

## 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; the former possesses simple, scattered to gregarious or with pairs slightly fascicled, white basidiomata, whereas the latter has simple, gregarious to caespitose clusters, rose-white to seashell-pink basidiomata. 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 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.

## Introduction

The genus *Clavaria* Vaill. ex L. is a member of the family Clavariaceae Chevall (1826). The name "Clavaria" was first proposed by Vaiallant (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 (2009) 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 clamped basidia.

Before the present study, eight species of *Clavaria* were recorded in China and approximately 35 species were recognized worldwide (Tai 1979; Olariage et al. 2017; Chen & Zhang 2019; Yan et al. 2020). Only *Clavaria acuta* Sowerby and *Clavaria gibbsiae* Ramsb. have a clamp at the base of the basidium; the others 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 I 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, was used for phylogentic 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 (Kornerup & 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  $(\mathbf{a}-)\mathbf{b}-\mathbf{c}(-\mathbf{d})$ , where the range  $\mathbf{b}-\mathbf{c}$  includes a minimum of 90% of the measured values, and extreme values (i.e.,  $\mathbf{a}$  or  $\mathbf{d}$ ) 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 fRPB2-5F, fRPB2-6F, and fRPB2-7.1R (Liu et al. 1999; Matheny *et al.* 2007) for *RPB*2 gene. Each PCR amplification was conducted using an Eppendorf Mastercyler thermal cycler (Eppendorf Inc., Germany) in a 25 µL reaction volume. The thermal-cycling protocol was as follows: initial denaturation at 94°C for 4min; 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 8min (Liu et al. 2017; Wu et al. 2019). The PCR products were separated by 1% agarose gel electrophoresis (Sangong 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 2005, 2006). The GTR + Gamma evolutionary model was used (Stamatakis 2008). A ML bootstrap analysis with 1000 replicates was performed to assess topological support. Bayesian inference (BI) was performed 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: This species is characterized by a simple, very pale orange-yellow to picric yellow basidiomata, gregarious or caespitose; ellipsoid hyaline basidiospores (4–6 × 2.5–4 µm); 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, 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)  $[60/5/4] 4.0-6.0(7.0) \times (2.0)2.5-4.0 \ \mu m [Q = 1.50-2.08(2.50), Q = 1.94 \pm 0.27]$ , narrowly ellipsoid or subcylindrical, thin-walled, hyaline, smooth, hilar appendage present  $(3.5-6.0 \ \mu m \ in length)$ , with granular contents, nonamyloid. Basidia (Fig. 2b)  $(30)35-50 \times (4)5-8 \ \mu m$ , clavate to subcylindrical, clampless, hyaline, many oily contents, four tapered sterigmata; 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  $3-8 \mu m$  wide; hyphae distant from subhymenium  $9-22 \mu m$  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 1956), Germany (Karich 2015), China.

Specimens examined: CHINA. Jilin Province: Jiaohe City, Hongye Valley, 43°44'07" N, 117°04'46" E, alt. 442 m, 25th Aug. 2019, P. Zhang (MHHNU10306); Tonghua City, Baijifeng National Forest Park, 41°33'56" N, 126°05'14" E, alt. 740 m, 28th Aug. 2020, P. Zhang (MHHNU10522; MHHNU10525; MHHNU10551).

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* Olariaga, Salcedo, P.P Daniëls & Kautman, 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 seems 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 basdia 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.

Figures 3 and 4

MycoBank: xxxxxx

Diagnosis: This species is characterized by simple, scattered to gregarious or with pairs slightly fascicled, white basidiomata; ellipsoid hyaline basidiospores ( $4-5 \times 2.5-4 \mu m$ ); basidia clavate to subcylindrical, clampless, 4-sterigmata.

Type: CHINA. Anhui Province: Huangshan City, Mount Huangshan, 30°10'59" N, 118°08'56" E, alt. 563 m, 1st Aug. 2020, Zuo H. Chen (MHHNU32157, holotype).

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

Basidiomata (Fig. 3a, b) simple, scattered to gregarious or with pairs slightly fascicled; 15–45 mm tall, 1– 4 mm wide. Fertile part cylindrical or claviform in outline, smooth, often slightly curved or flexuous, occasionally longitudinal depressions or grooves with age, white [1A1, White]. Apex off-white when young, yellowish or tawny when old. Sterile part distinct when young, pallid whitish, semitransparent, without tomentum at base, mycelial patch indistinct. Context frail, hymenium concolorous. Taste and odor, and macrochemical reactions not recorded.

Basidiospores (Fig. 4a) [60/5/3]  $(3.8)4-5 \times 2.5-4 \mu m$   $[Q = (1.14)1.25-1.52(1.60), Q = 1.36 \pm 0.13]$ , ellipsoid, thin-walled, hyaline, smooth, hilar appendage present  $(0.5-1.0 \mu m \text{ in length})$ , with granular contents, nonamyloid. Basidia (Fig. 4b)  $(25)35-50(60) \times 4-8(10) \mu m$ , clavate to subcylindrical, thin-walled, clampless, hyaline, many oily contents, four tapered sterigmata, long  $2-5 \mu m$  long; 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 the subhymenium  $4-10 \mu m$  wide; hyphae distant from subhymenium  $10-20 \mu m$  wide.

Habitat: Scattered to gregarious or with pairs slightly 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, 2rd Aug. 2019, Zuo H. Chen (MHHNU32750).

Comments:—*Clavaria aspersa* is primarily characterized by the simple, gregarious or with pairs slightly 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 septate; *Clavaria alliacea* Corner and *Clavaria fuscata* Oudem. are similar to *C. aspersa* in macromorphologicaly but the 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 septate, 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.

Figures 5 and 6

MycoBank: xxxxxx

Diagnosis: This species is characterized by simple, gregarious to caespitose clusters, rose-white to seashell-pink basidiomata, occasionally once furcate; ellipsoid hyaline basidiospores (4.0–6.0(7.0) × 3.5–5.0 µm); 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) simple, gregarious to caespitose clusters; clusters 35–70 mm tall, 35–60 mm wide, occasionally branched once, dichotomous towards apices. Fertile part simple, terete in outline, 2–4 mm wide, smooth, rose-white to seashell-pink [6A2, 7A2; Seashell pink]. Apices paler, concolorous. Sterile part 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)  $[40/2/1] 4.0-6.0(7.0) \times 3.5-5.0 \ \mu m [Q = (1.13)1.20-1.71, Q = 1.41 \pm 0.19]$ , slightly ellipsoid in profile, thin-walled, hyaline, smooth, hilar appendage present (0.5–1.0  $\mu$ m in length), with granular contents, nonamyloid. Basidia (Fig. 6b) (32)36–50 × 5–8  $\mu$ m, clavate, thin-walled, hyaline, many oily contents, clampless, four tapered sterigmata, long 2.5–5.0  $\mu$ m long; incrustations or crystals absent; subhymenium clearly delimited from the context, composed of densely interwoven hyphae. Hyphae of the context 3–15  $\mu$ m wide, cylindrical to inflated, thin-walled, parallel, secondarily septated, clampless, hyaline.

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 Hunnan 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* Weinm., *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 2019, Agnello & 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* can't form a sister lineage within the / fumosa clade.

# **Phylogenetic Analyses**

The alignment of concatenated sequences, which were 2,516-bp long, was used for BI and ML analyses. The matrix comprised 75 sequences (27 ITS, 26 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 Fig. 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 griseolilacina* P. Zhang, *C. hupingshanensis, C. sinensis,* and C. *zollingeri.* The results were consistent with the previous findings (Kautmanová et al. 2012; Birkebak et al. 2016). The two new species and one new record species are formed 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 sequence *C. amoenoides* accession. Therefore, we confirmed that the distribution range of *C. amoenoides* includes China. *Clavaria aspersa* and *C. hupingshanensis* is distinguishable from other species in the */ fumosa* clade. In the field, *C. aspersa* is scattered to gregarious or with pairs slightly fascicled, whereas *C. gibbsiae* and *C. fragilis* are densely caespitose. With regard to micromorpholoy, *C. aspersa* produces clampless basidia and the hyphae are not secondarily septated, whereas *C. gibbsiae* has a clamp at the base of *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 supported (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. griseolilacina, C. rosea, C. sinensis,* and *C. zollingeri* (Tai 1979; Chen and Zhang 2019; Yan et al. 2020). The species diversity of *Clavaria* is considerably lower than that elsewhere in the world. In addition, the limited availability of sequences for *Clavaria* taxa restricts assessment of 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*.

## Declarations

### Key to Clavaria species in China

1. Basidiomata branched2
1. Basidiomata unbranched4
2. Basidiomata sparsely branched
2. Basidiomata profusely branched3
3. Basidiomata purple to dark purpleC. zollingeri
3. Basidiomata pale purple
4. Basidia with a loop-like clamp at the base of the basidium5
4. Basidia without a clamp6
5. Basidiomata white, hyphae often secondarily septated
5.Basidiomata white to yellowish, hyphae not secondarily septated
6. Basidiomata white7
6. Basidiomata pigmented8

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**Author contribution** Conceptualization: Ping Zhang; methodology: Jun Yan and Gui-Wu Li; performing the experiment: Jun Yan, Gui-Wu Li and Wen-Hao Liu; resources: Ping Zhang, Zuo-Hong Chen, Gui-Wu Li and Wen-Hao Liu; writing—original draft preparation: Jun Yan; writing—review and editing: Ping Zhang; supervision: Ping Zhang; project administration: Ping Zhang; funding acquisition: Ping Zhang. All authors have read and agreed to the published version of the manuscript.

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

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

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## Tables

Table 1 is available in the Supplementary Files section.

## Figures

### Figure 1

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### Figure 2

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### Figure 3

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### **Supplementary Files**

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