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PHIALIDES WITH SOLITARY CONIDIA?

Remarks on condium ontogeny in some hyphomycetes

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(With six Text-figures)

Conidium formation in some species of *Aphanocladium* W. Gams, *Verticimonosporium* Matsushima, *Sibirina* Arnold, *Pseudofusarium* Matsushima and *Craspedodidymum* Hol.-Jech. is discussed and compared with other examples. Conidiogenous cells with solitary and with serial conidia may occur in apparently closely related species. It is questionable, whether the term phialides has to be restricted to the latter group.

The new species *Aphanocladium spectabile* and *Sibirina orthospora* are described.

Conidium ontogeny is becoming the dominating criterium in the taxonomy of hyphomycetes. One of the most common propagative structures is the phialide, which since Hughes (1951 and 1953) and more explicitly in Kendrick (1971) is defined as a 'cell producing from a fixed locus a basipetal succession of conidia whose walls arise de novo'. In the introduction to the monograph of *Cephalosporium*-like hyphomycetes (Gams, 1971a) several morphological types of phialides were distinguished according to the insertion in the subtending hyphae and designated with the nouns orthophialide, plagiophialide, schizophialide, etc. Although an adjectivic terminology is now preferable (Kendrick, 1971), the distinctions introduced have proved their usefulness.

The term phialide was introduced in a rather vague circumscription by Vuillemin (1910) to include also fungi with solitary conidia, such as *Beauveria* (cf. Mason, 1933). Whereas this type of conidium formation is now considered as holoblastic, some other fungi exist whose conidiogenous cells very strongly resemble phialides but produce only solitary conidia; they will be discussed in this contribution.

A. Aphanocladium W. Gams

The genus *Aphanocladium* (Gams, 1971a) was defined as having phialoconidia borne on either fully differentiated swollen phialides or on reduced narrow thread-like outgrowths ('aphanophialides') in heads or in chains. Further examination of numerous strains has, however, shown that in the type species *A. album* (Preuss) W. Gams conidia are always solitary; head-like agglomerations can arise when the conidia from neighbouring denticles contact one another. In this respect the generic diagnosis has to be corrected.

A similar and much more pronounced example of solitary conidia borne on phialide-like conidiogenous cells is found in the strain CBS 340.70, obtained from Mme J. Nicot, Paris, as 'Charpin 2' and originating from the atmosphere in Marseille; it will be described as a new species of *Aphanocladium*. The species with catenate conidia hitherto included in this genus will have to be accommodated in a new genus.

Aphanocladium spectabile W. Gams, *spec. nov.*—Fig. 1

Coloniae fere celeriter crescunt, ad 35 mm diametro post 10 dies, albae vel pallide roseae, floccosae-lanosae et conidiis pulverulentae; hyphae vegetativae 1.5–1.8 μ crassae, hyalinae, leves, saepe fasciculatae. Cellulae conidiogenae e hyphis aeriis singulae vel 3–5 verticillatae fere rectangulariter oriuntur, 9–11 μ longae, e basi 2.0–2.8 μ crassa sursum ad 0.5–0.8 μ attenuatae; nonnumquam proliferunt; raro conidiophora lateralia composita adsunt. Conidia semper singula, ellipsoidea, basi modice apiculata, hyalina, fere crassitunicata, levia, 7.2–9.2 × 2.9–3.3 μ. Chlamydosporae absunt.

TYPUS CBS 340.70, isolatus ex aere, Massiliae in Gallia.

Colonies on 2 % malt extract agar (and other media) spreading rather rapidly, attaining at room temperature a diameter of 30–35 mm within 10 days; white to very pale pink, deeply floccose-lanose and powdery from abundant conidia; margin lobulate, reverse pinkish. Hyphae of the aerial mycelium 1.5–1.8 μ wide, hyaline, smooth-walled. Hyphae bearing phialides nematogenous or plectonematogenous;

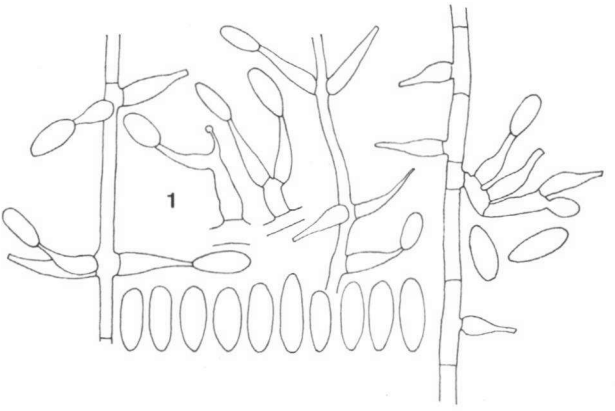

Fig. 1. *Aphanocladium spectabile*, from colony on malt-extract-agar (1000 : 1).

conidiogenous cells solitary or usually 3–5 in whorls, arising at almost right angles from the supporting hyphae, 9–11 μ long, tapering from a swollen, 2.0–2.8 μ wide base sigmoidally towards a narrow tip, 0.5–0.8 μ wide. Sometimes a proliferation occurs below the tip; rarely short lateral compound conidiophores are formed. Conidia always solitary, deciduous, ellipsoidal with slightly apiculate base, hyaline, rather thick-walled, smooth, 7.2–9.2 × 2.9–3.3 μ, length/width ratio 2.4–3.0. Chlamydospores not observed.

Although the conidiogenous cells are never reduced to conidium-bearing necks, this species is placed in *Aphanocladium* rather than in *Verticimonosporium* because of its spreading growth habit.

B. **Verticimonosporium** Matsushima

The genus *Verticimonosporium* is characterized by Matsushima (1971) as having verticillate conidiogenous cells (not phialides) with solitary conidia. The type strain of *V. diffractum* Matsushima (CBS 310.72) kindly supplied by T. Matsushima does not show much affinity with *Aphanocladium*. The growth is more restricted with a diameter less than 5 mm after 7 days; sporulation is rather scanty; conidiogenous cells are always fully differentiated and separated from the supporting hypha by a septum. The genus therefore is considered as sufficiently distinct; its affinities are not yet known.

C. **Sibirina** Arnold

Arnold (1970) described the genus *Sibirina* with the type species *S. fungicola* Arnold (Fig. 2) explicitly as producing conidia in slimy heads. Examinition of the type strain (CBS 458.71) and another isolate (CBS 821.70) collected on *Polyporus varius* Pers. ex Fr. near Abisko, Swedish Lappland, showed that conidia are strictly solitary; for each new conidium a new phialide is successively formed, leading eventually to extraordinarily dense verticils. By contact of neighbouring phialides, conidia may appear as if formed in heads. The phialides are 1.0–1.3 μ wide at the tip and almost imperceptably plugged with wall material. The conidia are two-celled, slightly curved and typically show a wall-thickening at the base and sometimes also at the tip. The smell is reminiscent of some agarics.

Another somewhat different strain, CBS 145.71, isolated by J. A. Stalpers from decaying wood at Schovenhorst near Putten, shows great similarity to *S. fungicola*, but has phialides with more than one conidium. Nevertheless, since no other appropriate genus and species name is available, it is described as a new species of *Sibirina*.

Sibirina orthospora W. Gams, *spec. nov.*—Fig. 3

Coloniae celeriter crescunt, ad 45 mm diam. post 5 dies, albidae, laxe floccosae-lanosae; conidiophora erecta e hyphis aeriis oriuntur. Hyphae aeriae hyalinae, leves, 2–3.5 μ crassae; conidiophora ad 400 μ alta, deorsum 4–4.5 μ crassa, sursum repetite verticillata, praecipue in ultimo verticillo ad 10 phialides ferunt. Phialides 20–26 μ longae, e 2.0–2.5 μ gradatim ad 0.7–1.0 μ attenuatae, conidia singula, rarius bina vel terna ferunt. Conidia cylindrica, recta, bicellularia, apice rotundata, basi fere apiculata, truncata, levia, hyalina, 16–21 × 4.0–6.0 μ. Chlamydosporae absunt.

TYPUS CBS 145.71, isolatus e ligno putrido, Putten.

Colonies spreading rapidly, on 2 % malt extract agar reaching a diameter of 45 mm within 5 days, whitish, loosely floccose-cottony. Odour absent. Moderately sporulating on erect conidiophores which arise from aerial hyphae. Aerial hyphae hyaline, 2–3.5 μ wide, smooth-walled. Conidiophores up to 400 μ high, 4–4.5 μ

Fig. 2. *Sibirina fungicola*, CBS 821.70, conidiophores and conidia (Left 500 : 1, right 1000 : 1).

Fig. 3. *Sibirina orthospora*, conidiophores and conidia (1000 : 1).

wide at the base, in the upper part repeatedly verticillately branched, especially in the uppermost whorl bearing up to 10 phialides. Phialides 20–26 μ long, gradually tapering from 2.0–2.5 μ towards 0.7–1.0 μ at the tip, bearing usually one, rarely 2 or 3 conidia. Conidia cylindrical, straight, two-celled, with rounded tip and slightly apiculate and truncate base, smooth-walled, hyaline; a very slight wall-thickening may be present only at the basal end; they measure 16–21 × 4.0–6.0 μ. Chlamydospores absent.

S. orthospora differs from *S. fungicola* not only by multiple conidium formation, but also by non-pigmented colonies and perfectly straight conidia without apical wall-thickening.

D. Pseudofusarium Matsushima

Matsushima (1971) defined the genus *Pseudofusarium* as having sympodially elongating, geniculate, denticulate, often proliferating conidiogenous cells and solitary septate conidia. These details would approach the genus to *Dactylaria* as redescribed by Bhatt & Kendrick (1968). The spreading growth habit, however, besides the sometimes sickle-shaped conidia, is sufficient evidence of an affinity with *Fusarium*. In this genus, *F. chlamydosporum* Wollenw. & Reinking has a similar structure with one-celled solitary microconidia borne on proliferating denticulate conidiogenous cells, while macroconidia are procuced at a very late stage serially on true phialides (Seemüller, 1968; Booth, 1971). In related species, such as *F. sporotrichioides* Sherb., similarly proliferating conidiogenous cells produce serial conidia. On the other hand there are strains with septate solitary conidia in which serial conidia have not yet been observed. It is likely that the conidium-bearing denticles in these *Pseudofusarium* species and in *F. chlamydosporum* are homologous to the phialide openings in *F. sporotrichioides*.

E. Craspedodidymum Hol.-Jech.

The genus *Craspedodidymum* was recently described by Holubová-Jechová (1972). The author regards the conidiogenous cells as phialides although they bear only solitary conidia. While releasing the conidium, the conidiogenous cell forms a cup-shaped collarette; after a subapical swelling the conidiogenous cell is somewhat constricted to 5–6 μ outer diameter with an internal wall-thickening and subsequently opens with flaring lips up to 12 μ in diam. The conidium develops endogenously. This may constitute a dematiaceous example of phialides with solitary conidia.

Discussion

In several cases a relationship between species with phialides producing serial conidia and others with solitary conidia can be demonstrated. In phialidic hyphomycetes so far investigated according to modern criteria the first formed conidium has a wall continuous with that of the conidiogenous cell, while the subsequently

formed conidia obtain a wall de novo. It would be attractive to assume that for some reason subsequent conidium formation has stopped, so that species with solitary conidia may be thought to be descendants from others with serial conidia. A possible mechanism for this interruption may be that the conidiiferous opening is too narrow and becomes blocked immediately; moreover it is a common phenomenon in phialidic fungi that the phialide opening is gradually plugged because with each subsequent conidium some wall material is left behind. In *Sibirina* such a plugging may occur already during the development of the first-formed conidium. This mechanism will require further study of the fine structure.

The relationship of *Aphanocladium* is unknown and it is premature to argue for or against a phialide nature of the conidiogenous cells. A similar genus with true phialides is *Tolypocladium* (Gams, 1971b), whereas holoblastic conidia are formed on geniculate conidiogenous cells in *Beauveria* (de Hoog, 1972). A relationship between these genera cannot theoretically be excluded; entomo-(araneo-)genous species or strains occur in all of them, and the growth pattern is similar. In the myco-

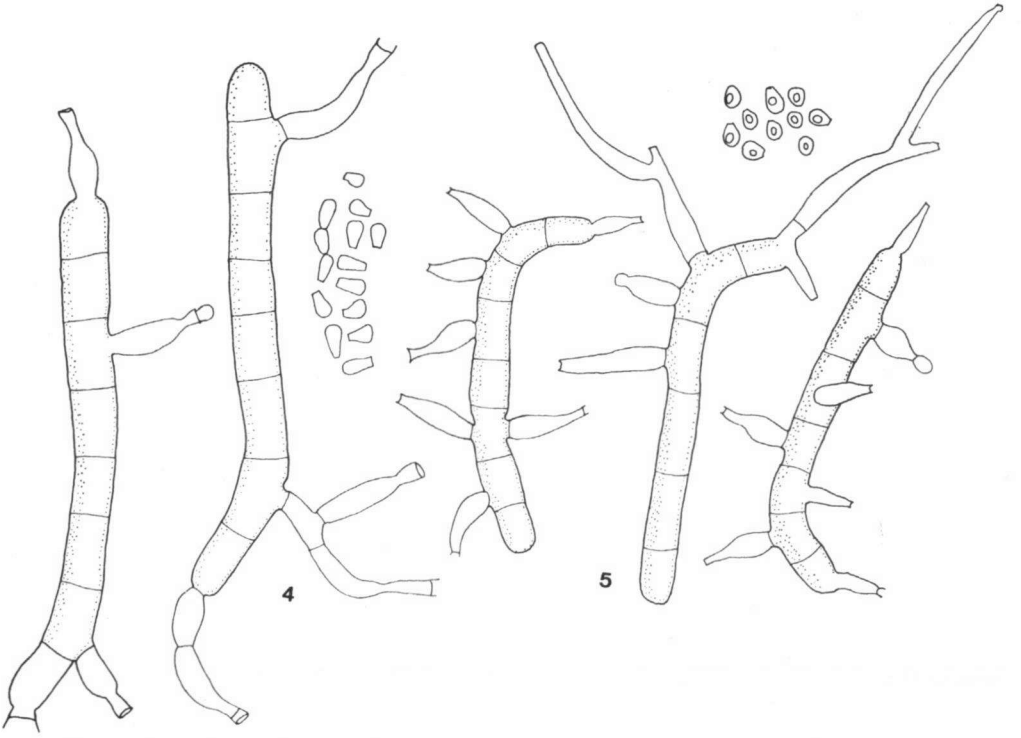

Fig. 4. *Lasiosphaeria hirsuta* and
Fig. 5. *Lasiosphaeria ovina*. Germinating ascospores in fresh collections from the Teutoburger Wald, W.-Germany, 1972 (1000 : 1).

and entomogenous genus *Cordyceps* conidial states of *Verticillium* (Gams, 1971a) and of other phialidic genera are known, while in *Ophiocordyceps clavulata* (Schw.) Petch a conidial state with sympodial conidiogenous cells and solitary (?) conidia has been observed. It was placed by Petch (1933) and Balazy (1971) in *Hirsutella* as *H. lecaniicola* (Jaap) Petch because of the conidiogenous cells with a strongly swollen basal part and slender apical elongations. Mains (1950) accommodated the species in *Hymenostilbe* Petch because of the absence of a slimy sheath on the conidia. The type species of this genus *H. muscaria* Petch, however, has conidiogenous cells of constant width with closely aggregated slightly prominent conidiiferous scars at the tip, and forms blastoconidia. Moreover, Balazy (l.c.) observed a thin slime sheath on the conidia in pure culture. Typical species of *Hirsutella* have non-proliferating phialides with usually more than one conidium. But more information from pure cultures is still lacking.

Another case of closely related species with phialides and with sympodial conidiogenous cells with holoblastic conidium formation has been found in the ascomycete genus *Lasiosphaeria*. In *L. hirsuta* (Fr.) Ces. & de Not. (Hughes, 1951; Fig. 4) and *L. ovina* (Fr.) Ces. & de Not. (Fig. 5) the germinating ascospores form *Phialophora*-like phialides, whereas in *L. spermoides* (Hoffm. ex Fr.) Ces. & de Not. (Fig. 6) the conidiogenous cells proliferate in a densely sympodial manner and each denticle apparently forms only a single conidium.

In this paper two species with solitary and serial phialoconidia have been merged into one genus. On the other hand it seems questionable, whether the species with solitary conidia borne on denticles placed by Deighton & Pirozynski (1972) in the genus *Sympodiophora* Arnold are really congeneric with the type species *S. stereicola* Arnold, the conidial state of *Hypomyces semitranslucens* Arnold; in this species the acrotonously proliferating conidiogenous cells bear star-like clusters of conidia on each opening (cf. Gams & Hoozemans, 1970, under *Cladobotryum* spec.). Some of

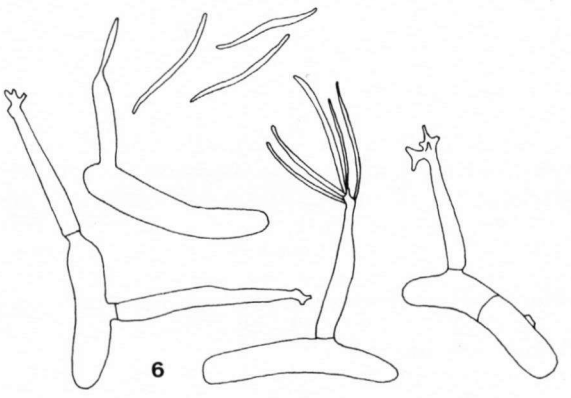

Fig. 6. *Lasiosphaeria spermoides*. Germinating ascospores in fresh collection from Rosenfelder See, Kr. Plön, W.-Germany (1000 : 1).

the species described by Deighton & Pirozynski might be better accommodated in *Pseudofusarium*.

The examples of the present study show a great deal of similarity between species with phialides forming serial conidia and others with solitary conidia. Further investigations are required to determine whether the term phialide should be refused to the latter group. Especially in the case of *Sibirina fungicola* the conidiogenous cells cannot be regarded as being of the holoblastic type.

Moreover, these examples show that conidium ontogeny is only one criterium amongst others which cannot be neglected in constructing a natural classification of Hyphomycetes. An illustrative example supporting this view is the genus *Cladobotryum* as redefined by Gams & Hoozemans (1970) in which conidial states of related *Hypomyces* species are combined. Study of conidium ontogeny, however, has shown (Cole & Kendrick, 1971), that in the type species *C. varium* Nees per Duby [= *Hypomyces aurantius* (Pers. per S. F. Gray) Tul., *stat. con.*] the conidia are abstricted in a retrogressive way, while in *C. mycophilum* (Oudem.) W. Gams & Hoozem. (= *Hypomyces odoratus* Arnold, *stat. con.*) the conidiogenous locus remains stable, and in the most closely related *C. dendroides* (Bull. per Mérat) W. Gams & Hoozem. [= *Hypomyces rosellus* (Alb. & Schw. per Fr.) Tul., *stat. con.*] it is progressive. Distribution of these species over different genera does not seem desirable not only for reasons of affinity in the perfect states, but also because of their great similarity in many other characters, such as pigmentation, mycelium structure and ramification of the conidiophores.

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