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PHELLINUS FRAGRANS SP. NOV. (APHYLLOPHORALES, HYMENOCHAETACEAE) ASSOCIATED WITH A WHITE ROT OF MAPLE

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Species of *Phellinus* are often difficult to determine because of the morphological similarity that frequently exists between currently recognized species. It has become apparent that several *Phellinus* taxa may be mixtures of two or more species. We present herein our studies of a segregate of *Phellinus* that is decidedly similar to *P. pouzarii* Kotl. and apparently closely related to, and in the past confused with, *P. ferrugineofuscus* (Karst.) Bourd.

Lowe (1946) stated that *Poria ferruganeofusca* Karst. (now *Phellinus ferruganeofuscus*) occurred "on the wood of coniferous trees." Later, a preference for coniferous substrata with rare occurrence on angiosperms was noted (Lowe, 1966). The host relationship with angiosperms apparently was based on a collection (M.J. Larsen 717 in SYRF, collected in 1964) from Cranberry Lake in the Adirondack Mountains, New York. The fungus was on *Acer* sp., associated with a white rot, and had an extremely fragrant and pungent odor. It appeared inseparable from the concept of *P. ferrugineofuscus* on the basis of basidiocarp morphology.

In 1969, an additional collection of the fungus (M. J. Larsen 3832 in CFMR) was found on *Acer saccharum* Marsh. just south of the Straits of Mackinac in Michigan, and also was associated with a fragrant odor. The fungus agreed in all respects with the collection from the Adirondack Mountains. Dr. M. A. Donk (personal communication) indicated at the time of the collection of the Michigan specimen that it was probably *Phellinus pouzarii* Kotl. (Kotlaba, 1968).

In this study we have examined in detail and evaluated the various characteristics associated with basidiocarps, culture, and habitat of the *Phellinus* on *Acer*. Also, careful comparisons were made with similar or apparently closely related species. We have concluded from our investigations that the polypore on *Acer* is heretofore unrecognized and is described herein as *Phellinus fragrans*.

Microscopic characters of basidiocarps were studied from freeze-microtome sections which were subsequently mounted in Hoyer's solution (Anderson, 1954). Other sections were mounted in Melzer's reagent (for formula see Slysh, 1960). The methods employed in studying the cultures and the arrangement of the descriptions, and explanation of the "Key Pattern" are the same as used in previous studies (Davidson et al., 1942). Mat descriptions and growth rates were based on 2- and 5-week-old cultures incubated in Petri dishes at 25°C on 1.5% malt extract agar (Davidson et al., 1942). Extracellular oxidase production was detected by the Bavendamm test described by Davidson et al. (1938), in which the cultures are grown on malt agar containing 0.5% gallic and tannic acids, and the gum guaiac test described by Nobles (1958), in which an alcoholic solution is applied to fungal mats grown on malt agar. For constant temperature studies, Petri dish cultures on malt agar, in triplicate, were placed in incubators as soon as

growth was visible (8 days after inoculation) and were measured at the end of 5 weeks incubation. Measurements of mat diameters represent averages of three replications of one isolate. Killing temperatures were determined by removing those cultures without observable growth from the higher test temperatures and incubating them at 25°C for 4 weeks. Those that did not grow were presumed killed at the test temperatures.

Microscopic structures were drawn with the aid of a camera lucida and a Zeiss drawing apparatus. Capitalized color names are from Ridgway (1912) and numerical color designations are from Munsell (1929–1942). Herbarium designations are those of Holmgren and Kueken (1974).

MORPHOLOGY OF BASIDIOCARPS

Phellinus fragrans M. J. Larsen & F. F. Lombard, sp. nov. (Aphyllophorales, Hymenochaetaceae) Figs. 1–5.

Etymology — from *fragrans* (L., part.) = scented pleasantly or fragrant.

Basidiocarpis effusis, non pileatis, fuscobrunneis; poris laceris, 5-7 per mm; setis adsunt in contextum et tramam; setis in hymenium nullis; basidiosporis 2.5–3(–3.5)× 1.5–2 μm, hyalinis, cylindraceo-ellipsoideis; conidiis 13–17 × 6–8μm, brunneis, globosis vel ovoideis, interdum irregularis, in contextum et tramam.

Fragrantibus.

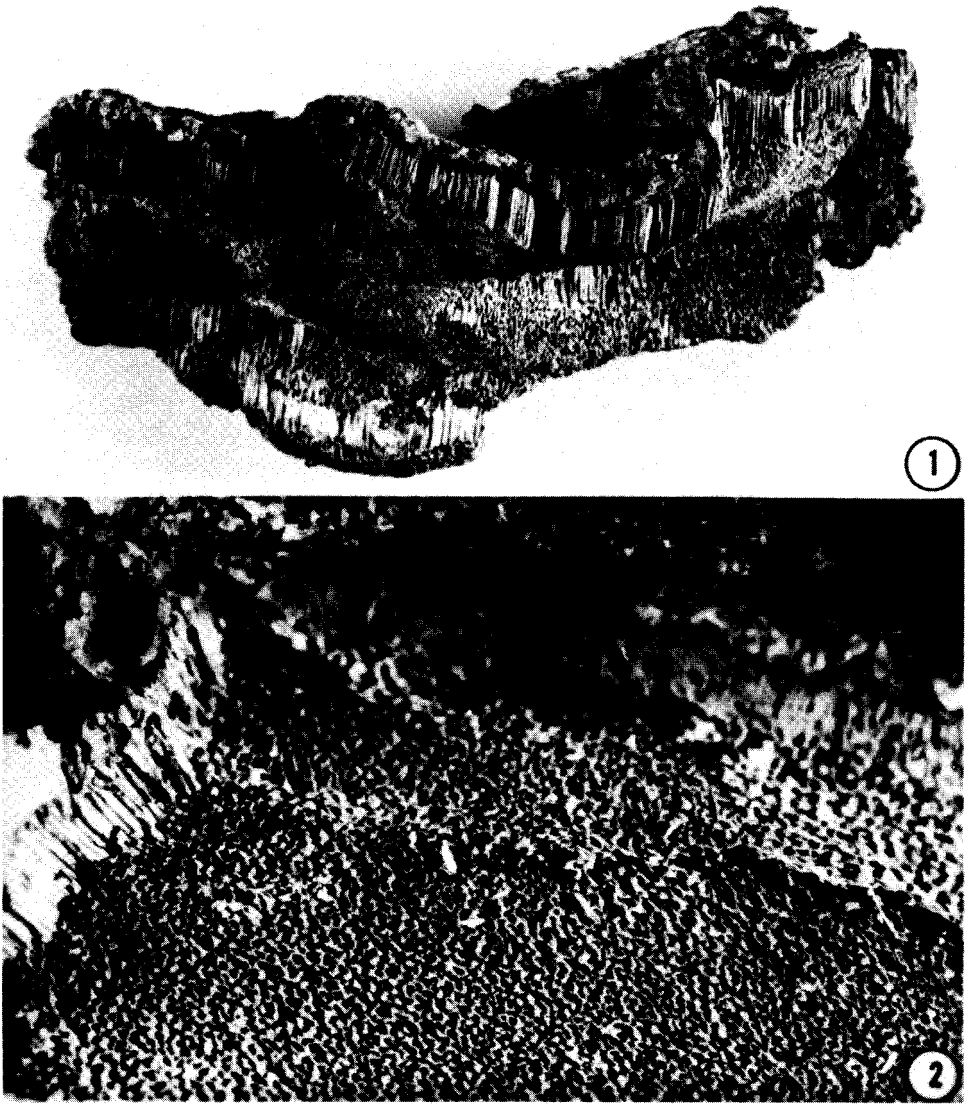
Typus-U.S.A., Michigan, Cheboygan County, Roxbury Creek, ad lignum putridum *Acer saccharum* Marsh., *M. J. Larsen 3832,* legit 28 X1969 (Holotypus in CFMR; isotypi in DAOM et BPI).

Basidiocarps (Figs. 1 & 2) effused and conforming to the substrate surface, annual or more rarely perennial, up to 12 mm thick; sterile margin narrow, yellowish brown (near 5.0 YR 4/6), fibrous, sometimes strigose; pore surface glancing slightly, fuscus brown when fresh with little change on drying (near 5.0 YR 3/4); pores round to somewhat angular, 5–7 per mm, soft-brittle, becoming noticeably lacerate, edges thin; tubes grayish brown to pale gray when fresh (near 5.0 YR 7/2), no change on drying, up to 8 mm long; projecting tramal setae barely visible at 70×; tissue of context and trama darkening noticeably in 2% KOH solution.

Hyphal system dimitic. Clamps absent throughout. Context hyphae of two kinds: generative, 2–3(–3.5) μm diam, septate, branched, thin-walled, hyaline to pale brown; skeletal, 3–5μm diam, rarely branched, thick-walled, septa rare, pale brown to dark ferruginous brown; contextual setae originating from skeletal hyphae, 4–6μm near apex, terminal part slightly broader and usually accuminate, less frequently rounded or blunt.

Trama continuous with the context. Tramal hyphae of two kinds: generative, 3–4 μm diam, septate, branched, thin-walled, yellow-brown to subhyaline; skeletal, 4–5μm diam, septate, dark ferruginous brown; tramal setae (Fig. 4) appearing to form the bulk of the trama, 4–5(–6)μm diam, aseptate, dark ferruginous brown, usually rounded, rarely pointed at the apex, thick-walled, wall thin at apex, projecting at a narrow angle into the pore volume and not usually abruptly bent (as in *P. ferrugineofuscus*), projecting 10–15 μm; subhymenial hyphae 1.5–2μm diam, septate, branched, hyaline.

Basidia 5–6× 2.5–3.5μm, clavate, septate at the base, 4-sterigmate; basidiospores (Fig. 3) hyaline, smooth, cylindric-ellipsoid and tapering slightly towards

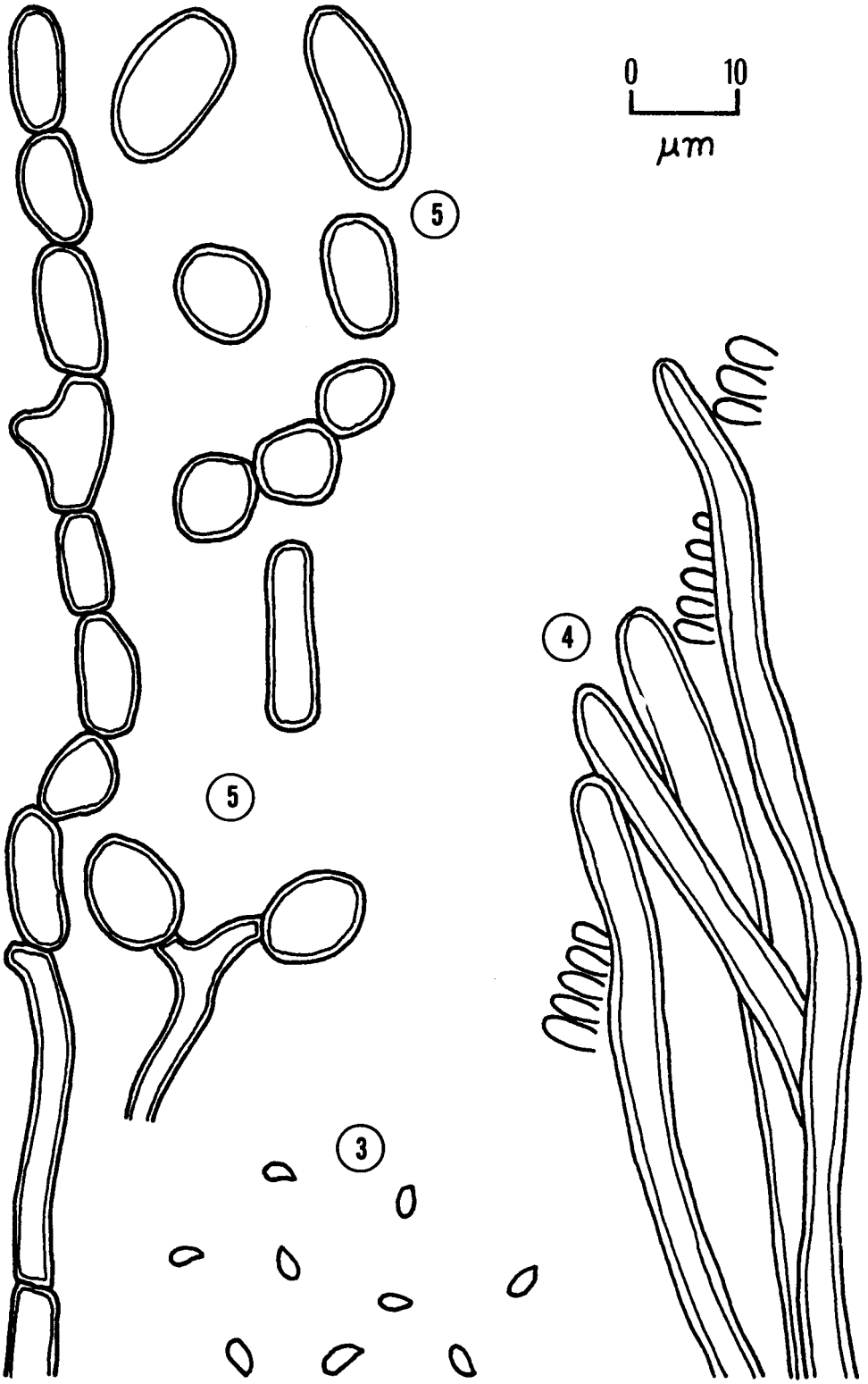


Figs. 1–2. Basidiocarps of *Phellinus fragrans.* 1. Detail of habit (×2). 2. Detail of pore surface (×6). Both from holotype (MJL-3832) in CFMR.

the apiculus (spores distinctly bent in fresh spore print), 2.5–3(–3.5) × 1.5–2 μm, acyanophilous, not amyloid or dextrinoid; conidia (Fig. 5) variable in size and shape, approximately 13–17 × 6–8 μm, globose to ovoid to less frequently irregular in outline, medium to dark yellowish brown, found frequently in context and less so in trama, with walls holothallic with conidiogenous cells apparently of the meristem type (indeterminate retrogressive), borne singly or in chains.

Basidiocarps and associated white stringy rot pungently fragrant.

Type-USA., Michigan, Cheboygan Co., Roxbury Creek, on decayed log of


0
10
μm
5
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4
3

Figs. 3–5. Microscopic characters of *Phellinus fragrans.* 3. Basidiospores. 4. Tramal setae. 5. Conidia. From holotype (MJL-3832) in CFMR.

Acer saccharum, *M. J. Larsen 3832,* 28 X 1969 (Holotype in CFMR; isotypes in DAOM and BPI).

Additional Specimen Examined.

U.S.A., New York, Cranberry Lake, State Univ. New York Forestry Summer Camp, on decayed log of *Acer* sp., *M. J. Larsen 717,* 27 VI 1964 (SYRF).

Specimens of Other Taxa Examined.

Phellinus ferrugineofuscus (Karst.) Bourd. Rabenhorst, Fungi Europaea 3645 (BPI, lectotype of *Poria ferrugineofusca* Karst.). Estonian SSR, District Johui, on *Picea excelsa* Link, *E. Parmasto,* 22 IX 1953 (SYRF), and District Rapina, Jarvselja, on *P. excelsa, E. Parmasto,* 16 IX 1956 (*Mycotheca Estonica,* 23, in SYRF). U.S.A. Colorado: White River National Forest, on conifer, *J. L. Lowe, R. W. Davidson,* & *T. E. Hinds* (*Lowe 6095* & *6126,* SYRF); Fraser Experimental Forest, on *Picea* sp., *R. W. Davidson, FP*[1] 104537 (CFMR). Idaho: Challis National Forest, China Creek, on *Picea* sp., *M. J. Larsen 1328* (SYRF); Payette National Forest, Lick Creek Summit, on conifer log, *J. L. Lowe* & *R. L. Gilbertson* (*Lowe 6833,* SYRF). New York: North Elba, on *Picea* sp., C. H. Peck, September (NYS, SYRF, holotype of *Polyporus marginellus* Pk. = *P. ferrugineofuscus*).

Phellinus nigrolimitatus (Rom.) Bourd. & Galz. U.S.A. Oregon: Siuslaw National Forest, Lincoln County, Cougar Mountain, on *Tsuga* sp., M. J. *Larsen, FP 133694* (CFMR); Siskiyou National Forest, Winchuk Campground, on conifer log, *M. J. Larsen, FP 133225* (CFMR).

Phellinus pouzarii Kotl. Czechoslovakia, near Jablunkov, Moravskoslézski Beskydy Mountains, on *Abies alba* Mill., *F. Kotlaba* & *Z. Pouzar,* 23 VIII 1966 (*PR 628130,* holotype).

Phellinus repandus (Overh.) Gilbertson. USA. Idaho: Clearwater National Forest, on *Pinus monticola* Dougl., *J. R. Weir 15134* (BPI). Montana: Darby, on *Pseudotsuga menziesii* (Mirb.) Franco, *J. R. Weir 18481* (BPI). Washington: Chehalis, on conifer log, *R. W. Davidson, FP 105605* (CFMR).

The outstanding features by which *P. fragrans* may be recognized are (1) presence and nature of conidia, (2) occurrence on *Acer,* (3) association with a fragrant odor, (4) size and shape of spores, and (5) presence of contextual and tramal setae and absence of hymenia1 setae.

At first, *P. fragrans* may be confused with *P. nigrolimitatus* and *P. repandus,* but the size and shape of the spores (Table I) readily separate both from *P. fragrans.* Basidiocarps (Fig. 6) of *P. ferrugineofuscus* are very similar to the newly described species. It appears that the only truly reliable criterion that can be used for separation is the basidiospore character: the spores in *P. ferrugineofuscus* are allantoid, whereas those in *P. fragrans* are cylindrical-ellipsoid when dry and distinctly bent in a fresh spore print. *Phellinus pouzarii* (Fig. 7) also possesses

[1] Designation for CFMR herbarium numbers.

Table I

Comparison of *Phellinus* species: *ferrugineofuscus, fragrans, nigrolimitatus, pouzarii,* and *repandus*

	Basidiocarps					
Species	Hymenial setae	Tramal setae (projecting beyond basidia)	Contextual setae	Basidiospores	Host	Rot
ferrugineofuscus[a]	very rare	to 25 μm	present	allantoid, 4–5.5 × 1–1.5μm	conifers	white, laminated, with small pits
fragrans	absent	10–15 μm	present	cylindrical-ellipsoid, tapering, 2.5–3.5 × 1.5–2μm	maple	white, soft, stringy, with fragrance
nigrolimitatus[a]	abundant 25–35 μm long	absent	absent	cylindrical, 6–8.5 × 2–3μm	conifers	white, large pockets, wood between pockets firm
pouzarii[b]	very rare	5–10 μm	present	short-ellipsoid, nearly flattened on ventral side, 2.8–3.5 × 1.5–1.8 μm	conifers	white (?), with fragrance
repandus[a]	rare, 20–25 μm long	absent	absent	ovoid to subglobose, 4–5.5 × 3–4.5μm	conifers	white, small pockets, wood between pockets fragile

[a] Data from Lombard et al. (1972).
[b] Data from Kotlaba (1968) with exception of length of projection of tramal setae and occurrence of hymenial setae.

several characters that are similar to *P. fragrans.* However, the pore size of *P. pouzarii* is considerably larger, and the context lacks conidia. Kotlaba (1968, p. 27) described the spores of *P. pouzarii* as "short-ellipsoid, on the vertical-side nearly flattened, narrowed at one side into a short apiculus."

The white rot produced by *P. fragrans* also presents some unique characteristics. In appearance and texture, the wood becomes stringy and is associated with a slimy or mucilaginous substance, the cause of which has not been ascertained, but we suggest that it may be bacterial in origin.

CULTURAL DESCRIPTION

Key Patterns

A-P-V-10 at 2 wks, (B-P-V-4-10, at 5 wks)

Growth Characteristics

Growth very slow, developing a barely visible trace of white mycelium on the inoculum block by 14 days; mat in 5 wks 38–41mm in diam (Fig. 8), Cinnamon-Brown over and immediately around the inoculum block, becoming Ochraceous-Tawny, and fading into Light Buff to creamy white at the margin, appressed,

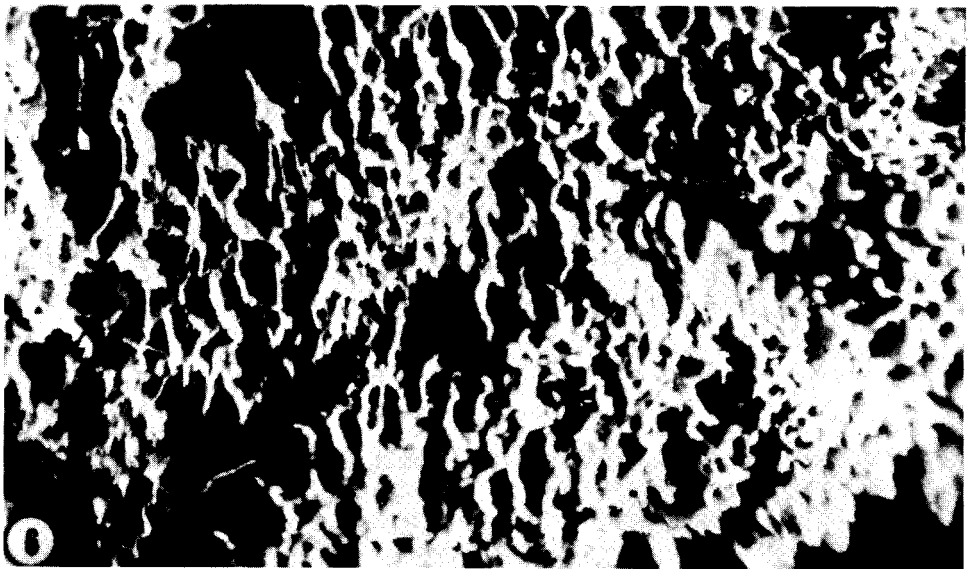


Fig. 6. Detail of pore surface of *Phellinus ferrugineofuscus* (×25; from FP 104537 in CFMR).

fine-downy, adherent, homogeneous, occasionally with fine, tawny brown skeins radiating a short distance from the inoculum; margin indistinct, uneven; reverse discoloration Bister under the inoculum surrounded by a narrow Tawny-Olive zone, fading into Chamois under most of the mat; odor strong, fragrant (similar

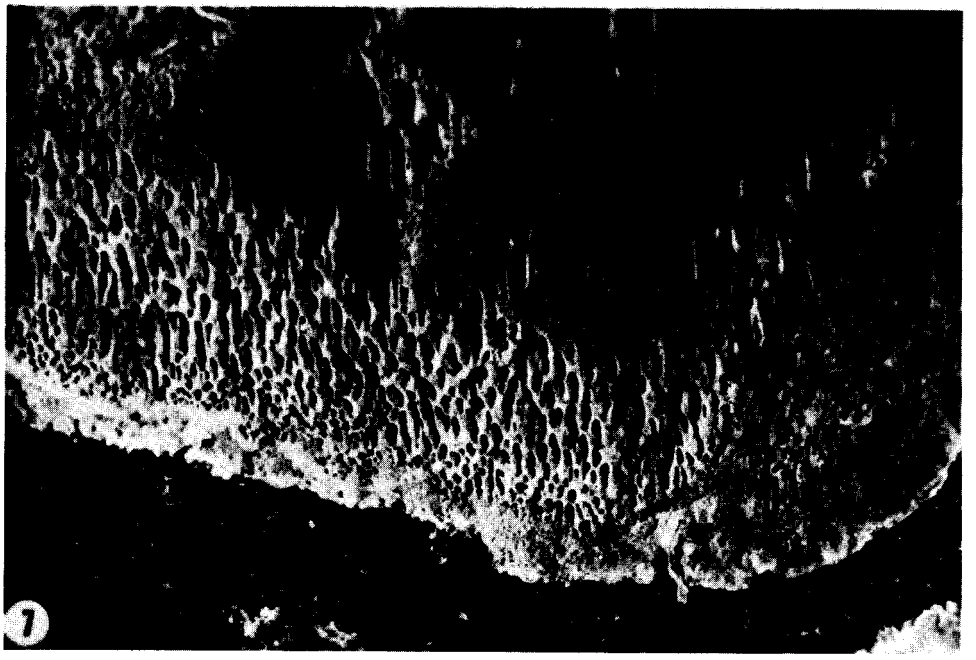
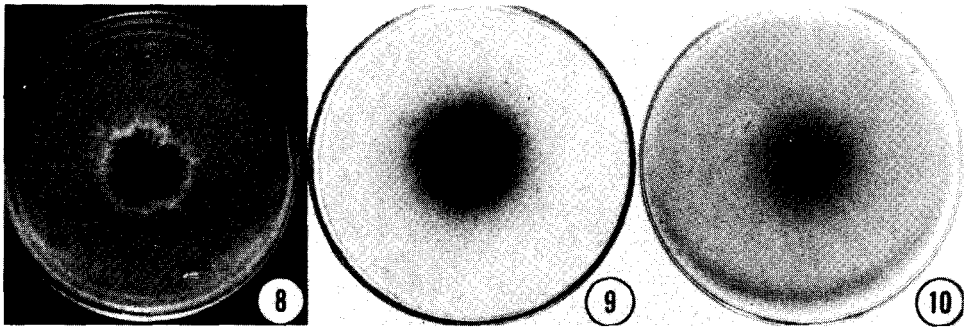


Fig. 7. Detail of pore surface of *Phellinus pouzarii* (×5; from holotype, PR 628130 in PRM)



Figs. 8–10. Cultures of *Phellinus fragrans* (MJL-3832-S). 8. Five weeks old on malt agar. 9. One week old on gallic acid agar. 10. One week old on tannic acid agar.

to oil of rose); oxidase reactions positive, making no growth and with a strong reaction on gallic acid (Fig. 9) and no growth with a weak reaction on tannic acid (Fig. 10) agars in 7 days, and moderately strong with the gum guaiac test, giving a reaction in 10 minutes varying in color from Dark Gobelin Blue on the dark colored parts of the mat to Light Glaucous-Blue on the paler mycelium.

Hyphal Characteristics

Hyphae from marginal areas staining in phloxine, some appearing to be vacuolate, simple-septate, branching, with thin hyaline walls, rarely sparsely encrusted. 1.5–3.5(–4.5)μm diam (Fig. 11), some branches curving back past the parent hyphae and appearing clamp-like, staining heavily in phloxine, slightly greater in diam than parent hyphae (Fig. 12); hyphae (from colored areas of mat) with yellowish-brown walls and contents, very rare, 2.5–3.5 μm diam (Fig. 13); "oidia" from marginal areas with thin hyaline walls, stainingin phloxine, free-floating, in chains or single (Fig. 14), later in great abundance (constituting almost the whole mat), with walls slightly thickened and walls and contents light brown, non-staining in phloxine, mostly cylindrical, a few ovoid or egg-shaped, 8.5–15 × 2–5.5 μm (Fig. 15); crystals octahedrons, some quite large and eroded (Fig. 16).

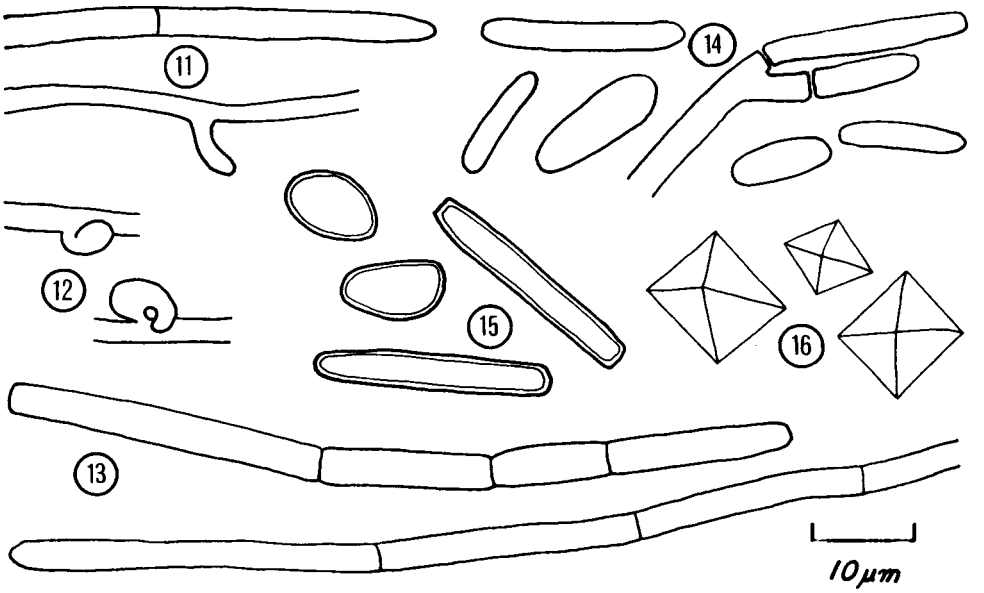
Temperature Relations

Average mat diameters of one isolate grown in triplicate on malt agar in the dark, measured at the end of 5 weeks at the following 11 constant temperatures: 12°C, 33.3 mm; 16°C, 45.6 mm; 20°C, 61.3 mm; 22°C, 52.3 mm; 24°C, 45.6 mm; 26°C, 36.3 mm; 28°C, 19.3 mm; 30°C, trace[2]; 32°C, trace; 36°C, trace; 40°C, 0. Optimum, 20°C; killing, 36°C.

Cultures Studied

From the type, MJL-3832, an isolate from basidiocarp context and one from conidial spore mass associated with the basidiocarp.

[2] Less than 11 mm.



Figs. 11–16, *Phellinus fragrans.* Microscopic elements from culture (MJL-3832-S). 11. Hyphae from marginal area. 12. Curved hyphal branches. 13. Brown hyphal elements. 14. "Oidia" from marginal areas. 15. Comparatively thick-walled "oidia" from older portions of mat. 16. Crystals.

Remarks

According to the system devised by Davidson et al. (1942) for uniformly describing and coding cultural characters of wood-inhabiting Hymenomycetes, the Key Pattern normally includes only those characters present in 14-day-old dish cultures. However, the cultures of *P. fragrans* are so slow-growing that only a trace of growth is visible on the inoculum block at 14 days. The first Key Pattern given reflects this condition. The second Key Pattern is based on the characters of 5-week-old dish cultures.

The asexual spores of *P.* fragrans are coded as "oidia" in conformity with the definitions used by Davidson et al. (1942) and Nobles (1965). By more recent definition (Kendrick, 1971, pp. 253–262), they are conidia of the meristem type.

The species is characterized in culture by its extremely slow growth rate and, in older cultures, by the tan- to rust-colored mat, presence of brown-walled oidia, and fragrance. The Species Code of Nobles (1965) based on 6-week-old dish cultures is 2.6.7.35.37.39.47.50.54.

At the time of the collection of the specimen MJL-3832, a polysporous isolate (basidiospores deposited on agar) was obtained. However, this isolate was not available at the time of and, therefore is not included in, the cultural studies. Subsequent examination of this polysporous isolate showed that it agrees with the microscopic characters exhibited by the contextual and conidial isolates used in the study.

SUMMARY

Phellinusfragrans, associated with a soft stringy white rot of down maple logs, is described as a new species. The relationships of the new taxon to other members

of the genus *Phellinus* are discussed, and significant comparative characteristics are tabulated. A cultural description is also provided.

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Trade or proprietary names are included for identification purposes only and imply no endorsement by the Forest Service of the U.S. Department of Agriculture.

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