

***Phellinus bicuspidatus***  
**(HYMENOCHAETALES, HYMENOCHAETACEAE), A NEW**  
**SPECIES ASSOCIATED WITH A WHITE SAP**  
**ROT OF OAK IN LOUISIANA**

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ABSTRACT

A new wood-inhabiting basidiomycete, *Phellinus bicuspidatus*, is described from oak in Louisiana. Characteristics of the fruiting body and culture are presented and compared to those of *P. johnsonianus* and *P. spiculosus*. Key patterns and species codes are given for the three species.

Key Words: *Phellinus*, cultures, white rot, taxonomy.

In the summer of 1931, Dr. L. O. Overholts of The Pennsylvania State University collected wood-decay fungi in Louisiana for the Division of Forest Pathology, Bureau of Plant Industry, U.S. Department of Agriculture. Cultures were isolated from many of the specimens. He sent portions of the specimens and cultures to Washington, D.C., for the Division's herbarium and culture collection. He kept other portions of the specimens for his own herbarium. During that summer, he made one collection of a brown "*Poria*" that he recognized as a new species and used the epithet "*cuspidata*" for the specimen in his herbarium. However, the epithet "*bicuspidatus*" seems to us to be more aptly descriptive. Our purpose here is to describe the basidiocarp and culture of this species of *Phellinus* from oak in the southern United States.

MATERIALS AND METHODS

Microscopic characters of the basidiocarps were studied from freehand sections mounted in 10% KOH stained with 1% aqueous Phloxine-B, in Melzer's reagent (Melzer, 1924), or in 1% cotton blue (Johansen, 1940).

TABLE I lists the specimens and cultures studied. The methods employed in studying the cultures were the same as used in previous studies (Davidson *et al.*, 1938, 1942). Cultures were grown on 1.5% malt extract agar (MEA), on 0.5% gallic acid agar (GAA), and on 0.5% tannic acid agar (TAA). The Key Patterns were based on 2-wk-old cultures. The Species Codes of Nobles (1965) were based on 6-wk-old cultures. For the constant temperature study, isolates on MEA were placed in incubators 24 h after plating and measured at the end of 4 da. Measurements of mat diameters represent averages of three replications of the individual isolates.

Microscopic structures were drawn with the aid of a Zeiss drawing tube. Color designations of the basidiocarp are from Munsell (1929–1942); color designations of the cultures are from Ridgway (1912), and herbarium designations are from Holmgren *et al.* (1981).

<sup>1</sup> Maintained at Madison, Wisconsin, in cooperation with the University of Wisconsin

TABLE I

SPECIMENS AND CULTURES OF *Phellinus bicuspidatus*, *P. johnsonianus*, AND *P. spiculosus* INCLUDED IN THE STUDY

Name	No.	Source of cultures	Substratum	Locality
<i>P. bicuspidatus</i>	FP <sup>a</sup> 55700	S <sup>b</sup>	<i>Quercus</i> sp.	Clayton. La.
<i>P. johnsonianus</i>	GHH-522	R	<i>Fraxinus</i> sp.	Holly Bluff, La.
	FP 50383	S	<i>Fraxinus</i> sp.	Ferriday, La.
	FP 52033	S	<i>Fraxinus</i> sp.	Fairfax. Va.
	FP 52046	S	<i>Fraxinus</i> sp.	Fairfax. Va.
	FP 55500	S	<i>Fraxinus</i> sp.	Willetts, La.
<i>P. spiculosus</i>	FP 94090	R	<i>Carya ovata</i> (Mill.) K. Koch	Saltsburg, Pa.
	FP 94091	R	<i>Carya glabra</i> (Mill.) Sweet	Red Lion, Pa.
	FP 94102	R	<i>Quercus falcata</i> Michx.	Waverly, Va.
	FP 94123	R	<i>Carya glabra</i> (Mill.) Sweet	Bent Creek. N.C.

<sup>a</sup> Designation for CFMR herbarium specimens and cultures.

<sup>b</sup> S, culture from basidiocarp tissue; R, culture from rot in host wood usually associated with a basidiocarp.

## DESCRIPTION OF BASIDIOCARP

***Phellinus bicuspidatus* Lombard et M. J. Larsen, sp. nov.**

FIGS. 1-4

(*Poria cuspidata* Overholts, *nom. in Herb.* Overholts)

Species differt setis saepe bicuspidatis a congeneribus diversa, 16–24(–27) × 8–14 μm: basidiosporis late ellipsoideis, 4–6 × 3–4 μm. depressis adaxiali ad apiculum; in culturis 90+ mm in 14 diebus crescens, temperaturis optimis 36 C.

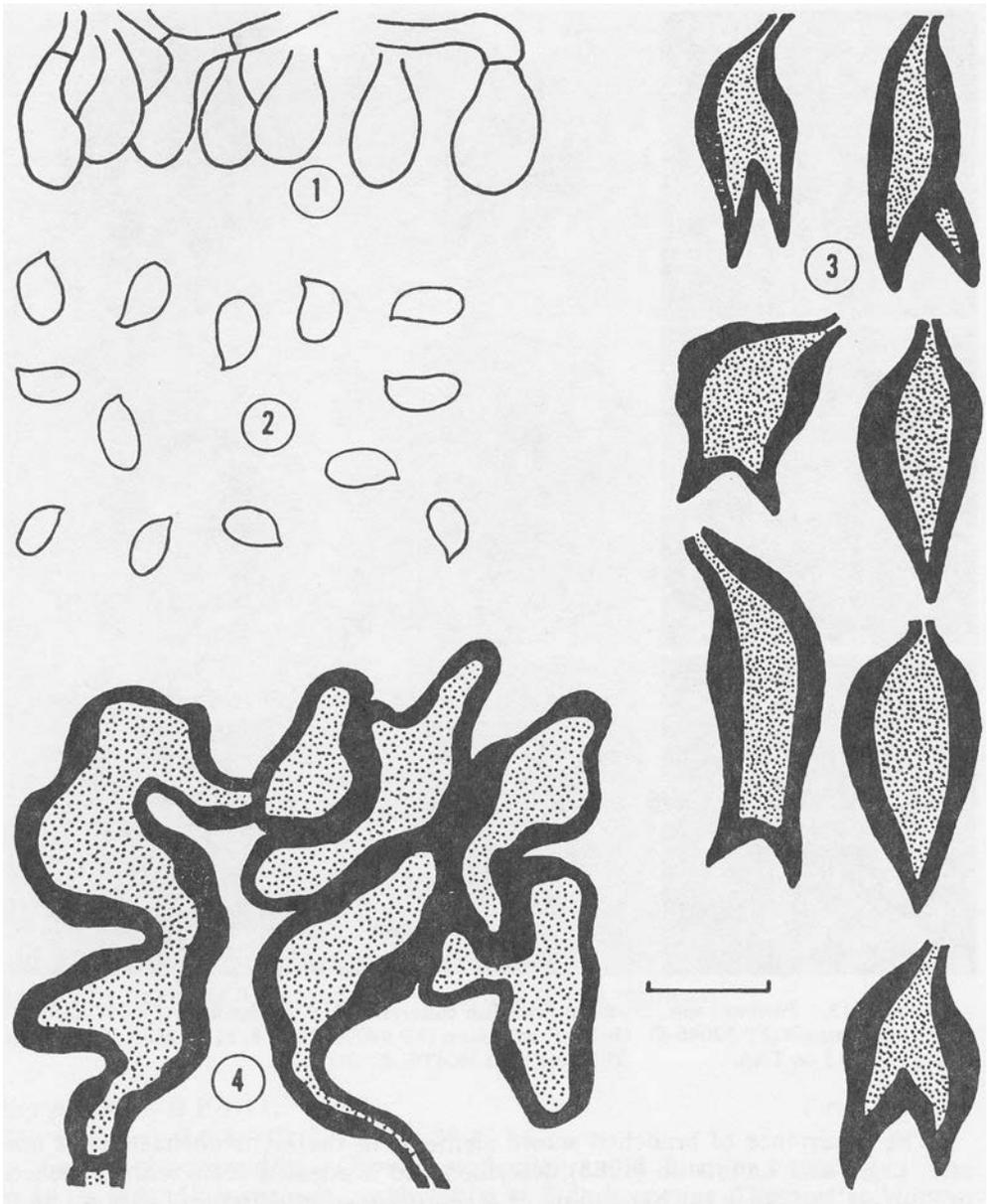
Holotypus: U.S.A., Louisiana, Clayton, ad lignum *Quercuum* sp., legit L. O. Overholts et F. H. Kaufert, 3 VIII 1931, Overholts No. 14064 (PAC), et isotypus in CFMR (FP 55700).

ETYMOLOGY: from *bi-* (L., comp., two) + *cuspidatus* (L., adj., tapering to a point) = with two points, referring to the morphology of apices of setae.

Basidiocarp effuse and conforming to the surface of the substratum, perennial, 10–15 mm thick; margin narrow, fuscus brown (near 5.0YR3/4), darker than pore surface, matted tomentose; pore surface dull ferruginous brown (near 7.5YR4/4); context 1–5 mm thick, ferruginous brown, infrequently with black layers: pores round to somewhat angular, sometimes elongate, 5–7 per mm, dissepiments entire: tubes 1–3 mm long, tube layers not distinctly stratified, older tubes appearing stuffed with white mycelium; setae not evident at 10 ×; tissue of context and trama darkening noticeably in 5% KOH solution (xanthochroic).

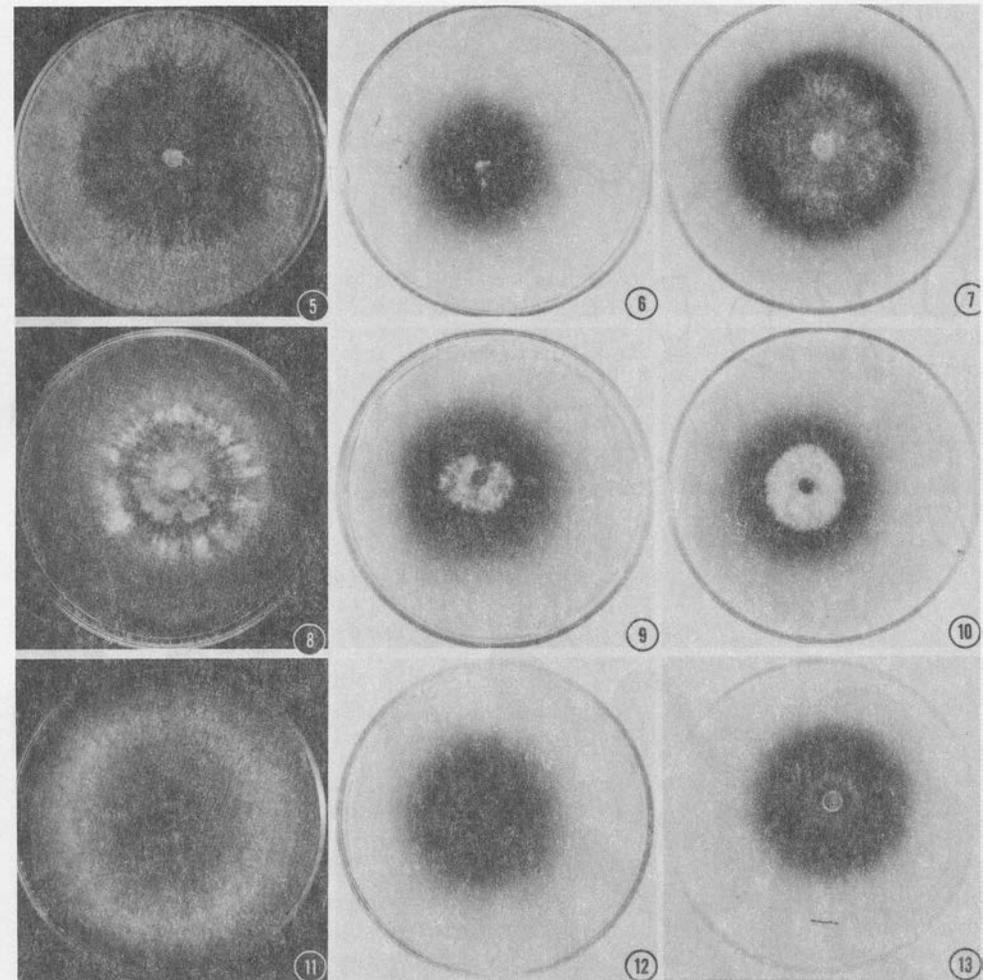
Hyphal system monomitic. Clamps absent throughout. Hyphae of context 2.5–4 μm diam, thick-walled, occasionally branched, dark ferruginous brown: tramal hyphae continuous and concolorous with the context, 1.5–2.5 μm diam. septate; subhymenial hyphae 2 μm diam. septate, branched, hyaline: basidia (FIG. 1) 8–11 × 4.5–6 μm, clavate. 4-sterigmate; basidiospores (FIG. 2) 4–6 × 3–4 μm. broadly ellipsoid and walls slightly depressed adaxially near the apiculum, thin-walled, hyaline, inamyloid, acyanophilous; setae (FIG. 3) 16–24(–27) × 8–14 μm. projecting 8–10 μm above the hymenial surface, short ventricose, often bicuspidate; plectenchymatous tissue (FIG. 4) comprising black layers in context and zone lines in wood substrate, hyphal elements swollen and contorted, tightly interwoven, branched, dark ferruginous brown, individual components difficult to separate.

HOLOTYPE: U.S.A., LOUISIANA, Clayton, growing on bark and associated with a white rot of a red oak (*Quercus* sp.) log on ground. collected by L. O. Overholts and F. H. Kaufert, 3 VIII 1931, Overholts No. 14064 (PAC), and isotype in CFMR (FP 55700).



FIGS. 1-4. *Phellinus bicuspidatus*. microscopic characters from holotype. 1. Basidia. 2. Basidiospores. 3. Setae. 4. Plectenchymatous hyphae. Scale bar = 10  $\mu$ m.

*Remarks.* —The basidiocarp of *P. bicuspidatus* is similar to those of *P. johnsonianus* (Murr.) Ryv. and *P. spiculosus* (Campbell et Davidson) Niemelä in color, pore size, and hyphal dimensions. *Phellinus johnsonianus* has smaller basidiospores ( $2.5-4 \times 2-3 \mu$ m) and larger setae [(13-)20-30(-40)  $\times$  (5-)6-8(-11)  $\mu$ m]. In *P. spiculosus*, the basidiospores are  $4.5-6 \times 3.5 \mu$ m, ovoid, and are not adaxially depressed near the apiculum. Also, the setae are somewhat smaller (11-20  $\times$  4-8  $\mu$ m). Neither of the latter possess setae that are apically branched. Basidiocarps of *P. bicuspidatus* should be readily recognized by the presence of bicuspidate setae.



FIGS. 5–13. *Phellinus* spp., 2-wk-old Petri dish cultures. 5–7. *P. bicuspidatus* (Fp 55700-S). 8–10. *P. johnsonianus* (FP 52046-S). 11–13. *P. spiculosus* (FP 94090-R). 5, 8, 11 on MEA; 6, 9, 12 on GAA; 7, 10, 13 on TAA.

The occurrence of branched setoid elements in the Hymenochaetales is not rare. Leger and Lanquetin (1983) described and illustrated setae with branched or “furcate” apices in the hymenium of *Hymenochaete paucisetosa* Leger et Lanq. Ten per cent of the setae they observed were of this form. Lindsey and Gilbertson (1978: 179, fig. 118) illustrated forked setae for *H. tabacina* (Sow. : Fr.) Lév. Ryvarden (1978) reported that setae are “very rarely forked dichotomously” in *P. pini* (Fr.) A. Ames. We have not been able to substantiate Ryvarden’s observation. Ryvarden and Johansen (1980: 157, fig. 48) illustrated a two-pointed hymenial seta in *P. extensus* (Lév.) Pat., but did not mention it in their description. Lindsey and Gilbertson (1978: 182, fig. 120) illustrated a forked seta for *P. conchatus* (Pers. : Fr.) Quél. Ryvarden (1978: 230, fig. 91) noted “subulate (rarely split)” setae in *Inonotus dryadeus* (Fr.) Murr. Also Gilbertson (1976: 75, fig. 5) illustrated a forked seta for the same species.

Non-hymenial, branched setoid elements are formed on the pileus cuticle in *I. cuticularis* (Fr.) Karst. and have been described and illustrated by Ryvarden

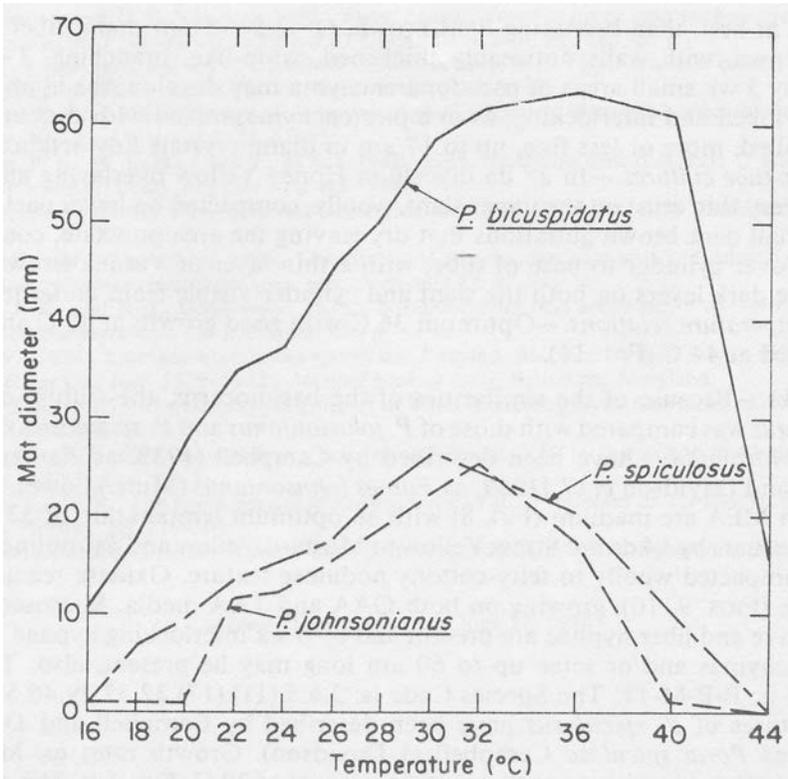


FIG. 14. Average mat diameters of *Phellinus* spp. grown on MEA after 4 da incubation at 12 constant temperatures.

(1978: 227, figs. 89 and 90 as “setigerous elements”) and Gilbertson (1976: 74, fig. 4). Campbell and Davidson (1942) recorded “setae-bearing hyphae” in culture and in mycelial plugs formed in wood decayed by *P. spiculosus*. These hyphae frequently have the appearance of branched setoid elements.

DESCRIPTION OF CULTURE

*Key pattern.* —B-P-I-11.

FIGS. 5–7, 14

*Species code.* - 2.6.8.(10).(11).32.37.39.43.54.

*Growth characteristics.* —Growth moderately rapid, forming a mat 90+ mm in 14 da (FIG. 5); mycelium nearly white at 7 da becoming Chamois to Honey Yellow, with or without Tawny-Olive spots around the periphery by 14 da, appressed, thin and short downy, homogeneous, becoming denser with small, slightly raised to intermediate nodulose patches scattered over the surface or around the edges of the dish by 6 wk; margin indistinct, fimbriate; reverse discoloration at 14 da Light Ochraceous-Buff, with Cinnamon-Brown under the darker spots on the surface; odorless; oxidase reactions positive, strong, making 0 to a trace<sup>2</sup> of growth on GAA (FIG. 6) and 68–74mm in diam on TAA (FIG. 7) in 14 da, the mat on TAA Cartridge Buff to Cream-Buff, fine downy-cottony with a distinct marginal zone.

*Hyphal characteristics.* —Hyphae staining in phloxine, simple septate, walls

<sup>2</sup>Less than 11 mm diam including 4 mm inoculum plug.

hyaline at first, then becoming light brown, (1–)2.5–6.5  $\mu\text{m}$  diam; fiber hyphae dark brown, with walls noticeably thickened, whip-like, branching, 1–1.5  $\mu\text{m}$  diam; by 5 wk small areas of pseudoparenchyma may develop, the hyphae thin-walled, lobed and interlocking, as in a plectenchyma, mixed with cuticular cells, thin-walled, more or less free, up to 17  $\mu\text{m}$  in diam; crystals tiny octahedrons.

Test-tube cultures.—In 28 da mycelium Honey Yellow overlaying an almost black, very thin crust on the upper slant, woolly, compacted on lower part of slant with small dark brown guttations that dry leaving the area punctate, continuous woolly over cylinder to base of tube, with a thin layer of Verona Brown under mat; the dark layers on both the slant and cylinder visible from underneath.

Temperature relations.—Optimum 36 C with good growth at 32 C and 40 C; not killed at 44 C (FIG. 14).

Remarks.—Because of the similarities of the basidiocarps. the culture of *P. bicuspидatus* was compared with those of *P. johnsonianus* and *P. spiculosus*. Cultures of *P. johnsonianus* have been described by Campbell (1938, as *Fomes densus* Lloyd) and Davidson et al. [1959, as *Fomes johnsonianus* (Murr.) Lowe]. Growth rates on MEA are medium (FIG. 8) with an optimum temperature of 32 C (FIG. 14). The mats by 14 da are Straw Yellow to Mustard Yellow and Primuline Yellow with compacted woolly to felty-cottony nodulose texture. Oxidase reactions are positive (FIGS. 9, 10), growing on both GAA and TAA media. Microscopically, generative and fiber hyphae are present and by 6 wk interlocking hyphae forming plectenchymas and/or setae up to 60  $\mu\text{m}$  long may be present, also. The Key Pattern is: B-P-M-11. The Species Code is: 2.6.8.(11).(17).32.37.39.46.54.

Cultures of *P. spiculosus* have been described by Campbell and Davidson (1942, as *Poria spiculosa* Campbell et Davidson). Growth rates on MEA are medium (FIG. 11) with an optimum temperature of 30 C (FIG. 14). The mats are Honey Yellow, Isabella Color to Antimony Yellow and Buckthorn Brown with a tough, leathery or chamois-like texture. Oxidase reactions are positive (FIGS. 12, 13), growing only on TAA medium. Microscopically, generative and fiber hyphae are present and, usually, subulate to ventricose setae borne on dark brown hyphae are conspicuous in the mounts. The Key Patterns are: B-P-M-7-11 and B-P-M-11. The Species Code is: 2.6.8.17.32.37.39.44–45.54.

The culture of *Phellinus bicuspидatus* is readily distinguished from those of *P. johnsonianus* and *P. spiculosus* by its faster growth rate on MEA, higher optimum temperature, and lack of setae. In addition, it differs from both species by the presence of cuticular cells in the areas of pseudoparenchyma. In common with cultures of *P. johnsonianus*, the culture of *P. bicuspидatus* develops interlocking hyphae in a plectenchyma.

#### ACKNOWLEDGMENTS

Dr. C. L. Fergus, The Pennsylvania State University, loaned us the Overholts specimen. Dr. R. B. Miller, Forest Products Laboratory, determined that the substratum of *P. bicuspидatus* belongs in the red-oak group.

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Accepted for publication June 27, 1984