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Contributed Paper

The Genus *Phylloporus* (*Boletaceae*, *Boletales*), from Mekong River Basin (Yunnan Province, China)

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ABSTRACT

The genus *Phylloporus* is a gilled bolete and a typical ectomycorrhizal mushroom which forms mycorrhizal associations with woody Fagaceae or Pinaceae. Collections of *Phylloporus* were made from Xiaozhongdian (Shangri-La), Haitang (Baoshan) and Mengsong (Xishuangbanna) from May to October 2012. Morphology and sequence analysis revealed that the *Phylloporus* collections belonged to seven groups and five species: *P. imbricatus* (Xiaozhongdian, Shangri-La), *P. pachcystidiatus* and *P. yunnanensis* (Haitang, Baoshan), *P. rubiginosus* (Mengsong, Xishuangbanna) and *P. megacystidiatus* sp. nov (Mengsong, Xishuangbanna). In this paper we introduce one new *Phylloporus* species: *Phylloporus megacystidiatus* sp. nov., and new record *P. rubiginosus* for the first time in Yunnan Province, China. The new species and the new record are described and illustrated with drawings and color photos. Also the species and new record are compared with similar taxa.

Keywords: biodiversity, ectomycorrhizal fungi, medicinal fungi, *Phylloporus*

1. INTRODUCTION

Most species of Boletaceae are economically important edible and medicinal fungi [1-3], and also ecologically important ectomycorrhizal fungi [4-6]. The genus *Phylloporus* is a gilled bolete and a typical ectomycorrhizal mushroom which forms mycorrhizal associations with woody Fagaceae or Pinaceae. There are 108 records of *Phylloporus* in Index Fungorum [7], and according to Neves *et al*. [8], 70 *Phylloporus* species have been described worldwide.

Mushroom diversity varies according to the vegetation type, biotic and biotic factors [9]. The Mekong River flows through four unique ecological areas as it travels through Yunnan Province, China: the region of Hengduan Mountains, the region of Jinsha River of Yunnan, the valleys region of North-western Yunnan and South-western Yunnan rain-forest region, which are diverse in terms of mushrooms [10]. There are rich higher fungi resources in southern China and nearly 1,200 genera and over 5,000 species of fungi that belong to Ascomycota and Basidiomycota can be found in this region [11].

Several species of *Phylloporus* have been recorded in Yunnan Province but these data have not been published. Some of the species, such as *P. rhodoxanthus* (Schwein.) Bres. can be found in Yunnan's wild mushroom markets. In addition, publications have discussed the nutritional values and symbiotic relationships of *Phylloporus* species. For example *P. bellus* (Massee) Corner has a symbiotic relationship with various trees [8, 12-14].

We are studying underground microbial diversity of the selected land use types in Yunnan Mekong River Basin (Shangri-La, Baoshan, Xishuangbanna), Laos and northern Thailand which expect to find numerous new species and records of mushrooms to China, Laos and Thailand [15,16]. Abundance of wild mushrooms are bioindicators of ecosystem health [17-21]. In Thailand and Laos and some parts of Yunnan, wild mushrooms are poorly studied and recent studies focusing on specific genera have resulted in a large number of new species [15,16, 22-39], indicating how little we actually know about the organisms and the role they play. Our main aim is to explore the ecologically and economically important genus *Phylloporus*, in Yunnan Province, in order to better understand the ecological importance of this genus.

In this paper, we report on collections of *Phylloporus* made in fungal biodiversity plots in Yunnan Province [40] using macro- and micro-characteristics, and phylogenetic analyses inferred from ITS rDNA sequences. We introduce *Phylloporus megacystidiatus* as a new species and *P. rubiginosus* as a new record, in this paper.

2. MATERIALS AND METHODS

2.1 Study Sites

Sample collection plots with different land use types were established in three areas of the Yunnan Mekong River Basin (8 plots in Shangri-La, 7 plots in Baoshan, 4 plots in Mengsong, Xishuangbanna). Each 100m×100m plot was randomly selected within each forest type and permanently established [41]. The plot types in which we found *Phylloporus* species were mixed forests dominated by Pinaceae and other broad-leaf trees. These included high land Ericaceae (Xiaozhongdian, Shangri-La and Haitang, Baoshan) and evergreen broad-leaf old forests dominated by large and old Fagaceae, such as *Castanopsis* sp. (Mengsong, Xishuangbanna) and mixed tea garden dominated by *Camellia sinensis* (Mengsong, Xishuangbanna).

2.2 Specimen Collections

Specimens were photographed *in situ*, gathered and wrapped in aluminium foil or kept separately in a collecting box in order to avoid mixing or crushing, and returned to the laboratory for further analysis and characterization. Odor and color change upon bruising were recorded at the time of collection. The description of macrocharacteristics, chemical testing and further photographing of fresh samples were carried out as soon as possible after returning from the field. This was done in accordance with the methodology described by Largent [42]. Color terms described by Kornerup and Wanscher [43] were used. Specimens were dried in a food drier, sealed in plastic bags, and deposited in the Kunming Institute of Botany herbarium.

2.3 Micromorphological Observations

Micromorphological features were documented by examining dried specimens following the protocols of Largent [42]. For micro-morphological examination, sections were cut with a razor blade from dried specimens and mounted on slides in 5% KOH and Congo red, and then observed, measured and illustrated using a compound microscope (Zeiss Axioskop 40). In the description of the basidiospores, "n" indicates the number of basidiospores (20 basidiospores per one collection) which were measured; L_m = mean basidiospore length over a population of basidiospores; W_m = mean basidiospore width over a population of basidiospores; Q = "length/width ratio" (L/W) of a basidiospore in side view; Q_m = average Q of all basidiospores measured.

2.4 Sequence Alignment and Phylogenetic Analysis

The taxon information and GenBank accession numbers used in the molecular analysis are listed in Table 1. Sequences for each strain were aligned using Clustal X [44]. Alignments were manually adjusted to allow for maximum sequence similarity. Gaps were treated as missing data. Maximum Likelihood (ML) and unweighted maximum Parsimony (MP) analyses were performed using PAUP* 4.0b10 [45]. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Clade stability of the trees resulting from the parsimony analyses were assessed by bootstrap analysis with 1,000 replicates, each with 10 replicates of random stepwise addition of taxa [46]. Trees were viewed in Treeview and exported to graphics programs [47].

3. RESULTS

3.1 Sequence Analysis

The ITS dataset of the maximum parsimony tree includes 40 sequences of the genera *Phylloporus* and *Xerocomus*. *Xerocomus magniporus* was chosen as the outgoup (Figure 1). Phylogenetic analysis revealed five clades of *Phylloporus*: Clade I with 90% bootstrap support, comprised four collections of *P. yunnanensis*. Clade II was basal to the four *P. yunnanensis* samples and comprised *P. megacystidiatus* sp. nov. with 69% bootstrap support. Clade III comprised four collections of *P. imbricatus*, (100% bootstrap support). Clade IV comprised five collections of *P. rubiginosus* with 100% bootstrap support. Clade V, which was supported by 70% bootstrap support, consisted of six collections of the genus *Phylloporus,* which included two sequences of *P. pachcystidiatus* from Baoshan and three sequences of *P. pachcystidiatus,* which showed 81% similarity to the two sequences of *P. centroamericanus*.

The equally weighted maximum parsimony tree, by contrast, showed a consistency index (CI) of 0.541, a retention index (RI) of 0.739, a rescaled consistency index (RC) of 0.399, and a homoplasy index (HI) of 0.459. There were 1708 characters in this analysis, of which 681 were constant, 205 were parsimony-uninformative and 822 were parsimony-informative.

Supplementary Table 1: Species used in molecular phylogenetic analyses, and GenBank accession numbers.

Species	Voucher	Locality	GenBank accession numbers
Phylloporus alborufus	MAN022	Costa Rica	JQ003624
P. bellus	HKAS 56763	Yunnan, SW China	JQ967239
P. bellus	HKAS 42850	Yunnan, SW China	JQ967240
P. brunneiceps	HKAS 56903	Yunnan, SW China	JQ967241
P. castanopsidis	MAN107	Thailand	JQ003643
P. centroamericanus	MAN016	Costa Rica	JQ003637
P. centroamericanus	MAN037	Costa Rica	JQ003634
P. cyanescens	REH8681	Australia	JQ003621
P. foliiporus	JLMI1677	eastern USA	JQ003641
P. imbricatus	HKAS 54647	Yunnan, SW China	JQ967245
P. imbricatus	HKAS 54859	Yunnan, SW China	JQ967246
P. imbricatus	HKAS 54860	Yunnan, SW China	JQ967247
P. leucomycelinus	MB00-043	eastern USA	JQ003628
P. luxiensis	HKAS 57037	Yunnan, SW China	JQ967251
P. luxiensis	HKAS 57048	Yunnan, SW China	JQ967252
P. maculatus	HKAS 56683	Yunnan, SW China	JQ967253
P. orientalis	REH8756	Australia	JQ003652
P. pachycystidiatus	HKAS 54540	Yunnan, SW China	JQ967254
P. pachycystidiatus	HKAS 54541	Yunnan, SW China	JQ967255
P. parvisporus	HKAS 54768	Yunnan, SW China	JQ967257
P. pelletieri	K 128205	England	JQ967258
P. rhodoxanthus	JLMI1808	eastern USA	JQ003654
P. rubeolus	HKAS 54543	Yunnan, SW China	JQ967261
P. rubiginosus	MAN117	Thailand	JQ003645
P. rubiginosus	MAN117	Thailand	JQ003647
P. rubrosquamosus	HKAS 54559	Yunnan, SW China	JQ967262
P. rufescens	HKAS 59723	Hainan, southern China	JQ967264
P. scabripes	REH8531	Belize	JQ003623
P. yunnanensis	HKAS 52225	Yunnan, SW China	JQ967265
P. yunnanensis	HKAS 52527	Yunnan, SW China	JQ967266
P. yunnanensis	HKAS 56999	Yunnan, SW China	JQ967267
P. imbricatus	HKAS 78355	Shangri-La, NW Yunnan	**KF052997**
P. yunnanensis	HKAS 78353	Baoshan, western Yunnan	**KF052998**
P. megacystidiatus sp. nov.	HKAS 78351	Mengsong, south Yunnan	**KF052999**
P. rubiginosus	HKAS 78349	Mengsong, south Yunnan	**KF053000**
P. rubiginosus	HKAS 78350	Mengsong, south Yunnan	**KF053001**
P. pachycystidiatus	HKAS 78354	Baoshan, western Yunnan	**KF053002**
P. pachycystidiatus	HKAS 78354	Baoshan, western Yunnan	**KF053003**
P. rubiginosus	HKAS 78352	Mengsong, south Yunnan	**KF053004**
Xerocomus magniporus	HKAS 59820	Yunnan, SW China	JQ678697

● Newly generated sequences are in bold

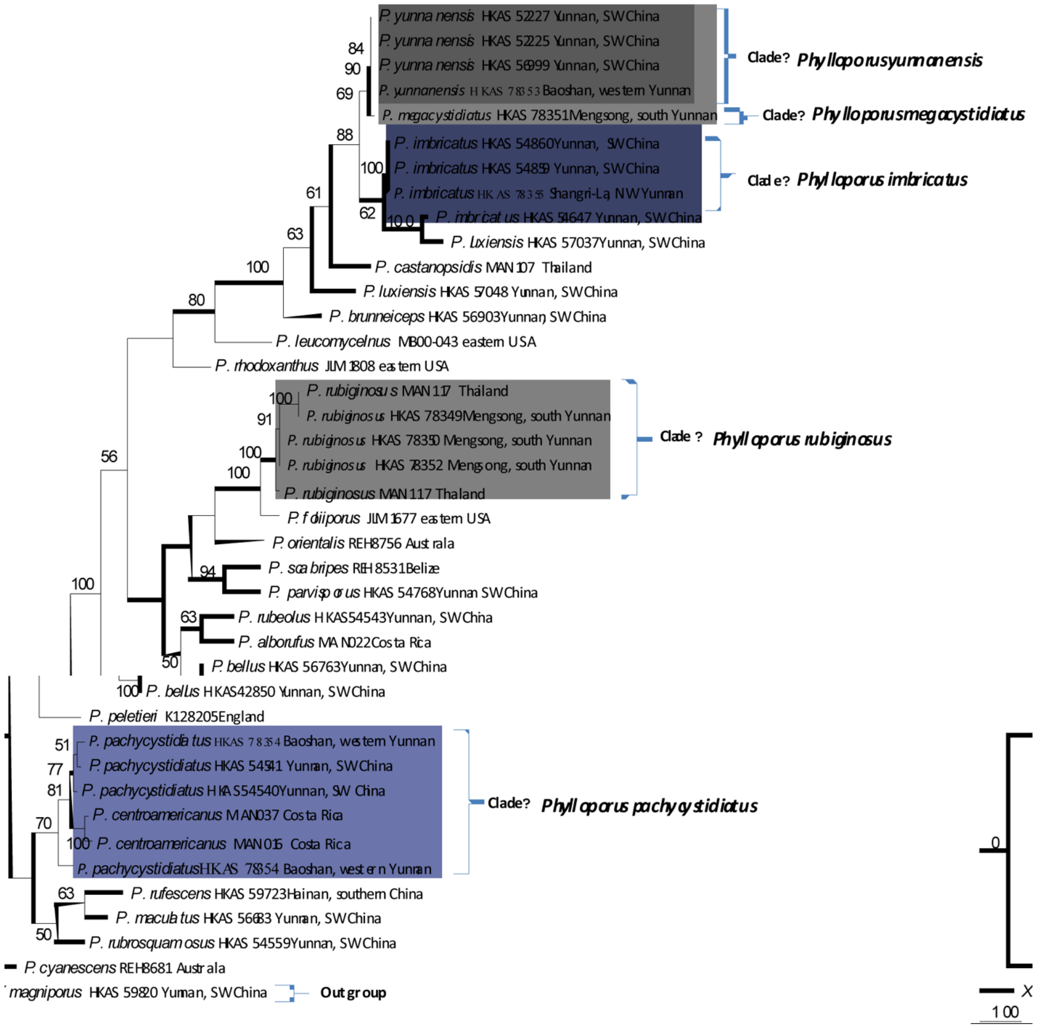

Figure 1. Maximum parsimony phylogram showing the phylogenetic position of *Phylloporus megacystidiatus*, *P. rubiginosus*, *P. imbricatus*, *P. pachcystidiatus* and *P. yunnanensis* with some selected *Phylloporus* species based on ITS2 rDNA sequences. Data were analysed with random addition sequence, unweighted parsimony and gaps were treated as missing data. Values above the branches are parsimony bootstrap (≥ 50%). The tree is rooted with *Xerocomus magniporus* M. Zang & R.H. Petersen.

Taxonomy

Phylloporus rubiginosus M.A. Neves & Halling, Fungal Divers, 55: 118-119, 2012 Figures 2a-e, 4a-c

Pileus: 40-59 mm in diameter, convex to plano-convex, applanate with age, slightly depressed at the centre and inflexed in age. The surface is dry, smooth, then squamulose or becoming cracked with age. It is dark brown (6F8) when young and becomes reddish brown (8E8) or mahogany (8E7) and light brown (6D8) or deep red orange (7F6) with age. The margin is inrolled when young. Context: 7-11 mm thick and soft. It is light yellowish generally but turns a greenish-blue when injured.

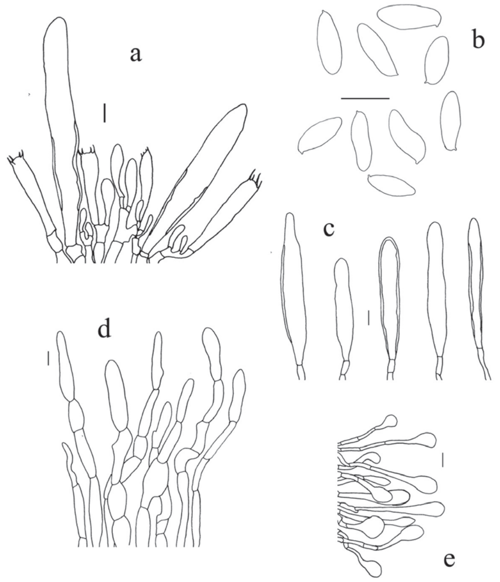

Figure 2. Micromorphological features of *P. rubiginosus*: a. Basidia and pleurocystidia, b. Basidiospores, c. Cheilocystidia, d. Pilleipellis, e. Stipitipellis (scale bars a, b, c, d and e = 10 μm).

Annulus and volva: absent. Basidia: 33.5-51×8-10.5 μm, four spored, clavate and thin-walled. It is colorless or yellowish in KOH. The sterigmata is 4-6 μm in length. Basidiospores: [120/4/2] 10-13×4-5 μm, some spores are slightly thick-walled and subfusiform to subclavate. They are yellowish and appear smooth under a light microscope. Pleurocystidia: 98.5-125×13-16.5 μm, abundant, subfusiform to fusiform, thick-walled, yellowish and possess no encrustations. Cheilocystidia: 74.5-110× 10.5-15 μm, abundant, clavate, slightly thick-walled, and colorless in KOH, no encrustations. Pilleipellis: a trichoderm composed of compact, branched hyphae with some positioned vertically. It is smooth and yellowish in KOH. The terminal cells are 32-58×5.5-13 μm and narrowly clavate. Stipitipellis: a trichoderm-like structure composed of thin to slightly thick walled hyphae. The terminal cells are 20-68×8.5-11.5 μm, clavate and colorless. Taste and smell: No special odor or taste. Spore print: yellow.

Habitat and distribution: Solitary or as a group, mostly found on old and living *Castanopsis* tree bases. This is a new record to China.

Material examined: CHINA, Yunnan province: Xishuangbanna, Mengsong Village; 47R UTM E 656217.753714; UTM N 2379244.485281, alt. 1600 m; 15 September. 2012; Lei Ye (HKAS78349) *ibid.* 10 Oct. 2012; Lei Ye (HKAS78350) *ibid.* 30 Aug. 2012; *ibid.* Lei Ye; (HKAS78352) *ibid.*

Notes: *P. rubiginosus* is a sister group in the *P. foliiporus* and *P. orientalis* clade, and compared to these two species *P. rubiginosus* has a typical reddish-brown or brown fruiting bodies. The basidia and basidiospores of *P. foliiporus* are similar to *P. rubiginosus*, but the basidiospores of *P. foliiporus* are larger (11-15×4.5-5.8 μm) than *P. rubiginosus* (10-13×4-5 μm). *P. rubiginosus* is also phylogenetically related to *P. luxiensis*, *P. yunnanensis* and *P. megacystidiatus* sp. nov. (Figure 1).

Phylloporus megacystidiatus L. Ye, P.E. Mortimer, J.C. Xu & K.D. Hyde sp. nov., Figures. 3a-e; 4d-f.

MycoBank: MB804643

Holotype: HKAS78351

Etymology: *megacystidiatus* refers to the long and big Cheilocystidia.

Pileus: 30-55 mm diameter, convex to applanate and depressed or infundibuliform at the centre. The surface is dry, smooth and then squamulose with age. The pileus has light yellow (2A5) and light brown (6D8) scales points. The margin is inrolled when young and becomes uplifted with age. Context: 5-6 mm thick and soft. The creamy to yellowish color does not change with injury. Lamellae: 6.5-12 mm wide, subdistant and commonly anastomosing. The lamellae are light yellow (2A5) and deeper yellow (2A8) in color, with older fruiting bodies exhibiting an orange yellow (4A8). They become greenish-blue when injured. The lamellulae are crowded, common and the same color as the lamellae. Stipe: 25-31×5-7 mm, central, subcylindric to cylindric and solid to fistulose. The surface is dry, smooth to tomentose, white (A1) and orange white (5A2). Annulus and volva: absent. Basidia: 32-42×9-12 μm, four spored, clavate, thin-walled, colorless or yellowish in KOH; sterigmata 4-5 μm in length. Basidiospores: [50/2/2] 10-12×3-5 μm. Some spores are slightly thick-walled and subfusiform to subclavate. They are yellowish and appear smooth under a light microscope. Pleurocystidia: 84-110×11-14 μm, abundant and subfusiform to fusiform. They are thick-walled and yellowish with no encrustations. Cheilocystidia: 94-181× 10-17 μm, abundant, subfusiform and thick-walled. They are colorless in KOH and have no encrustations. Pilleipellis: a trichoderm composed of compact, branched hyphae, which are smooth and yellowish or colorless in KOH. The terminal cells are 112-127×25-49 μm and narrowly clavate. Stipitipellis: a trichoderm-like structure composed of thin to slightly thick walled hyphae. The terminal cells are 156-255×32-54 μm, narrowly clavate or clavate and colorless. Taste and smell: No special odor or taste. Spore print: light yellow and yellow.

Habitat and distribution: Solitary and found in areas planted with mixed tea.

Material examined: CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, UTM N2379744.485281, UTM E653217.753714, alt. 1600 m, 19 Sep. 2012, Lei Ye, (HKAS78351, **holotype**).

Notes: Phylogenetic analysis showed that *P. megacystidiatus* is closely related to *P. yunnanensis*, but the fruiting bodies and a number of microscopic characteristics were disparate. The pileus of *P. megacystidiatus* is light yellow and the cap tissues are softer than *P. yunnanensis*. This new species has similar basidiospores to *P. yunnanensis*, but the basidia are wider (width: 9-12 μm), and the pleurocystidia are narrower. The cheilocystidia of *P. megacystidiatus* are also longer (length: 94-181 μm) and narrower (width: 10-17 μm) than *P. yunnanensis*. The terminal cells of the pileipellis in *P. megacystidiatus* are large (length: 112-127 μm), while *P. yunnanensis* has smaller (length: 52-76 μm) pileipellis terminal cells. The terminal cells of the stipitipellis in *P. megacystidiatus* are longer (length: 156-255 μm) and larger (size: 156-255×32-54 μm) than those of *P. yunnanensis*, furthermore the stipitipellis cells in these two species always have round apices.

4. DISCUSSION

Seven different *Phylloporus* species were collected from Shangri-La, Mengsong and Baoshan in Yunnan Province between September and October 2012. The specimens of *Phylloporus* were obtained from Alpine, temperate and subtropical regions of the Yunnan Province, indicating the wide habitat range of *Phylloporus* (Table 2).

In the phylogenetic tree (Figure 1), Clade II was basal to the four *P. yunnanensis* samples and comprised *P. megacystidiatus* sp. nov. with 69% bootstrap support. The morphological data (Figure 3) together with phylogenetic results also support *P. megacystidiatus* as a distinct new species. The *P. rubiginosus* samples form a distinct group in clade IV in our phylogenetic tree (Figure 1). *P. rubiginosus* was recorded in a sub-tropical rain forest in Xishuangbanna,

Table 2. List of *Phylloporus* species found in different forest types.

Species name of *Phylloporus*	Voucher	Forest type
P. rubiginosus	HKAS78349, HKAS78350, HKAS78352	Tropical broad-leaf forest dominated by *Castanopsis mekongensis*
P. megacystidiatus	HKAS78351	Tropical mixed tea plant garden dominated by *Camellia sinensis* with few *Castanopsis mekongensis*
P. yunnanensis	HKAS78353	Alpine broad-leaf forest dominated by *Casternopsis* spp. and *Lithorcarpus* spp.
P. pachcystidiatus	HKAS78354	Alpine pine forest dominated by *Pinus yunnanensis*
P. imbricatus	HKAS78355	Alpine mixed forest dominated by *Picea likiangensis*, *Pinus densata* and *Rhododendron decorum*

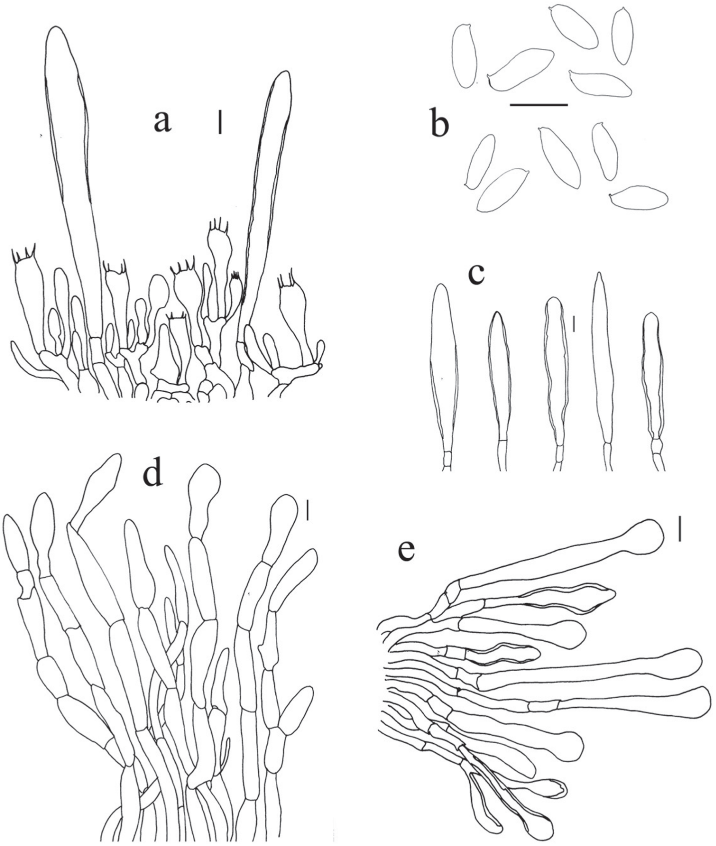

Figure 3. Micromorphological features of *P. megacystidiatus*: a. Basida and Pleurocystidia, b. Basidiospores, c. Cheilocystidia, d. Pilleipellis, e. Stipitipellis (scale bars a, b, c, d and e = 10 μm).

Figure 4. Basidiomata of *Phylloporus* species a–c. *P. rubiginosus*; d–f. *P. megacystidiatus* sp.nov; g–h. *P. imbricatus*; i–j. *P. pachcystidiatus*; k–l. *P. yunnanensis*.

China; which is a new record for this species in China. This species has previously only been recorded from northern Thailand [8]. Clade I comprises four samples of *P. yunnanensis* with 90% bootstrap support, while Clade III comprises four samples of *P. imbricatus* with 100% bootstrap support. Clade V has 70% bootstrap support, and comprises six samples from *Phylloporus,* including four samples of *P. pachcystidiatus* with 81% similarity, plus the two samples of *P. centroamericanus*.

According to the published literature this genus is considered to be best represented in Australia and Malaysia where most of the described species are distributed [8, 48-50], and also in Africa [51,52]. The genus is also known from North America [53-56], and Europe [57]. However *Phylloporus* species have not been well-studied in many regions of the world including China. Further research on the ecology, taxonomy and phylogeny on this relatively poorly known genus is needed.

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