Anamorphs of pyrenomycetous Ascomycetes III. The *Sporoschisma* and *Chalara* anamorphs of *Melanochaeta aotearoae*

E. Müller

Mikrobiologisches Institut, Eidg. Technische Hochschule, Universitätstr. 2, CH-8092 Zürich, Switzerland

&

G. J. Samuels

Plant Diseases Division, DSIR, Private Bag, Auckland, New Zealand

Zusammenfassung. — Die erstmals gelungene Reinkultur des Pyrenomyceten *Melanochaeta aotearoae* (Hughes) Müller, Harr & Sulmont brachte den Beweis für seine Zusammengehörigkeit mit *Sporoschisma mirabile* Berkeley & Broome und *Chalara* sp. als Syn-Anamorphe. Die Taxonomie von *Melanochaeta*, *Sporoschisma*, *Chalara* und verwandten Pilzen wird diskutiert und der Ascomycet zusammen mit seinen Anamorphen beschrieben und abgebildet.

Summary. — *Melanochaeta aotearoae* (Hughes) Müller, Harr & Sulmont was grown in culture for the first time. *Melanochaeta aotearoae* is proven to have *Sporoschisma mirabile* Berkeley & Broome and *Chalara* sp. synanamorphs. Taxonomy of *Melanochaeta*, *Sporoschisma*, *Chalara* and related fungi is discussed. *Melanochaeta aotearoae* and its *Sporoschisma* and *Chalara* synanamorphs are redescribed and illustrated.

Introduction

Sporoschisma Berkeley & Broome, a genus of dematiaceous Hyphomycetes, is characterized by cylindrical, monophialidic conidiophores; integrated, tubular phialides; pale brown to nearly black, cylindrical, catenate phragmoconidia and erect setae, each of which has a somewhat swollen tip that is enclosed within mucilaginous material ("capitate hyphae"). It is a genus of four known species (Hughes, 1966; Nag Raj & Kendrick, 1975), two of which were first linked through juxtaposition to teleomorphs: *Sporoschisma saccardoi* Hughes & Mason to *Melanochaeta hemipsila* (Berkeley & Broome) Müller, Harr & Sulmont (1969) and *S. mirabile* Berkeley & Broome to *Melanochaeta aotearoae* (Hughes) Müller, Harr & Sulmont (1969). The connection of *S. saccardoi* to *M. hemipsila* was established through cultural work (Müller & al., 1969) and the relationship of *S. mirabile* to *M. aotearoae* is proven here

for the first time. Although the connections between these ascomycetes and their anamorphs has been demonstrated in pure culture, the morphological similarities between the sexual and asexual stages are so great, however, as to render cultural proof of the connection almost superfluous. Ascomata of *Melanochaeta* species bear capitate hyphae on their walls that are morphologically indistinguishable from the capitate hyphae arising from the substrate along with conidiophores and also ascospores and conidia are phaeophragmospores. A similar case of morphological similarity between teleomorphs and anamorphs in this group is found in *Porosphaerellopsis sporoschismophora* (SAMUELS & MÜLLER) MÜLLER & SAMUELS and its *Sporoschismopsis* HOLUBOVÁ-JECHOVÁ & HENNEBERT anamorph (SAMUELS & MÜLLER, 1978; as *Porosphaeria sporoschismophora* SAMUELS & MÜLLER). Ascospores of *P. sporoschismophora* and conidia of its *Sporoschismopsis* anamorph are phaeophragmospores that have a pore at each end; sterile hairs are lacking in both phases. *Porosphaerellopsis* and *Melanochaeta* are closely related genera within the Trichosphaeriaceae. *Sporoschisma* and *Sporoschismopsis* are also apparently closely related, differing in the presence of capitate hyphae in the former and the absence of sterile hairs in the latter. The fact that these two genera have distinct teleomorphs emphasizes their generic separation even though they are separated on the basis of what is apparently a single character, the presence or absence of setae.

We cultured a recent collection of *M. aotearoae* found in New Zealand and it produced a *Chalara* (CORDA) RABENHORST phase in addition to the *Sporoschisma mirabile* anamorph. With the possible exception of *S. juvenile* BOUDIER (ELLIS, 1971), no species of *Sporoschisma* is known to be polymorphic. *Sporoschisma juvenile* is redescribed by ELLIS (1971) as having "conidia in very long chains, often remaining hyaline and non-septate for a long time, eventually becoming 3-septate, pale brown and minutely verruculose; ..." (ELLIS, 1971; p. 206, fig. 363 B). The illustration provided by ELLIS suggests that *S. juvenile* has a *Chalara* synanamorph with oblong, unicellular, hyaline conidia. The *Chalara* synanamorph of *M. aotearoae* is known only from culture; it is not present on either of the recent collections that were grown in pure culture (see below) or on the type specimen of *M. aotearoae* (PDD). It is not one of the species given by NAG RAJ & KENDRICK (1975) in their monograph of *Chalara*.

Chalara species are often polymorphic (NAG RAJ & KENDRICK, 1975) and these synanamorphs are often given their own names. In *Thielaviopsis* WENT, dark, thick-walled, catenate conidia are predominantly enteroblastic-phialidic; some may be holoblastic. *Chalaropsis* PEYRONEL is characterized by dark, holoblastic-sympodial aleuriospores. *Chaetochalara* SUTTON & PIROZYNSKI is the setose counterpart of *Chalara*, bearing the same relationship to *Chalara* as *Sporoschisma*

does to *Sporoschismopsis*. Pleomorphism in hyphomycetes related to *Chalara* is apparently not uncommon. Booth (1957) described "micro-" and "macroconidial" phases for the *Codinaea* anamorph of *Chaetosphaeria callimorpha* (Montagne) Saccardo. The two conidial forms differed only in their conidial measurements and in the more robust, typically *Codinaea*, conidiophores of the macroconidial phase. Both phases, however, were recognizeable as *Codinaea*. In a more extreme case Toyazaki & Udagawa (1981) found that *Codinaea dimorpha* produces a typical *Codinaea* phase with septate, setose conidia and a *Chloridium* phase with acicular, aseptate conidia. As in *Melanochaeta aotearoae*, pleomorphism in *Chaetosphaeria callimorpha* and *Codinaea dimorpha* is known only *in vitro*, whether such alternate phases occur in nature is unknown.

Various ascomycetes are known to have *Chalara* and its segregates as anamorphs (Nag Raj & Kendrick, 1975). *Chalara* has been linked

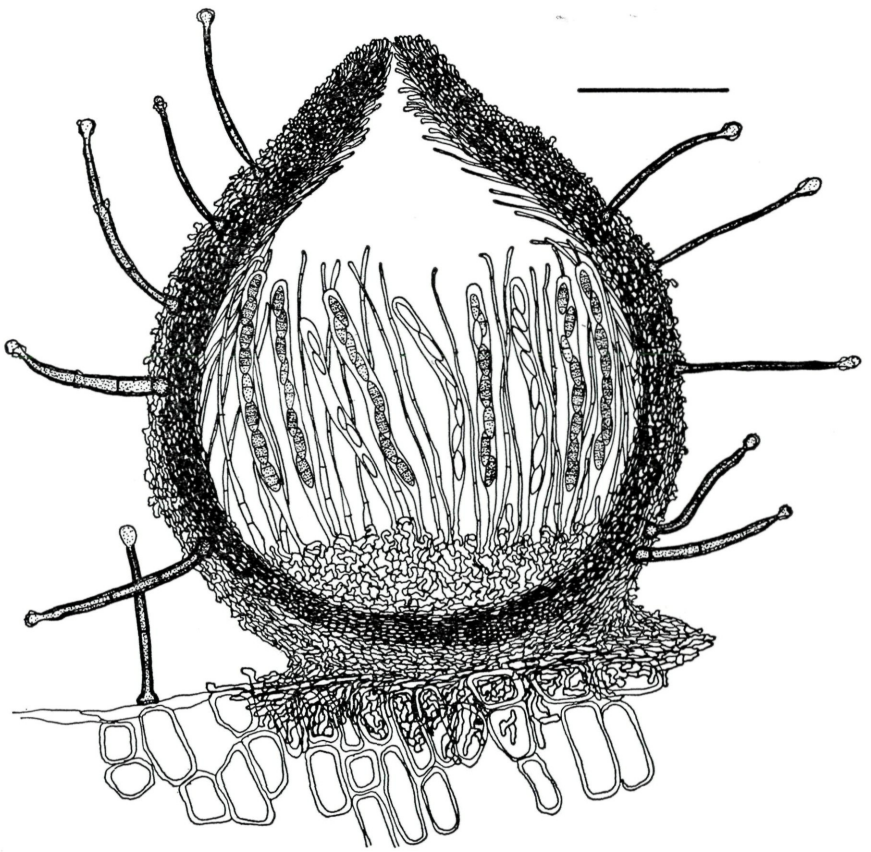

Fig. 1. Lontitudinal section through a mature ascoma (PDD 41943, line = 100 μm)

to *Pyxidiophora* Brefeld & Tavel (Lundquist, 1980) and, now, *Melanochaeta*. In addition, *Chalara* — as well as *Chalaropsis* and *Thielaviopsis* — anamorphs have been proven for *Ceratocystis* Ellis & Halsted. *Chaetochalara* is the anamorph of two inoperculate discomycetes, viz. *Calycellina cardinensis* Nag Raj & Kendrick and *Hyaloscypha cladii* Nag Raj & Kendrick. These teleomorphs represent three developmental groups with *Ceratocysis* and *Pyxidiophora* in one, *Melanochaeta* in a second and the two cup fungi in a third. The *Chalara* phases may also be divided into three groups. Conidiophores of the *Chalara* anamorphs of the *Ceratocystis* group (i. e. *Chalara*, s. str.) tend to have a swollen venter. The *Chalara* anamorph of *Melanochaeta aotearoae* is cylindrical or with a constricted base; this may be a reduced form of the *Sporoschisma* phase which it resembles. Conidiophores of the *Chaetochalara* states of *Calycellina* and *Hyaloscypha* tend to have enlarged venters as in the *Chalara* states of *Ceratocystis* but these anamorphs are separated from *Chalara* by the presence of setae.

Material and Methods

Ascospores were germinated on cornmeal dextrose agar (CMD, Difco); characteristics in culture were observed on CMD. Dried material was rehydrated in 3% KOH. Microscopic observations were made on material mounted in 100% lactic acid.

Description of Melanochaeta Aotearoae

Melanochaeta aotearoae (Hughes) Müller, Harr & Sulmont, Rev. Mycol. (Paris). 33: 378. 1969.

= *Chaetosphaeria aotearoae* Hughes, New Zealand J. Bot. 4: 78. 1966.

Synanamorphs. *Sporoschisma mirabile* Berkeley & Broome in Berkeley, Gardeners' Chronicle 1847: 540. 1847.

Chalara sp.

Colonies black, consisting of tufts of *Sporoschisma* conidiophores and capitate hyphae borne on small, pseudoparenchymatous stromata, scattered capitate hyphae, and ascomata. Ascomata solitary, scattered, superficial, anchored to the substrate by an indistinct basal stroma, broadly pyriform with a short, conical papillum, 240—470 μm high × 220—400 μm wide; ascomatal wall smooth and shining; stiff, erect unbranched, dark brown to black, 100—200 μm long × 5—6 μm wide hairs arising from all over surface of ascomatal wall, each with a slightly swollen, light brown to colorless cap; ascomata not collapsing when dry; cinereous with a black ostiolar area or entirely black, especially when aged. Ascomatal wall ca. 40 μm wide, entirely opaque, details of cells at surface of wall not visible in whole mount. Ascomatal wall in longitudinal section comprising 2 regions; outer

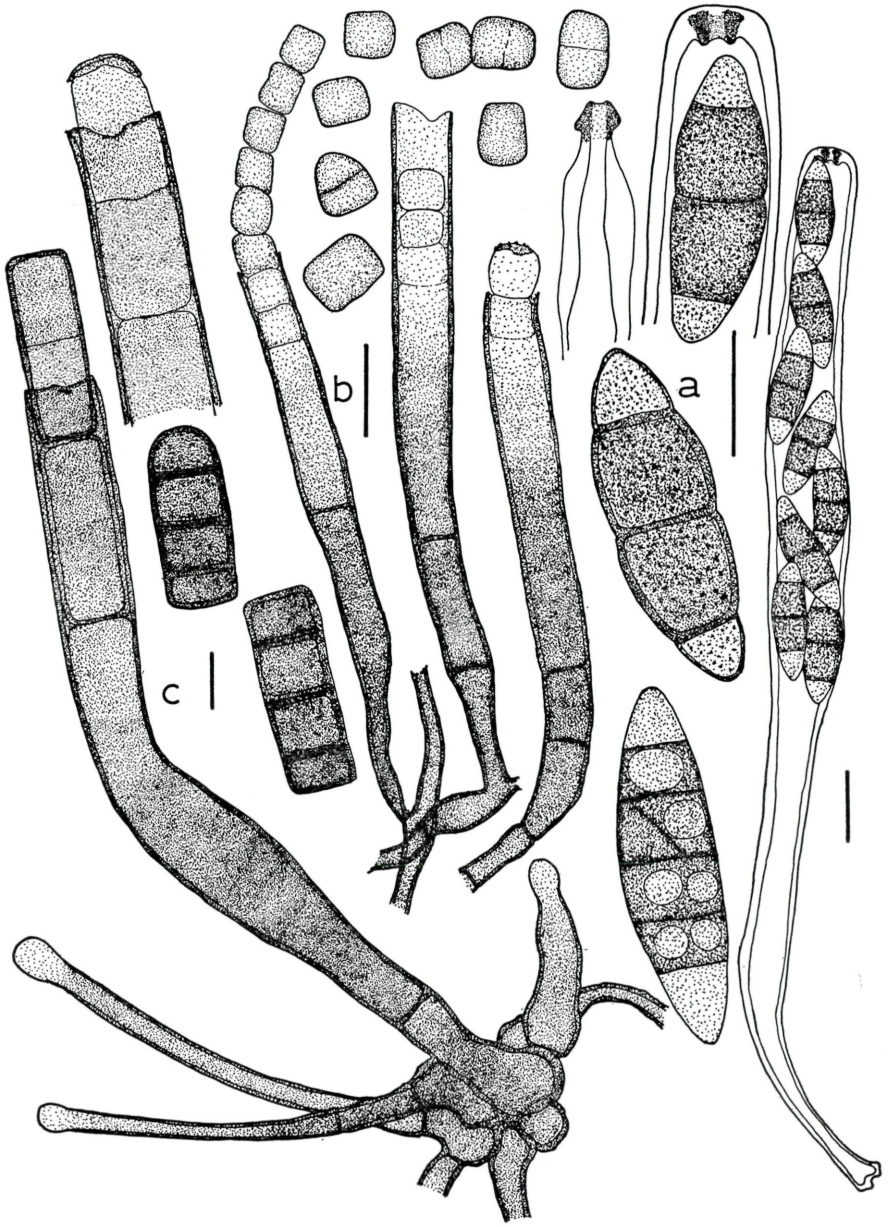

Fig. 2. a. Asci and ascospores, the ascus tip on the left as seen following discharge of ascospores (PDD 41943); 100% lactic acid; asci as seen in phase contrast microscopy, discharged ascospores as seen with bright field microscopy
b. *Chalara* anamorph formed on CMD (PDD 41943, 100% lactic acid/bright field microscopy)
c. *Sporoschisma* anamorph formed on CMD (PDD 41942, 100% lactic acid / bright field microscopy, all lines = 10 μm)

region *ca.* 10 μm wide, formed of densely matted, lightly pigmented, short-celled, 3—5 μm wide hyphae with walls *ca.* 0.5 μm thick, cells of outer region at ascomatal base 5—7 μm long ×2—4 μm wide and continuous with the basal stroma; capitate hyphae arising from cells at surface of ascomatal wall. Inner region of wall *ca.* 20 μm wide, cells ± elliptical in outline, ca. 5 μm long ×2—3 μm wide with heavily pigmented, ≤ 5 μm thick walls; 1—2 layers of thin-walled, fusiform cells lining the locule. Ostiolar region not structurally distinct from the wall below; ostiolar canal lined with periphyses, periphyses continuous with the paraphyses.

Asci cylindrical to narrowly clavate, (150—)170—230×10—13(—15) μm, unitunicate, apex with a refractive, non-amyloid ring; 8-spored, ascospores partially biseriate; asci arising in a hymenium in base of ascoma, interspersed with unbranched, 3—5 μm wide, septate paraphyses. Paraphyses forming continuously from ascomatal base to ostiolar canal, approximately the same length as asci at the hymenium, becoming progressively shorter toward the ostiolar canal; subhymenial tissue thin-walled, hyaline, short-celled hyphae.

Ascospores fusiform, (21.0—)22.8—29.8(—33.0)×(5.0—)7.0—9.5(—10.5) μm; with 3 transverse septa, rarely 1—2 longitudinal or diagonal septa also forming, central 2 cells darker brown and with thicker walls than the lighter brown end cells, finely punctate.

Characteristics in Culture. Ascospores germinating on CMD within 12 h at 20° C.

Colonies (grown on CMD 1 mo. at 18—21° C in diffused daylight 5—7 cm diam.) velvety with short aerial hyphae, opaque, black with a white margin. *Chalara* conidiophores inconspicuous, forming profusely throughout the colony arising usually as terminations of hyphae rather than as lateral branches, macronematous, unbranched, brown-olivaceous (in 100% lactic acid), smooth, tapering gently and uniformly from base to tip, (0—)1—2-septate, (40—)45—65(—73) μm long, (2—)3—6(—7) μm wide basally, walls *ca.* 1 μm thick; terminating in a single phialide. Phialides cylindrical, 37—49(—55) μm long, without a morphologically distinct venter; collarette up to 25 μm deep, containing up to 5 conidia, (5—)6—7 μm wide at the opening. Conidia extruded in long chains, first conidium rounded, conidia otherwise rectangular to cuboidal, (5.0—)5.6—9.2(—11.0)×5.0—6.6(—8.0) μm, 0(—1) septate, pale brown (in 100% lactic acid).

Sporoschisma synanamorph forming after 6 weeks (on CMD at 18—21° C, diffused daylight). Conidiophores arising together with capitate hyphae in scattered fascicles of a few, from a small, pseudoparenchymatous base, macronematous, unbranched, black (in 100% lactic acid), smooth, cylindrical, abruptly narrowed at the base, 1—2-septate, (140—)160—205 μm long terminating in a single phialide. Phialides cylindrical, 125—155 μm long, collarette ca. 40 μm deep,

containing 1—2 conidia, (11—)13—15 µm wide at the torn opening. Conidia extruded in long chains, the first conidium with a rounded apex, conidia otherwise rectangular, (25—)32—40(—42)×(11—)12—14(—15) µm, 3-septate, entirely dark brown to black (100% lactic acid).

Capitate hyphae scattered throughout the colony, standing singly or joined in fascicles of conidiophores of *Sporoschisma* or *Chalara* synanamorphs, 70—130 µm long, unbranched, black by reflected light, brown by transmitted light (in 100% lactic acid), cylindrical, 2—3-septate, (76—)81—119(—130) µm long, 5—6 µm wide basally, tip swollen, clavate to globose, 7—10 µm wide with a gelatinous (?) sheath, lighter in color; sometimes proliferating through the swollen tip.

Habitat. — Decaying wood.

Known Distribution. — Cosmopolitan (Holubová-Jechová 1979, Hughes 1966, Ellis 1971, Matsushima 1980).

Specimens examined. — New Zealand: Waikato, Ngaruawahia, Hakarimata Trig Track, on decorticated wood, leg. Samuels, Dingley & Johnston, 23 Jan 1981 (PDD 41944); Auckland, Waitakere Ranges, Waitemata City, Huia, Parau Track, on decorticated wood, leg. Samuels (80—148) & Johnston, 23 Oct 1980 (PDD 41942); second collection, same data, Samuels (80—152) (PDD 41943).

References

Booth, C. (1957). Studies of Pyrenomycetes: I. Four species of *Chaetosphaeria*, two with *Catenularia conidia*. II. *Melanopsamma pomiformis* and its *Stachybotrys* conidia. — Mycol. Pap. 68: 1—27.

Ellis, M. B. (1971). Dematiaceous Hyphomycetes. — Commonwealth Mycological Institute. Kew, Surrey. 608 pp.

Holubová-Jechová, V. (1979). Lignicolous and some other saprophytic Hyphomycetes from Hungary. — Česká Mykol. 33: 138—149.

Hughes, S. J. (1966). New Zealand Fungi 6. *Sporoschisma* Berk. & Br. — New Zealand J. Bot. 4: 77—85.

Lundquist, N. (1980). On the genus *Pyxidiophora* sensu lato (Pyrenomycetes). — Bot. Not. 133: 121—144.

Matsushima, T. (1980). Saprophytic microfungi from Taiwan. Part 1. Hyphomycetes. — Matsushima Mycological Memoires No. 1. 82 p.

Müller, E., J. Harr & P. Sulmont (1969). Deux Ascomycetes dont le stade conidien presente des conidies phaeophragmiées endogènes. — Rev. Mycol. (Paris) 33: 369—378.

Nag Raj, T. R. & B. Kendrick (1975). A monograph of *Chalara* and allied genera. — Wilfrid Laurier University Press, Waterloo, Ontario. 200 pp.

Samuels, G. J. & E. Müller 1978 (1979). Life history studies of Brazilian Ascomycetes 1. Two new genera of the Sphaeriaceae having, respectively, *Sporoschisma*-like and *Codinaea* anamorphs. — Sydowia 31: 126—136.

Toyazaki, N. & S.-I. Udagawa (1981). An undescribed pleomorphic species of *Codinaea*. — Mycotaxon 13: 450—456.