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ORIGINAL ARTICLE

Hourangia, a new genus of Boletaceae to accommodate *Xerocomus cheoi* and its allied species

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Abstract *Hourangia*, a new genus in the Boletaceae, is erected to accommodate *Xerocomus cheoi* and allied species based on molecular phylogenetic analyses and morphological study. This genus can easily be distinguished from other genera of Boletaceae by the combination of the context of the stipe becoming first bluish, then reddish to brownish red, and finally brownish to blackish when cut, the thick hymenophore being 3–5 (7) times that of the pileal context, and the basidiospore surface with bacillate ornamentation. The phylogenetic analyses based on five gene markers (ITS, nrLSU, *tef1-α*, *rpb1* and *rpb2*) recognized five distinct taxa. Four of them are treated here, with one undescribed due to lack of adequate material. A key to the four species of *Hourangia* is provided. *Xerocomus punctilifer* is treated as a synonym of *H. cheoi* according to the macro-morphological and micro-morphological comparative study. Epitypes are designated for *H. cheoi*, *H. microcarpa* and *H. nigropunctata*. *Phylloporus pumilus*, originally described from Indonesia, is transferred to *Hourangia*.

Keywords New clade · Synonym · Phylogeny · Taxonomy · New combination

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Introduction

With the development and utilization of molecular techniques, phylogenetic analyses based on multigene sequences have strongly contributed to modern concepts in fungal taxonomy (Taylor et al. 2000; Hibbett et al. 2011; Yang 2011), and more and more new taxa representing monophyletic groups in the Boletaceae have been recognized and documented (Binder and Bresinsky 2002; Halling et al. 2007; Desjardin et al. 2008; Desjardin et al. 2009; Orihara et al. 2010; Lebel et al. 2012; Li et al. 2011; Halling et al. 2012; Zeng et al. 2012; Hosen et al. 2013; Li et al. 2014; Zhao et al. 2014; Zhu et al. 2014; Wu et al. 2015).

Based on recent molecular phylogenetic analyses of Boletaceae using multi-gene sequences, 22 new generic clades were revealed (Wu et al. 2014). Among them, clade 8 was studied morphologically and phylogenetically with additional specimens and sequences. Morphological studies showed that all species of this clade share a unique combination of features in the family Boletaceae, indicating that the clade indeed represents a new genus in the Boletaceae.

This study is to characterize the species of clade 8 and to erect a new genus to accommodate them.

Materials and methods

Morphological studies

Macroscopic descriptions are based on detailed field notes and photos of fresh basidiomata. Microscopic structures were observed on dried material with light microscopy. Tissues from dried material were rehydrated in 5 % potassium hydroxide (KOH); the color of microstructures was examined in water. Sections of the pileipellis were cut radially and vertically at a

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position halfway from the margin to the center of the pileus. Sections of the squamules on the stipe were taken from the middle part along the longitudinal axis of the stipe. Basidiospores were examined with a Hitachi S-4800 scanning electron microscope (SEM). Specimens examined are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany of the Chinese Academy of Science (HKAS), the Herbarium of the Institute of Microbiology of the Chinese Academy of Science (HMAS) and the New York Botanical Garden (NY). The notations '(n/m/p)' indicate that the measurements are made on 'n' basidiospores from 'm' basidiomata of 'p' collections. The expressions '(a) b–c (d)' stand for the dimensions of the basidiospores, in which 'b–c' contains a minimum of 90 % of the measured values, and 'a' and 'd' within parentheses stand for the extreme values. Q is the length/width of the basidiospores and Q_m is the average Q±standard deviation.

DNA isolation, PCR amplification and DNA sequencing

Total DNA was extracted from silica-gel dried herbarium material using the CTAB procedure of Doyle and Doyle (1987). The primer pairs used in this study are as follows: ITS1 and ITS4 for ITS (White et al. 1990), LR0R and LR5 for nrLSU (Vilgalys and Hester 1990), EF1-B-F1 and EF1-B-R for *tef1*-α, RPB1-B-F and RPB1-B-R for *rpb1*, RPB2-B-F1 and RPB2-B-R for *rpb2* (Wu et al. 2014).

PCR amplifications were conducted on an ABI 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA), containing 1 μl DNA template, 2.5 μl PCR reaction buffer, 0.5 μl dNTP mix (0.2 mM), 1 μl per primer (5 μm), 1 U Taq polymerase. The final volume was adjusted to 25 μl with distilled H_2O. The PCR conditions were set as follows: pre-denaturation at 95 °C for 4 min, 35 cycles of denaturation 1 min at 94 °C, annealing 40 s (for ITS) or 80 s (for nrLSU, *tef1*-α, *rpb1* and *rpb2*) at 50 °C (for ITS and nrLSU) or 53 °C (for *tef1*-α, *rpb1* and *rpb2*), elongation 80 s at 72 °C, and a final elongation of 8 min at 72 °C was included after the cycles. The PCR products were purified using the Bioteke Purification Kit (Bioteke Corporation, Beijing, China), and then the purified products were sequenced on an ABI-3730-XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA), using the same primer pairs as in the original PCR amplifications.

Phylogenetic analyses

DNA sequences were edited with SeqMan (DNASTAR in Laser gene 9) and Bioedit v7.0.9 (Hall 1999), then aligned with MUSCLE v3.6 (Edgar 2004). The aligned sequences were manually adjusted when necessary. The five-locus (ITS, nrLSU, *tef1*-α, *rpb1* and *rpb2*) combined data set was analyzed in order to confirm the systematic position of *Phylloporus pumilus* and to recognize the species in clade 8. *Xerocomus subtomentosus* and *X. ferrugineus*, which are close to *Phylloporus* and clade 8 in Wu et al. (2014), were selected as outgroups.

Bayesian inference (BI) and Maximum likelihood (ML) methods were used to analyze the combined data set. Substitution models of partition in the data set were determined using the Akaike Information Criterion (AIC) implemented in MrModeltest v2.3 (Nylander 2004).

BI and ML were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) and RAxML v7.2.6 (Stamatakis 2006), respectively. The partitioned mixed model was used to estimate the model parameters for each gene separately. The selected substitution models for these five partitions are as follows: GTR+I+G for nrLSU, *tef1*-α and *rpb1*, K80+I for ITS, HKY+I+G for *rpb2*. All parameters in the ML analysis were kept at default except for the model choosing as GTRGAMMAI, and statistical support values were obtained using nonparametric bootstrapping with 1000 replications. BI analysis using selected models and four chains were conducted by setting generations to 3 million and stoprul command with the value of stopval set to 0.01. Trees were sampled every 100 generations. The first 25 % generations were discarded as burn-in, and Bayesian posterior probabilities (PP) were then calculated from the posterior distribution of the retained Bayesian trees.

Results

Molecular data

A total of 88 sequences was newly generated in this study, of which 17 were for ITS, 21 for nrLSU, 22 for *tef1*-α, ten for *rpb1* and 18 for *rpb2* (Table 1).

The five-locus data set consists of 36 taxa and 4199 nucleotide sites (including gaps) for each taxon, of which 1150 characters were from ITS, 847 from nrLSU, 685 from *tef1*-α, 666 from *rpb1* and 851 from *rpb2*. Five lineages within clade 8 were recognized (Fig. 1).

Morphological observations

A total of 38 specimens was examined, including the type specimens of *Xerocomus cheoi* (HMAS 08963) and *X. punctilifer* (HMAS 03860), and 36 new collections of clade 8 from China. Clade 8 can easily be distinguished from other genera in the Boletaceae by the combination of the following characters: the context of the stipe becoming first bluish, then reddish to brownish red, and finally brownish to blackish

Table 1 Specimens used in the five-gene combined data set, their vouchers and GenBank accession numbers

Species Name	Voucher	Locality	GenBank Accessions				
			ITS	nrLSU	*tef1-α*	*rpb1*	*rpb2*
Hourangia cheoi	HKAS 52269	China	–	KF112385	KF112286	KF112628	KF112773
Hourangia cheoi	HKAS74744	China	–	–	KF112285	KF112772	KF112772
Hourangia cheoi	HKAS 56182	China	KP136995	KP136952	KP136939	–	KP136981
Hourangia cheoi	HKAS 54803	China	KP136998	KP136953	KP136943	KP136971	KP136985
Hourangia cheoi	HKAS 68306	China	KP137001	KP136950	KP136929	KP136972	KP136980
Hourangia cheoi	HKAS 54450	China	KP136997	KP136947	KP136924	KP136966	KP136975
Hourangia cheoi	HKAS 68318	China	KP136999	KP136957	KP136937	–	–
Hourangia cheoi	HKAS 59790	China	–	KP136948	KP136939	KP136974	–
Hourangia cheoi	HKAS 68342	China	KP136996	KP136946	KP136941	–	KP136983
Hourangia cheoi	HKAS 53378	China	KP137000	–	KP136936	–	–
Hourangia cheoi	HKAS 68161	China	–	KP136954	KP136931	–	–
Hourangia cheoi	HKAS 69077	China	–	KP136951	KP136935	–	–
Hourangia cheoi	HKAS 67885	China	–	KP136955	KP136934	–	–
Hourangia cheoi	HKAS 61644	China	KP136994	KP136956	KP136930	–	–
Hourangia cheoi	HKAS 68284	China	KP137002	KP136949	KP136928	KP136970	KP136979
Hourangia microcarpa	HKSA 53378	China	KP136986	KF112452	KF112300	–	KF112775
Hourangia microcarpa	HKAS 83763	China	–	KP136945	KP136923	–	–
Hourangia nigropunctata	HKAS 76657	China	–	KF112388	KF112287	KF112774	KF112287
Hourangia nigropunctata	HKAS 53468	China	KP136990	KP136962	KP136942	–	–
Hourangia nigropunctata	HKAS 59849	China	–	KP136959	KP136932	–	–
Hourangia nigropunctata	HKAS 53431	China	KP136988	KP136960	KP136933	–	–
Hourangia nigropunctata	HKAS 52623	China	–	KP136958	KP136938	–	–
Hourangia nigropunctata	HKAS 57427	China	KP136992	KP136964	KP136927	KP136969	KP136978
Hourangia nigropunctata	HKAS 55357	China	KP136989	KP136965	KP136926	KP136968	KP136977
Hourangia nigropunctata	HKAS 53355	China	KP136991	KP136963	KP136940	KP136973	KP136982
Hourangia nigropunctata	HKAS 53383	China	KP136993	KP136961	KP136925	KP136967	KP136976
Hourangia sp.	HKAS 68178	China	KP136987	KF112453	KF112301	–	KF112776
Phylloporus imbricatus	HKAS 68642	China	–	KF112398	KF112299	KF112637	KF112786
Phylloporus luxiensis	HKAS 75077	China	–	KF112490	KF112298	KF112636	KF112785
Phylloporus pelletieri	Pp1	Germany	–	AF456818	JQ327036	KF030390	–
Phylloporus pumilus	REH8062	Indonesia	JQ003627	JQ003681	–	–	–
Phylloporus pumilus	REH8063	Indonesia	JQ003626	JQ003682	–	–	–
Phylloporus rubrosquamosus	HKAS 52552	China	–	KF11239	KF112289	–	KF112780
Xerocomus ferrugineus	MB00-005	USA	JQ003657	JQ003702	KF030438	–	–
Xerocomus subtomentosus	KM 167686	England	KC215201	KC215222	KC215248	–	–

Accessions numbers in boldface indicate sequences obtained in this study

when cut, the thick hymenophore being 3–5 (7) times than that of the context of pileus and the basidiospores with bacillate ornamentations (under SEM). The five lineages recognized within clade 8 represent five species easily characterized by morphological features. Among them, *X. cheoi*, *Boletus microcarpus*, *X. nigropunctatus* and *P. pumilus* are transferred to the new genus as new combinations in the taxonomy section below, with one represented by HKAS 68178 to be formally described in the future, due to the paucity of materials available.

Taxonomy

Hourangia Xue T. Zhu & Zhu L. Yang, **gen. nov.**

MycoBank: MB 810695.

Etymology: "*Hou*" and "*rang*" mean "thick" and "hymenium" in Chinese, respectively, referring to the relatively thick hymenophore.

Basidioma stipitate-pileate with tubular hymenophore. *Pileus* hemispherical, convex to applanate, sometimes umbonate; surface densely covered with yellow-brown,

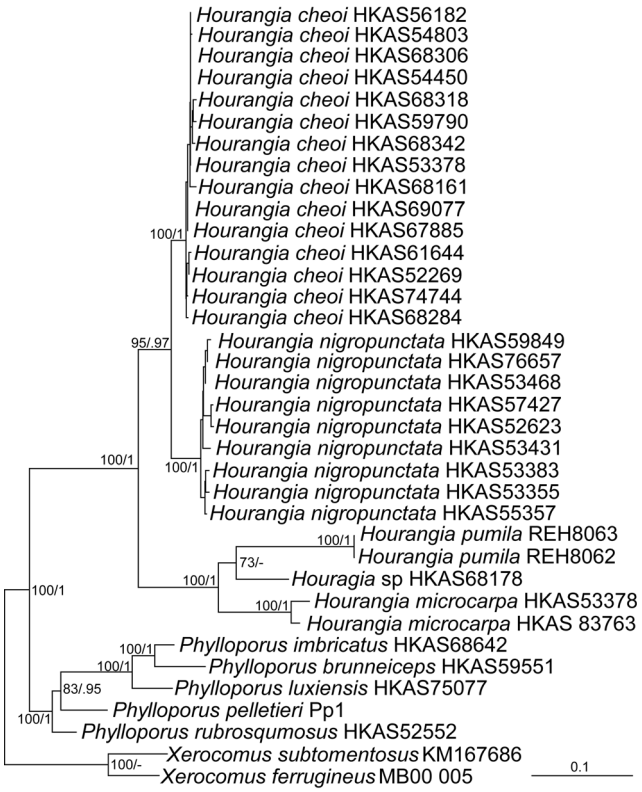

Fig. 1 Phylogram resulting from the multi-gene (ITS, nrLSU, *tef1-α*, *rpb1* and *rpb2*) data set using RAxML. RAxML likelihood bootstrap support values (>50 %) and Bayesian posterior probabilities (PP >0.95) are indicated above or below the branches as RAxML BS/PP. Herbarium voucher or isolate number is provided behind the species name

Fig. 2 Basidiomata of *Hourangia* species. **a.** *H. cheoi* (HKAS 52269, *epitype*) the surface of pileus is rimose-diffract to punctiform. **b.** *H. cheoi* (HKAS 68284) with an umbonate pileus. **c.** *H. cheoi* (HKAS 54450) on the bark of the stem base of *Pinus* sp. **d.** *H. cheoi* (HKAS 68342) showing the pileus staining dark brown to blackish. **e.** *H. cheoi* (HKAS 68161) the long tubes and the color change of the context of the pileus and stipe. **f.** *H. nigropunctata* (HKAS 76657, *epitype*) the surface of pileus is tomentose, somewhere rimose-diffract. **g** and **h.** *H. microcarpa* (HKAS 83763, *epitype*)

red-brown or dull brown granular squamules when young, becoming rimose-diffract to small tufted squamulose with age, dry; context whitish, cream-colored to yellowish, first bluish or indistinctly bluish, then reddish to brownish red, finally brownish to blackish when injured. *Hymenophore* adanate, sinuate or slightly decurrent; thickness of hymenophore 3–5 (7) times that of pileal context at the position halfway to the pileus center (Fig. 2e); hymenophoral surface flesh yellow to dull yellow, staining blue when injured; pores compound, angular; tubes concolorous with hymenophoral surface, staining blue when injured. *Stipe* central, pale yellow-brown, pale red-brown to dirty pale brown, nearly smooth, sometimes finely fibrillose; context dirty white to yellowish, first typically becoming bluish, then reddish to brownish red, and finally brownish to blackish when exposed (Fig. 2e); basal mycelia whitish. *Pileipellis* a trichoderm composed of cylindrical or tumid cells; terminal cells short cylindrical or subglobose. *Pleuro-* and *cheilocystidia* lanceolate to clavate or ventricose, thin-walled. *Basidiospores* subfusiform, brownish yellow, with bacillate ornamentation (under SEM), rarely only partially ornamented (Fig. 3). *Clamp connections* absent in all tissues.

Typus species: *Hourangia cheoi* (W.F. Chiu) Xue T. Zhu & Zhu L. Yang ≡ *Boletus cheoi* W.F. Chiu.

Phylogenetic position: Clade 8 in Wu et al. (2014).

Hourangia cheoi (W.F. Chiu) Xue T. Zhu & Zhu L. Yang, **comb. nov.** (Figs. 2a–e, 3a and 4).

MycoBank: MB 810696.

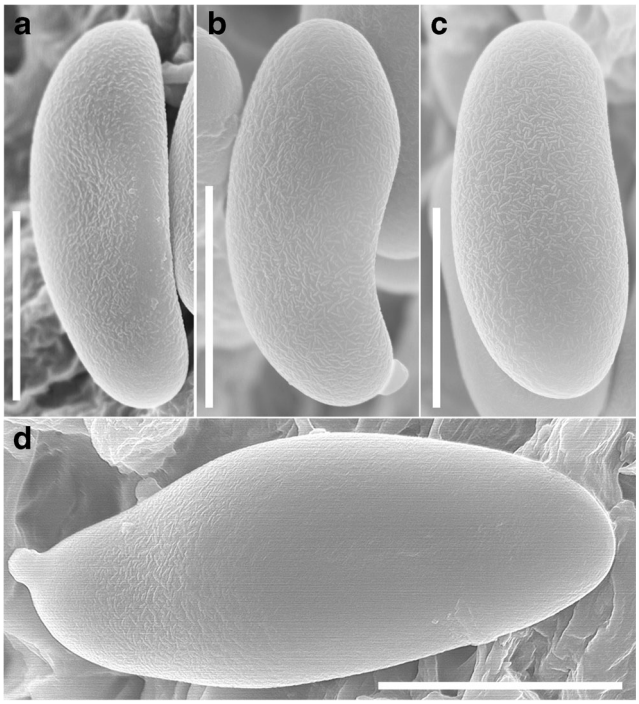

Fig. 3 Basidiospores of *Hourangia* species under SEM. **a.** *H. cheoi* (HMAS 03860, *holotype*). **b.** *H. nigropunctata* (HKAS 55357, *epitype*). **c.** *H. microcarpa* (HKAS 53378). **d**. *H. pumila* (REH8062, *holotype*). Note the whole surfaces of basidiospores of *H. cheoi*, *H. nigropunctata* and *H. microcarpa* are with bacillate ornamentation, whereas only the basal portions of basidiospores of *H. pumila* are ornamented

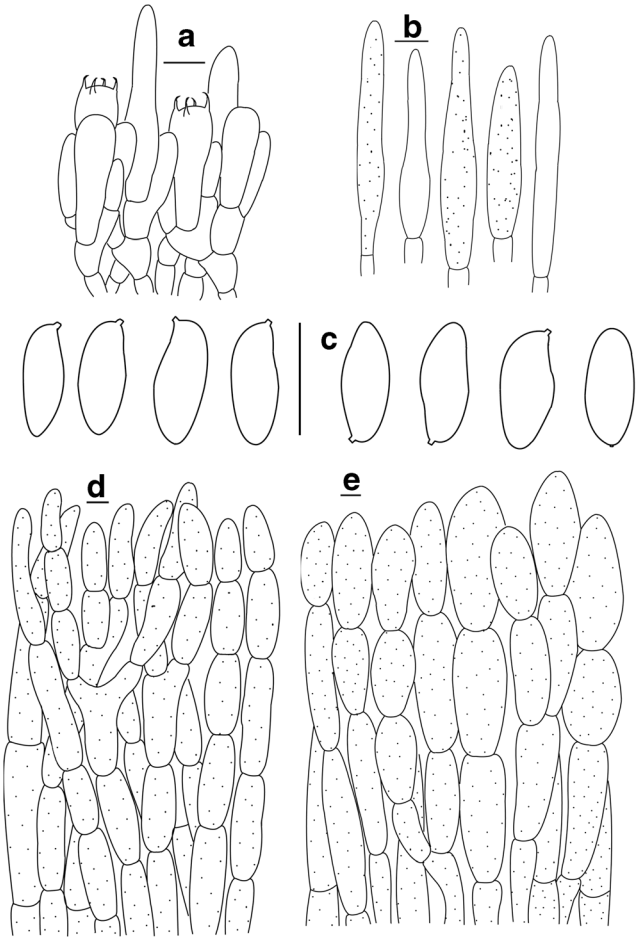

Fig. 4 Microscopic features of *Hourangia cheoi* (HKAS 52269). **a**. Basidia with basidioles. **b.** Pleuro- and cheilocystidia. **c**. Basidiospores. **d**. Pileipellis from a young basidioma. **e**. Pileipellis from a mature basidioma. (bar=10 μm)

Basionym: *Boletus cheoi* W.F. Chiu, Mycologia 40: 215 (1948) [≡ *Xerocomus cheoi* (W.F. Chiu) F.L. Tai, Syll. fung. sinicorum: 813 (1979)].

Synonym: *Boletus punctilifer* W.F. Chiu, Mycologia 40 (2): 216 (1948) [≡ *Xerocomus punctilifer* (W.F. Chiu) F.L. Tai, Syll. fung. sinicorum: 814 (1979)].

Basidioma small to medium-sized. *Pileus* 2–8 cm in diam., hemispherical, convex to applanate, sometimes umbonate (Fig. 2b); surface densely covered with red-brown or dull brown granular squamules when young, becoming rimose-diffract to small tuft squamulae with age, dry, sometimes staining blackish (Fig. 2d); context dirty white, bluing quickly, then changing to reddish or reddish brown in a few minutes, finally becoming brownish to blackish slowly. *Hymenophore* adanate, sinuate or slightly decurrent; surface flesh yellow, becoming dull yellow when mature, bluing quickly when injured, 3–5 (7) times thick of pileal context at the position halfway to the center of the pileus; pores compound, angular, 0.5–2 mm wide; tubes 5–12 mm long, concolorous with hymenophoral surface, staining blue when injured. *Stipe* cylindrical, 5–8×0.3–0.6 cm, solid, brown, pale red-brown to dirty pale brown, nearly smooth, sometimes finely fibrillose; context dirty white, changing bluish slowly at the upper part, then changing to reddish to reddish-brown, other parts becoming reddish to reddish-brown directly, finally all becoming brownish to blackish slowly when cut (Fig. 2e); basal mycelia dirty white. *Taste* and *odor* mild.

Basidia 27–34×8–11 μm, clavate, hyaline in KOH, 4-spored, sterigmata 4–5 μm long. *Basidiospores* [360/21/21] (8.5) 10–12.5 (14.5)×(3.5) 4–4.5 (5.5) μm [Q=(1.81) 2.26–2.71 (3.13), Q_m=2.48±0.23], subfusiform in side view with slight suprahilar depression, narrowly oblong to subfusoid in ventral view, brownish yellow, inamyloid, with bacillate surface ornamentation under SEM. *Hymenophoral trama*, phylloporoid; *Pleuro-* and *cheilocystidia* scattered, 50–90×7–17 μm, lanceolate to clavate or ventricose, thin-walled, hyaline, sometime spale yellow-brown. *Caulocystidia* 60–80×5–9 μm, clavate. *Pileipellis* a trichoderm, composed of cylindrical cells with terminal cells 20–55×7–13 μm in youth, of moniform hyphal elements with subglobose terminal cells 35–70×17–30 μm with age; pileipellis elements with pale yellow-brown to pale brown vacuolar pigments. *Clamp connections* absent in all tissues.

Habitat: On the ground in the forests of *Pinus*, *Castanopsis*, *Lithocarpus* and *Quercus*, sometimes on the bark of the stem base of *Pinus* spp. (Fig. 2c).

Known distribution: Currently known from southwestern China and Japan.

Specimens examined: CHINA, YUNNAN PROVINCE: Binchuan County, Jizushan Mountain, 11 September 1938, C.C. Cheo 7692 (HMAS 08963, *holotype*); Dali City, 24, August 1938, C.C. Cheo 7700 (HMAS 09021); Kunming City, Dapuji, 8 July 1942, W.F. Chiu 7860 (HMAS 03860, *holotype* of *Boletus punctilifer*); same location and time, W.F. Chiu 7873 (HMAS03873); Xishan Mountain, 19 July 1938, T.K. Yien 7699 (HMAS 08996); same location, August 1938, W.F. Chiu 7748 (HMAS 03748); Heilongtan Park, alt. 1990 m, 8 September 2007, Z.L. Yang 4952 (HKAS 52269, *epitype,* designated here); same location, 16 August 2008, Z.L. Yang 5153 (HKAS 54450); Lijiang City, Yulong Snow Mountain, 10 August 2008, L.P. Tang 572 (HKAS 54803); Same location, 23 July 2008, Y.C Li 1328 (HKAS 55366); Yulong County, Laojunshan Mountain, alt. 2900 m, 23 July 2008, G. Wu 203 (HKAS 59790); Lanpin County, alt. 2500 m, 14 August 2010, X.T. Zhu 142 (HKAS 68318); same location and time, X.T. Zhu 166 (HKAS 68342); Same location, 13 August 2010, X.T. Zhu 130 (HKAS 68306); Lushui County, alt. 2000 m, 7 August 2010, Y.J. Hao 189 (HKAS 68161); same location and time, T. Guo 85 (HKAS 69077); Gongshan County, 29 July 2010, G. Wu 433 (HKAS 74744); Shizong County, alt. 2260 m, 7 August 2010, X.T. Zhu 108 (HKAS 68284); Pu'er City, Caiyang River Natural Reserve, alt. 1500 m, 28 July 2008, B. Feng 230 (HKAS 53378); Jingdong County, alt. 2500 m, 20 July 2006, Z.L. Yang 4683 (HKAS 50480); Chuxiong City, Zixishan Mountain, 5 September 2010, Z.W. Ge 2721 (HKAS 61644); Baoshan City, Longyang District, Baihua Mountain, alt. 1900 m, 25 July 2003, Z.L. Yang 3878 (HKAS 43022); Same location, 9 August 2010, Q. Cai 323 (HKAS 67885).

Notes: The quickly bluing context of pileus and large spores (10–12.5×4–4.5 μm) can be used to distinguish *H. cheoi* from other species of *Hourangia*. Chiu (1948) described both *Boletus cheoi* and *B. punctilifer* in the same paper. According to Chiu (1948, 1957), *B. cheoi* was separated from *B. punctilifer* by the umbonate cap, the broad adnate tubes and the glabrous stipe. Reexamination of the types and newly collected materials indicated that all these characters are unstable, and there is no distinction of macro-morphologica and/or micro-morphological features between *B. cheoi* and *B. punctilifer*. Chiu (1948) cited three additional collections under *B. punctilifer* besides the type. Two of them are *H. cheoi*. The third collection C.C. Cheo 7735 (HMAS 03735) has much broader basidiospores (12–15.5×5–6 μm) than those of the collections cited above, and may represent a different species.

Xerocomus nigromaculatus (Hongo 1966) is similar to *H. cheoi*. However, *X. nigromaculatus* differs from *H. cheoi* by the blackish staining of the pileus and the stipe.

Hourangia microcarpa (Corner) G. Wu, Xue T. Zhu & Zhu L. Yang, **comb. nov.** (Figs. 2g, h, 3c and 5).

MycoBank: MB 810795.

Basionym: *Boletus microcarpus* Corner, *Boletus* in Malaysia (Singapore): 209 (1972).

Pileus 1.5–3 cm in diam., hemispherical to applanate; surface brownish to brown, sometimes with reddish tinge, subtomentose, usually cracked on the surface, with sterile marginal veil; context whitish to cream, 4 mm thick, staining pale blue very slowly when cut. *Hymenophore* adnate, sinuate to decurrent; surface light yellow to yellow, staining dull blue when bruised; pores irregular to roundish, 1–2 mm wide, surface toothy; tubes concolorous with the pores, 6 mm long, staining dull blue when cut. *Stipe* central, subcylindrical to cylindrical, nearly smooth, 2–4×0.2–0.5 cm, brown to sun brown at the upper part, and brown to cinnamon at the lower part; context brown and sometimes whitish to cream at the central part, staining pale blue very slowly when cut; basal mycelia white.

Basidia 24–34×7.5–11 μm, clavate, 4-spored, rarely 2-spored or 3-spored. *Basidiospores* 8–10×3.5–4 (4.5) μm

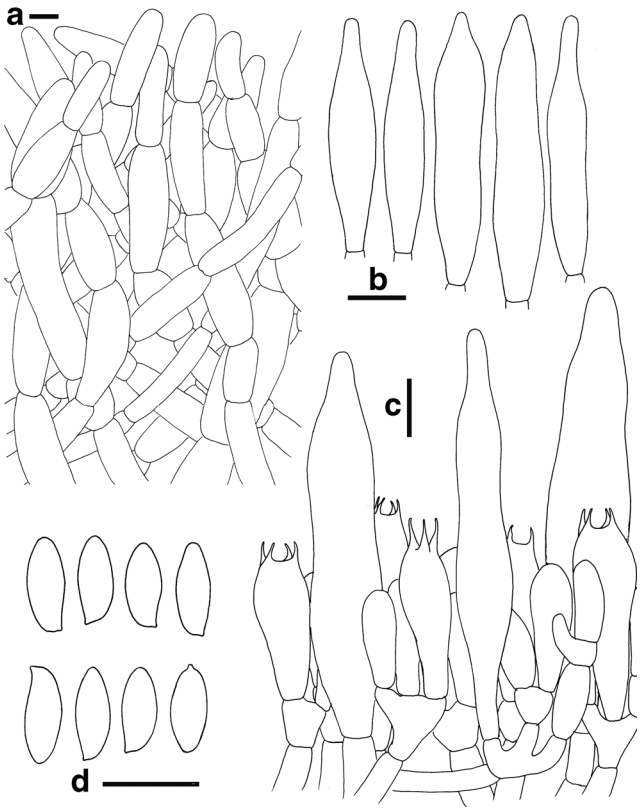

Fig. 5 Microscopic features of *Hourangia microcarpa* (HKAS 83763, *epitype*). **a**. Pileipellis. **b.** Pleuro- and cheilocystidia. **c**. Basidia with basidioles. **d**. Basidiospores. (bar=10 μm)

[Q=(1.97) 2–2.5 (2.52), Q_m=2.24±0.14], subfusiform and inequilateral in side view with suprahilar depression, subfusoid in ventral view, brownish, smooth, inamyloid. *Hymenophoral trama* phylloporoid; hyphae subcylindrical to cylindrical, 7–14 μm wide. *Cheilocystidia* 40–60×7–10 μm, fusoid-ventricose often with subacute apex, sometimes constricted at the submedian part, thin walled. *Pleurocystidia* 60–79×9–15 μm, fusoid-ventricose, often with subacute apex, often constricted at the submedian part, thin walled. *Pileipellis* a trichodermium to intricate trichodermium, up to 150 μm thick, composed of inflated hyphae 8.5–20 μm wide, with terminal cells 25–50×8.5–19 μm, which are subcylindrical or sometimes subacute towards the apex. *Pileal trama* composed of interwoven hyphae 10–18 μm wide. *Stipitipellis* composed of turfs of clavate cells, 35–50×6–10 μm. *Stipe trama* composed of parallel hyphae 6–15 μm wide at the inner part and 3.5–10 μm at the outer part. *Clamp connections* absent.

Habitat: scattered, in subtropic to tropic forests dominated by Fagaceae (*Castanopsis, Lithocarpus*, *Quercus*, etc.).

Known distribution: Currently known only from southwestern and southeast China and Malaysia.

Specimens examined: CHINA, YUNNAN PROVINCE: Pu'er City, Caiyanghe National Reserve, 11 July 2014, alt. 1300 m, G. Wu (HKAS 83763, *epitype,* designated here); FUJIAN PROVINCE: Sanming City, Sanyuan National Forest Park, 26 August 2007, alt. 250 m, Y.C. Li 1033 (HKAS 53378).

Notes: *Hourangia microcarpa* was originally described as *Boletus microcarpus* by Corner (1972), and is characterized by its small basidioma, thick hymenophore with bluing reaction when bruised, thin context with very slow bluing reaction when bruised, and small basidiospores.

Phylogenetically, *H. microcarpa* is close to *Hourangia pumila* (≡ *Phylloporus pumilus*, see below) and an undescribed species (HKAS 68178 in Fig. 1), but *H. pumila* has an alveolate hymenophore (Neves et al. 2012), and the undescribed species has a larger basidioma with a pileus 4–5 cm in diam. and basidiospores measuring 8–10×4–5 μm. Morphologically, *Xerocomus parvulus* Hongo (1963), *Boletus pseudo-parvulus* Bi et al. (1982) and *Boletus subparvulus* Smith & Thiers (1971) are somewhat similar to *H. microcarpa* due to their small basidioma, and *Boletus subparvulus* has a similar trichodermium pileipellis with inflated terminal cells. However, *X. parvulus* is different from *H. microcarpa* by its wider basidiospores (7.5–11×5–6.5 μm); *B. pseudo-parvulus* is distinct by its browning reaction of context when cut and narrower basidiospores (6.6–10×3–3.3 μm); *B. subparvulus* differs from *H. microcarpa* by its longer basidiospores (10–13×4–4.5 μm).

Hourangia nigropunctata (W.F. Chiu) Xue T. Zhu & Zhu L. Yang, **comb. nov.** (Figs. 2f, 3b and 6).

MycoBank: MB 810697.

Basionym:

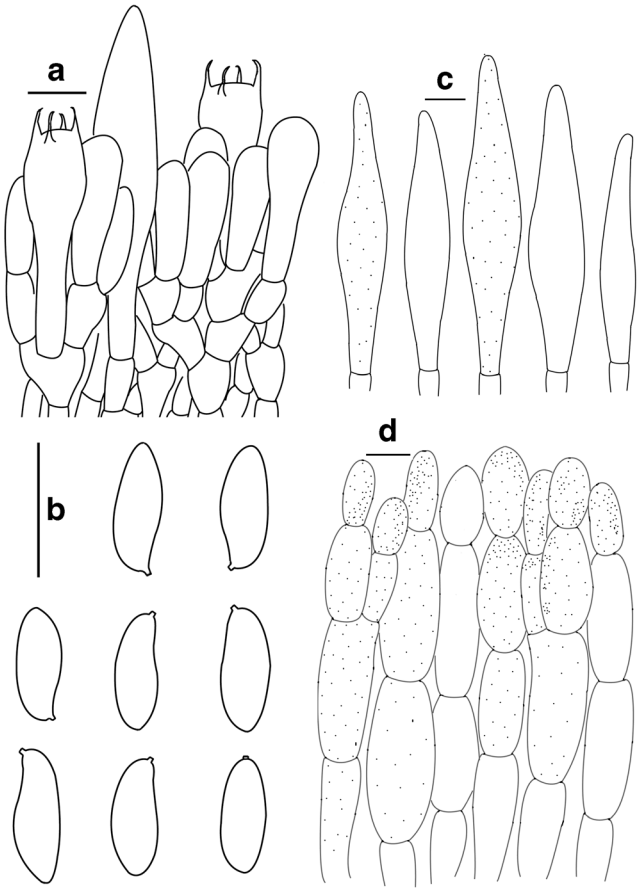

Fig. 6 Microscopic features of *Hourangia nigropunctata* (HKAS 76657, *epitype*). **a**. Basidia with basidioles. **b.** Basidiospores. **c**. Pleurocystidia and cheilocystidia. **d**. Pileipellis. (bar=10 μm)

Boletus nigropunctatus W.F. Chiu, Mycologia 40 (2): 214 (1948) [≡ *Xerocomus nigropunctatus* (W.F. Chiu) F.L. Tai, Syll. fung. sinicorum: 813 (1979)].

Basidioma small to medium-sized. *Pileus* 3–7 cm in diam., at first hemispherical, then convex to plano-convex; surface tomentose when young, densely covered by yellow-brown, red-brown or dull brown squamules, becoming rimose-diffract to granular with age, dry; context cream to yellowish, turning bluish or indistinctly bluish firstly, then reddish to brownish red, finally brownish to blackish when injured. *Hymenophore* adanate to sinuate, sometimes depressed around the stipe, surface yellowish firstly, becoming ochraceous with age, bluing immediately when bruised, then dull brown, 3–5 times thick that of pileal context at the position halfway to the pileus center; pores compound, angular, 1–2 mm in diam.; tubes 7–12 mm long, concolorous with hymenophoral surface, staining blue when injured. *Stipe* 2–8×0.3–1.2 cm, clavate, enlarged downwards; surface yellow brown to brownish, sometimes with red tinge; context dirty white to yellowish, becoming reddish to brownish red in a few minutes when exposed,

finally brownish to blackish slowly when exposed. *Basal mycelia* dirty white. *Taste* and *odor* mild.

Basidia 27–40×9–11 μm, clavate, 4-spored, sterigmata 4–5 μm long. *Basidiospores* [280/10/9] (6.5) 7.5–9 (11)×(3) 3.5–4 (5) μm, Q=(1.88) 2.07–2.31 (2.83), Q_m=2.19±0.18, subfusiform in side view with slight suprahilar depression, subfusoid in ventral view, brownish yellow, inamyloid, with bacillate surface ornamentation under SEM. *Hymenophoral trama* phylloporoid. *Pleuro-* and *cheilocystidia* scattered, 45–95×9–17 μm, lanceolate to clavate or ventricose, thin-walled, colorless or sometimes pale yellow-brown. *Caulocystidia* 40–60×9–13 μm, clavate. *Pileipellis* a trichoderm composed of moniform hyphal elements with pale yellow-brown to pale brown vacuolar-pigmented, short cylindrical or subglobose terminal cells, 20–70×15–36 μm. *Clamp connections* absent in all tissues.

Habitat: In forests of *Pinus*, *Castanopsis*, *Lithocarpus*, and *Quercus*.

Known distribution: Currently known from southwestern, central and southeastern China.

Materials examined: CHINA, YUNNAN PROVINCE, Jiangcheng County, alt. 1500 m, 29 July 2008, B. Feng 247 (HKAS 55357); Jinghong County, alt. 1300 m, 18 July 2009, Y.C. Li 936 (HKAS 52623); Tengchong County, alt. 1900 m, Y.C. Li 1717 (HKAS 57427); HUNAN PROVINCE, Yizhang County, Mangshan, Y.C. Li 1068 (HKAS 53468); same location and time, Y.C. Li 1086 (HKAS 53431); FUJIAN PROVINCE, Sanming City, Sanyuan National Forest Park, 25 August 2007, Y.C. Li 1010 (HKAS 53355); same location, 27 August 2007, Y.C. Li 1038 (HKAS 53383); HAINAN PROVINCE, Ledong County, Jianfengling, 5 August 2009, N.K. Zeng 450 (HKAS 59849); GUIZHOU PROVINCE, Daozhen County, Dashahe Natural Reserve, alt. 1400 m, 27 July 2010, X.F. Shi 390 (HKAS 76657, *epitype,* designated here).

Notes: *Hourangia nigropunctata* is characterized by its small to medium-sized basidioma and small spores (7.5–9×3.5–4.0 μm). The size and the color of the basidioma of *H. nigropunctata* are very similar to those of *H. cheoi*. However, the latter can be distinguished by its larger spores (10–12.5×4–4.5 μm). The type of *H. nigropunctata*, W.F. Chiu 262, is lost. In order to interpret the concept of the species consistently, the HKAS 76657 was designated as the epitype of this species.

Hourangia pumila (M.A. Neves & Halling) Xue T. Zhu, Halling & Zhu L. Yang, **comb. nov.** (Figs. 3d and 7).

MycoBank: MB 810796.

Basionym:

Phylloporus pumilus M.A. Neves & Halling, Fungal Diversity 55 (1): 118 (2012).

Basidioma tiny. *Pileus* 0.5–1 cm in diam., convex to plano-convex; surface cocoa brown or dark brown, subtomentose,

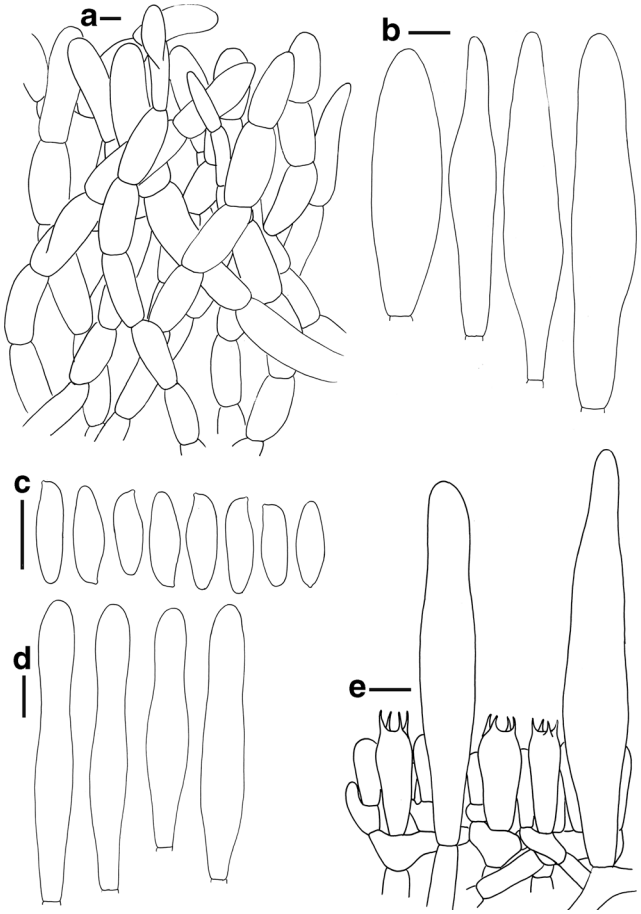

Fig. 7 Microscopic features of *Hourangia pumila* (Halling 8062, *holotype*). **a**. Pileipellis. **b.** Pleurocystidia. **c**. Basidiospores. **d**. Cheilocystidia. **e**. Basidia with basidioles. (bar=10 μm)

usually becoming tufted squamulose with age, with sterile marginal veil, dry; context less than 1 mm in thickness, white to cream, staining absent when cut. *Hymenophore* alveolate, decurrent; surface dull yellow firstly, becoming wax yellow with age, unchanging when bruised; pores compound, angular, 1–3 mm in diam.; tubes concolorous with hymenophoral surface, unchanging when bruised, 1–2.5 mm long. *Stipe* slender, 0.7–1 cm long, less than 0.1 cm in width, concolorous with pileus, densely covered with dark brown squamules, with apical part cream to pinkish brown; basal mycelia white.

Basidia 17–25×6–10 μm, clavate, 4-spored, rarely 2-spored or 3-spored. *Basidiospores* 10–14 (16)×3.5–4.5 (5) μm [Q=2.3–2.8 (3), Q_m=2.55±0.14], subfusiform and inequilateral in side view with suprahilar depression, subfusoid in ventral view, brownish, with fine bacillate surface ornamentation under SEM. *Hymenophoral trama* phylloporoid; hyphae subcylindrical to cylindrical, 3–5 μm wide. *Pleuro-* and *cheilocystidia* abundant, 50–85×5–17 μm, lanceolate to subfusoid-mucronate, thin walled. *Pileipellis* a trichodermium to intricate trichodermium, composed of inflated hyphae 8–23 μm wide, with terminal cells 30–70×8–20 μm, which are subcylindrical or sometimes

subacute towards the apex. *Stipitipellis* composed of turfs of clavate cells, 35–50×3.5–7 μm. *Clamp connections* absent.

Habitat: In forests of *Dipterocarpus*.

Known distribution: Currently known from Indonesia (Java) and only known from the type locality.

Material examined: INDONESIA. Java: Haurbentes Park, alt. 300 m, 14 January 2001, Halling 8062 (*holotype*).

Notes: *Hourangia pumila* was originally described as *Phylloporus pumilus* by Neves et al. (2012) and is characterized by its small basidioma and alveolate hymenophore. In the phylogenetic analyses (Fig. 1), *H. pumila*, *H. microcarpa* and an undescribed species constitute a lineage in *Hourangia* with high support in ML (100 %) and Bayesian analyses (1.0). Considering its angular pores and pinkish brown stipe context, we transferred it to *Hourangia. Hourangia pumila* is distinct from the other species of *Hourangia* by its small basidioma with an alveolate hymenophore (Neves et al. 2012). The basidiospores of *H. pumila* were described to be smooth under SEM in the protologue. Reexamination on the holotype of *H. pumila* under SEM, however, indicated that the basidiospores are indeed with a fine bacillate ornamentation, which is yet restricted to the basal part of the basidiospores above the hilar appendix (Fig. 3d).

For the convenience of identification of the species, a dichotomous key is given below.

Key to the species in *Hourangia*.

1. Hymenophore tubular, pores 0.5–2 mm in diam.; whole surface of basidiospores with bacillate ornamentation under SEM……..... 2
1. Hymenophore alveolate, pores 1–3 mm in diam.; part surface of basidiospores with bacillate ornamentation under SEM…….....……***H. pumila***
2. Context of pileus changing bluish slowly or indistinctly bluish when injured; basidiospores smaller (7.5–10× 3.5–4 μm)…….....…… 3
2. Context of pileus changing blue quickly and distinctly when injured; basidiospores larger (10–12.5×4–4.5 μm)…….....***H. cheoi***
3. Pileus 3–7 cm in diam., associated with subalpine to alpine coniferous trees ……..... ***H. nigropunctata***
3. Pileus 1.5–3 cm in diam., associated with subtropical or tropical broad-leaved trees…..... ***H. microcarpa***

Discussion

Phylloporus, a unique group with predominantly lamellate instead of poroid hymenophore in the Boletaceae, is a monophyletic group and has a close relationship with *Xerocomus* s. str. (Neves et al. 2012; Zeng et al. 2013). The phylogenetic analyses based on a multi-gene database showed that the genera *Hourangia*, *Xerocomus* s. str. and *Phylloporus* constitute a lineage with high support in ML (100 %) and Bayesian analyses (1.0). Meanwhile, they are separated from each other by long genetic distances (Wu et al. 2014), and *Phylloporus* is more closely related to *Hourangia* (Fig. 1).

The three genera share a unique feature in the Boletaceae: the surface of the basidiospores possess a bacillate ornamentation. In addition, they are similar to each other in their dry, tomentose pileus, with a trichodermal pileipellis. However, the species of *Hourangia* can be distinguished from the species of *Xerocomus* s. str. and *Phylloporus* by their surprisingly thicker hymenophore (3–7 times the thickness of the pileal context), and the bluing, then reddish to reddish brown, and finally brownish to blackish color changes of the context. Meanwhile, the combination of macro-morphology and micro-morphology, as well as geographical distribution and host range, can be used as informative characters to distinguish them.

The appearances of *H. cheoi* are variable: the basidiomata are small to medium-sized, the pileus is hemispherical, convex, to applanate, sometimes umbonate (Fig. 2b); pileal color is pale yellow-brown, brownish, dull brown, red, red-brown, and sometimes staining blackish (Fig. 2d); squamules on the pileal surface are granular (Fig. 2b), rimose-areolate, rimose-diffract (Fig. 2a, b and d) and punctiform (Fig. 2c). Basidiomata in different forms and different colors may be regarded as different species in the field. However, the results of phylogenetic analyses and micro-morphological studies indicated that all of the 21 collections are *H. cheoi* (Fig. 1).

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