Anamorphs of pyrenomycetous Ascomycetes I. *Rhamphoria* NIESSL and *Trichosphaerella* BOMMER, ROUSSEAU & SACCARDO

E. MÜLLER

Mikrobiologisches Institut, Eidg. Technischen Hochschule, Universitätstr. 2, CH-8006 Zürich, Switzerland

&

G. J. SAMUELS

Plant Diseases Division, D. S. I. R., Private Bag, Auckland, New Zealand

Zusammenfassung. — Erstmals sind Teleomorph-Anamorph Beziehungen der unitunicaten Pyrenomyceten *Rhamphoria pyriformis* (Anamorph: *Phaeoisaria*) und *Trichosphaerella ceratophora* (Anamorph: ähnlich *Tritirachium*) mit Hilfe von Kulturversuchen nachgewiesen worden.

Introduction

The ascomycetous family Trichosphaeriaceae WINTER 1855 (= Sphaeriaceae FRIES sensu MÜLLER & v. ARX, 1962) exhibits two types of conidial ontogeny: phialidic and holoblastic-sympodial. Phialidic development is found in *Chaetosphaeria* L.-R. & C. TULASNE [*Catenularia* GROVE, *Chloridium* LINK, *Codinaea* MAIRE, *Menispora* PERSOON, *Stachybotrys* CORDA, *Zanclospora* HUGHES & KENDRICK (KENDRICK, 1979)], *Niesslia* AUERSWALD (*Monocillium* SAKSENA; GAMS, 1971), *Melanochaeta* MÜLLER et al. (*Sporoschisma* BERKELEY & BROOME; MÜLLER et al., 1969), *Porosphaerellopsis* SAMUELS & MÜLLER [1]) (*Sporoschismopsis* HOLUBOVÁ-JECHOVÁ & HENNEBERT, 1972; SAMUELS & MÜLLER, 1978, as *Sporoschisma*-like) and *Striatosphaeria* SAMUELS & MÜLLER (*Codinaea*, SAMUELS & MÜLLER, 1978). In *Chaetosphaerella* BOOTH & MÜLLER both phialidic (*Phialocephala* KENDRICK) and holoblastic-sympodial (*Oedemium* LINK, see HUGHES & HENNEBERT, 1963) phases are known. Finally, the only known anamorph of *Helminthosphaeria* FUCKEL is holoblastic-sympodial

[1]) *Porosphaerellopsis* SAMUELS & MÜLLER, nom. nov., with its type species *P. sporoschismophora* (SAMUELS & MÜLLER) MÜLLER & SAMUELS, comb. nov. (= *Porosphaeria sporoschismophora* SAMUELS & MÜLLER, Sydowia 31: 127. 1978.), is introduced as a substitute for *Porosphaeria* SAMUELS & MÜLLER (Sydowia 31: 127. 1978.) which is a later homonym for *Porosphaera* DUMORTIER (Commentaires Botaniques p. 31. 1822).

(*Diplococcum* GROVE; KENDRICK 1979). In the present paper two further genera exhibiting holoblastic-sympodial conidial ontogeny are added: *Rhamphoria* NIESSL and *Trichosphaerella* BOMMER, ROUSSEAU & SACCARDO.

Descriptions

1. *Rhamphoria pyriformis* (FRIES) HÖHNEL — Fig. 1

Rhamphoria is characterized by black, solitary superficial ascomata that form on rotting wood; unitunicate, cylindrical asci (fig. 1,a), filiform paraphyses and hyaline, muriform ascospores (fig. 1,b). The twelve species accepted by SIVANESAN (1976) differ mainly in the number of ascosporal septa and the size and shape of ascospores. Three species, *R. pyriformis* (FRIES) v. HÖHNEL, *R. thelecarpoidea* HÖHNEL and *R. tympanidispora* REHM, are known to produce conidia directly from ascospores still held within asci.

A *Rhamphoria* species collected in spring 1981 in Switzerland on rotten wood of *Carpinus betulus* L. (Switzerland, Kt. Zürich, Zweidlen, near railway station, 4. 5. 1981, ZT) was difficult to identify with any of the described species. Ascospores found in just one ascoma are so variable in shape, size and septation (fig. 1,b) as to include most of the spore types described for the various species in the genus (SIVANESAN, 1976). Because none of the described species has been collected more than a few times, and some have been reported only once, we suspect that the number of species will ultimately be much lower than is currently accepted. Even the ability of ascospores to produce conidia directly does not seem to be a taxonomically useful character. We observed asci with and without budding ascospores and in any one ascus it was possible to observe the entire range of variation in ascospore morphology described for the genus, including the elongate-clavate shape of the ascospores found in type material of *R. tympanidispora* and *R. thelecarpoidea* and drawn by SIVANESAN (1976) for *R. pyriformis*. We have therefore identified the collection as *R. pyriformis*, the oldest available epithet within the genus. This species has not previously been reported for Switzerland.

CHARACTERISTICS IN CULTURE.

Single ascospores of the Swiss collection of *R. pyriformis* readily produced a germ tube. Growth of the colony was slow, 1—1.5 mm after 4 weeks on malt agar at 18° C; the mycelium was colorless at first but gradually became yellowish and finally — after 2—3 months — brown. Within three weeks, short conidiogenous cells formed from apparently undifferentiated hyphae. Later erect, unbranched or branched, brown, 2 µm wide conidiophores formed; the conidiogenous areas were somewhat swollen or elongated, 2—5 µm wide, at first terminal but became intercalary as the conidiophore elongated

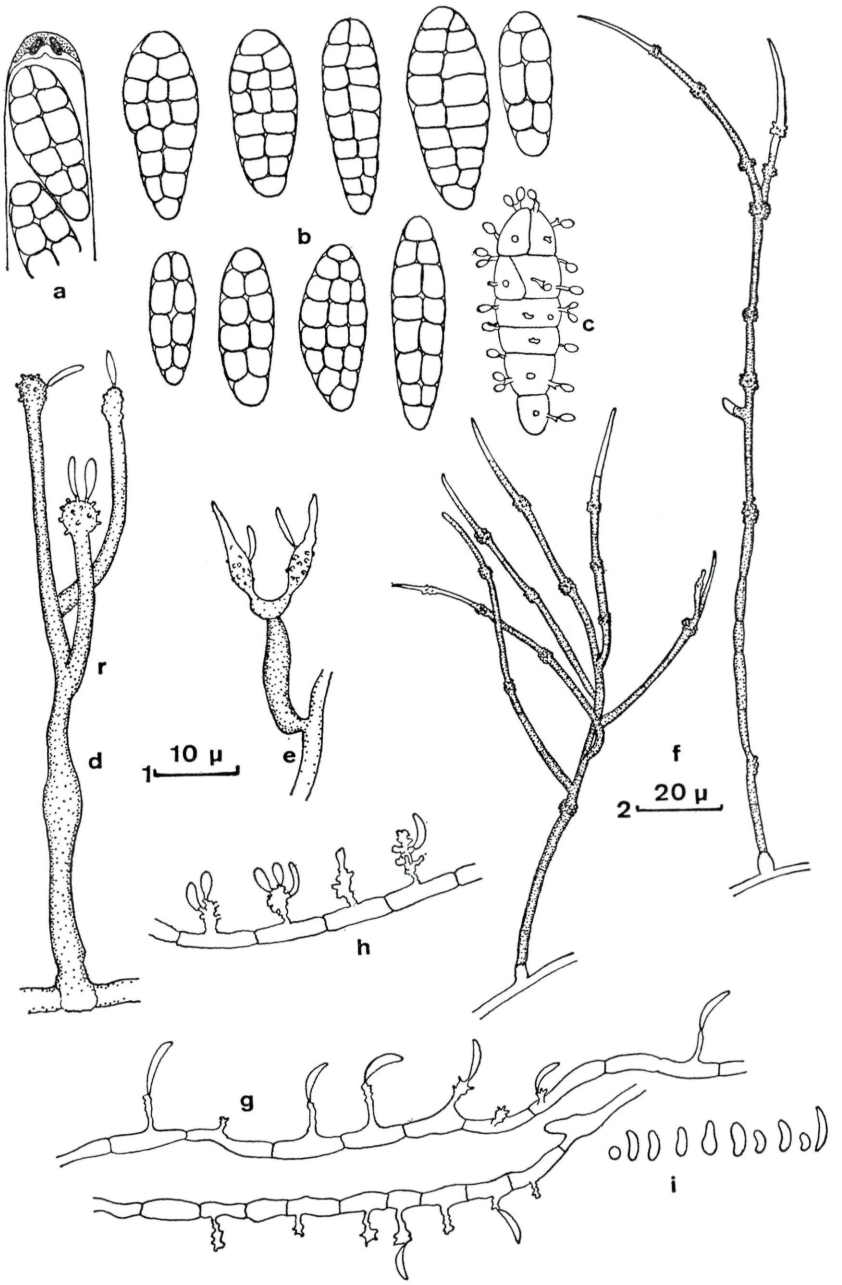

Fig. 1. *Rhamphoria pyriformis*; a: apical portion of ascus with ascospores; b: ascospores of one single perithecium demonstrating variation in shape, size and septation; c: denticulate ascospore (from the type of *R. tympanidispora*); d: conidiophore with globose fertile portions; f: conidiophores in culture after 5 months; g: anamorph in young cultures (3 weeks); h: conidiogenous cells on vegetative hyphae in old cultures (5 months); i: conidia from all anamorphic structures (scale 1: a, b, c, d, e, g, h, i; scale 2: f)

and ultimately became up to 200 µm long (fig. 1,f). Conidia were borne holoblastically on denticles and were at first lunate, unicellular, hyaline, 2—3×1 µm (fig. 1, g—i) but conidia formed later were irregular in shape, fusoid or even globose, ellipsoid or clavate (fig. 1,d,i).

None of the species of *Rhamphoria* has been linked to an anamorph apart from *R. pyriformis* with its ascoconidia. *R. pyriformis* therefore has three kinds of conidial structures: denticles produced directly on ascospores (fig. 1,c); small, laterally produced conidiogenous protrusions of vegetative hyphae (fig. 1,g—h), and brown, erect conidiophores with intercalary conidiogenous zones (fig. 1,d—f). Conidial production is denticulate, holoblastic-sympodial throughout.

The anamorph of *R. pyriformis* fits best with *Idriella* Nelson & Wilhelm (1956) or *Phaeoisaria* v. Höhnel sensu de Hoog & Papendorf (1976). It does not agree with any of the known species of either genus (Sutton et al., 1972; Nicot & Mouchacca, 1972; Mouchacca & Samson, 1973; de Hoog & Papendorf, 1976; v. Arx, 1982). Only the youngest conidia are lunate, a characteristic of *Idriella*, whereas with age the conidia are irregular in shape and are more typical of *Phaeoisaria*. A further argument against assigning this anamorph to *Idriella* is that Kimbrough & Atkinson (1972) reported an *Idriella* anamorph for *Hymenoscyphus caudatus* (Karsten) Dennis (Helotiales). Following the advice of Dr. J. A. v. Arx (Baarn) we have concluded that the anamorph of *R. pyriformis* is a species of *Phaeoisaria* distinguished from all other species by its small conidia.

2. *Trichosphaerella ceratophora* (v. Höhnel) E. Müller — fig. 2

Trichosphaerella is based on *T. decipiens* Bommer, Rousseau & Saccardo (Synonyms: *Bresadolella aurea* v. Höhnel, *Larseniella major* Munk). The following species were added later: *T. arecae* (Sydow) E. Müller (= *Oplotheca arecae* Sydow), *T. ceratophora* (v. Höhnel) E. Müller (= *Neorehmia ceratophora* v. Höhnel), *T. foliicola* Batista & Bezerra, *T. inaequalis* (Grove) E. Müller (= *Melanopsamella inaequalis* Grove).

The genus, considered to be closely related to *Trichosphaeria* Fuckel, is characterized by small, solitary ascomata that have dark, often branched setae (fig. 2,a), unitunicate asci and two-celled ascospores that disarticulate at the septum; paraphyses are lacking at maturity. It is generally accepted that *Bresadolella* v. Höhnel, *Neorehmia* v. Höhnel, *Larseniella* Munk and *Oplothecium* Sydow are generic synonyms, whereas *Melanopsamella* Grove, which also has disarticulating ascospores but lacks setae and is aparaphysate, has been placed in *Chaetosphaeria* (Gams & Holubová-Jechová, 1976). *Chaetosphaeria inaequalis* (Grove) Gams & Holubová-Jechová has a *Chloridium* anamorph. No true *Trichosphaerella* species has ever

been connected to an anamorph through cultural work [the *Acremonium*-like anamorph reported for *T. arecae* has not been proved by cultural studies (MÜLLER & DENNIS, 1965; GAMS, 1971)].

We collected *T. ceratophora* on rotting wood of *Carpinus betulus* (Switzerland, Kt. Zürich, Zweidlen, near railway station, 4. 5. 1981, ZT). This species has not previously been reported for Switzerland. The ascomata are dark, superficial, solitary, globose, measure 80—110 μm in diameter and are easily overlooked. There are stout, erect, unbranched or often apically branched setae scattered over the surface of the ascomatal wall (fig. 2,a—b). The ascomatal wall is up to 15 μm wide and comprises elongated, dark-walled cells; the ostiolar canal is periphysate. Asci are unitunicate, cylindrical to narrowly clavate, 28—32×4—5 μm, 8-spored (fig. 2,c). Paraphyses are lacking. Ascospores are elliptical, 6—8×3—4 μm; at first

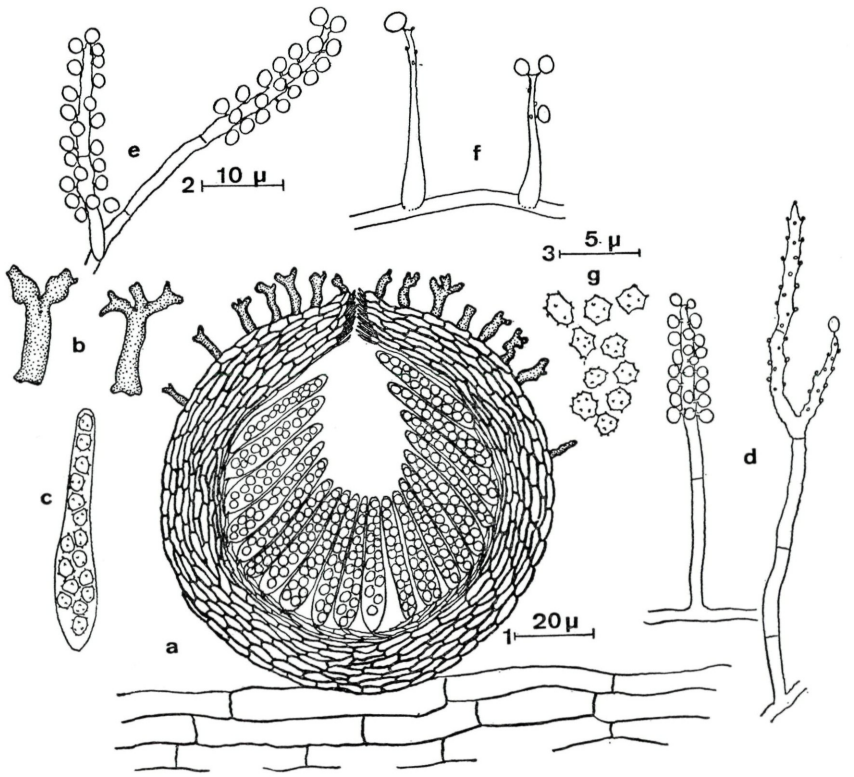

Fig. 2. *Trichosphaerella ceratophora*; a: perithecium, median section; b: perithecial setae; c: ascus with disarticulated ascospores; d: conidiogenous cell on vegetative hyphae (simple or slightly branched); e: branched conidiophores; f: small conidiogenous cells on vegetative hyphae, slightly inflated at base; g: mature conidia (scale 1: a; scale 2: b, c, d, e, f; scale 3: g)

bicellular but early disarticulating at the septum into two spinulose, hyaline subglobose part spores of equal size. The part spores are morphologically similar to the conidia.

CHARACTERISTICS IN CULTURE.

Isolated ascospores germinated slowly. Growth of the colony was slow, attaining only 1 mm after four weeks, but by that time secondary colonies had become established indicating the early production of conidia. Colonies grown in darkness were white at first but became roseus to light brown when exposed to light and comprised radially growing, irregularly branched, densely intertwined hyphae. Beginning in the center of the colony and progressing toward the margin, conidiogenous branches of hyphae formed, each of which bore a terminal rachis of denticles (fig. 2,d—f). Conidia were at first globose but gradually enlarged, becoming up to 1.5—2.5 µm diam. irregular in outline and conspicuously roughened (fig. 2,h).

It is difficult to satisfactorily place the anamorph of *T. ceratophora* into any of the known genera of Hyphomycetes. Several apparently unrelated genera produce simple conidiophores with denticulate, sympodial conidiogenesis including *Sporothrix* HEKTOEN & PERKINS ex NICOL & MARIAT (DE HOOG, 1974). *Rhinocladiella* PREUSS, *Rhinotrichiella* ARNAUD ex DE HOOG, *Ramichloridium* STAHEL ex DE HOOG (DE HOOG & HERMANIDES-NIJHOF, 1977), *Beauveria* VUILLEMIN and *Tritirachium* (DE HOOG, 1972). It differs from *Rhinocladiella*, *Rhinotrichiella* and *Ramichloridium*, which have dark colonies, in being entirely colorless or at most lightly pigmented; furthermore, the conidia of *Rhinotrichiella* are much larger than those of the anamorph of *T. ceratophora*. This anamorph bears some morphological resemblance to *Beauveria*, which also has white colonies, but the bases of the conidiogenous cells are inflated and the known *Beauveria* species are parasites of insects. The closest overall morphological fit of the anamorph of *T. ceratophora* is with *Tritirachium*. Colonies of *Tritirachium* species are white or lightly pigmented; the main disagreement with *Tritirachium* lies with the fact that conidiogenous cells of *Tritirachium* generally have a verticillate arrangement on an erect conidiophore and have a rather narrow conidiogenous rachis. Some strains accepted by DE HOOG (1972), however, have unbranched conidiophores and a broad rachis. In its cultural features, the anamorph of *Trichosphaerella ceratophora* suggests *Tritirachium oryzae* (VINCENS) DE HOOG, but it differs in growing more slowly and in having smaller and roughened conidia.

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Literature

ARX, J. A. von (1982). Notes on *Microdochium* and *Idriella*. — Sydowia 34: 30—38.

GAMS, W. (1971). *Cephalosporium*-artige Schimmelpilze (Hyphomyceten). — Gustav Fischer Verlag, Stuttgart, 262 p.

— & HOLUBOVA-JECHOVA, V. (1976). *Chloridium* and some other dematiacious hyphomycetes growing on decaying wood. — Studies in Mycology 13: 1—99.

HOLUBOVÁ-JECHOVÁ, V. & HENNEBERT, G. L. (1963). *Sporoschismopsis*, a new genus of lignicolous Hyphomycetes. — Bull. Jard. Bot. Nat. Belge 42: 385—391.

HOOG, G. S. de (1972). The genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. nov. — Studies in Mycology 1: 1—41.

— (1974). The genera *Blastobotrys*, *Sporothrix*, *Calcarisporium and Calcarisporiella* gen. nov. — Studies in Mycology 7: 1—84.

— & HERMANIDES-NIJHOFF, E. J. (1977). The black yeasts and allied Hyphomycetes. — Studies in Mycology 15: 1—222.

— & PAPENDORF, M. C. (1976). The genus *Phaeoisaria*. — Persoonia 8: 407—414.

HUGHES, S. J. & HENNEBERT, G. L. (1963). Microfuni X. *Oedemium*, *Dimera*, *Diplosporium*, *Ganglyocladium* and *Cladotrichum*. — Canad. J. Bot. 41: 773—809.

KENDRICK, B. (1979). The Whole Fungus. — National Museum of Natural Sciences, National Museums of Canada, Ottawa and the Kananaskis Foundation. Vol. I & II, 793 p.

KIMBROUGH, J. & ATKINSON, M. (1972). Cultural features and imperfect stage of *Hymenoscyphus caudatus*. — Amer. J. Bot. 59: 165—171.

MOUCHACCA, J. & SAMSON, R. A. (1973). Deux nouvelles espèces du genre *Microdochium* SYDOW. — Revue Mycol. 37: 367—275.

MÜLLER, E. & von ARX J. A. (1962). Die Gattungen der didymosporen Pyrenomyceten. — Beitr. Kryptogamenfl. Schweiz II (2): 1—922.

— & DENNIS, R. W. G. (1965): Fungi venezuelani: VIII. Plectascales, Sphaeriales, Loculoascomycetes. — Kew Bull. 19: 357—386.

—, HARR, L. & SULMONT, P. (1969). Deux ascomycètes dont le stade conidien presente des conidies phaeophragmiées endogènes. — Rev. Mycol. (Paris) 33: 369—378.

NICOT, J. & MOUCHACCA, J. (1972). Une nouvelle espèce du genre *Idriella*. — Rev. Mycol. (Paris) 36: 185—193.

SAMUELS, G. J. & MÜLLER, E. (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.

SIVANESAN, A. (1976). New British Species of *Rhamphoria*, *Trematosphaeria* and *Chaetosphaerella*. — Trans. Brit. Mycol. Soc. 67: 469—467.

SUTTON, B., PIROZYNSKI, K. A. & DEIGHTON, F. C. (1972). *Microdochium* SYD. — Canad. J. Bot. 50: 1899—1907.

WINTER, G. (1887). Die Pilze Deutschlands, Oesterreichs und der Schweiz. — Dr. L. Rabenhorst's Kryptogamen-Flora, 2. Aufl. 1, ii. Abt.: Ascomyceten, Gymnoasceen und Pyrenomyceten (E. Kummer, Leipzig, 928 S.).