basidia clavate to subcylindrical, clampless, 4 sterigmata.

Type: China. Anhui Province: Huangshan City, Mount Huangshan, 30°10′52″ N, 118°08′56″ E, alt. 563 m, 1st Aug. 2020, Zuo H. Chen (MHHNU32157, holotype).

**Etymology**: *aspersus* (Lat.): from the Latin *aspersus*, refers to the scattered growth habit of this species.

Basidiomata (Fig. 3a, b) white [1A1. White], fragile, simple, scattered to gregarious or weakly fascicled, 15–45 mm tall, 1–4 mm wide; hymenium amphigenous, cylindrical, clavate to slightly curved or flexuous, smooth to occasionally longitudinal depressions or grooves in age; apex off-white when young, yellowish or tawny in age; stipe sterile, distinct when young, pallid, semi-translucent; base without tomentum; context frail, hymenium concolorous; taste, odor, and macrochemical reactions not recorded.

Basidiospores (Fig. 4a) [60/5/3] (3.8)4–5 × 2.5–4 μm [\(Q = (1.14)1.25–1.52(1.60), Q = 1.36 \pm 0.13\)], ellipsoid, thin-walled, hyaline, smooth, inamyloid; hilar appendage 0.5–1.0 μm long, with granular contents. Basidia (Fig. 4b) (25)35–50(60) × 4–8(10) μm, clavate to subcylindrical, clampless, hyaline, multiguttulate, sterigmata four, tapered, 2–5 μm long; subhymenium clearly delimited from trama; hyphae densely interwoven; trama hyphae cylindrical to inflated, thin-walled, parallel, clampless, without secondary septa.

Habitat: Scattered to gregarious or weakly fascicled in humus layers of soil in broad-leaved forest or on the ground covered with moss. Basidiomata produced in summer or autumn, usually throughout July to September.
Distribution: Known only from the type locality, China.

Additional specimens examined: China. Hunan Province: Zhangjiajie City, Zhangjiajie National Forest Park, 29°35′78.73″ N, 110°41′99.83″ E, alt. 1033 m, 5th Sep. 2020, Zuo H. Chen (MHHNU32397); Zhangjiajie City, Zhangjiajie National Forest Park, 29°38′55.10″ N, 110°48′17.52″ E, alt. 1033 m, 11th Jul. 2020, Zuo H. Chen (MHHNU32698); Zhangjiajie City, Zhangjiajie National Forest Park, 29°35′89.71″ N, 110°41′99.27″ E, alt. 1004 m, 2nd Aug. 2019, Zuo H. Chen (MHHNU32750).

Comments:—Clavaria aspersa is primarily characterized by the simple, gregarious or weakly fascicled, white basidiomata and ellipsoid hyaline basidiospores. Before this study, more than 10 white species have been reported in Clavaria, which represents the largest group of species in the genus. Clavaria gibbsiae and Clavaria tenuipes Berk. & Broome are consistent with C. aspersa in lacking secondarily septate hyphae, but they have a loop-like basal clamp at the base of the basidium and belong to subg. Holocoryne; Clavaria acuta is also classified in subg. Holocoryne and is secondarily sepaate; Clavaria alliacea Corner and Clavaria fascata Oudem. are similar to C. aspersa macromorphologically but they differ in producing 2-spored basidia; and Clavaria filiola Corner and Clavaria fossicola Corner are white tone species, but both are extremely small species and easy to distinguish (Corner 1950, 1970).

In the field, Clavaria fragilis Holmsk. is the most difficult species to distinguish from C. aspersa because the two species are similar in color and size. In comparison, C. fragilis is usually densely caespitose, secondarily sepaate, and without an indistinct stem (Burt 1922, Corner 1950, 1970). Based on macro- and micromorphological examination and phylogenetic analyses, the present results revealed that C. aspersa is a distinct species new to science.

Clavaria hupingshanensis P. Zhang & Ju. Yan, sp. nov.
MycoBank: 844450
Diagnosis: 1. basidiomata simple, occasionally once furcate, rose-white to seashell-pink, gregarious to caespitose clusters; 2. basidiospores ellipsoid, hyaline, smooth, 4.0–6.0(7.0) × 3.5–5.0 μm; 3. basidia clavate, clampless, 4 sterigmata.

Type: China. Hunan Province: Changde, Shimen County, Hupingshan Natural Reserve, 30°2′11.77″ N, 110°33′48.39″ E, alt. 1500 m, 1st Sep. 2010, P. Zhang (MHHNU7362, holotype).

Etymology: hupingshanensis (Lat.): refers to the currently known distribution of the species in China.

Basidiomata (Fig. 5) rose-white to seashell-pink [6A2, 7A2; Seashell pink], fragile, simple, occasionally branched once, dichotomous towards apices, gregarious to caespitose clusters, clusters 35–70 mm tall, 35–60 mm wide; hymenium amphigenous, terete to slightly curved or flexuous, 2–4 mm wide, smooth; apices paler, concolorous; stipe sterile, indistinct, smooth, without tomentum or mycelial patch at base; context frail, hymenium concolorous but slightly paler; taste and odor, and macrochemical reactions not recorded.
Basidiospores (Fig. 6a) [40/2/1] 4.0–6.0 (7.0) × 3.5–5.0 μm [Q = (1.13)1.20–1.71, Q = 1.41 ± 0.19], slightly ellipsoid in profile, thin-walled, hyaline, smooth, inamyloid; hilar appendage 0.5–1.0 μm long, with granular contents. Basidia (Fig. 6b) (32)36–50 × 5–8 μm, clavate, clampless, multiguttulate, ste- rigmata four, tapered, 2.5–5.0 μm long; subhymenium clearly delimited from trama; hyphae densely interwoven; tramal hyphae cylindrical to inflated, thin-walled, parallel, clampless, secondarily septated.

Habitat: Fasciculate to caespitose in humus layers on soils in coniferous forest. Basidiomata generally produced from August to September.

Distribution: Known only from the type locality in Hunan Province, China.

Comments: Clavaria hupingshanensis is mainly characterized by the simple, gregarious, or caespitose clusters of rose-white to seashell-pink basidiomata. Within the genus Clavaria, pink tone species are not uncommon. Corner (1950) recognized and summarized five species with pink tones, namely Clavaria barlae Bres., Clavaria helicoides Pat. & Demange, Clavaria incarnata Weim., Clavaria rosea Fr., and Clavaria zollingeri Lév. Between 2014 and 2020, four species with pink tones, Clavaria appendiculata Franchi & M. Marchetti, Clavaria apulica Agnello & Papetti, Clavaria messapica Agnello, Kautman. & M. Carbone, and Clavaria pseudoincarnata Franchi & M. Marchetti, were described from Italy (Agnello et al. 2014, Franchi and Marchetti, 2021, A gnello & Papetti 2020). In 2020, we described one new species, Clavaria sinensis P. Zhang, with pink basidiomata from central China (Yan et al. 2020). However, C. hupingshanensis is clearly distinct from these species. In contrast to C. apulica, C. barlae, and C. zollingeri, C. hupingshanensis does not produce branched basidiomata and the basidiomata color is paler. Compared with C. appendiculata, C. incarnata, C. messapica, and C. pseudoincarnata, C. hupingshanensis, as a member of subg. Syncoryne, lacks basidia with a loop-like clamp. Clavaria helicoides is the most unique among the pink species; its spores are pink, which can be distinguished from C. hupingshanensis with white spores. Clavaria rosea and C. sinensis have been reported in China (Tai 1979; Yan et al. 2020). In morphology, C. rosea is darker than C. hupingshanensis, and C. sinensis can produce branched basidiomata. With regard to phylogenetic relationships, C. rosea is closely related to C. fragilis within /Clavaria sensu stricto (Birkebak et al. 2016), and C. hupingshanensis and C. sinensis cannot form a sister lineage within the /fumosa clade.

Phylogenetic analyses

The alignment of concatenated sequences, which were 2516-bp long, was used for BI and ML analyses. The matrix comprised 77 sequences (28 ITS, 27 nrLSU, and 22 RPB2) representing 11 species. Clavaria taxa and two species of Mucronella Fr. as the outgroups. The ML analysis yielded the phylogeny shown in Figure 7. The BI phylogeny (not shown) was extremely similar in topology to the ML tree. Bayesian posterior probabilities greater than 0.90 and bootstrap values exceeding 50% are shown at the relevant nodes. The ML and BI analyses resolved two clades among the species of Clavaria: /Clavaria sensu stricto and /fumosa clade. Clavaria aspersa, C. fragilis, and C. rosea were grouped in the well-supported /Clavaria sensu stricto (BI 1/ML 97%). The /fumosa clade (BI 1/ML 100%) comprised six clampless species: C. amoenoides, Clavaria fumosa Pers, Clavaria
grieseolilacina P. Zhang, C. hupingshanensis, C. sinensis, and C. zollingeri. The results were consistent with the previous findings (Kautmanová et al. 2012; Birkbak et al. 2016). The two new species and one newly recorded species form a distinct monophyletic lineage in the tree.

Discussion

In this study, two new Clavaria species and one newly recorded Clavaria species in China (C. amoenoides) were identified in China. Yellow Clavaria species are rare in China. Morphologically, C. amoenoides is similar to other yellow Clavaria or Clavulinopsis species, but can be distinguished from most of these species because it lacks a clamp at the base of the basidium. It is distinguishable from C. straminea by the basidiospores shape and stem characteristics. Phylogenetically, our specimens formed a well-supported (BI 1/ML 100%) lineage with a previously sequenced C. amoenoides accession. Therefore, we confirmed that the distribution range of C. amoenoides includes China. Clavaria aspersa and C. hupingshanensis are described here as new species. The former is similar to C. gibbsiae and C. fragilis, and C. hupingshanensis is distinguishable from other species in the /fumosa clade. In the field, C. aspersa is scattered to gregarious or weakly fascicled, whereas C. gibbsiae and C. fragilis are densely caespitose. With regard to micromorphology, C. aspersa produces clampless basidia and the hyphae are not secondarily septated, whereas C. gibbsiae has a clamp at the base of the basidium and C. fragilis has secondarily septated hyphae. In the present phylogenetic reconstructions, C. aspersa was closely related to C. fragilis and C. rosea, which formed a sister lineage with stronger support (BI 1/ML 97%). Clavaria hupingshanensis may be mistaken for other species in the /fumosa clade, but the macromorphological data and molecular analyses confirmed that the specimen belonged to a species new to science. In the phylogenies, C. hupingshanensis formed a distinct early-diverging monophyletic lineage in the /fumosa clade.

In China, before this study, eight Clavaria species were formally reported, namely C. acuta, C. fragilis, C. fumosa, C. gibbsiae, C. grieseolilacina, C. rosea, C. sinensis, and C. zollingeri (Tai 1979; Chen and Zhang 2019; Yan et al. 2020). Thus, so far, the species diversity of Clavaria is considerably lower than that elsewhere in the world, of which more species need uncovering. In addition, the limited availability of sequences for Clavaria taxa restricts assessment of

Fig. 7 Phylogenetic relationships of Clavaria species inferred from a concatenated sequence (ITS, nrLSU, and RPB2) dataset under the maximum likelihood optimality criterion. Bayesian posterior probabilities over 0.90 and bootstrap values over 50% are reported at nodes (BI/MP); the sign ‘–’ means under the reported level. Two new species and one newly recorded species are shown in boldface text.
phylogenetic relationships within the genus. In this study, two new species and one newly recorded species of *Clavaria* from China are documented, which enriches the species diversity of this genus in China; forty-five newly generated sequences (14 ITS, 17 nrLSU, and 16 RPB2) for *Clavaria* taxa have been deposited in GenBank, which provides reliable data for future phylogenetic studies of *Clavaria*. Notably, the present study is the first to use a DNA region other than ITS and nrLSU to explore phylogenetic relationships in *Clavaria*.

**Key to Clavaria species in China**

1. Basidiomata branched........................................ 2
2. Basidiomata unbranched................................... 4
3. Basidiomata sparsely branched.......................... 3
4. Basidiomata purple to dark purple..................... 5
5. Basidiomata white, hyphae often secondarily septated...... 
6. Basidiomata white to yellowish, hyphae not secondarily septated.................................................. 7
7. Basidiomata usually densely caespitose, hyphae secondarily septeate, and without an indistinct stem....................... 8
8. Basidiomata pink............................................. 9
9. Basidiomata rose pink, with a white or pallid stem.................................................. 10
10. Basidiomata roséwhite to seashell pink, stem indistinct.............................................. 11
11. Basidiomata pale cream to fuliginous.................. 12
12. Basidiomata pale orange-yellow to picric yellow................................. 13

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**Data availability** The sequence data generated in this study are deposited in NCBI GenBank.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

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