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# The sclerotia of *Polyporus squamosus*

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Summary. – A secondary mycelium isolate of *P. squamosus* developed sclerotia in culture. This isolate was incompatible with monosporic isolates of *P. tuberaster*, but compatible with other *P. squamosus* isolates. Mating compatibility tests performed with cultures of geographically isolated individuals either with or without sclerotia also showed that incompatibility was correlated with macro- and micromorphological characters of the basidiocarps, and not with the ability to form sclerotia.

Résumé. – Une souche isolée à partir d'un mycélium secondaire de *P. squamosus* a développé des sclérotés en culture. Cette souche présente une incompatibilité lorsqu'on la confronte à des isolements monosporiques de *P. tuberaster* mais est compatible avec d'autres souches de *P. squamosus*. Les tests d'interfertilité menés avec des cultures d'individus – avec ou sans sclérotés – de différentes provenances géographiques montrent que l'incompatibilité est bien corrélée avec les caractères morphologiques macro- et microscopiques des basidiocarpes mais pas avec la faculté à produire des sclérotés.

Sclerotia are resting structures that act as nutrient reservoirs. They have been reported in Deuteromycetes (Rudolph, 1962), Ascomycetes (Le Torneau, 1966; Amir *et al.*, 1992) and Basidiomycetes (Lentz & McKay, 1970; Ginns & Weresub, 1976; Pegler, 1983; Henderson, Elliott & Ross, 1983; Grenville, Peterson & Piche, 1985; Hutchison, 1991).

Within the genus *Polyporus* five out of the six endemic species in Australia form sclerotia in nature (Cunningham, 1965) as well as *P. umbellatus* Fr. and *P. tuberaster* (Jacq.) Fr. from the Northern hemisphere (Jahn, 1969; Gilbertson & Ryvarden, 1987). Basidiocarps of the last species are sometimes found far from where their sclerotia develop (Jahn, 1980; Boertmann, 1984) as those structures can give rise to mycelia independently of basidiocarp formation (Snell & Dick, 1971).

*P. squamosus* (Huds.) Fr. is similar to *P. tuberaster* by its scaly pileus, usually a black stipe base, and similar pore and spore size. The absence of sclerotia in

*P. squamosus* has been one of the basic characters used to separate both species (Pilåt, 1936; Jahn, 1969; Gilbertson & Ryvarden, 1987). Nevertheless, Campbell & Munson (1936) reported formation of sclerotia-like structures of *P. squamosus* on elm wood blocks in laboratory studies. Donk (1960) suggested that both taxa could be conspecific. Jahn (op. cit.) separated the species because of the different microstructures of the pilear scales.

One secondary mycelium isolate from Duke University, North Carolina, received as *P. squamosus* (D804) formed sclerotia in a drying culture in Oslo (Fig. 1). Mating compatibility tests performed with available isolates of *P. tuberaster* were negative, but D804 was able to dikaryotize monosporic isolates of *P. squamosus*. The corresponding voucher specimen was borrowed to compare basidiocarps of the two incompatibility groups.

### Materials and Methods

Macro-, microanatomical and cultural terms are from Snell & Dick (1971). Herbaria abbreviations are taken from Holmgren, Holmgren & Barnett (1990). The concept of biological species is the same as in Esser & Hoffmann (1977).

The specimens and cultures used here are deposited in herb. O and TFM, except for the voucher specimen of D804 (ABSM).

### Light microscopy

Basidiocarp tissues were dissected apart under a WILD M3B binocular using a needle and a razor blade. Both basidiocarps and cultures were mounted in 5% potassium hydroxide (KOH) and observed at  $\times 400$  and  $\times 1000$  magnifications with a Zeiss microscope provided with phase contrast. Measurements were made in 5% KOH.

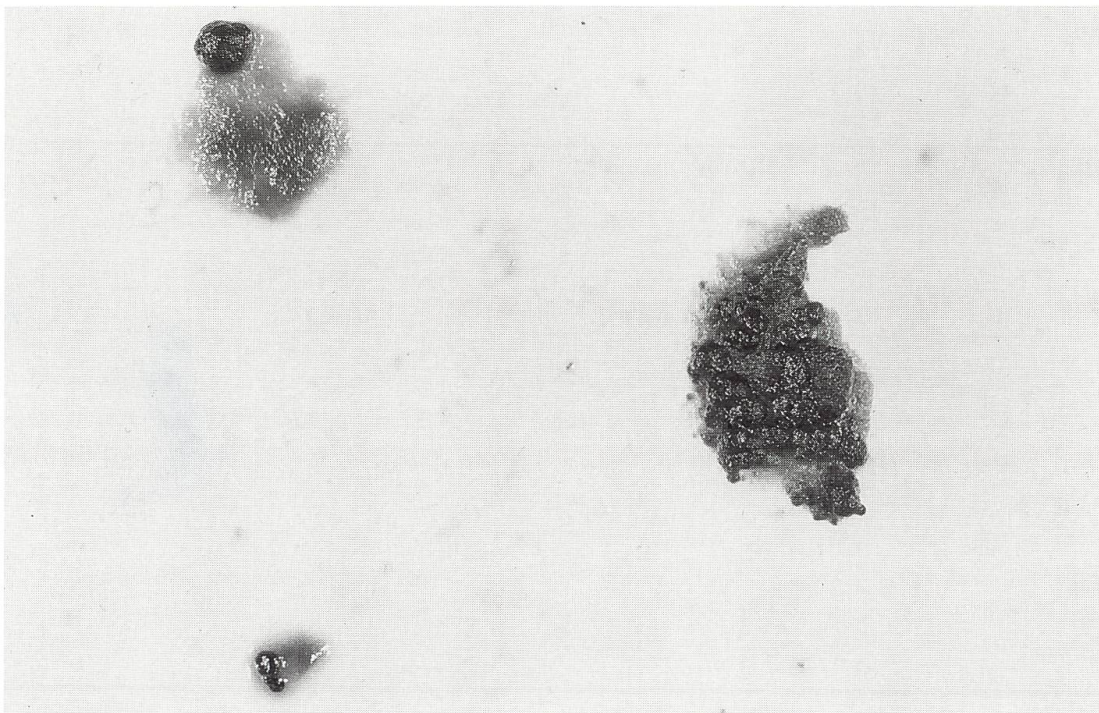
### Cultures

Fresh specimens were inverted on plastic slides, covered with a humid cloth and left overnight to sporulate. Twelve hours later the plastic slide was recovered and wrapped in paper for further plating at Oslo University.

To obtain monosporic isolates, a suspension of basidiospores in 10 ml distilled water was poured onto a 9 cm Petri dish with water agar (WA, 15 g Kebo Lab Agar in 1 l sterile distilled water). Germlings were picked with a sterile needle under a binocular microscope ( $\times 40$ ) and transferred to 2% malt extract agar (MEA, 15 g Kebo Lab Agar and 20 g malt extract in 1 l sterile, distilled water). The cultures were marked with the herbarium number of the voucher specimen. All subsequent cultural work was performed in Petri dishes with 2% MEA at room temperature (18–20 °C).



*Fig. 1. Basidiocarp of P. squamosus collected from a sclerotium in the ground (LR27136, Estonia).*



*Fig. 2. Sclerotia of P. squamosus formed in culture (Coll. D804).*

Table 1 shows the isolates used here and their collecting locality.

Secondary mycelium isolates were obtained from spore germlings that had already contacted and where clamps could be observed. Only one secondary mycelium isolate per specimen was kept.

### Compatibility tests

Monosporic isolates (5 mm<sup>3</sup>) were paired by placing them 3 cm apart in a Petri dish. Two weeks after the contact between monosporic mycelia had been established, pairings were assessed for the presence of clamps as a sign of successful mating (Vandendries, 1936).

Two pieces of secondary mycelium from the interface zone of each positive pairing were subcultured to reveal possible mating irregularities (Ainsworth *et al.*, 1992).

One extra isolate of each monosporic mycelium was always kept as unmated control. Di-mon tests (Kühner 1977) were performed when a monosporic mycelium was not available for one of the paired isolates.

### Results

Two incompatibility groups were identified after the mating compatibility tests (Table 2). Basidiocarps of both groups could be characterized by a different microstructure of the pilear scales (Jahn, 1969) and of the stipe cuticle, but not by the presence or absence of sclerotia.

The pilear microstructure of *P. squamosus* and *P. tuberaster* is a cutis. While the pilear scales of *P. tuberaster* are formed by parallel tufts of hyphae embedded in an amorphous matter, the scales of *P. squamosus* are formed by the break of the pilear cutis as basidiocarps enlarge, and the amorphous matter is absent.

The stipe surface is also embedded in an amorphous matter in *P. tuberaster*. The heavy accumulation of this matter results in a black cuticle on the stipe. In *P. squamosus*, the black cuticle on the stipe is formed by a palisade of strongly melanized hyphae free of amorphous matter.

Both biological species could be identified by the following key:

- Pilear and stipe surface embedded in an amorphous matter. Pilear scales formed of agglutinated tufts of parallel hyphae. Stipe surface a cutis:  
..... *P. tuberaster*.
- Pilear and stipe surfaces not embedded in an amorphous matter. Pilear scales formed by the break of the pilear surface. Stipe surface a palisade:  
..... *P. squamosus*.

**Table 1. Voucher specimens and culture isolates of *P. tuberaster* and *P. squamosus* used in this work.**

SPECIES	ISOLATE	LOCALITY
<i>P. tuberaster</i>	99 (4)	Kåfjord, Troms. Norway
	102 (6)	Lullesletta, Troms. Norway
	31307 (4)	Wyrdclyffe, Wales. U. K.
	366 (6)	Kashima, Ibaraki. Japan
	367 (6)	Kashima, Ibaraki. Japan
	TFM1821 (8)	Kamikawa, Hokkaido. Japan
	647 (5) (S)	Takinoshita, Hokkaido. Japan
	<b>TFM756 (S)</b>	Ogawa, Ibaraki. Japan
<i>P. squamosus</i>	<b>D804</b>	North Carolina. U.S.A.
	97 (2)	Oslo. Norway
	<b>103</b>	Lullesletta, Troms. Norway

The number of monosporic isolates obtained from each voucher specimen is indicated by parentheses. Secondary mycelium isolates are indicated in bold face. (S) indicates the presence of sclerotium in the corresponding basidiocarp.

**Table 2. Compatibility tests among the isolates from Table 1.**

	97	<b>103</b>	99	102	307	366	367	1821	647	<b>756</b>
<b>804</b>	+	*	-	-	-	-	-	-	-	-
97		+	-	-	-	-	-	-	-	-
<b>103</b>			-	-	-	-	-	-	*	*
99				+	+	+	+	+	+	+
102					+	+	+	+	+	+
307						+	+	+	+	+
366							+	+	+	+
367								+	+	+
1821									+	+
647										+

Biological species are separated by lines between isolates. A + sign indicates formation of secondary mycelium after the test, and thus compatibility between the isolates. A - sign indicates incompatibility with no formation of a secondary mycelium. Secondary mycelium isolates are shown in bold face. Pairings not performed are indicated with a \* sign. Only the number of the voucher specimen is specified as all monosporic isolates from the same specimen gave the same compatibility result (data not shown).

## Discussion

Basidiocarps determined as *P. tuberaster* based upon the presence of a sclerotium should be re-examined for the stipe and pilear microstructure. These microscopical characters correlate consistently with mating incompatibility, the basic argument to separate biological species (Boidin, 1986; Hallenberg, 1988).

The presence of sclerotia cannot be used to taxonomically separate *P. tuberaster* from *P. squamosus*. The ability of the last species to form sclerotia in culture could be a reflect of what occurs in nature. Corner (1993) observed that the sclerotia of *Lentinus tuber-regium* (Fr.) Fr. were developed in large rotten trunks from which they dislodged. The sclerotium of *Lignosus rhinocerus* (Cke.) Ryv. was recently found inside a rotten log from which basidiocarps developed (personal data). One of the collections of *Polyporus squamosus* in O grew from a sclerotium in the ground (Fig. 2).

The basidiocarps of most *Polyporus* species where sclerotia have been reported occur in sandy soils, as is the case with the Australian species (Cunningham, 1965) and with *P. umbellatus* (Guo & Xu, 1991). Sandy soil is a substrate easy to tear apart where sclerotia are likely to be found. On the contrary, sclerotia embedded in harder substrates such as wood are easily overlooked (Müller, Hutch & Herschel, 1978; Jahn, 1980; Boertmann, 1984).

Fungal sclerotia are especially extended among necrotroph parasites (Christias & Lockwood, 1973). *P. squamosus* is a necrotroph parasite (Buller, 1906; Campbell & Munson, 1936). As the host dies, the fungus can continue its life-cycle even if a new host is not available.

*P. radicans*, close to *P. squamosus*, and characterized by a long, radicating stipe (Gilbertson & Ryvarden, 1987) could also be a case where basidiocarps arise from a subterranean sclerotium. Its scurfy, greyish pileus and the smaller pores make this species distinct.

I wish to thank E. Bendiksen for supplying the photograph in Fig. 2; the curator of the herbarium at Duke University for the loan of *P. squamosus* D804, Dr. D. Hibbett for sending a culture of this collection, and Mr. T. Hattori for providing Japanese isolates of *P. tuberaster*. Leif Ryvarden suggested corrections that improved the manuscript.

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