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A new species in the *Skeletocutis subincarnata* complex (Polyporales, Basidiomycota) from southwestern China

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Abstract

A new polypore species, *Skeletocutis pseudo-odora*, was collected on fallen branches of *Pinus armandii* in Guizhou Province, southwestern China. It is described based on molecular data and morphological characteristics. The species belongs to the *Skeletocutis subincarnata* complex, and macro-morphologically resembles *S. albocream* and microscopically is similar to *Skeletocutis odora*. However, *S. albocream* can be readily distinguished from *S. pseudo-odora* by having hyphal pegs and wider basidiospores (4–5 × 1.3–1.7 µm). *S. pseudo-odora* differs from *S. odora* in having corky basidiocarps without any odour or taste when fresh, smaller pores (6–8 per mm), thick and entire dissepiments. Phylogeny inferred from the ITS dataset indicates that *Skeletocutis* under the current concept was not a monophyletic genus and *S. pseudo-odora* was distinct from other sampled species of *Skeletocutis*, including *S. odora*.

Key words – Phylogeny – polypore – taxonomy – wood-decaying

Introduction

The polypore genus *Skeletocutis* Kotl. & Pouzar, typified by *Skeletocutis amorpha* (Fr.) Kotl. & Pouzar, was established in 1958 (Kotlába & Pouzar 1958). Species of the genus mostly have resupinate basidiocarps although its type species has pileate or effused-reflexed basidiocarp, many species have tiny basidiospores and are found on gymnosperm wood in boreal and temperate forests from the northern hemisphere (Gilbertson & Ryvarden 1987, Niemelä 1998, Dai 2002, Dai & Wu 2004, Dai 2012, Ryvarden & Melo 2014, Bian et al. 2016). During the survey of lignicolous fungi in southwestern China, four specimens were collected on fallen branches of *Pinus armandii* from Leigongshan Nature Reserve, Guizhou Province, and they have resupinate basidiocarps with distinct cottony sterile margin, small pores, a dimitic hyphal structure with scanty skeletal hyphae at dissepiment edges, tramal generative hyphae bearing fine, sharp-pointed encrustations, and allantoid basidiospores. These characters make these collections to represent a taxon in the *Skeletocutis subincarnata* complex, but no suitable existing name is available for them. After phylogenetic analysis and morphological examination, they turn out to be an undescribed species, and here we describe them as new.

Materials & Methods

Morphological studies

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The studied specimens are deposited in the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). The microscopic procedure follows Dai (2010). Microscopic measurements were made from slide preparations stained with Cotton Blue, Melzer's reagent and 5% potassium hydroxide. In the text the following abbreviations were used: KOH = 5% potassium hydroxide, IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, CB = Cotton Blue, CB– = cyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the ratios of L/W between specimens studied, n = number of spores measured from given number of specimens. Colour terms followed Petersen (1996).

Molecular procedures and phylogenetic analyses

The methods of DNA extraction and amplification in this study followed Chen et al. (2016). CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to extract total genomic DNA from dried specimens of the new collections according to the manufacturer's instructions with some modifications. The primers ITS5 /ITS4 was used for PCR amplifications (primer sequences used in this study were obtained from http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR products were sequenced in Beijing Genomics Institute, China, with the same primers. The newly generated sequences were deposited at GenBank and labelled in Fig. 1.

Besides the newly generated ITS sequences, the current ITS dataset included sequences from all available species of the three genera, viz. *Piloporia*, *Skeletocutis* and *Tyromyces*, in the tyromyces clade (Binder et al. 2013). The ITS sequences of *Cinereomyces lindbladii* from the gelatoporia clade was selected as outgroup (Binder et al. 2013). This dataset was aligned using MAFFT 7.110 (Katoh & Standley 2013) with the Q-INS-I opinion (Katoh et al. 2005).

Maximum likelihood (ML) and maximum parsimony (MP) were used to perform phylogenetic analysis. ML analysis was conducted using raxmlGUI 1.2 (Stamatakis 2006, Silvestro & Michalak 2012) under GTR + I + G evolutionary model. Bootstrap (BS) values were calculated under the auto FC option (Pattengale et al. 2010). MP tree was constructed using PAUP* version 4.0b10 (Swofford 2002) with the heuristic search option and default parameters. All characters were equally weighted and gaps were treated as missing data. Clade robustness was assessed using a BS analysis with 1,000 replicates (Felsenstein 1985).

Results

Phylogeny

The ITS dataset, including 26 sequences, resulted in an alignment with 784 characters, of which 396 characters are constant, 112 parsimony-uninformative and 276 parsimony-informative. BS searches for the ML analysis stopped after 350 replicates. MP analysis yielded six equally parsimonious trees (CI = 0.557, RI = 0.601). The ML and MP analyses generated congruent topologies in main clades, and thus only that from ML analysis was presented with BS values from both analyses.

The ITS-based phylogeny indicated the monophyly of either *Skeletocutis* or *Tyromyces* is questionable (Fig. 1). The newly sequenced specimens formed a distinct lineage (97% in ML, 100% in MP) from other sampled species.

Taxonomy

Skeletocutis pseudo-odora L.F. Fan & Jing Si, *sp. nov.* Figs 2–3

Index Fungorum number: IF553635; Facesoffungi number: FoF02916

Type – CHINA, Guizhou Province, Leishan County, Leigongshan Nature Reserve, on fallen branch of *Pinus armandii*, alt, 1800 m, 13 June 2016, Yu-Cheng Dai 16525 (Holotype, BJFC022513)

Etymology – *Pseudo-odora* (Lat.): referring to the similarity to *Skeletocutis odora.*

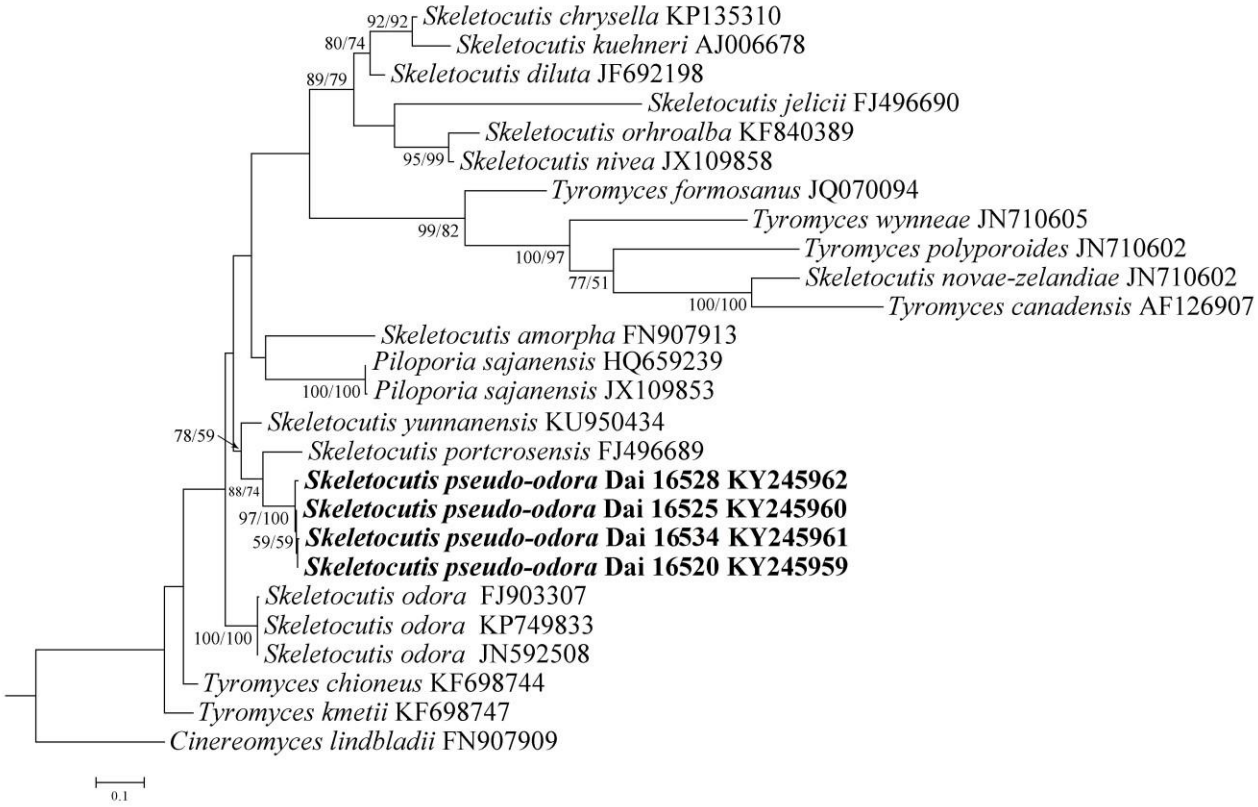

Fig.1 – Phylogenetic position of *Skeletocutis pseudo-odora i*nferred from the ITS dataset. The topology resulted from the maximum likelihood analysis is presented with bootstrap values from maximum likelihood (before the slash) and maximum parsimony (after the slash) analyses, if simultaneously higher than 50%, at the nodes. The newly sequenced specimens are labeled in boldface

Fruitbody – Basidiocarps annual, resupinate, very difficult to separate from substrate, soft leathery and without odour or taste when fresh, becoming hard corky upon drying, up to 6 cm long, 1.8 cm wide, and 1 mm thick at centre. Pore surface snow white to white when fresh, brownish buff and sometimes cracked upon drying; margin distinct, consistent white, byssoid to cottony, up to 2 mm; pores round, freely arranged, 6–8 per mm; dissepiments thick, entire. Subiculum white, soft corky, very thin, less than 0.1 mm thick. Tubes concolorous with poroid surface, hard corky, up to 0.9 mm long.

Hyphal structure – Hyphal system dimitic, generative hyphae with clamp connections, hyaline, thin-walled, dominant at dissepiment edge; skeletal hyphae thick-walled with a narrow lumen to subsolid; all hyphae IKI–, CB–, unchanged in KOH.

Subiculum – Generative hyphae frequent, hyaline, thin-walled, smooth, occasionally branched and smooth, 2.5–3.5 µm diam; skeletal hyphae dominant, thick-walled, flexuous, unbranched, interwoven, 3–4 µm diam.

Tubes – Generative hyphae frequent, thin-walled, rarely branched, usually covered by fine, sharp-pointed encrustations, especially at dissepiment edge, 2–3 µm diam; skeletal hyphae dominant at upper part of tube, thick-walled with a narrow lumen to subsolid, unbranched, interwoven, agglutinated, 2.5–3.5 µm diam. Dissepiment edge with scanty smooth skeletal hyphae and abundant, spirally winding, encrusted generative hyphae. Cystidia absent; cystidioles abundant, spindle-shaped to almond-shaped, with a conical apex or rudimentary neck, almost as size as basidia, 8–10 × 3–4 µm. Basidia broadly clavate to barrel-shaped, with a basal clamp connection and four sterigmata, 7–9 × 4–5 µm; basidioles in shape similar to basidia, but slightly smaller.

Spores – Basidiospores mostly allantoid, hyaline, thin-walled, smooth, with one or two guttules, CB–, IKI–, (3.9–)4–5(–5.7) × (0.9–)1.0–1.1(–1.2) μm, L = 4.43 μm, W = 1.06 μm, Q = 4.18 (n = 30/1).

Notes – *Skeletocutis pseudo-odora* is characterized by resupinate, snow white pores when fresh, but brownish buff when dry, distinct byssoid to cottony sterile margin, circular pores mostly 6–8 per mm with entire mouths, a dimitic hyphal structure in subiculum and upper part of tube, generative hyphae in trama covered by fine crystals, skeletal hyphae unchanged in KOH, allantoid basidiospores which measure 3.5–4.5 × 1.0–1.2 μm, and growing on pine wood.

Additional specimens examined (Paratypes) – CHINA, Guizhou Province, Leishan County, Leigongshan Nature Reserve, on fallen branch of *Pinus armandii*, alt. 1800 m, 13 June 2016, Y.C. Dai 16520 (BJFC022512), 16528 (BJFC022514), 16534 (BJFC022515).

Key to species of *Skeletocutis* in China

1. Basidiospores < 1 μm in diameter 2
1. Basidiospores >1 μm in diameter 11
2. Tramal hyphae monomitic 3
2. Tramal hyphae dimitic 4
3. Growing on angiosperms; pore surface usually with bluish-grey tint .....*S. nivea*
3. Growing on gymnosperms; pore surface with salmon or amber tint .....*S. ochroalba*
4. Basidiocarps with fimbriate to rhizomorphic margin .....*S. fimbriata*
4. Basidiocarps with entire margin 5
5. Basidiocarps pileate .....*S. inflata*
5. Basidiocarps resupinate 6
6. Pore surface violaceous .....*S. lilacina*
6. Pore surface white to cream .....
7 7. Basidiospores < 0.7 μm in width .....*S. luteolus*
7. Basidiospores > 0.7 μm in width 8
8. Pores angular, 5–6 per mm 9
8. Pores round, 7–11 per mm 10
9. Basidiocarps thin, fragile; accompanying *Trichaptum* species .....*S. kuehneri*
9. Basidiocarps sturdy, tough; accompanying *Phellinus* species .....*S. chrysella*
10. Basidiocarps perennial; skeletal hyphae unchanged in KOH .....*S. stellae*
10. Basidiocarps annual; skeletal hyphae swollen in KOH .....*S. substellae*
11. Rhizomorphs present 12
11. Rhizomorphs absent 13
12. Basidiospores < 5 μm in length .....*S. alutacea*
12. Basidiospores > 5 μm in length .....*S. percandida*
13. Basidiospores ellipsoid 14
13. Basidiospores cylindrical, allantoid to lunate 16
14. Growing on bamboo; pores 8–11 per mm .....*S. bambusicola*
14. Growing on hardwoods; pores 4–7 per mm 15
15. Basidiocarps perennial; basidiospores < 2.5 μm in width .....*S. perennis*
15. Basidiocarps annual; basidiospores > 2.5 μm in width .....*S. sensitive*
16. Basidiocarps effused-reflexed to pileate 17
16. Basidiocarps resupinate 18
17. Fresh pores orange; basidiospores > 1.3 μm in width .....*S. amorpha*
17. Fresh pores pinkish grey; basidiospores < 1.3 μm in width .....*S. carneogrisea*
18. Dissepiment edge almost monomitic 19
18. Dissepiment edge distinctly dimitic 22
19. Basidiocarps thick and fleshy, with odor when fresh .....*S. odora*
19. Basidiocarps thin and fragile, without odor when fresh 20

20. Basidiospores 1.3–1.7 μm in width .....*S. albocremea*
20. Basidiospores 1–1.2 μm in width 21
21. Pores mostly 5–6 per mm; generative hyphae in subiculum and trama covered by fine crystals ..... *S. yunnanensis*
21. Pores mostly 6–8 per mm, generative hyphae in trama covered by fine crystals ....*S. albocremea*
22. Basidiospores curved 23
22. Basidiospores fairly straight 24
23. Pore surface lemon yellow when dry; accompanying *Phellinidium* species .....*S. brevispora*
23. Pore surface yellowish cream when dry; on rotten *Pinus* wood .....*S. subvulgaris*
24. Skeletal hyphae unchanged in KOH .....*S. biguttulata*
24. Skeletal hyphae dissolved in KOH .....*S. papyracea*

Discussion

Skeletocutis pseudo-odora is characterized by resupinate basidiocarp with distinct sterile margin, snow white poroid surface when fresh which becomes brownish buff when dry, small pores (6–8 per mm) with entire dissepiments, a dimitic hyphal structure with encrusted generative hyphae at tube trama only, cylindrical to allantoid basidiospores (4–5 × 1–1.1 μm), and growing on fallen wood of *Pinus*.

Macro-morphologically *Skeletocutis pseudo-odora* resembles *S. albocream* A. David by having thin basidiocarp with very thin subiculum, distinct, byssoid to cottony margin, similar pores (6–8 per mm) and entire pore mouths. However, *S. albocream* has frequent hyphal pegs, dominant generative hyphae in subiculum, thicker basidiospores (4–5 × 1.3–1.7 μm, Niemelä 1998); while *S. pseudo-odora* lacks hyphal pegs, its skeletal hyphae are dominant in subiculum, basidiospores are thinner (4–5 × 1–1.1 μm).

Microscopically *Skeletocutis odora* is very similar to *Skeletocutis pseudo-odora*, and both species occur on gymnosperm wood. In fact the latter comes to the former when identifying our specimens follow the key by Niemelä (1998). However, *S. odora* has soft, juicy and acidic smell fresh basidiocarp, bigger pores (4–6 per mm), thin and lacerate dissepiments. In contrast, *S. pseudo-odora* has corky fresh basidiocarp without any odour or taste, smaller pores (6–8 per mm), thick and entire dissepiments.

Skeletocutis yunnanensis L.S. Bian, C.L. Zhao & F. Wu was recently described from China (Bian et al. 2016), and it is similar to *S. odora* in having resupinate and white hymenophores and allantoid basidiospores (3.5–4.5 × 1–1.2 μm), but the former lacks sterile margin when mature, has bigger and angular pores (5–6 per mm), its generative hyphae in subiculum and trama are covered by fine crystals, and grows on angiosperm wood (Bian et al. 2016).

Phylogenetically, the currently accepted species of *Skeletocutis* did not clustered into a wellsupported clade and *S. pseudo-odora* was not closely related to *S. amorpha*, the generic type (Fig. 1). However, according to morphological concept, we place *S. pseudo-odora* in *Skeletocutis* for now. Obviously, a combination of morphological examinations on more samples of *Skeletocutis* and comprehensive phylogenetic analysis with the aid of multiloci is needed to construct the phylogenetic framework for *Skeletocutis*.

Southwest China is very rich biodiversity (Yang 2010, Li et al. 2011). Although Guizhou located in southwest China, is a mountainous province, but more new fungal taxa, including species of *Skeletocutis* (Zhou & Qin 2012, Bian et al. 2016), were found in Yunnan Province, the neighbor of Guizhou Province, e.g. more than 20 wood-decaying fungal species have been described from Yunnan during last ten years (Dai et al. 2007, 2009, Yuan & Dai 2008, He & Li 2011, 2013, Zhou & Dai 2012, Tian et al. 2013, Jia et al. 2014, Li et al. 2014, Zhou 2014, Zhao et al. 2015, Zhou 2015, Zhou et al. 2016), but very few new taxa were found from Guizhou Province (Wu et al. 2015, Zhou 2016). One reason is that Guizhou has the Karst landform, and its vegetation is less rich for species than that in Yunnan Province. Another reason may be less investigation and study on mycota in Guizhou, and the fungal diversity in the area is still not well known.

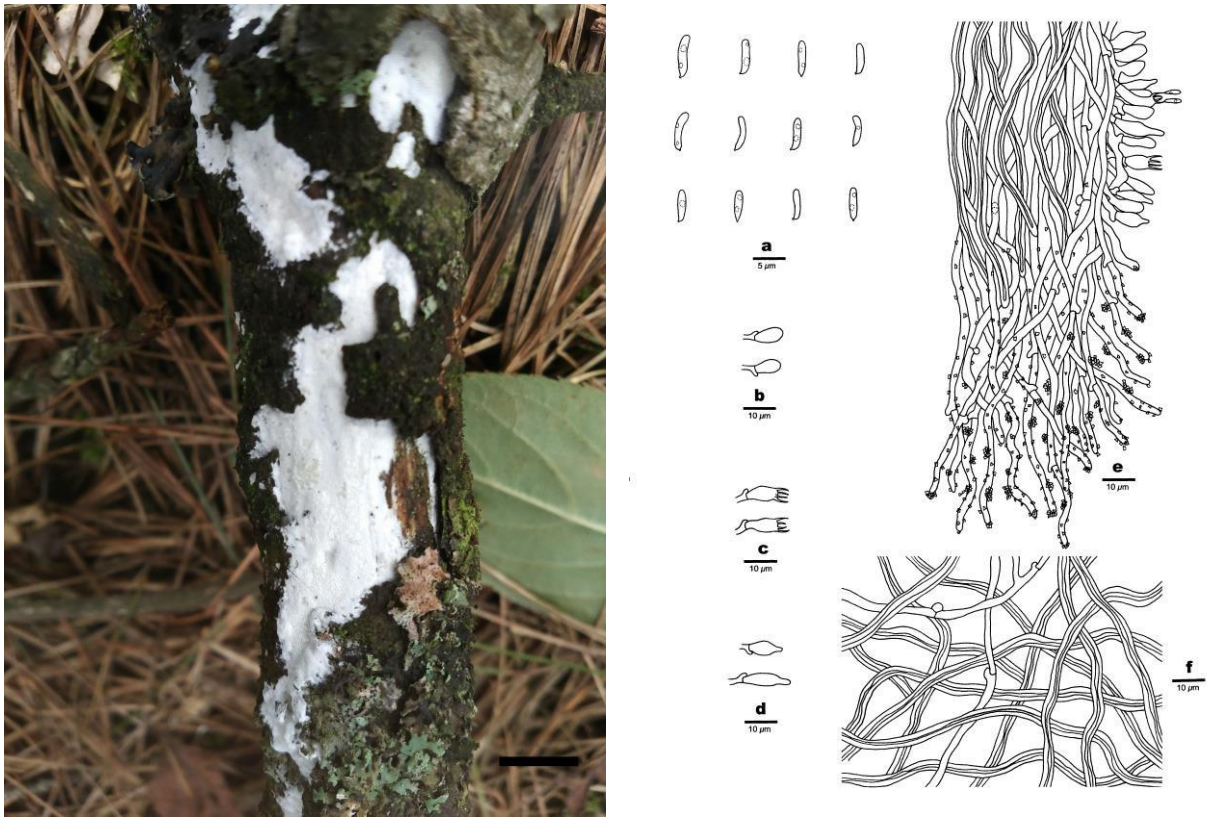

Fig. 2 – A basidiocarps of *Skeletocutis pseudo-odora, Dai 16534* (BJFC022515). – Bar: 1 cm.
Fig.3 – Microscopic structures of *pseudo-odora* (Holotype). a Basidiospores. b Basidioles. c Basidia. d Cystidioles. e A section from tube trama. f Hyphae from subiculum. Scale bars: a = 5 µm; b–f = 10 µm.

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