

## The genus *Ponticulomyces* (Physalacriaceae, Agaricales) from Japan

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**Abstract** Two species of the genus *Ponticulomyces* collected from Japan for the first time are described and illustrated. *Ponticulomyces kedrovayae* is characterized by its lamellae staining yellow when bruised and in age, stipe lacking an annulus and a pseudorhiza, scattered pileal hairs, and large amygdaliform basidiospores. It mainly occurs on dead wood of *Fagus crenata*. *Ponticulomyces orientalis* is characterized by its scattered pileal hairs and broadly ellipsoid to ellipsoid basidiospores. It was collected mostly on dead wood of *Camelia japonica*.

**Keywords** Agaricomycetidae · New geographic distribution · *Oudemansiella* · Taxonomy · *Xerula*

*Ponticulomyces* R.H. Petersen (in Petersen and Hughes 2010) is an agaric genus recently proposed as new in the *Oudemansiella/Xerula* complex, accommodating two species, *Ponticulomyces kedrovayae* R.H. Petersen from the

Far East of Russia (as the type) and *Ponticulomyces orientalis* (Zhu L. Yang) R.H. Petersen ( $\equiv$  *Oudemansiella orientalis* Zhu L. Yang) from China based on morphological and molecular data. The genus is mainly characterized by subviscid to glutinous pileus, fruiting directly on woody substrates, and two types of pileocystidia.

In our field surveys of the *Oudemansiella/Xerula* complex, two species belonging to *Ponticulomyces* were newly collected from Honshu (Tottori Pref., Okayama Pref., and Kanagawa Pref.) and/or Kagoshima Pref. (Yakushima Island) of Japan. They are reported herein.

Specimens examined in this study were deposited in the herbaria of Fungus/Mushroom Resource and Research Center, Tottori University (TUMH), and the Tottori Mycological Institute (TMI). Color and configuration of basidiomata were examined using fresh specimens. Color names in double quotation marks are from the color identification chart of the Royal Botanical Garden (1969). In descriptions,  $Q$  = spore length divided by spore width,  $Q^m$  = median  $Q$  of a population of spores,  $L^m$  = median spore length over a population of spores, and  $W^m$  = median spore width over a population of spores. For microscopic observations, pieces of the dried specimens were hand sectioned vertically using razor blades. Sections were mounted in Melzer's reagent, 3–5% KOH solution, 1% Congo red solution, and distilled water. DNA extraction was carried out using FTA card (GE Healthcare Japan, Tokyo, Japan) methods following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification was carried out for the internal transcribed spacer (ITS) region, using the primers ITS1-F and ITS4-B (Gardes and Bruns 1993). PCR amplification was carried out using Ampdirect plus/NovaTaq (Shimadzu, Kyoto, Japan), under the following conditions: 10 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C, with a final

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extension of 7 min at 72°C. Sequencing was performed on an ABI 3130 genetic analyzer (Applied Biosystems Japan, Tokyo, Japan) according to the manufacturer's protocols. Sequences were edited with FinchTV (<http://www.geospiza.com/Products/finchtv.shtml>) and aligned with Clustal X ver. 2.0. All sequences used in this study were deposited in the DNA Data Bank of Japan (DDBJ), their accession numbers are listed in Table 1. Identity (%) of all sequences was analyzed by GENETYX ver. 10.

***Ponticulomyces kedrovayae*** R.H. Petersen. Nova Hedwigia, Beiheft, 137:311, figs. 323–328, 2010

Figs. 1, 2, 4, 5, 6, 7, 8, 9, 10, 18

Basidiomata (Fig. 1) collybioid, gregarious or in small groups. Pileus 9–50 mm broad, hemispherical to conico-

convex when immature, convex to plano-convex when mature; surface “Cigar brown” to “Sepia” or dark brown at first, often with an olivaceous tinge, becoming paler with age (in particular toward margin) at times, “Grey olivaceous” with whitish margin when old, moderately or weakly radially rugose to rugulose, dry and velutinous when young, glabrous, slippery to subviscid when wet and old; margin often with translucent striations in mature specimens. Context of pileus white, color unchanging when cut; odor negligible, taste mild. Lamellae white, adnate to adnexed, subdistant, with 1–2 tiers of lamellulae, not marginate, edges entire, staining “Luteous” to “Lemon chrome” on bruising or with age (see Fig. 2, arrows). Stipe 10–30 × 3–5 mm, whitish at first, becoming yellowish brown to “Dark brick” from the base upward, fine

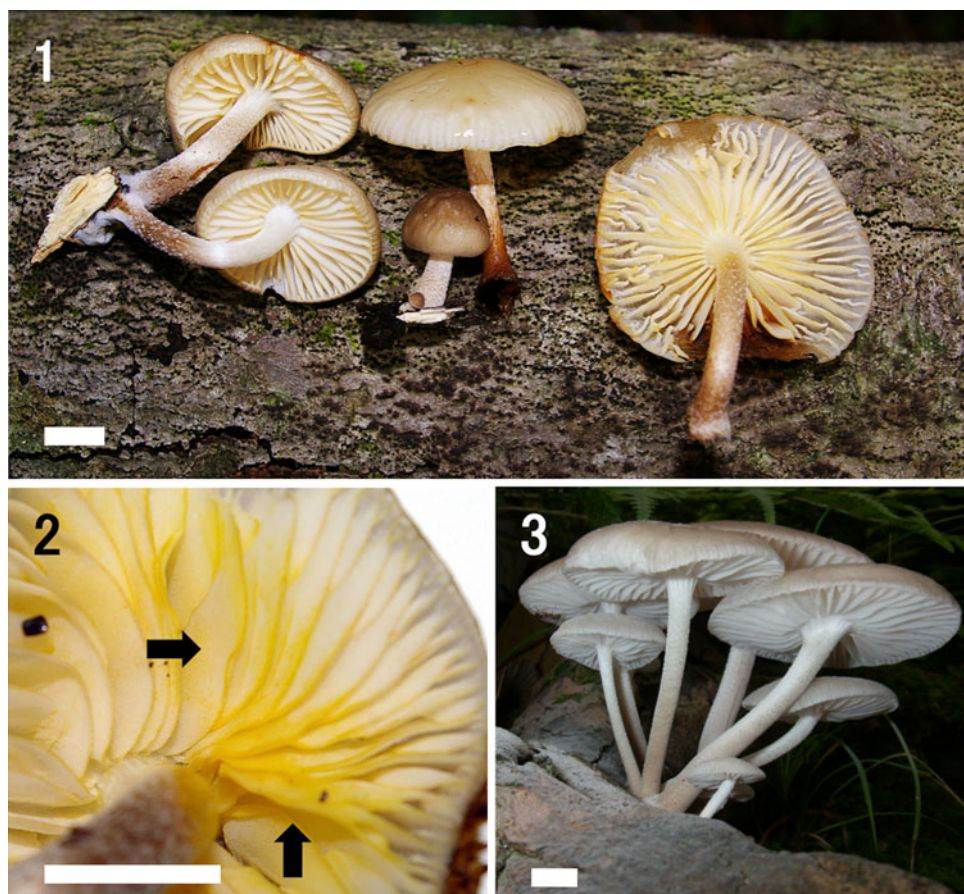
**Table 1** Comparison of internal transcribed spacer (ITS) sequences between *Ponticulomyces kedrovayae* (type) and the Japanese *Ponticulomyces* species

Species (strain no.)	Accession no. <sup>a</sup>	Number of nucleotides used in this study (bp)	Identity (%) with <i>P. kedrovayae</i> (type)	
			HM005145 <sup>a</sup>	HM005146 <sup>a</sup>
<i>P. kedrovayae</i> (TUMH40166)	AB607173	878	99.8	99.6
<i>P. kedrovayae</i> (TUMH40167)	AB607174	867	100	99.7
<i>P. orientalis</i> (TUMH40159)	AB607171	783	83.0	83.0
<i>P. orientalis</i> (TUMH40177)	AB607172	783	83.0	83.0

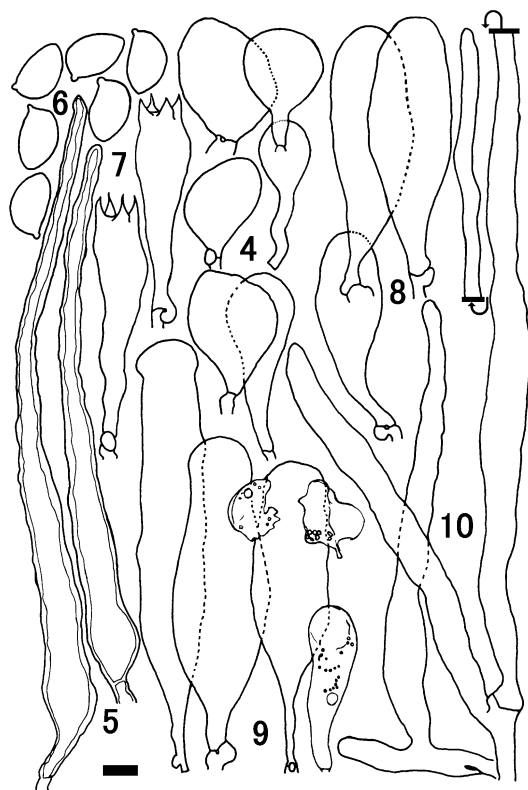
<sup>a</sup> DDBJ/GenBank database accession number

**Figs. 1–3** *Ponticulomyces kedrovayae* (TUMH40167) and *P. orientalis* (TUMH40164).

**1** Basidiomata of *P. kedrovayae* on a dead trunk of *Fagus crenata*. **2** Lamellae of *Ponticulomyces kedrovayae* stained yellow when bruised (arrows). **3** Basidiomata of *P. orientalis* on dead wood of *Cameria japonica*. Bars 10 mm



furfuraceous with minute whitish scales in the lower portion, lacking annulus and pseudorhiza. Spore print white. Pileipellis (Fig. 18) a hymeniderm, 150–200  $\mu\text{m}$  thick, made up of two different cellular elements: (1) dominant broadly clavate to sphaeropedunculate cells (Fig. 4), 37–58  $\times$  16–30  $\mu\text{m}$ , hyaline, sometimes with intercellular brown pigments, thin walled, occasionally with clamp connection; and (2) scattered pileocystidia (Fig. 5) 103–355  $\times$  13–19  $\mu\text{m}$ , gradually tapering to apex from an enlarged base, hair like, narrowly subfusiform to lanceolate, shortly stipitate, hyaline, slightly thick walled, without clamp connection. Basidiospores (Fig. 6) 14.5–25  $\times$  8–15  $\mu\text{m}$ ,  $Q = 1.5$ –2.2 (180 spores from 6 basidiomata of six collections,  $L^m = 20$ ,  $W^m = 11$ ,  $Q^m = 1.8$ ), amygdaliform or mango shaped, smooth, hyaline, with slightly thickened walls. Basidia (Fig. 7) 45–73  $\times$  13–15  $\mu\text{m}$ , clavate, with 4 sterigmata, clamped. Cheilocystidia (Fig. 8) 59–85  $\times$  19–31  $\mu\text{m}$ , cylindric-clavate to obpyriform, tapering to a pedicellate base, hyaline, thin walled, with clamp connection. Pleurocystidia (Fig. 9) 80–136  $\times$  21–30  $\mu\text{m}$ , subcylindric to cylindric-fusiform, often capitate, tapering to a pedicellate base, hyaline, thin walled, with clamp connection, sometimes with dark olive-colored mucous substances at the apex in dry specimens mounted by 3% KOH. Caulocystidia



**Figs. 4–10** *Ponticulomyces kedrovayae* (TUMH40168). **4** Dominant pileipellis element cells. **5** Pileocystidia. **6** Basidiospores. **7** Basidia. **8** Cheilocystidia. **9** Pleurocystidia; one (third from left) shows mucous substance at the apex. **10** Caulocystidia. Bar 10  $\mu\text{m}$

(Fig. 10) 57–302  $\times$  12–22  $\mu\text{m}$ , slender and hair like, slender-fusiform to cylindrical, tapering to an obtuse apex, hyaline, thin walled, with clamp connection.

Specimens examined: TMI22362, on fallen trunk of a broad-leaved tree (*Fagus* aff. *crenata* Blume), Mt. Daisen, Saihaku-gun, Tottori Pref., 19 Oct. 1996, collected by M. Tanaka; TUMH40165, on fallen trunk of a broad-leaved tree (*F. aff. crenata*), same locality, 4 Oct. 2003, collected by S. Ushijima; TUMH40166, on dead trunk of *F. crenata*, same locality, 1 Oct. 2005, collected by N. Shimomura and N. Maekawa; TUMH40167, on dead trunk of *F. crenata*, same locality, 13 Oct. 2007, collected by S. Ushijima; TUMH40168, on dead trunk of *F. crenata*, same locality, 10 Oct. 2009, collected by S. Ushijima; TUMH40178, on dead wood of *F. crenata*, same locality, 31 Oct. 2009, collected by Y. Ando; TMI26116, on dead wood of a broad-leaved tree (*F. aff. crenata*), Nishiawakura-son, Okayama Pref., 17 Nov. 2004, collected by K. Fujinawa; TMI26117, on dead wood of a broad-leaved tree (*F. aff. crenata*), same locality, 17 Oct. 2007, collected by K. Fujinawa.

Additional specimens examined: TENN60767 (holotype), on rotting, exposed hardwood, south side of Kedrovaya River, Kedrovaya Pad Preserve, Khasansky Dist., Primorsky Reg., Russia, 22 Oct. 2005, collected by A. Kovalenko.

*Ponticulomyces kedrovayae* has been known only from the Far East of Russia growing on rotting exposed hardwood (Petersen and Hughes 2010). The Japanese specimens represent the first records of *P. kedrovayae* outside Russia. In Japan it has been mainly found on dead wood of *F. crenata* in Honshu (Tottori Pref. and Okayama Pref.). This species is characterized by its dark brown to yellowish brown, radially wrinkled pileus that is slippery to subviscid when wet (Fig. 1), the fine furfuraceous stipe lacking an annulus and a pseudorhiza at the base, the lamellae turning “Luteous” to “Lemon chrome” on bruising or with age, and microscopically by the large amygdaliform basidiospores measuring 14.5–25  $\times$  8–15  $\mu\text{m}$  (Fig. 6). Because of the habit of growing directly on wood, the nonannulate stipe, and the rugose to rugulose subviscid pileus, this species would be most comparable to *P. orientalis*. However, the latter species differs from *P. kedrovayae* by unchanging lamellae on bruising or with age, and by smaller broadly ellipsoid to ellipsoid basidiospores measuring 12–16.5(–17.5)  $\times$  9.5–13  $\mu\text{m}$  in this study; (10.5–)11.5–14.5(–16.5)  $\times$  (9.0–)9.5–11.0(–12.0)  $\mu\text{m}$  in Yang (2000). Most macro- and micromorphological characters of the Japanese specimens were identical with those of the type specimen of *P. kedrovayae* (TENN60767), with a few exceptions. According to Petersen and Hughes (2010), the lamellae of the Russian material are described “pale orange buff” with “orange buff tints,” “minutely marginate to sayal brown” in color, but the lamellae of the Japanese



specimens are not marginate and are primarily white, although they stain yellow on bruising and/or with age. Perhaps when the lamellae edges are first touched, the edges seem marginate, and are stained apparently yellow or luteous at bruising or age (entirely yellow with age). Furthermore, the ITS sequences of the Japanese specimens, TUMH40166 and TUMH40167, showed high levels of similarity, >99.6% and >99.7%, respectively, to those of the type of *P. kedrovayae*, whereas their similarity to the ITS sequences of *P. orientalis* were low, 83.0% and 83.0% (Table 1). The analyses of the ITS sequence data support that the two Japanese specimens and *P. kedrovayae* (type) are conspecific.

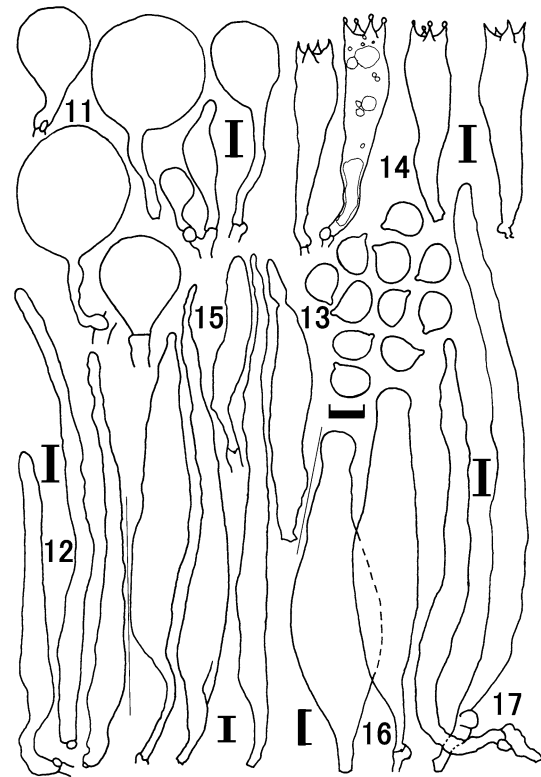
***Ponticulomyces orientalis*** (Zhu L. Yang) R.H. Petersen. Nova Hedwigia, Beiheft 137: 315, figs. 329–332, 2010.

≡ *Oudemansiella orientalis* Zhu L. Yang. Mycotaxon 74:357, figs. 1–6, 2000.

Figures 3, 11, 12, 13, 14, 15, 16, 17, 19

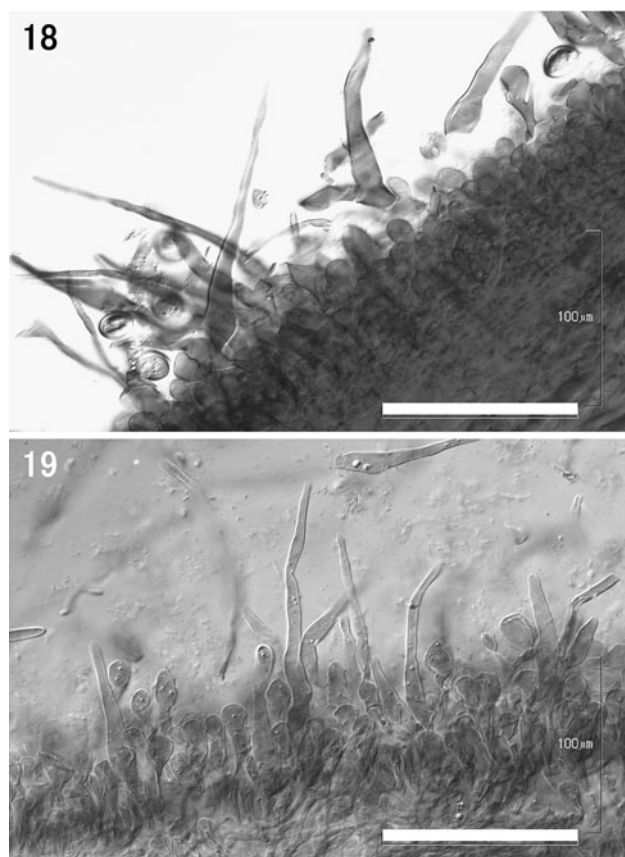
Basidiomata (Fig. 3) collybioid, often in small groups. Pileus 25–100 mm broad, hemispherical to conico-convex, convex to plano-convex, color when young “Snuff brown” to “Drab,” when mature “Smoke grey” to “Mouse grey,” much paler at margin, radially rugulose, fine pubescent (when young) to slightly velutinous, viscid when wet, margin with short translucent striations. Context of pileus white. Lamellae white, sinuate to adnexed, subdistant, not marginate. Stipe 10–60 × 4.5–5 mm, white (upward) to brown (downward), with small whitish scales, lacking an annulus and a pseudorhiza. Spore print white. Pileipellis a hymeniderm (Fig. 19), made up of two different cellular elements: (1) dominant broadly clavate, pyriform to shortly pedicellate sphaeropedunculate, occasionally subfusiform cells (Fig. 11) 37–68 × 11–38 μm, hyaline, thin walled, with clamp connection; and (2) scattered pileocystidia (Fig. 12) 108–161 × 8–13 μm, narrowly fusiform to lanceolate, hyaline, slightly thick walled, occasionally with clamp connection. Basidiospores (Fig. 13) 12–16.5 (–17.5) × 9.5–13 μm,  $Q = 1.16–1.5$  (120 basidiospores from 6 basidiomata of six collections,  $L^m = 14.5$ ,  $W^m = 11$ ,  $Q^m = 1.3$ ), broadly ellipsoid to ellipsoid, rarely broadly amygdaliform, smooth, nonamyloid. Basidia (Fig. 14) 41–71 × 10–16 μm, clavate, with 4 sterigmata, with clamp connection. Cheilocystidia (Fig. 15) 73–298 × 6–25 μm, subclavate to fusiform, or narrowly elongate-fusiform to lanceolate, with clamp connection. Pleurocystidia (Fig. 16) 80–140 × 18–32 μm, subfusiform, capitate, slightly thick walled, with clamp connection. Caulocystidia (Fig. 17) 57–301 × 12–22 μm, narrowly lanceolate, subfusiform to subcylindrical, hyaline, thin walled, with clamp connection.

Specimens examined: TMI2393, on dead wood of *Castanopsis* sp., Uemachi, Tottori City, Tottori Pref., 17



**Figs. 11–17** *Ponticulomyces orientalis* (TUMH40164). **11** Dominant pileipellis element cells. **12** Pileocystidia. **13** Basidiospores. **14** Basidia. **15** Cheilocystidia. **16** Pleurocystidia. **17** Caulocystidia. Bars 10 μm

July 1976, collected by T. Ikeda and M. Kuwana; TUMH40177, on dead standing tree of *Cameria japonica* L., same locality, 27 June 2007, collected by S. Ushijima; TUMH40176, on dead wood of *C. japonica*, same locality, 5 June 2008, collected by S. Ushijima; TUMH40164, on dead trunk of *C. japonica*, same locality, 9 June 2009, collected by S. Ushijima; TUMH40174, on dead wood of *C. japonica*, same locality, 3 July 2009, collected by H. Suhara; TUMH40159, on dead wood of *C. japonica*, Kurayoshi City, Tottori Pref., 1 June 2006, collected by S. Ikemoto; TUMH40161, on dead wood of *C. japonica*, Iwatsubo, Tottori City, Tottori Pref., 5 June 2008, collected by S. Ushijima; TUMH40163, on fallen wood of *C. japonica*, Ikuta-ryokuchi, Kawasaki City, Kanagawa Pref., 15 June 2008, collected by Y. Shibata; TUMH40175, on dead wood of broad-leaved tree, near Arakawa-dam, Yakushima Island, Kagoshima Pref., 19 July 2009, collected by S. Ushijima; TMI18605, on dead stump of *C. japonica*, Kokoge, Tottori City, Tottori Pref., 7 July 1993, collected by E. Nagasawa; TUMH40157, on dead stump of *C. japonica*, same locality, 23 May 2006, collected by S. Ushijima; TUMH40172, on dead stump of *C. japonica*, same locality, 24 May 2006, collected by S. Ushijima; TUMH40158, on dead trunk of *C. japonica*,



**Figs. 18, 19** Pileipellis of *Ponticulomyces* species (radial sections in Congo red). **18** *Ponticulomyces kedrovayae* (TUMH40168). **19** *Ponticulomyces orientalis* (TUMH40164). Bars 100 µm

same locality, 26 May 2006, collected by S. Ushijima; TUMH40169, on dead trunk of *C. japonica*, same locality, 6 June 2006, collected by S. Ushijima; TUMH40160, on dead wood of *C. japonica*, same locality, 5 June 2008, collected by S. Ushijima; TUMH40162, on fallen trunk of *C. japonica*, same locality, 9 June 2008, collected by S. Ushijima.

Additional specimens examined: HKAS32021 and HKAS32127 (holotype), Sanjiangkou Nature Reserve

(1,800 m alt.), Mugu Township, Yiliang County, Yunnan Prov., China, 21 September 1998, collected by Zhu L. Yang.

*Ponticulomyces orientalis* is recognized by its pale gray to pale grayish brown pileus, the surface of which is pubescent when young and viscid when wet; white to whitish stipe lacking an annulus and a pseudorhiza, the pileipellis made up of two types of cells; and broadly ellipsoid to ellipsoid basidiospores measuring  $12\text{--}16.5\text{--}(17.5) \times 9.5\text{--}13\text{ }\mu\text{m}$ . The Japanese specimens examined in this study were macro- and micromorphologically identical with those of the type specimen (HKAS32127) in most features. This species was originally reported from Yunnan Province of southwestern China growing on dead wood or stumps of broad-leaved trees in subtropical evergreen broad-leaved tree forest (Yang 2000). In Japan, it has been mainly known on dead trunks or stumps of *C. japonica* in intermixed evergreen broad-leaved tree forests. The Japanese specimens represent the first records of *P. orientalis* outside China.

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