Two new species of *Chalciporus* (Boletaceae, Boletales) from tropical China

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Abstract
Two new species of *Chalciporus* (Boletaceae, Boletales), viz. *C. sinensis* and *C. vulparius*, are described based on morphological and molecular data. Detailed descriptions, color photographs of fresh basidiomata, and line drawings of microscopic features of the two new taxa are presented. In addition, a key to all known species of *Chalciporus* from China is provided.

Keywords New taxa · Molecular phylogeny · Morphology · Taxonomy

Introduction

*Chalciporus* Bataille (Boletaceae, Boletales), typified by *C. piperatus* (Bull.) Bataille, was erected to accommodate species with a peppery taste, reddish hymenophore, solid stipe, and smooth basidiospores (Moreno and García-Bona 1976; Pegler 1981, 1983; Baroni and Both 1991; Gómez 1996; Klofac and Krisai-Greilhuber 2006; Halling et al. 2004). *Chalciporus* diverged early in the evolution of the Boletaceae (Nuhn et al. 2013; Wu et al. 2014). In this genus, *C. piperatus* was thought to be an ectomycorrhizal symbiont but was later proven to be a mycoparasite colonizing *Amanita muscaria* (L.) Lam. ectomycorrhizae (Dickie et al. 2010, 2016).

Although the genus *Chalciporus* is small, its species are distributed worldwide (Desjardin et al. 2009; Nuhn et al. 2013; Raspé et al. 2016; Zhang et al. 2016, 2017; Wu et al. 2016; Vadhanarat et al. 2019; Chai et al. 2019; Klofac and Krisai-Greilhuber 2020). Several metabolites from members of this genus were identified and characterized. For instance, sclerocitrin (the yellow pigment in the stipe base and mycelium), variegatic acid, variegatorubin, chalcitrin, and the unusual 2H-azepine derivative chalciporone (responsible for the pungent taste of the basidioma) were isolated from *C. piperatus* (Winner et al. 2004). Collections identified as *C. piperatus* from China are believed to be edible (Li and Song 2002) and are used as a condiment in many countries (Antonio 2003). In addition, the basidiome of *C. piperatus* is used for dyeing (Roberts and Evans 2011).

In China, five species of the genus *Chalciporus*, viz. *C. citrinaurantius* Ming Zhang & T.H. Li, *C. hainanensis* Ming Zhang & T.H. Li, *C. piperatus* (Bull.) Bataille, *C. radiatus* Ming Zhang & T.H. Li, and *C. rubinelloides* G. Wu & Zhu L. Yang, have been described (Zhang et al. 2016, 2017; Chai et al. 2019; Wu et al. 2016; Deng et al. 2018). Herein, we describe two new species of *Chalciporus* from Hainan Province, a hotspot for discovering new fungal taxa in tropical China, based on morphological and phylogenetic analyses, and demonstrate the diversity of Boletaceae in China.

Materials and methods

Morphological studies

The specimens were described and photographed in the field. Dried specimens were deposited in the Fungal Herbarium of...
Hainan Medical University (FHMU), Haikou City, Hainan Province, China. Color codes are based on Kornerup and Wanscher (1981). Sections of the pileipellis were cut longitudinally and halfway between the center and margin of the pileus. Sections of the stipitpellis were obtained from the middle part along the longitudinal axis of the stipe. Samples were rehydrated with 5% KOH and mounted on microscope slides. All microscopic structures were drawn freehand. The number of basidiospores is given as n/m/p, i.e., n basidiospores from m basidiomata from p collections were counted. The basidiospore dimensions are given as (a)b−c(d), where b and c represent the 5th and 95th percentile, respectively, and extreme values are shown in parentheses. Q refers to the length/width ratio of basidiospores, and Qm is the average Q and standard deviation. The terms related to the size of basidia are based on the study by Bas (1969).

Molecular procedures

Total genomic DNA was obtained using the Plant Genomic DNA Kit (KANGWEI Company, China) according to the manufacturer’s instructions from material dried in silica gel. The primer pairs used for amplification were LR0R/LR5 (Vilgalys and Hester 1990; James et al. 2006) for the nuc 28S rDNA D1-D2 domains (28S), ITS5/ITS4 (White et al. 1990) for the nuc rDNA region encompassing the internal transcribed spacers 1 and 2 and for 5.8S rDNA (ITS), EF1-2F/EF1-2R (Zeng et al. 2013) for the translation elongation factor 1-α gene (TEF1), and RPB2-B-R/RPB2-B-F1 (Wu et al. 2014) for the gene for the RNA polymerase II second largest (RPB2) subunit. PCR was performed in a reaction volume of 25 μl containing 13 μl of 2× Taq PCR MasterMix (KANGWEI Company, China), 2 μl of each primer (10 μM), 8 μl of nuclease-free water, and 2 μl of DNA template. Amplification reactions included one cycle of denaturation at 95 °C for 4 min, followed by 34 cycles of denaturation at 94 °C for 30 s, annealing at the appropriate temperature (50 °C for 28S and ITS, 53 °C for TEF1, and 52 °C for RPB2) for 30 s, extension at 72 °C for 120 s, and one extension cycle at 72 °C for 7 min. PCR products were separated in 1% (w/v) agarose gels, and amplicons were purified and sequenced using an ABI 3730xl DNA Analyzer (Guangzhou Branch of BGI, China) with the same primers used in PCR. Forward or reverse sequences were compiled with BioEdit (Hall 1999).

Dataset assembly

Twenty sequences (six of 28S, four of ITS, six of TEF1, and four of RPB2) from six collections were generated, and edited sequences were deposited in GenBank. The GenBank accession numbers of ITS sequences are MW917178 to MW917181, and those of 28S, TEF1, and RPB2 are listed in Table 1. 28S, TEF1, and RPB2 sequences were aligned with sequences obtained from GenBank and previous studies (Table 1). Buchwaldoboletus xylophilus (Petch) Both & B. Ortiz was chosen as the outgroup based on phylogeny (Xie et al. 2021). Single-locus phylogenetic trees based on 28S, TEF1 (exons only), and RPB2 (exons only) sequences were aligned using MUSCLE (Edgar 2004). There were no conflicts in the topologies of these trees, indicating that phylogenetic signals from different gene fragments were congruent. Then sequences were concatenated using Phyutility version 2.2 (Smith and Dunn 2008).

Phylogenetic analyses

The combined nuclear dataset (28S + TEF1 + RPB2) was analyzed using maximum likelihood (ML) and Bayesian inference (BI) methods. The ML tree was generated, and bootstrap analyses were performed using RAxML version 7.2.6 (Stamatakis 2006). ML searches were performed using RAxML 7.2.6 program and involved 1000 repetitions under the GTR+GAMMA model, and all model parameters were estimated by this software. BI was performed using the Markov Chain Monte Carlo technique in MrBayes version 3.1 (Huelsenbeck and Ronquist 2005), and parameters were predetermined using MrModeltest version 2.3 (Nylander 2004). The evolution model used in BI was determined with MrModeltest 2.3 (Nylander 2004). For the combined dataset, GTR+I+G, GTR+I+G, and SYM+G were chosen as the best-fit likelihood models for 28S, TEF1, and RPB2, respectively. Bayesian analysis of the combined dataset (28S + TEF1 + RPB2) was repeated for 0.4 million generations and sampled every 100th generation. Trees sampled from the first 25% of the generations were discarded as burn-in, and Bayesian posterior probabilities (PP) were calculated for the majority consensus of the retained Bayesian trees.

Results

The combined Chalciporus dataset (28S + TEF1 + RPB2) consisted of 33 taxa and 2159 nucleotide sites, and alignments were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S28091). Phylogenetic trees based on the combined dataset generated from ML and BI analyses were congruent except for slight differences of statistical support. The phylogram generated from RAxML, including support values, is shown in Fig. 1. Molecular data indicated that the Chinese collections of Chalciporus were distributed in eight distinct species-level lineages (Fig. 1), representing eight species. Lineage 1 comprised four materials of C. citrinaaurantius (GDGM44480, GDGM44717, GDGM44481, and GDGM44776) from central China, with high statistical
| Taxon            | Voucher | Locality            | GenBank accession nos  | References                  |
|-----------------|---------|---------------------|------------------------|----------------------------|
|                 |         |                     | 28S        | TEF1  | RPB2      |                        |
| Buchwaldoboletus xylophilus | FHMU5932 | Yunnan, SW China | MW783421 | MW897334 | MW820943 | Xie et al. (2021)     |
| Buchwaldoboletus xylophilus | FHMU5933 | Yunnan, SW China | MW783423 | MW897336 | MW820945 | Xie et al. (2021)     |
| Chalciporus africanus | JD0517  | Cameroon            | —         | KT824029 | KT823996 | Raspé et al. (2016)   |
| Chalciporus amarellus | DS4640-3 | Germany            | KF030283 | KF030440 | —         | Nuhn et al. (2013)    |
| Chalciporus citrinaurantius | GDGM44480 | Hunan, Central China | MZ157128 | —      | —         | Zhang et al. (2017)   |
| Chalciporus citrinaurantius | GDGM44481 | Hunan, Central China | MZ157129 | —      | —         | Zhang et al. (2017)   |
| Chalciporus citrinaurantius | GDGM44717 | Hunan, Central China | MZ157130 | —      | —         | Zhang et al. (2017)   |
| Chalciporus citrinaurantius | GDGM44776 | Hunan, Central China | MZ157131 | —      | —         | Zhang et al. (2017)   |
| Chalciporus hainanensis | GDGM46161 | Hainan, Southern China | MZ157126 | —      | —         | Zhang et al. (2017)   |
| Chalciporus hainanensis | GDGM44464 | Hainan, Southern China | MZ157127 | —      | —         | Zhang et al. (2017)   |
| Chalciporus piperatus | VDK01063 | Belgium             | —         | MH614713 | MH614759 | Vadthanarath et al. (2019) |
| Chalciporus piperatus | HKAS84882 | Germany            | KT990562 | KT990758 | KT990397 | Wu et al. (2016)      |
| Chalciporus pseudorubinellus | 4302     | USA                | KF030284 | KF030441 | —         | Nuhn et al. (2013)    |
| Chalciporus radiatus | GDGM43285 | Hunan, Central China | KP871800 | —      | —         | Zhang et al. (2016)   |
| Chalciporus radiatus | GDGM50080 | Hunan, Central China | KP871801 | —      | —         | Zhang et al. (2016)   |
| Chalciporus radiatus | GDGM43305 | Guangdong, Southern China | KP871802 | —      | —         | Zhang et al. (2016)   |
| Chalciporus radiatus | N.K. Zeng1379 (FHMU930) | Fujian, SE China | MH879710 | MH879738 | —         | Chai et al. (2019)    |
| Chalciporus radiatus | N.K. Zeng1414 (FHMU959) | Fujian, SE China | MH879711 | MH879739 | —         | Chai et al. (2019)    |
| Chalciporus radiatus | N.K. Zeng1808 (FHMU2494) | Hainan, southern China | MH879737 | —      | —         | Chai et al. (2019)    |
| Chalciporus rubinelloides | HKAS74952 | Yunnan, SW China | KT990565 | KT990761 | KT990400 | Wu et al. (2016)      |
| Chalciporus rubinelloides | HKAS58728 | Yunnan, SW China | KT990564 | KT990760 | KT990399 | Wu et al. (2016)      |
| Chalciporus rubinelloides | HKAS57362 | Yunnan, SW China | KT990563 | KT990759 | KT990398 | Wu et al. (2016)      |
| Chalciporus rubinellus | 191/81   | USA                | EU685106 | —      | —         | Desjardin et al. (2009) |
| Chalciporus sinensis | N.K. Zeng4478 (FHMU4701) | Hainan, Southern China | MW917171 | MW925928 | MW925934 | This study             |
| Chalciporus sinensis | N.K. Zeng4479 (FHMU4691) | Hainan, Southern China | MW917172 | MW925929 | MW925935 | This study             |
| Chalciporus sp. | X.T. Zhu134 (FHMU2721) | Yunnan, SW China | MW917175 | MW925932 | —         | This study             |
| Chalciporus sp. | S. Jiang81 (FHMU4573) | Hainan, Southern China | MW917176 | MW925933 | —         | This study             |
| Chalciporus sp. | HKAS53400 | Hunan, Central China | KF112352 | KF112279 | KF112821 | Wu et al. (2014)      |
| Chalciporus sp. | OR0363   | Thailand            | —         | MH645594 | MH645602 | Vadthanarath et al. (2019) |
| Chalciporus sp. | GDGM43250 | Southern China      | MZ157125 | —      | —         | Zhang et al. (2017)   |
| Chalciporus vulparius | N.K. Zeng4978 (FHMU5554) | Hainan, Southern China | MW917173 | MW925930 | MW925936 | This study             |
support (BS = 100%, PP = 1.0); lineage 2 included three collections of *C. rubinelloides* (HKAS57362, HKAS58728, and HKAS74952) from southwestern China and one specimen (*C. sp.*, FHMU2721) from southwestern China, with strong statistical support (BS = 97%, PP = 1.0); lineage 3 included six specimens of *C. radiatus* (GDGM43285, GDGM43305, GDGM50080, FHMU930, FHMU959, and FHMU2494) from central, southern, and southeastern China, with high statistical support (BS = 100%, PP = 1.0); lineage 4 comprised one collection (HKAS53400) from central China; lineage 5 contained three collections of *C. hainanensis* (GDGM46161, GDGM44464, and FHMU4573) from southern China, with strong statistical support (BS = 100%, PP = 1.0); lineage 6 included two specimens (*C. sp.*) from southern China and one collection (*C. sp.*) from Thailand, with high statistical support (BS = 100%, PP = 1.0); lineage 7 included two collections (FHMU5560 and FHMU5554) from southern China, with high statistical support (BS = 100%, PP = 1.0); lineage 8 comprised two materials (FHMU4701 and FHMU4691) from southern China, with high statistical support (BS = 100%, PP = 1.0).

**Taxonomy**

*Chalciporus sinensis* N.K. Zeng, Chang Xu, S. Jiang & Zhi Q. Liang, sp. nov.

Figures 2a–c and 3

Mycobank: MB839317.

*Etymology:* Latin “sinensis” meaning China, holotype locality.

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**Table 1 (continued)**

| Taxon           | Voucher          | Locality          | GenBank accession nos | References                  |
|-----------------|------------------|-------------------|-----------------------|-----------------------------|
| *Chalciporus vulgaris* | N.K. Zeng4979   | Hainan, Southern China | MW911714 MW925931 MW925937 | This study                   |

GenBank numbers in bold indicate the newly generated sequences; SW Southwest, SE Southeast

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**Fig. 1** Phylogram inferred from a three-locus (28S, TEF1, and RPB2) dataset using RAxML. BS ≥ 50% and PP ≥ 0.95 are indicated above or below the branches as RAxML BS/PP. SW, Southwest; SE Southeast

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Holotype: CHINA. Hainan Province: Yinggeling, Hainan Tropical Rainforest National Park, elev. 650 m, 5 July 2020, N.K. Zeng4478 (FHMU4701).

**Basidiomata** very small to small. Pileus 1.5–3.2 cm in diameter, sub-hemispherical, convex to planate, margin usually curved upward; surface dry and covered with brown (2D8) to grey-brown (2D5) scales; context approximately 0.4 cm thick in the center of the pileus, white (1A1), unchanging in color when bruised. **Hymenophore** poroid, nearly adnate to slightly depressed around the stipe apex; pores subrounded to angular, 0.5–1 mm in diameter, reddish orange or light orange (4A6–4A8), unchanging in color when bruised; tubes approximately 0.3 cm long, pale yellow to yellowish orange (5A4–5A6), unchanging in color when bruised. **Stipe** 1.7–2.8 × 0.2–0.4 cm, central, solid, subcylindrical, base slightly enlarged; surface dry, densely covered with squamules, squamules reddish (8A6) at apex and dark brown (1C4) or brown (2B3) to yellowish brown (2B6) elsewhere; context white (1A1), unchanging in color when bruised; basal mycelium yellow (2A3). **Odor** indistinct.

**Basidia** 17–22 × 7.5–11 μm, clavate, thin- to slightly thick-walled (up to 0.5 μm), four-spored, yellowish to hyaline in KOH; sterigmata 1.5–3 μm long. **Basidiospores** [40/2/2] (3.5–)4–7(–7.5) × (3–)3.5–5(–6) μm, Q = (1–)1.11–1.57 (–1.75), Qm = 1.39 ± 0.17, subglobose to ellipsoid, slightly thick-walled (up to 0.7 μm), smooth, pale yellowish brown in KOH. **Sublamellate hymenophore** composed of hyphae 3–4.5 μm in width, thin-walled, hyaline to pale yellow in KOH. **Hymenophoral trama** boletoid, composed of thin- to slightly thick-walled (up to 0.4 μm) hyphae, 4–11 μm in diameter, hyaline to yellowish in KOH. **Cheilocystidia** 45–52 × 9–14 μm, fusiform or subfusiform, thin-walled, hyaline to yellowish in KOH. **Pleurocystidia** 41–74 × 10–13.5 μm, fusiform or subfusiform, thin- to slightly thick-walled (up to 0.5 μm), hyaline to yellowish in KOH. **Pileipellis** a trichodermium 300–450 μm in thickness, composed of hyphae 4.5–11 μm in diameter, bright lemon yellow in KOH, occasionally branched, thin- to slightly thick-walled (up to 0.5 μm); terminal cells 35–77 × 5.5–10 μm, subcylindrical or subclavate, with obtuse apex. **Pileal trama** composed of hyphae 2–8 μm in diameter, slightly thick-walled (up to 0.5 μm), pale yellow in KOH. **Stipitipellis** a 190–500 μm thick trichoderm, composed of thin- to slightly thick-walled (up to 0.5 μm) hyphae 2.5–7 μm in diameter, pale yellow in KOH; terminal cells 11–50 × 2.5–6 μm, subclavate, subcylindrical, or subfusiform. **Stipe trama** composed of parallel hyphae 4–9 μm in diameter, cylindrical, thin- to slightly thick-walled (up to 0.5 μm), hyaline to light yellow in KOH. **Clamp connections** absent in all tissues.

**Fig. 2** Basidiomata of *Chalciporus* species. a–c *Chalciporus sinensis* (a from FHMU4691; b, c from FHMU4701, holotype). d–f *Chalciporus vulparius* (d from FHMU5554; e, f from FHMU5560, holotype). Photos by N.K. Zeng
Habitat: Solitary on the ground in forests dominated by Castanopsis indica (Roxburgh ex Lindley) A. DC.

Known distribution: Southern China (Hainan Province).

Additional specimen examined: CHINA. Hainan Province: Yinggeling, Hainan Tropical Rainforest National Park, elev. 650 m, 5 July 2020, N.K. Zeng4479 (FHMU4691).

Notes: Chalciporus sinensis is well characterized by a pileus covered with brown, dark brown to black-brown scales, a context unchanging in color when injured, a stipe covered with reddish, dark brown, or brown to yellowish brown squamules, a yellow basal mycelium, and small basidiospores. Chalciporus sinensis is morphologically similar to C. citrinoaurantius, C. hainanensis, C. radiatus, C. rubinelloides, and C. vulparius from China (Zhang et al. 2016, 2017; Wu et al. 2016) and C. cervinococcineus (Corner) Klofac & Krisai from Singapore (Corner 1972; Klofac and Krisai 2006). However, C. citrinoaurantius has a velvety, tomentose, light yellow, lemon yellow, light orange, and grayish yellow to grayish orange pileus and large basidiospores measuring 9.5–12.5 × 3.5–4 μm (Zhang et al. 2017); C. hainanensis has a grayish yellow to olive-yellow or grayish orange to brownish orange pileus and large (8–10.5 × 4–5 μm) basidiospores, and context initially changes to blue and then to grayish orange, brownish orange, or grayish red when bruised (Zhang et al. 2017); C. radiatus has grayish yellow, grayish orange, grayish brown to brownish orange, radially arranged pores, a yellow context, and large basidiospores measuring 7–8 × 3.5–4 μm (Zhang et al. 2016; Chai et al. 2019); C. rubinelloides has a large, light orange, orange, orange-red to brownish orange pileus and distinctively large basidiospores measuring 11.5–15 × 4–5.5 μm (Wu et al. 2016); C. vulparius has a reddish brown to pale reddish brown pileus, a white basal mycelium, and slightly large basidiospores measuring 5.5–9 × 3–5 μm (see below); C. cervinococcineus has a large (5–9 cm) pileus, a yellow stipe with orange-red apex, and large basidiospores measuring (11–)13–16.5 (–18) × 5–6.3 (–6.7) μm (Corner 1972; Klofac and Krisai 2006).

Phylogenetically, C. sinensis is closely related to previously described species, viz. C. africanaus Degreef & De Kesel and C. hainanensis (Fig. 1). Chalciporus africanaus, native to Africa, has a dull red pileus and large (7.5–9.9 × 3.4–4.2 μm) basidiospores (Degreelf and Kesel 2008). The morphological differences between C. sinensis and C. hainanensis have been discussed above.

Chalciporus vulparius N.K. Zeng, Chang Xu & Zhi Q. Liang, sp. nov.

Figures 2d–f and 4
Mycobank: MB839318.
**Etymology:** Latin “*vulparius*” meaning that the pileal surface is reddish brown.

**Holotype:** CHINA, Hainan Province: Bawangling, Hainan Tropical Rainforest National Park, elev. 650 m, 3 September 2020, N.K. Zeng4979 (FHMU5560).

**Basidiomata** very small. **Pileus** 1.5–2 cm in diameter, sub-hemispherical, then convex to applanate, margin curved downward, occasionally upward; surface dry, tomentose, reddish brown (7B5) to pale reddish brown (8B4); context approximately 0.4 cm thick in the center of the pileus, yellowish white (1A2), unchanging in color when bruised. **Hymenophore** poroid, slightly decurrent; pores subrounded, pink (8A3), unchanging in color when bruised; tubes approximately 0.2 cm long, pink (8B4–8B6), unchanging in color when bruised. **Stipe** 1.5–2 × 0.25–0.4 cm, central, solid, subcylindrical; surface dry and densely covered with squamules, squamules white (1A1) or pale brown to brown (3B2–3B3); context yellowish brown (1B3), unchanging in color when bruised; basal mycelium white. **Odor** indistinct.

**Basidia** 22–31 × 10–14 μm, clavate, thin- to slightly thick-walled (up to 0.5 μm), four-spored, hyaline in KOH; sterigmata 1.5–3 μm long. **Basidiospores** [40/2/2] 5.5–9 × 3–5 μm, $Q = (1.38–)1.50–2.29 (–2.57)$, $Q_m = 1.94 ± 0.24$, subfusiform to ellipsoid, slightly thick-walled (up to 0.7 μm), smooth, pale yellowish brown in KOH. **Sublamellate hymenophore** composed of hyphae 12–30 × 3–5 μm in diameter, thin-walled, hyaline to pale yellow in KOH. **Hymenophoral trama** boletoid, composed of thin- to slightly thick-walled (up to 0.4 μm) hyphae, 5–9 μm in diameter, hyaline to yellowish in KOH. **Cheilocystidia** 32–63 × 9–15 μm, abundant, fusiform or subfusiform, thin- to slightly thick-walled (up to 0.5 μm),

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Fig. 4 Microscopic features of *Chalciporus vulparius* (FHMU5560, holotype). a Basidiospores. b Basidia. c Cheilocystidia. d Pleurocystidia. e Pileipellis. f Stipitipellis. Bars = 10 μm. Drawings by C. Xu
hyaline in KOH. Pleurocystidia 45–72×10–13 μm, abundant, fusiform or subfusiform, thin- to slightly thick-walled (up to 0.6 μm), hyaline in KOH. Pileipellis a trichodermium 390–500 μm in thickness, composed of hyphae 6–11 μm in diameter, bright lemon yellow in KOH, thin- to slightly thick-walled (up to 0.5 μm); terminal cells 23–52×7–10 μm, cylindrical, subcylindrical or narrow clavate, with obtuse apex. Pileal trama made up of hyphae 4–9 μm in diameter, thin- to slightly thick-walled (up to 0.5 μm), yellowish in KOH. Stipitipellis a 90–200 μm thick trichoderm, composed of thin- to slightly thick-walled (up to 0.5 μm) hyphae 3.5–7.5 μm in diameter, light yellow in KOH; terminal cells 21.5–31×3–7 μm, clavate or subcylindrical, occasionally sub fusiform. Stipe trama composed of parallel hyphae 3–10 μm in diameter, thin- to slightly thick-walled (up to 0.5 μm), hyaline to light yellow in KOH. Clamp connections absent in all tissues.

**Habitat:** Scattered on the ground in forests dominated by fagaceous trees.

**Known distribution:** Southern China (Hainan Province).

**Additional specimen examined:** CHINA. Hainan Province: Bawangling, Hainan Tropical Rainforest National Park, elev. 650 m, 3 September 2020, N.K. Zeng 4978 (FHMU5554).

**Notes:** Chalciporus vulparius is well characterized by a reddish brown pileus, a brown stipe with a white basal mycelium, a context unchanging in color when injured, and relatively small basidiospores. Morphologically, C. vulparius is similar to C. cervinococcineus, C. citrinoaurantius, C. hainanensis, and C. piperatus. However, C. cervinococcineus has a large (5–9 cm) pileus, a yellow stipe with orange-red apex, and large basidiospores measuring (11–)13–16.5 (–18)×5–6.3 (–6.7) μm (Corner 1972; Klofac and Krisai-Greilhuber 2006); C. citrinoaurantius has a light yellow, lemon yellow, light orange, grayish yellow to grayish orange pileus and large basidiospores measuring (9–)9.5–12.5(–13)×3–3.5–4(–4.5) μm (Zhang et al. 2017); C. hainanensis has a grayish yellow, olive yellow, grayish orange to brownish orange pileus and large basidiospores measuring 8–10.5(–11)×4–5(–5.5) μm, and a context turns reddish brown when injured (Zhang et al. 2017); C. piperatus has a distinctly large pileus and large basidiospores measuring 7–12(–13)×3.2–5 μm, and it is distributed in Europe (Moser 1983).

Phylogenetically, C. vulparius is closely related to C. africanaus and C. hainanensis and C. sinensis (Fig. 1). However, C. africanaus has a reddish brown, grayish orange, cinnamon to cognac-brown stipe, a yellow basal mycelium, and a large value of Qm, and it is distributed in Africa (Degreve and Kesel 2008). The morphological differences between C. vulparius and the two Chinese taxa, viz. C. sinensis and C. hainanensis, were discussed above.

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### Key to the species of Chalciporus from China

1a. Pores radially arranged when young, seemingly sublumellate at maturity………………………………………C. radiatus
1b. Pores not radially arranged………………………………………2
2a. Pileal context initially changes to blue and then to grayish orange, brownish orange, or grayish red when bruised… ………………………………………………………………C. hainanensis
2b. Pileal context does not change color when injured…………………………………………………………………………………3
3a. Basidiomata large (pileus up to 7 cm), length of basidiospores up to 16 μm………………………………………C. rubinelloides
3b. Basidiomata small (pileus up to 5 cm), length of basidiospores up to 13 μm………………………………………4
4a. Pileal surface black brown………………………………………C. sinensis
4b. Pileal surface light yellow, grayish orange to reddish brown……………………………………………………………5
5a. Length of basidiospores (9–)9.5–12.5(–13) μm…………………5b. Length of basidiospores 5.5–9 μm…………………C. vulgaris

### Discussion

Molecular data indicate that the newly collected specimens were nested into the genus *Chalciporus* with high statistical support (Fig. 1), and morphological features of the two new species, viz. *C. sinensis* and *C. vulparius*, are consistent with those of *Chalciporus*.

The present study showed high species diversity in China and identified eight lineages of *Chalciporus* (Fig. 1). Lineages 7 and 8 (C. vulgaris and C. sinensis) are new; lineages 1, 2, 3, and 5 (C. citrinoaurantius, C. rubinelloides, C. radiatus, and C. hainanensis) were described previously, and lineages 4 and 6 were not described because of the paucity of material. *Chalciporus piperatus* was described previously (Li and Song, 2002); however, its occurrence in China needs to be confirmed.

The present data indicate that the species of *Chalciporus* in China are distributed in subtropical and tropical areas, and no species of this genus were identified in temperate regions of the country (Fig. 1). With more field investigations, more tropical *Chalciporus* species are expected to be discovered in China. Moreover, we noted that the affinities of *Chalciporus* species from East Asia and southeast Asia are evident (Fig. 1), and both regions shared one common taxon (lineage 5, Fig. 1). Similar scenarios have been documented for many other boletes (Zeng et al. 2013, 2018; Wu et al. 2019).

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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.

References

Antonio C (2003) The complete mushroom book. Quadrille, London, p 34
Baroni TJ, Both EE (1991) Chalciporus piperatoides in North America. Mycologia 83:559–564. https://doi.org/10.2307/3760211
Bas C (1969) Morphology and subdivision of Amanita and a monograph of its section Lepiella. Persoonia 5:285–579
Chai H, Liang QZ, Xue R, Jiang S, Luo SH, Wang Y, Wu LL, Tang LP, Chen Y, Hong D, Zeng NK (2019) New and noteworthy boletes from subtropical and tropical China. MycoKeys 46:55–96. https://doi.org/10.3897/mycokeys.46.31470
Corner EJH (1972) Boletus in Malaysia. Botanic Gardens, Singapore, pp 263
Degweer J, Kesel AD (2008) Chalciporus africusus, a new bolete species from Africa. Mycota 10:329–335
Deng CY, Kang C, Li Q, Xia YP (2018) Research on macrofungi in Caohai I: species diversity. Guizhou Sci 36(1):5–15
Desjardin DE, Binder M, Roekring S, Flegel T (2009) Spongiforma, a new genus of gasteroid boletes from Thailand. Fungal Divers 37:1–8. https://doi.org/10.3852/10-433
Dickie IA, Bolstridge N, Cooper JA, Peltzer DA (2010) Co-invasion by Pinus and its mycorrhizal fungi. New Phytol 187:475–484. https://doi.org/10.1111/j.1469-8137.2010.03277.x
Dickie IA, Nuñez MA, Pringle A, Lebel T, Tourtellot SG, Johnston PR (2016) Towards management of invasive ectomycorrhizal fungi. Biol Invasions 18:3383–3395. https://doi.org/10.1007/s10530-016-1243-x
Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340
Gómez LD (1996) Basidiomycetes de Costa Rica: Xerocomus, Chalciporus, Pulveroboletus, Boletellus, Xanthoconium (Agaricales: Boletaceae). Rev Biol Trop 44(Suppl 4):59–89
Halling RE, Mata M, Mueller GM (2004) Three new boletes for Costa Rica. Mem N Y Bot Gard 89:141–147
Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analyses program for Windows 95/98/NT.
Nucleic Acids Symp Ser 41:95–98. https://doi.org/10.1016/S1468-1614(01)60416-1
Huelsenbeck JP, Ronquist F (2005) Bayesian analysis of molecular evolution using MrBayes. In: Nielsen R (ed) Statistical methods in molecular evolution. Springer, New York, pp 183–226. https://doi.org/10.1007/0-387-27733-1_7
James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox C, Celio G, Gueidan C, Fraker E, Smilack J, Joy JC, Hosaka K, Sun GH, Johnson D, O’Rourke B, Crockett M, Binder M, Rauhut A, Reeb V, Arnold AE, Amao A, Stajich JE, Hosaka K, Sun GH, Johnson D, O’Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüller A, Longcore JE, O’Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmyer J, Vollmann Kohlmyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett D, Lutzoni F, McLaughlin D, Spatafora J, Vilgalys R (2006) Reconstructing the early evolution of the fungi using a six gene phylogeny. Nature 443:818–822. https://doi.org/10.1038/nature05110
Klofac W, Krisai-Greilhuber I (2006) The genus Chalciporus, a world-wide survey. Öst Z Pilzk 15:31–65
Klofac W, Krisai-Greilhuber I (2020) Chalciporus pseudopiperatus, a new taxon in the Chalciporus piperatus species complex. Öst Z Pilzk 28:75–78
Kornerup A, Wanscher JH (1981) Taschenlexikon der Farben, 3rd edn. Muster-Schmidt Verlag, Göttingen, p 242
Li TH, Song B (2002) Species and distributions of Chinese edible boletes. Acta Edulis Fungi 9(2):22–30. https://doi.org/10.16488/j.cnki.1005-9873.2002.02.006
Moreno G, García-Bona LM (1976) Macromycetes interesantes de Navarra. I. Anal Inst Bot Cavanilles 33:125–133
Moser M (1983) Keys to agarics and boletes (Polyporales, Boletales, Agaricales, Russulales). Roger Phillips, London, pp 535
Nuhn ME, Binder M, Taylor AFS, Halling RE, Hibbett DS (2016) Phylogenetic overview of the Boletinae. Fungal Biol 117:1–33. https://doi.org/10.1016/j.funbio.2013.04.008
Nylander JAA (2004) MrModeltest 2.3, program distributed by the author. Evolutionary Biology Center, Uppsala University
Pegler DN (1981) A natural arrangement of the boleteles, with reference to spore morphology. Trans Br Mycol Soc 76:103–146
Pegler DN (1983) Agaric flora of the Lesser Antilles. Additional Series IX. Kew Bulletin, London, pp 668
Raspé O, Vadhanarat S, Kesel AD, Hyde KD, Lumyong S (2016) Pulveroboletus fragrans, a new Boletaceae species from northern Thailand, with a remarkable aromatic odor. Mycol Prog 15:38. https://doi.org/10.1007/s11557-016-1179-7
Roberts P, Evans S (2011) The book of fungi. University of Chicago Press, Chicago, p 343
Smith SA, Dunn CW (2008) Phyutility: a phyloinformatics tool for trees, alignments and molecular data. Bioinformation 24:715–716. https://doi.org/10.1039/biointformatics/016-btum19
Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690. https://doi.org/10.1093/bioinformatics/btl446
Vadhanarat S, Amalfi M, Halling RE, Bandula V, Lumyong S, Raspe O (2019) Two new Erytrophyllophorus species (Boletaceae) from Thailand, with two new combinations of American species. MycoKeys 55:29–57. https://doi.org/10.3897/mycokeys.55.34570
Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.28.4238-4246.1990
White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322. https://doi.org/10.1016/B978-0-12-372180-850042-1

Winner M, Giménez A, Schmidt H, Sontag B, Stefan B, Steglich W (2004) Unusual pulvinic acid dimers from the common fungi Scleroderma citrinum (common earthball) and Chalciporus piperatus (Peppery Bolete). Angew Chemie 43:1883–1886. https://doi.org/10.1002/anie.200352529

Wu G, Feng B, Xu JP, Zhu XT, Li YC, Zeng NK, Hosen MI, Yang ZL (2014) Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. Fungal Divers 69:93–115. https://doi.org/10.1007/s13225-014-0283-8

Wu G, Li YC, Zhu XT, Zhao K, Han LH, Cui YY, Li F, Xu JP, Yang ZL (2016) One hundred noteworthy boletes from China. Fungal Divers 81:25–188. https://doi.org/10.1007/s13225-016-0375-8

Wu LL, Liang ZQ, Xue R, Fan YG, Jiang S, Fu YQ, Zeng NK, Su MS (2019) The genus Crocinoboletus (Boletaceae Boletales): a new species and updated information for previously described species. Phytotaxa 419:91–99. https://doi.org/10.11646/phytotaxa.419.1.6

Xie HJ, Zhang CX, He MX, Liang ZQ, Deng XH, Zeng NK (2021) Buchwaldoboletus xylophilus and Phlebopus portentosus, two non-ectomycorrhizal boletes from tropical China. Phytotaxa 520:137–154. https://doi.org/10.11646/phytotaxa.520.2.2

Zeng NK, Tang LP, Li YC, Tolgor B, Zhu XT, Zhao Q, Yang ZL (2013) The genus Phylloporus (Boletaceae, Boletales) from China: morphological and multilocus DNA sequence analyses. Fungal Divers 58:73–101. https://doi.org/10.1007/s13225-012-0184-7

Zeng NK, Chai H, Jiang S, Xue R, Wang Y, Hong D, Liang ZQ (2018) Retiboletus nigrogriseus and Tengioboletus fujianensis two new boletes from the south of China. Phytotaxa 367:45–54. https://doi.org/10.11646/phytotaxa.367.1.5

Zhang M, Wang CQ, Li TH, Song B (2016) A new species of Chalciporus (Boletaceae, Boletales) with strongly radially arranged pores. Mycoscience 57:20–25. https://doi.org/10.1016/j.myc.2015.07.004

Zhang M, LI TH, Song B (2017) Two new species of Chalciporus (Boletaceae) from southern China revealed by morphological characters and molecular data. Phytotaxa 327:47–56. https://doi.org/10.11646/phytotaxa.327.1.2

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