THE SYNNEMATOUS ANAMORPH OF *EXIDIA SACCHARINA* (AURICULARIALES, BASIDIOMYCOTA): MORPHOLOGY, CONIDIIOGENESIS AND ASSOCIATION WITH BARK BEETLES*

ROLAND KIRSCHNER

Abstract. The paper describes a synnematous anamorph of *Exidia saccharina*, its natural occurrence in the field, and the ultrastructure of conidiogenesis. Conidiogenesis was revealed as annellidic by transmission electron microscopy. The anamorph was found rarely in galleries of the bark beetles *Ips typographus* and *Pityogenes chalcographus* (Coleoptera, Scolytinae) in the bark of *Picea abies* in Germany. The anamorph–teleomorph connection was confirmed by morphological comparison of the anamorph from bark beetle galleries with the anamorph grown from basidiospores on natural medium. The newly discovered synnematous growth and annellidic conidiogenesis are similar to those of *Gloeosynnema* species (basidiomycetous hyphomycetes), which differ by details of septal clamp connections.

Key words: basidiomycetes, mitosporic fungi, synnemata, Europe

INTRODUCTION

Anamorphs of Auriculariales usually are characterized by hyaline, one-celled, mostly allantoid conidia which are produced from germinating basidiospores or mycelia derived from basidiospores. The anamorph of *Exidia glandulosa* (Bull.) Fr. is illustrated as an example in Figure 1 (from dead branch; GERMANY. Baden-Württemberg, Tübingen, Schönblick, 22 Febr. 1998, leg. R. Kirschner 308, FR). The conidiogenous cells have sometimes been called phialide-like (Ingold 1982a, b, 1984, 1992, 1995), but the exact mode of conidiogenesis is usually not evident by light microscopy and has not been investigated with transmission electron microscopy. Brefeld (1888) found considerable morphological differences among anamorphs of certain species of Auriculariales in culture, but this approach has not been used consistently for monographs of this group. Production of secondary spores and conidia from germinating basidiospores can be observed often on freshly collected basidiomata of Auriculariales, but the anamorphs alone are rarely found in nature (Olive 1952; Ingold 1991), or if they occur their association with a teleomorph has not been recognized. Recently an anamorph with a relationship to the Hyaloriaceae (Auriculariales) was described from material on a dead branch in Taiwan and in culture as *Helicomyxa everhartioides* R. Kirschner & Chee J. Chen (Kirschner & Chen 2004). Another anamorph was found on dead bamboo in China (Kirschner et al., in press). Both species form superficial pustulate or cupulate conidiomata on dead plant material, and their asexual propagules probably are dispersed by rain. Another habit connected to a cryptic habitat hidden beneath tree bark and probable dispersal by insects is demonstrated in this paper.

MATERIALS AND METHODS

Bark of *Picea abies* (L.) Karst. with galleries of the bark beetles *Crypturgus pusillus* (Gyllenhal), *Ips typographus* L., and *Pityogenes chalcographus* (L.) was...
collected in Germany in 1994–2000. For details see the section on specimens examined; for general collection strategies see Kirschner (1998, 2001). Bark beetle galleries were immediately examined with a dissecting microscope. In order to obtain cultures, conidia were transferred from conidiophores in the galleries to a natural medium (1.5% water agar containing autoclaved pieces of twigs of *Picea abies*) with a flame needle. Parts of a basidioma of *Exidia saccharina* (Alb. & Schwein.) Fr. (leg. R. Kirschner 88 and 333) were fixed under the lids of Petri dishes and allowed to disperse basidiospores onto 1.5% water agar containing autoclaved pieces of twigs of *Picea abies* or 2% malt extract agar. Polyspore cultures were deposited under accession numbers F 3274 (ex-teleomorph), F 3275 and F 3468 (both ex-anamorph) in the culture collection of the Botanical Institute of the University of Tübingen, Germany. Dried specimens were deposited in the Herbarium Senckenbergianum in Frankfurt am Main, Germany (FR). Transmission electron microscopy (TEM) was used to study material from a gallery of an unidentified bark beetle in *Picea abies*, Germany, Bad Waldsee, 7 Apr. 1995, leg. R. Kirschner 11, using methods described in Kirschner et al. (2001a). For microscopic examination, specimens were mounted.
in water or 5–10% aqueous KOH, with and without 1% aqueous phloxine staining.

RESULTS

Anamorph of *Exidia saccharina* (Alb. & Schwein.) Fr. Figs 2–4

The anamorphic fungus described and illustrated below from material found in bark beetle galleries was indistinguishable from the anamorph derived from the polysporous cultures of the teleomorph of *E. saccharina* (leg. R. Kirschner 88, Fig. 5) on natural medium. For synonyms of the teleomorph see Neuhoff (1935) and Wojewoda (1981). Septal ultrastructure revealed dolipores with continuous parenthesomes.

Conidiophores on autoclaved spruce twigs and in bark beetle galleries predominantly synnematos, but also mononematous, erect, hyaline, sparsely and irregularly branched and septate, 120–150 × 2–3 μm. Clamps at the septa often with a retrorse projection which in some cases is delimited by a septum. One to three conidiogenous cells arising terminally from branches of the conidiophore, cylindrical, 50–63 × 2–3 μm. Mode of conidiogenesis unclear when viewed by LM, but visible by TEM as annellidic, because longitudinal sections through conidiogenous cells show lateral wall remnants of previous conidium dehiscence. Conidia accumulating in a slimy mass on the apex of the conidiogenous cells, hyaline, one-celled, smooth, cylindrical with truncate base and rounded apex, cytoplasm strongly granulate, 10–16 × 3 μm.

SPECIMENS EXAMINED

*Exidia saccharina* anamorph: In galleries of *Pityogenes chalcographus* in bark of *Picea abies*: GERMANY. Baden-Württemberg, near Bad Waldsee, ca 600 m a.s.l., 12 Sept. 1994, leg. R. Kirschner (Fig. 2), not deposited; in abandoned galleries of an unidentified bark beetle in bark of *Picea abies*: GERMANY. Baden-Württemberg, near Bad Waldsee, ca 600 m a.s.l., 7 Apr. 1995, leg. R. Kirschner 11 (Fig. 4), FR; in galleries of *Ips typographus* in bark of *Picea abies*: GERMANY. Baden-Württemberg, near Bad Waldsee, ca 600 m a.s.l., 19 Oct. 1995, leg. R. Kirschner 10 (Fig. 3), FR, culture F 3275 in Tübingen; in abandoned bark beetle galleries secondarily colonized by *Crypturgus pusillus* in bark of felled stem of *Picea abies*, GERMANY. Bavaria, Oberjoch, Iseler, ca 1200 m a.s.l., 26 Sept. 2000, leg. R. Kirschner 756, FR, culture F 3468 in Tübingen.

*Exidia saccharina* teleomorph: On felled stems of *Pinus sylvestris*: GERMANY. Hessen, Darmstadt-Eberstadt, Malcher Schneise, ca 100 m a.s.l., 9 Nov. 1996, leg. R. Kirschner 88, FR, LSU rDNA sequence available under GenBank accession number AF291323 (Weiß & Oberwinkler
Fig. 4. Ultrastructure of the anamorph of *Exidia saccharina* (Alb. & Schwein.) Fr. from a gallery of an unidentified bark beetle in *Picea abies*, Germany, Bad Waldsee, 19 Oct. 1995, leg. R. Kirschner II. a – longitudinal section through the apex of a conidiogenous cell with a developing conidium, b – longitudinal section through subapical part of a conidiogenous cell showing lateral wall remnants of previous conidium dehiscence indicating annellidic conidiogenesis, c – longitudinal section through a clamp connection showing the dolipore with parenthesomes in the regular septum (black arrow) and the retraction septum delimiting the retrorse, plasma-free elongation of the clamp (white arrow), d – median section through a dolipore with continuous parenthesomes. Scale bars: a = 1.5 μm, b = 0.5 μm, c = 0.75 μm, d = 0.25 μm.

DISCUSSION

Brefeld (1888) described the conidia of *E. saccharina* in culture as cylindrical, measuring 10–15 × 3–5 μm, and accumulating in heads. Comparing these conidia to the curved conidia in most other Auriculariales, he considered this characteristic important enough to propose the genus *Ulocolla* for the holomorph. This concept was not followed by later researchers (e.g., Neuhoff 1935). The germination of basidiospores and production of cylindrical or slightly curved conidia in culture were also reported by Neuhoff (1935) and Wojewoda (1981), but with smaller sizes reported for the conidia (5–7 × 1–2 μm). Brefeld (1888) used water and liquid media for his observation of conidium development. Neuhoff (1935) probably observed
conidia developing from hyphae of germinating basidiospores on teleomorph specimens from nature. It is unclear whether the conidal dimensions differ because of different environmental or genetic conditions. Neuhoff (1935) also found different sizes of basidiospores among different collections of the same species. The different sizes of basidiospores and conidia might indicate some genetically fixed variability to be tested by mating experiments and DNA analyses. The occurrence of synnematous conidiophores with clamped stipe hyphae in nature or in culture was not observed by previous authors. This finding resulted only from field collection and cultivation on natural media (Kirschner 1998).

Anamorphs of other species of Auriculariales could be distinguished from the anamorph of _Exidia saccharina_ based on the literature. Straight or nearly straight cylindrical conidia have been demonstrated clearly for only a few other _Exidia_ species, namely _E. badioumbrina_ (Bres.) Neuhoff, _E. cartilaginea_ S. Lundell & Neuhoff, and _E. recisa_ (Ditmar) Fr. (Neuhoff 1935; Pilát 1957; Ingold 1995). However, these species are restricted to angiosperms and the conidia are smaller, up to 6 μm long (Pilát 1957; Ingold 1995). As far as known, the conidia of the majority of Auriculariales _s.str._ are allantoid: _Auricularia_ Bull. _ex_ Juss. _spp._ (Brefeld 1888; Ingold 1982a), _Ductifera sucina_ (Möller) K. Wells (Möller 1895), _E. glandulosa_ (Bull.) Fr. (Brefeld 1888; Ingold 1982b), _E. pithya_ Fr. (Neuhoff 1935; Pilát 1957; Wojewoda 1981), _E. thuretiana_ (Lév.) Fr. [Neuhoff 1935, as _E. albida_ (Huds. _ex_ Louds.) Bref.; Ingold 1995], _E. truncata_ Fr. (Neuhoff 1936), _E. umbrinella_ Bres. (Pilát 1957), _Exidiopsis cerina_ Möller (Möller 1895), _Exidiopsis ciliata_ Möller (Möller 1895), _Exidiopsis effusa_ (Bref.) Möller (Brefeld 1888; Ingold 1992), _Exidiopsis glabra_ Möller (Möller 1895), _Heterochaete hirneoloides_ (Pat.) K. Wells (Möller 1895, as _Exidiopsis tremellispora_ Möller), _H. shearii_ (Burt) Burt (Kisimova-Horovitz _et al._ 1997), _H. verruculosa_ (Möller) Bodman (Möller 1895, as _Exidiopsis verruculosa_ Möller), _Mxyarium grilletii_ (Boud.) D. A. Reid [Neuhoff 1935, as _Exidia grilletii_ (Boud.) Neuhoff], _M. laccatum_ (Bourd & Galzin) D. A. Reid (Kisimova-Horovitz _et al._ 2000) and _M. nucleatum_ Wallr. (Brefeld 1888, as _Exidia corrugativa_ Bref. – synonymy according to Neuhoff 1935; Ingold 1984). In some of these cases, conidiogenous cells were described as phialide-like. As demonstrated here for the anamorph of _E. saccharina_, conidiogenesis is annellidic with percurrent proliferations not visible by LM but only by electron microscopy, in which longitudinal sections through conidiogenous cells show lateral wall remnants of previous conidium dehiscence. Phialidic development with apical replacement wall-building indicated by apical wall-thickening in the conidiogenous cell (Minter _et al._ 1983) has been confirmed in Basidiomycota only for spermatia in spermogonia of rust fungi (Mims _et al._ 1976). The clear distinction between phialidic and annellidic conidiogenesis, difficult to detect with LM but obvious with TEM, supported segregation of the genus _Leucogloea_ R. Kirschner with annellidic conidium development, and basidiomycetous affinities from _Pleurocolla_ Petr. with phialidic conidiogenesis (Kirschner 2004). Within ascomycetous anamorphs, detailed studies of conidiogenesis based on electron microscopy have led to consider-
able taxonomic changes on the generic level in the former *Leptographium* complex (Wingfield et al. 1987). Conidiogenous cells of additional species of Auriculariales should therefore be investigated by electron microscopy. *Helicomyxa everhartioides* R. Kirschner & Chee J. Chen was described for a sporodochial anamorph with a close phylogenetic relationship to *Hyaloriaceae* and conidia developing from clamps (Kirschner & Chen 2004). Compared to the anamorph of *Myxarium tremelloides* (L. S. Olive) Wojewoda, for which separate conidiomata were also recorded from nature (Olive 1952), *H. everhartioides* might be closely related or identical, but the details of conidiophores and conidiogenesis of *M. tremelloides* are unknown (Olive 1952). Conidium development from clamps as in *H. everhartioides* appears to be unique within the Auriculariales.

The synnematous anamorph of *E. saccharina* is morphologically similar to species of *Gloeosyn- nema* Seifert & G. Okada, hitherto comprising the synnematous type species *G. ochroleucum* (Penz. & Sacc.) Seifert & G. Okada and the sporodochial species *G. roseum* Matsush. (Seifert & Okada 1988; Matsushima 1995). These species share the characters of hyaline or colored conidiomata, anellidic conidiogenesis, and clamps at the septa. The sporodochial *G. roseum* appears identical to the anamorph of *Typhula micans* (Pers.) Berthier (Agaricales) as described by Koske and Perrin (1971) and Berthier (1976). Clamps of *Gloeosyn- nema* species lack the retrorse projection typical for *E. saccharina*. Clamps with a posterior appendage, in some cases delimited by a retraction septum (‘spurred clamp’), are a phylogenetically significant characteristic of the Auriculariales (Bandoni & Wells 1992). The spurred clamp is also illustrated for the teleomorph of *E. saccharina* (Fig. 5a). Other basidiomycetous synnematous anamorphs with anellidic conidiogenesis without clamps have been recorded for *Basidiopycnis* Oberw., R. Kirschner, R. Bauer, Begerow & Arenal (Atractiellales) and *Stilbotulasnella* Oberw. & Bandoni (Cantharel- lales) (Bandoni & Oberwinkler 1982; Oberwinkler et al. 2006). These anamorphs were not named separately by the original authors because they could be connected to the named teleomorph beyond any doubt. This practice is followed here for the distinct anamorph of *E. saccharina*. Though it would be nomenclaturally correct to create a new taxon, I consider it unnecessary for several reasons given in Kirschner and Oberwinkler (2009).

*Exidia saccharina* is known as a teleomorph from conifers with a preference for pine (Neuhoff 1935). Though bark beetle galleries in pine were also examined, the anamorph was found in this study only in spruce (Kirschner 1998). On these comparatively rare occasions, the anamorph appeared abundantly in the galleries but was never directly isolated from bark beetles. No teleomorph was found at the places where the anamorph of *E. saccharina* was collected. *Exidia saccharina* appears considerably less common than *E. pithya*, which can be found frequently on the bark of spruce after infestation by bark beetles (pers. ob- servation). It may be that different environmental conditions are required for the development of both morphs of *E. saccharina*. As mentioned by Neuhoff (1935), basidiomata of this species in Europe appear very frequently in certain regions but are conspicuously rare in others. Although this paper adds a further species to the known basidiomycteous associates of bark beetles (e.g., Kirschner 2001; Kirschner et al. 2001a, b; Kirschner & Chen 2003; Kolafik et al. 2006; Oberwinkler et al. 2006), the complex ecological relationships, life cycles and systematic diversity of these fungi are not well understood, and more insights are to be expected from future work.

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