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CULTURAL CHARACTERISTICS AND TAXONOMY OF SOME POLYPORES (APHYLLOPHORALES) FROM SANTA CATARINA ISLAND, SC, BRAZIL

MARIA ALICE NEVES
CLARICE LOGUERCIO-LEITE

Laboratório de Micologia, Departamento de Botânica
Universidade Federal de Santa Catarina / Centro de Ciências Biológicas
88040-900, Florianópolis, SC, Brazil
e-mail: cllcite@ccb.ufsc.br

ABSTRACT

Cultural characters of seven wood-rotting polypores (Aphyllophorales) collected in Santa Catarina Island (Southern Brazil) were studied according to Nobles' system (1965). Little or nothing was known of the cultural characteristics of these polypores. The species are *Phellinus apiahynus* (Speg.) Rajchenb. & Wright, *Antrodiella multipileata* Loguercio-Leite & Wright, *Fomitella supina* (Sw.: Fr.) Murr., *Megasporoporia cavernulosa* (Berk.) Ryv., *Perenniporia stipitata* Ryv., *Rigidoporus microporus* (Fr.) Overeem and *Trametes pavonia* (Hook.) Ryv. The cultural characteristics were helpful in distinguishing the species, specially in *Fomitella supina*, *Antrodiella multipileata* and *Trametes pavonia*.

KEY WORDS: cultural studies, Aphyllophorales, wood rotting

INTRODUCTION

Santa Catarina Island (27° 10' and 27° 30' S lat. and 48° 25' and 48° 35' W long.) is a subtropical area, whose vegetation corresponds to the Atlantic Rain Forest in a secondary stage (IBGE, 1992). During the last ten years collecting of wood-rotting fungi has resulted in new records of polypores to Santa Catarina (Loguercio-Leite & Wright, 1991a, 1991b; Gerber & Loguercio-Leite, 1997, Loguercio-Leite & Gerber, 1997).

In this paper cultural studies are reported for 7 polypores, in order to better understand their biology, and consequently, their taxonomy. The cultural features of five species were unknown. Six of the species are well known by mycologists, while *A. multipileata* has been reported only once (Loguercio-Leite & Wright, 1991b).

MATERIAL AND METHODS

Cultures were isolated from tissues of fresh fruitbodies collected on angiosperms in Santa Catarina Island. They were studied and described according to Nobles (1965). The number after 'cc' indicates the culture while the number after FLOR indicates the voucher specimen.

Dicaryotic cultures were inoculated in the center of 10 cm Petri dishes with malt extract agar (MEA), differing from Nobles (1965) which inoculated the dishes at the side. For this reason the code symbol to rate of growth was not included. Cultures were grown on MEA in the dark at 25° C and studied during 6 weeks. Microscopic preparations were mounted in KOH 5% plus phloxine 1% and in Melzer's reagent. Drawings were made with a camera lucida (1000X).

Tests on gallic acid (GAA) and on tannic acid (TAA) agar media were recorded at 1 week after inoculation at the same conditions (dark, 25° C) and were made according to Nobles (1965) while the intensity was evaluated based on Bettucci and Guerrero (1971). Biochemical enzymatic tests were performed according to Käärik (1965), Harkin and Obst (1973), Taylor (1974) and Stalpers (1978). The cultures were 7 days old when the biochemical tests were performed. The intensity of reactions are classified in: negative (-), weak (+), medium (++) and strong (+++).

RESULTS AND DISCUSSION

This work allows to improve the knowledgement from the mycelian characteristics of the species described here in culture and increases the number of characters that can be used in traditional taxonomy. Species are in two families, one of them belongs to the Hymenochactaceae (*Phellinus apiahynus*) while the six others belong to the Polyporaceae (*Antrodia multipileata*, *Fomitella supina*, *Megasporoporia cavernulosa*, *Perenniporia stipitata*, *Rigidoporus microporus* and *Trametes pavonia*).

Phellinus apiahynus (Speg.) Rajchenb. & Wright
Mycologia, 72 (2): 251. 1987.

Fig. 1

Cultures studied: cc 470 (≡ FLOR 11000; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Gerber & Cabral; 30/V/1994).

Macroscopic characters: Mat cottony, yellowish brown to pale yellow at the margin; margin regular; growing very slowly, 7,5 - 8,0 cm diam. at the 6th week. Reverse darkened. Odor faint.

Microscopic characters: Marginal hyphae simple-septate, thin-walled, with some small ramifications (1,5 - 4,0 µm diam.). After three weeks the number of ramifications decrease. Fiber hyphae unbranched, hyaline, thick walled, mostly present at the center of the culture (2,0 - 4,0 µm diam.), sometimes presenting small projections. Sexual structures and setae absent.

Enzymatic tests: GAA: ++++; TAA: ++++; Laccase: ++(α-naphtol), -(guaiacol), ++(syringaldazine); Cytochromo oxidase: -(tmpda); Peroxidase: +(pyrogallol); Tyrosinase: -(p-cresol)

Species code: 2. 6. 8. 32. 37. 39. 50. 54.

Remarks: The cultural features of *P. apiahynus* are quite similar to those of *P. robustus* (Karst.) Bourd. & Galz., a species which basidiome is macroscopically similar to *P. apiahynus*. Stalpers' (1978) description of cultures of *P. robustus* differed from ours only in the formation of fewer fiber hyphae. The former species is

distinguished by the small triangular basidiomes, and smaller spores (Rajchenberg and Wright, 1987). We could ask if there is real difference between these two species.

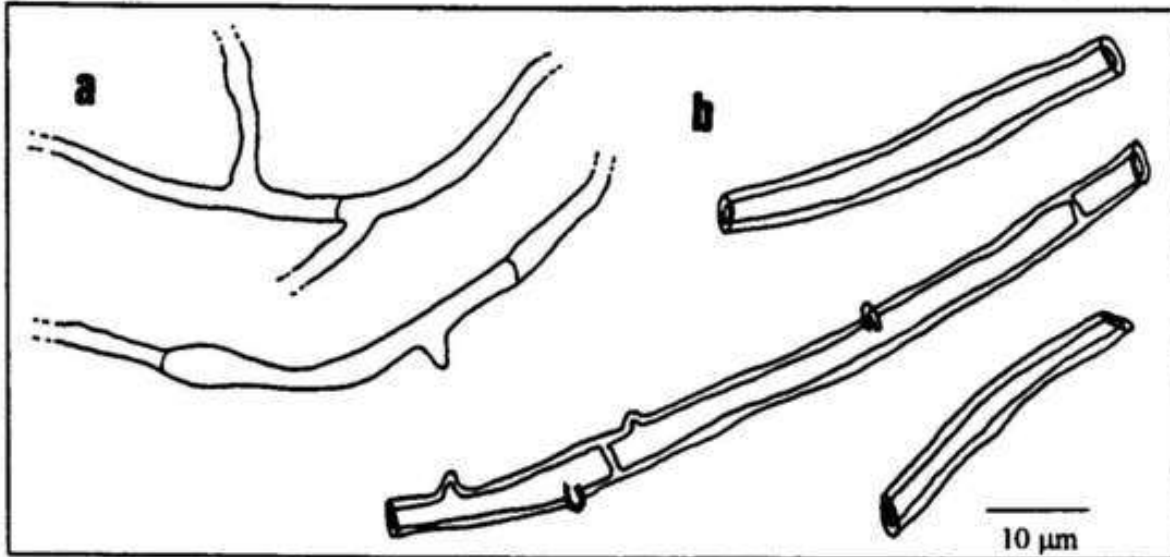


Fig. 1: *Phellinus apiahymus*. a) simple-septate hyphae; b) fiber hyphae

Antrodiella multipileata Loguercio-Leite & Wright
Mycotaxon, 41 (1): 167. 1991.

Fig. 2

Culture studied: cc 746 (\equiv FLOR 11284; BRAZIL, SC, Ilha de Santa Catarina, Lagoa do Peri; Althoff, Gerber & Neves; 26/VII/1995).

Macroscopic characters: Mat cottony becoming felty in the center; white to pale yellow; margin regular; growing rapidly, dish covered by the 2nd week. Reverse unchanged. Odor strong, unpleasant.

Microscopic characters: Mycelium formed by clamped generative hyphae (3.0 - 6.0 μ m diam.), aerial mycelium composed of fiber hyphae, with thin walls, not observed near the agar. After the 3rd week, branched binding hyphae develop in the aerial mycelium. After the 4th week some hyphae becoming wider forming a pseudoparenchymatous layer by the margin of the culture. Sexual structures absent.

Enzymatic tests: GAA: ++; TAA: ++++; Laccase: +(α -naphthol), -(guaiacol), ++(syringaldazine); Cytochromo oxidase: +(tmpda); Peroxidase: +(pyrogallol); Tyrosinase: -(p-cresol)

Species code: 2. 3. 8. 10. 32. 36. 38. 53. 54.

Remarks: *A. multipileata* presents an effused-reflexed to multipileate basidiomata with an irpicioid hymenial configuration, and broadly ellipsoid spores (3.6-5.1 μ m long) (Loguercio-Leite and Wright, 1991b). In culture *A. multipileata* is trimitic as are *A. semisupina* (Berk. & Curt.) Ryv. and *A. citrinella* Niem. & Ryv. studied by David & Tortic (1986). Microscopically, *A. semisupina* and *A. citrinella* are distinguished mainly by the spores size (2.5-3.5 μ m long and 3-3.5 μ m long, respectively). In these three species the generative hyphae differentiate into binding and skeletal hyphae and cultures produce laccase and peroxidase. In culture *A. multipileata* differs from the other two species in forming a pseudoparenchyma similar to figures 52 - 55 in Nobles (1965).

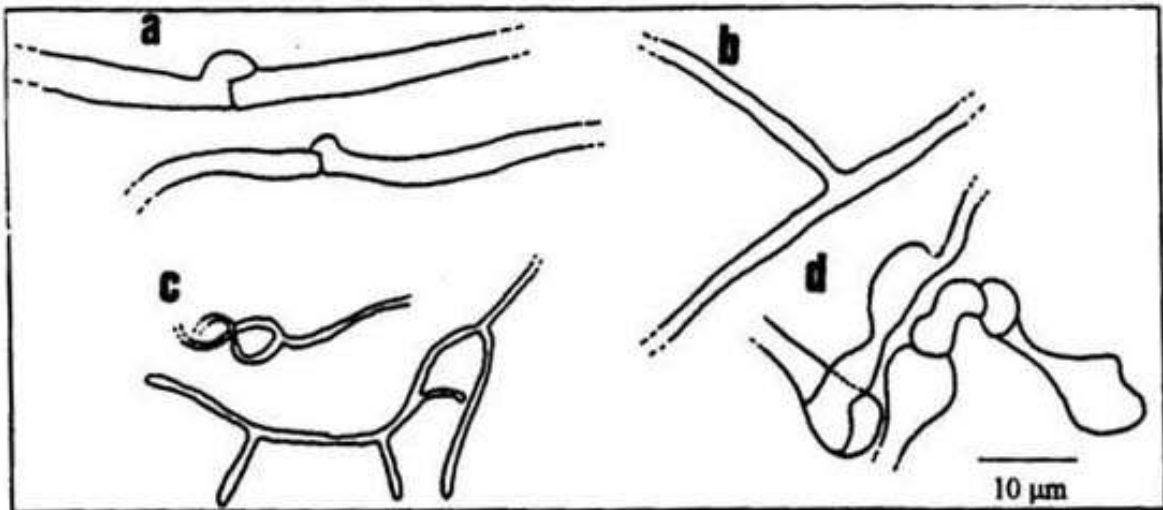


Fig. 2: *Antrodiella multipileata*. a) clamped generative hyphae; b) fiber hyphae; c) binding hyphae; d) pseudoparenchyma

Fomitella supina (Sw.: Fr.) Murr.
Bull. Torrey Bot. Club, 32: 365. 1905.

Fig. 3

Cultures studied: cc 549 (\cong FLOR 11095; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Degenhardt, Halmenschlager & Neves; 22/IX/1994); cc 663 (\cong FLOR 11213; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Althoff, Gerber & Foresti; 30/V/1995).

Macroscopic characters: Mat cottony and white, becoming crustose and pale brown after the 3rd week; margin regular, growing rapidly, dish covered by the 2nd week. Reverse unchanged. Odor sweet.

Microscopic characters: Mycelium formed by generative clamped hyphae (3,0 - 6,0 μ m diam.) thin and thick walled. Fiber hyphae thick walled (3,0 - 5,0 μ m diam.) in the center of the colony and, after the 3rd week, also in the margin. Sexual structures absent.

Enzymatic tests: GAA: +++; TAA: ++++; Laccase: +++(α -naphthol), -(guaiacol), +++(syringaldazine); Cytochromo oxidase: +++(tmpda); Peroxidase: +++(pyrogallol); Tyrosinase: -(p -cresol)

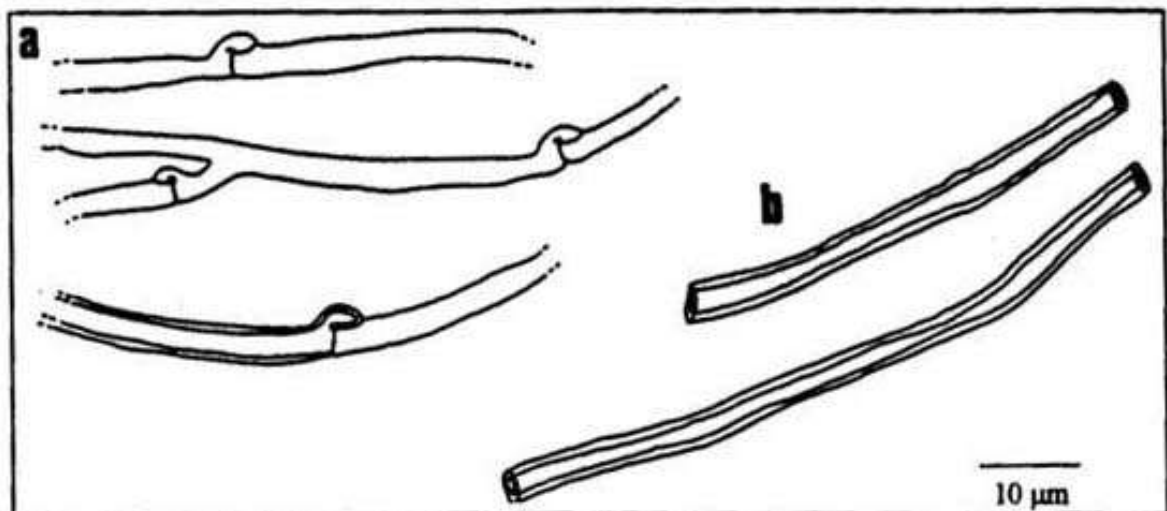


Fig. 3: *Fomitella supina*. a) clamped generative hyphae; b) fiber hyphae

Species code: 2. 3. 8. 32. 36. 38. 50. 54.

Remarks: *Fomitella supina* is the only species in this genus and it is morphologically similar with species of *Fomitopsis*. In fact, the character that distinguishes them is the type of rot (Gilbertson & Ryvarden, 1986), which in culture is seen as positive reaction in the enzymatic tests (GAA, TAA) for *Fomitella*. Cultures of some *Fomitopsis* species studied by Stalpers (1978) are very similar to our culture of *Fomitella supina*. Generally, *Fomitopsis* species produce chlamydospores in culture, but our cultures of *Fomitella supina* did not, thus this structure may be another character to distinguish these species, besides the type of rot.

Megasporoporia cavernulosa (Berk.) Ryv.
Mycotaxon, 16: 174. 1982.

Fig. 4

Cultures studied: cc 575 (\cong FLOR 11083; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Degenhardt & Halmenschlager; 17/X/1994); cc 579 (\cong FLOR 11096; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Degenhardt & Halmenschlager; 17/X/1994); cc 608 (\cong FLOR 11341; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Althoff, Gerber & Foresti; 16/III/1995).

Macroscopic characters: Colony crustose and appressed in the center, cottony and raised in the margin, white to pale yellow, margin regular; growing slowly, dish covered by the 3rd week. Reverse unchanged. Odor musty.

Microscopic characters: Mycelium formed by clamped generative hyphae (3,0 - 8,0 μ m diam.) that differentiate to dextrinoid fiber hyphae (2,0 - 5,0 μ m diam.) after the 2nd week. At the 3rd week the fiber hyphae are predominant in the culture, generative hyphae being present only in aerial mycelium. Some few crystals are formed near the agar. Sexual structures absent.

Enzymatic tests: GAA: +++; TAA: +++; Laccase: ++(α -naphthol), -(guaiacol), +(syringaldazine); Cytochromo oxidase: -(tm ρ da); Peroxidase: +(pyrogallol); Tyrosinase: -(p -cresol)

Species code: 2. 3. 8. 32. 36. 38. 51. 54.

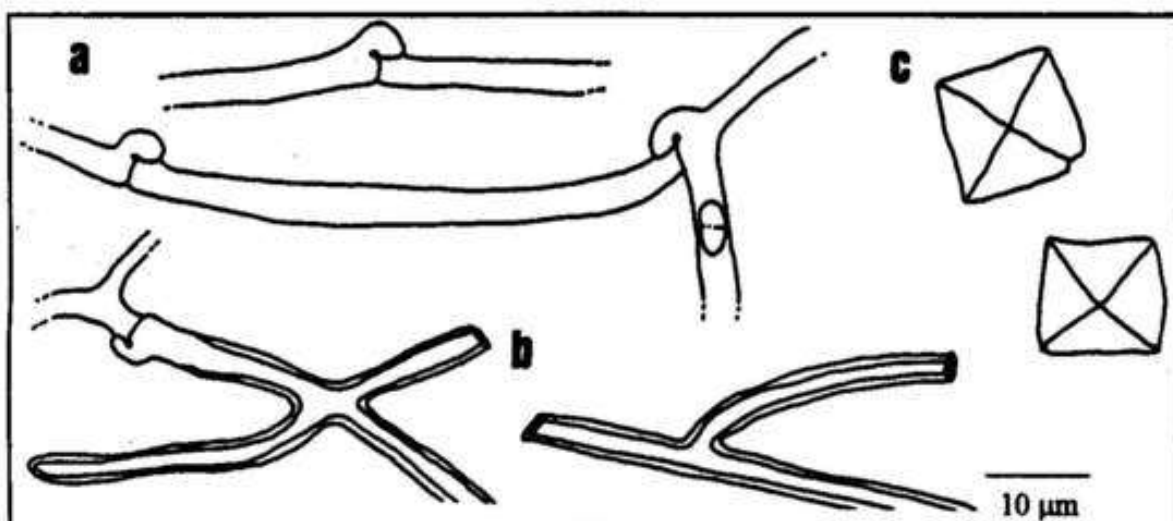


Fig. 4: *Megasporoporia cavernulosa*. a) clamped generative hyphae; b) fiber hyphae; c) crystals

Remarks: Our cultures of *M. cavernulosa* produced laccase and peroxidase, formed crystals, and the generative hyphae differentiated into skeletal hyphae, as happened with *Megasporoporia hexagonoides* (Speg.) Wright & Rajchenb. studied by Stalpers

(1978). These species are distinguished in their basidiomes by the presence or not of dendrohyphidia along the dissepiments and by the spores size. According to Ryvarden (1982) *M. cavernulosa* presents dendrohyphidia and has smaller spores (10,0-16,0 (18,0) μm) while *M. hexagonoides* does not present dendrohyphidia and the spores are longer (16,6-21,8 μm). It is important to emphasize that *M. cavernulosa* maintains a conspicuous dextrinoid reaction in skeletal hyphae, which is a distinctive characteristic of this genus. This reaction is lacking in cultures of *M. hexagonoides*, and could be useful character to distinguish them.

Perenniporia stipitata Ryv.
Mycotaxon, 28 (2): 535. 1987.

Fig. 5

Culture studied: cc 499 (\cong FLOR 11015; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Cabral, Gerber & Halmenschlager, 28/VI/1994).

Macroscopic characters: Mat crustose and dense, white to the center, hyaline to the margin. After the 4th week the mat formed crustose elevations. Margin regular, growth very slow, 4,0 - 4,5 cm diam. at 6th week. Reverse unchanged. Odor faint.

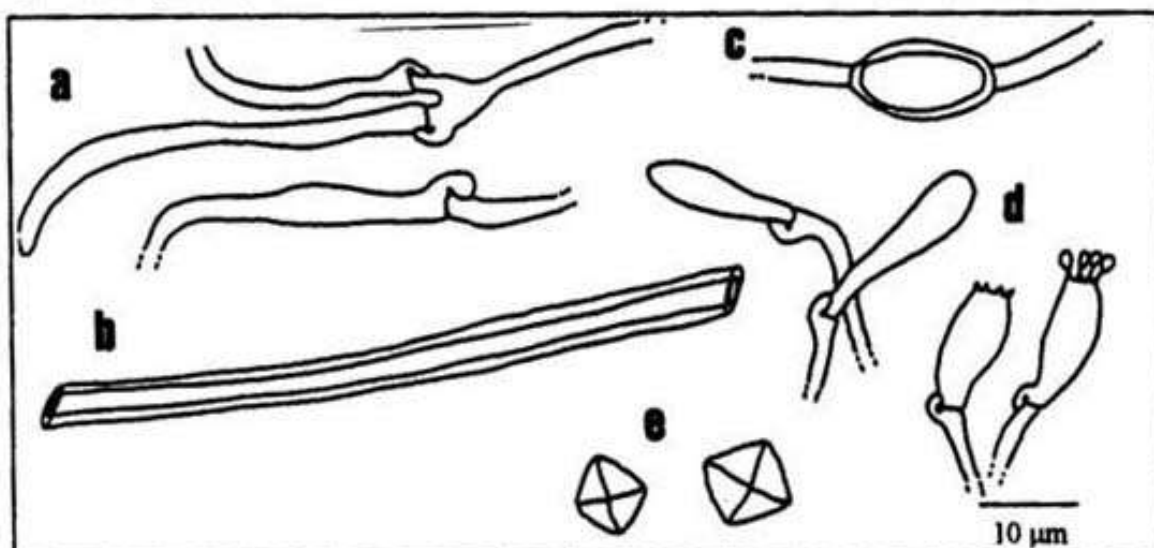


Fig. 5: *Perenniporia stipitata*. a) clamped generative hyphae; b) fiber hypha; c) chlamydospore; d) basidioles and basidia; e) crystals

Microscopic characters: Aerial and submerged mycelium formed by clamped generative hyphae (3,0 - 6,0 μm diam.). Fiber dextrinoid thick-walled hyphae (3,0 - 7,0 μm diam.) develop in the center submerged mycelium. Ellipsoid intercalary chlamydospores (17,0 x 25,0 μm diam.) present in crustose areas. Basidioles, basidia with sterigmata and, sometimes, with young basidiospores (1,0 μm) formed in the crustose areas after the 4th week. Marginal pyramidal crystals are present near the agar (12,0 - 15,0 x 12,0 - 15,0 μm diam.).

Enzymatic tests: GAA: +; TAA: +

Species code: 2. 3. 8. 34. 36. 38. 50. 54.

Remarks: Our culture of *P. stipitata* had a slow growth, not covering the plate within six weeks, produced skeletal dextrinoid hyphae and chlamydospores, all characteristics observed also to *P. martius* (Berk.) Ryv. studied by Wright and Deschamps (1975). The difference is that culture of *P. martius* produced binding hyphae. When we compared these cultures with a culture of *P. medulla-panis* (Fr.) Donk., also studied by Wright and Deschamps (*op cit*) we note that the last species

had faster growth and did not produce sexual structures. These three species can be separated by the following features of their fruitbodies: the first (*P. stipitata*) has a stipitate basidiomes, while the second (*P. martius*) is dimidiate, unguulate to lightly effused-reflexed, and the third (*P. medulla-panis*) is usually resupinate, becoming widely effused. *P. stipitata* was the only that produced basidioles and basidia in culture within six weeks.

Rigidoporus microporus (Fr.) Overeem
Icon. Fung. Malayensium, 2: 1. 1924.

Fig. 6

Culture studied: cc 797 (\equiv FLOR 11353; BRAZIL, SC, Ilha de Santa Catarina, Canto dos Araçás; Althoff, Gerber & Neves; 22/XI/1995).

Macroscopic characters: Mat hyaline, apressed without differentiation, excepting few little cottony tufts by the dish margin. Margin irregular; growth very rapid, dish covered by the first week. Reverse unchanged. Odor musty.

Microscopic characters: Mycelium formed by generative, simple-septate, thin or thick-walled hyphae (1,5 - 6,0 μm diam.). Fiber hyphae absent. Pyramidal (3,0 - 5,5 μm diam.) or irregular crystals present in the center of the mat, the last ones free or incrustated on generative hyphae, seen after the 3rd week. Cottony tufts at the margin composed of generative, simple-septate hyphae and few hyphae with clamps on a few of the septa (1,5 - 4,5 μm) seen only at last week. Sexual structures absent.

Enzymatic tests: GAA: +++; TAA: +++; Laccase: ++(α -naphthol), -(guaiacol), +++(syringaldazine); Cytochromo oxidase: +(tmpda); Peroxidase: ++(pyrogallol); Tyrosinase: -(p -cresol)

Species code: 2. 5. 7. 32. 36. 38. 51. 54.

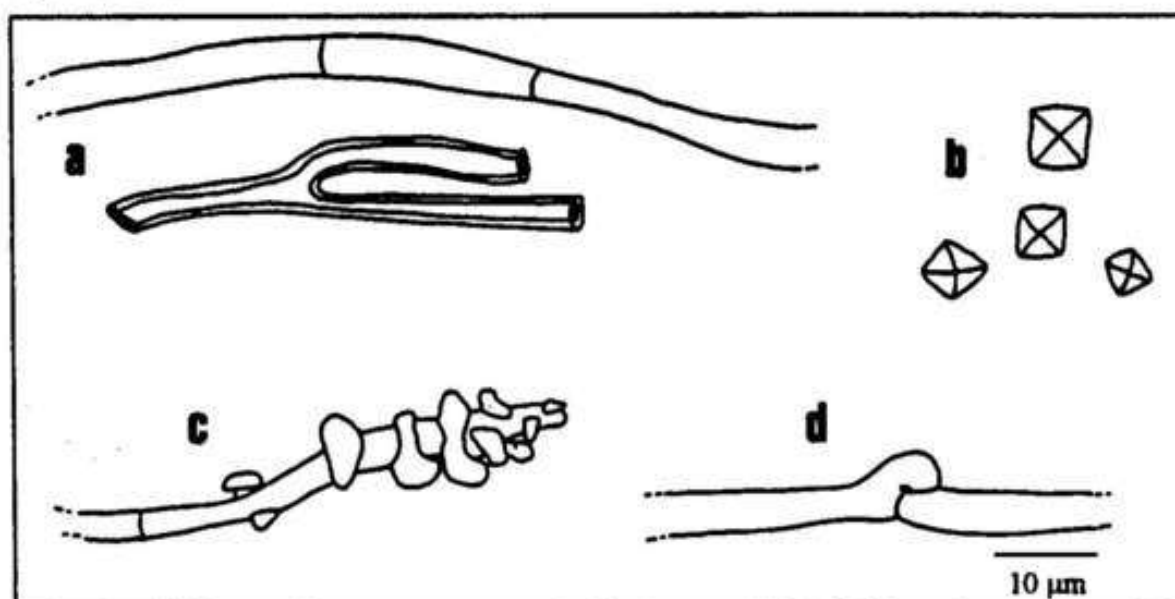


Fig. 6: *Rigidoporus microporus*. a) simple-septate hyphae; b) crystals; c) hyphae incrustated with crystals; d) clamped generative hypha

Remarks: This genus is characterized in the carpophore by simple-septate hyphae. *R. microporus* does not have cystidia and spore size is 3.5 - 5.0 μm long and differs from *R. lineatus* (Pers.) Ryv. that has cystidia and bigger spores (4.5 - 6.0 μm long), both species are pileate. *R. vinctus* (Berk.) Ryv. is resupinate, does not have cystidia and the spores has an intermediate size (4.0 - 5.5 μm long). *R. microporus* was studied in culture before by Bakshi *et al.* (1963) and Wright and Deschamps (1975) without the

mention of clamped hyphae. In our study, a conspicuous characteristic in the culture was the presence of simple-septate and clamped hyphae together. Studies with *R. lineatus* (Bakshi *et al.*, *op. cit.*; Neves, 1996) and with *R. vinctus* (Hood & Dick, 1988) showed clamped hyphae in culture. Thus, clamped hyphae may be a useful taxonomic feature in *Rigidoporus* cultures.

Trametes pavonia (Hook.) Ryv.
Norw. J. Bot., 19: 237. 1972.

Fig. 7

Culture studied: cc 545 (\cong FLOR 11088; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Degenhardt, Halmenschlager & Neves; 22/IX/1994); cc 601 (\cong FLOR 11063; BRAZIL, SC, Ilha de Santa Catarina, Morro da Lagoa; Althoff, Foresti & Gerber, 16/III/1995).

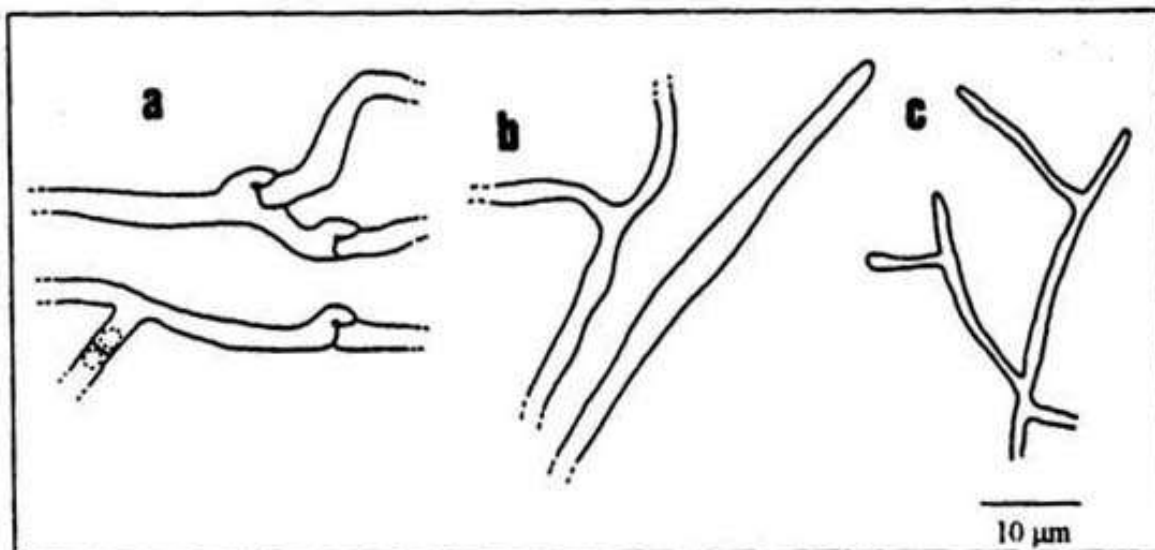


Fig. 7. *Trametes pavonia*. a) clamped generative hyphae; b) fiber hyphae; c) binding hyphae

Macroscopic characters: Mat cottony by the margin, crustose by the center and white. Margin regular; growth slow, dish covered by the 3rd week. Reverse unchanged. Odor musty.

Microscopic characters: Mycelium formed by generative clamped hyphae (3,5 - 7,0 (10,0) μ m diam.). Fiber hyphae (2,0 - 5,0 μ m diam.) formed after the 2nd week, most of them present in the center of the culture. Submerged binding hyphae present in the margin (0,5 - 1,0 μ m diam.). Sexual structures absent.

Enzymatic tests: GAA: ++++; TAA: +++; Laccase: ++(α -naphthol), -(guaiacol), ++(syringaldazine); Cytochromo oxidase: -(tmpda); Peroxidase: -(pyrogallol); Tyrosinase: -(p -cresol)

Species code: 2. 3. 8. 32. 36. 38. 51. 54.

Remarks: *T. pavonia* has been confused with *Trametes villosa* (Fr.) Kreisel. In the former the basidiocarp is flabeliform to dimidiate, zonate, hirsute to glabrous while the second has a hirsute, thin and pliable basidiocarp. In culture *T. pavonia* has a trimitic hyphal system. Another culture of this species studied by Rajchenberg (1982) had the same results. Small differences include swellings on hyphae and the reverse bleached in the culture studied by Rajchenberg (*op. cit.*). Bettucci and Guerrero (1971) studied *T. villosa* in culture describing faster growing mycelium, smaller diameter generative and fiber hyphae, and presence of crystals. These differences can be useful to distinguish the two species in culture.

The cultural studies are an important complement to the traditional taxonomy based upon basidiome morphology and, the enzymatic tests are an auxiliary tool to distinguish the type of rot. Nevertheless basidiomata can display individual behavior according to the conditions of the natural or the artificial environment. Thus the same species can give different responses, in nature or in culture, depending on the place, temperature and mainly the individual genetic characters.

Biochemical tests were negative in all the cases for tyrosinase (ρ -cresol) and guaiacol. Taylor (1974) performed biochemical tests with several species, in cultures of different ages. In his work we could notice that the reactions to ρ -cresol and to guaiacol were observed only for cultures that were 10 days old or more while our cultures were seven days old when tests were performed.

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