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Phylogenetic and biogeographic relationships of *Chroogomphus* species as inferred from molecular and morphological data

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The relationships among species of the genus *Chroogomphus* were inferred from morphology and the nucleotide sequences of the internal transcribed spacer (ITS) regions of the nuclear ribosomal gene cluster. Our analysis revealed that: (1) the monophyly of *Chroogomphus* was well supported but a previous two-section infrageneric division of *Chroogomphus* is not valid. (2) Geographical divergences of *Chroogomphus* in the Northern Hemisphere were evident. Most species had relatively narrow distribution ranges, and taxa with intercontinental distributions were uncommon. In Far East Asia and central Europe, *C. rutilus* had a sympatric distribution pattern as *C. purpurascens* but with plant hosts in different subgenera of *Pinus*. Between East Asia and North America vicariously paired or closely related species of *Chroogomphus* were present. (3) During the co-evolution of *Chroogomphus* with its mycorrhizal hosts, host specificity generally increased yet host-switches between the two subgenera of *Pinus* took place. (4) Seven species were collected from China, four of which were described as new and illustrated with line drawings. In addition, a new combination, *C. albipes*, was proposed, and a key to the *Chroogomphus* species from China was provided.

Key words: *Boletales*, ectomycorrhiza, *Gomphidiaceae*, phylogeny, rDNA, taxonomy

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Introduction

Chroogomphus (Singer) O.K. Mill. was originally regarded as one of the three subgenera of the genus *Gomphidius* Fr. (Singer, 1949). Miller (1964) elevated *Gomphidius* subgen. *Chroogomphus* Singer to the level of a genus. Recent studies of the family *Gomphidiaceae* based on morphology and molecular data have verified the generic status of *Chroogomphus* (Miller and Aime, 2001; Miller, 2003). The genus *Chroogomphus* is mainly characterized by pale orange to ochraceous lamellae when young, an ochraceous pileal trama, a moist to viscid pileipellis and a mycelium at the base of the stipe consisting of amyloid hyphae. In contrast, the genus *Gomphidius* is primarily characterized by its pallid to whitish lamellae in young basidiomata, a white to pallid pileal trama, a glutinous pileipellis and a mycelium at the base of the stipe with dextrinoid to non-amyloid hyphae (Miller, 1964; Miller and Aime, 2001; Miller, 2003). While Index Fungorum listed twenty-nine named taxa at various levels in genus *Chroogomphus*, including species, subspecies and varieties (http://www.indexfungorum.org/-Name/Names.asp), only sixteen taxa have been accepted by recent studies (Villarreal and Heykoop, 1996; Miller and Aime, 2001; Miller, 2003; Watling, 2004).

Based on the records in herbaria in China and our recent field observations, species of *Chroogomphus* are widely distributed and highly diverse within China. The basidiomata of the genus are economically very important and often collected as food or herbal medicine (Xie *et al*., 1986; Mao *et al*., 1993; Mao, 1998, 2000; Wang *et al*., 2004; Yu and Liu, 2005; Dai and Tolgor, 2007). However, up to now only two species, i.e., *C. tomentosus* (Murrill) O.K. Mill. (≡ *Gomphidius tomentosus* Murrill) and *C. rutilus* (Schaeff.: Fr.) O.K. Mill. [≡ *G. rutilus* (Schaeff.: Fr.) S. Lundell], have been reported from China (Xie *et al.*, 1986; Mao *et al.*, 1993; Mao, 1998, 2000; Yu and Liu, 2005; Dai and Tu, 2007). As has been found in other groups of macrofungi (Yang *et al.*, 2001; Yang, 2005), the scarcity of report on this groups of mushrooms in China is likely due to our limited understanding and poor documentation, not due to the lack of materials in natural environment in China. For example, a recent re-analysis showed that some materials of "*C. tomentosus*" from China were in fact *C. pseudotomentosus* O.K. Mill. & Aime (Li, 2007). Whether *C. tomentosus*, originally described from western North America, occurs in China is still uncertain. Similarly, the distribution of *C. rutilus* in China or in East Asia as a whole has not been verified by molecular phylogenetic approach.

In this study, a phylogenetic investigation of *Chroogomphus* species and their related taxa was conducted using both morphological and molecular data. The ITS regions were used for systematics and biogeography in various fungal genera including *Chroogomphus* (e.g. Kretzer *et al.*, 1996; Miller and Aime, 2001; Mueller *et al.*, 2001; Miller *et al.*, 2002; Miller, 2003; Chen *et al.*, 2007; Alves *et al.*, 2008; Ge *et al.*, 2008; Paul *et al.*, 2008; Peláez *et al.*, 2008). The maximum parsimony approach was used to analyze the internal transcribed spacer (ITS) sequences. We attempt to (1) evaluate the phylogenetic relationships of the species within the genus *Chroogomphus*; (2) examine the geographic diversity and evolution of plant host specificity of *Chroogomphus*; (3) and elucidate the species diversity of *Chroogomphus* in China.

Materials and methods

Morphological studies

In the descriptions, macroscopic characters were based on the field notes and colour slides of the specimens. Colour codes of the form "11A3" indicate the plate, row, and colour block in Kornerup and Wanscher (1981). Microscopic characters were from the observations of the specimens through light microscopy. For microscopic observations, tissue fragments of dried materials were mounted in a 5% KOH aqueous solution and observed under the light phase. Melzer's reagent was used for testing colour reactions of the tissue fragments to the solution. All measurements were made in the KOH mounts. Specimens examined have been deposited in six herbaria: HKAS, HMAS, HMJAU, IFP, MB and WU. Herbarium codes followed Holmgren *et al.* (1990) with two exceptions: HKAS = Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences, and HMJAU = Herbarium of Jilin Agriculture University. Neither herbaria were listed in the index or relevant publications. The descriptions of species appear in alphabetical order by species epithet. The notations "basidiospores (n/m/p)" indicate that the measurements were made on n basidiospores from m basidiomata of p collections. The expressions (a)b–c(d) stand for the dimensions of basidiospores; the range b–c contains a minimum of 90% of the measured values, a and d in the brackets stand for the extreme values. The following abbreviations are used: Q (length/width of basidiospores) and Q_m (average Q ± standard deviation).

Molecular procedures and phylogenetic analysis

Total DNA was extracted from silica-gel-dried or herbarium materials using a modified CTAB procedure of Doyle and Doyle (1987). The internal transcribed spacer (ITS) regions were amplified by the polymerase chain reaction (PCR) with the primers ITS1-F and ITS4 (White *et al.*, 1990; Gardes and Bruns, 1993). PCR reactions contained 2 μl DNA solution (adjusted to approximately 20 ng), 5 μl PCR reaction buffer, 5 μl dNTP mix (0.2 mM), 2 μl each of the primers ITS1-F and ITS4 (5 μM), and 1.5 U TaqDNA polymerase. The final volume was adjusted to 50 μl with distilled sterile H_2O. The amplification conditions were set as follows: denaturation at 95 ºC for 4 min, 34 cycles of 30 s at 94 ºC, 2 min at 50 ºC, 1 min at 72 ºC, and a final extension of 5 min at 72 ºC. The PCR products were purified using the Bioteke's Purification Kit (Bioteke Corporation, Beijing, China), and sequenced with an ABI 3730 DNA analyzer and an ABI bigdye3.1 terminator cycle sequencing kit (Sangon Co., Ltd, Shanghai, China); the same

Table 1. Specimens used in molecular phylogenetic studies and their GenBank accession numbers.

Taxon	Voucher	Locality	Accession
Brauniellula albipes^{a}	VPI-CLC 339	Gallatin Co., MT, USA	AF205637
B. albipes^{b}	VPI-OKM 2542	Valley Co., ID, USA	AF205651
Chroogomphus asiaticus^{a}	VPI-VC1479	Nepal	AF205666
C. asiaticus^{b}	VPI-VC1466	Nepal	AF205664
C. confusus^{a}	HKAS 50537	Yunnan, SW China	*EF423622
C. confusus^{b}	HKAS 50407 (Holotype)	Yunnan, SW China	*EF423621
C. confusus^{c}	HKAS 49658	Yunnan, SW China	*EF423620
C. filiformis^{a}	HKAS 52934 (Holotype)	Yunnan, SW China	*EU706323
C. filiformis^{b}	HKAS 52935	Yunnan, SW China	*EU706324
C. filiformis^{c}	HKAS 54927	Yunnan, SW China	*FJ513326
C. filiformis^{d}	HKAS 54945	Yunnan, SW China	*FJ513327
C. helveticus^{a}	VPI-OKM 21376	Klausoden, Austria	AF205642
C. helveticus^{b}	VPI-OKM 24410	Alp Claire, Switzerland	AF205650
C. helveticus^{c}	HKAS 55293	Neustupov, Czech Republic	*FJ652070
C. jamaicensis^{a}	VPI-OKM 27493	Jamaica	AF205660
C. jamaicensis^{b}	VPI-OKM 26852	Dominican Republic	AF205658
C. leptocystis	PK 5361	Canada	FJ157000
C. loculatus	VPI-VTMH 3996	Lane Co., OR, USA	AF205668
C. ochraceus	VPI-OKM 25472	Ashland, VA, USA	AF205653 AF205654
C. orientirutilus^{a}	HKAS 52933	Yunnan, SW China	*EU706326
C. orientirutilus^{b}	HKAS 52671 (Holotype)	Yunnan, SW China	*EU706328
C. orientirutilus^{c}	HKAS 51180	Yunnan, SW China	*EU706327
C. orientirutilus^{d}	HKAS 52932	Yunnan, SW China	*EU706325
C. pseudotomentosus^{a}	HKAS 48726	Yunnan, SW China	*EU706334
C. pseudotomentosus^{b}	HKAS 50386	Yunnan, SW China	*EU706335
C. purpurascens^{a}	HMJAU 4633	Nikitintsy, Kyrov, Russia	*EU706332
C. purpurascens^{b}	HMJAU 4634	Falyonsky, Kyrov, Russia	*EU706333
C. purpurascens^{c}	HMJAU 3687	Jilin, NE China	*EU706331
C. purpurascens^{d}	HMJAU 3489	Jilin, NE China	*EU706330
C. purpurascens^{e}	HKAS 54925	Marburg, Germany	*FJ481128
C. purpurascens^{f}	HKAS 55295	Roudnice nad Labem, Czech Republic	*FJ652072
C. roseolus^{a}	HKAS 50552 (Holotype)	Yunnan, SW China	*EF423620
C. roseolus^{b}	HKAS 52901	Yunnan, SW China	*EU706329
C. roseolus^{c}	HKAS 52912	Yunnan, SW China	*EU791579
C. rutilus^{a}	HMJAU 3681	Jilin, NE China	*EU791580
C. rutilus^{b}	HMJAU 1197	Jilin, NE China	*EU791581
C. rutilus^{c}	HMJAU 4665	Zuyevsky, Kyrov, Russia	*EU791582
C. rutilus^{d}	VPI-OKM 24401	Switzerland	AF205649
C. rutilus^{e}	HKAS 55294	Roudnice nad Labem, Czech Republic	*FJ652071
“*C. rutilus*^{a}”	VPI-OKM 17238	Finland	AF205639
“*C. rutilus*^{b}”	Herbarium of Aberdeen Mycorrhiza Group IA09	Culbin Forest, Scotland, UK	UDB001530
“*C. rutilus*^{c}”	Herbarium of Aberdeen Mycorrhiza Group DG 56	Culbin Forest, Scotland, UK	UDB001529
C. sibiricus	VPI-OKM 21628	Gwongnlung, Korea	AF205643 AF205644
C. tomentosus	VPI-OKM 22998	CA, USA	AF205648
C. vinicolor^{a}	OUC 97213	CA, USA	L54095
C. vinicolor^{b}	VPI-OKM 22514	CA, USA	AF205645
Gomphidius borealis^{a}	VPI-IBNR 0726	Siberia, Russia	AY077468
G. borealis^{b}	VPI-IBNR 0532	Siberia, Russia	AY077469
G. aff. *glutinosus*	HKAS 52879	Yunnan, SW China	*EU791578
G. glutinosus	VPI-OKM 22997	CA, USA	AF205647
G. maculatus^{a}	VPI-OKM 24408	Switzerland	AY077470

Table 1 (continued). Specimens used in molecular phylogenetic studies and their GenBank accession numbers.

Taxon	Voucher	Locality	Accession
G. maculatus^{b}	Herbarium, Uppsala University, AT2004063	Uppsala, Sweden	UDB001187
G. nigricans	VPI-OKM 27445	NC, USA	AF205659
G. oregonensis	TDB 953b	Unknown	DQ533976
G. roseus	CUW-MB 95-038	Bavaria, Germany	DQ534570
G. smithii^{a}	UBC F16262	CA, USA	EF530941
G. smithii^{b}	VPI-OKM 25757	UT, USA	AY077471
G. subroseus	OUC 99229	Columbia, Canada	DQ099900
Rhizopogon rubescens	Unknown	Unknown	AF158018
R. salebrosus	AHS 69273	Unknown	AF351874
R. vulgaris	GP 38	USA	DQ822823
Suillus cavipes	TDB-646	MI, USA	L54105
S. paluster	SugaSp	Sugadaira, Japan	AB284451

[*] Sequences obtained in this study. AB284451, DQ533976, EF530941 and FJ157000 were from GenBank. AF15808 was from Taylor and Bruns (1999). AF351874 was from Bidartondo and Bruns (2001). DQ822823 was from Peay *et al.* (2007). L54105 was from Kretzer *et al.* (1996). DQ534570 was from Binder and Hibbett (2007). UDB001187, UDB001529 and UDB001530 were retrieved from UNITE database. The rest sequences were from Miller and Aime (2001), and Miller *et al.* (2002). Superscripts (a, b, c, d, e and f) are used to relate individual collections of the same taxon to their corresponding sequence data shown in Fig. 1. SW = southwestern; NE = northeastern.

primers described above for PCR were used for the sequencing reactions. The ITS sequences obtained in this study have been deposited in the GenBank (Table 1, those with an asterisk). From the GenBank, high quality sequences of *Chroogomphus* and *Gomphidius* were retrieved and included in our analysis (Table 1). DNA sequences were edited and aligned with SeqMan and MegAlign (DNASTAR Package) and manually modified where necessary.

The phylogenetic relationships among the taxa were constructed using the maximum parsimony (MP) method with the heuristic search option of the computer package: Phylogenetic Analysis Using Parsimony (PAUP*) version 4.0 (Swofford, 2002). Gaps were treated as missing data. All unambiguous characters and character-transformations were weighted equally. Bootstrap values were calculated from 1000 replicates using the heuristic search option. As *Gomphidiaceae* is nested in the *Suillineae* clade with *Suillus* Gray, and *Rhizopogon* Fr. (Bruns *et al*., 1998; Binder and Bresinsky, 2002; Binder and Hibbett, 2002; Yang *et al*., 2006; Binder and Hibbett, 2007), sequences of *Rhizopogon rubescens* (Tul. & C. Tul.) Tul. & C. Tul., *R. salebrosus* A.H. Sm., *R. vulgaris* (Vittad.) M. Lange, *Suillus cavipes* (Opat.) A.H. Sm. & Thiers and *S. paluster* (Peck) Kretzer & T.D. Bruns, were downloaded from the GenBank and included for rooting purpose.

Results

Morphologic observations

Sixty-six specimens were examined, including twenty-four collections of the four new species of the genus *Chroogomphus* and nine collections of the genus *Gomphidius*. 22 known *Chroogomphus* taxa were revealed based on our and other studies (Villarreal and Heykoop, 1996; Miller and Aime, 2001; Miller, 2003; Watling, 2004). *Chroogomphus* could be delimited from *Gomphidius* by its ochraceous pileal trama, pale orange to ochraceous lamellae and the mycelium on the base of the stipe consisting of amyloid hyphae.

Basidiomata of different species of *Chroogomphus* may have similar colours when aged or dried. For example, *C. filiformis* and *C. roseolus* have identically coloured pileus and basal mycelium in aged specimens. On the other hand, basidiomata of the same species, e.g. those belonging to *C. confusus*, *C. orientirutilus* or *C. rutilus*, may vary in coloration among different stages of development or among those grown in different habitats. As a result, it is often difficult to recognize species based only on macroscopic

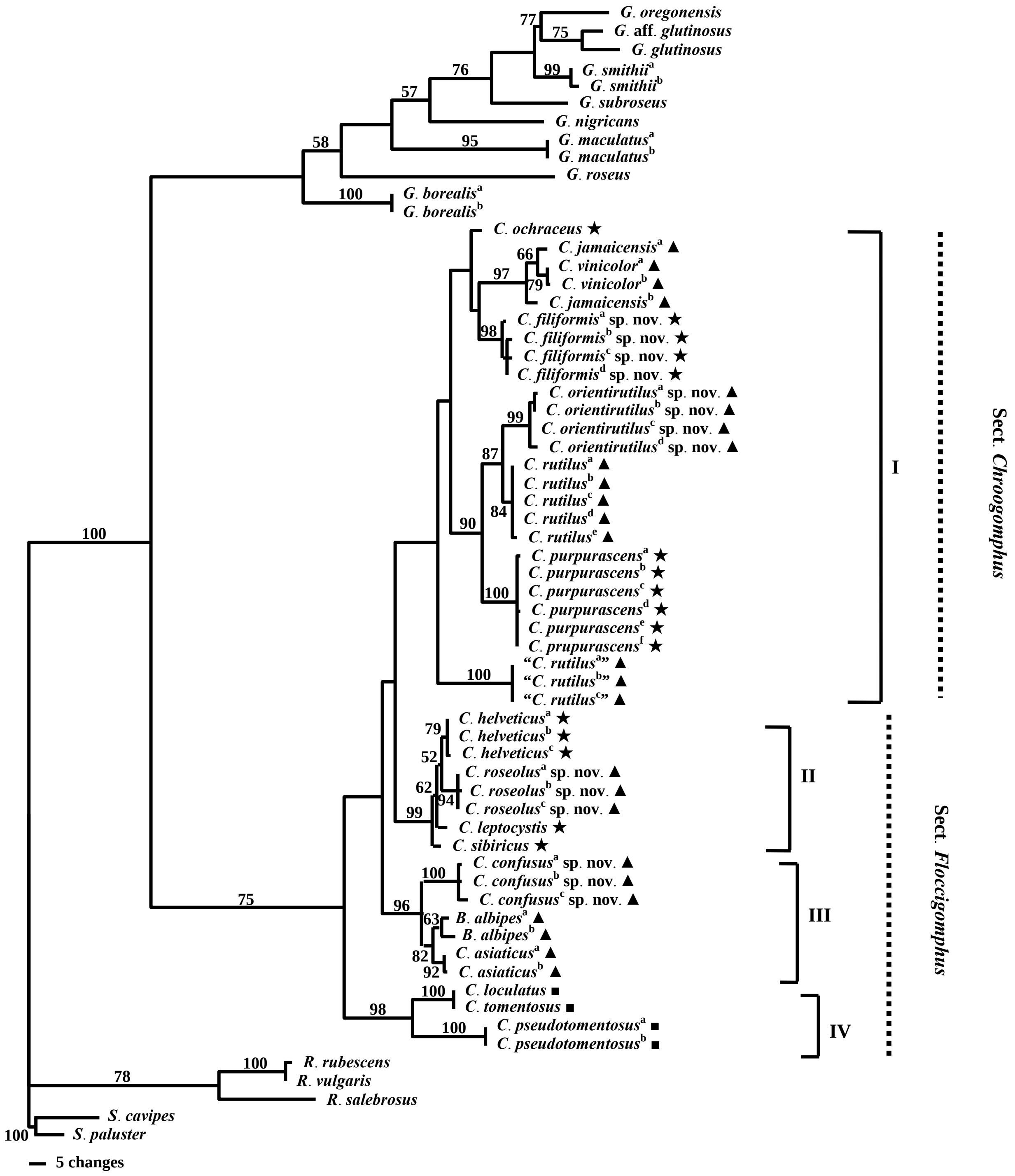


Fig. 1. Phylogeny of the *Chroogomphus* resulted from Maximum Parsimony analysis of nuclear ITS region sequences data. The bootstrapping values > 50 percent are indicated at individual branches. The previous infrageneric two-section division, viz. *Chroogomphus* and *Floccigomphus*, as suggested by Miller (1964), and Miller and Aime (2001), is shown on the tree. The mycorrhizal hosts in *Pinus* subgen. *Pinus*, *Pinus* subgen. *Strobus* and various genera in *Pinaceae* are indicated with geometric forms ▲, ★ and ■ respectively. During subsequent evolution, the mycorrhizal host ranges became narrower and restricted to the two subgenera of *Pinus*, but host switches within very closely related species between the two subgenera of *Pinus* took place.

characters. Fortunately, microscopic characters in combination with macroscopic ones generally allow unambiguous identifications of species.

Molecular data

The ITS sequence data of 63 sequences (28 sequences were newly generated, 32 sequences were download from GenBank and 3 sequences were from UNITE database), representing 17 of the 22 known *Chroogomphus* taxa, were analyzed. The final alignment contained 801 nucleotide sites, of which 23 sites (72–81, 182–185, 299–300, 711–717) were excluded due to alignment ambiguities. In the analyzed 778 nucleotides, 456 were constant, 65 were variable but parsimony uninformative, and 257 were variable and parsimony informative. Parsimony analysis resulted in two most parsimonious trees of 698 steps, with CI = 0.605, RI = 0.839.

The monophyly of *Chroogomphus* was moderately supported (bootstrap = 75%) in our analyses (Fig. 1). Four main clades (I–IV) were recovered within *Chroogomphus*. Clade I corresponds to section *Chroogomphus* circumscribed by Miller and Aime (2001) and Miller (2003), but is not significantly supported by statistic analysis. Within clade I, several subclades with high bootstrap support were recovered: samples of *C. filiformis* formed a well supported subclade with 98% bootstrap support; *C. vinicolor* (Peck) O.K. Mill. and *C. jamaicensis* (Murrill) O.K. Mill. were clustered together in a subclade with 97% bootstrap support; materials of *C. purpurascens* (Lj.N. Vassiljeva) M.M. Nazarova from eastern Russia, northeastern China and central Europe, grouped as a monophyletic group with its sister taxa *C. rutilus* and *C. orientirutilus* with 90% bootstrapping support; three samples of "*C. rutilus*" from Finland and UK were clustered together, and their sequences were identical.

In clade II, the sister relationships of *C. helveticus* (Singer) M.M. Moser, *C. roseolus* and *C. leptocystis* (Singer) O.K. Mill. were moderately supported (with bootstrap value of 62%). In addition, their monophyletic relationship with *C. sibiricus* (Singer) O.K. Mill. was strongly supported (bootstrap value 99%).

In clade III, the monophyly of *C. confusus* with its sister taxa *C. asiaticus* O.K. Mill. & Aime and *Brauniellula albipes* (Zeller) A.H. Sm. & Singer was supported with 96% boots-trap analysis.

Clade IV included *C. tomentosus* (including *C. loculatus*) and *C. pseudotomentosus* with 98% bootstrap support. These two species have similar macro-morphological characters but can be distinguished by having different microscopic characters, ectomycorrhizal associations and geographic distributions.

Taxonomy

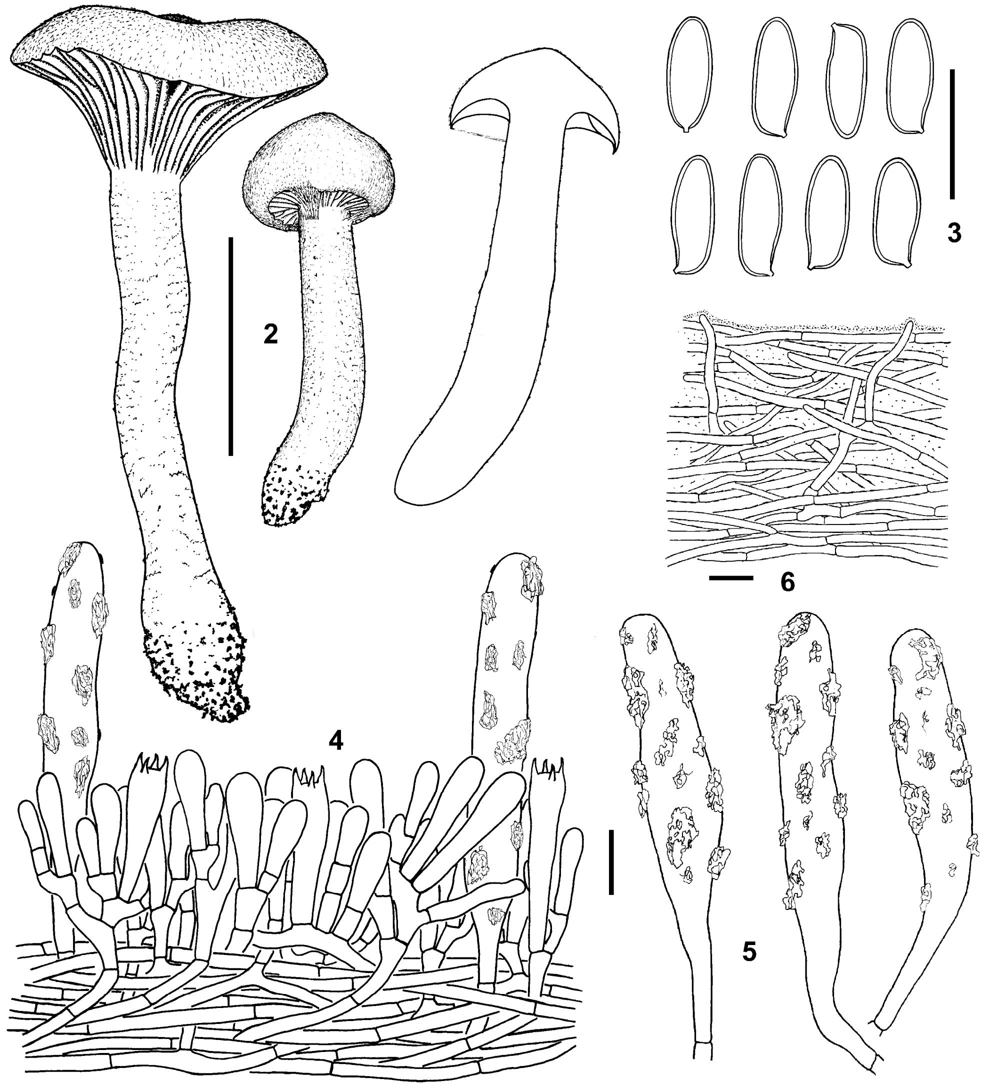
In this study, seven species of *Chroogomphus* from China were recognized and recorded based on morphological and molecular data. *Chroogomphus rutilus*, originally described from Europe, indeed occurs in China, but only in its northeastern region. The previous records of *C. tomentosus* from China were based on misidentification of *C. pseudotomentosus*, *C. confusus* or even taxa of *Gomphidius*.

Chroogomphus confusus Yan C. Li & Zhu L. Yang, **sp. nov.** (Figs 2–6)
MycoBank: 512125

Etymology: Named because it is easy to be confused with *C. asiaticus*.

Pileus 1.3–4 cm latus, convexus vel subconicus, subglabrus, brunneolo-aurantiacus, aurantiacus vel luteo-aurantiacus. *Lamellae* decurrentes, distantes, luteo-aurantiacae, dein griseolo-aurantiacae. Stipes 2.5–8 × 0.5–1 cm, subcylindricus, aurantiacus vel griseo-aurantiacus; *mycelium* ad basem griseolum vel pallidum. *Basidia* 38–60 × 9–18 µm, clavata, tetraspora. *Basidiosporae* (12.0)15.0–20.0(22.0) × 5.0–7.0 µm, subfusoideae vel ellipsoideae, dextrinoideae. *Cystidia* 62–150 × 13–21 µm, subclavata.

Pileus 1.3–4 cm broad, convex to subconical, sometimes slightly umbonate, shining when dry, nearly glabrous, brownish orange (6C6-7) to orange (5A6) or orange yellow (5A4-5) some times with reddish tinge; margin inrolled. *Lamellae* decurrent, distant, orange yellow (5A4-5), then grayish orange (6B3-5); *lamellulae* common, attenuate, concolourous with lamellae. *Stipe* 2.5–8 × 0.5–1 cm, subcylindric, sometimes slightly attenuate downwards, fibrillose, yellowish orange (5A4-5) to grayish orange (4A4-5) with reddish tinge, but pale orange to orange yellow at apical part and brownish to orange-brown downwards; *annulus* can be observed in younger basidiomata, cortinous, whitish to pale orange, fugacious;



Figs 2–6. *Chroogomphus confusus* (from holotype). **2**. Basidiomata. **3**. Basidiospores. **4**. Basidia and pleurocystidia. **5**. Pleurocystidia. **6**. Pileipellis. Bars: 2 = 2 cm; 3–6 = 20 µm.

mycelium on the base of the stipe gray to whitish. *Trama* of pileus and stipe orange yellow, without brown to dark brown tinge at base, unchanging in colour when bruised; taste and odor mild.

Basidia 38–60 × 9–18 µm, clavate, 4-spored, thin-walled, hyaline in KOH, yellowish to yellowish brown in Melzer's reagent. *Basidiospores* [310/7/6] 15.0–20.0(21.0) × 5.5–7.0(7.5) µm, [Q = (2.20)2.46–3.33(3.5), Q_m = 2.82 ± 0.25], subfusiform to ellipsoid, slightly thick-walled (ca. 0.5 µm in thickness), dextrinoid, hyaline or with brownish tinge in KOH. *Lamellar trama* consists of hyphae 3–11 µm in width, hyaline to yellowish in KOH, with weakly scattered amyloid cell walls and cytoplasm. *Pleuro-* and *cheilocystidia* 62–150 ×13–21 µm, numerous, thin-walled, subclavate to subfusiform, with one or two constrictions at upper parts, hyaline to brownish in KOH, yellow to yellowish brown in Melzer's, always with concolourous encrustations. *Pileipellis* made up of loosely interwoven, viscid, non-amyloid, colourless to yellowish hyphae 3.5–7 µm in diameter and encrusted with yellow to yellowish brown pigment but soon dissolved in KOH solution; terminal cells 23–82 × 4–6 µm, subcylindrical. *Pileal trama* hyphae 7–12 µm in diameter, brownish to brown in KOH, with weakly amyloid cytoplasm, and encrusted with amyloid granules, often with scattered oleiferous yellowish brown hyphae. *Mycelium* on the base of the stipe composed of deeply amyloid hyphae 5–13 µm in diameter; clamp connections occasionally present.

Habitat: Solitary on the ground. In southwestern China in the forest of *Pinus yunnanensis* or *P. densata*, while in northeastern China in mixed forests of *P. densiflora*, *P. koraiensis*, *Abies* spp. and *Picea* spp.

Known distribution: Southwestern and northeastern China.

Material examined: CHINA. TIBET: Bomi, Daxing, alt. 3000 m, 27 August 1983, X.L. Mao 1385 (HMAS 51441, as *C. rutilus* in Mao *et al*., 1993 and in Mao, 1998, 2000); JILIN PROVINCE: Chanbaishan Mountain, 25 August 1981, Y. Wang 811571 (IFP 811571, as *C. tomentosus* in Xie *et al*., 1986); YUNNAN PROVINCE: Kunming, Jindian, alt. 2000 m, 4 November 2005, Z.L. Yang 4631 (HKAS 49658); Shangri-La, alt. 3200 m, 6 August 2005, Y.C. Li 345 (HKAS 48578); Shangri-La, alt. 3400 m, 26 July 2006, Y.C. Li 653 (HKAS 50407, **holotype**); same location and date, Z.L. Yang 4740 (HKAS 50537).

Notes: *Chroogomphus confusus* has a brownish orange to orange pileus, a non-amyloid pileipellis and reduced amyloidity in the pileal trama. Such traits are very similar to those of *C. asiaticus*. Interestingly, *C. confusus* is indeed clustered with *C. asiaticus*, and, additionally, *Brauniellula albipes* with much high support (Fig. 1). However, *C. asiaticus*, originally described from Nepal but not found in China yet, has a distinctly smaller basidiomata with a sharply umbonate pileus, an orange yellow lower part of the stipe and non-amyloid lamellar trama. *Brauniellula albipes*, originally described from California as *Secotium albipes* Zeller (1948), has a secotioid basidioma and statismosporic basidiospores, which can easily be separated from *C. asiaticus* and *C. confusus* by morphology. In addition, *B. albipes* possesses thick-walled cystidia and amyloid hyphae in trama (Miller, 2003). Miller (2003) transferred *Brauniellula albipes* to *Chroogomphus*. However, according to Vienna Code, Art. 33.4, the new combination is invalid because of the shortage of literature citation of the basionym (McNeill *et al*., 2006). As the generic name *Brauniellula* has priority over *Chroogomphus*, Aime and Miller (2006) proposed to conserve *Chroogomphus* against *Brauniellula*. Consequently, a new combination is proposed here: ***Chroogomphus albipes*** (Zeller) Yan C. Li & Zhu L. Yang, **comb. nov.** [Basionym: *Secotium albipes* Zeller, Mycologia 40: 657 (1948), MycoBank: MB 512129].

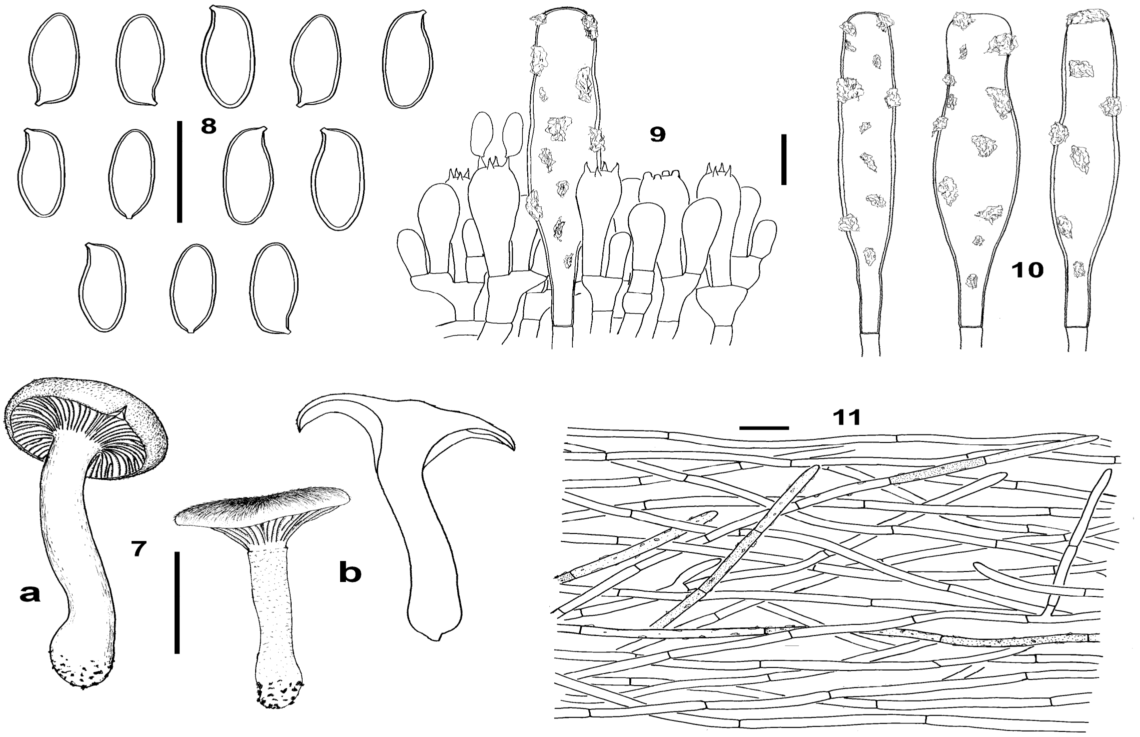
Chroogomphus filiformis Yan C. Li & Zhu L. Yang, **sp. nov.** (Figs 7–11)
MycoBank: 512126

Etymology: "*filiformis*" referring to the narrow hyphae in the pileipellis.

Pileus 1–6 cm latus, subconicus vel applanatus, siccus, fibrillosus vel tomentosus, olivaceus vel griseolo-aurantiacus vel aurantiacus, deinde roseus vel rubeolo-roseus. *Lamellae* decurrentes, distantes, aurantiacae dein griseolo-aurantiacae. *Stipes* 2–7 × 0.4–1 cm, subcylindricus, clavatus, roseus vel luteolo-roseus, apicis aurantiacis, basis roseis to roseolis; *mycelium* ad basem luteolum deinde vivido-roseum. *Basidia* 30–55 × 10–15 µm, clavata, tetraspora. *Basidiosporae* (15.0)16.0–19.0 (21.5) × (5.5)6.0–7.0(7.5) µm, subfusoideae, dextrinoideae. *Cystidia* 90–170 × 14–21 µm, subclavata vel subfusoidea.

Pileus 1–6 cm broad, subconical to applanate, sometimes slightly umbonate, margin always inrolled, dry, with radially arranged fibrillose to fine tomentose squamules, olive gray (3E2) to orange gray (5B3-5) when young and grayish orange (6B3-5) to orange (5A4-5) in age, but pink (11A3-4) to purplish pink (4A3-5) when dried. *Lamellae* decurrent, distant, orange yellow (5A4-5) when young, grayish orange (6B3-5) when mature, not forking; *lamellulae* common, attenuate, concolourous with lamellae. *Stipe* 2–7 × 0.4–1 cm, subcylindric, sometimes slightly enlarged at base, orange yellow (5A4-5) then pink (11A3-4) in age, but golden yellow at apical part, and pink to pinkish at base, with pinkish remnants of annulus at the apical part; *mycelium* on the base of the stipe yellowish but vivid pink when dried. *Trama* of pileus and stipe orange yellow, without brown to dark brown tinge at the base of the stipe, unchanging in colour when bruised; taste and odor mild.

Basidia 30–55 × 10–15 µm, clavate, 4-spored, sometimes 2-spored, hyaline or with pink to pinkish vacuolar pigment in KOH, yellowish to yellowish brown in Melzer's reagent. *Basidiospores* [300/10/6] (15.0)16.0–19.0(21.5) × (5.5)6.0–7.0(7.5) µm, [Q = (2.36) 2.50–3.08(3.33), Q_m = 2.77 ±0.17], subfusiform, thin- to slightly thick-walled (up to 0.7 µm in thickness), dextrinoid, yellowish, yellowish brown or pinkish brown in KOH. *Lamellar trama* made up of hyphae 4–14 µm in width, colourless to yellowish in KOH, with weakly scattered amyloid cell walls and cytoplasm. *Pleuro-* and *cheilocystidia* 90–170 × 14–21 µm, numerous, thin- to slightly thick-walled (up to 1.5 µm), subclavate with apical part attenuate or obtuse, with one to three constrictions at upper parts, hyaline or with pink to brownish pink vacuolar pigment in KOH, yellow to yellowish brown in Melzer's, always with concolourous encrustations. *Pileipellis* compo-



Figs 7–11. *Chroogomphus filiformis* (7b from HKAS 52935, others from holotype). **7**. Basidiomata. **8**. Basidiospores. **9**. Basidia and pleurocystidium. **10**. Pleurocystidia. **11**. Pileipellis. Bars: 7 = 2 cm; 8–11 = 20 µm.

sed of more or less radially arranged to loosely interwoven, non-amyloid, somewhat viscid, colourless to yellowish, filamentous hyphae 4–7 µm in diameter and often encrusted with yellow to yellowish brown pigment but soon dissolved in KOH solution; terminal cells 37–100 × 4.5–7 µm, subcylindric. *Pileal trama* made up of hyphae 7–21 µm in diameter with weakly amyloid cell walls and cytoplasm, and encrusted with amyloid granules, colourless to yellowish in KOH but yellowish brown to pinkish brown beneath pileipellis. *Mycelium* on the base of the stipe composed of deeply amyloid hyphae 4–10 µm in diameter; clamp connections frequent.

Habitat: Solitary on the ground. Under *Pinus armandii* or in mixed forests dominated by *P. armandii* and *P. yunnanensis*.

Known distribution: Southwestern China.

Material examined: CHINA. YUNNAN PROVINCE: Shangri-La County, Haba Mountain, alt. 2800 m, 30 September 2007, B. Feng 179 (HKAS 52900); Shangri-La County, Shangri-La Valley, alt. 3030 m, 20 August 2008, Y.C. Li 1529 (HKAS 54927); same location and date, Y.C. Li 1531 (HKAS 54945); Kunming, Panlong District, alt. 2400 m, 10 November 2007, Y.C. Li 1115 (HKAS 52934, **holotype**); Same location and same day, Y. C. Li 1116 (HKAS 52935); Kunming, Yeyahu, alt. 2100 m, 8 November 2008, Y.C. Li 1599 (HKAS 54928).

Notes: *Chroogomphus filiformis* is characterized by its primitively olive gray to orange gray or orange then pink to purplish pink pileus, yellowish then vivid pink mycelium on the base of the stipe, non-amyloid, somewhat viscid narrow hyphae in the pileipellis and slightly thick-walled cystidia. *Chroogomphus helveticus* has a pale orange yellow to somewhat rose-coloured pileus and a pinkish mycelium on the base of the stipe, which are similar to those of *C. filiformis*. However, *C. helveticus* has non-viscid, much broader hyphae (up to 15 µm in diam.) in the pileipellis and thin-walled cystidia (Singer, 1950; Singer and Kuthan, 1976; Courtecuisse and Duhem, 1995; our own observation on a collection of *C. helveticus* made from the Czech Republic, HKAS 55293).

In the phylogenetic analysis (Fig. 1), *C. filiformis* was clustered with *C. ochraceus* (Kauffman) O.K. Mill., *C. vinicolor* and *C. jamaicensis* yet without bootstrap support. The last two species differed significantly from *C. filiformis* by their differentially coloured pileus, much thicker-walled cystidia (5-7.5 µm thick in *C. vinicolor* and 4-5 µm in *C. jamaicensis*) (Miller, 1964; Singer and Kuthan, 1976). *Chroogomphus ochraceus* has a brightly coloured pileus ranging from yellowish orange to orange-ochraceous or salmon, a pinkish mycelium on the base of the stipe and a typically amyloid pileipellis (Singer, 1949; Miller, 1964; Singer and Kuthan, 1976; Miller and Aime, 2001).

Chroogomphus orientirutilus Yan C. Li & Zhu L. Yang, **sp. nov.** (Figs 12–16)

MycoBank: 512127

Etymology: *orientirutilus* is proposed because of the eastern Asian mushroom's similarity to *C. rutilus*.

Pileus 2–6 cm latus, subconicus vel applanatus, glabrus dein tomentosus vel fibrillosus, rufus vel sanguineus quam rufobrunneus vel rubidus. *Lamellae* decurrentes, distantes, aurantiacae dein griseolae aurantiacae. *Stipes* 3–6 × 0.6–1.3 cm, subcylindricus, brunneolo-aurantiacus, luteus ad basem; mycelium ad basem albidum vel griseolum. *Basidia* 33–75 × 10–17 µm, tetraspora, clavata. Basidiosporae (14.0)15.5–19.5(22.5) × (5.0)5.5–7.0(7.5) µm, subfusoideae vel ellipsoideae. *Cystidia* 100–225 × 11–20µm, auguste clavata vel subfusoidea.

Pileus 2–6 cm broad, subconical to applanate, slightly umbonate, viscid when wet, glabrous when young and tomentose to fibrillose in age, red (10B7-8) to blood red (11B7-8) or brownish red (9C7-8) to dark red (7B3-5), margin inrolled. *Lamellae* decurrent, distant, orange (5A4-5) when young and grayish orange (6B3-5) when old; *Lamellulae* common, attenuate, concolourous with lamellae. *Stipe* 3–6 × 0.6–1.3 cm, subcylindric, fibrillose, grayish orange (5B3-6) to reddish orange (7A6-8), but light orange (5A4-5) to orange (5A6-7) at apical part, yellow to yellowish orange (4A6-7) at base; *mycelium* on the base of the stipe whitish to salmon. *Trama* of pileus and stipe yellowish to orange yellow but much bright at the base of the stipe, unchanging in colour when bruised; taste and odor mild.

Basidia 33–75 × 10–17 µm, clavate, 4-spored, thin-walled, hyaline in KOH, yellowish to yellowish brown in Melzer's reagent. *Basidiospores* [630/18/8] (14.0)15.5–19.5(22.5) × (5.0)5.5–7.0(7.5) µm, [Q = (2.38)2.50–3.17 (3.46), Q_m = 2.82 ± 0.19], subfusiform to ellipsoid, slightly thick-walled (up to 0.8 µm), weakly dextrinoid, yellowish brown to olive brown in KOH. *Lamellar trama* made up of hyphae 4-17 µm in width with weakly amyloid cell walls and cytoplasm, and encrusted with some amyloid granules, colourless to yellowish in KOH. *Pleuro*- and *cheilocystidia* 100–225 × 11–20 µm, numerous, thin- to slightly thick-walled (up to 2 µm in thickness), narrowly clavate to subfusiform, always with distinct constrictions at upper parts, colourless to brownish in KOH, yellow to yellowish brown in Melzer's with concolourous encrustations. *Pileipellis* composed of more or less radially arranged to loosely interwoven, non-amyloid, viscid hyphae (3.5–8 µm in diameter), which are colourless to yellowish often encrusted with yellow to yellowish brown pigment but soon dissolved in KOH solution; terminal cells 26–95 × 5–10.5 µm, subcylindrical to narrowly clavate, apex obtuse or attenuate. *Pileal trama* made up of hyphae 16–25 µm in diameter, brownish to brown in KOH and weakly amyloid in Melzer's reagent. *Mycelium* on the base of the stipe composed of deeply amyloid hyphae 4.5–12 µm in diameter; clamp connections relatively common.

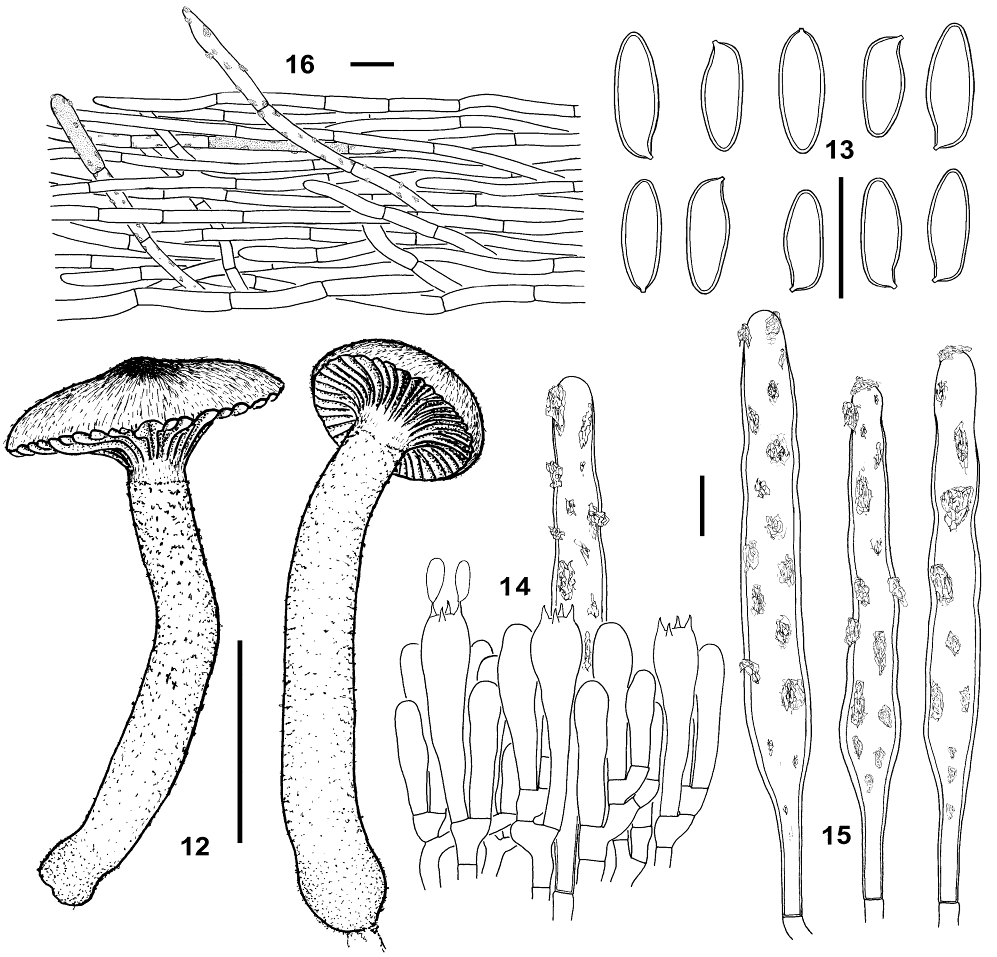
Habitat: Solitary to gregarious on the ground. In conifer forests dominated by *Pinus yunnanensis*.

Known distribution: Southwestern China.

Material examined: CHINA. YUNNAN PROVINCE: Anning County, free market, 5 September 1997, X.H. Wang 73 (HKAS 31719, as *C. rutilus* in Wang *et al*., 2004); Kunming, Ciba free market, 20 June 1998, X.H. Wang 264 (HKAS 32694, as *C. rutilus* in Wang *et al*., 2004); same location, 30 August 2002, H.D. Zheng 146 (HKAS 42223, as *C. rutilus* in Wang *et al*., 2004) and 16 October 2007, Y.C. Li 1113a and 1113b (HKAS 52932 and 52933 respectively); Songming, Aziying, 25 August 2002, F.Q. Yu 937 (HKAS 45000, as *C. rutilus* in Wang *et al*., 2004); Kunming Botanical Garden, 11 October 2006, Y.C. Li 743 (HKAS 51180); Kunming, Xishan, 10 August 2007, Y.C. Li 984 (HKAS 52671, **holotype**).

Notes: *Chroogomphus orientirutilus* is characterized by its red to blood red or brownish red to grayish red, often only slightly umbonate pileus, whitish to salmon mycelium on the base of the stipe, a viscid pileipellis with non-amyloid hyphae, slightly thick-walled cystidia and an association with *P. yunnanensis*, a 3-needle pine of subgen. *Pinus*. This taxon was regarded as *C. rutilus* by some Chinese mycologists (Wang *et al*., 1999; Wang *et al*., 2004; Yu and Liu, 2005).

Phylogenetically, *C. orientirutilus* is indeed clustered with *C. rutilus* and *C. purpurascens* with a strong bootstrap support (bootstrap value 90%). However, *C. rutilus* has a vinaceous to vinaceous brown or ochraceous-buff to vinaceous red and mostly distinctly umbonate pileus, a cream to buff or yellow mycelium on



Figs 12–16. *Chroogomphus orientirutilus* (from holotype). **12**. Basidiomata. **13**. Basidiospores. **14**. Basidia and pleurocystidium. **15**. Pleurocystidia. **16**. Pileipellis. Bars: 12 = 2 cm; 13–16 = 20 µm.

the base of the stipe (Singer, 1949; Miller, 1964; Singer and Kuthan, 1976; Gerhardt, 1984; Breitenbach and Kränzlin, 1991; Villarreal and Heykoop, 1996; Horak, 1968, 2005; also see our brief description of *C. rutilus* below). *Chroogomphus purpurascens* has a gray to leaden gray then purple to dark purple pileus, a salmon-ochraceous stipe, salmon to purple pink mycelium on the base of the stipe and thin-walled cystidia (Vassiljeva, 1950, 1973; Azbukina, 1990; also see our brief description of *C. purpurascens* below).

Chroogomphus purpurascens (Lj.N. Vassiljeva) M.M. Nazarova, Plant. Non-Vasc. Fungi Bryops. Orient. Extr. Soviet. 1: 378 (1990); *Gomphidius purpurascens* Lj.N. Vassiljeva, Not. Syst. Crypt. Inst. Bot. Acad. Sci. USSR 6: 193 (1950).

Pileus gray, grayish red to brownish red then purple to dark purple, at first umbonate and then depressed. *Lamellae* decurrent, distant, salmon-ochraceous then grayish; *Lamellulae* common, attenuate, concolourous with lamellae. *Stipe* salmon-ochraceous. *Mycelium* on the base of the stipe salmon to purple pink. *Trama* of pileus and stipe incarnadine to salmon ochraceous, unchanging in colour when bruised; taste and odor mild.

Basidia 40–62 × 11–13 µm. *Basidiospores* [330/15/9] (15.0)15.5–19.0(21.0) × (5.5)6.0–6.5(7.5) µm, [Q = (2.38)2.50–3.0 (3.45), $Q_m = 2.76 \pm 0.16$], slightly thick-walled (up to 0.8 µm), dextrinoid. *Lamellar trama* hyphae with weakly amyloid cell walls and cytoplasm. *Pleuro-* and *cheilocystidia* 95–185 × 13–19 µm, thin-walled, always with distinct constrictions at their upper parts. *Pileipellis* composed of non-amyloid, viscid hyphae 3–7 µm in diameter; terminal cells 58–105 × 5–7 µm, subcylindrical to narrowly clavate, apex obtuse or attenuate. *Pileal trama* made up of hyphae 10–18 μm in diameter, deeply amyloid in Melzer's. *Mycelium* on the base of the stipe composed of deeply amyloid hyphae; clamp connections occasionally present.

Habitat: Solitary to scattered, in forests dominated with *Pinus cembra* or *P. koraiensis*, sometimes in mixed forests with *P. koraiensis* and *P. tabuliformis*.

Known distribution: Eastern Russia, northeastern China and Central Europe.

Material examined: CHINA. JILIN PROVINCE: Jilin, Zuojia, 16 September 2000, B. Tolgor (HMJAU 1950 as *C. rutilus* in Li and Tolgor, 2003); Changchun, Jingyuetan National Forest Park, 14 August 2003, J.R. Wang (HMJAU 3587); same location, 12 July 2003, J.R. Wang (HMJAU 3650); same location, 24 August 2004, J.R. Wang (HMJAU 3687); same location, 20 September 2004, J.R. Wang (HMJAU 3489). RUSSIA. Kyrov: Nikitintsy, 12 August 2006, B. Tolgor (HMJAU4633); Kyrov: Falyonsky, 15 August 2006, B. Tolgor (HMJAU 4634). GERMANY. Marburg, KR8344 (HKAS 54925, duplicate of MB). CZECH REPUBLIC. Near Roudnice nad Labem, 14 September 2008, J. Borovička (HKAS 55295).

Notes: Studies on collections from far eastern Russia, where the type was collected, central Europe and northeastern China add more information to the recognition of *C. purpurascens*. This taxon has a primary gray to leaden gray then purple to dark purple pileus, a salmon-ochraceous stipe, a salmon to purple pink mycelium on the base of the stipe and thin-walled cystidia. Because of its similarities to *C. rutilus*, *C. purpurascens* was regarded as a synonymy of *C. rutilus* by Miller (1964, 2001), and collections of *C. purpurascens* from northeastern China were also usually identified as *C. rutilus* (Mao, 1998, 2000; Li and Tolgor, 2003; Dai and Tolgor, 2007). But they differ in the colour of the basidiomata, the morphology of the cystidia, and the mycorrhizal associations (Vassiljeva, 1950, 1973; Azbukina, 1990; see also discussion below). It is noteworthy that this species also occurs in central Europe.

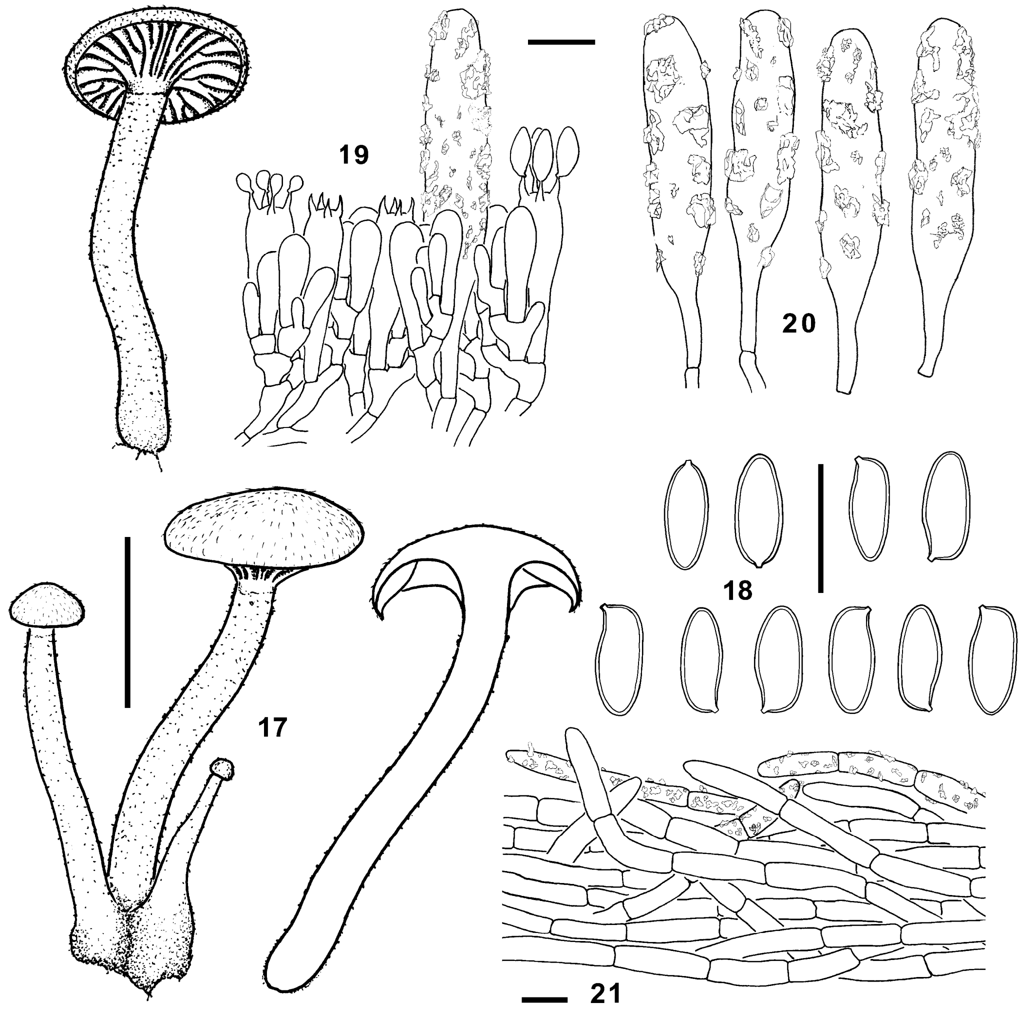
Chroogomphus roseolus Yan C. Li & Zhu L. Yang, **sp. nov.** (Figs. 17–21)
MycoBank: 512128

Etymology: "*roseolus*" referring to the colour of the basidiomata when mature or dried.

Pileus (1)2–2.5 cm latus, subhemisphaericus vel convexo-applanatus, fibrillosus vel tomentosus, roseus, carmineo-roseus vel aurantio-roseus, margine purpureo-roseus. *Lamellae* decurrentes, distantes, aurantiacae dein griseo-aurantiacae. *Stipes* 3–6 × 0.3–0.6 cm, subcylindricus, pallido-aurantiacus vel pallido-roseus; mycelium ad basem roseolum. *Basidia* 38–52 × 9–14 μm, clavata, tetraspora. *Basidiosporae* (14.0)15.0–19.0(20.5) × (5.0) 6.0–7.5(8.0) μm, ellipsoideae vel subfusoideae, dextrinoideae. *Cystidia* 75–155 × 12–21 μm, subclavata.

Pileus (1)2–3.5 cm in diam., subhemispherical to convexo-applanate, non-umbonate, pink (11A4) to carmine-rose coloured (12A3-5), or with pinkish tinge on the orange ground, dry, with fibrillose to tomentose squamules; margin purplish pink (14A3-5) often with concolourous remnants of annulus in younger basidiomata. *Lamellae* decurrent, distant, yellowish orange (5A4-5) when young, then gray orange (5B3-5) in age; *lamellulae* abundant, attenuate, not forking. *Stipe* 3–6 × 0.3–0.6 cm, subcylindric, pale yellowish orange (6A4-5) but pale rose coloured (13A3-4) towards base, covered with pinkish squamules; *mycelium* on the base of the stipe pinkish (13A2). *Trama* of pileus and stipe dull orange (5A2) but much deeper at the base of stipe; taste and odor mild.

Basidia 38–52 × 9–14 μm, clavate, 4-spored, rarely 2-spored, thin-walled, hyaline in KOH, yellowish to yellowish brown in Melzer's reagent. *Basidiospores* [190/9/4] (14.0) 15.0–19.0(20.5) × 6.0–7.5(8.0) μm, [Q = (2.27) 2.33–2.92(3.17), $Q_m = 2.58 \pm 0.17$], ellipsoid to subfusiform, slightly thick-walled (up to 0.7 μm), dextrinoid, brownish to yellowish brown in KOH. *Lamellar trama* composed of non-amyloid hyphae 4–9 μm in width and encrusted with amyloid granules. *Pleuro-* and *cheilocystidia* 75–155 × 12–21 μm, thin-walled, subclavate, with one to three constrictions at upper parts, colourless or with pinkish to brownish vacuolar pigment in KOH, often with brown to brownish encrustations. *Pileipellis* composed of subrepent, non-viscid hyphae 6–17 μm in width with scattered amyloid cell walls and cytoplasm, surface of hyphae covered with weakly amyloid encrustations; terminal cells 26–108 × 8–15 μm, subcylindric or slightly attenuate upwards. *Pileal trama* consisting of yellow brown to ochraceous, deeply amyloid, 10–22 μm wide hyphae, encrusted with deeply amyloid substance. *Stipitipellis* composed of yellowish to yellowish brown, more or less longitudinally arranged hyphae 6.5–13 μm in diam. with amyloid cell walls and cytoplasm; *mycelium* on the base of the stipe composed of loosely interwoven, deeply amyloid hyphae 4–13 μm in diam., encrusted with abundant deeply amyloid substance; clamp connections present but very rare.



Figs 17–21. *Chroogomphus roseolus* (from holotype). **17**. Basidiomata. **18**. Basidiospores. **19**. Basidia and pleurocystidium. **20**. Pleurocystidia. **21**. Pileipellis. Bars: 17 = 2 cm; 18–21 = 20 µm.

Habitat: Solitary to scattered, in mixed forests of *Pinus densata* and *Quercus* spp.

Known distribution: Southwestern China.

Material examined: CHINA. YUNNAN PROVINCE: Shangri-La County, Haba Mountain, alt. 3000 m, 30 September 2007, B. Feng 180 and 191 (HKAS 52901 and 52912 respectively). Lijiang County, Yulong Mountain, alt. 2900 m, 29 July 2006, Z.L. Yang 4755 (HKAS 50552, **holotype**); same location, October 1916, Handel-Mazzetti 12647 (WU, as *Psilocybe uda* in Lohwag, 1937 and *Chroogomphus* sp. in Horak, 1987).

Notes: *Chroogomphus roseolus* is characterized by its pink to purplish pink pileus, pinkish mycelium on the base of the stipe, broad and weakly amyloid hyphae up to 17 µm in width in the non-viscid pileipellis. It is mainly associated with *P. densata*, a 2-needle pine in subgen. *Pinus*. It is similar to *C. filiformis* phenetically. However, the latter species has a large basidioma with much narrower, non-amyloid hyphae (up to 7 µm in width) in the pileipellis and slightly thick-walled (up to 1.5 µm) cystidia.

Phylogenetically, *C. roseolus* grouped with *C. helveticus*, *C. leptocystis* and *C. sibiricus* with a very high statistic support (Fig. 1). Although *C. helveticus* also has a somewhat roseolate pileus and a pinkish mycelium on the base of the stipe when dried, it has a significantly larger, umbonate, pale orange yellow pileus (4–7 cm in diam.), brownish squamules on pileus, somewhat larger basidiospores (17–23× 6.8–8 µm), and an association with *P. cembra*, a 5-needle pine of subgen. *Strobus* (Singer, 1950; Singer and Kuthan, 1976; Courtecuisse and Duhem, 1995; Miller and Aime, 2001). *Chroogomphus leptocystis* differs from *C. roseolus* in the empire yellow base of the stipe, occasionally thick-walled cystidia (up to 1.5 µm), much narrower pileipellis hyphae (up to 10 µm in width) and an association with *P. monticola*, a 5-needle pine of subgen. *Strobus* (Singer, 1949; Miller, 1964; Singer and Kuthan, 1976). *Chroogomphus sibiricus* has an orange to fuscus-gray pileus, a vivid yellow to saturated orange stipe context without any brown tinge especially at the stipe base, relatively broader terminal cells (8–21.5 µm in width) in the pileipellis and much longer cystidia (up to 200 µm in length). Additionally, it is known from Siberia and associates with *P. sibirica*, a 5-needle pine of subgen. *Strobus* (Singer, 1938; Miller, 1964; Singer and Kuthan, 1976; Miller and Aime, 2001).

Chroogomphus rutilus (Schaeff.: Fr.) O.K. Mill., Mycologia 56: 543 (1964); *Agaricus rutilus* Schaeff., Fung. Bavar. Palat. 4: 24 (1774); Fr., Syst. Mycol. 1: 315 (1821).

Pileus vinaceous to vinaceous brown or ochraceous-buff to vinaceous red, mostly distinctly umbonate, margin inrolled. *Lamellae* grayish orange then gray in age. *Stip*e orange to reddish orange, yellow to orange yellow at base. *Mycelium* on stipe base cream to yellowish. *Trama* of pileus and stipe ochraceous but much more yellowish to orange yellow at the base of the stipe.

Basidia 40–50 × 12–14 µm. *Basidiospores* [300/18/15] (15.5)16.5–19 (21) × (5.5) 6.0–6.5 µm, [$Q = (2.36)2.62–3.15(3.42)$, $Q_m = 2.89 \pm 0.16$], subfusiform to ellipsoid, slightly thick-walled (up to 0.8 µm), weakly dextrinoid. Lamellar trama hyphae with weakly amyloid cell walls and cytoplasm. *Pleuro-* and *cheilocystidia* 118–170 × 16–23 µm, slightly thick-walled (up to 2.5 µm in thickness), subclavate to subfusiform, always with distinct constrictions at upper parts. *Pileipellis* composed of non-amyloid, viscid hyphae (4–8 µm in diameter); terminal cells 45–105 × 4–7 µm, subcylindrical to narrowly clavate, apex obtuse or attenuate. *Pileal trama* made up of hyphae 9–24 µm in diameter, weakly amyloid in Melzer's. *Mycelium* on the base of the stipe composed of deeply amyloid hyphae 5–9 µm in diameter; clamp connections occasionally present.

Habitat: Solitary to scattered, under *Pinus sylvestris* or in mixed forests of *P. densiflora* and *P. tabuliformis*.

Known distribution: Europe, eastern Russia and northeastern China.

Material examined: CHINA. BEIJING: Jietaisi, 17 September 1996, X.L. Mao *et al*., 96012 (HMAS 73264S); Dongling Mountain, 12 September 2001, H.A. Wen and M.R. Ding 18 (HMAS 63546S); Forest Observation Station, 13 September 2003, Q.B. Wang 273 (HMAS 84758); Huairou District, 27 August 2000, B. Tolgor (HMJAU 0410, 0431, 0460 and 0714); same location, 1 September 2000, B. Tolgor (HMJAU 0875 and 1179); JILIN PROVINCE: Jilin City, Zuojia, 16 September 2000, B. Tolgor (HMJAU 1197); Changchun, Jingyuetan National Forest Park, 13 September 2003, J.R. Wang (HMJAU 3658); same location, 21 September 2005, J.R. Wang (HMJAU 5283); Jilin Agriculture University, 8 September 2004, J.R. Wang (HMJAU3681). RUSSIA. Kyrov: Zuyevsky, 16 August 2006, B. Tolgor (HMJAU4665). CZECH REPUBLIC. In the vicinity of Roudnice nad Labem, 30 August 2008, J. Borovička (HKAS 55294).

Notes: *Chroogomphus rutilus*, originally described by Schaeffer (1774) based on materials from Bavaria, Germany, is a name widely used in European mycological literature. There was no problem with its application in central Europe. However, with the development of molecular phylogeny, it is turned out that there are more than one species of this complex in Europe. To understand the species concept of *C. rutilus*, it would be ideal to study the type or authentic materials, even only morphologically. Unfortunately, the type (and additional materials) of *C. rutilus* studied by Schaeffer could not be traced (Singer, 1949). Our collections HKAS 55294 made from the Czech Republic, HMJAU 4665 from far eastern Russia, and HMJAU 1197 and HMJAU 3681 from northeastern China, generally have similar morphological characters of *C. rutilus* as provided in modern descriptions (e.g. Horak, 1968, 2005; Breitenbach and Kränzlin, 1991; Villarreal and Heykoop, 1996). Our phylogenetic study showed that these four collections possess almost the same ITS sequences as the Swiss collection of *C. rutilus*, VPI-OKM 24401, does (Fig. 1). *Chroogomphus rutilus* is characterized by a vinaceous to vinaceous brown or ochraceous-buff to vinaceous red, mostly distinctly umbonate pileus, a yellow to orange yellow stipe base with a cream to buff or yellow mycelium attached to it, which is not pink when dried.

The sequences UDB001529, UDB001530 and AF205639 labeled as "*C. rutilus*" were based on materials from Finland and UK, which might be *C. britannicus* A.Z.M. Khan & Hora (1978), a species originally described from Britain but regarded as a synonym of *C. rutilus* by Miller (2003) without any comments or molecular evidence. According to Khan and Hora (1978), *C. britannicus*, also growing under trees of *Pinus sylvestris*, differs from *C. rutilus* by its yellowish orange or ochraceous orange pileus and stipe, broader spores (up to 8 µm) and thin-walled cystidia.

Key to the species of Chroogomphus in China

For the convenience of identification of the species, a key is given below. We also included *C. asiaticus* and *C. sibiricus* because these two species have been found in East Asia and likely exist in China even though neither species have been recorded from China yet.

1. Pileipellis hyphae broad, non-viscid, 6-21µm in width 2
1. Pileipellis hyphae narrow, mostly viscid, 3.5-8 µm in width 4

2. Pileus pale orange to orange brown; basidio-spores 13.5–18.2 × 7.5–9.9 µm; cystidia thick-walled (up to 5.5 µm thick)........ ***C. pseudotomentosus***
2. Basidiospores narrow (≤ 8 µm in width); cystidia thin-walled 3

3. Pileus orange pink to pinkish or rose coloured; stipe base and mycelium attached to it rose coloured; cystidia up to 155 µm in length .... ***C. roseolus***
3. Pileus orange to fuscus gray; stipe orange yellow but salmon orange at base; cystidia up to 200 µm in length .... ***C. sibiricus***

4. Cystidia thick-walled (≤ 3 µm in thickness) 5
4. Cystidia thin-walled (≤ 1 µm in thickness) 7

5. Pileus without pink to purple tinge; base of stipe yellow to orange yellow; mycelium on the base of the stipe without any pinkish tinge even when dried 6
5. Pileus primitively olive gray to orange gray then pink to purple pink; base of stipe pink to pinkish; basal mycelium yellowish but vivid pink when dried .... ***C. filiformis***

6. Pileus often distinctly umbonate, ochraceous, vinaceous to vinaceous brown or ochraceous-buff to vinaceous red; mycelium on stipe base cream to yellowish; cystidia relatively wide, 118–170 × 16–23 µm; temperate .... ***C. rutilus***
6. Pileus slightly umbonate, red to blood red or brownish red to grayish red; mycelium on the base of the stipe whitish to salmon; cystidia narrow, 100–225 × 11–20 µm; subtropical .... ***C. orientirutilus***

7. Basidiomata with orange to brownish orange tinge; mycelium on the base of the stipe whitish to gray or grayish yellow 8
7. Pileus mostly distinct umbonate, gray, grayish red to brownish red then purple to dark purple; mycelium on the base of the stipe salmon to purplish pinkish; under pines of subgen. *Strobus*, temperate .... ***C. purpurascens***

8. Basidiomata distinctly small; pileus applanate with a distinctly sharp umbo; stipe light orange becoming orange yellow below; lamellar trama hyphae non-amyloid .... ***C. asiaticus***
8. Basidiomata large; pileus conical to convex sometimes with indistinct umbo; stipe yellowish orange to grayish orange with reddish tinge; lamellar trama hyphae with scattered amyloid cell walls and cytoplasm .... ***C. confusus***

Discussion

Phylogeny and infrageneric divisions of Chroogomphus

Recent phylogenetic studies of *Gomphidiaceae* have delimited the genus *Chroogomphus* from *Gomphidius* (Miller and Aime, 2001; Miller, 2003). In our parsimony analysis with an extensive ITS sequence dataset, *Chroogomphus* was clearly shown as a monophyletic group with a moderate bootstrap (75%). However, whether *Gomphidius* is monophyletic remains unclear. Considering the morphological differences between the two groups and their strong mycorrhizal host specificity, *Chroogomphus* associates with genus *Pinus* of *Pinoideae* while *Gomphidius*, with minor exceptions, forms mycorrhizae with taxa of *Piceoideae*, *Laricoideae* and *Abietoideae* within the *Pinaceae* (Miller, 1964; Miller and Aime, 2001; Miller, 2003), we follow Miller (1964, 2003) and treat *Chroogomphus* and *Gomphidius* as separate genera for the time being.

In the genus *Chroogomphus*, two sections were proposed, namely *C.* sect. *Chroogomphus*, typified by *C. rutilus* with a pileipellis composed of appressed gelatinous hyphae, and *C.* sect. *Floccigomphus* (S. Imai) O.K. Mill., typified by *C. tomentosus* with a pileipellis consisting of non-gelatinous, tomentose to innately fibrillose filamentous hyphae (Singer, 1949; Miller, 1964; Miller and Trappe, 1970; Miller and Aime, 2001). Miller (1964) and Miller and Aime (2001) placed *C. asiaticus*, *C. helveticus*, *C. leptocystis*, *C. sibiricus* besides *C. tomen-*

tosus and *C. pseudotomentosus* in sect. *Floccigomphus*. The similar infrageneric delimitation was adapted by Singer and Kuthan (1976), Singer (1986), and Azbukina (1990). The sect. *Chroogomphus* in Miller (1964) and Miller and Aime (2001) covers only the *C. rutilus*-*C. ochraceus* complex, which nested among the subclades of the remaining taxa of the genus, making sect. *Floccigomphus* paraphyletic (see Fig. 1 of Miller 2003). Our analysis suggested that neither sect. *Chroogomphus* nor sect. *Floccigomphus* is monophyletic. The high level divergence within clade IV as revealed by our current study and earlier studies of others (Miller and Aime, 2001; Miller 2003) using both molecular and morphological evidences, clearly suggests a very early split of *C. tomentosus* and *C. pseudotomentosus* from the rest of the genus. Alternatively, clade IV might represent an ancestral stock of the genus.

Because clades II, III, and IV all had significant statistic supports for monophyly and morphological features indicated clear distinctions, we think it may be appropriate to divide the genus into at least three monophyletic groups or sections and abandon the original two-sectioned infrageneric treatment of *Chroogomphus*. However, formal change of the infrageneric grouping within this genus should await more molecular and morphological data from additional taxa.

Geographical divergence in Chroogomphus

The geographic distribution of *Chroogomphus* generally coincides with that of its mycorrhizal host tree genus *Pinus* (Dahlberg and Finlay, 1999; Wang *et al*., 1999; Liu *et al*., 2002). Our phylogenetic results support a strong geography-based pattern of *Chroogomphus* evolution (Fig. 1). In the *C. rutilus/C. ochraceus* complex, *C. ochraceus* was clustered with *C. filiformis*, *C. vinicolor* and *C. jamaicensis* (however without bootstrap support). In this assemblage, *C. vinicolor* is widely distributed in North America and *C. jamaicensis* fruits in the regions of southern and southeastern United States and Jamaica. Both of them associate with 2- or 3-needle pines of *Pinus* subgen. *Pinus* (Miller, 1964; Miller and Aime, 2001). The other two species, *C. filiformis* and *C. ochraceus*, are symbionts of 5-needle pines of *Pinus* subgen. *Strobus*. *Chroogomphus filiformis* occurs in forests dominated by *P. armandii* in southwestern China, while *C. ochraceus* is widely distributed in North America from the Atlantic eastern coast states to the Pacific western coast regions and fruits preferably under *P. strobus* and *P. monticola* (Singer, 1949; Miller, 1964; Miller and Aime, 2001; Miller, 2003). The latter species has been found from Europe under 2-needle pines, such as *P. pinaster*, *P. nigra* ssp. *salzmannii* etc. (Villarreal and Heykoop, 1996). It should be cautioned that *C. ochraceus* from Europe might not be the same as those from North America. Molecular analyses of specimens from both regions should be able to settle this issue.

In the four closely related species within clade II, *C. sibiricus* occurs in Far East Asia, while *C. leptocystis*, *C. helveticus* and *C. roseolus* fruit in western North America, Europe and southwestern China respectively. Because both morphological and molecular divergences among the four taxa were limited, they likely started diverging from each other very recently. Both mycorrhizal host specificity or preference and geographic separation could contribute to their ongoing divergence.

In clades III and IV vicariously paired or closely related species (*C. asiaticus*-*C. albipes*, and *C. pseudotomentosus*-*C. tomentosus*) between Eastern Asia and North American were uncovered. Similar distribution patterns in other groups of fungi have also been elucidated in the last few years (Wu and Mueller, 1997; Wu *et al*., 2000; Mueller *et al*., 2001; Zhang *et al*., 2004; Yang, 2005; Petersen and Hughes, 2007).

Host-switch in Chroogomphus species

Chroogomphus can either form typical ectomycorrhiza with *Pinus* or parasitize on ectomycorrhizae of *Suillus* and *Rhizopogon* associated with pines (Agerer, 1990 and 1991). For the parasitic life style, the biological relationships among pine, *Suillus*/*Rhizopogon* and *Chroogomphus* are not well understood. As the mycorrhizal associations of *Chroogomphus* with *Pinus* are constantly observed, the mycorrhizal host specificity or preference and its evolutionary tendency are discussed briefly below.

In Fig. 1, clade IV is basal to other clades of *Chroogomphus*. In clade IV, *C. tomentosus* seemed limited to North America and were mostly collected from mixed forests of *Pinus monticola*, *P. contorta* and *Thuja plicata* (Miller, 1964), while its sister species *C. pseudotomentosus* occurred in East Asia and was found in mixed forests of *Pinus*, *Abies*, *Picea*, and *Quercus* in China, of *Abies*, *Larix*, *Thuja* and *Fagus* in Korea and Japan, and of *Larix* and *Tsuga* in Nepal (Miller, 2001; Li, 2007).

During subsequent evolution, it seems that mycorrhizal host ranges of *Chroogomphus* species became progressively narrower and with stronger specificity or preference. In clades I, II, and III of Fig. 1, most species were found to form mycorrhizal associations with *Pinus*. Species in clade III formed ectomycorrhizal associations with 2- or 3-needle pines of subgen. *Pinus*, while species of clade II were associated with both 2-needle pines in subgen. *Pinus* (e.g. *C. roseolus* with *P. densata*) and 5-needle pines of subgen. *Strobus* (e.g. *C. helveticus* with *P. cembra*, *C. leptocystis* with *P. monticola*, and *C. sibiricus* with *P. sibirica*) (Singer, 1950; Miller, 1964; Singer and Kuthan, 1976; Pacioni and Fogel, 1990; Wang *et al*., 1999; Miller, 2001). A mycorrhizal host-switch within the closely related species of clade II could be inferred (Fig. 1).

In the *C. rutilus/C. ochraceus* complex (Clade I of Fig. 1), a well-supported lineage (with bootstrap value 90%) was uncovered and this lineage included three taxa, namely *C. orientirutilus*, *C. rutilus* and *C. purpurascens*. *C. purpurascens* occurs in forests dominated with 5-needle pines in the *Pinus* subgen. *Strobus*, such as *P. koraiensis* in Far East Aisa and *P. cembra* in Central Europe, while *C. rutilus* and *C. orientirutilus* grow in forests dominated with 2- or 3-needle pines, such as *P. sylvestris* in Europe, *P. tabuliformis* and *P. densiflora* in northeastern China, and *P. yunnanensis* and *P. densata* in southwestern China, all being members of the *Pinus* subgen. *Pinus* (Singer, 1949; Miller, 1964; Singer and Kuthan 1976; Azbukina, 1990; Wang *et al*., 1999). The results here again indicate that a host-switch between the two subgenera of *Pinus* during the evolution of the allied mushroom species. Such host-shifts might be a relaxation to the evolution of strict mycorrhizal host specificity and can be common in certain symbiotic relationships (Halling and Ovrebo, 1987; Mueller and Strack, 1992; Kretzer *et al*., 1996; Hibbett *et al*., 2000). These mycorrhizal symbioses appear to be unstable, and can even switch to parasitism and saprophytism in the *Boletales* (Binder and Hibbett, 2001). In central Europe, eastern Russia and northeastern China *C. rutilus* is distributed sympatrically with *C. purpurascens* but with plant hosts in two different subgenera of *Pinus*. The divergence between *C. orientirutilus* and *C. rutilus* observed here could be the result of geographic separation and/or ecological niche differentiations such as plant host specialization and climate differences.

Concluding remarks

The phylogenetic relationships among taxa in the genus *Chroogomphus* from Europe are not as well understood as those from East Asia and North America. Although a few taxa of this genus were described from Europe in the last century (Singer 1950; Miller and Watling 1970; Singer and Kuthan, 1976; Kahn and Hora 1978; Trimbach, 1988), they have often been treated as synonyms or infraspecific taxa of other older names recently (see Miller and Aime, 2001; Miller, 2003; Watling, 2004). Because diagnostic morphological characters among the species of C. *rutilus/C. ochraceus* are often subtle, future studies would require more molecular sequence data to help fully resolve their evolutionary relationships.

Chroogomphus exhibits strong mycorrhizal host specificity with the tree genus *Pinus* (Miller, 2003). This type of specific mycorrhizal association with *Pinus* is also found in the genus *Suillus*. Moreover, both *Chroogomphus* and *Suillus* are phylogenetically related (Kretzer *et al*., 1996; Bruns *et al*., 1998; Dahlberg and Finlay, 1999; Wu *et al*., 2000; Binder and Hibbett, 2007). The interesting three-way association among pine, *Suillus*/*Rhizopogon* and *Chroogomphus*/*Gomphidius* has received considerable attention (Agerer 1990, 1991; Olsson *et al*., 2000). Our field investigations of the Chinese *Chroogomphus*, especially those from southwestern China, show that species of this genus may also be associated with different *Suillus* species. Further field investigations, careful morphological observations and extensive molecular analysis using multiple genes should help better understand the biological and ecological relationships among the different groups of fungi and their hosts.

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