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## A NEW *PHANEROCHAETE* WITH A *CHRYSOSPORIUM* IMPERFECT STATE

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### SUMMARY

A new species is proposed in the genus *Phanerochaete* for a wood decaying fungus that is extremely important in the destruction of wood chips in storage. The names *Chrysosporium pruinatum*, *C. lignorum*, *Sporotrichum pruinatum*, and *S. pulverulentum* have all been applied to this species in its imperfect state. Complete descriptions of basidiocarps and cultures are presented and the use of the species in wood decay studies is discussed.

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While collecting wood-rotting fungi in the Sonoran Desert, Arizona, in 1971, the senior author found an undescribed species of *Phanerochaete*. This species, which has a *Chrysosporium* imperfect state in culture, was represented at that time by this single Arizona collection. However, other specimens have since been encountered from New York and Maryland.

Earlier, a fungus identified as *Sporotrichum* sp. had been isolated from test wood chip storage piles in Georgia (Lindgren and Eslyn 1961) and Maine (Eslyn 1967). To conform to Carmichael's concept (1962), it was re-identified as *Chrysosporium* sp. When some of these isolates developed basidiocarps in culture they were sent to Dr. H. McKay, of the Forest Disease Laboratory, Laurel, Md. After examining the basidiocarps she designated the species "*Peniophora* G."

Comparisons of basidiocarps of the undescribed *Phanerochaete* species and those produced in culture by "Peniophora G" isolates showed them to be conspecific. In addition, the cultures derived from germinating spore prints of the *Phanerochaete* basidiocarps were almost identical in every way to the "Peniophora G" (*Chrysosporium* sp.) isolates from decay. The asexual state was identified as *Chrysosporium pruinosum* (Gilman et Abbott) Carmichael and this determination was subsequently confirmed by Dr. J. W. Carmichael, University of Alberta, Alberta, Canada.

Comparison of our cultures with Nilsson's wood chip pile isolate, which was first identified as *C. pruinosum* (Nilsson 1965) and later as *C. lignorum* Bergman et Nilsson (1966) *nomen nudum*, and Shields' (1969) isolate of *Ptychogaster* sp. from wood chips, showed all to be conspecific. Nilsson (Shields 1969) also considered his isolate to be identical to Shields' isolate as did Smith and Ofozu-Asiedu (1972), who noted that both isolates were probably *C. pruinosum*. Hofsten and Hofsten (1973) indicated that the Nilsson isolate is conspecific with *Sporotrichum pulverulentum* Novobranova.

In addition, Shields (1969) and Bergman and Nilsson (1971) believed their isolates to be the imperfect state of a basidiomycete, Shields indicating *Hyphodontia* and *Peniophora* as possible placements. The perfect state, however, has not been precisely identified to date. The present study has revealed the fungus to be a new species of *Phanerochaete* which is described herein.

*Phanerochaete chrysosporium* Burds., sp. nov. FIGS. 1-12.

Basidiocarpis late effusis, pallidoluteis vel luteo-brunneis. Hyphis subiculis absque fibulatis. Cystidiis ab oriundis subhymenio aut subiculis, 60-150(-250) x 6.5-9  $\mu$ m, cylindraceis, tenuibus parietibus, laevibus, obtusis apicibus. Basidiis 22-35 x 5-6  $\mu$ m. Basidiosporis (5.5-) 6-7.5 (-9) x 3-4  $\mu$ m, depressis ovoideis.

HOLOTYPE--U.S.A., Arizona: HHB 6251, on dead wood of *Platanus wrightii* S. Wats. (Arizona sycamore), Guadalupe Canyon, Peloncillo Mts., Cochise County, August 25, 1971 (CFMR). Isotype in BPI and ARIZ.

CON.STAT. --*Chrysosporium pruinosum* (Gilman et Abbott) Carmichael, Can. J. Bot. 40: 1166. 1962.

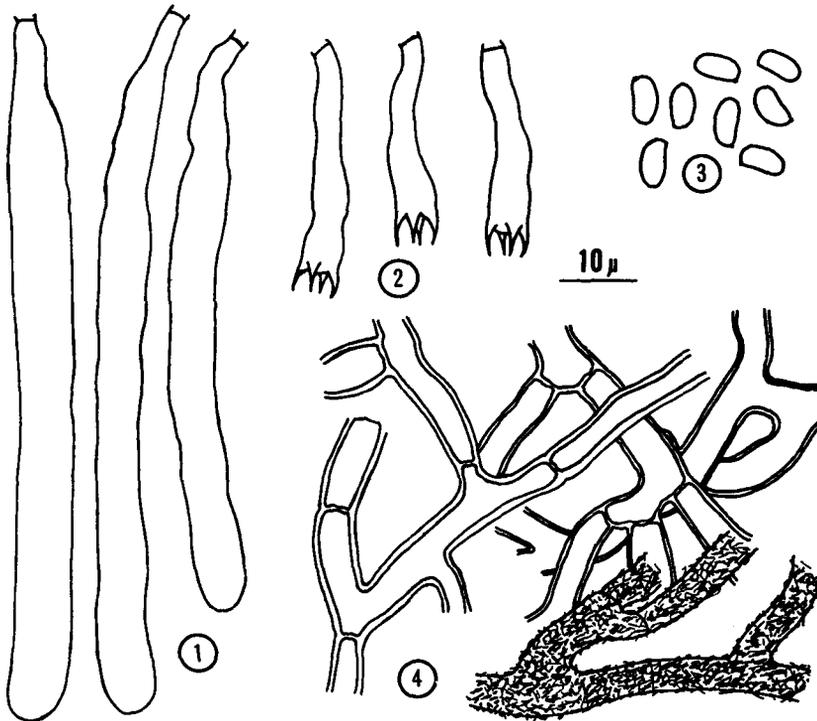
≡ *Sporotrichum pruinosum* Gilman et Abbott, Iowa State Coll. J. Sci. 1: 306. 1927.

= *Chrysosporium lignorum* Bergman et Nilsson (*nom. nud.*), Dept. Forest Prod., Roy. Coll. Forest., Stockholm, Res. Notes No. 53, p. 28. 1966.

=(?) *Sporotrichum pulverulentum* Novobranova, Akad. Nauk U.S.S.R., Botan. Inst. Nov. Sist. Niz. Rastanii 9: 180. 1972.

Basidiocarps broadly effused, (indeterminate), membranous, moist when fresh, yellowish-white (3A2)<sup>1/</sup> to light brown (5D5), up to 0.25 mm thick, loosely attached or separable in small pieces, smooth, continuous, not cracking; margin rather abrupt or up to 2 mm broad, sterile, very thin, powdery or finely granular, irregular in outline, white, non-rhizomorphic; *subiculum* byssoid, white. Hyphal system monomitic; *subiculum* a textura intricata, hyphae (Fig. 4) (3.5-)4.5-6(-7)  $\mu\text{m}$  diam, hyaline, thin walled or with walls up to 1  $\mu\text{m}$  thick, lacking clamps, branched at nearly right angles, smooth near substrate to heavily encrusted with large hyaline crystals near subhymenium, sometimes with oval to spherical chlamyospore-like thick walled cells near substrate; *subhymenium* up to 75  $\mu\text{m}$  thick, a compact textura intricata-porrecta, hyphae 2.5-4  $\mu\text{m}$  diam, thin walled or with slight wall thickening, somewhat agglutinated; hymenium composed of cystidia (Fig. 1) and basidia (Fig. 2); cystidia (Fig. 1) arising at various levels of the subhymenium and subiculum, 60-150 (-250)  $\times$  6.5-9  $\mu\text{m}$ , cylindrical, thin walled or with slight wall thickening, hyaline, smooth, obtuse at apex, septate only at base, lacking clamps, protruding up to 70  $\mu\text{m}$ ; basidia (Fig. 2) 22-35  $\times$  5-6  $\mu\text{m}$ , clavate, hyaline, thin walled, septate at base, lacking clamps, 4-sterigmate, sterigmata 4-5  $\mu\text{m}$  long; *basidiospores* (Fig. 3) (5.5-) 6-7.5(-9)  $\times$  3-4  $\mu\text{m}$ , depressed ovoid, hyaline, thin walled, smooth, not reacting with Melzer's reagent.

<sup>1/</sup> Color notations are those of Kornerup and Wanscher (1967), indicating plate number, vertical column, and horizontal column, respectively.



Figs. 1-4. Zeiss drawing tube drawings of microscopic characters of *Phanerochaete chrysosporium*, HHB 6251.  
 1. cystidia. 2. basidia. 3. basidiospores.  
 4. subicular hyphae.

Etymology: from its *Chrysosporium* imperfect state.

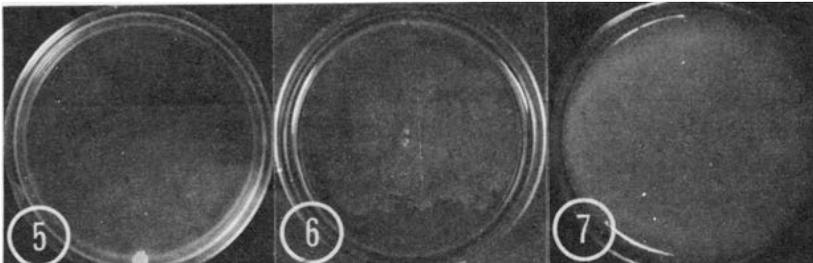
Additional Specimens Examined: U.S.A., New York - MJL 98, on dead wood of *Ulmus americana* L., Monroe County (?); Maryland-104297, on dead wood of *Liriodendron tulipifera* L., Montgomery County. Both in CFMR.

Remarks: *Phanerochaete chrysosporium* basidiocarps are distinguished by their long, broad, cylindrical, smooth cystidia and complete lack of clamp connections. They are similar to *P. arizonica* Burds. et Gilbertson, which possesses much smaller cystidia arising in the subhymenium

only and to *P. cremea* (Bres.) Parm. which also possesses smaller cystidia that are thick walled and usually encrusted. Neither of these is known to possess a *Chryso sporium* imperfect state.

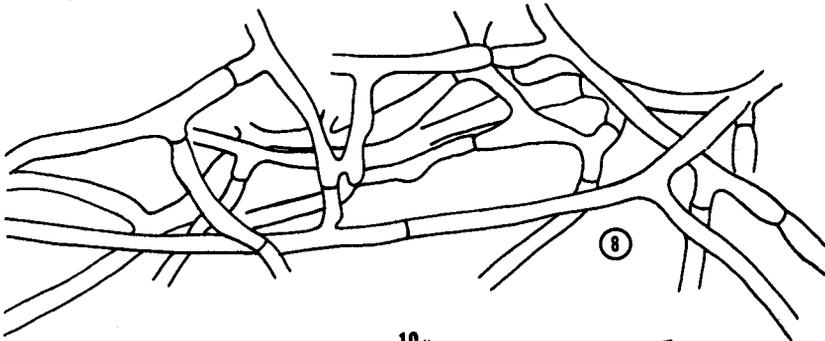
#### DESCRIPTION OF CULTURES

Growth Characters: Radial growth on 1.5% Difco Bacto Malt Extract Agar 45–60 mm in three days at 25C; mat (Fig. 5) very thin, white, appressed radiating, some isolates with granular surface 1–2 cm behind the advancing margin, others with granular surface after margin reaches edge of plate, granular aspect spreading from inoculum until entire plate is covered; agar bleached below inoculum and often over whole plate; some isolates producing cream-colored, effused hymenial surface, usually in 18–28 days, forming all structures typical of fruiting in nature; other isolates never fruit. On gallic acid agar (Davidson, et al. 1938) growth 24–30 mm diam/week, mat thin-woolly, margin irregular, agar not stained (Fig. 6); on tannic acid agar (Davidson, et al. 1938) no growth or staining of agar (Fig. 7); tests with syringaldazine and gum guaiac (Harkin and Obst 1973) negative, indicating lack of laccase, peroxidase and tyrosinase.

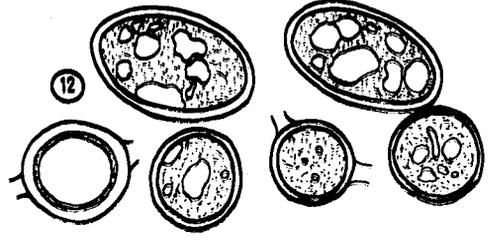
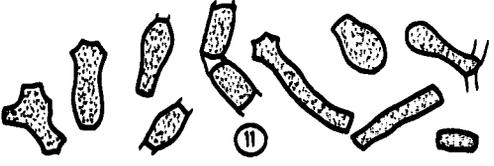
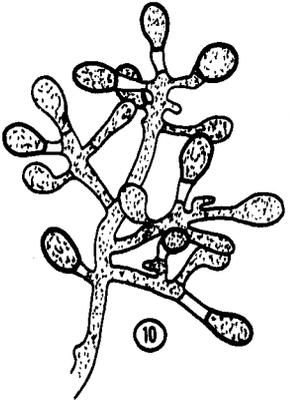
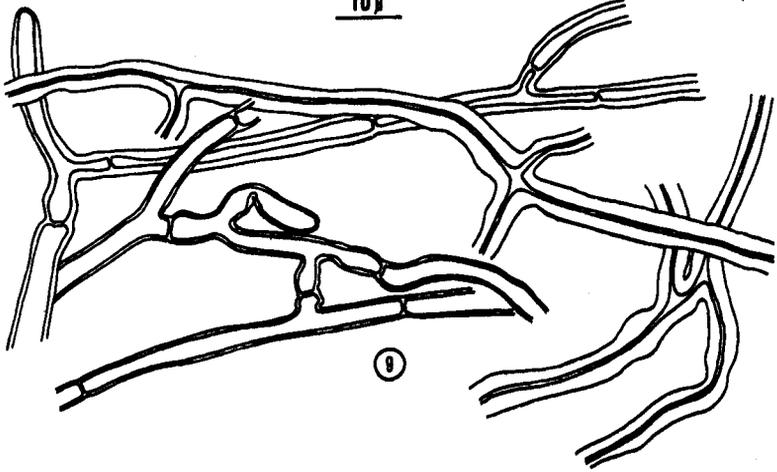


Figs. 5–7. Cultures of *Phanerochaete chrysosporium* grown at 25C, HHB 6251. 5. on malt agar after three days growth. 6. on gallic acid agar after one week. 7. on tannic acid agar after one week.

Microscopic Characters: Hyphae of margin hyaline, thin walled, 3.5–7  $\mu\text{m}$  diam, smooth, infrequently branched, densely stained in phloxine, first septum usually occurring more than 1 mm from tip, clamps lacking.



10  $\mu$



Legend  $\longrightarrow$

*Aerial hyphae* (Fig. 8) 2–4  $\mu\text{m}$  diam, hyaline, thin walled, smooth or occasionally encrusted with long narrow crystals, septate, lacking clamps, branched frequently, these branches often developing as conidiophores; *submerged hyphae* (Fig. 9) (2.5–) 3.5–6 (–8)  $\mu\text{m}$  diam, mostly thick walled, occasionally thin walled in narrower hyphae, hyaline, smooth, septate, lacking clamps, frequently branched, usually at nearly right angles.

Conidia only on aerial hyphae; terminal persistent conidia (*aleuriospores* of Carmichael, 1962) (Fig. 10) 5–10 x 3–6  $\mu\text{m}$ , ovate to elliptical, truncate at attachment to poorly differentiated conidiophore, smooth, hyaline, densely granular, sometimes guttulate, with slight wall thickening, not reacting with Melzer's reagent; arthric, retrogressive, fragmenting conidia (*arthrospores* of Carmichael, 1962) (Fig. 11) variable in size and shape (cylindrical, ovoid, clavate, branched, or fusoid), hyaline, densely granular, with slightly thickened walls, not reacting with Melzer's reagent; a third type of conidia (*chlamydospores* of Carmichael, 1962) (Fig. 12) terminal or intercalary, pyriform, spherical, to truncate-fusiform, hyaline, smooth, with densely granular contents and thick walls, up to 60  $\mu\text{m}$  in longest dimension.

Temperature Relations: Average growth rates (nun radius/day) for the temperatures indicated are: 12C, 2–3 mm; 16C, 3–5mm; 20C, 9–13 mm; 24C, 14–19 mm; 28C, 18–24 mm; 32C, 26–32 mm; 36C, 30–36 mm; 40C, 35–42 mm; 44C, 28–33 mm; 50C, 0–6 mm. According to the method of Davidson, et al, (1942) the key pattern would be A–O–F 2–3–4–10–14 or B–O–F 2–3–4–5–6–10–14–16 Using the method of Nobles (1965) the following species code is obtained: 1.6.14.24.32.33.34.35.36.40.41. (48). 54.55.

Cultures Examined: Polysporous isolates from basidiocarps: HHB 6251 (Holotype), MJL 98, and FP 104297. Isolates from decayed wood that have fruited in culture: ME–8 (from *Nyssa sylvatica* Marsh, [blackgum] wood chip, Brunswick, Ga.); ME–446 (from *Fagus grandifolia* Ehrh. [American beech] wood chip, Winslow, Me.); ME–450 (from

Fig, 8-12. Zeiss drawing tube drawings of microscopic characters of cultures of *Phanerochaete chrysosporium*, HHB 6251, 8, aerial hyphae, 9, submerged hyphae. 10, aleuriospores and conidiophores. 11, arthrospores. 12, chlamydospores.

mixed *F. grandifolia*, *Betula* sp. [birch], *Acer* sp. [maple] wood chips, Winslow, Me.); ME-461 (from *Pinus* sp. [pine] wood chip, Fargo, Ga.); ML-20, ML-21 and ML-26 (all from *Sequoia sempervirens* (D. Don) Endl. [redwood] cooling tower, Kan.); FPL-V 170G (Shields' isolate from mixed *Abies balsamea* (L.) Mill.-*Picea mariana* (Mill.) B.S.P. [balsam fir-black spruce] wood chips, New Brunswick, Canada); QM 9145 (Nilsson isolate P127-1, from *Pinus* sp. wood chips, Sweden).

Cultures exhibiting only the imperfect state: ME-PC-8 (from *Pinus* sp. wood chip, Fargo, Ga.); ME-GC-15 (from *N. sylvatica* wood chip, Brunswick, Ga.); ME-OC-11 (from *Quercus falcata* Michx. var. *falcata* [southern red oak] wood chip, Brunswick, Ga.); ME-BC-10 (from *F. grandifolia* wood chip, Winslow, Me.); ME-BIC-6 (from *Betula* sp. wood chip, Winslow, Me.); ME-MC-7 (from *Acer* sp. wood chip, Winslow, Me.).

Remarks: Culturally this species differs from other Phanerochaete species studied in culture because of its *Chrysosporium pruinosum* imperfect state, its rapid growth rate and its high optimum temperature for growth, ca 40C. It is unusual in that it produces a white rot of wood but tests negative for extracellular oxidases as indicated by the Bavendamm test and tests with syringaldazine and gum guaiac (Harkin and Obst 1973).

## DISCUSSION

The *Chrysosporium* state of this species has been repeatedly isolated from wood chip storage piles in North America, (as *Sporotrichum* sp. by Lindgren and Eslyn 1961; Saucier and Miller 1961; and Eslyn 1967; as *Ptychogaster* sp. by Shields and Unligil 1968, and Shields 1969; and as *C. pruinosum* by Smith and Ofosu-Asiedu 1972); in Sweden (as *C. pruinosum* by Nilsson 1965; and as *C. lignorum* by Bergman and Nilsson 1966, 1967, 1968, and 1971; Bergman, et al. 1970; Assarsson, et al. 1970); and in Poland (as *C. lignorum* by Zielinski 1973). In Sweden (Nilsson 1973) *C. lignorum* was noted to be the most common white rot fungus in all types of chip piles studied. Zielinski (1973) reported it to be one of the most frequently found fungi in two birch piles studied in Poland. The perfect state has been reported also (as "*Peniophora mollis* (Bres.) Bourd. et Galz.?" from redwood in cooling towers (Duncan and Lombard 1965).

Because of its frequency of occurrence in wood chip storage piles and affirmative results of laboratory decay appraisals (Eslyn 1969), the species has been utilized extensively as a wood-rot test organism. Research by members of this laboratory based on the use of the isolates designated *Peniophora* "G" (ME-461) and *Chrysosporium* sp. (ME-PC-8) included: evaluation of chemicals for controlling biodeterioration in wood chip storage piles (Eslyn 1973a, b, and Springer, et al. 1969); determination of decay resistance of alkaline-treated wood (Highley 1973a, b); investigation of the chemistry of wood decay (Highley 1973c, and Kirk and Highley 1973); and evaluation of two methods of determining wood loss after fungal attack (Feist, et al. 1971).

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