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SCYTINOSTROMA GALACTINUM SPECIES COMPLEX IN THE UNITED STATES

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ABSTRACT

Scytinostroma galactinum is a species complex and contains two inter-incompatible species with similar basidiocarp and cultural morphology. The two species can be distinguished by substrate and isozyme analysis. *Scyrinostroma galuctinum* occurs on woody gymnosperms and occasionally on angiosperms throughout North America. *Scytinostroma protrusum, comb. nov.*, is found on angiospermous wood. Two subspecies of *S. protrusum* are recognized based on physiological and distributional data. *Scytinostroma protrusum* subsp. *septentrionale, subsp. nov.*, occurs primarily in the northern United States and is less tolerant of gallic acid than *S. protrusum* subsp. *protrusum*, which occurs in the southern United States. Basidiocarp and cultural descriptions of *S. galactinum* are provided. ***Scytinostroma protrusum*** is tetrapolar and multiallelic at the mating type locus.

Key Words: *Scytinostroma galactinum, S. protrusum,* cultural description, species complex, sibling species.

Scytinostroma galactinurn (Fr.) Donk, also known as *Corticium galactinum* (Fr.) Burt, is a common and widely distributed white-rot fungus in North America (Burt, 1926; Lentz and Burdsall, 1973). Fries described *Thelephora galactina* Fr. in 1851 from a specimen fruiting on pine roots in South Carolina. Von Shrenk (1902) reported that *T. galactina* caused a root rot disease of apple trees in West Virginia, Kentucky, Illinois, Missouri, Arkansas, and Oklahoma. Since then, *S. galactinum* has been implicated many times as a pathogen and saprobe of trees and shrubs throughout North America (see Lentz and Burdsall, 1973, for a summary). Cooley and Davidson (1940) described the symptoms and established the pathogenicity of *C. galactinum* on apple trees. They also described the fruiting stage and cultures of this fungus. White (1951) reviewed the early literature on *C. galactinum* and compared *C. galactinum* with *C. odoratum* (Fr.) Bourdot & Galzin and included basidiocarp descriptions of both species. He also showed that *C. galactinurn* is heterothallic with a tetrapolar multiallelic mating system. In their comprehensive report on *S. galactinum*, Lentz and Burdsall (1973) included basidiocarp and cultural descriptions, a host list, and pathogenicity data.

Scytinostroma galactinum has been treated as a single species by plant pathologists and mycologists until recently. Boidin (1977) reported that cultures of *S. galactinurn* from France, Kamchatka (U.S.S.R.), Canada, Guiana, and Ivory Coast (Africa) were intercompatible. However, after examining additional intercompatibility tests, Boidin and Lanqueth (1987) now report that some of these cultures in fact are wholly or partially inter-incompatible. They propose that S. ultraspecies *galactinum* consists of four sibling species that have distinct geographical distributions: *S. galactinurn sensu stricto* in North America; *S. eurasiaticogalactinum* Boidin *et* Lanquetin in Europe and Asian U.S.S.R.; S. *africanogalactinum* Boidin *et* Lanquetin in tropical Africa, and *S. neogalactinum* Boidin *et* Lanquetin in Central America. Even earlier, in 1960, Francis F. Lombard (pers. comm.) and the late Dr. Hazel H. McKay were able to identify two forms of *S. galactinum* using cultural characters. One form grew exclusively on wood of gymnosperms and the other on angiosperms. Recently, after studying dikaryon-monokaryon pairings between cultures of *S. galactinum* held in the culture collection at the Center for Forest Mycology Research (CFMR), we were able to distinguish three forms. Although the basidiocarp characters of the three forms are nearly identical, they can be distinguished by cultural characters, host, geographic distribution, and isozyme analysis.

This study resolves the *S. galactinum* species complex in North America and provides detailed basidiocarp and cultural descriptions of the taxa

TABLE I

CULTURES AND SPECIMENS OF *SCYTINOSTROMA* STUDIED

Species and Isolate No.	Substrate	State/Country
Scytinostroma galactinum		
MB 65[a]	*Picea rubens* Sarg.	New Hampshire
ME 123(-R)[b]	*Thuja plicata* Donn. *ex* D. Don	Washington
MD 223(-R)[c]	*Magnolia virginiana* L.	Mississippi
HHB 513	conifer	New York
MB 1880	*Pinus contorta* Douglas *ex* Loud.	Wyoming
RLG 2681[c]	*Populus tremuloides* Michx.	New York
RLG 5830	Pinus *ponderosa* Laws.	Montana
HHB 7546[a]	*Betula papyrifera* Marsh.	Michigan
HHB 7576	*Thuja occidentalis* L.	Michigan
HHB 8822[c]	*Pinus* sp.	Mississippi
HHB 10427	*T. occidentalis*	Michigan
HHB 11626	*Pinus resinosa* Ait.	Minnesota
FP 94412	*P. rubens*	Maine
FP 101874	conifer	Minnesota
FP 101918[c]	conifer	Wisconsin
FP 105384[a]	*P. resinosa*	Massachusetts
FP 105442	*Abies lasiocarpa* (Hook.) Nutt.	Colorado
FP 105520[c]	conifer	Oregon
FP 105675[c]	*Pinus* sp.	Georgia
FP 133172	*Abies balsamea* (L.) Mill.	Wisconsin
FP 134644[c]	*Juniperus* sp.	Idaho
FP 134683	*Pseudotsuga menziesii* (Mirb.) Franco	Idaho
Scytinostroma protrusum subsp. *septentrionale*		
MD 227(-R)[c]	*Pinus* sp.	Wisconsin
MJL 266[c]	*Fagus grandifolia* Ehrh.	New York
MJL 1146[a]	*P. tremuloides*	New York
MJL 3678	*Acer* sp.	Michigan
MJL 3806[c]	*Populus* sp.	Michigan
LY 9562[cd]	*Populus* sp.	Lyon, France
HHB 11175[c]	*B. papyrifera*	Minnesota
HHB 11244[c]	*P. tremuloides*	Minnesota
HHB 11612	*B. papyrifera*	Minnesota
HHB 11663[c]	*B. papyrifera*	Minnesota
JLL 15558	*Acer* sp.	New York
FP 100733	hardwood	Minnesota
FP 101506[c]	*P. tremuloides*	Idaho
FP 101898	*B. papyrifera*	Wisconsin
FP 133181	*Populus* sp.	Wisconsin
Scytinostroma protrusum subsp. *protrusum*		
Black alder(-R)[ac]	*Alnus* sp.	Kentucky
Filer 2(-R)[c]	*Liquidambar styraciflua* L.	Mississippi
CS-65-98-14(-R)	*Carya* sp.	Indiana
PLL 2402[c]	*Prunus* sp.	Maryland
HHB 5023	*Prunus* sp.	Maryland
HHB 5026[c]	*Quercus rubra* L.	Maryland
HHB 6410[c]	*Quercus* sp.	Florida
FP 70852[c]	*L. styraciflua*	Georgia
FP 71383[c]	*Malus* sp.	Virginia
FP 102138[a]	hardwood	Illinois
FP 102142[a]	*Quercus* sp.	Illinois
FP 105036	*Quercus* sp.	Mississippi
FP 105244[c]	*P. tremuloides*	Indiana

[a]Isolates not used in pH and gallic acid growth experiments.

[b]R indicates that the cultures were isolated from rotted wood. Otherwise all cultures are from mass basidiospore deposits.

[c]Isolates used in isozyme analysis.

[d] LY 9562 is a culture from the holotype of *Scytinostroma eurasiaticogalactinum* Boldin *et* Lanquetin.

involved. In addition, isozymes are analyzed to explore the genetic relationships within the *S. galactinum* species complex.

MATERIALS AND METHODS

Cultures and specimens examined are listed in TABLE I. Unless otherwise indicated, specimens and cultures are deposited at the Center for Forest Mycology Research (CFMR). Microscopic examinations of basidiocarps were made from freehand sections mounted in 2% KOH and 1% aqueous phloxine, in Melzer's reagent (Hawksworth *et al.,* 1983), in sulfobenzaldehyde (Boidin, 1951), or in 0.1% Poirrier's blue in 60% lactic acid. Scientific names of plant hosts are from Little (1979). Herbarium abbreviations are from Holmgren *et al.* (1981). Color names are from Ridgway (1912).

All cultures studied are of polysporous origin unless otherwise indicated. 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) at 25 C in the dark (Davidson *et al.,* 1938). Cultures were examined at weekly intervals. Key patterns, describing 2-week-old cultures, are based on the system of Davidson *et al.* (1942). Species codes, describing 6-week-old cultures, are based on the system of Nobles (1965).

Pairings of monokaryons and dikaryons were done on 2% malt extract agar and were checked microscopically for the presence of clamp connections after 3 weeks incubation at 25 C. In the dikaryon-monokaryon pairings, the monokaryons were examined for clamp formation. The presence of clamp connections, indicating that dikaryotization occurred, was interpreted as interfertility or more accurately as inter-compatibility (Boidin, 1986).

In order to study the effect of pH on the growth of Scytinostroma species, 2 g gallic acid (GA) was dissolved in 150 ml H_2O over a hotplate. The pH of the GA solution was raised to 4, 4.5, 5, 5.5, 6. or 7 with 1 N and 10 N NaOH. In a 2-L flask 15 g agar and 15 g malt extract were suspended in 850 ml H_2O. The solutions were sterilized separately at 15 psi for 15 min. After cooling slightly, the GA solution was added to the malt extract solution, mixed well, then poured into sterile glass Petri dishes. The pH of the gallic acid agar (GAA) was within 0.1 to 0.2 units of the original value. Dishes were inoculated with hyphae near the margin of 10-day-old cultures grown on 2% malt extract agar. The isolates used are listed in TABLE I. Inocula, 5-mm-diam disks, were cut out with sterilized cork borers and placed upside down so that the mycelia were in direct contact with the medium. Each isolate was inoculated onto two plates. The inoculated plates were incubated at 25 C in the dark, and diameters were measured at 7, 10, and 14 days.

In another experiment, the effect of different concentrations of GA on radial growth of the fungal isolates was examined. The procedure described above was followed except that 0.5, l, 2, 3, 4, and 5 g of GA were used, the pH adjusted to 5.5, and radial growth measured.

Isozyme analysis was conducted with eight dikaryotic isolates each of *S. galactinum, S. protrusum* subsp. protrusum, and *S. protrusum* subsp. septentrionale; the individual isolates are specified in TABLE I. Cultures were grown in 50 ml of 2% malt extract for 7–10 da at 24 C the mycelium was then lyophilized, ground in a mortar and pestle, and stored at −20 C. The mycelial powder was suspended in 0.05 M Tris-Cl, pH 7.1 at the rate of 100 µg/ml. Starch gel electrophoresis was performed as described by Micales *et al.* (1986); gels were stained for the enzymes listed in TABLE II. The genetic nomenclature of May *et al.* (1979) was utilized. Capital-letter abbreviations refer to enzymes; abbreviations with only the first letter capitalized refer to the putative genetic loci that code for the enzymes. Alleles at a given locus are characterized by the relative mobility of their protein products compared to the movement of the protein coded by the most common allele (designated 100). For example, a heterozygous or heterokaryotic isolate would produce a three-banded pattern for a dimeric enzyme, such as glucose phosphate isomerase (GPI), due to the production of an intermediate, heteromeric band. A culture with GPI staining at positions 100 and 1 18 would be heterozygous for alleles 1 18 and 100 and would be designated 118/100. A culture designated GPI 118/118 would be homozygous at the GPI locus for allele 118 and would appear on the gel as a single band.

RESULTS

Determination of sexuality. - *Scytinostroma galactinum* (FP 101874). on conifer from Minnesota. is tetrapolar. Fourteen monosporous cul-

TABLE II

ABBREVIATIONS, E.C. NUMBERS, AND BUFFER SYSEMS OF ENZYMES USED IN THIS STUDY

Enzyme	Abbreviation	E.C. number	Buffer system
Acid phosphatase	ACP	3.1.3.2	C[a], R[b]
Adenylate kinase	AK	2.7.4.3	C
Alcohol dehydrogenase	ADH	1.1.1.1	R
Diaphorase	DIA	1.6.4.3	C, R
Fructose bisphosphate aldolase	ALD	4.1.2.13	R
Glucokinase	GK	2.7.1.2	R
B-D-Glucosidase	B-GLU	3.2.1.21	C
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	C
Glucose phosphate isomerase	GPI	5.3.1.9	C
Glutamate dehydrogenase	GDH	1.4.1.3	S[c]
Glutathione reductase	GR	1.6.4.2	R
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	C
Mannose phosphate isomerase	MPI	5.3.1.8	S, R
Peptidases:		3.4.11 or 3.4.13	
Leucyl-leucyl-leucine	PEP-LLL		
Phenylalanyl-proline	PEP-PAP		C

[a] Electrode buffer according to Ridgway et al. (1970). Electrophoresis run at ≤250 V, 75 mA for 3 h.
[b] Electrode buffer as described by Clayton and Tretiak (1972), diluted 1:10 for gel buffer. Electrophoresis run at ≤200 V, 90 mA for 3 h.
[c] Gel and electrode buffer as described by Steiner and Joslyn (1979). Electrode buffer diluted 1:3 (cathode tray) or 1:4 (anode tray). Electrophoresis run at ≤90 mA for 3 h.

tures were paired in all combinations, and four mating types were identified A_1B_1 = 15,16,17; A_2B_2 = 2,4,10,11,14; A_1B_2 = 5,7,8,12,13; A_2B_1 = 9. These results agree with White's (1951) work. He found that TRT 23337, on *Pinus resinosa* and TRT 23338, on *Thuja occidentalis,* were tetrapolar and established that *S. galactinurn* was multiallelic at the mating-type locus.

Scytinostroma protrusum subsp. *septentrionale* (HHB 11244), on *Populus tremuloides* from Minnesota, is tetrapolar. Twenty monosporous cultures were paired in all combinations, and three mating types were identified A_1B_1 = 5,6,7,11,13,16,17,21,22; A_2B_2 = 1,2,4,9,10,12,14; A_1B_2 = 18,19,20,23; A_2B_2 = none. The sexuality of another isolate, HHB 11663, on *Betula papyrifera* was also determined: A_3B_3 = 1,6,7,9,16, 17,18,19,20,22,24; A_4B_4 = 2,4,5,8,14,21,23,25; A_3B_4 = 10,11; A_4B_3 = 12,13. This species is also multiallelic at the mating-type locus.

Scytinostroma protrusum subsp. *protrusum* (FP 102138), on hardwood from southern Illinois, is tetrapolar: A_1B_1 = 1,2,8,9,13,19,20,22,27,29; A_2B_2 = 3,5,10,11,16,24,32; A_1B_2 = 21,23; A_2B_1 = 18,25,26,31.

Pairings between monokaryotic isolates. - Scytinostroma galactinum is incompatible with both subspecies of S. *protrusum* (TABLE III). From a total of 308 pairings which were examined, in only two pairings were clamp connections observed between monokaryons of *S. galactinum* and *S. protrusum.* The subspecies of *S. protrusum* are compatible with each other. From 308 pairings between monokaryons of subsp. *septentrionale* and subsp. *protrusum.* clamp connections were observed in 307 pairings. Also the isolate from France (LY 9562) is shown to be compatible with both subspecies of *protrusum* (TABLE III).

Pairings between dikaryon and monokaryon isolates. —Dikaryon-monokaryon pairings provide further evidence that the two species are biologically distinct. TABLE IV shows that monokaryotic isolates of *S. galactinurn* were dikaryotized successfully only by dikaryons of *S. galactinum.* Seventy-eight percent (82 of 105) of these pairings were positive. Monokaryons of *S. protrusum* subsp. *septentrionale* were dikaryotized only by dikaryons of subsp. *septentrionale* and represented 83% positive pairings. However, results with *S. protrusum* subsp. *protrusum* were unexpected. Only 11 (11.5%) of the pairings between different isolates of this subspecies resulted in the dikaryotization of the monokaryons.

Host and geographic distribution. - Scytinostro-

TABLE III

INTRASPECIFIC AND INTERSPECIFIC PAIRINGS BETWEEN MONOKARYONS OF *SCYTINOSTROMA GAUCTINUM* AND THE SUBSPECIES OF *S. PROTRUSUM*

Species			Species			
Name and isolate number	Number of monokaryons[a]	Paired with (×)	Name and isolate number	Number of monokaryons[a]	Total number of pairings	Number of positive pairings
galactinum FP 101874	7	×	protrusum subsp. septentrionale HHB 11244	9	63	2
			HHB 11663	23	161	0
			LY 9562	4	28	0
			protrusum subsp. protrusum FP 102138	8	56	0
protrusum subsp. *protrusum* FP 102138	8	×	protrusum subsp. septentrionale HHB 11244	9	72	72
			HHB 11663	8	64	64
			LY 9562	4	32	31
protrusum subsp. *septentrionale* HHB 11244	9	×	protrusum subsp. septentrionale HHB 11663	8	72	72
			LY 9562	4	36	36
HHB 11663	8	×	LY 9562	4	32	32

[a] At least one isolate of each mating type was used except with HHB 11244 where only three mating types were recovered.

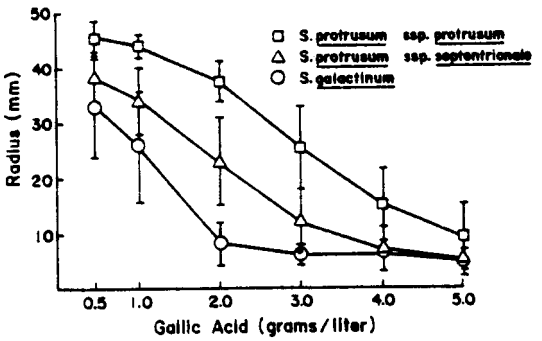


FIG. 1. Radial growth of *Scytinostroma* species growing for 10 days on different concentrations of gallic acid. (ML87 5472)

ma galactinum occurs primarily on gymnospermous trees and wood throughout the United States. Of the 21 isolates studied, only three were isolated from angiospermous wood. In contrast, *S. protrusum* subsp. *septentrionale* is found exclusively in the northern states, such as Idaho, Minnesota, Wisconsin, Michigan, and New York It occurs on woody angiosperms, especially *Populus* and *Betula* species. Only one isolate (MD 227) was isolated from a gymnospermous host. Finally, *S. protrusum* subsp. *protrusum* is found in the midwestem and southeastern United States on angiospermous trees and shrubs, especially *Quercus* and *Malus* species.

Effect of GA on growth.—The two species are differentially tolerant to concentrations of GA. *Scytinostroma galactinum* has the least tolerance to GA (FIG. 1). The growth rate of *S. galactinum* drops sharply between 0.1% and 0.2% concentration of GA. The most tolerant organism is *S. protrusum* subsp. *protrusum* which shows a gradual decrease in radial growth as the concentration of GA increases. The response of *S. protrusum* subsp. *septentrionale* is intermediate to that of the other two taxa. The greatest differences in growth between the taxa is on 0.2% GA. Isolates of *S. protrusum* subsp. *protrusum* have an average radial growth of 37.5 (±3.5) mm compared to 23 (±8) mm for *S. protrusum* subsp. *septentrionale* and 8 (±4) mm for *S. galactinum.*

Effect of pH on growth.—Both subspecies of *S. protrusum* respond similarly when grown between pH 4—7 on 0.2% GA (FIG. 2). They have similar growth patterns and grow best at pH 5-5.5. *Scytinostroma protrusum* subsp. *protrusum.* however, grows 14-23 mm faster than *S. ga-*

TABLE IV

INTRASPECIFIC AND INTERSPECIFIC PAIRINGS BETWEEN MONOKARYONS AND DIKARYONS OF *SCYTINOSTROMA GALACTINUM* AND THE SUBSPECIES OF *S. PROTRUSUM*

Monokaryotic isolates			Dikaryotic isolates			Number of positive pairings
Species	Number	Paired with (×)	Species	Number[a]	Total number of pairings	
galactinum FP 101874	5[b]	×	*galactinum*	21	105	82
			protrusum subsp. *septentrionale*	14	70	0
			protrusum subsp. *protrusum*	12	60	0
protrusum subsp. *septentrionale* HHB 11244	6[c]	×	*galactinum*	21	126	0
			protrusum subsp. *septentrionale*	14	84	70
			protrusum subsp. *protrusum*	12	72	0
protrusum subsp. *protrusum* FP 102138	8[d]	×	*galactinum*	21	168	0
			protrusum subsp. *septentrionale*	14	112	1
			protrusum subsp. *protrusum*	12	96	11

[a] These isolates are listed in TABLE I.
[b] The five monokaryons used were 15 A_1B_1, 2 and 4 A_2B_2, 5 A_1B_2, 9 A_2B_1.
[c] The six monokaryons used were 5 and 7 A_1B_1, 1 and 2 A_2B_2, 18 and 23 A_1B_2.
[d] The eight monokaryons used were 1 and 2 A_1B_1, 5 and 10 A_2B_2, 21 and 23 A_1B_2, 18 and 25 A_2B_1.

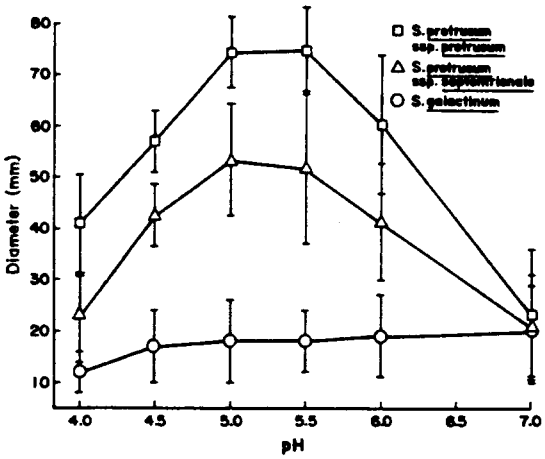


FIG. 2. Diameter growth of *Scytinostroma* species growing for 10 days on 0.2% gallic acid agar at different pH. (ML7 5473)

lactinum subsp. *septentrionale* between pH 4-6 at 10 days. *Scytinostroma galactinum* is not affected by pH when grown on 0.2% GA.

Isozyme analysis. — Fifteen presumed genetic loci, representing alleles to 15 different enzymes, were detected by isozyme analysis. These enzyme systems, and the buffers that provided for optimal resolution, are presented in TABLE II. No detectable levels of the following enzymes were observed in four different buffers: aconitase (E.C. 4.2.1.3), fructose bisphosphatase (E.C. 3.1.3.11), isocitrate dehydrogenase (E.C. 1.1.1.42), malic enzyme (E.C. 1.1.1.40), and mannitol dehydrogenase (E.C. 1.1.1.67).

With the exception of glucose phosphate isomerase (GPI), there was no correlation between enzyme banding patterns and the presumed taxonomic groupings of these isolates. Most of the enzymes were polymorphic with two or more alleles being shared by isolates of all three taxa. Glyceraldehyde-3-phosphate dehydrogenase was the only enzyme that appeared to be monomorphic. The banding pattern observed for GPI (FIG. 3) showed distinct differences between S. galactinurn and s. protrusum. Most isolates of s. protrusum were homozygous for allele 100 (genotype 100/100); however, isolate PLL 2402 was homozygous for allele 10 (genotype 10/10). Isolates "Black alder," HHB 11 175, LY 9565, and HHB 11663 were heterozygous for alleles 100 and 118 (genotype 100/118), as demonstrated by the production of an intermediate, heteromeric band. Isolates of *S. protrusum* subsp. *septentrionale* exhibited a larger percent of heterozygosity (37.5%) than did isolates of *S. protrusum* subsp. protrusum (12.5%). Most isolates of *S. galactinum* were homozygous for allele 118 (genotype 118/118); isolate RLG 2681 was homozygous for allele 139 (genotype 139/139). No heterozygosity was detected among isolates of *S. galactinum* for this enzyme system.

TABLE V

CHARACTERS THAT SEPARATE TAXA IN THE *SCYTINOSTROMA GALACTINUM* SPECIES COMPLEX IN THE UNITED STATES

Characters	*S. galactinum*	*S. protrusum* subsp. *septentrionale*	*S. protrusum* subsp. *protrusum*
Basidiospore size			
Length (µm)	4–5.5(-8)	(4–)4.5–5.5(-6)	(4–)4.5–5(-6)
Width (µm)	(2–)2.5–3	(2–)2.5–3	2–2.5(–3)
Growth on 0.5% tannic acid agar			
At 1 wk (mm diam)	tr–19(–34)	(16)21-30	25–38(-44)
At 2 wk (mm diam)	tr–34(–54)	(32–)40-60(–72)	60-80
Growth on 0.2% gallic acid agar			
At 10 da (mm radius)	8 ± 4	23 ± 8	37.5 ± 3.5
Woody substrate	gymnosperms and occasionally angiosperms	angiosperms, rarely on gymnosperms	angiosperms
Distribution	throughout U.S.	northern U.S.	southeastern U.S.
Pathogenicity	parasitic and saprophytic	saprophytic	parasitic and saprophytic

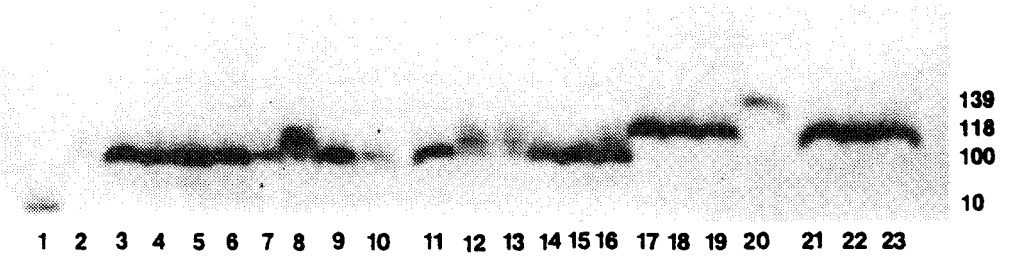


FIG. 3. Electrophoretic banding pattern for glucose phosphate isomerase (GPI). Allelic designations appear on right. Sequence of isolates by species and lane number: *Scytinostroma protrusum* subsp. *protrusum* 1–PLL 2402; 2—Filer2; 3—FP 105244; 4—FP71383; 5—HHB5026; 6—FP70852; 7—HHB5026; 8—"Blackalder"; *S. protrusum* subsp. *septentrionale*; 9—HHB 11244; 10—FP 101506; 11 –MJL 3806; 12—HHB 11 175; 13—HHB 11663; 14—MD227; 15—MJL266; 16—LY9562; *S. galactinum;* 17—FP101918; 18—HHB8822; 19—MD 223; 20—RLG 2681; 21—FP 134644; 22—FP 105520; 23—FP 105675.

DESCRIPTIONS OF BASIDIOCARPS AND CULTURES

SCYTINOSTROMA GALACTITNUM (Fr.) Donk. *Fungus* 26: 20. 1956.

≡ *Thelephora galactina* Fr., Nova Acta Regiae Soc. *Sci. Upsal.* 111, **1:** 136. 1851.
≡ *Corticium galactinum* (Fr.) Burt, *Ann. Missouri Bot. Gard.* **13:** 199. 1926.

HOLOTYPE *Thelephora galactina* Fr., *In radicibus ad latera fossarum.* South Carolina, *legit* Ravenel, M. A. Curtis 1601, UPS; ISOTYPE FH.

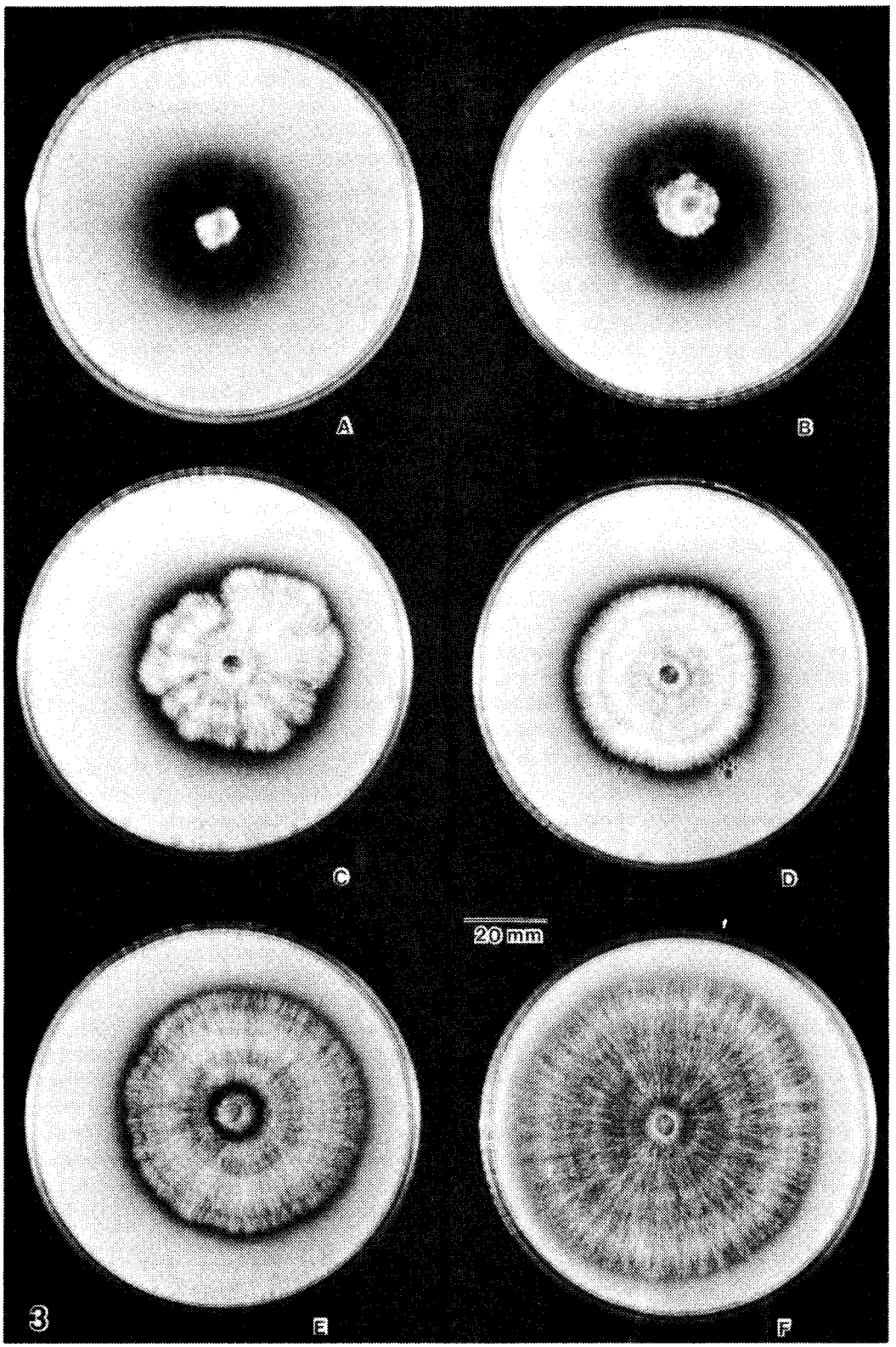
Basidiocarps annual, appressed, adherent, broadly effused, occasionally occurring as small circular patches about 1 mm diam, up to 1 mm thick, stratose or not, when fresh pliant, tough-coriaceous and peeling away easily from substrate, when dry coriaceous or ligneous and brittle; hymenial surface white or Light-Buff to Cream-Buff, sometimes Chamois or Ochraceous-Buff, rarely darkening to Ochraceous Tawny, smooth to tuberculate; margin concolorous or lighter, adnate, abrupt or thinning out.

Hyphal system dimitic. *Subiculum* consisting of fiber hyphae, generative hyphae and cystidia in a dense, tightly interwoven tissue; subicular *hyphae* 2–8 µm diam, thin-walled, nodose septate, moderately to frequently branched, hyphae next to substrate arranged horizontally, otherwise vertical; fiber hyphae 1–3.5 µm diam, hyaline, thick-walled, aseptate, sparingly branched, dextrinoid, abundant throughout basidiocarp, best observed in Melzer's reagent. *Cystidia* cylindrical, with rounded or tapered apex, 40-60 (–120) × 2–5 µm, hyaline, thin-walled, clamped at base, flexuous, often with refractive, globular contents, positive or negative in sulfobenzaldehyde, scattered to numerous throughout subiculum and hymenium, embedded or slightly exerted, best observed in phloxine. *Basidia* clavate, 20–35 × 3-4 µm, hyaline, thin-walled, clamped at base, 4-sterigmate. *Basidiospores* ellipsoid to cylindrical, flattened on adaxial side, 4-5.3-8) × (2–)2.5–3 µm, hyaline, thin-walled, smooth, amyloid or not, amyloid reaction distributed evenly or limited to a small part of the adaxial surface, acyanophilous.

SPECIMENS EXAMINED: UNITED STATES. CALIFORNIA: W. B. and V. G. Cooke 20358, on *Abies magnifica* var. *shartensis* Lemm. (BPI). COLORADO: FP 105369 on *Pinus contorta* Dougl. *ex* Loud. MICHIGAN: HHB 3622 on *Thuja occidentalis.* MONTANA: HHB 538 1 on *Larix* sp. In addition, the specimens Listed in TABLE I were also examined.

Cultural description: Macroscopic characters. - Growth on 2% malt extract agar moderately rapid, dishes covered (>90 mm diam when inoculated in center) by 2 wk. Mats white, zonate or azonate, around inocula moderately thick, slightly raised to raised, downy to cottony, then becoming thicker, raised, cottony-woolly toward margins at 2 wk, by 6 wk moderately thick, appressed to slightly raised, felty to woolly throughout; margins even, appressed no odor at 2 and 6 wk; agar unchanged at 2 wk, unchanged or partially bleached by 6 wk not fruiting by 6 wk. Reactions with GAA at 1 and 2 wk strong, growth 0–10 mm diam, with TAA at 1 wk strong, growth 5–19(–34) mm diam, at 2 wk strong, 5–34(–54) mm diam (FIG. 4).

Microscopic characters. —Marginal hyphae 2–5.5 µm diam, thin-walled, nodose septate, sparingly branched. Submerged hyphae 1.5-6(–7) µm diam, thin to slightly thick-walled, nodose septate, moderately branched. Aerial hyphae: (a) similar


A
B
C
D
20 mm
E
F
3

to submerged hyphae, sometimes coated with resinous materials; (b) fiber hyphae 1–2µm diam, hyaline, thick-walled, aseptate, sparingly branched, dextrinoid or not in Melzer's reagent, rare to scattered at 2 wk, abundant by 6 wk. Gloeocystidia or gloeoplerous hyphae cylindrical, 2–3 µm diam, often with lateral or terminal enlargements and apical papillae, hyaline or golden yellow from oil-like inclusions, terminal or intercalary, numerous in aerial mats at 2 and 6 wk.

Species code: 2.3c.8(d).15.21.32.36.38.(40).44-45.(54).55.60. Key patterns: A-P-I-1-10-16; A-P-1-1-11-16.

Remarks. - *Scytinostroma galactinum* is associated with a white rot of gymnospermous and occasionally of angiospermous wood throughout North America. It is associated with root, butt, and heartrot of various gymnospermous trees (Lentz and Burdsall, 1973). Boidin (1958: 81) also describes this species in culture. Descriptions by other researchers are of S. galactinurn and *S. protrusum* combined or of *S. protrusum.*

Fresh isolates of *S. galactinum* will sometimes grow significantly on 0.2% TAA and may resemble isolates of *S. protrusum.* However, the mats of these isolates are always thin and sparse and do not resemble the much thicker and denser mats of *S. protrusum* (FIG. 4).

Scytinostroma protrusum (Burt) Nakas., *comb. nov.,* subsp. *protrusum.*

BASIONYM: *Corticium protrusum* Burt, *Ann. Missouri Bot. Gard.* **13:** 260. 1926.

ISOTYPE: *Corticium protrusum* Burt, Jalapa, Mexico, *Legit* W. A. & E. L. Murrill, Dec. 12–20, 1909, FH.

This species is similar to basidiocarps of *S. galactinum* in all morphological characters except that the basidiospores of *S. protrusum* subsp. *protrusum* are slightly smaller and narrower on average, (4–)4.5–5(-6)× 2–2.5(–3)µm, than those of *S. galactinum,* and the amyloid reaction is limited to a small part of the adaxial spore surface of S. *protrusum.* In addition, *S. protrusum* subsp. *protrusum* is found exclusively on woody angiosperms in the southeastern United States. Cultures of subsp. *protrusum* grow significantly on 0.5% TAA, 25-38(44) mm diam at 1 wk and 60–80 mm diam at 2 wk.

Scytinostroma protrusum (Burt) Nakas. subsp. **septentrionale** Nakas., subsp. nov.

Differt a forma typicis subsp. *protrusum* basidiosporis leviter latis, (4–)4.5–5.5(-6)× (2–)2.5–3µm; crescenti tardiore 0.5% agaro tannico acido [(32–)40–60(–70) mm diam 14 diebus] et 0.2% agaro gallico acido [(8–)23(-8) mm diam 10 diebus]; distributionis in Aetate Unita septentrionale; HOLOTYPO HHB 11244 in herbario CFMR et ISOTYPO in BPI.

Differing from typical *S. protrusum* with its slightly wider basidiospores, (4-)4.5-5.5(4) × (2–)2.5–3µm, slower growth rates on 0.2% GAA (23 ± 8 mm radius at 10 da, FIG. 1) and 0.5% TAA [(32-)40-60(-72) mm diam at 2 wk] and distribution in northern United States.

HOLOTYPE: HHB 11244, on *Populus tremuloides* Michx., Observation Tower intersection, Itasca State Park, Clearwater County, Minnesota, United States, 31.VII.81, Coll. H. H. Burdsall, Jr. in herb. CFMR; ISOTYPE in BPI.

We believe that *Corticium protrusum* is the best available name for this segregate of the *Scytinostroma galactinum* species complex. A living culture from the type specimen of *C. protrusum* is necessary to prove conclusively that it is similar to other isolates of *S. protrusum* and different from *S. galactinum;* however, no culture from the type exists. Nevertheless, the isotype specimen of *C. protrusum,* although very similar morphologically to *S. galactinum,* has basidiospores which are slightly narrower on average (2–2.5µm diam) than those of *S. galactinum.* Additionally, the isotype specimen occurs on angiospermous wood, which is not the typical substrate for *S. galactinum.*

There are no significant morphological differences between basidiocarps of s. *protrusum* and *S. galactinum.* However, these two species have

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FIG. 4. Two-week-old growth on 0.5% tannic acid agar of *Scytinostroma* species. A, B. Scytinostroma *galactinum* (FP 101874 and HHB 7576, respectively). C, D. *S. protrusum* subsp. *septentrionale* (FP 101506 and MJL 3678, respectively). E, F. *S. protrusum* subsp. *protrusum* (FP 105244 and HHB 6410, respectively). (M870126)

distinct preferences in substrates. *Scytinostroma protrusum* is almost exclusively found on woody angiosperms whereas *S. galactinum* is typically found on wood of gymnosperms and only occasionally on angiosperms in the United States (TABLE V). Culturally, these species are also morphologically similar. Differential growth on 0.5% TAA, however, can often be used to distinguish between them. Typically, *S. protrusum* grows significantly more than *S. galactinum* at 1 and 2 wk (TABLE V; FIG. 4).

Two subspecies of *S. protrusum* are recognized. *Scytinostroma protrusum* subsp. *septentrionale* is distinguished from *S. protrusum* subsp. *protrusum* primarily by geographic distribution, tolerance to GA and growth response to pH (TABLE V, FIGS. 1,2). In addition, subsp. *septentrionale* is found typically on woody angiosperms such as *Acer, Alnus, Betula.* and *Populus* in the northern United States. Subspecies *protrusum* tends to have slightly narrower basidiospores and is often associated with a root rot of various angiospermous trees, especially apple (see Lentz and Burdsall, 1973, Appendix II; Sutton *et al.,* **1981).** The TAA mats of both subspecies of *S. protrusum* are quite characteristic and distinctive. The mats of subsp. *septentrionale* are moderately thick to thick, raised and woolly, occasionally silky, azonate or zonate, with raised even to bayed margins (FIG. 4C, D). Mats of subsp. protrusum, on the other hand, are thinner and more appressed with consistently even margins (FIG. 4E, F).

With the exception of LY 9562 from France, the specimens and cultures used in this study are from the United States. The dikaryotic and four monokaryotic cultures (representing the four mating types) of LY 9562 were originally named *S. galactinum.* However, based on GA tolerance, growth response to pH, isozymic analysis, and compatibility tests, isolate LY 9562 was found to be similar to *S. protrusum* and in particular to subsp. *septentrionale.* From these results, we know that *S. protrusum* occurs in Europe. Recently, Boidin and Lanquetin **(1987)** described the new species, *S. eurasiaticogalactinum,* from Europe and designated LY 9562 as the holotype specimen. Therefore, based on the culture from LY 9562, we also know that *S. eurasiaticogalactinum* is similar to the *S. protrusum* group. We feel, however, that it is premature to state that *S. eurasiaticogalactinum* and *S. protrusum* are conspecific based on a single culture of *S. eurasiaticogalactinum.* Additional cultures and specimens of *S. eurasiaticogalactinum* must be compared with *S. protrusum.* Before sound taxonomic decisions on this matter can be made, further cultural, physiological, enzymatic, and compatibility studies of these new materials are necessary.

DISCUSSION

Scytinostroma galactinum and *S. protrusum* are cryptic or sibling species that appear to have arisen sympatrically. Sibling species that arise sympatrically are not unusual in fungi, and somatic or vegetative incompatibility factors may be responsible for the speciation process (Burnett, **1983).** Both species are widely distributed throughout the United States and also occur in Canada but prefer different woody substrates. The two species are reproductively isolated but are nearly identical with respect to basidiocarp and cultural morphology. In addition, both species have tetrapolar (bifactorial), multiallelic incompatibility systems. Through compatibility tests between monokaryons, we demonstrated that the two species, *S. galactinum* and *S. protrusum,* are distinct entities. Furthermore, we show that *S. protrusum* consists of two morphologically identical subspecies, namely subsp. *protrusum* and subsp. *septentrionale.* The two subspecies can be distinguished in culture by differential growth rates on gallic and tannic acid agars and mat texture on tannic acid agar. In addition, the subspecies have different geographical distributions, substrate preferences, and pathogenic ability; however, these apparent differences may not obtain as more specimens are collected and studied. Pairings among monokaryons of both subspecies demonstrated that subsp. *septentrionale* and subsp. *protrusum* are completely intercompatible. However, in the dikaryon-monokaryon pairings, monokaryons of subsp. *septentrionale* were dikaryotized successfully only by dikaryons of subsp. *septentrtonale.* We expected these monokaryons to be dikaryotized also by dikaryons of subsp. *protrusum.* Similarly, monokaryons of subsp. *protrusum* were dikaryotized successfully only by dikaryons of subsp. *protrusum*; however, only a very few (1 1%) of these pairings were successful. We had expected about 80% successful pairings as was demonstrated for the other two taxa. Perhaps other factors, such as heterogenic

or somatic incompatibility factors, may have interfered with the dikaryotization process.

TABLE V summarizes the pertinent characters which can be used to distinguish the species in this complex. In culture, *S. galactinum* and *S. protrusum* are readily differentiated by growth on 0.5% TAA (FIG. 4) and 0.2% GAA (FIG. 1). However, subsp. *septentrionale* is intermediate in growth rate and blurs the distinction between the taxa. Thus, slow-growing isolates of subsp. *septentrionale* resemble *S. galactinum* while fast-growing isolates resemble subsp. *protrusum.* The best separation of the three taxa in culture is achieved by growth on 0.2% GAA (FIG. 1).

Isozyme analysis, as used for chemotaxonomy, can be used to study fungal relationships when morphological distinctions are unclear. This procedure, performed with starch gel electrophoresis, has been used to clarify the taxonomy and genetics of a number of fungal genera, including *Peronosclerospora* (Bonde *et al.,* **1984),** *Agaricus* (Royse and May, **1982),** *Puccinia* (Burdon and Roelfs, **1985),** and ***Phytophthora*** (Tooley *et al.*. 1985). Enzymes that are coded by different alleles or separate genetic loci frequently display different electrophoretic mobilities. Such differences are due to variations in the amino acid content of the molecule, which in turn is dependent on the sequence of the nucleotides in the DNA. Crossing experiments may be necessary to confirm genetic interpretations of electrophoretic data, but certain banding patterns are easily recognizable from comparable studies in human and animal genetics (Hams and Hopkinson, **1976).**

Scytinostroma galactinum and *S. protrusum* appear very similar genetically, despite their reproductive isolation. The taxa shared common alleles at all 15 loci which were tested. It is likely that their reproductive isolation is relatively recent. There are some genetic differences, as demonstrated by the banding patterns of GPI. Certain isolates of *S. protrusum* were heterozygous for GPI, as demonstrated by the production of an intermediate. heteromeric band; all isolates of *S. galactinum* were homozygous for this enzyme. The subspecies of *S. protrusum* could not be distinguished by isozyme analysis. Such failure to separate subspecies is quite common in isozyme analysis. especially when differences in Pathogenicity or host are major criteria for separation (Burdon and Marshall, 1981; Micales *et al.*. 1983; Schmidt *et al.,* 1977). It is possible that different enzyme systems, such as those directly involved in pathways for mating, host selection, and pathogenicity, may have revealed greater genetic diversity.

Boidin and Lanquetin **(1987),** in their recent monograph, also dealt with the *S. galactinurn* problem. They recognize four sibling species which have distinct geographical distributions and are wholly or partly inter-incompatible. In their treatment, *S. galactinum sensu stricto* occurs only in North America on woody gymnosperms as well as angiosperms. However, their concept is too broad as we have demonstrated in this study. *Scytinostroma eurasiaticogalactinum,* typified by LY 9562, occurs in France, Hungary, Czechoslovakia, and Asian U.S.S.R. on *Populus, Betula, Fagus, Aesculus, Pinus,* and *Abies.* We found that the culture of LY 9562 is similar to cultures of *S. protrusum* subsp. *septentrionale* when grown on gallic and tannic acid agars and examined enzymatically. Furthermore, monokaryons of LY 9562 paired successfully with monokaryons of subsp. *septentrionale.* Thus, *S. eurasiaticogalactinum* and *S. protrusum* subsp. *septentrionale* are conspecific. However, additional cultures of *S. eurasiaticogalactinum* need to be studied enzymatically and physiologically and more compatibility tests are needed between *S. eurasiaticogalactinum* and subsp. *septentrionale* before they are proven to be synonymous. Boidin and Lanquetin also described two additional species: *S. africanogalactinum* from Gabon and Ivory Coast in Africa and *S. neogalactinum* from Guadeloupe, Guyana, and Martinique in tropical America. Compatibility tests among these species and *S. protrusum* subsp. *protrusum* are needed to determine if they are related.

EXCLUDED TAXA

THELEPHORA ALNEA Fries. *Syst. Mycol.* I, p. 446. 1821.

≡ *Srereum alneum* (Fr.) Fr.. *Epicr. Syst. .Mycol.,* p. 554. 1838.

= *Corticium odoratum* Fr. var. *alni* (Fr.) Bresadola, *Ann Mycol.* **18:** 63, 1920.

This name refers either to *Phanerochaete velutina* (DC. *per* Pers.: Fr.) Karsten or to *Scytinostroma galactinum* (Fr.) Donk. Because there is no material which typifies *T. alnea* in Fries's Herbarium, Burdsall **(1977)** considered this name

a nomen dubium. We examined a specimen called S. alneum from E. Fries's Herbarium (Finlande: Vasa, 28.IV.1865, leg. P. A. Karsten). This specimen, however, appears to be *Scytinostroma hemidichophyticum* Pouzar as determined by Nils Hallenberg.

THELEPHORA SUAVEOLEUS Fries. *Elenchus Fung.* I, p. 208. 1828.

≡ *Stereum suaveoleus* (Fr.) Fr., *Epicr. Syst. Mycol.*, p. 553. 1838.

This name often refers to *S. galactinum.* We examined a specimen called *Xylostroma suaveoleus,* Gallia, leg. Mougeot, from E. Fries's Herbarium. This may have been the specimen Fries referred to in 1828. The specimen is sterile but does have frequently branched skeletal hyphae. These skeletal hyphae, however, do not react with Melzer's reagent; thus, it does not belong in *Scytinostroma.*

CORTICIUM RIGESCENS Berk. *et* Curtis *in* Cooke. *Grevillea* **20:** 12. 1891.

This name is included by Burt **(1926)** as a questionable synonym of C. galactinurn. White **(1951)** examined three collections (186, 188, and 191) from the Farlow Herbarium (FH) which were cited by Cooke in 189 1. We examined the same specimens, and we agree with White that they represent three different species. Specimen 186 appears to be *Phanerochaete sordida* (P. Karsten) John Eriksson & Ryv. in Eriksson et al., while 188 (cited in Cooke as 1 18) appears to be *Hypochnicium bombycinum* (Sommerf.: Fr.) John Eriksson. Specimen 191 may be Scytinostroma galactinum except that no basidia or basidiospores were seen. We also examined four specimens (186, 187, 188, and 191) from the Kew Herbarium called *C. rigescens.* They all represent different species and only 186 and 191 agree with the FH specimens of the same number. Number 187, which is only represented by the Kew Herbarium specimen, is also a Phanerochaete species, but is different from 186. Number 188, from Kew, is a *Hypochnicium*-like species, but it has basidiospores that are much smaller than those of the FH specimen. Number 191 is similar to the FH specimen, and again no basidia or basidiospores were seen.

Because none of the four specimens from Kew are alike or comfortably fit the original description, we consider the name *Corticium rigescens* a *nomen dubium* as well as a *nomen confusum.*

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