

TAXONOMY OF THE FUNGUS ASSOCIATED WITH *SIREX NOCTILIO*

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Summary

The fungus associated with *Sirex noctilio* (F.) wood wasps which attack *Pinus radiata* Don in Australia and New Zealand, is described and has been determined as a species of *Amylostereum* Boidin. Its specific determination awaits the production of monosporous cultures to be used in interfertility tests with cultures of known species of *Amylostereum*.

I. INTRODUCTION

Little has been recorded on the taxonomy of fungi associated with species of *Sirex*. In England, a Basidiomycete determined by Cartwright as *Stereum sanguinolentum* (Alb. & Schw. ex Fr.) Fr. was cultured from oidia in the hypopleural sacs of *Sirex gigas* (L.) and *S. cyaneus* (F.) (Cartwright 1929, 1938; Parkin 1941, 1942). In Europe, Francke-Grosmann (1939) has suggested that *Stereum sanguinolentum*, *Stereum chailletii* (Pers. ex Fr.) Fr., and *Trametes odorata* (Wulf.) Fr. may be associated with species of *Sirex*. Stilwell (1960) has reported that *Stereum sanguinolentum* and *S. chailletii* are associates of *Sirex* in living fir trees. Rawlings (1948) recorded that the fungus found with *Sirex noctilio* in New Zealand could be a species of *Stereum* but was not *Stereum sanguinolentum*, while Orman (1958) expressed the opinion that it was a species of *Peniophora* Cooke.

As no natural fructifications of the fungus have yet been found in Australia and New Zealand, it has had to be studied from fragments occurring in the hypopleural sacs of wood wasps and otherwise entirely in culture. On agar media it does not produce characteristic fructifications, but by using the wood block method of culturing (Tamblyn and Da Costa 1958) reasonably mature fructifications may be obtained. Most of the difficulties in determining the *Sirex* fungus have arisen from having immature stages to study. In young cultures, even on wood blocks, the fructification has no definite cuticle and tomentum, has relatively thin-walled and branched cystidia of great length, and has non-amyloid spores; all these features change to the exact opposites in mature fructifications.

II. STRUCTURE OF THE SIREX FUNGUS

(a) From Hypopleural Sacs of *Sirex noctilio*

In the hypopleural sacs the fungus occurs as mycelium fragmented into irregularly shaped segments comprising one to four short cells, each of which is an arthrospore (or "oidium") measuring 12-28 by 3-9 μ (Fig. 1). Most septa separating the arthrospores are clamped, and when two cells are parted the clamp may remain

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Figs. 1-7.—*Amylostereum* species. Fig. 1.—Arthrospores and blastospores from hypopleural sacs of *Sirax*. Fig. 2.—Hymenial elements. Fig. 3.—Basidia and basidiospores. Fig. 4.—Arthrospores. Fig. 5.—Transverse section of a mature basidiocarp. Fig. 6.—Transverse section of a young basidiocarp. Fig. 7.—Cystidia and generative hyphae. Figs. 2, 5, and 6 are from wood block cultures; Figs. 3, 4, and 7 from agar cultures.

as a conspicuous lateral attachment at the end, or at both ends, of a cell. The arthrospores may bud off globose to ellipsoidal blastospores, 5-6 by 3-6 μ , singly or in short chains (Fig. 1). On agar media the arthrospores and blastospores are able to germinate by means of one or more germ tubes.

(b) *From Agar Culture*

On corn meal agar the fungus produces branched, thin-walled, hyaline generative hyphae, 2-4.5 μ wide, with abundant simple clamp connexions. The generative hyphae (Fig. 7) have binucleate cells (Boidin, personal communication). From the generative hyphae, arthrospores and skeletal hyphae are formed.

The arthrospores (Fig. 4) are oblong to cylindrical, one to three per chain, 4.5-17 by 2-3 μ . They differ from those found in the hypopleural sacs by being narrower, more regular in shape, and lacking clamp connexions at the transverse septa.

The skeletal hyphae are brownish, 2.5-3.5 μ wide, with slightly thickened walls, some becoming modified into brown cystidia towards their apices or in lateral branches. At first the cystidia (Fig. 7) are relatively thin-walled with a wide lumen, but later become thick-walled with a narrower lumen and densely encrusted with hyaline mineral matter especially near the apex; they measure 55-170 by (3.5-5-8 (-12) μ and the wall may be up to 3 μ thick. Some of the cystidia branch once or twice near the apex or further back. Both skeletal hyphae and cystidia may have occasional secondary septa and may bear curious lateral small and dark knob-like structures at intervals. A clamp connexion often marks the junction of a cystidium or skeletal hypha with its parent generative hypha.

No basidiocarp or definite hymenium is formed in agar cultures, but here and there groups of basidia (Fig. 3) may be found; they are homobasidiate, irregularly clavate, 8.5-23 by 4.5-6.5 μ , with four straight or slightly curved sterigmata reaching 5.5 μ in length. The basidiospores are hyaline, smooth, amyloid (only when mature), broadly ellipsoid, and attenuated to a small apiculus or subcylindrical with a flattened side, 4-6.3 by 2.5-3 μ ; they are also uninucleate (Boidin, personal communication).

(c) *From Fructifications on Wood Block Cultures*

The fructification is resupinate, with a smooth or undulating or tuberculate hymenium which varies in colour from light date brown to deep umber; the margin is lighter-coloured and appressed. In section the fructification is 70-500 μ thick.

In the underlying wood (Fig. 6), clamped generative hyphae and occasional skeletal hyphae are seen, some of the latter curving out towards the hymenium to become modified into cystidia in the trama. The connexion between skeletal hypha and cystidium is not always clear and is progressively more difficult to determine as the fructification ages, so that eventually the cystidia appear quite short.

The trama gradually thickens and becomes stereoid in its tissue distribution (Fig. 5); there is a poorly defined cuticle and tomentum both composed of mixed

skeletal and generative hyphae, then a medullary layer composed of horizontally arranged skeletal and generative hyphae, and finally the subhymenial and hymenial layers where the hyphae are arranged almost vertically. The skeletal hyphae are good indicators of the directions of various growth forces: horizontal and radial towards the margin and in the medulla, and curving outward towards the hymenium on the inferior surface and towards the trichoderm on the superior surface.

The cystidia formed in or near the hymenium are erect and relatively short, but by their method of formation in the older parts they are clearly tramal cystidia which are among the first-formed elements in the fructification and arise as apical modifications of skeletal hyphae. They continue to be formed during subsequent growth in thickness of the fructification and thus terminate at all levels, including in or slightly beyond the level of basidia. Their apical part is differentiated by being encrusted with easily detersite hyaline mineral granules, by being somewhat inflated up to 9–(12) μ , and by being slightly rugose. The long hyphoid pedicel is traceable back to a parent generative hypha, often with a clamp connexion at their junction. Cystidia and skeletal hyphae are brown and, as in agar cultures, may be secondarily septate and bear small lateral knob-like structures (Fig. 5). They stain deeply and quickly with safranin.

The generative hyphae in the medulla may be loosely intertexted and easily seen or may form short-celled, compact tissue. They bear simple clamp connexions.

Basidia (up to 35 μ long) and basidiospores are essentially as described from agar culture. In addition basidioles may be present; they are of the same dimensions as basidia but terminate in an apical constriction bearing a small globose nipple, 1–1.5 μ in diameter. They contain vacuoles or oil globules of appreciable size. The basidia and accompanying elements (Fig. 2) are formed in a thickening hymenium and may often be clamped at the base.

III. CLASSIFICATION AND DETERMINATION

The presence of homobasidia borne in a smooth, open hymenium of a resupinate basidiocarp indicates that the fungus is one of the Thelephoraceae *sensu lato*. Two features of its construction should be emphasized: the presence of cystidia and the stereoid distribution of tissues. The presence of cystidia in a wholly resupinate Hymenomycete is usually taken as indicative of the genus *Peniophora*, although it is now well known that no attempt to characterize genera on the presence or absence of a single conspicuous feature has ever been entirely successful. Moreover the term "cystidium" has been applied to a diversity of hyphal terminations which are clearly not homologous. The stereoid tissue distribution traditionally indicates the genus *Stereum* Pers. ex S. F. Gray, whether the fructifications are resupinate or effuso-reflexed and whether or not they contain some type of cystidium.

Both *Peniophora* and *Stereum* have become meaningless genera by having innumerable unrelated species associated with the type species over the years. The recent approach to systematics in Hymenomycetes has been to study generic types in as many aspects as possible and to align with them only such species as seem closely related in external and internal morphology, development, cytogenetics, biochemistry.

and other relevant features as far as they are known. The resulting genera are smaller and far more natural than previously, but there is still a large residue of heterogeneous species remaining to be placed in new generic groupings.

In *Peniophora sensu stricto* (Eriksson 1950), the fructification is resupinate or narrowly reflexed at the margin, with a smooth hymenium coloured red, blue, grey, or brownish; embedded or emergent metuloid cystidia and/or gloecystidia are present; the spores are uninucleate, non-amyloid, smooth, cylindrical-curved to allantoid, pinkish in a mass; the monomitic context is dense in texture and consists of short-celled, laterally cemented, vertical hyphae with brownish, binucleate cells, above a horizontal basal layer of cemented or subgelatinous hyphae; clamp connexions are present in the fructifications; the fungus produces a white rot and a positive oxidase reaction. Clearly the *Sirex* fungus differs from a true *Peniophora* in several respects, particularly in its type of cystidium, its spore characters, and its tissue formation.

In *Stereum sensu stricto*, the growth form is resupinate or effuso-reflexed; the hymenium is smooth and inferior; colourless generative hyphae and hyaline to brownish skeletal hyphae form a dimitic system, and there is a cuticle with a well-developed tomentum on the superior surface. A horizontal layer of medullary hyphae adjoins the cuticle, while the hyphae are vertically arranged towards the hymenium. The generative hyphae have multinucleate cells (Boidin 1954) and bear simple, opposite or whorled clamp connexions in the mycelium, but none in the fructification. The basidiospores are smooth, binucleate (Boidin 1954), hyaline, amyloid, ellipsoid to cylindrical-curved. The fungus produces a white rot and a strongly positive oxidase reaction. Those skeletal hyphae that approach the hymenium become somewhat inflated and contain accumulations of yellowish to brown tannin-like substances (Boidin 1958); they may be termed "pseudocystidia". In a few species such as *Stereum sanguinolentum* the tannin-like contents are abundant in the pseudocystidia, and on exposure to air by wounding immediately exude as a red fluid, a phenomenon commonly known as "bleeding". Apart from pseudocystidia, and the presence of pseudoacanthophyses (Boidin 1958) in some species of *Stereum s. str.*, there are no other notable sterile hyphae associated with the hymenium. It is clear that the *Sirex* fungus could be loosely classified in *Stereum*; in its external morphology, stereoid structure, amyloid spores, and strongly positive oxidase reaction it closely resembles *Stereum*, but several notable features make its segregation from *Stereum* desirable. These include the brown, encrusted tramal cystidia without tannin-like contents, the presence of simple clamp connexions in the fructification, and the presence of uninucleate spores and binucleate hyphae.

Recent studies of stereoid fungi include those of Boidin (1954, 1958, 1959a, 1959b), Lentz (1955, 1960), Pouzar (1959), Eriksson (1950, 1958), and Talbot (1954). Boidin, in particular, has studied these fungi both in culture and from natural fructifications. About 14 genera containing species which at some time have been placed in *Stereum* have now been proposed. In only eight of these genera are the fungi resupinate-reflexed with a dimitic hyphal system. Tramal cystidia are present in *Columnocystis* Pouzar, *Duportella* Pat., and *Amylostereum*, while smooth amyloid spores are present in *Stereum s. str.*, *Xylobolus* Karst., and *Amylostereum*. The

Sirex fungus is clearly a species of *Amylostereum*. It corresponds with the three species of *Amylostereum* known in Europe in general morphology, in possession of binucleate clamped hyphae in the fructification, in its brown tramal cystidia with crystals, in its uninucleate spores, its type of woodrot, and its strong phenoloxidase activity. In addition, the European species of *Amylostereum* all inhabit conifers: *A. chailletii* (Fr.) Boidin is known on *Picea* and *Abies*, rarely on *Pinus*; *A. areolatum* (Fr.) Boidin on various *Abies* spp.; and *A. laevigatum* (Fr.) Boidin on *Juniperus*, rarely on *Taxus*. Arthrospores have been found in a species of *Amylostereum* near to or identical with *A. areolatum* from Asia (Boidin, personal communication). Septation of the skeletal hyphae and cystidia, also the peculiar knob-like attachments that they sometimes bear, are further points in common between *A. chailletii* and the Sirex fungus. In *A. chailletii* the fructifications eventually become narrowly reflexed and the context vaguely stratified; such features have not been noted in the relatively young wood block cultures of the Sirex fungus.

It is considered that the fructifications yielded by wood block culture, although reasonably characteristic, do not afford a good basis for direct comparison with natural fructifications of the other species of *Amylostereum*, since marked differences occur with age of the fructification. Consequently it is necessary to obtain monosporous cultures for interfertility tests with cultures of the known species of *Amylostereum* before one can be sure of the specific identity of the Sirex fungus. Preliminary experiments by colleagues at the Waite Institute have shown that although basidiospores are shed from agar and wood block cultures and may be isolated readily, they do not germinate under the cultural conditions used so far; thus the problem of obtaining monosporous isolates will evidently take some time to solve. Since the discovery of Sirex infestation of *Pinus radiata* in Victoria and the establishment of a Sirex Research Committee, the identity of the fungus has become a matter of concern to many workers in this field, and perhaps justifies the publication of these incomplete results.

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