



***Crocinoletus*, a new genus of Boletaceae (Boletales) with unusual boletocrocin polyene pigments**

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Abstract

Crocinoletus is described as a new genus of Boletaceae to accommodate *Boletus rufoaureus* and *B. laetissimus*, characterized by its brilliant orange color of basidiomata caused by the presence of unusual boletocrocin polyene pigments, bluish olivaceous staining of all parts when bruised, smooth basidiospores, and the pileipellis which has an interwoven trichoderm at the middle part of the pileus and a cutis at the margin of the pileus. Prior molecular phylogenetic analyses also confirmed the two taxa are not members of the genus *Boletus* s.s., but form a well-supported generic lineage within Boletaceae. Consequently a description, color photos of fresh basidiomata, line-drawings of microstructures and a comparison of *Crocinoletus* with allied taxa are presented.

Key words: boletes, molecular phylogenetics, mycorrhizal fungi, taxonomy

Introduction

During a study of Chinese boletes (Li *et al.* 2011, 2014; Zeng *et al.* 2012, 2013), several collections of *Boletus rufoaureus* Masee (Boletaceae, Boletales), a species originally described from Singapore (Masee 1909; Corner 1972), were found in subtropical and tropical regions of China. The species is characterized by the brilliant orange color of the pileus, hymenophore and stipe, which readily distinguishes this species from many other species of Boletaceae. Interestingly, Kahner *et al.* (1998) detected unusual boletocrocin polyene pigments from the fruit bodies of the fungi, which account for the intense color. Like the genus *Retiboletus* Manfr. Binder & Bresinsky with retipolides (Binder & Bresinsky 2002), *B. rufoaureus* probably is representative of a new genus of Boletaceae due to the characteristic secondary metabolites.

Our recent molecular phylogenetic analyses based on the nuclear ribosomal large subunit (nrLSU), the gene encoding the largest subunit of RNA polymerase II (*rpb1*), the second largest subunit of RNA polymerase II (*rpb2*), and the translation elongation factor 1 α (*tef1- α*) indicated that *B. laetissimus* Hongo (as “*Boletus* sp. HKAS 59701” in Wu *et al.* 2014) and *B. rufoaureus* cluster together and represent a well-supported distinct lineage (named “clade 41” in Wu *et al.* 2014) within Boletaceae. And, thus, a new genus, *Crocinoletus*, is proposed herein to accommodate *B. rufoaureus* and *B. laetissimus*.

Materials and Methods

Specimens were photographed and described in the field, and deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Sections of the pileipellis on the pileus from the middle

and marginal parts were made by cutting tangential to the pileus. Sections of the squamules on the stipe were taken from the middle part along the longitudinal axis of the stipe. 5% KOH was used as a mounting medium for microscopic studies. All microscopic structures were drawn free hand. The notations “basidiospores (n/m/p)” indicate that the measurements were made on *n* basidiospores from *m* basidiomata of *p* collections. Dimensions of basidiospores are given using the notation (a)b–c(d), where the range b–c represents a minimum of 90% of the measured values, and extreme values (a and d), whenever present, are given in parentheses. Q refers to the length/breadth ratio of basidiospores; Q_m refers to the average Q of basidiospores \pm sample standard deviation. All line-drawings of microstructures were made from rehydrated material.

Taxonomy

Crocinoboletus N.K. Zeng, Zhu L. Yang & G. Wu, *gen. nov.*

Mycobank: MB 809228

Etymology: “*Crocino*” refers to the presence of the boletocrocins.

Basidiocarp stipitate-pileate with tubular hymenophore. *Pileus* 3.8–8 cm in diameter, convex to applanate, surface yellowish orange, bright orange to reddish orange, covered with minute, reddish brown squamules, turning bluish olivaceous quickly, then blackening when bruised. *Hymenophore* poroid, adnate or slightly depressed around apex of stipe; tubes 3–5 mm in depth, with pores 0.2–0.7 mm in diameter; orange, turning bluish olivaceous quickly, then blackening when bruised. *Stipe* 5–11 \times 1–3 cm, centrally attached, subcylindric; surface concolorous with the pileus, covered with reddish orange squamules, turning bluish olivaceous quickly, then blackening when bruised. *Context* vivid golden yellow, turning bluish olivaceous quickly when bruised. *Basidiospores* subfusiform to ellipsoid, smooth. *Pleuro-* and *cheilocystidia* present. *Pileipellis* an interwoven trichoderm at the middle part of the pileus but a cutis at the margin of the pileus. *Clamp connections* absent. *Polyene pigments* boletocrocins present.

Type species: *Boletus rufoaureus* Masee, Bulletin of Miscellaneous Information of the Royal Botanic Gardens Kew 1909: 204, 1909

Crocinoboletus rufoaureus (Masee) N.K. Zeng, Zhu L. Yang & G. Wu, *comb. nov.*

Figs. 1, 2

Mycobank: MB 809229

Boletus rufoaureus Masee, Bulletin of Miscellaneous Information of the Royal Botanical Gardens Kew 1909: 204, 1909

Pileus 4–8 cm in diameter, subhemispherical when young, then convex to applanate; surface dry, yellowish orange, bright orange to reddish orange, covered with minute, reddish brown squamules, turning bluish olivaceous quickly, then blackening when bruised; margin incurved; context 0.9–1.4 cm in thickness in the center of the pileus, vivid golden yellow, turning bluish olivaceous quickly when bruised. *Hymenophore* poroid, adnate or slightly depressed around apex of stipe; pores subcircular, minute, 0.2–0.5 mm in diameter, orange, turning bluish olivaceous quickly, then blackening when bruised; tubes 3–5 mm in depth, orange, turning bluish olivaceous quickly, then blackening when bruised. *Stipe* 5–8 \times 1–3 cm, centrally attached, subcylindric, solid; surface dry, concolorous with the pileus, covered with minute, reddish orange squamules, sometimes ribbed by the fine anastomosing longitudinal rugulae, turning bluish olivaceous quickly, then blackening when bruised; context vivid golden yellow, turning bluish olivaceous quickly when bruised; annulus absent. *Basal mycelium* orangish yellow. *Odor* not distinct. *Taste* not distinct.

Basidia 23–34 \times 7–10 μ m, clavate, thin-walled, 4-spored, colorless to yellowish in KOH; sterigmata 5–6 μ m in length. *Basidiospores* [160/8/2] (9–)11–14(–15) \times 4–5(–5.5) μ m, Q = (1.80–)2.20–3.25(–3.75), Q_m = 2.65 \pm 0.38, subfusiform to ellipsoid, slightly thick-walled (up to 0.5 μ m), olive brown to yellowish brown in KOH, smooth, inamyloid. *Hymenophoral trama* subboletoid with weakly gelatinous lateral strata. *Cheilocystidia* 27–42 \times 6–9 μ m, abundant, subfusiform or fusiform, thin-walled, colorless, brownish yellow to yellowish brown in KOH, no encrustations. *Pleurocystidia* 28–41 \times 5.5–9 μ m, abundant, fusiform or subfusiform, thin-walled, colorless, brownish yellow to yellowish brown in KOH, no encrustations. *Pileipellis* an interwoven trichoderm at the middle part of the pileus and a cutis at the margin of the pileus; composed of thin-walled hyphae 3–7 μ m wide, occasionally branched,

with terminal cells $24\text{--}50 \times 5\text{--}6 \mu\text{m}$, narrowly clavate or subcylindrical, with obtuse apex; yellowish to yellowish brown in KOH. *Pileal trama* composed of thin-walled hyphae $3\text{--}7 \mu\text{m}$ in diameter, colorless to yellowish in KOH. *Stipitipellis* a trichoderm-like structure composed of thin- to slightly thick-walled (up to $0.5 \mu\text{m}$) hyphae $3\text{--}14$ wide, with narrowly or broadly clavate, occasionally subfusiform terminal cells measuring $15\text{--}30 \times 9\text{--}14 \mu\text{m}$, yellowish to yellowish brown in KOH. *Stipe trama* composed of cylindrical, parallel hyphae, $4\text{--}10 \mu\text{m}$ wide, thin- to slightly thick-walled (up to $1 \mu\text{m}$), colorless to yellowish in KOH. *Clamp connections* absent in all tissues.

Habitat and habitat: Solitary, scattered to gregarious on the ground in broadleaf forests dominated by Fagaceae.

Known distribution: Singapore, Malaysia (Corner 1972, 1974; Horak 2011), subtropical and tropical region of China. Possibly Indonesia, Papua New Guinea (type of *Boletus flammeus*) (Heim 1966; Corner 1972; Horak 2011).

Materials examined: CHINA. Hainan Province: Qiongzong County, Yinggeling Nature Reserve, elev. 850 m, 26 July 2009, N.K. Zeng 327 (HKAS 82333, as “HKAS 59820” in Wu *et al.* 2014); Same location, alt. 900 m, 28 July 2009, N.K. Zeng 371 (HKAS 59821); Wuzhishan County, Wuzhishan Nature Reserve. elev. 1175 m, 2 August 2009, N.K. Zeng 420 (HKAS 82334); Qiongzong County, Limushan Nature Reserve, elev. 850 m, 3 August 2010, N.K. Zeng 808 (HKAS 82335); Hunan Province: Yizhang County, Mangshan National Forest Park, elev. 880 m, 3 September 2007, Y.C. Li 1079 (HKAS 53424).

Crocinoletus laetissimus (Hongo) N.K. Zeng, Zhu L. Yang & G. Wu, *comb. nov.*

Figs. 1, 3

Mycobank: MB 809230

Boletus laetissimus Hongo, *Memoirs of the Faculty of Liberal Arts and Education, Shiga University* 18: 49, Fig. 20/1–3, 1968

Pileus $3.8\text{--}7$ cm in diameter, subhemispherical when young, then convex to applanate; surface dry, golden yellow, bright orange to reddish orange, covered with minute, dark reddish brown squamules, turning bluish olivaceous quickly, then blackening when bruised; margin decurved; context vivid golden yellow, turning bluish olivaceous quickly when bruised. *Hymenophore* poroid, adnate or slightly depressed around apex of stipe; pores subcircular, minute, about 0.7 mm in diameter, orange, turning bluish olivaceous quickly, then blackening when bruised; tubes $3\text{--}4$ mm in depth, golden yellow to orange, turning bluish olivaceous quickly, then blackening when bruised. *Stipe* $6\text{--}11 \times 1.2\text{--}2$ cm, centrally attached, subcylindric, solid; surface dry, concolorous with the pileus, sometimes covered with dark reddish orange squamules, turning bluish olivaceous quickly, then blackening when bruised; context vivid golden yellow, turning bluish olivaceous quickly when bruised; annulus absent. *Basal mycelium* orangish yellow. *Odor* not distinct. *Taste* not distinct.

Basidia $24\text{--}35 \times 7\text{--}10 \mu\text{m}$, clavate, thin-walled, 4-spored; colorless to yellowish in KOH; sterigmata $5\text{--}6 \mu\text{m}$ in length. *Basidiospores* $[56/2/2](9\text{--})9.2\text{--}12(-14) \times 4\text{--}5 \mu\text{m}$, $Q = (1.84\text{--})1.98\text{--}2.60 (-3.00)$, $Q_m = 2.23 \pm 0.22$, subfusiform to ellipsoid, slightly thick-walled (up to $0.5 \mu\text{m}$), olive brown to yellowish brown in KOH, smooth, inamyloid. *Hymenophoral trama* subboletoid with weakly gelatinous lateral strata. *Cheilocystidia* $19\text{--}28 \times 3.5\text{--}5 \mu\text{m}$, subfusiform or fusiform, thin-walled, colorless, brownish yellow to yellowish brown in KOH, no encrustations. *Pleurocystidia* $28\text{--}44 \times 4\text{--}8 \mu\text{m}$, fusiform or subfusiform, thin-walled, colorless, brownish yellow to yellowish brown in KOH, no encrustations. *Pileipellis* an interwoven trichoderm at the middle part of the pileus and a cutis at the margin of the pileus; composed of thin-walled hyphae $3\text{--}6 \mu\text{m}$ wide, occasionally branched, with terminal cells $18\text{--}80 \times 2.5\text{--}4 \mu\text{m}$, narrowly clavate or subcylindrical, with obtuse apex; yellowish to yellowish brown in KOH. *Pileal trama* made up of hyphae $3\text{--}6 \mu\text{m}$ in diameter, thin-walled, colorless to yellowish in KOH. *Stipitipellis* a trichoderm-like structure composed of thin- to slightly thick-walled (up to $0.5 \mu\text{m}$) hyphae $3\text{--}12.5$ wide, with narrowly or broadly clavate, subfusiform terminal cells measuring $13\text{--}30 \times (3\text{--})5\text{--}12.5 \mu\text{m}$, yellowish to yellowish brown in KOH, and occasionally with clavate, 4-spored basidia at the apex of the stipe. *Stipe trama* composed of cylindrical parallel hyphae $4\text{--}12 \mu\text{m}$ wide, thin- to slightly thick-walled (up to $1 \mu\text{m}$), colorless to yellowish in KOH. *Clamp connections* absent in all tissues.

Habitat and habitat: Solitary on the ground in mixed forests of Fagaceae and Pinaceae.

Known distribution: Japan (Hongo 1968, 1984), subtropical and tropical regions of China.

Materials examined: CHINA. Yunnan Province: Dai Autonomous Prefecture of Xishuangbanna, 9 July 2006, Y.C. Li 478 (HKAS 50232); Nanhua County, bought from agriculture supermarket, 2 August 2009, Y.C. Li 1953 (HKAS 59701).



FIGURE 1. Basidiomata of *Crocinoboletus* species. **a–g.** *Crocinoboletus rufoaurantum* [**a** from HKAS 82334; **b, e** from HKAS 82333; **c, e** from HKAS 59821; **d, f, g** from HKAS 82335 (**f** showing the surface of the pileus turning bluish olivaceous, then blackening when bruised; **g** showing the surface of the stipe turning bluish olivaceous, then blackening when bruised)]. **h, i.** *Crocinoboletus laetissimus* (**h** from HKAS 50232; **i** from HKAS 59701) (**a–g** photo N.K. Zeng; **h, i** photo Y.C. Li).

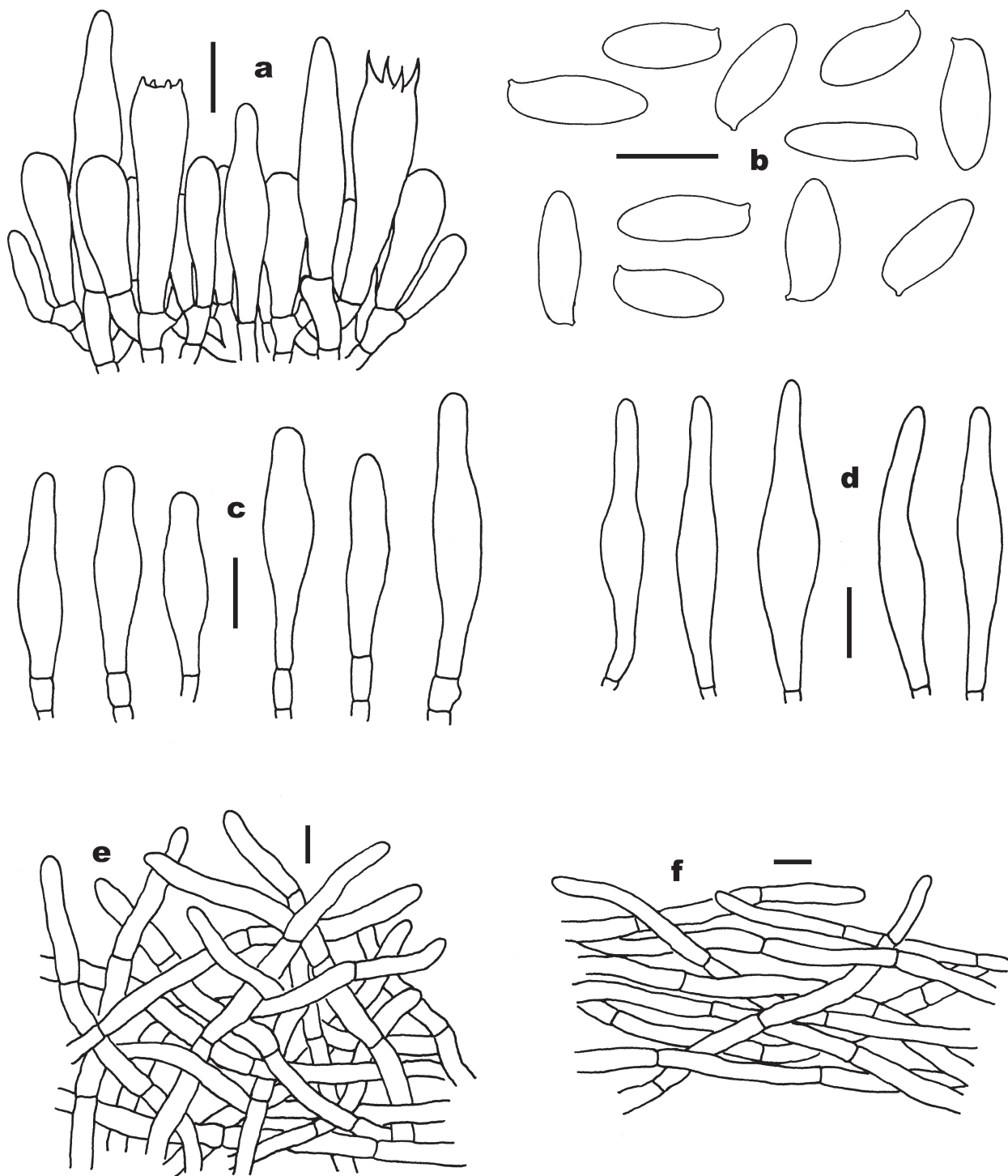


FIGURE 2. Microscopic features of *Crocinoboletus rufoaureus* (a, c from HKAS 82333; b from HKAS 82333 and 82335; d–f from HKAS 82335. drawn by N.K. Zeng). a. Basidia and pleurocystidia. b. Basidiospores. c. Cheilocystidia. d. Pleurocystidia. e. Pileipellis (from the middle part of the pileus). f. Pileipellis (from the marginal part of the pileus). Bars = 10 μ m.

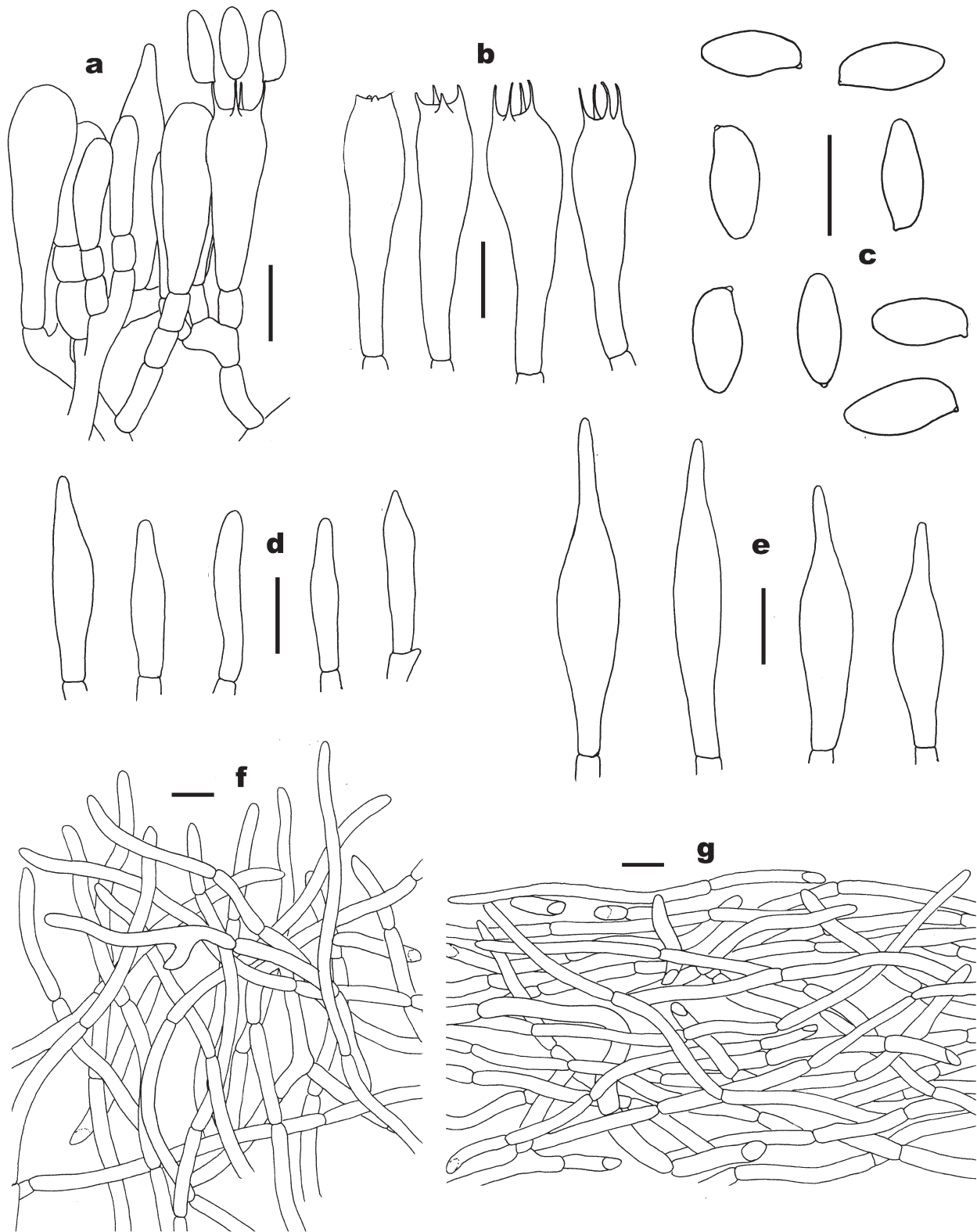


FIGURE 3. Microscopic features of *Crocinoboletus laetissimus* (**a–c, f, g** from HKAS 59701; **d, e** from HKAS 50232. drawn by G. Wu). **a.** Basidia and a pleurocystidium. **b.** Basidia. **c.** Basidiospores. **d.** Cheilocystidia. **e.** Pleurocystidia. **f.** Pileipellis (from the middle part of the pileus). **g.** Pileipellis (from the marginal part of the pileus). Bars = 10 μ m.

Discussion

Rapid progress in molecular phylogenetic methods has revealed a lot of new generic lineages within Boletaceae, providing a better understanding of the relationships within the family (Binder & Hibbett 2007; Nuhn *et al.* 2013; Wu *et al.* 2014). However, many of them were difficult to delimit just based on morphological characters alone, and, thus, the chemical analysis of pigments was also used to recognize lineages of boletes (Binder & Bresinsky 2002). The boletocrocin polyene pigments, responsible for the brilliant orange color (Kahner *et al.* 1998) in *Crocinoletus* can also help to delimit.

Crocinoletus rufoaureus and *C. laetissimus* were originally placed in the genus *Boletus* (Masse 1909; Hongo 1968, 1984; Corner 1972; Horak 2011). Corner (1972) suspected *C. rufoaureus* was a member of *Leccinum* Gray on account of the coarse scurfy particles on the surface of the stipe. However, *Crocinoletus* is clearly different from the modern concept of *Boletus* and *Leccinum* (den Bakker 2004; Dentinger *et al.* 2010; Feng *et al.* 2012; Wu *et al.* 2014) on account of the brilliant orange color, the bluish olivaceous staining of all parts when bruised, and the presence of boletocrocins.

Phylogenetically, *Crocinoletus* nested in the major clade “*Pulveroboletus* group” (Wu *et al.* 2014). *Crocinoletus* formed a monophyletic lineage with a high statistical support in the group (Wu *et al.* 2014), but its relationship to other genera was not resolved. The genera *Boletus* s.s. and *Leccinum* s.s., belonging to the subfamily Boletoidae and Leccinoideae, respectively, are distinct from *Crocinoletus* (Wu *et al.* 2014).

So far, *Crocinoletus* contains at least two species, viz. *C. rufoaureus* and *C. laetissimus*, both containing boletocrocins (Kahner *et al.* 1998). Morphologically, the two taxa are so highly similar to each other that Horak (2011) suspected that they were conspecific. The phylogenetic distance is 0.161 between the two taxa (Wu *et al.* 2014), which is significantly greater than the value for the inter-specific variation of other boletes (Li *et al.* 2013). Morphologically, *C. laetissimus* has relatively smaller basidiospores, as previously noted by Hongo (1968; 1984). Consequently, we treated them as two different species. *Boletus flammeus* R. Heim, first described from Papua New Guinea (Heim 1966; Corner 1972; Horak 2011), is also very similar to *C. rufoaureus*, and was regarded as a possible synonym of *C. rufoaureus* based on morphological studies (Corner 1972; Horak 2011).

Acknowledgments

We are grateful to Mr. H.S. Wang, Yinggeling Nature Reserve, Hainan, and Mr. G. Lu, Kadoorie Farm and Botanic Garden, Kadoorie Conservation China, for the arrangement of field work in Baisha County, Hainan. We sincerely thank Mr. J.Q. Li, Hainan Medical University, for accompanying the first author in the field. Special thanks are due to a subeditor and two anonymous reviewers for their valuable suggestions and comments which improved our manuscript. The study was supported by the National Natural Science Foundation of China (No. 31210103919, and No. 31360008), and the Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences (KLBB 201303).

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