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SHORT COMMUNICATION

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Pseudolagarobasidium calcareum: Japanese records and cultural characteristics

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Abstract *Pseudolagarobasidium calcareum* (Basidiomycotina, Corticiaceae s.l.) is newly reported for Japan, and its cultural characteristics are described for the first time. Seven of nine Japanese and Taiwanese specimens examined produce arthroconidia associated with the basidiomata. Variability in color of the hymenial surface is also observed among the specimens. In culture, this species is characterized by fiber hyphae (quasi-binding hyphae) and arthroconidia. Mating tests reveal a multiallelic, bifactorial incompatibility system of this fungus and intercompatibility between Japanese and Taiwanese strains. This species resembles *Pseudolagarobasidium subvinosum* but differs in producing the fiber hyphae not only in culture but also in the basidiomata.

Key words Corticioid fungi · Cultural characteristics · Morphology · Taxonomy

Pseudolagarobasidium calcareum (Cooke & Massee) Sheng H. Wu is a resupinate wood-decaying fungus belonging to the family Corticiaceae (s.l.). It is characterized by a hydnoid hymenial surface and cylindrical or tubular leptocystidia. This species is reported from Australia (Jülich 1978), Malaysia and Sierra Leone (Reid 1956), and Taiwan (Wu 1990). Recently, we collected several specimens of this species in southeastern Japan. In this study, *P. calcareum* is described based on the Japanese specimens, and its cultural features are provided for the first time.

Light microscopic observations of basidiomata and cultured mycelia were made from freehand sections mounted in 2% KOH and in Melzer's reagent. Color names and herbarium abbreviations are from Rayner (1970) and Holmgren et al. (1990), respectively. Specimens for scanning electron microscopy were prepared using the method described in Maekawa (1987).

All cultures studied are of polysporous origin unless otherwise indicated. Cultures were grown on malt extract agar (MEA, 1.5% malt extract agar, Difco; 2% Bacto agar, Difco, Detroit, MI, USA), on 0.5% gallic acid agar (GAA), on 0.5% tannic acid agar (TAA) (Davidson et al. 1938), and on 0.2% tyrosine agar (TyA) (Nakasone 1990) at 25°C in the dark. To determine optimum growth temperature, the strains were grown on MEA plates at 13 different temperatures (4°–42°C). The strains examined in this study are deposited in the Tottori Mycological Institute Culture Collection.

For inducing basidioma, each dikaryotic strain was inoculated to saw-dust medium (containing 0.5% malt extract solution) in a Petri dish (90 mm in diameter, 50 mm in depth). After 30-day incubation at 27°C in the dark, a sterilized twig (50 mm long, 10 mm in diameter) of *Castanea crenata* Sieb. & Zucc. was placed on the medium colonized by *P. calcareum*, and the Petri dishes were placed on a laboratory bench at room temperature (15°–25°C). After 30–60 days of incubation, basidiomata were produced on the twigs covered with a mycelial mat.

Pseudolagarobasidium calcareum (Cooke & Massee in Cooke) Sheng H. Wu, Acta Bot. Fenn. 142:112, 1990.
≡*Hydnum calcareum* Cooke & Massee in Cooke, Grevillea 21:38, 1892.

Distribution: Africa (Sierra Leone), Asia (Japan, Malaysia, Taiwan), and Australasia (Australia).

Basidiomata resupinate, adnate, at first orbicular and then becoming widely effused; hymenial surface pale grayish-cream, "buff," "ochreous" to "hazel," smooth, odontoid or hydnoid, sometimes cracked when dried, 100–300 μm thick (excluding spines); aculei 3–10/mm^2, conical, to 3 mm long, 100–300 μm wide at the base; margin white to "buff," thinning out, macroscopically determinate, sometimes finely fibrillose under the lens (20×), without hyphal

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strands. In vertical section subhyaline, membranous with a basal layer (15–40 μm thick). Hyphal system dimitic; generative hyphae subhyaline, 1.5–3.5 μm in diameter, smooth, thin-walled, nodose-septate; sometimes encrusted with subhyaline to pale brownish crystalline materials in the trama, subhymenia, and hymenia; quasi-binding hyphae subhyaline, 0.5–2 μm in diameter, smooth, thin- to thick-walled (to 1 μm), aseptate, usually diverging at right angles; leptocystidia clavate to tubular, 30–110 × 5.5–7 μm, thin-walled, with a basal clamp; basidia narrowly clavate to suburniform, slightly sinuous, 13–24 × 4.5–5 μm with a basal clamp, producing four sterigmata; basidiospores broadly ellipsoid to subglobose, 4–6 × 3–4 μm, smooth, thin-walled, nonamyloid; arthroconidia sometimes present on the hymenial surface.

Specimens examined: Japan: Kagoshima, TMI16198 (TMI) on decaying branch of *Castanea crenata*, Hirakawa (Mt. Eboshidake), Kagoshima City, 25 Sept. 1992, col. & det. by N. Maekawa; Okinawa, TFM-F-16295 (TFM) on decaying hardwood, Nakura, Ishigaki City, 19 Sept. 1991, col. by T. Hattori, det. by N. Maekawa; TFM-F-16917 (TFM) on decaying hardwood, Taketomi-cho (riverside of Nakama River, Iriomote Island), Yaeyama-gun, 24 Sept. 1993, col. by T. Hattori, det. by N. Maekawa; TMI19856 (TMI) on decaying wood of a broad-leaved tree, Taketomi-cho (near Kanpira Cascade, Iriomote Island), Yaeyama-gun, 12 June 1996, col. & det. by N. Maekawa; TMI19890 (TMI) on decaying branch of a broad-leaved tree, Ishigaki City (Banna Park), 14 June 1996, col. & det. by N. Maekawa. Taiwan: Nantou, Wu881026-18 (TNM) on decaying branch of *Castanopsis kusanoi* Hayata, around Sun-Moon Lake, 26 Oct. 1988, col. & det. by S.H. Wu; Pintung, Wu9409-2 (TNM) on decaying branch of an angiosperm, Kenting National Park (Chufengshan, 300 m altitude), 1 Sept. 1994, col. & det. by S.H. Wu; Wu9206-67 (TNM, as *P. subvinosum*) on decaying branch of an angiosperm, Kenting (250 m altitude), 17 June 1992, col. & det. by S.H. Wu; Taichung, Wu9311-1 (TNM, as *P. subvinosum*) on decaying

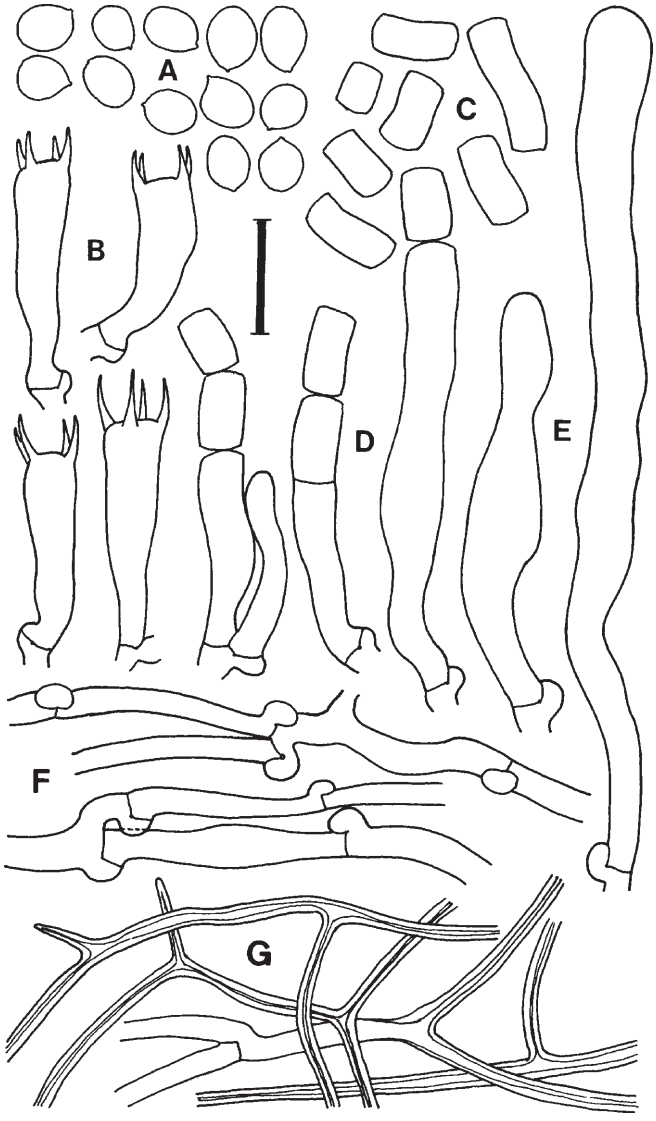


Fig. 1. Microscopic morphology of a basidioma (TMI16198) of *Pseudolagarobasidium calcareum*. **A** Basidiospores, **B** basidia, **C** arthroconidia, **D** conidiogenous cells, **E** leptocystidia, **F** subicular hyphae, **G** quasi-binding hyphae. *Bar* 10 μm

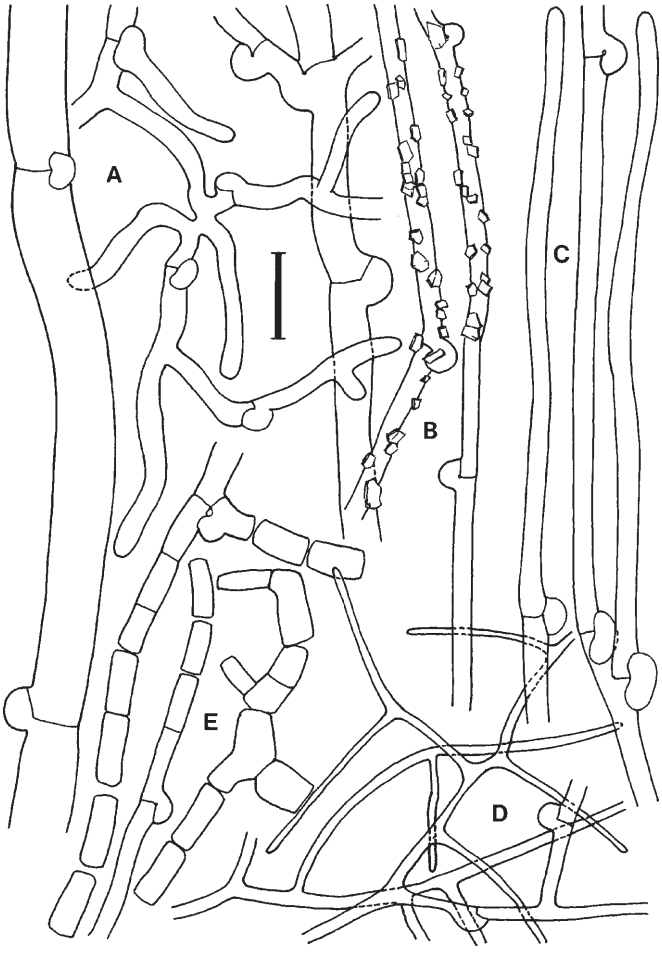
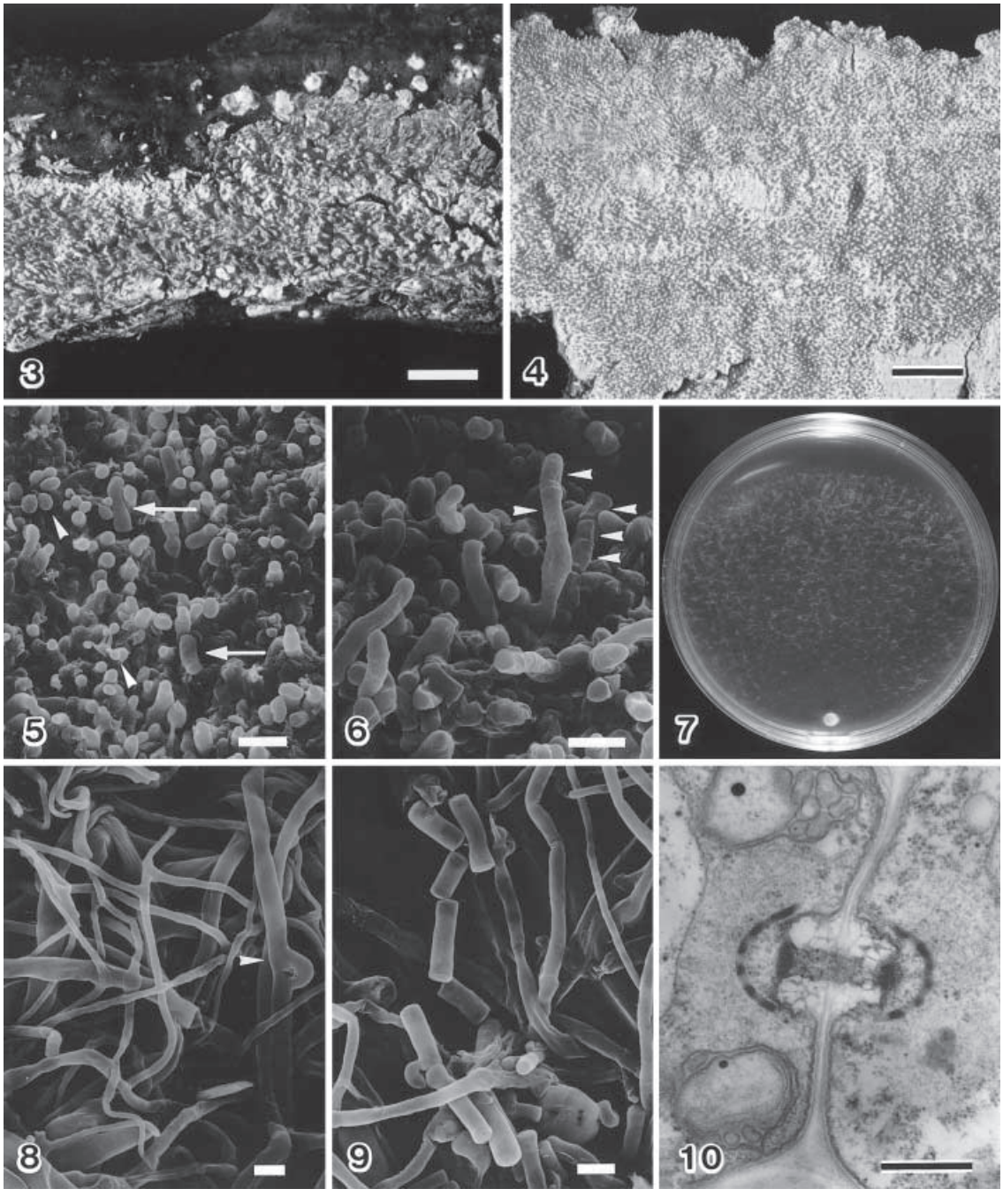


Fig. 2. Microscopic morphology of a dikaryotic isolate (TMIC32218) of *Pseudolagarobasidium calcareum* cultured for 2 weeks on malt extract agar (MEA). **A** Submerged hyphae, **B** surface and aerial hyphae, **C** advancing hyphae, **D** fiber hyphae, **E** arthroconidia. *Bar* 10 μm

branch of an angiosperm, Takeng, 7 Nov. 1993, col. by S.Z. Chen, det. by S.H. Wu.

Cultural characteristics: Optimal temperature for the three dikaryotic strains examined was between 27° and 30°C. These strains could grow between 12° and 36°C, but no visible growth was observed at 4°, 8°, 39°, and 42°C. Growth on MEA 30–48 mm in radius at 27°C for 5 days. Mycelial mats at 1 week subhyaline to white, by 6 weeks white; around inocula and surrounding area appressed and subfelty, then becoming moderately thin, slightly raised, arachnoid to cottony–woolly toward margin at 2 weeks (Fig. 7); margin even, appressed to raised; odor absent at 6 weeks; agar bleached around inocula at 1 week, partially or entirely bleached at 3 weeks; not fruiting by 6 weeks.



Figs. 3–10. *Pseudolagarobasidium calcareum*. **3** Basidioma of TMI16198. **4** Basidioma of TMI19890. **5** Hymenial surface of TMI16198, showing arthroconidia (*arrows*) and basidiospores (*arrowheads*). **6** Conidiogenous cells arising from a hymenium of TMI16198. Arthroconidia (*arrowheads*) developing through fragmentation of a hyphal tip. **7** Colony of TMIC32218 on MEA. **8** Fiber hyphae and generative hyphae (*arrowhead*) of TMIC32218. **9** Arthroconidia produced in culture (TMIC32218). **10** Septal pore ultrastructure of a generative hypha in culture (TMIC32218), showing dolipore parenthesomes. *Bars* **3**, **4** 5 mm; **5**, **6**, **8**, **9** 10 μm; **10** 0.5 μm

Oxidase reactions on GAA +++, no growth at 2 weeks; on TAA +++ or ++++, radial growth 0–28 mm at 1 week; TyA−, 51–69 mm at 1 week.

Advancing hyphae hyaline, 1.5–3.5 μm in diameter, smooth, thin-walled, nodose-septate, sparsely branched (Fig. 2C). Submerged hyphae hyaline, 1.5–7 μm in diameter, smooth, thin-walled, nodose-septate, sparsely to moderately branched (Fig. 2A). Surface and aerial hyphae: (1) similar to the submerged hyphae, but sometimes encrusted with crystalline materials (Fig. 2B); (2) fiber hyphae 1–2 μm in diameter, thin-walled to slightly thick-walled (to 0.5 μm), often branches diverging at right angles (Figs. 2D, 8). Arthroconidia doliform to cylindrical, 4–20 × 2–4 μm, hyaline, thin-walled, rarely branched (Figs. 2E, 9), produced from the surface and aerial hyphae. Species code (Nakasone 1990): 2. 3c. 8.19. (21.) 35. 36. 40. 42. 54. 60.

Cultures examined: Three dikaryotic isolates of *P. calcareum* TMIC32218, TMIC33324, and TMIC33356 were obtained from natural specimens TMI16198, TMI19856, and TMI19890, respectively. A dikaryotic isolate from Wu9409-2 was provided by S.H. Wu.

Septal structure: Ultrastructure of septal pore apparatus of a dikaryotic strain (TMIC33324) shows a perforated dolipore-parenthesome (holes of parenthesome, ~85 nm in diameter) (Fig. 10).

Incompatibility system: Twelve single spore isolates lacking clamp connections, from a basidioma derived from a Japanese strain (TMIC32218) of *P. calcareum*, were paired in all combinations. After 5 days the pairings were examined for clamp connections, and four mating types were obtained: A_1B_1 = 1, 5, 8, 10, 12; A_1B_2 = 2, 3, 6, 9; A_2B_1 = 7, 11; and A_2B_2 = 4. Similar results were obtained from a Taiwanese strain (Wu9409-2): A_3B_3 = 1, 2, 5, 7; A_3B_4 = 3, 8, 11; A_4B_3 = 4, 6, 10; and A_4B_4 = 9, 12. In intraspecific parings, monokaryons derived from TMIC32218 were compatible with those from Wu9409-2 and TMIC33356.

Pseudolagarobasidium calcareum is characterized by its hydnoid basidiomata (Figs. 3, 4), narrow and much branched hyphae (quasi-binding hyphae) (Fig. 1G), cylindrical and thin-walled leptocystidia (Fig. 1E), and subglobose, thin-walled, smooth, and inamyloid basidiospores measuring 4–6 × 3–4 μm (Fig. 1A).

In all the Japanese and Taiwanese specimens examined in this study, except for TMI19890 and Wu9409-2, we found arthroconidia (Fig. 1C) associated with the basidiomata. The production of arthroconidia associated with the basidiomata has not been noted in the previous descriptions (Jülich 1978; Wu 1990; Nakasone 2001). The scanning electron microscopic observations of TMI16198 revealed that arthroconidia occurred on the hymenium (Fig. 5) and that their conidiogenous cells arose from the hymenium (Fig. 6). In the corticiaceous species, arthroconidium formation from their hymenia has not been reported, although several species that belong to some corticiaceous genera such as *Phanerochaete*, *Phlebia*, and *Resinicium* produce arthroconidia in culture (Kendrick and Watling 1979; Nakasone 1990). Thus, this is the first observation on arthroconidium formation from the hymenia of a corticiaceous fungus.

There is variation in color of the hymenial surface among the specimens; e.g., TMI16198 (Fig. 3) has a brownish ("buff," "ochreous" to "hazel") hymenial surface, whereas that of TMI19890 (Fig. 4) is pale-colored (pale grayish-cream to "buff"). However, basidiomata derived in the laboratory from the dikaryotic cultures of these two specimens had a white to pale-cream hymenial surface in the early stage that became brownish after 2 months. These observations suggest that color of the hymenial surface may be influenced by environmental factors (temperature, humidity, etc.) during the development of basidiomata. *Pseudolagarobasidium calcareum* resembles *P. subvinosum* (Berk. & Br.) Sheng H. Wu. According to Wu (1990), *P. calcareum* has a whitish or pale-colored hymenial surface, whereas *P. subvinosum* produces a brownish one. Color of the hymenial surface has been recognized as a helpful characteristic for distinguishing *P. calcareum* from *P. subvinosum* (Stalpers 1998; Wu 1990). As stated earlier, however, *P. calcareum* possesses a variably colored (whitish to brownish) hymenial surface. Therefore, we consider that color of the hymenium is not reliable for distinguishing basidiomata of the two species.

In culture, all the isolates of *P. calcareum* examined in this study, including dikaryotic isolates from TMI19890 and Wu9409-2 without arthroconidia, produced many arthroconidia (Fig. 2E) and fiber hyphae (Figs. 2D, 8), which are quite similar to quasi-binding hyphae. According to Jang and Chen (1985), *P. subvinosum* (as *P. leguminicola*) also produced arthroconidia in culture and has a heterothallic and tetrapolar mating system. However, the development of the fiber hyphae (quasi-binding hyphae) in culture distinguishes *P. calcareum* from *P. subvinosum*. Their basidiomata also provide an important criterion for discriminating between the two species.

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