

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

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## Research Article

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# Abstract

Two new species of *Chalciporus* (Boletaceae, Boletales), viz. *C. nigrofuscus* 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* in China is provided.

## Introduction

*Chalciporus* Bataille (Boletaceae, Boletales), typified by *C. piperatus* (Bull.) Bataille, was erected to accommodate species with peppery taste, reddish hymenophore, no-ornamental stipe, and smooth basidiospores (Moreno and García-Bona 1976; Pegler 1981, 1983; Baroni and Both 1991; Gómez 1996; Klofac and Krisai 2006; Halling et al. 2004). It is an early branching lineage in the Boletaceae (Nuhn et al. 2013; Wu et al. 2014). Among species of *Chalciporus*, the well-known *C. piperatus* was thought to be an ectomycorrhizal symbiont, nowadays it is believed to be a mycoparasite, colonizing *Amanita muscaria* (L.) Lam. ectomycorrhizae (Dickie et al. 2010, 2016).

Although *Chalciporus* is a small genus, the species of this genus are distributed worldwide (Desjardin et al. 2009; Nuhn et al. 2013; Raspé et al. 2016; Zhang et al. 2015, 2017; Wu et al. 2016; Vadthanarat et al. 2019; Chai et al. 2019; Klofac and Krisai-Greilhuber 2020). People paid much attention to the species of the genus, for example, some interesting metabolites including sclerocitrin (the major pigment of the bright yellow stalk base and the mycelium), variegatic acid, variegatorubin, chalcitrin, and the unusual 2H-azepine derivative chalciporone (responsible for the pungent taste of the fruit bodies) have been isolated from the peppery bolete (*C. piperatus*) (Winner et al. 2004). Furthermore, collections identified as *C. piperatus* in China are believed to be edible (Li and Song 2002), and can be used as a condiment in many countries (Antonio 2003). In addition, fruit body of *C. piperatus* can also be used for dyeing (Roberts and Evans 2011).

In China, five species of the genus, viz. *C. citrinoaurantius* 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 were described/reported in previous studies (Zhang et al. 2015, 2017; Chai et al. 2019; Wu et al. 2016; Deng et al. 2018). Herein, we describe another two new species of *Chalciporus* from Hainan Province, an important area of tropical China, based on morphological and molecular phylogenetic analyses, aiming to further demonstrate the diversity of Boletaceae in China.

## Materials And Methods

### Morphological studies

The specimens were described and photographed in the field. Then, dry specimens were deposited at the Fungal Herbarium of Hainan Medical University (FHMU), Haikou City, Hainan Province, China. Color codes are from Kornerup and Wanscher (1981). Sections of the pileipellis were cut perpendicularly and halfway between the center and margin of the pileus. Sections of the stipitipellis were taken from the middle part along the longitudinal axis of the stipe. KOH (5%) was used as

the mounting medium for the microscopic studies. All microscopic structures were drawn freehand from materials rehydrated in 5% KOH. The number of measured basidiospores is given as  $n/m/p$ , indicating that the measurements were taken on  $n$  basidiospores from  $m$  basidiomata of  $p$  collections. The basidiospore dimensions are given as  $(a)b-c(d)$ , where the range  $b-c$  represents a minimum of 90% of the

measured value (5th to 95th percentile), and extreme values ( $a$  and  $d$ ) ( $a < 5$ th percentile,  $d > 95$ th percentile) are in parentheses.  $Q$  refers to the length/width ratio of the basidiospores;  $Q_m$  refers to the average  $Q$  of the basidiospores and is given

with a sample standard deviation.

## Molecular procedures

Total genomic DNA was obtained with Plant Genomic DNA Kit (KANGWEI Company, China) from materials dried with silica gel according to the manufacturer's instructions. The primer pairs used for amplification were the nuc 28S rDNA D1-D2 domains (28S) with LR0R/LR5 (Vilgalys and Hester 1990; James et al. 2006), the nuc rDNA region encompassing the internal transcribed spacers 1 and 2, along with the 5.8S rDNA (ITS) with ITS5/ITS4 (White et al. 1990), the translation elongation factor 1- $\alpha$  gene (*TEF1*) with EF1-2F/EF1-2R (Zeng et al. 2013), and the gene for RNA polymerase II second largest (*RPB2*) with RPB2-B-R/RPB2-B-F1 (Wu et al. 2014). The PCR analysis was performed in a total volume of 25  $\mu$ L containing 13  $\mu$ L 2  $\times$  Taq PCR MasterMix (KANGWEI Company, China), 2  $\mu$ L of each primer (10  $\mu$ M), 8  $\mu$ L of nuclease-free water, and 2  $\mu$ L of DNA template. The PCR reactions were performed with 4 min initial denaturation at 95  $^{\circ}$ C, followed by 34 cycles of denaturation at 94  $^{\circ}$ C for 30 s, annealing at appropriate temperature (50  $^{\circ}$ C for 28S and ITS, 53  $^{\circ}$ C for *TEF1*, 52  $^{\circ}$ C for *RPB2*) for 30 s, extension at 72  $^{\circ}$ C for 120 s, and a final extension at 72  $^{\circ}$ C for 7 min. PCR products were checked in 1% (w/v) agarose gels, and positive reactions with a bright single band were purified and directly sequenced using an ABI 3730xl DNA Analyzer (Guangzhou Branch of BGI, China) with the same primers used for PCR amplifications. Forward or reverse sequences were compiled with BioEdit (Hall 1999) and deposited in GenBank (Table 1).

## Dataset assembly

Twenty sequences (six of 28S, four of ITS, six of *TEF1*, and four of *RPB2*) from six collections were newly generated. The 28S, ITS, *TEF1* and *RPB2* sequences of the concatenated multilocus dataset were aligned with selected sequences from GenBank and previous studies (Table 1). *Paxillus vernalis* Watling and *P. obscurusporus* C. Hahn were chosen as outgroup based on the phylogeny in Wu et al. (2016). Single-gene phylogenetic trees based on the 28S, ITS, *TEF1*, *RPB2* fragments were analyzed to test for phylogenetic conflict. The topologies of the phylogenetic trees based on a single gene were almost identical, indicating that the phylogenetic signals present in the different gene fragments were not in conflict. Then, the sequences of the different genes were aligned using MUSCLE (Edgar 2004). The sequences of the different genes were concatenated using Phyutility v2.2 for further analyses (Smith and Dunn 2008).

## Phylogenetic analyses

The combined nuclear dataset (28S + ITS + *TEF1* + *RPB2*) was analyzed using maximum likelihood (ML) and Bayesian Inference (BI) methods. The ML tree was generated and bootstrap analyses were performed with the RAxML 7.2.6 program (Stamatakis 2006) by running 1000 replicates combined with an ML search. The Bayesian analysis was performed with MrBayes 3.1 (Huelsenbeck and Ronquist 2005) implementing the Markov Chain Monte Carlo technique and parameters predetermined with MrModeltest 2.3 (Nylander 2004). The evolution model used in the Bayesian analysis was determined with MrModeltest 2.3 (Nylander 2004). For the combined dataset, GTR+I+G, GTR+G, GTR+I+G, SYM+G were chosen as the best-fit likelihood models of 28S, ITS, *TEF1* and *RPB2*, respectively. The Bayesian analyses were run with one cold and three heated chains and sampled every 100 generations; trees sampled from the first 25% of generations were discarded as burn-in; the average standard deviation of the split frequencies was restricted to below 0.01, and Bayesian posterior probabilities (PP) were calculated for the majority consensus tree of the retained Bayesian trees.

## Results

The combined *Chalciporus* dataset (28S + ITS + *TEF1* + *RPB2*) consisted of 58 taxa and 2893 nucleotide sites, and the alignment was deposited in TreeBASE (S28091). The topologies of the phylogenetic trees based on the combined dataset generated from ML and BI analyses were almost identical, but statistical support showed slight differences. The phylogram with branch lengths generated from RAxML, including the support values, is shown in Fig. 1.

The present molecular data indicate that the Chinese collections of *Chalciporus* were grouped into eight independent lineages (1, 2, 3, 4, 5, 6, 7 and 8) (Fig. 1). Lineage 1, with strong statistical support (BS = 100%, PP = 1.0), included one collection (GDGM46161) and the holotype (GDGM44464) of *C. hainanensis* and one specimen labeled as *C. sp.* (FHMU4573), all from southern China; lineage 2 included one specimen named as *C. sp.* from southern China; lineage 3, with high statistical support (BS = 100%, PP = 1.0), included two collections (FHMU5560 and FHMU5554), both from southern China; lineage 4, with high statistical support (BS = 100%, PP = 1.0), also included two materials (FHMU4701 and FHMU4691), both from southern China; lineage 5, with high statistical support (BS = 100%, PP = 1.0), included one specimen (GDGM50080) from central China, two collections (GDGM43305 and FHMU2494) from southern China and two specimens (FHMU930 and FHMU959) from southeastern China and the holotype (GDGM43285) of *C. radiatus* from central China; lineage 6, one collection numbered HKAS53400 from central China; lineage 7, with high statistical support (BS = 100%, PP = 1.0), included three materials (GDGM44480, GDGM44717 and GDGM44481) from central China and the holotype (GDGM44776) of *C. citrinoaurantius* also from central China; lineage 8, two collections numbered as HKAS57362 and HKAS58728 from southwestern China, one specimen labeled as *C. sp.* (FHMU2721) from southwestern China, and the holotype (HKAS74952) of *C. rubinelloides* grouped together with strong statistical supported (BS = 97%, PP = 1.0).

## Taxonomy

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

Figures 2 a and c and 3

MycoBank: MB839317

*Etymology.* Latin “*nigrofuscus*” meaning the pileal surface of the the new species colored with black brown.

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

**Basidiomata** small. **Pileus** 1.5–3.2 cm diam, subhemispherical, then convex to applanate, margin usually upwards; surface dry, covered with brown (2D8), dark brown (2D5) to black brown (2E5) scales; context about 0.4 cm in thick in the center of the pileus, white (1A1), unchanging in color when bruised.

**Hymenophore** poroid, nearly adnate to slightly depressed around apex of stipe; pores subround to angular, 0.5–1 mm diam, reddish orange or light orange (4A6–4A8), unchanging in color when bruised; tubes about 0.3 cm in length, 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, dark brown (1C4), brown (2B3) to yellowish brown (2B6) at other parts; 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), 4-spored, yellowish to hyaline in KOH; sterigmata 1.5–3 µm in length. **Basidiospores** [40/2/2] (3.5–)4–7(–7.5) × (3–)3.5–5(–6) µm,  $Q = (1–)1.11–1.57 (–1.75)$ ,  $Q_m = 1.39 \pm 0.17$ , subglobose to ellipsoid, slightly thick-walled (up to 0.7 µm), smooth, pale yellowish brown in KOH. **Hymenophoral trama** boletoid, composed of thin- to slightly thick-walled (up to 0.4 µm) hyphae, 4–11 µm wide, yellowish to hyaline 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 300–620 µm in thickness, composed of hyphae 4.5–11 µm in diam, bright lemon yellow in KOH, occasionally branch, 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** made up of hyphae 2–8 µm in diam, slightly thick-walled (up to 0.5 µm), pale yellow in KOH. **Stipitipellis** a trichoderm-like structure 190–500 µm in thickness, composed of thin- to slightly thick-walled (up to 0.5 µm) hyphae 2.5–7 µm in diam, 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 wide, cylindrical, thin- to slightly thick-walled (up to 0.5 µm), hyaline to light yellow in KOH. **Clamp connections** absent in all tissues.

*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).

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

Figures 2 e–f and 4

MycoBank: MB839318

*Etymology*: Latin “*vulparius*” meaning the pileal surface colored with reddish brown .

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

**Basidiomata** small. **Pileus** 1.5–2 cm diam, subhemispherical, then convex to applanate, margin decurved, occasionally upwards; surface dry, tomentose, reddish brown (7B5) to pale reddish brown (8B4); context about 0.4 cm in thick in the center of the pileus, yellowish white (1A2), unchanging in color when bruised.

**Hymenophore** poroid, slightly decurrent; pores subround, reddish pink (8A3), unchanging in color when bruised; tubes about 0.2 cm in length, pale reddish pink (8B4–8B6), unchanging in color when bruised. **Stipe** 1.5–2 × 0.25–0.4 cm, central, solid, subcylindrical; surface dry, densely covered with squamules, squamules white (1A1) at upper part, pale brown to brown (3B2–3B3) at other parts; 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), 4-spored, hyaline in KOH; sterigmata 1.5–3 µm in length. **Basidiospores** [40/2/2] 5.5–9 × 3–5 µm,  $Q = (1.38- )1.50-2.29 (-2.57)$ ,  $Q_m = 1.94 \pm 0.24$ , subfusiform to ellipsoid, slightly thick-walled (up to 0.7 µm), smooth, pale yellowish brown in KOH. **Hymenophoral trama** boletoid, composed of thin- to slightly thick-walled (up to 0.4 µm) hyphae, 5–9 µm wide, yellowish to hyaline in KOH. **Cheilocystidia** 32–63 × 9–15 µm, abundant, fusiform or subfusiform, thin- to slightly thick-walled (up to 0.5 µm), 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 390–900 µm in thickness, composed of hyphae 6–11 µm in diam, 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 diam, thin- to slightly thick-walled (up to 0.5 µm), yellowish in KOH. **Stipitipellis** a trichoderm-like structure 90–200 µm in thickness, composed of thin- to slightly thick-walled (up to 0.5 µm) hyphae 3.5–7.5 µm in diam, light yellow in KOH; terminal cells 21.5–31 × 3–7 µm, clavate or subcylindrical, occasionally subfusiform. **Stipe trama** composed of parallel hyphae 3–10 µm wide, cylindrical, 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. Zeng4978* (FHMU5554).

## Discussion

Molecular data indicate our newly collected specimens were placed into the genus *Chalciporus* with high statistical support (Fig. 1), and morphological features of the two new species, viz. *C. nigrofuscus* and *C. vulparius*, also match well with those of *Chalciporus*.

*Chalciporus nigrofuscus* 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, brown to yellowish brown squamules, a yellow basal mycelium, and smaller basidiospores. Morphologically, *C. nigrofuscus* is somewhat similar to *C. citrinoaurantius*, *C. hainanensis*, *C. radiatus*, *C. rubinelloides*, *C. vulparius*, all originally described from China (Zhang et al. 2015, 2017; Wu et al. 2016). However, *C. citrinoaurantius* has a velvety tomentose, light yellow, lemon yellow, light orange, grayish yellow to grayish orange pileus, larger basidiospores measuring 9.5–12.5 × 3.5–4 µm (Zhang et al. 2017); *C.*

*hainanensis* has a grayish yellow, olive yellow, grayish orange to brownish orange pileus, a context changing blue at first, then gradually changing to grayish orange to brownish orange or grayish red when bruised, and larger basidiospores measuring 8–10.5 × 4–5 µm (Zhang et al. 2017); *C. radiatus* has a grayish yellow, grayish orange, grayish brown to brownish orange, a radially arranged pores, a yellow context, and larger basidiospores measuring 7–8 × 3.5–4 µm (Zhang et al. 2015; Chai et al. 2019); *C. rubinelloides* has a larger, light orange, orange, orange-red to brownish orange pileus, and distinctively larger 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 larger basidiospores measuring 5.5–9 × 3–5 µm. Phylogenetically, *C. nigrofuscus* is closely related to previous described taxa, viz. *C. africanus* Degreef & De Kesel and *C. hainanensis* (Fig. 1). *Chalciporus africanus*, a species originally described from Africa, has a dull red pileus, and larger basidiospores measuring 7.5–9.9 × 3.4–4.2 µm (Degreef & Kesel 2008). The morphological differences between *C. nigrofuscus* and *C. hainanensis* have been discussed above.

*Chalciporus vulparius* is well characterized by its reddish brown pileus, a brown stipe with a white basal mycelium, a context unchanging in color when injured, and relatively smaller basidiospores. Morphologically, *C. vulparius* is similar to *C. citrinoaurantius*, *C. hainanensis*, *C. piperatus*. However, *C. citrinoaurantius* has a pileus colored with light yellow, lemon yellow, light orange, grayish yellow to grayish orange, and larger basidiospores measuring (9–)9.5–12.5(–13) × (3–)3.5–4(–4.5) µm (Zhang et al. 2017); *C. hainanensis* has a pileus colored with grayish yellow, olive yellow, grayish orange to brownish orange, a context turning reddish brown when injured, and slightly larger basidiospores measuring 8–10.5(–11) × 4–5(–5.5) µm (Zhang et al. 2017); *C. piperatus* has a distinctly larger pileus, and larger basidiospores measuring 7–12(–13) × 3.2–5 µm, and a distribution in Europe (Moser 1983). Phylogenetically, *C. vulparius* is closely related to previous *C. africanus* and *C. hainanensis*, and the new species *C. nigrofuscus* (Fig. 1). However, *C. africanus* has a reddish brown, grayish orange, cinnamon to cognac brown stipe, a yellow basal mycelium, a larger value of  $Q_m$ , and a distribution in Africa (Degreef & Kesel 2008). As to the morphological differences between *C. vulparius* and the two Chinese taxa, viz. *C. nigrofuscus* and *C. hainanensis*, they have been discussed above.

In the present study, unexpected abundant species diversity was revealed in China, and eight lineages of *Chalciporus* were uncovered (Fig. 1). Two (lineages 3 and 4) are described as new: *C. vulparius* and *C. nigrofuscus*; four (lineages 1, 5, 7 and 8) are previously described taxa: *C. citrinoaurantius*, *C. hainanensis*, *C. radiatus* and *C. rubinelloides*; two (lineages 2 and 6) were not described due to the paucity of material. Besides the aforementioned species, there is one additional taxon reported from China, viz. *C. piperatus* (Li & Song, 2002), yet its occurrence in the country remains to be confirmed.

## Key To The Species Of *Chalciporus* Known From China

1. Pores strongly radially arranged.....*C. radiatus*
1. Pores not radially arranged.....2

2. Pileal context changing blue at first, then gradually changing to grayish orange to brownish orange or grayish red when bruised.....*C. hainanensis*
2. Pileal context unchanging in color when injured.....3
3. Basidioma larger (pileus up to 7 cm), basidiospores longer (up to 16  $\mu\text{m}$ )..... *C. rubinelloides*
3. Basidioma smaller (pileus up to 5 cm), basidiospores shorter (up to 13  $\mu\text{m}$ ).....4
4. Pileal surface black brown.....*C. nigrofuscus*
4. Pileal surface light yellow, grayish orange to reddish brown.....5
5. Basidiospores larger [(9–)9.5–12.5(–13)  $\times$  (3–)3.5–4(–4.5)  $\mu\text{m}$ ],  $Q_m = 2.96$ .....*C. citrinoaurantius*
5. Basidiospores smaller (5.5–9  $\times$  3–5  $\mu\text{m}$ ),  $Q_m = 1.94$ .....*C. vulparius*

## Declarations

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**Authors' contributions** Conceptualization: Zhi-Qun Liang and Nian-Kai Zeng; Methodology: Chang Xu and Hui-Jing Xie; Performing the experiment: Chang Xu and Hui-Jing Xie; Formal analysis: Chang Xu and Zhi-Qun Liang; Resources: Nian-Kai Zeng, Shuai Jiang, Xin-Hua Fu; Writing—original draft preparation: Chang Xu; Writing—review and editing: Zhi-Qun Liang and Nian-Kai Zeng; Supervision: Nian-Kai Zeng; Project administration: Nian-Kai Zeng; Funding acquisition: Nian-Kai Zeng. 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.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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## Tables

**Table.1** Taxa, vouchers, locations and GenBank accession numbers of the DNA sequences used in the study

| Taxon                               | Voucher                  | Locality               | GenBank accessions no. |                 |                 |                 | References                |
|-------------------------------------|--------------------------|------------------------|------------------------|-----------------|-----------------|-----------------|---------------------------|
|                                     |                          |                        | 28S                    | ITS             | TEF1            | RPB2            |                           |
| <i>Buchwaldoboletus lignicola</i>   | KA14-0907                | South Korea            | —                      | MH170897        | —               | —               | Jo et al. (2019)          |
| <i>Buchwaldoboletus lignicola</i>   | KA14-0711                | South Korea            | —                      | MH170896        | —               | —               | Jo et al. (2019)          |
| <i>Buchwaldoboletus lignicola</i>   | —                        | Italy                  | —                      | HM003619        | —               | —               | Unpublished               |
| <i>Buchwaldoboletus lignicola</i>   | —                        | Sweden                 | —                      | HM003618        | —               | —               | Unpublished               |
| <i>Buchwaldoboletus lignicola</i>   | —                        | United Kingdom         | —                      | HM003617        | —               | —               | Unpublished               |
| <i>Buchwaldoboletus lignicola</i>   | HKAS:76674               | Heilongjiang, China    | NE KF112350            | —               | KF112277        | KF112819        | Wu et al. (2014)          |
| <i>Buchwaldoboletus lignicola</i>   | VDKO1140                 | Belgium                | —                      | —               | MH614710        | MH614756        | Vadthananat et al. (2019) |
| <i>Buchwaldoboletus lignicola</i>   | 3533                     | Canada                 | —                      | KM248950        | —               | —               | Jo et al. (2019)          |
| <i>Buchwaldoboletus lignicola</i>   | HKAS84904                | Germany                | KT990538               | —               | KT990740        | KT990377        | Wu et al. (2016)          |
| <i>Buchwaldoboletus lignicola</i>   | Pul1                     | Germany                | JQ326997               | —               | JQ327040        | —               | Nuhn et al. (2013)        |
| <i>Buchwaldoboletus lignicola</i>   | KM157323                 | England                | —                      | GQ981493        | —               | —               | Jo et al. (2019)          |
| <i>Buchwaldoboletus lignicola</i>   | —                        | —                      | —                      | MH234512        | —               | —               | Unpublished               |
| <i>Buchwaldoboletus</i> sp.         | JLF_X22                  | USA                    | —                      | KU144820        | —               | —               | Frank et al. (2020)       |
| <i>Buchwaldoboletus xylophilus</i>  | X.H. Deng1 (FHMU5848)    | Hainan, southern China | MW783425               | —               | MW897338        | MW820947        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | X.H. Deng2 (FHMU5849)    | Hainan, southern China | MW783426               | —               | MW897339        | MW820948        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | FHMU5930                 | Yunnan, SW China       | MW783417               | MW783439        | MW897330        | MW820939        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | FHMU5930-1               | Yunnan, SW China       | MW783418               | MW783440        | MW897331        | MW820940        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | FHMU5931                 | Yunnan, SW China       | MW783419               | MW783441        | MW897332        | MW820941        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | FHMU5931-1               | Yunnan, SW China       | MW783420               | MW783442        | MW897333        | MW820942        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | FHMU5932                 | Yunnan, SW China       | MW783421               | MW783443        | MW897334        | MW820943        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | FHMU5932-1               | Yunnan, SW China       | MW783422               | MW783444        | MW897335        | MW820944        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | FHMU5933                 | Yunnan, SW China       | MW783423               | MW783445        | MW897336        | MW820945        | Xie et al. (2021)         |
| <i>Buchwaldoboletus xylophilus</i>  | FHMU5933-1               | Yunnan, SW China       | MW783424               | MW783446        | MW897337        | MW820946        | Xie et al. (2021)         |
| <i>Chalciporus africanus</i>        | JD0517                   | Cameroon               | —                      | —               | KT824029        | KT823996        | Raspé et al. (2016)       |
| <i>Chalciporus amarellus</i>        | DS4640-3                 | Germany                | KF030283               | —               | KF030440        | —               | Nuhn et al. (2013)        |
| <i>Chalciporus citrinoaurantius</i> | GDGM44480                | Hunan, central China   | —                      | —               | —               | —               | Zhang et al. (2017)       |
| <i>Chalciporus citrinoaurantius</i> | GDGM44481                | Hunan, central China   | —                      | —               | —               | —               | Zhang et al. (2017)       |
| <i>Chalciporus citrinoaurantius</i> | GDGM44717                | Hunan, central China   | —                      | —               | —               | —               | Zhang et al. (2017)       |
| <i>Chalciporus citrinoaurantius</i> | GDGM 44776 e             | Hunan, central China   | —                      | —               | —               | —               | Zhang et al. (2017)       |
| <i>Chalciporus hainanensis</i>      | GDGM46161                | Hainan, southern China | —                      | —               | —               | —               | Zhang et al. (2017)       |
| <i>Chalciporus hainanensis</i>      | GDGM44464e               | Hainan, southern China | —                      | —               | —               | —               | Zhang et al. (2017)       |
| <i>Chalciporus nigrofuscus</i>      | N.K. Zeng4478 (FHMU4701) | Hainan, southern China | <b>MW917171</b>        | <b>MW917178</b> | <b>MW925928</b> | <b>MW925934</b> | This study                |

| Taxon                               | Voucher                       | Locality                          | GenBank accessions no. |                 |                 |                 | References   |
|-------------------------------------|-------------------------------|-----------------------------------|------------------------|-----------------|-----------------|-----------------|--|
|                                     |                               |                                   | 28S                    | ITS             | TEF1            | RPB2            |  |
| <i>Chalciporus nigrofuscus</i>      | N.K. Zeng4479 (FHMU4691)      | Hainan, southern China            | <b>MW917172</b>        | <b>MW917179</b> | <b>MW925929</b> | <b>MW925935</b> | This study   |
| <i>Chalciporus piperatus</i>        | VDKO1063                      | Belgium                           | —                      | —               | MH614713        | MH614759        | Vadthhanarat et al. (2019)                         |
| <i>Chalciporus piperatus</i>        | HKAS84882                     | Germany                           | KT990562               | —               | KT990758        | KT990397        | Wu et al. (2016)                                   |
| <i>Chalciporus pseudorubinellus</i> | 4302                          | USAws, Tulet, NY                  | KF030284               | —               | KF030441        | —               | Nuhn et al. (2013)                                 |
| <i>Chalciporus radiatus</i>         | GDGM43285                     | Hunan, central Chinana            | KP871800               | KP871804        | —               | —               | Zhang et al. (2015)                                |
| <i>Chalciporus radiatus</i>         | GDGM50080                     | Hunan, central Chinana            | KP871801               | KP871806        | —               | —               | Zhang et al. (2015)                                |
| <i>Chalciporus radiatus</i>         | GDGM43305                     | Guangdong, southern Chinarn China | KP871802               | KP871805        | —               | —               | Zhang et al. (2015)                                |
| <i>Chalciporus radiatus</i>         | N.K. Zeng1379 (FHMU930)       | Fujian, SE China                  | MH879710               | MH885367        | MH879738        | —               | Chai et al. (2019)                                 |
| <i>Chalciporus radiatus</i>         | N.K. Zeng1414 (FHMU959)MU959) | Fujian, SE China                  | MH879711               | —               | MH879739        | —               | Chai et al. (2019)                                 |
| <i>Chalciporus radiatus</i>         | N.K. Zeng1808 (FHMU 2494)     | Hainan, southern China            | —                      | —               | MH879737        | —               | Chai et al. (2019)                                 |
| <i>Chalciporus rubinelloides</i>    | HKAS74952                     | Yunnan, SW Chinaaa                | KT990565               | —               | KT990761        | KT990400        | Wu et al. (2016)                                   |
| <i>Chalciporus rubinelloides</i>    | HKAS58728                     | Yunnan, SW Chinaaa                | KT990564               | —               | KT990760        | KT990399        | Wu et al. (2016)                                   |
| <i>Chalciporus rubinelloides</i>    | HKAS57362                     | Yunnan, SW Chinaaa                | KT990563               | —               | KT990759        | KT990398        | Wu et al. (2016)                                   |
| <i>Chalciporus rubinellus</i>       | 191/81                        | USA                               | EU685106               | EU685111        | —               | —               | Desjardin et al. (2009)                            |
| <i>Chalciporus</i> sp.              | X.T. Zhu134 (FHMU2721)        | Yunnan, SW China                  | <b>MW917175</b>        | <b>MW917180</b> | <b>MW925932</b> | —               | This study   |
| <i>Chalciporus</i> sp.              | S. Jiang81 (FHMU4573)         | Hainan, southern China            | <b>MW917176</b>        | <b>MW917181</b> | <b>MW925933</b> | —               | This study   |
| <i>Chalciporus</i> sp.              | HKAS53400                     | Hunan, central China              | KF112352               | —               | KF112279        | KF112821        | Wu et al. (2014)                                   |
| <i>Chalciporus</i> sp.              | OR0363                        | Thailand                          | —                      | —               | MH645594        | MH645602        | Vadthhanarat et al. (2019)                         |
| <i>Chalciporus</i> sp.              | GDGM43250                     | —                                 | —                      | —               | —               | —               | Zhang et al. (2017)                                |
| <i>Chalciporus vulparius</i>        | N.K. Zeng4978 (FHMU5554)      | Hainan, southern China            | <b>MW917173</b>        | —               | <b>MW925930</b> | <b>MW925936</b> | This study   |
| <i>Chalciporus vulparius</i>        | N.K. Zeng4979 (FHMU5560)      | Hainan, southern China            | <b>MW917174</b>        | —               | <b>MW925931</b> | <b>MW925937</b> | This study   |
| <i>Gyrodon</i> sp.                  | HKAS57588                     | China                             | KF112348               | —               | KF112275        | KF112817        | Wu et al. (2014)                                   |
| <i>Gyrodon</i> sp.                  | HKAS59448                     | China                             | KF112349               | —               | KF112276        | KF112818        | Wu et al. (2014)                                   |
| <i>Paxillus obscurosporus</i>       | Po1                           | Germany                           | AY177256               | —               | KF030442        | —               | Nuhn et al. (2013)                                 |
| <i>Paxillus vernalis</i>            | AFTOL-ID 715                  | China                             | AY645059               | DQ647827        | DQ457629        | —               | Matheny et al. (2006)<br>Binder and Hibbett (2006) |

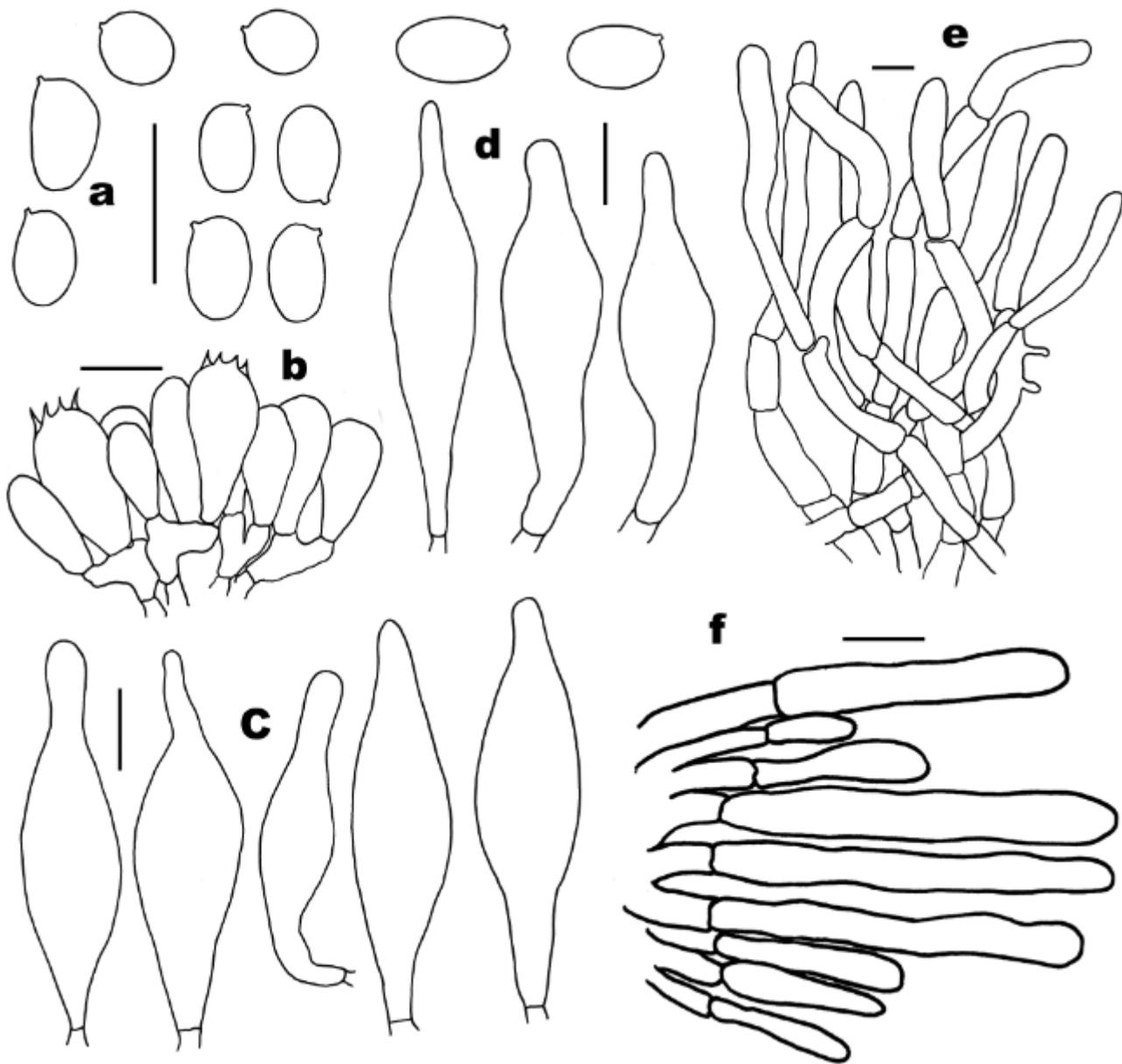
Note. Bold GenBank numbers indicate the sequences newly generated; NE = Northeast, SW = Southwest, SE = Southeast.

## Figures



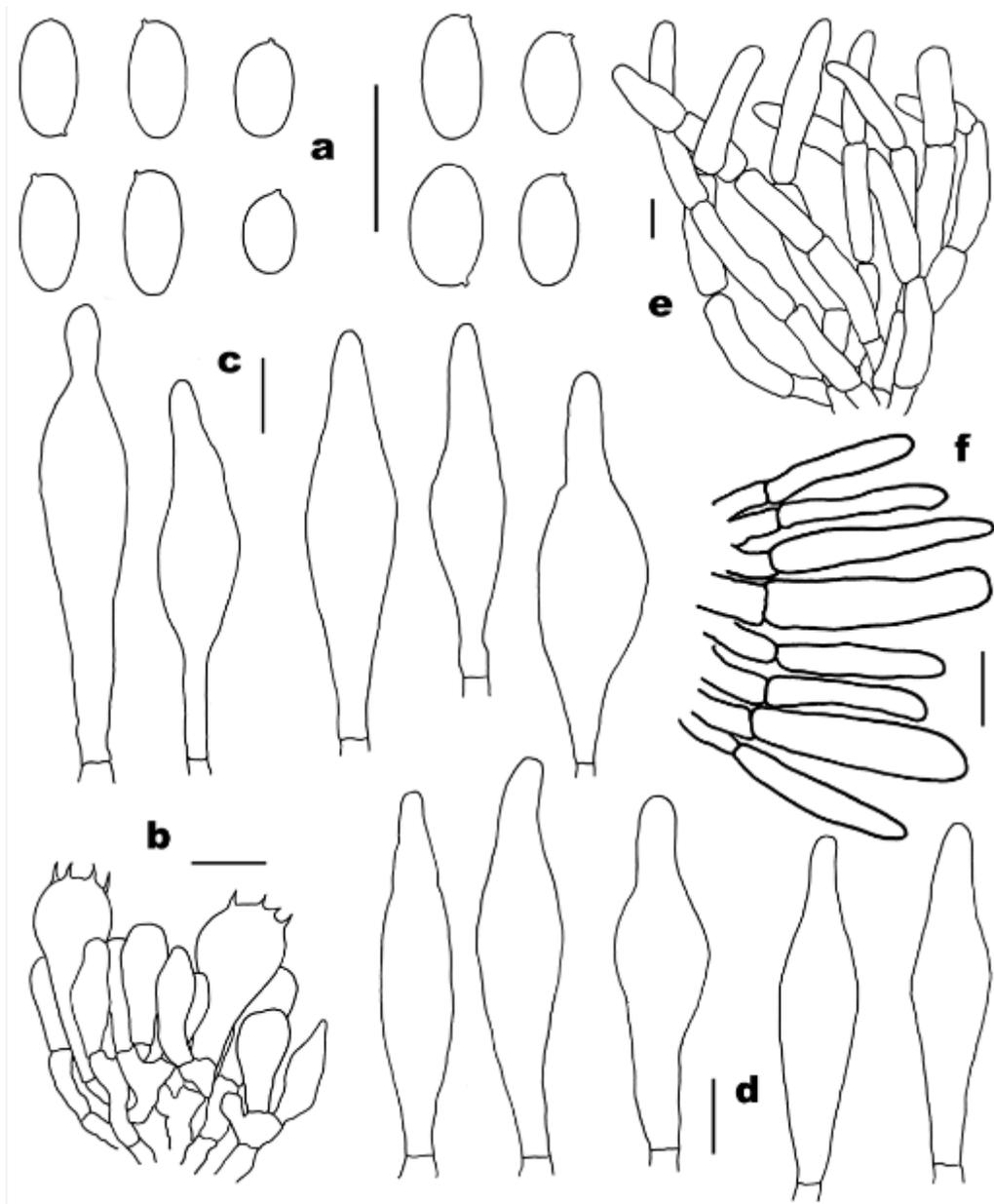
**Figure 2**

Basidiomata of *Chalciporus* species. a–c *Chalciporus nigrofuscus* (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.



**Figure 3**

Microscopic features of *Chalciporus nigrofuscus* (FHMU4701, holotype). a Basidiospores. b Basidia. c Cheilocystidia. d Pleurocystidia. e Pileipellis. f Stipitipellis. Bars =10  $\mu$ m. Drawings by C. Xu.



**Figure 4**

Microscopic features of *Chalciporus vulparius* (FHMU5560, holotype). a Basidiospores. b Basidia. c Cheilocystidia. d Pleurocystidia. e Pileipellis. f Stipitipellis. Bars = 10  $\mu\text{m}$ . Drawings by C. Xu.