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Zangia, a new genus of Boletaceae supported by molecular and morphological evidence

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Abstract A new distinct genus of Boletales, *Zangia*, with phenotypic similarities to the genus *Tylopilus*, is proposed based on molecular and morphological data. The monophyly of *Zangia* was highly supported using two nuclear and three mitochondrial genes based on Maximum Parsimony, Maximum Likelihood and Bayesian analyses. Morphologically, *Zangia* is distinguished from other boletoid lineages by its combination of rugose pileus, pinkish to pink hymenophore, pink to pinkish brown spore deposit, pink scabrous squamules on the stipe, chrome yellow to golden yellow stipe base, chrome yellow to golden yellow mycelia on the base of the stipe, ixohyphoepithelium pileipellis, glabrous spores and bluish colour changes in the stipe in some species. Geographically, *Zangia* is currently only known from southern, southeastern and southwestern China under forests dominated by Fagaceae mixed with Pinaceae. Six species, including 4 new ones and 2 new combinations, are fully documented with taxonomic descriptions and illustrations. A key to the species in *Zangia* is provided. It is suggested that some of the species might have started diverging from each other relatively recently with the uplifts of the eastern Himalayas and Hengduan Mountains, and both the mycorrhizal host specificity or preference and geographic separation could contribute to their ongoing divergence.

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Introduction

Boletes with pinkish to pinkish brown or reddish to vinaceous brown hymenophores have been placed historically in two genera: those with ornamented spores were grouped in the genus *Austroboletus* (Corner) Wolfe (Wolfe 1979), while the rest with smooth spores were put in *Tylopilus* P. Karst. (Singer 1945; Smith and Thiers 1971; Wolfe and Petersen 1978; Wolfe 1979). However, the taxonomic positions of some species with pink hymenophores have been the subject of debate. Specifically, *Boletus chromapes* Frost is characterized by a pink to pinkish hymenophore, pink scabrous dots on the stipe, chrome yellow coloured stipe base, interwoven pileipellis, and ecological association with birch, conifers or aspen (Singer 1947; Smith and Thiers 1971; Both 1993). It has been placed in several genera based on different morphological characters. Singer (1947) transferred it to the genus *Leccinum* Gray based on the stipe with coarsely verrucose warts, which was accepted by others (Snell and Dick 1970; Singer 1986; Binder and Besl 2000). Smith and Thiers (1968, 1971) regarded it as a member of *Tylopilus* due to the pink to pinkish hymenophore, which was followed by Wolfe and Bougher (1993) and Watling and Li (1999). Likewise, many species in *Tylopilus* s.l. have been placed in a number of other genera in Boletaceae including *Porphyrellus* E.-J. Gilbert (Singer 1945), *Fistulinella* Henn. (Pegler and Young 1981; Singer 1986; Watling and Gregory 1989), and *Leccinum* (Singer 1986; Binder and Besl 2000). Moreover, some new genera were established to accommodate some of these species, such as *Retiboletus*

(R. Heim) Manfr. Binder et Bresinsky (Binder and Bresinsky 2002b) and *Veloporphyrellus* L.D. Gómez et Singer (Gómez and Singer 1984).

Nearly 500 species belonging to Boletales have been reported from China, of which over 40 species in different genera possess pink hymenophores (Chiu 1948; Tai, 1979; Wolfe and Bougher 1993; Zang 1996; Li et al. 2002; Li and Song 2003; Yang 2005; Fu et al. 2006). Working on these species, we have found a unique generic lineage, that does not fit the existing genera in Boletaceae. We thus propose a new genus, *Zangia*, to accommodate the species in this lineage. We used both morphological data and molecular sequences of five genes, together with ecological data, in order to (1) compare the morphological features between *Zangia* and the similar genera *Austroboletus*, *Fistulinella*, *Leccinum*, *Porphyrellus* and *Tylopilus* s.s.; (2) investigate the phylogenetic position and sister groups of *Zangia*; (3) evaluate the relationships among the species in *Zangia*; and (4) provide insights about the distribution and mycorrhizal hosts of the genus.

Materials and methods

Morphological studies

The macroscopic descriptions are based on detailed field notes made on fresh basidiocarps. Microscopic structures were revived in 5% KOH. Sections of the pileipellis were cut anticlinally and halfway between center and margin of the pileus. Sections of the squamules on the stipe were taken from the middle part along the longitudinal axis of the stipe. All microscopic features were drawn by hand. For explanations of spore data see Li et al. (2009). Colour codes are from Kornerup and Wanscher (1981). In scanning electron microscopy (SEM), spores were scraped from the dried hymenophores and mounted in distilled water on a cover glass; after the water was vapoured, the cover glass was pasted directly onto an SEM stub with double-sided tape, coated with gold-palladium and photographed with an AMRAY 1000B SEM. Specimens examined are deposited in three herbaria: HKAS, HMAS and TENN. Herbarium acronyms followed Thiers (2010) with one exception: HKAS = Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences. The descriptions of species appear in alphabetical order by species epithet.

Molecular procedures and phylogenetic analysis

Protocols for DNA extraction, PCR, cloning, sequencing and sequence alignment followed those in Li et al. (2009), Gao and Yang (2010) and references therein. The phylogenetic analyses were based on two nuclear genes (nrLSU and EF1-α) and three mitochondrial genes (mtSSU, mtLSU and ATP6). Two datasets were analyzed: the nrLSU single-locus dataset and the combined nuclear and mitochondrial dataset. Both datasets were analyzed using Maximum Parsimony (MP), Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian methods respectively.

The primer pair used for amplifying the nrLSU region was LROR and LR7 (Vilgalys and Hester 1990). However, for sample TENN 47220 (the holotype of *Tylopilus chlorinosmus* Wolfe et Bougher), we failed to amplify the nrLSU region using the above primer pair. Thus, two primer pairs LROR and LR3, and LR3R and LR5 (Vilgalys and Hester 1990) were used to amplify the nrLSU region. In order to amplify EF1-α, the primers EF1-α-F and EF1-α-R were used (Mikheyev et al. 2006). The primers for amplifying the mtLSU gene fragment were ML5 and ML6 (Bruns et al. 1998). For amplifying the mtSSU gene fragment, we used the primer pair MS1 and MS2 (White et al. 1990). As the two universal primer pairs atp6-1, atp6-2 and atp6-3, atp6-4 (Kretzer and Bruns 1999) failed to amplify the entire atp6 gene fragments, we designed an additional primer pair atp6-5 (5′ -CATCAAAATAGTGGTAAAG- 3′) and atp6-R (5′ -GATACAGCTAATTCTAAWCC- 3′), based on the sequences download from GenBank and those obtained in this study. This additional primer pair allowed us to amplify most of the remaining sequences for the present study.

To test for phylogenetic conflict among the five genes, the partition homogeneity (PH) or incongruence length difference (ILD) test was performed with 1000 randomized replicates, using heuristic searches with simple addition of sequences in PAUP* 4.0b10 (Swofford 2002). The result of the partition homogeneity test showed that the phylogenetic signals present in the different gene fragments were not in conflict (P=0.86) and could therefore be combined. In the preliminary analysis, *Zangia* was found nested in the Boletaceae clade, thus, sequences from samples *Suillus cavipes* (Opat.) A.H. Sm. et Thiers, *S. granulatus* (L.) Roussel and *S. luteus* (L.) Roussel of the suborder Suillineae were downloaded from GenBank and included for outgroup rooting in both analyses. The scientific names, collection information and GenBank accession numbers for the sequences used in the combined nuclear and mitochondrial DNA dataset are presented in Table 1.

MP analyses phylogenetic relationships were estimated using the MP method in PAUP*4.0b10 (Swofford 2002). The analysis used a heuristic search strategy with the following settings: gaps as missing data; multistate taxa interpreted as uncertainty; starting tree(s) obtained via stepwise addition; 1,000 random addition sequences; one tree held at each step during stepwise addition; tree-bisection-reconnection (TBR) branch-swapping; steepest

Table 1 Specimens used in the combined nuclear and mitochondrial DNA dataset and their GenBank accession numbers

Taxon	Voucher	Coll. No.	Locality	GenBank accession				
				nrLSU	atp6	mtSSU	mtLSU	EF1-α
Boletellus projectellus	–	–	–	AY684158	DQ534604	–	DQ534521	AY879116
Boletinellus merulioides	–	–	–	AY684153	DQ534601	–	DQ534581	DQ056287
**Boletus edulis*[a]	HKAS 55836	Z. L. Yang 061124	Marburg, Germany	HQ326927	HQ326839	HQ326903	HQ326879	HQ326860
B. edulis[b]	–	–	–	AF336240	AF002141	AY615903	AD001562	–
B. satanas	–	–	–	AF071528	–	M91009	AD001566	–
Chamonixia caespitosa	–	–	–	AF336245	AF114444	AF213145	AF114464	–
**Leccinum holopus*	HKAS 53417	Y. C. Li 1072	Hunan, central China	HQ326928	–	HQ326904	HQ326880	HQ326861
**L. manzanitae*	HKAS 51277	J. F. Liang 566	Tibet, SW China	HQ326929	–	HQ326905	HQ326881	HQ326862
Paragyrodon sphaerosporus	–	–	–	AF071531	–	M91014	AD001613	–
Paxillus involutus	–	–	–	AY612815	AF114447	AY615912	AD001615	–
Phylloporus rhodoxanthus	–	–	–	DQ534631	AF114443	M91013	AD001618	–
Strobilomyces floccopus	–	–	–	DQ534626	DQ534607	AY615918	AD001640	AY883428
Suillus cavipes	–	–	–	AF071535	–	M91016	AD001641	–
S. granulatus	–	–	–	AB284479	AF002137	AY615920	DQ534592	–
S. luteus	–	–	–	AY612825	AF002135	–	L47587	–
**Tylopilus chromapes*[a]	HKAS 49416	Z. W. Ge 921	Sichuan, SW China	HQ326930	HQ326840	HQ326906	HQ326882	HQ326863
**T. chromapes*[b]	HKAS 59217	Z. W. Ge 2134	Vermont, USA	HQ326931	HQ326841	HQ326907	HQ326883	HQ326864
**T. chromapes*[c]	HKAS 59218	Z. W. Ge 2241	Hampshire, USA	HQ326932	HQ326842	HQ326908	HQ326884	HQ326865
**T. felleus*[a]	HKAS54926	K. H. Rexer 8989	Marburg, Germany	HQ326933	HQ326843	HQ326909	HQ326885	HQ326866
**T. felleus*[b]	HKAS 55832	Y. C. Li 1167	Jilin, NE China	HQ326934	HQ326844	HQ326910	HQ326886	HQ326867
**T. violatinctus*	HKAS 50279	Y. C. Li 525	Yunnan, SW China	HQ326935	HQ326845	HQ326911	HQ326887	HQ326868
**T. neofelleus*	HKAS 50319	Y. C. Li 565	Yunnan, SW China	HQ326936	–	HQ326912	HQ326888	HQ326869
**T. plumbeoviolaceoides*	HKAS 50210	Y. C. Li 456	Yunnan, SW China	HQ326937	HQ326846	HQ326913	HQ326889	HQ326870
Xanthoconium affine	–	–	–	AY612838	–	AY615926	AD001561	–
Xerocomus chrysenteron	–	–	–	AF514808	AF002143	M91018	AD001659	–
**Zangia chlorinosma*[a]	TENN 47220	R. H. Petersen 56400	Yunnan, SW China	HQ326938	HQ326847	HQ326914	HQ326890	–
**Z. chlorinosma*[b]	HKAS 48695	Z. L. Yang 4531	Yunnan, SW China	HQ326939	HQ326848	HQ326915	HQ326891	–
**Z. citrina*[a]	HKAS 52677	Y. C. Li 990	Fujian, SE China	HQ326940	HQ326849	HQ326916	HQ326892	HQ326871
**Z. citrina*[b]	HKAS 52684	Y. C. Li 997	Fujian, SE China	HQ326941	HQ326850	HQ326917	HQ326893	HQ326872
**Z. erythrocephala*[a]	HKAS 48696A	Z. L. Yang 4532A	Yunnan, SW China	HQ326942	HQ326851	HQ326918	HQ326894	–
**Z. erythrocephala*[b]	HKAS 52843	B. Feng 122	Yunnan, SW China	HQ326943	HQ326852	HQ326919	HQ326895	–
**Z. erythrocephala*[c]	HKAS 52844	B. Feng 123	Yunnan, SW China	HQ326944	HQ326853	HQ326920	HQ326896	–
**Z. olivacea*[a]	HKAS 55830	Z. W. Ge 1086	Yunnan, SW China	HQ326946	HQ326855	HQ326922	HQ326898	HQ326874
**Z. olivacea*[b]	HKAS 45445	Z. L. Yang 3960	Yunnan, SW China	HQ326945	HQ326854	HQ326921	HQ326897	HQ326873
**Z. olivaceobrunnea*[a]	HKAS 52275	Z. L. Yang 4960	Yunnan, SW China	HQ326947	HQ326856	HQ326923	HQ326899	HQ326875
**Z. olivaceobrunnea*[b]	HKAS 52272	Z. L. Yang 4955	Yunnan, SW China	HQ326948	HQ326857	HQ326924	HQ326900	HQ326876
**Z. roseola*[a]	HKAS 51137	Y. C. Li 700	Yunnan, SW	HQ326949	HQ326858	HQ326925	HQ326901	HQ326877

Table 1 (continued)

Taxon	Voucher	Coll. No.	Locality	GenBank accession				
				nrLSU	atp6	mtSSU	mtLSU	EF1-α
Zangia roseola[b]	HKAS 52649	Y. C. Li 962	China Yunnan, SW China	HQ326950	HQ326859	HQ326926	HQ326902	HQ326878

* Sequences obtained in this study. AY612815, AY612825, AY612838, AY615903, AY615912, AY615918, AY615920, AY615926, AY684153, AY684158, AY879116, AY883428, AF071528, AF071531, AF071535, AF213145, AB284479 and L47587 were from GenBank. M91009, M91013, M91014, M91016 and M91018 were from Bruns and Szaro (1992). AD001561, AD001562, AD001566, AD001613, AD001615, AD001618, AD001640, AD001641 and AD001659 were from Bruns et al. (1998). AF002135, AF002137, AF002141, AF002143, AF114443, AF114444 and AF114447 were from Kretzer and Bruns (1999). AF336240 and AF336245 were from Binder and Bresinsky (2002a). AF514808 was from Peintner et al. (2003). DQ534521, DQ534581, DQ534592, DQ534601, DQ534604, DQ534607, DQ534626 and DQ534631 were from Binder and Hibbett (2006). DQ056287 was from Matheny and Hibbett (2007). SW = southwestern; NE = northeastern; SE = southeastern. Superscripts (a, b and c) are used to relate individual collections of the same taxon to their corresponding sequence data shown in Figs. 1 and 2

descent and "Mul-Trees" options not in effect. 100 MP bootstrap replicates were completed using heuristic searches with the same search parameters as above.

ML analyses We used the RAxML web-server program available at the CIPRES portal in San Diego (http://8ball.sdsc.edu:8889/cipres-web/Home.do). This online version implements a very efficient and rapid bootstrap heuristic in RAxML (Stamatakis et al. 2008). For each analysis, the "Maximum likelihood search" and "Estimate proportion of invariable sites" options were selected, with a total of 100 bootstrap replicates performed.

Bayesian Estimation Both datasets were analyzed further with a Bayesian approach (Metropolis-coupled Markov chain Monte Carlo or MC3) using MrBayes 3.1 (Huelsenbeck and Ronquist 2005). The parameter model was selected by the Akaike information criterion (AIC) as the best-fit likelihood model with Modeltest 3.7 (Posada and Buckley 2004). To perform the Bayesian analysis of the combined dataset, the model employed for each of the five partitions were: TrN + I+G for nrLSU, TrNef+G for EF1-α, and mtLSU, GTR+I + G for mtSSU, and TVM + G for ATP6. The substitution rate matrix, transition/transversion ratio, character state frequencies, gamma shape parameter (α) and proportion of invariant sites were unlinked across nuclear and mitochondrial partitions and calculated independently by MrBayes. Posterior probabilities (PP) were determined twice by running one cold and three heated chains in parallel mode, saving trees every 100th generation. Runs were terminated once the average standard deviation of split frequencies went below 0.01 (Huelsenbeck and Ronquist 2005). The first 500 iterations were used for the burn-in period. The 50% majority-rule consensus tree of the remaining trees was calculated using PAUP* to determine the Bayesian posterior probability (PP) of each clade.

Results

Morphologic observations

Fifty-three specimens were examined, including 47 collections of *Zangia*, three collections of *Tylopilus* s.s., two collections of *Leccinum* and one collection of *Boletus*. *Zangia* is characterized by having a rugose pileus, pinkish to pink hymenophore, pink to pinkish brown spore deposit, pink to purple pink scabrous squamules on the stipe, chrome yellow to golden yellow stipe base, chrome yellow to golden yellow mycelia on the base of the stipe, ixohyphoepithelium pileipellis, glabrous spores, and bluish colour changes in the stipe in some species. The ixohyphoepithelium of the pileipellis is a consistent and unique character among the species within *Zangia*. Six species of *Zangia* can be recognized, and will be described in the Taxonomy section below.

Molecular data

For the nrLSU dataset, sequences generated in our study and sequences from newly reported genera in Boletales, i.e. *Bothia* Halling, T.J. Baroni et Manfr. Binder, *Durianella* A.W. Wilson et Manfr. Binder, *Spongiforma* Desjardin, Manfr. Binder, Roekring et Flegel (Castellano et al. 1992; Halling et al. 2007; Desjardin et al. 2008; Desjardin et al. 2009), were aligned manually with the nrLSU dataset of Binder and Hibbett (2006) that includes 301, or roughly 30%, of the described species in the order Boletales. The dataset was then narrowed by running heuristic searches in Paup* 4.0b10, pruning redundant sequences until a comprehensive dataset including 81 species remained.

One hundred and two sequences were newly generated for this study including 24 nrLSU sequences, 19 EF1-α sequences, 24 mtLSU sequences, 24 mtSSU sequences and 21 ATP6 sequences. The nrLSU dataset included 81 nrLSU

sequences, and the alignment contained 1384 nucleotide sites (308 were parsimony informative). Parsimony analysis resulted in 7 most parsimonious trees of 1410 steps, with Consistency Index (CI)=0.404, Retention Index (RI)=0.665 and Rescaled Consistency Index (RC)=0.268. The combined (nrLSU, EF1-α, mtLSU, mtSSU, and ATP6) dataset consisted of 1411, 402, 621, 392, and 529 characters, respectively (including gaps). The final alignment consisted of 107 amplified sequences and 24 downloaded from GenBank, and contained 3355 nucleotides (697 sites were parsimony informative). Parsimony analysis resulted in 14 parsimonious trees of 2064 steps, with CI=0.631, RI=0.709 and RC=0.447.

ML, MP and Bayesian analyses produced very similar estimates of tree topologies (Figs. 1 and 2); the analyses differed in that ML and Bayesian yielded greater resolution within and among clades. The most significant finding was that *Zangia* was nested into the clade Boletaceae and clustered as a monophyletic group in all analyses on both datasets.

Taxonomy

Zangia Yan C. Li et Zhu L. Yang, **gen. nov.**

MycoBank: MB 517380

Etymology Named after Prof. Mu Zang, in honour of his over 40 years' contribution to mycology, on the occasion of his 80th birthday.

Genus Boletacearum. Basidiomata pileata, stipitata. Pileus hemisphericus, convexus vel applanatus, viscidus, puberulus, verrucosus vel rugosus; contextus albidus, immutatus cum contusus. Hymenophorum tubuliforme circum stipitem depressum, albidum vel pallidum cum immaturum, incarnatum vel purpureum cum maturum. Stipes centralis, scabrosus, pallidus, luteolus, vel rufus, sed purpureus ad apicem, aureus ad basem; contextus albidus, pallidus ad apicem, aureus ad basem, immutatus vel cyanescens cum contusus. Pileipellis ixohyphoepithelium est. Pleuro- atque cheilocystidia subcylindrica, longi-fusiformia vel elongato-clavata. Basidiosporae glabrae, subfusiformes vel ellipsoideae, pallido-incarnatae vel incoloratae. Fibulae in carposomate nullae.

Typus generis ***Zangia roseola*** (W.F. Chiu) Yan C. Li et Zhu L. Yang ≡ *Boletus roseolus* W.F. Chiu

Basidiocarps stipitate-pileate with tubular hymenophore. Pileus hemispherical, convex or applanate, rugose and pubescent when young, viscid when wet; context white to pallid, unchanged in colour when injured. Hymenophore white when young, and becomes pinkish or pink to purplish when mature, depressed around apex of stipe, unchanged in colour when injured. Spore print pinkish, pink to purplish pink or pale purple. Stipe central, pallid to yellowish or reddish but pinkish sometimes at apical part and chrome to golden yellow at base, always with red to purplish red scabrous squamules; context white to cream at apex, cream to yellowish in middle part, golden yellow at base; unchanging in colour or slowly becoming bluish in some species when injured (Fig. 4a, c, d, e and f); mycelia on base of stipe chrome yellow to golden yellow. Pileipellis an ixohyphoepithelium. Pleuro- and cheilocystidia subcylindrical, subfusiform or clavate. Basidiospores smooth, subfusiform or ellipsoid, pinkish, light olivaceous to nearly colourless (Fig. 3). Clamp connections absent.

1. *Zangia chlorinosma* (Wolfe et Bougher) Yan C. Li et Zhu L. Yang, **comb. nov.** Figures 3a, 4a and 5

Basionym: *Tylopilus chlorinosmus* Wolfe et Bougher, Aust. Syst. Bot. 6(3): 207, 1993

MycoBank: MB 518866

Pileus 5–8 cm in diam., hemispherical to convex or applanate, dry or slightly viscid when wet, rugose, brownish olivaceous to yellowish brown or orange yellow to honey yellow with olivaceous tinge; context whitish to brownish, unchanging in colour when bruised. Hymenophore free to subfree or sinuate around apex of stipe, pinkish to brownish pink; pores angular, about 0.5–1 mm in diam., tubes relatively long, up to 16 mm in length, pinkish to dirty pink. Stipe 4–9× 0.7–1.2 cm, tapering upwards and enlarge downwards, pink to vinaceous pink, but cream to yellowish at apical part and bright yellow to chrome yellow at base, with pinkish to pink scabrous squamules, lower parts sometimes indistinctly reticulated, bluish slowly when injured; mycelia on base of stipe chrome yellow to golden yellow; context yellowish to yellow but empire yellow at base of stipe, asymmetrically bluish when injured. Taste and odor mild.

Basidia 22–42×9.5–14 μm, clavate, hyaline in KOH and yellowish in Melzer's reagent, 4-spored, sometimes 2-spored. Basidiospores (320/9/5) (12)13–15(17)×(5.5)6–7 (7.5) μm, [Q = (1.86)2.07–2.5(2.67), Q = 2.27±0.16], ellipsoid to somewhat oblong, smooth, slightly thick walled (0.5–1 μm in thickness), sub hyaline to light olivaceous in KOH and yellowish brown in Melzer's reagent. Pleuro- and cheilocystidia 61–75×8.5–12 μm, lanceolate to subfusoid-mucronate, or ventricose-mucronate with a long pedicel, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Caulocystidia forming squamules over stipe surface, ventricose, fusoid ventricose, clavate, lanceolate. Pileipellis an ixohyphoepithelium, outer layer con-

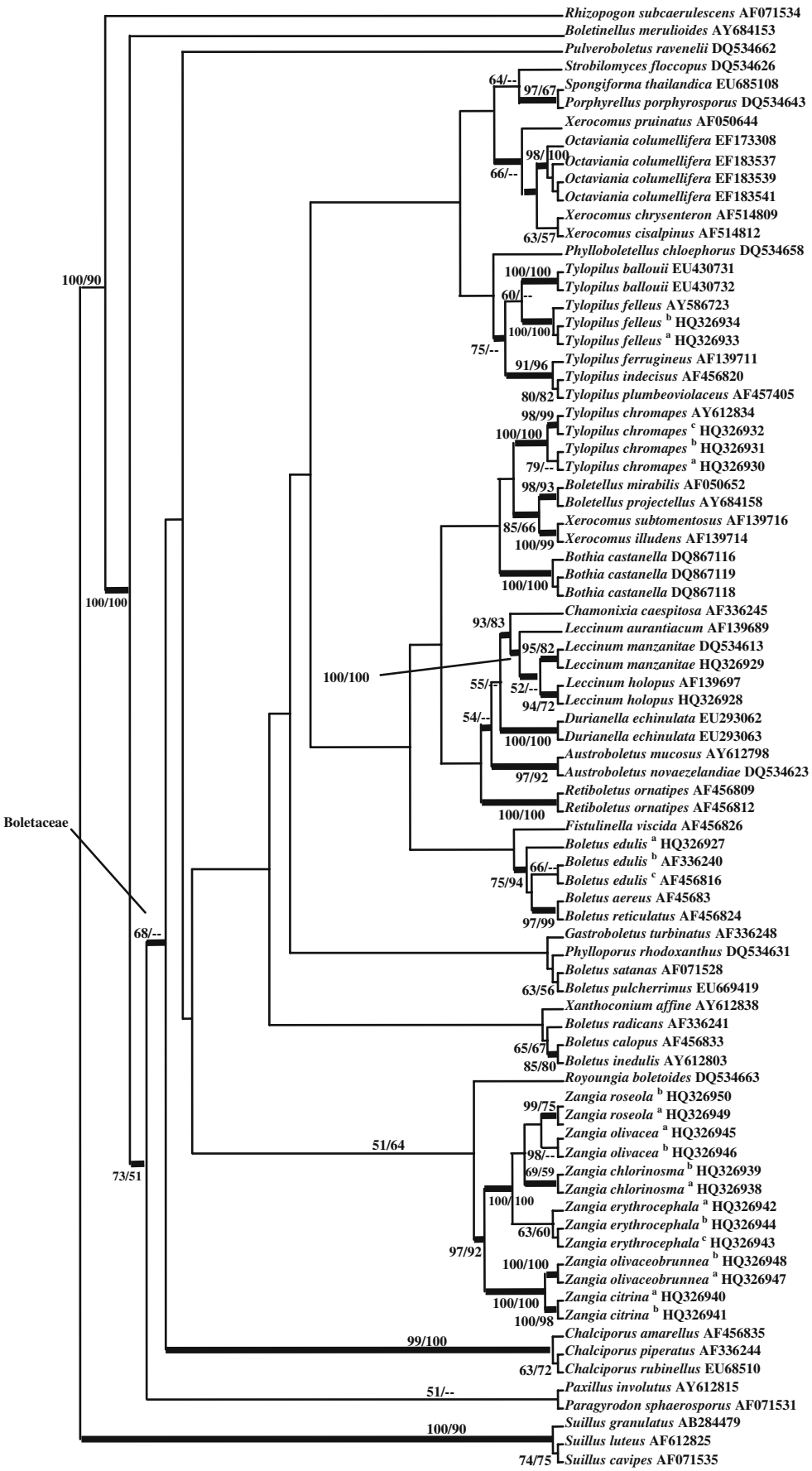

Fig. 1 Cladogram resulting from the nrLSU rDNA dataset using RAxML. RAxML and MP BS support values (>50%) are indicated above or below the branches as RAxML BS/MP BS. In Bayesian analysis, PP> 0.95 are indicated with thick branches. GenBank accession numbers are provided behind the species name

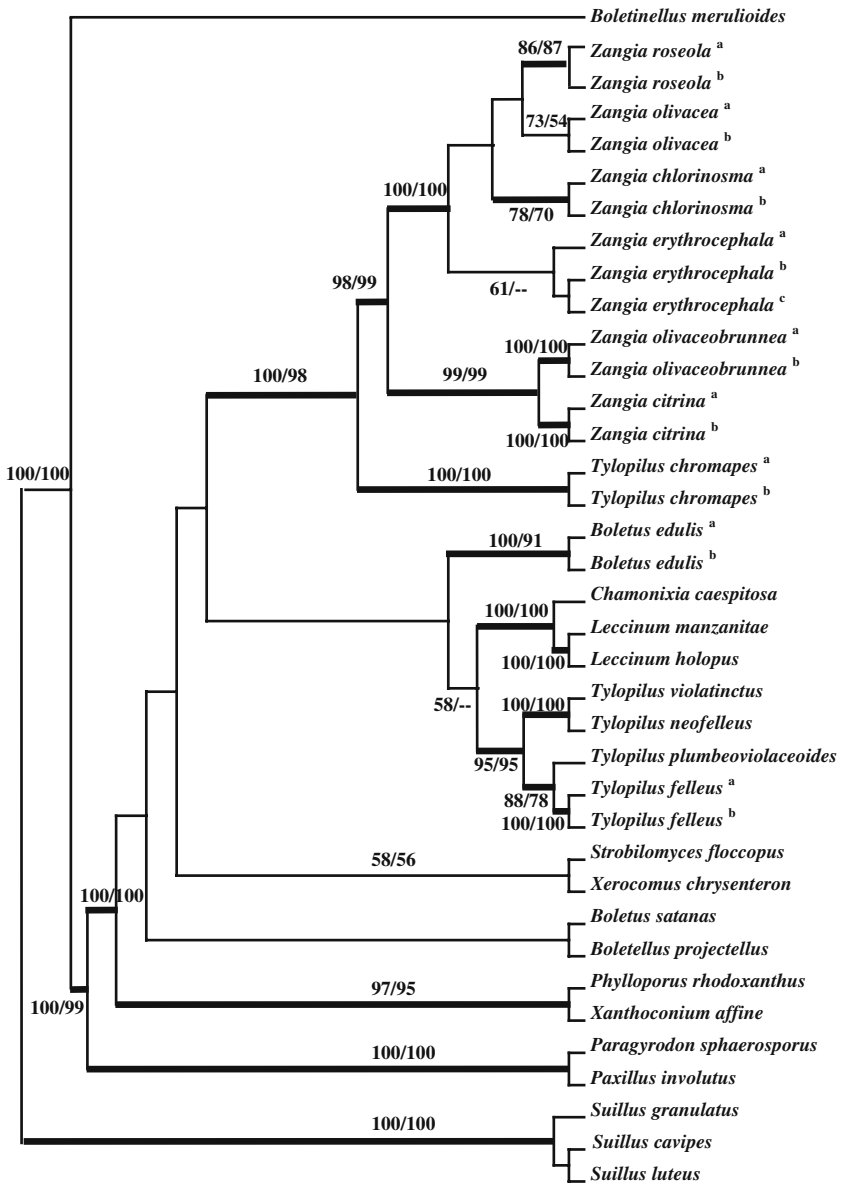

Fig. 2 Cladogram resulting from the combined nuclear (nrLSU, EF1-α) and mitochondrial (mtLSU, mtSSU and ATP6) DNA dataset using RAxML. RAxML and MP BS support values (>50%) are indicated above or below the branches as RAxML BS/MP BS. In Bayesian analysis, PP> 0.95 are indicated with thick branch. GenBank accession numbers for the five genes of each species were presented in Table 1

sisting of yellow to yellowish filamentous hyphae 3.5–8 μm in width with terminal cells 8–31×4–7 μm, inner layer composed of inflated cells in short chains up to 30 μm in width arising from radially arranged, repent filamentous hyphae 3–4 μm in width. Pileal trama composed of hyaline to yellowish interwoven hyphae up to 15 μm in width. Clamp connections absent.

Habitat solitary to scattered, in mixed forests of *Pinus densata*, *Picea* spp. and *Quercus semicarpifolia*.

Known distribution Currently only known from southwestern China (Yunnan Province).

Materials examined CHINA, YUNNAN PROVINCE: Jianchuan County, alt. 2700 m, 7 September 1983, W. K. Zheng 83124 (HKAS 12079); Lijiang County, Ganhaizi, alt. 3000 m, 3 September 1986, R. H. Petersen 56400 (TENN 47220, holotype of *T. chlorinosmus*); Lijiang County, Tiejia Mountain, alt. 2800 m, 30 September 2000, M. Zang 13723 (HKAS 37032); Lijiang County, Yulong Snow Mountain, Heibaishui, alt. 3200 m, 5 August 1985, M. Zang10351 (HKAS 15229); and same location, alt. 3100 m, 4 August 2005, Z. L. Yang 4531 (HKAS 48695).

Notes Zangia chlorinosma was originally described by Wolfe and Bougher (1993) as a new species of *Tylopilus* subgen. *Roseoscabra* Smith et Thiers. It is characterized by the brownish olivaceous to yellowish brown or olivaceous yellow pileus, the whitish to pinkish or pink to purplish

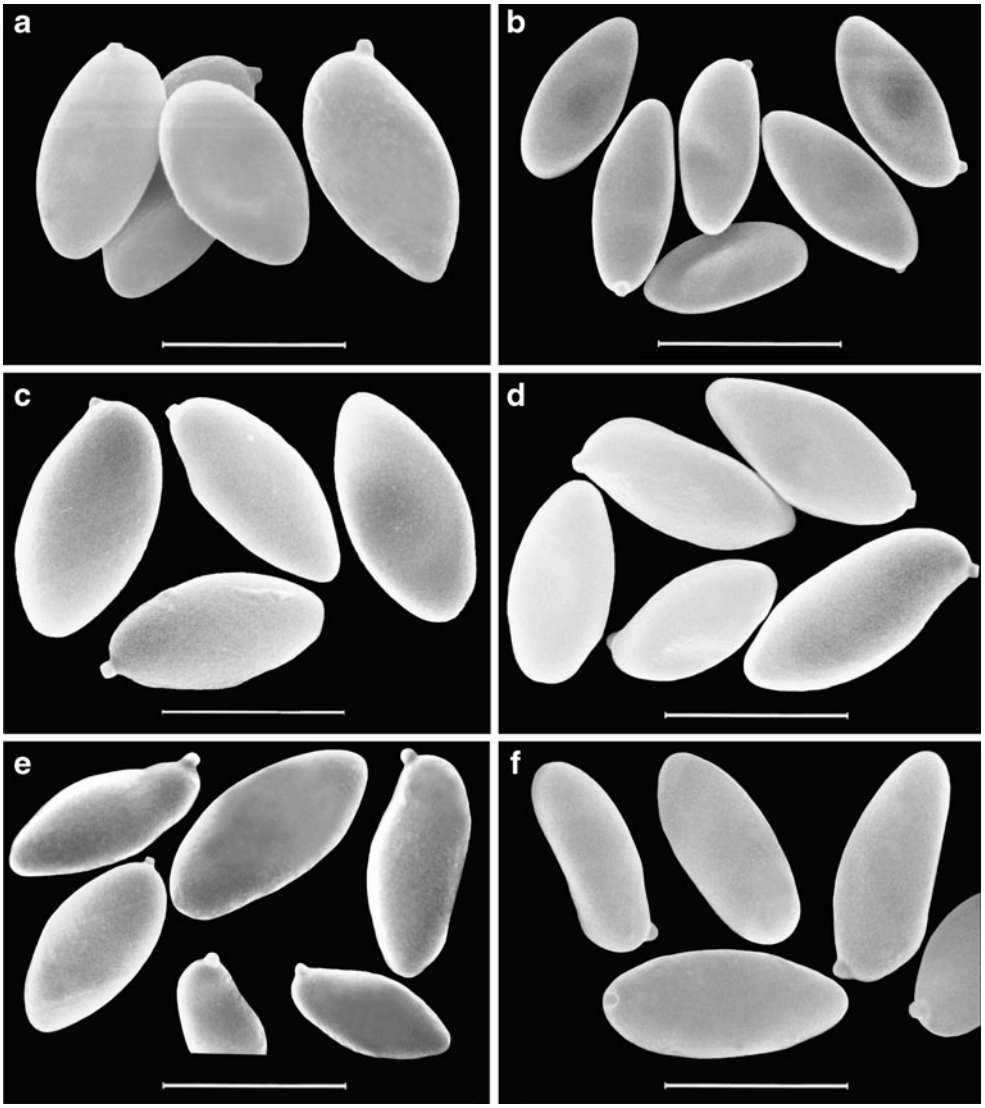

Fig. 3 Spores of *Zangia* under SEM. **a.** *Z. chlorinosma* (TENN 47220). **b.** *Z. citrina* (HKAS 52684, holotypus). **c.** *Z. erythrocephala* (HKAS 48696A, holotypus). **d.** *Z. olivacea* (HKAS 45445, holotypus). **e.** *Z. olivaceobrunnea* (HKAS 52275, holotypus). **f.** *Z. roseola* (HKAS 51137). *Bars*=10 μm

pink hymenophore, the bluish colour changes when injured and broad spores up to 7.5 μm in width. It is easy to separate this species from the remaining taxa in *Zangia*, and the species in *T.* subgen. *Roseoscabra*.

2. *Zangia citrina* Yan C. Li et Zhu L. Yang, **sp. nov.** Figures 3b, 4b and 6

MycoBank: MB 518867

Etymology citrina, lemon-yellow, on account of the colour of the pileus.

Pileus 2–5 cm latus, hemisphaericus vel applanatus, viscidus, levis vel rugosus, citrinus, luteolus vel pallido-luteolus; contextus albidus, immutatus cum contusus. Hymenophorum pallidum cum immaturum, incarnatum cum maturum, depressum ad stipitem. Stipes 4–7×0.4–0.7 cm, scabrosus, purpureus ad apicem, aureus ad basem; contextus albidus vel pallidus ad apicem, aureus ad basem, immutatus cum contusus. Pileipellis ixohyphoepithelium est. Basidiosporae (10)11–13.5(14)×(4)4.5–5.5 μm, subfusiformes vel ellipsoideae, incoloratae vel pallido-incarnatae, glabrae. Pleuro- and cheilocystidia 23–61×8.5–13 μm, subfusiformia vel elongato-clavata. Fibulae in carposomate nullae.

Holotypus CHINA, FUJIAN PROVINCE: Sanming Nature Reserve of *Castanopsis kawakamii*, alt. 260 m, 24 August 2007, Y. C. Li 997 (HKAS 52684).

Pileus 2–5 cm in diam., hemispherical to applanate, viscid when wet, nearly smooth to rugose, lemon-yellow (2A7-8), yellowish (2A4-5) to pale yellow (3A3) but margin much paler; context white to pallid white, unchanged in colour when bruised. Hymenophore free to subfree, depressed around apex of stipe, white (1A1)

Fig. 4 Habitat of *Zangia* species. **a.** *Z. chlorinosma* (HKAS 48695). **b.** *Z. citrina* (HKAS 52684, holotypus). **c.** *Z. erythrocephala* (HKAS 43032). **d.** *Z. olivacea* (HKAS 45445, holotypus). **e.** *Z. olivaceobrunnea* (HKAS 54442). **f.** *Z. roseola* (HKAS 51137)

initially, to pink (12A3-4) or grayish rose coloured (11B3-5) when mature; pores small, up to 0.5 mm in diam., unchanging in colour when injured. Spore print pinkish (13A2) to pink (12A3-4). Stipe 4–7×0.4–0.7 cm, clavate, sometimes enlarged downwards, yellow but white to yellowish at apex and bright yellow at base, sparsely or densely covered with pink to purple pink scabrous squamules; mycelia on base of stipe chrome yellow to golden yellow; context yellowish to yellow or bright yellow, but white to cream at apex and golden yellow to chrome yellow at base, unchanged in colour when bruised or when injured. Taste and odor mild.

Basidia 18–37×10–15 μm, clavate, hyaline in KOH and yellowish in Melzer's reagent, 4-spored, rarely 2-spored. Basidiospores (250/8/8) (10)11–13.5(14)×(4)4.5–5.5 μm, [Q = (2)2.18–2.86(3), Q = 2.53±0.19], ellipsoid to somewhat oblong, smooth, slightly thick walled (up to 0.5 μm in thickness), subhyaline to light olivaceous in KOH and yellowish brown in Melzer's reagent. Pleuro- and cheilocystidia 23–61×8.5–13 μm, subfusiform, to ventricose or ventricose-rostrate to strangulated, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Caulocystidia forming scabrous squamules over surface of stipe, clavate or subfusoid-mucronate to ventricose-mucronate, some with 1–2 fibrillous or flagelliform terminal cells. Pileipellis an ixohyphoepithelium, outer layer consisting of yellow to yellowish brown interwoven hyphae 4–8 μm in width with terminal cells 22–76×4–8 μm, inner layer made up of hyaline to yellowish inflated cells up to 25 μm in width arising from radially arranged, repent filamentous hyphae 2.5–4.5 μm in width. Pileal trama composed of hyaline to brownish yellow interwoven hyphae up to 11 μm in width. Clamp connections absent.

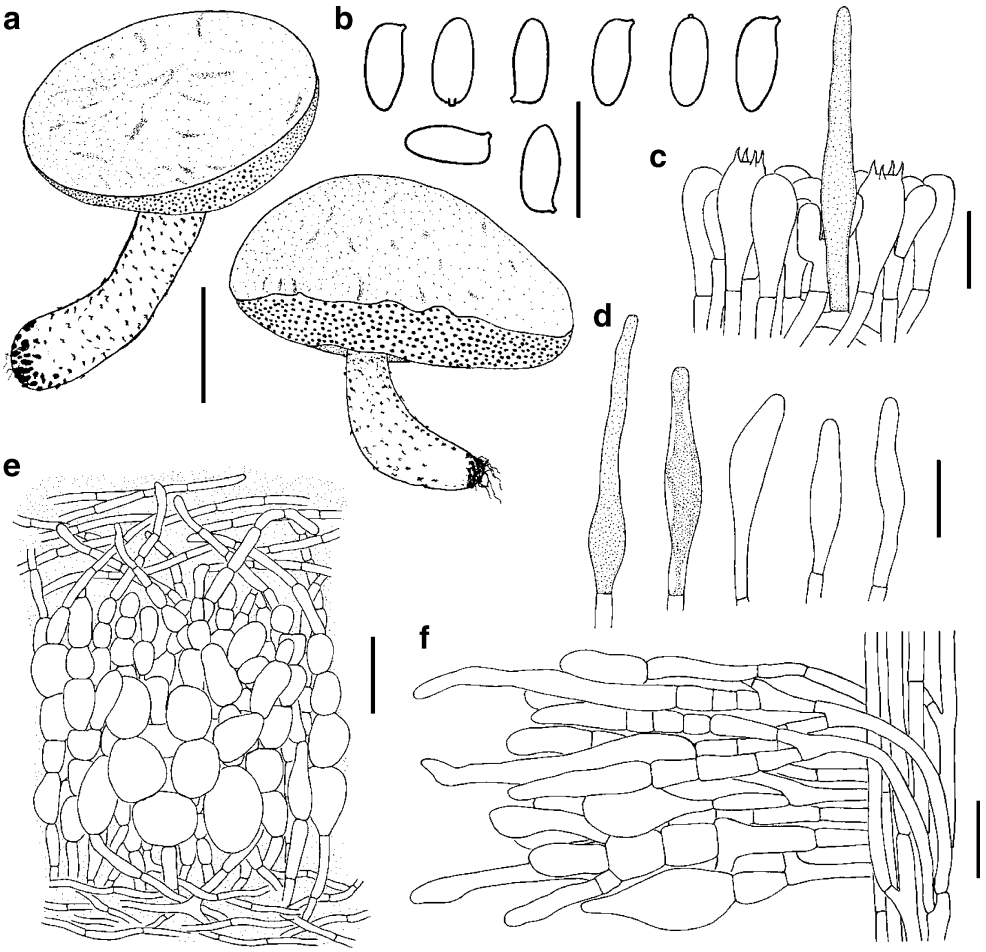

Fig. 5 *Zangia chlorinosma* (from holotype of *T. chlorinosmus*). **a.** Basidiomata. **b.** Basidiospores. **c.** Basidia and pleurocystidium. **d.** Pleuro- and cheilocystidia. **e.** Pileipellis. **f.** Stiptipellis. *Bars*: a=2 cm; b–d, f=20 μm; e=40 μm

Habitat solitary to scattered, in forest of *Castanopsis kawakamii*, or in the forest of *Quercus acutissima*, or in the mixed forests dominated with *Castanopsis* spp.

Known distribution Currently only known from southern and southeastern China.

Additional materials examined CHINA, FUJIAN PROVINCE: Sanming Nature Reserve of *Castanopsis kawakamii*, 24 August 2007, alt. 260 m, Y. C. Li 990 (HKAS 52677), and same location, 25 August 2007, Y. C. Li 1000 (HKAS 53345) and Y. C. Li 1001 (HKAS 53346), and 27 August 2007, Y. C. Li 1039 (HKAS 53384). GUANGDONG PROVINCE: Zhaoqing, 6 September 1998, alt. 300 m, M. Zang and Z. L. Yang 12901 (HKAS 32756). HUNAN PROVINCE: Liuyang County, Daweishan National Forest Park, 10 July 2003, alt. 1000 m, H. C. Wang 296 (HKAS 42457); Yizhang County, Mangshan National Forest Park, 29 September 1981, Y. C. Zong and X. L. Mao 52 (HMAS 42680).

Notes Zangia citrina has a distinct lemon-yellow, yellowish to pale yellowish pileus, a pinkish to pink hymenophore and a white to yellowish stipe without colour changes when injured. Such traits are very similar to those of *Fistulinella lutea* Redeuilh et Soop. However, the latter species has a nearly glabrous stipe, a white to grayish white stipe context, a white mycelium on the base of the stipe, a cuticle-like pileipellis without inflated cells and larger spores measuring 13–17.5×4.5–6.5 μm (Redeuilh and Soop 2006).

3. *Zangia erythrocephala* Yan C. Li et Zhu L. Yang, **sp. nov.** Figures 3c, 4c and 7

MycoBank: MB 518868

Etymology erythrocephala, red cap, named after the red coloured cap.

Pileus 3–8 cm latus, hemisphaericus, convexus vel subconvexus, viscidus vel siccus, verrucosus ad puberulus vel rugosus, coccineus, rubidus, brunneolo-purpureus vel purpureus, interdum rufobrunneus; contextus albidus, immutatus cum contusus. Hymenophorum pallidum cum immaturum, incarnatum vel purpureum cum maturum, depressum ad stipitem. Stipe 4–9×0.5–1.2 cm, centralis,

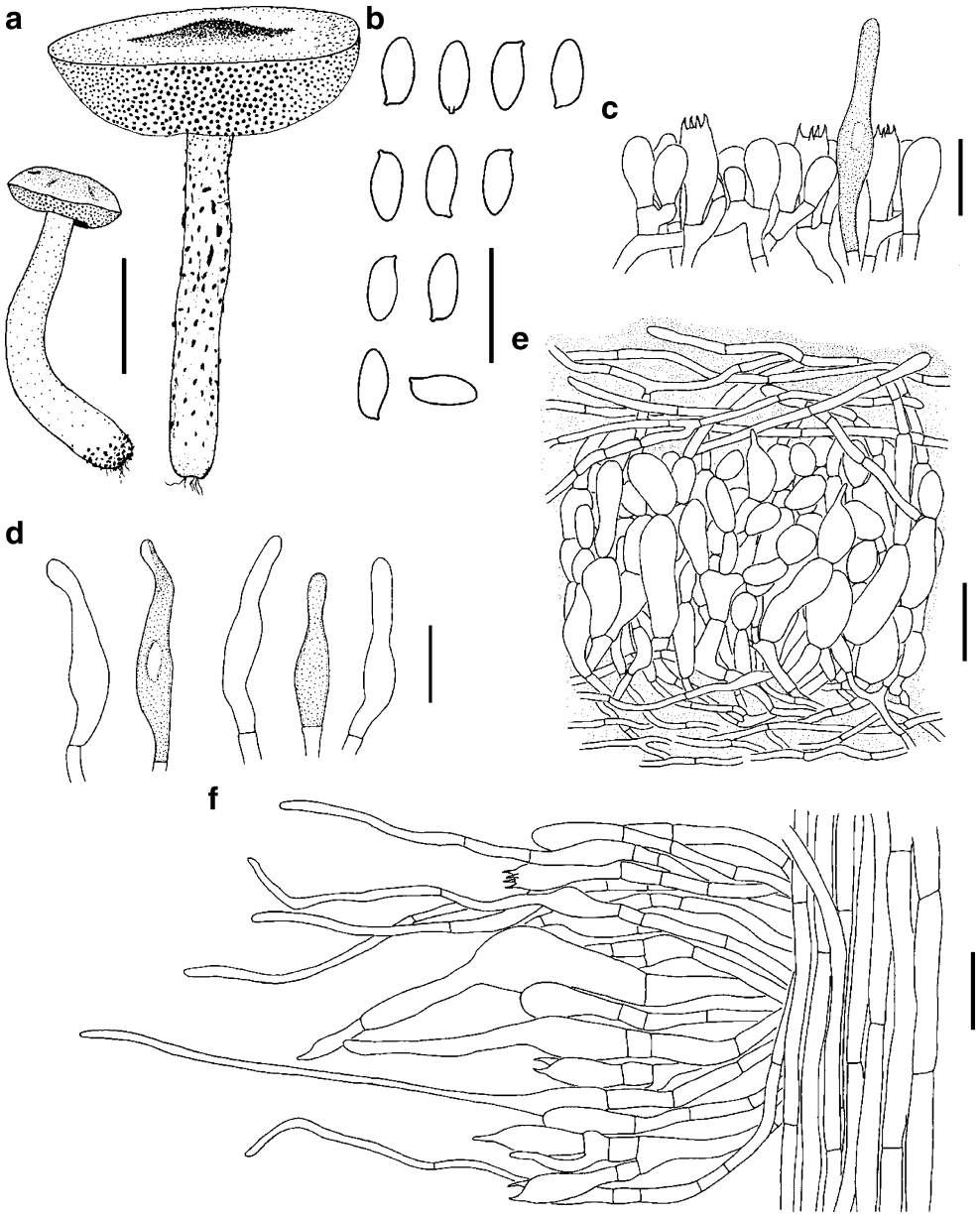

Fig. 6 *Zangia citrina* (from holotype). **a**. Basidiomata. **b.** Basidiospores. **c.** Basidia and pleurocystidium. **d.** Pleuro- and cheilocystidia. **e.** Pileipellis. **f.** Stiptipellis. *Bars*: a=2 cm; b–d, f=20 μm; e=40 μm

scabrosus, purpureus ad apicem, aureus ad basem; contextus pallidus vel lacteus, sed albidus vel pallidus ad apicem, aureus ad basem, cyanescens cum expositus. Pileipellis ixohyphoepithelium est. Basidiosporae (11.5) 12–15(16.5)×5.5–6.5(7) μm, ellipsoideae, incoloratae vel pallido-incarnatae, glabrae. Pleuro- atque cheilocystidia 26–75×5–11 μm, subcylindrica vel elongato-clavata. Fibulae in carposomate nullae.

Holotypus CHINA, YUNNAN PROVINCE: Shangri-La County, Geza, Hong Mountain, 9 September 2007, alt. 3400 m, B. Feng 123 (HKAS 52844).

Pileus 3–8 cm in diam., hemispherical to convex or applanate, viscid when wet, finely rugose and pubescent, red (10A8), dark red (11C7-8) or brownish purple (9C6-8) to purple (11D7-8) with reddish tinge, sometimes reddish brown (12C7-8), margin often with pale yellowish tinge; context white to cream, unchanged in colour when injured. Hymenophore free to subfree or sinuate, depressed around apex of stipe, white (1A1) initially, pinkish (13A2) to pink (12A3-4) when mature; pores 0.5–1 mm in diam., with scattered rust brown stains when old or bruised, without bluish colour changes when injured; tubes relatively long, up to 20 mm in length, dirty white to pinkish. Spore print pinkish (13A2), pink (12A3-4) to purplish pink (14A3-5). Stipe 4–9×0.5–1.2 cm, subcylindrical, attenuated upwards, enlarged downwards, yellowish purplish or pinkish to pink upwards but apical part white and base bright yellow to chrome yellow, with purplish

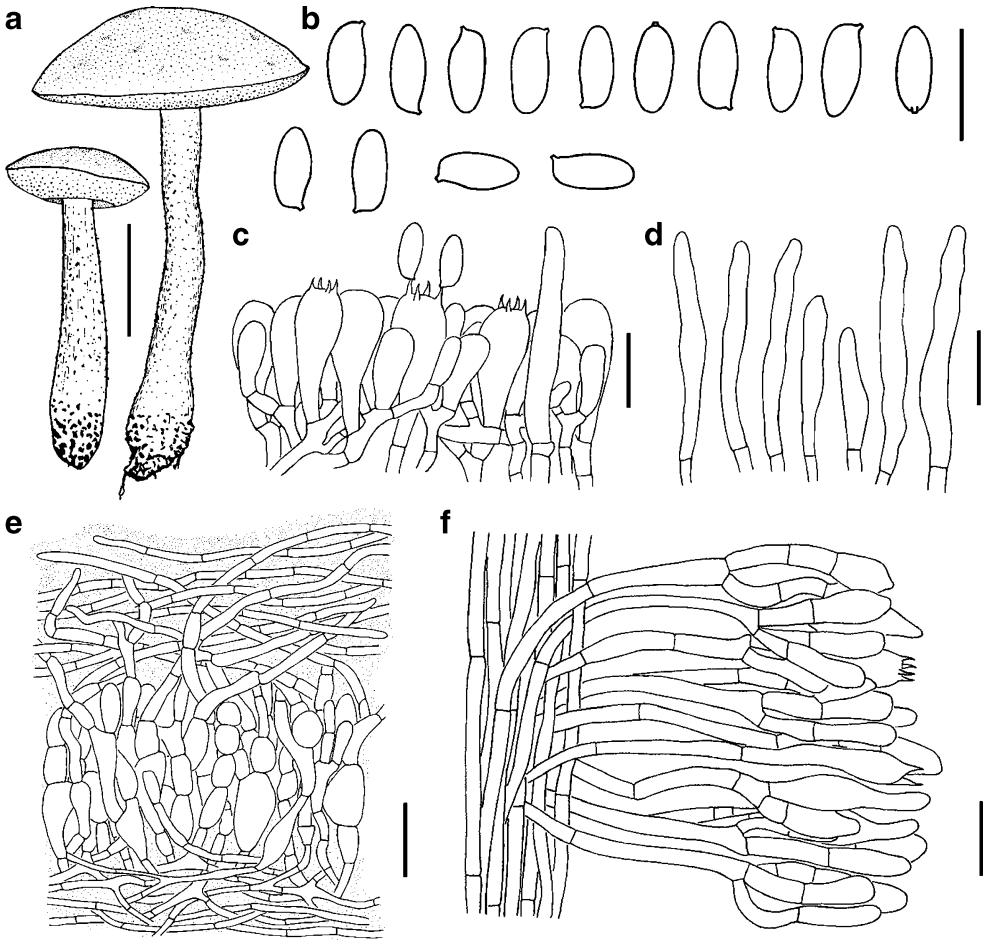

Fig. 7 *Zangia erythrocephala* (from holotype). **a.** Basidiomata. **b.** Basidiospores. **c.** Basidia and pleurocystidium. **d.** Pleuro- and cheilocystidia. **e.** Pileipellis. **f.** Stiptipellis. *Bars:* a=2 cm; b–d, f=20 μm; e=40 μm

to pink scabrous squamules, sometimes lower part with indistinct shallow reticulation; mycelia on base of stipe golden yellow; context cream to yellowish in upper part and bright yellow at base, asymmetrically bluish when bruised. Taste and odor mild.

Basidia 32–41×13–16 μm, clavate, hyaline in KOH and yellowish in Melzer's reagent, 4-spored, sometimes 2-spored. Basidiospores (220/11/11) (11.5)12–15(16.5)×5.5–6.5(7) μm, [Q = (1.79)1.92–2.5(2.64), Q = 2.24±0.20], ellipsoid to somewhat oblong, smooth, wall slightly thickened but less than 1 μm, yellowish brown or brownish or nearly colourless in KOH and yellow brown in Melzer's reagent. Pleuro- and cheilocystidia 26–75×5–11 μm, subcylindrical to lanceolate, or ventricose-mucronate with a long pedicel, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Caulocystidia forming scabrous squamules over surface of stipe, similar to pleuro- and cheilocystidia morphologically but much shorter. Pileipellis an ixohyphoepithelium, outer layer consisting of yellow to yellowish brown interwoven hyphae 4–6 μm in width with terminal cell 18–63×3–5 μm, inner layer made up of inflated cells up to 20 μm in width arising from radially arranged, repent filamentous hyphae 2–5 μm in width. Pileal trama composed of broad interwoven hyphae up to 20 μm in width. Clamp connections absent.

Habitat solitary to scattered, in mixed forests of *Pinus densata*, *Picea* spp. and *Quercus aquifolioides*.

Known distribution Currently only known from southwestern China.

Additional materials examined CHINA, YUNNAN PROVINCE: Jianchuan County, Shibaoshan, 11 September 2001, alt. 2600 m, X. H. Wang 1417 (HKAS 39313), and same location, 14 August 2003, alt. 2600 m, Z. L. Yang 4002 (HKAS 43032); Lijiang County, Heibaishui, 30 July 2001, alt. 2900 m, Z. L. Yang 3121 (HKAS 38298), and same location, 22 July 2008, alt. 2900 m, Y. C. Li 1325 (HKAS 56179); Lijiang County, Yulong Snow Mountain, September 1986, alt. 3100 m, R. H. Petersen 56400 (HKAS 20036) and same location, 4 August 2005, Z. L. Yang 4532A (HKAS 48696A); Shangri-La County, Geza, Hong Mountain, 9 September 2007, alt. 3400 m, B. Feng 122 (HKAS 52843); Shangri-La County, Haba Snow Mountain, 12 August. 2008, alt. 2800 m, Y. C. Li 1433 (HKAS 56273)

and Y. C. Li 1434 (HKAS 56274), same location, 14 August 2008, alt. 3475 m, Y. C. Li 1472 (HKAS 56312).

Notes Zangia erythrocephala is characterized by the red to dark red or brownish red pileus, which is similar to *Z. roseola*. However, *Z. erythrocephala* has a much broader pileus (up to 8 cm), narrower spores (6–6.5 μm in width) and cystidia (5.5–8 μm in width), and mainly distributed in subalpine to alpine regions and associated with *Pinus densata*, *Picea* spp. and *Quercus aquifolioides*, while *Z. roseola* has much smaller pileus (up to 4 cm), broader spores up to 8 μm and cystidia (5–18 μm in width), and is distributed in subtropical regions and associated with *Pinus armandii*, *P. yunnanensis*, *Quercus variabilis*, *Lithocarpus* spp., *Castanopsis* spp, and *Keteleeria* spp.

4. *Zangia olivacea* Yan C. Li et Zhu L. Yang, **sp. nov.** Figures 3d, 4d and 8

MycoBank: MB 518869

Etymology olivacea, olive green, referring to the colour of the pileus.

Pileus 4–7 cm latus, hemisphaericus, convexus vel applanatus, siccus, sed humidus viscidus, rugosus, olivaceus, viridifuscus vel brunneolo-olivaceus; contextus albidus, immutatus cum contusus. Hymenophorum albidum vel pallidum cum immaturum, incarnatum cum maturum, depressum ad stipitem. Stipes 8–13×1–2 cm, centralis, scabrosus, purpureus ad apicem, aureus ad basem; contextus albidus vel pallidus ad apicem, aureus ad basem, cyanescens cum expositus. Pileipellis ixohyphoepithelium est. Basidiosporae (12)12.5–15.5(17)×6–7 μm, ellipsoideae, incoloratae vel pallido-incarnatae, glabrae. Pleuro- atque cheilocystidia 32–61×6–12 μm, subfusiformia vel elongato-clavata. Fibulae in carposomate nullae.

Holotypus CHINA, YUNNAN PROVINCE: Shangri-La County, Daxueshan Mountain, alt. 3100 m, 6 July 2004, Z. L. Yang 3960 (HKAS 45445).

Pileus 4–7 cm in diam., hemispherical to convex or applanate, dry, but viscid when wet, rugose, olive green (30D5-7) to greenish brown (28E5-6) or brownish olivaceous (28 F8); context white to cream. Hymenophore free to subfree, depressed around stipe, white (1A1) initially, pinkish (13A2) to pink (12A3-4) when mature; pores small,

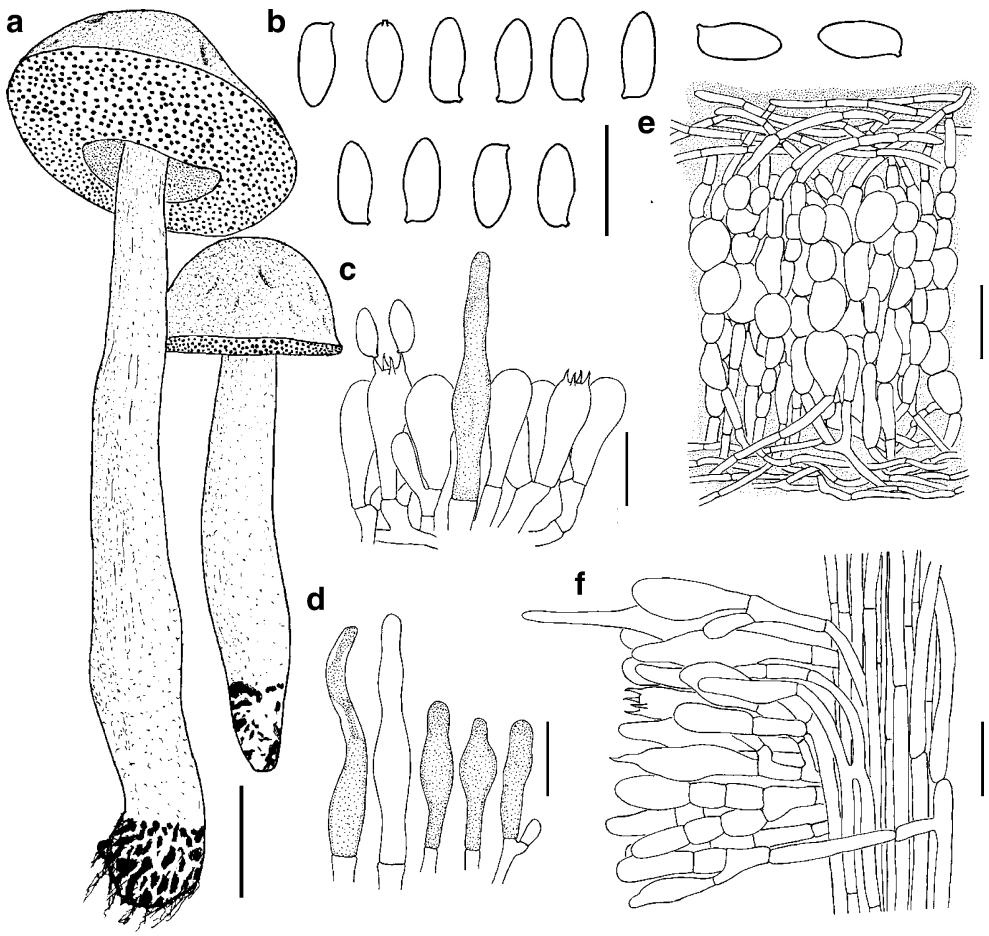

Fig. 8 *Zangia olivacea* (from holotype). **a.** Basidiomata. **b.** Basidiospores. **c.** Basidia and pleurocystidium. **d.** Pleuro- and cheilocystidia. **e.** Pileipellis. **f.** Stiptipellis. *Bars:* a=2 cm; b–d, f=20 μm; e=40 μm

about 0.5 mm in diam., with scattered rust brown stains when old or bruised; tubes relatively long, up to 15 mm in length. Spore print pinkish (13A2) to pink (12A3-4). Stipe 8–13×1–2 cm, clavate, attenuated upwards, enlarged downwards, white to cream but apical part white and base bright yellow to chrome yellow, with purplish to pink scabrous squamules, unchanging in colour when injured; mycelia on base of stipe golden yellow; context cream to yellowish but bright yellow at base, becoming bluish slowly when injured. Taste and odor mild.

Basidia 23–37×10–15 μm, clavate, hyaline in KOH and yellowish in Melzer's reagent, 4-spored, sometimes 2-spored. Basidiospores (117/3/3) (12)12.5–15.5(17)×6–7 μm, [Q = (1.85)1.92–2.5(2.58), Q = 2.18±0.16], ellipsoid to somewhat oblong, smooth, walls slightly thickened (up to 0.5 μm in thickness), light olivaceous to nearly colourless in KOH and yellowish brown in Melzer's reagent. Pleuro- and cheilocystidia 32–61×6–12 μm, subfusoid-mucronate to lanceolate, or ventricose-mucronate with a long pedicel, thin-walled, hyaline in KOH and yellowish to yellow in Melzer's reagent. Caulocystidia forming scabers over surface of stipe, 20–56×6–11 μm, similar to pleuro- and cheilocystidia morphologically. Pileipellis an ixohyphoepithelium, outer layer consisting of yellow to yellowish brown interwoven hyphae 3.5–7 μm in width, with terminal cell 20–44×4–5 μm, inner layer made up of inflated cells up to 23 μm in width arising from radially arranged, repent filamentous hyphae 3–6 μm in width. Pileal trama composed of broad interwoven hyphae up to 10 μm in width. Clamp connections absent.

Habitat solitary to scattered, in mixed forests of *Quercus semicarpifolia*, *Pinus densata* and *Picea* spp.

Known distribution Currently only known from southwestern China.

Additional materials examined CHINA, YUNNAN: Shangri-La County, 26 July 2006, alt. 3300 m, Z. W. Ge 1086 (HKAS 55830); Lijiang County, Tianwentai, 20 July 2008, alt. 3200 m, Y. C. Li 1294 (HKAS 55831).

Notes Zangia olivacea is characterized by its ixohyphoepithelium pileipellis, the white to cream context in the pileus and the yellow to bright yellow context in the stipe which is asymmetrically bluish when hurt, and broader spores up to 7 μm in width and pink to red scabrous squamules on the stipe. The olive green to greenish brown or brownish olive coloured pileus in *Z. olivacea* is somewhat similar to that in *Tylopilus virens* (W.F. Chiu) Hongo. However, the latter species has a nearly glabrous pileus with filamentous interwoven hyphae in the pileipellis, a yellow to bright yellow context which is unchanged in colour when injured and narrower spores up to 5 μm in width (Chiu 1948; Hongo 1964; our own unpublished data).

5. *Zangia olivaceobrunnea* Yan C. Li et Zhu L. Yang, **sp. nov.** Figures 3e, 4e and 9

MycoBank: MB 518870

Etymology olivaceobrunnea, olivaceous brown, referring to the colour of the pileus.

Pileus 4–6 cm latus, convexus vel hemisphaericus, viscidus vel siccus, verrucosus ad puberulus, brunneolo-olivaceus, olivaceo-brunneus, rufobrunneus rufo-olivaceus; contextus albidus, immutatus cum contusus. Hymenophorum pallidum cum immaturum, incarnatum cum maturum, depressum ad stipitem. Stipes 6–12×0.4–1 cm, centralis, scabrosus, purpureus ad apicem, aureus ad basem; contextus albidus vel pallidus ad apicem, aureus ad basem, cyanescens cum expositus. Pileipellis ixohyphoepithelium est. Basidiosporae (12)12.5–15.5(17)×(4.5)5–6(6.5) μm, ellipsoideae, incoloratae vel pallido-incarnatae, glabrae. Pleuro- atque cheilocystidia 19–70×4–12.5 μm, subfusiformia vel elongato-clavata. Fibulae in carposomate nullae.

Holotypus CHINA, YUNNAN PROVINCE: Kunming, Heilongtan Park, alt. 2000 m, 9 September 2007, Z. L. Yang 4960 (HKAS 52275).

Pileus 4–6 cm in diam., convex to hemispherical, dry, slightly viscid when wet, finely rugose, brownish olivaceous (28 F8) with reddish tinge or dull purplish brown (11D7-8) to reddish brown (9D7-8) or olive red to olive reddish brown, with grayish tinge, paler in colour towards pileal margin, grayish green (1C4-5) to greenish grey (1B2) or grayish white (1B1); context white to cream or yellowish, rarely pale blue. Hymenophore free to subfree, deeply depressed around apex of stipe, white (1A1) initially, pinkish white (13A2) to pink (12A3-4) or grayish rose coloured (11B3-5) when mature, without bluish colour changes; pores small, about 0.5–1 mm in diam., concolourous with surface of hymenophore, often with rust brown stains when old or bruised; tubes relatively long, up to 12 mm in length. Spore print pink (12A3-4) to purplish pink (14A3-5) or pale purple (14A2). Stipe 6–12×0.4–1 cm, subcylindrical or tapering upwards, dirty white to cream or pale yellow but much paler at apical part and bright yellow to chrome yellow at base, bluish when injured; surface densely covered with pink to red or purple scabrous squamules, becoming blue slowly when injured; mycelia on base of stipe golden yellow; context yellowish

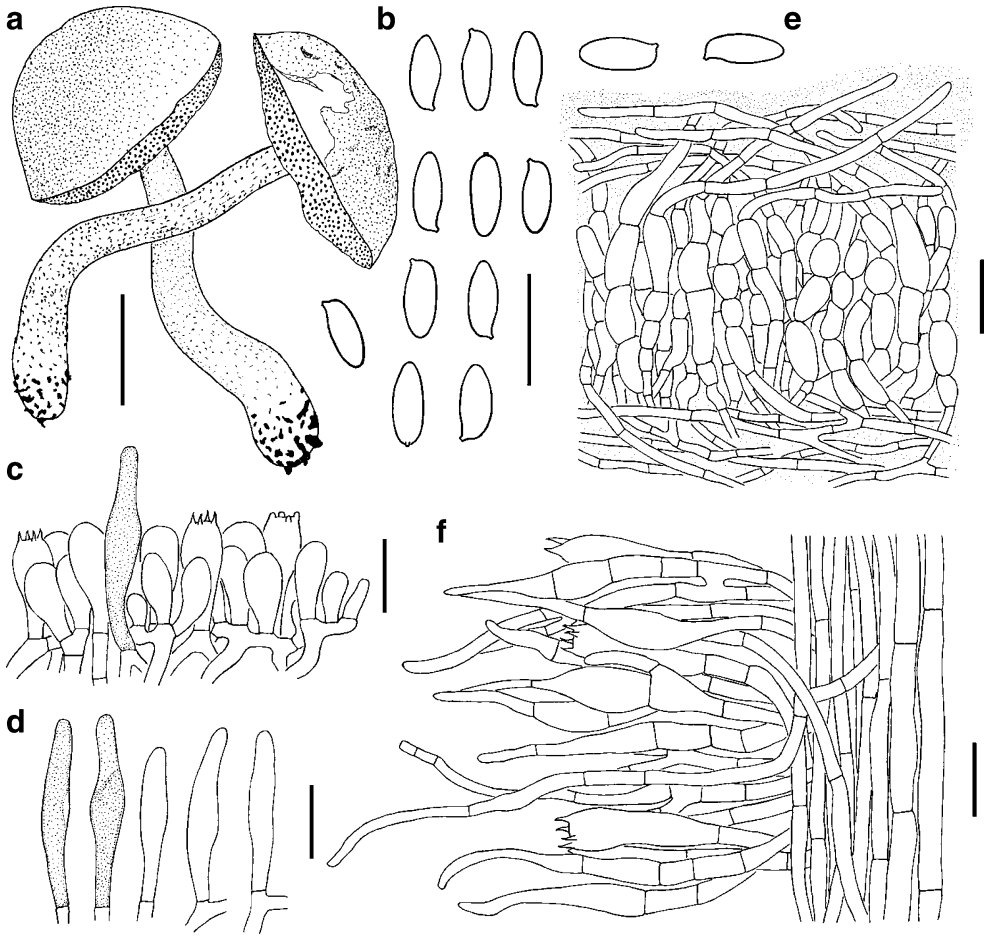

Fig. 9 *Zangia olivaceobrunnea* (from holotype). **a.** Basidiomata. **b.** Basidiospores. **c.** Basidia and pleurocystidium. **d.** Pleuro- and cheilocystidia. **e.** Pileipellis. **f.** Stiptipellis. *Bars*: a=2 cm; b–d, f=20 μm; e=40 μm

to yellow but golden yellow at base, becoming blue slowly when injured. Taste and odor mild.

Basidia 21–36×10–15 μm, clavate, hyaline in KOH and pale yellow in Melzer's reagent, 4-spored, sometimes 2-spored. Basidiospores (280/12/8) (12)12.5–15.5(17)×(4.5) 5–6(6.5) μm, [Q = (2)2.36–2.91(3.11), Q=2.62±0.19], ellipsoid to somewhat oblong, smooth, slightly thick walled (up to 0.5 μm in thickness), light olivaceous to nearly colourless in KOH and yellowish brown in Melzer's reagent. Pleuro- and cheilocystidia 19–70×4–12.5 μm, subfusoid-mucronate to lanceolate, or ventricose-mucronate with a long pedicel, thin-walled, hyaline in KOH and pale yellow to yellow in Melzer's reagent. Caulocystidia forming scabers over surface of stipe, clavate to narrowly fusoid ventricose or sublanceolate, many with distal secondary septum. Pileipellis an ixohyphoepithelium, outer layer consisting of yellow to yellowish brown interwoven hyphae 4–13 μm in width with terminal cells 36–63×4–6 μm, inner layer made up of inflated cells up to 17 μm in width arising from radially arranged, repent filamentous hyphae 4–7 μm in width. Pileal trama composed of broad interwoven hyphae up to 11 μm in width. Clamp connections absent.

Habitat solitary to scattered, in mixed forests of *Quercus variabilis* and *Keteleeria* spp. or in mixed forests of *Quercus variabilis*, *Pinus yunnanensis* and *Keteleeria* spp.

Known distribution currently only known from southwestern China.

Additional materials examined CHINA, YUNNAN PROVINCE: Chuxiong, Nanhua Wild Mushroom Market, 3 August 2009, Y. C. Li 1961 (HKAS 59220) and Y. C. Li 1962 (HKAS 59221); Kunming, Heilongtan Park, 8 September 2007, alt. 1980 m, Z. L. Yang 4955 (HKAS 52272); same location, 16 August 2008, alt. 1980 m, Z. L. Yang 5145 (HKAS 54442); same location, 28 August 2008, alt. 1980 m, Y. C. Li 1575 (HKAS 55511) and Y. C. Li 1576 (HKAS 55512); same location, 6 August 2007, alt. 1980 m, Y. C. Li 961 (HKAS 52648).

Notes Zangia olivaceobrunnea has a reddish olivaceous brown or dull purplish brown to reddish brown or olive reddish brown pileus, bluish colour changes in the stipe when injured and narrow spores most ranging from 5 to 6 μm in width. It is associated with subtropical trees.

Zangia olivacea is somewhat similar to *Z. olivaceobrunnea* in the colour of the pileus. However, *Z. olivacea* has broader spores up to 7 μm in width, and associated with alpine to subalpine trees. Although the morphological divergences between these two taxa were limited, the molecular divergences between them are great. They likely started diverging from each other very early.

6. *Zangia roseola* (W.F. Chiu) Yan C. Li et Zhu L. Yang, **comb. nov.** Figures 3f, 4f and 10

Basionym: *Boletus roseolus* W.F. Chiu, Mycologia 40: 208, 1948

Tylopilus roseolus (W.F. Chiu) F.L. Tai, Syll. Fung. Sinic.: 758, 1979

MycoBank: MB 518871

Pileus 2–4 cm in diam., hemispherical to convex, dry or slightly viscid, finely rugose, carnelian purplish red to purple red or dull red but becoming pinkish or even white towards the pileal margin, covered with white to cream or brownish pubescence when young but soon glabrous; context white to cream with purplish tinge beneath pileipellis rarely bluish when injured. Hymenophore free to subfree or sinuate around apex of stipe, white initially, pinkish to pink when mature; pores small, about 0.5 mm in diam., with scattered rust brown stains when old or bruised; tubes 5–10 mm in depth, concolourous with the pores. Spore print pinkish, pink or pale purple. Stipe 4–7×0.3–0.6 cm, subcylindrical or slightly attenuate upwards, base sometimes with globose bulb; surface pinkish to purplish or purple, but white to cream or yellowish at apical part and bright yellow to chrome yellow at base, whole surface covered with minute squamules, the colour corresponding to that of the stipe surface; mycelia on base of stipe chrome yellow to golden yellow; context cream to yellowish but golden yellow at base, becoming blue slowly when injured. Taste and odor not distinctive.

Basidia 26–38×10–15 μm, clavate, hyaline in KOH and yellowish in Melzer's reagent, 4-spored, sometimes 2-spored. Basidiospores (550/20/11) 13–16(17)×(5.5)6–7(8) μm, [Q = (2)2.14–2.54(2.83), Q=2.31±0.19], ellipsoid to somewhat oblong, smooth, light olivaceous to pale mellow in KOH and yellowish brown in Melzer's reagent. Pleuro- and cheilocystidia 30–83×5–18 μm, clavate to subfusiform, thin-walled, colourless and hyaline in KOH and yellowish to yellow in Melzer's reagent. Pileipellis an ixohyphoepithelium, outer layer consisting of yellow to yellowish brown interwoven hyphae 4–6 μm in width, lower layer made up of inflated cells up to 20 μm in width arising from radially arranged, repent filamentous hyphae 3–6 μm in width. Pileal trama composed of broad interwoven hyphae up to 5–10 μm in width. Stipe dermatocystidia ventricose, fusoid ventricose, clavate, or lanceolate, pale yellow in KOH. Clamp connections absent.

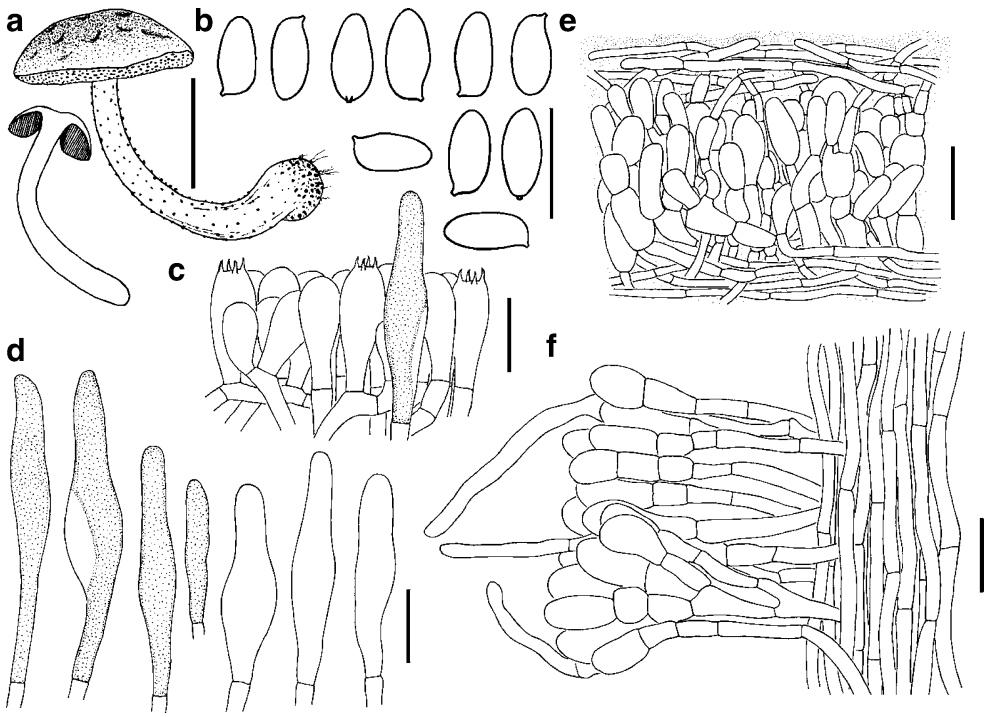

Fig. 10 *Zangia roseola* (from HKAS 51137). **a.** Basidiomata. **b.** Basidiospores. **c.** Basidia and pleurocystidium. **d.** Pleuro- and cheilocystidia. **e.** Pileipellis. **f.** Stiptipellis. *Bars*: a=2 cm; b–e=20 μm; f=40 μm

Habitat solitary to scattered, in mixed forests of *Pinus armandii*, *P. yunnanensis*, *Quercus variabilis*, *Lithocarpus* spp., *Castanopsis* spp., and *Keteleeria* spp.

Known distribution Currently only known from southwestern China.

Materials examined CHINA, YUNNAN PROVINCE: Binchuan, Jizushan, 5 August 1989, Y. C. Zong and Y. Li y113 (HMAS 72099); Chuxiong, Nanhua Wild Mushroom Market, 3 August 2009, Y. C. Li 1958 (HKAS 59219); Kunming, free market, 27 July 1938, JC. Zhou 7889 (HMAS 3889, isotype of *Boletus roseolus* in Chiu 1948); Kunming, Heilongtan Park, 8 September 2007, alt. 1900 m, Z. L. Yang 4956 (HKAS 52273), same location, 9 September 2007, alt. 1900 m, Z. L. Yang 4962 (HKAS 52277); Kunming, Qiongzhu Temple, 21 September 2006, alt. 2100 m, Y. C. Li 700 (HKAS 51137), same location, 8 August 2007, Y. C. Li 962 (HKAS 52649) and 8 August 2007, Z. L. Yang 4903 (HKAS 52220); Kunming Xishan, 10 August 2007, alt. 2050 m, Y. C. Li 980 (HKAS 52667); Lijiang City, Xiangshan Mountain, 17 July 2008, alt. 2450 m, Y. C. Li 1245 (HKAS 56099); Lijiang City, Yulong County, 17 July 2008, alt. 2400 m, Y. C. Li 1281 (HKAS 56135).

Notes Zangia roseola is characterized by the small (2–4 cm in diam.), carnelian purplish red to purple red or dull red pileus, the pinkish to purple pink scabrous stipe, the pinkish to pink or purple hymenophore, and broad basidiospores (up to 7 μm). Chiu (1948) described *Boletus roseolus* as a new species based on the collections from Binchuan and Kunming, Yunnan Province. This species was then transferred to *Tylopilus* due to the colour of the hymenophore and basidiospores (Tai 1979). However, the ixohyphoepithelium pileipellis, the coarsely verrucose stipe and bluish colour changes in the stipe are different from those in the genus *Tylopilus* typified by *T. felleus* (Bull.) P. Karst. or in *T.* subgen. *Roseoscabra* typed by *T. chromapes*. Thus, a new combination is proposed.

In this study, six species of *Zangia* from China could be recognized and identified. For the convenience of identification of the species, a key to them is given below.

Key to the species in *Zangia*

1. Pileus neither lemon-yellow nor pale yellow; stipe bluish slowly when injured; associated with alpine, subalpine or sometimes subtropical tree hosts..... 2
1. Pileus lemon-yellow to pale yellow; stipe without colour changes when injured; subtropical, associated with subtropical trees of *Castanopsis* and *Quercus*..... *Z. citrina*
2. Pileus with red, bright red or purple red tinge.....3
2. Pileus without red, bright red or purple red tinge.....4
3. Pileus up to 8 cm in diam., red, dark red or brownish red; spores up to 6.5 μm and cystidia up to 8 μm in width; mainly associated with subalpine to alpine trees.....*Z. erythrocephala*
3. Pileus ≤4 cm in diam., carnelian purplish red to purple red or dull red; basidiospores up to 8 μm and cystidia up to 18 μm in width; associated with subtropical trees.....*Z. roseola*
4. Pileus with olivaceous tinge, without yellowish brown or orange yellow to honey yellowish tinge.....5
4. Pileus with yellowish brown or orange yellow to honey yellowish tinge; basidiospores (12)13–15 (17)×(5.5)6–7(7.5) μm; mostly associated with subalpine to alpine trees.....*Z. chlorinosma*
5. Pileus reddish olivaceous-brown or dull purplish brown to reddish brown or olive reddish brown; basidiospores (12)12.5–15.5(17)×(4.5)5–6(6.5) μm; associated with subtropical trees....*Z. olivaceobrunnea*
5. Pileus olive green to greenish brown or brownish olivaceous; basidiospores (12)12.5–15.5(17)×6–7 μm; associated with subalpine to alpine trees.....*Z. olivacea*

Discussion

Zangia generally shares the same coloured hymenophore and spore deposit with *Austroboletus*, *Fistulinella*, *Tylopilus* s.s., and some species in *Porphyrellus*, but is characterized by pink scabrous squamules on the stipe which do not darken in age, the chrome yellow coloured base of the stipe, bluish colour changes on the stipe in some species, the ixohyphoepithelium pileipellis and smooth spores. *Austroboletus* is characterized by lightly to heavily ornamented spores with pits, warts or reticulations which are significantly different from the above genera (Wolfe 1979; Singer 1986; Fulgenzi et al. 2010). *Fistulinella* has a viscid to glutinous basidioma with a strongly gelatinized lateral stratum in the tube trama, a trichoderm, ixotrichoderm or ixocutis (Redeuilh and Soop 2006; Fulgenzi et al. 2010) but never an ixohyphoepithelium pileipellis. *Porphyrellus* has a trichoderm, an ixotrichoderm, subhymeniform pileipellis or trichoderm pileipellis formed from chains of short, cylindrical to submoniliform cells with cylindrical to clavate terminal cells (Singer 1986; Desjardin et al. 2009). Furthermore, *Porphyrellus* differs from *Zangia* in the

morphology of the stipe, the colour of the context and colour changes when bruised. The pink scabrous squamules on the stipe, and the chrome yellow coloured base of the stipe in *Zangia* are very similar to those in *Tylopilus* subgen. *Roseoscabra*. But species in *T.* subgen. *Roseoscabra* have an interwoven trichoderm pileipellis, and a context unchanging when bruised. The coarsely verrucose stipe in *Zangia* can be observed in species of *Leccinum*, which, however, possesses grayish, brownish or yellowish but never pink to pinkish hymenophores, a trichoderm to intricate trichoderm pileipellis and the ornamentations on the surface of the stipe usually become darker with age (den Bakker and Noordeloos 2005).

The genus *Zangia* was nested into the clade Boletaceae and clustered as a monophyletic group in all analyses on both datasets. Two main clades with significant support values were recovered within *Zangia*. There are four species, *Z. chlorinosma*, *Z. erythrocephala*, *Z. olivacea* and *Z. roseola*, in the first clade. The first three taxa of this clade are restricted in subalpine to alpine regions of the eastern Himalayas and Hengduan Mountains, and the last taxon was found both in subtropical environments and in montane temperate to alpine forests. The relationships among the four taxa are not well solved by the ML, MP and Bayesian analyses in both datasets. In addition, the diagnostic morphological characters among them are often subtle. It seems that these species might have started diverging from each other relatively recently with the uplifts of the eastern Himalayas and Hengduan Mountains (Yang 2005), and both the mycorrhizal host specificity or preference and geographic separation could contribute to their ongoing divergence. In the second clade consisting of *Z. citrina* and *Z. olivaceobrunnea*, the monophyly of these two species was highly supported in both datasets by the ML, MP and Bayesian analyses. Species in this clade have narrower spores (≤6 μm in width) and relatively lower distributions ranging from 250 m to 2000 m in altitude, and they are usually associated with subtropical to southern subtropical tree hosts.

In nrLSU dataset, the monophyly of *Zangia* with its sister group *Royoungia* Castellano, Trappe et Malajczuk was supported with 51% and 64% bootstrap (BS) value in the ML and MP analyses (Fig. 1). *Royoungia* is a truffle-like genus in Boletales, and different from *Zangia* by the bright golden yellow peridium and rhizomorphs, and the rubescent colour changes when bruised. The clade represented by the type species of *T.* subgen. *Roseoscabra*, *T. chromapes*, may well be in its own clade representing an independent genus, although the monophyletic relationship of *Zangia* with *T. chromapes* was strongly supported in the combined dataset with selected genera (Fig. 2). The trichoderm pileipellis and no discolourations in the stipe make *T.* subgen. *Roseoscabra* different from those of *Zangia*.

So far, the genus *Zangia* was only found in southern, southeastern and southwestern China in forests dominated by Fagaceae and Pinaceae. Species of the *T. chromapes* complex occur under *Betula*, *Quercus*, *Abies*, *Picea*, *Pinus*, *Tsuga*, *Populus* and *Salix* in eastern North America, and under *Acacia*, *Allocasuarina*, *Eucalyptus*, *Syncarpia*, *Xanthostemon*, and *Melaleuca* in Australia (Singer 1947; Smith and Thiers 1971; Corner 1972; Wolfe and Bougher 1993) and in mixed forests of *Pinus* and *Quercus* in China. *Tylopilus chromapes* can be found not only in temperate northeastern China at low elevations (up to 2000 m), but also in subalpine to alpine regions with high altitudes (up to 4300 m) in southwestern China, where a few species of the *Zangia* and *T. chromapes* complex have a sympatric distribution. As these species can form ectomycorrhizal associations with Fagaceae and Pinaceae, the same distribution range and host associations might induce the similarities in macro morphology.

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