

Taxonomy and phylogeny of the genus *Mycosphaerella* and its anamorphs

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Summary

The genus *Mycosphaerella* has been linked to more than 30 anamorphic form genera, which together represent several thousand species, the majority of which are plant pathogens. Historically species have been regarded as novel based on their hosts, with fungal morphology accepted as important among taxa occurring in specific plant families. Host specificity and anamorph-teleomorph connections have proven difficult to study, largely due to the relatively few fungal cultures available. During the course of the past 20 years a concerted effort has been made to collect these fungi, and devise methods to cultivate them to enable these questions to be addressed. Major findings from this work are that *Mycosphaerella* is polyphyletic, incorporating several anamorphic genera with formerly unknown affiliations. Teleomorph morphology was shown to be too narrowly defined in some cases, and again too widely in others. Species of *Cladosporium*, which have a characteristic conidial hilum and scar structure (coronate-type), were excluded from *Mycosphaerella*, and placed in a new genus, *Davidiella*, which is distinguished from *Mycosphaerella* by having irregular, somewhat angular lumens inside the ascospore cells, versus the normal guttules found in *Mycosphaerella*. Species of *Sphaerulina* that have 3-septate ascospores, but form *Pseudocercospora* anamorphs, were found to belong to *Mycosphaerella*, suggesting ascospore septation to be more variable. Several taxa that occur in extreme environments (especially on hosts with hard, leathery leaves), were shown to belong to *Teratosphaeria*, distinguished from *Mycosphaerella* by having hamathecial remnants, a multi-layered ascad endotunica, and ascospores with sheaths, that frequently turn brown while still in their asci. Anamorphs were shown to also differ between *Mycosphaerella* and *Teratosphaeria*. Among *Mycosphaerella* anamorphs, *Passalora* was shown to include species formerly placed in *Mycovellosiella* and *Phaeoramularia*, while *Pseudocercospora* was again shown to include species placed in *Cercostigmina*, *Stigmina*, and *Phaeoisariopsis*. Anamorph genera newly linked to *Mycosphaerella* include *Trochophora*, *Verrucisporota*, *Ramichloridium*, *Periconiella* and *Phaeophleospora*. *Teratosphaeria*, on the other hand, had anamorph genera such as *Batcheloromyces*, *Catenulostroma*, *Cibiessia*, *Colletogloeopsis*, *Davisoniella*, *Kirramyces*, *Nothostrasseria*, *Phaeothecoidea* and *Readeriella*. *Mycosphaerella*-like species with *Dissoconium* anamorphs appeared to represent a separate lineage. Finally, several species were revealed to not be host specific, while others were again strictly host specific, suggesting that no general rule can be applied. By designing degenerate mating type primers, proof could also be obtained that several apparently asexual species such as *Cercospora beticola*, *C. zaeae-maydis*, *C. zeina* and *Septoria passerini* are apparently undergoing cryptic sex, while others such as *Dothistroma septospora*, *D. pini* and *Passalora fulva* are heterothallic, with both mating types only occurring in some continents. Even though being polyphyletic, *Mycosphaerella* remains the largest genus of ascomycetous fungi known, with current species numbers of around 10 000 taxa shown to be conservative. Because most species are plant pathogens, detailed knowledge of their host specificity, sexual cycle and distribution will remain of paramount importance to plant pathologists and quarantine officers who must control the diseases associated with *Mycosphaerella* on the one hand, and enhance free trade in agricultural and forestry produce on the other.

What is *Mycosphaerella*?

Species of *Mycosphaerella* have adapted in various ways to different ecosystems, and vary from being saprobic, plant pathogenic to hyperparasitic (de Hoog et al. 1991, Goodwin et al. 2001, Jackson et al. 2004, Arzanlou et al. 2007b). *Mycosphaerella* spp. are among the most common and destructive plant pathogens known, causing considerable economic losses on a wide variety of host plants worldwide, including economically important crops such as banana, cereals, sugar beet, strawberry, soybean, citrus, eucalypts, acacia, pines and many others (Farr et al. 1995, Crous & Braun 2003). Plant pathogenic *Mycosphaerella* species are mainly foliicolous, although some are associated with stem cankers (Cortinas et al. 2006), fruit lesions (Pretorius et al. 2003) or blemishes, spots and specks (Batzer et al. 2008). Damage is usually due to defoliation, which reduces the photosynthetic capacity of the crop, leading to growth loss. Some species, such as *M. citri*, affect both leaves and fruits. Others such as *M. fijiensis*, infect banana leaves, thereby reducing the photosynthetic capacity of the crop, and also induces physiological changes resulting in premature ripening of fruit (Carrier et al. 2000, Marin et al. 2003).

The first generic description for *Mycosphaerella* (1884) was that of *Sphaerella* (1882). Saccardo placed all species of *Sphaeria* with presumably 1-septate, hyaline ascospores in *Sphaerella*. The genus *Sphaerella* was, however, already in use for green algae, and thus all these taxa had to be placed in *Mycosphaerella* (Aptroot 2006), which is based on *M. punctiformis* (Verkley et al. 2004). Despite the hyaline, 1-septate ascospores reported in the type by Persoon (1794), most authors at the beginning of the 19th century worked without microscopes, and thus what they described as a *Sphaeria* or *Sphaerella* species, literally meant a 'spherical' fruiting body (Aptroot 2006). Soon it became standard to also describe collections from different hosts as new species, which later led to many taxa being reduced to synonymy (Von Arx 1949, Barr 1972, Tomilin 1979, Corlett 1991, Aptroot 2006). In the recent revision of *Mycosphaerella* names, Aptroot (2006) treated close to 10 000 taxa, recognising around 3 000 species.

In her treatment of North American taxa, Barr (1972) recognised two subgenera, *Eu-Mycosphaerella* and *Didymellina* (including the section *Cymadothea*), and 10 sections. The sub-genera were separated on the basis of the shape of their asci and anamorphs, and the sections based on ascospore shape, and/or parasitic or saprobic habit. Von Arx (1983) found the subdivision unsatisfactory, because the characters were inordinately divergent.

The sections of Barr were refined by Crous et al. (2000) as follows:

Section *Mycosphaerella*: cylindrical asci and mostly uniseriate, thin-walled, often small ascospores that are constricted at the septum and inequilateral, with rounded upper ends. Anamorphs: typically *Ramularia* with *Asteromella* spermatial states. Representative species: the common polyphagous *M. punctiformis*.

Section *Tassiana*: pyriform asci and irregularly arranged, thick-walled ascospores that are often large and constricted at the septum and nearly equilateral, relatively broad with rounded ends, containing irregular lumina. Anamorph: *Cladosporium* s. str. Representative species: the common polyphagous species *Davidiella tassiana*. Further research supported the decision of David (1997) to place *Heteroconium* anamorphs in *Cladosporium*, while section *Tassiana* was elevated to generic level as *Davidiella* (*Cladosporium* anamorphs) (Braun et al. 2003, Crous et al. 2007a, Schubert et al. 2007a, b, Zalar et al. 2007), for which the family

Davidiellaceae was established (Schoch et al. 2006).

Section *Caterva*: cylindrical asci and irregularly arranged, thin-walled, often medium-sized ascospores that are rarely constricted at the septum and inequilateral, with more or less pointed ends. *Asteromella* spermatial forms are typical. Representative species: the common polyphagous *M. subradians*.

Section *Longispora*: cylindrical asci with aggregated, thin-walled, long and slender ascospores that are rarely constricted at the septum and mostly equilateral, long but slender ascospores, characteristically with rounded upper and pointed lower ends. Anamorphs: *Phloeospora* or *Septoria* s. lat. Representative species: *M. eryngii* (with short spores), *M. latebrosa* and *M. populi* (with longer spores). The phylogenetic position of *Sphaerulina*, which differs by having additional ascospore septa, still needs to be resolved.

Section *Fusispora*: pyriform asci and irregularly arranged, thin-walled ascospores that are rarely constricted at the septum and mostly equilateral, fusiform, pointed ascospores. Anamorphs have not been proven. Representative species: the common *M. lineolata* on *Poaceae*.

Section *Plaga*: (incl. Section *Macula*) incorporates endophytic species sporulating on leaf spots, many of which are described as plant pathogens. This section is characterised by obovoid to ellipsoidal or cylindrical asci, small to medium sized ascospores, fusiform to obovoid with rounded ends. Many species have been described in this section, the majority of which originate from warm-temperate and tropical areas. Anamorphs include *Colletogloeopsis*, *Kirramyces*, *Passalora*, *Phaeophleospora*, *Pseudocercospora*, *Pseudocercospora*, *Sonderhenia*, *Stenella*, etc. Several representative species are listed by Crous (1998) on *Eucalyptus*.

Section *Cymadothea*: This section is now accepted as the genus *Cymadothea* (*Polytrincium* anamorphs) (Simon et al. 2009). *Cymadothea* has superficial ascomata situated on a stroma of pseudoparenchymatal cells, and ascospores that can become pale brown with age. Representative species: the genus is monotypic, with *C. trifolii* occurring on *Trifolium*.

Von Arx (1949) proposed separating species with separate ascomata immersed within the host tissue, and those with ascomata occurring in pseudoparenchymatous stromata. This idea certainly has merit, but too few taxa in the latter category have been subjected to DNA analyses to fully test this proposal. The original hypothesis of separating species with pigmented ascospores into *Phaeosphaerella*, while retaining those with hyaline ascospores in *Mycosphaerella*, should also be reinvestigated. This separation was also followed by Tomilin (1979). However, Müller and von Arx (1962) found that the type species of *Phaeosphaerella*, *P. maculosa* was identical to *Venturia macularis*. A new generic name would thus have to be introduced for species with pigmented ascospores. Species with hyaline and slightly pigmented ascospores are currently retained in *Mycosphaerella*, though some are now placed in *Teratosphaeria* (see below). Although ascospore germination patterns have thus far only been used at species level (Crous 1998, Crous et al. 2004a), many species with ascospores that turn dark and verruculose during germination (Crous et al. 1993a, b, Crous & Wingfield 1996, Crous et al. 2008a, b) have in fact been shown to belong to *Teratosphaeria*, not *Mycosphaerella*, suggesting that this character may have value at the generic level as well.

Klebahn (1918) and Laibach (1922) proposed that species be classified in different genera according to their anamorphs, and proposed *Septorisphaerella* (*Septoria* anamorphs), *Ramularisphaerella* (*Ramularia* anamorphs), *Cercosphaerella* (*Cercospora* anamorphs) and *Ovosphaerella* (*Ovularia* anamorphs),

= *Ramularia* fide Braun 1998). This approach was not accepted by subsequent workers. Von Arx (1983) stated that the presence or absence of anamorphs should not be used to separate genera, subgenera or sections in *Mycosphaerella*. Crous (1998) suggested that anamorph morphology, rather than features such as ascus and ascospore shape be used to separate genera within *Mycosphaerella*, though initial DNA phylogenies based on ITS sequence data refuted this (Crous et al. 2000, Goodwin et al. 2001, but see below). Although Sutton and Hennebert (1994) suggested that different anamorph conidiogenous events and conidiomatal types could prove useful in grouping species at some subgeneric level, the presence of synanamorphs (Crous et al. 2007a, c), and general plasticity observed in conidiogenesis and conidiomatal structure when studied in culture, suggests that these characters should be used with caution.

Barr (1996) placed two species occurring on pine needles in a new genus, *Eruptio* (*Lecanosticta* and *Dothistroma* anamorphs) based on their elongate, erumpent ascostromata that open via schizogenously formed ostioles. The taxa presently accommodated in *Eruptio* are, however, not congeneric, and thus genus will have to be evaluated further in future studies.

Although *Sphaerulina* was established to accommodate taxa with primarily 3-septate ascospores, Crous et al. (2003b) showed that some elements of '*Sphaerulina*' clearly belong in *Mycosphaerella*, and that ascospore septation is not as definitive as previously thought. The most significant finding, however, was that *Mycosphaerella* is an assemblage of numerous genera that are morphologically similar. These genera are more easily separated based on their anamorphs (Crous 1998, Crous et al. 2007a), though the same anamorph morphology could also evolve in more than one lineage (genus), making morphological identifications cumbersome in certain groups.

The separation of *Davidiella* teleomorphs (Braun et al. 2003) with their angular lumens, remnants of hamathecial tissue, and *Cladosporium* s.str. anamorphs was a significant step in redefining *Mycosphaerella*. The next was the separation of *Teratosphaeria* from *Mycosphaerella* (Crous et al. 2007a). Species of *Teratosphaeria* are distinguished from *Mycosphaerella* by frequently having hamathecial remnants, a multi-layered ascus endotunica, and ascospores with sheaths that frequently turn brown while still in their asci. Anamorph genera were shown to also not overlap between *Mycosphaerella* and *Teratosphaeria*. In a recent paper by Schoch et al. (2006), further support was provided for the separation of the *Teratosphaeriaceae* from the *Mycosphaerellaceae* and *Davidiellaceae*, while all families were shown to be members of the *Capnodiales* (*Dothideomycetes*).

The genus *Mycosphaerella* s.str. *Ascomata* are submerged, separate, somewhat erumpent, small, simple, globose, black, with walls of 2–4 layers of textura angularis. *Ostioles* are central, frequently lined with periphyses. *Asci* are bitunicate, fasciculate, lacking hamathecial filaments. *Ascospores* are 1-septate, hyaline, lacking appendages and sheaths. *Anamorphs* are accommodated in *Ramularia*.

Contemporary taxonomy of *Mycosphaerella* is based on a range of characters, including symptoms on host plants, morphological characters of both anamorphs and teleomorphs, cultural characteristics, and DNA phylogeny (Crous 1998, Stewart et al. 1999, Crous et al. 2000).

Symptomatology: Lesions vary in shape from being angular to circular or irregular, and in size from specks to spots or larger coalescing blotches, causing a distortion of the leaf lamina. Lesions also vary in colour at different stages of development,

and can be smooth and amphigenous, or corky and not extending through the lamina (Fig. 1). Borders of lesions can be raised, and frequently darker in colour, and margins can be absent, or vary from a chlorotic yellow to red or red-purple. Many *Mycosphaerella* spp. seem to occur only on foliage of defined age, namely on juvenile, intermediate or mature foliage. Lesions can also occur on fruit (spots or rot), or on twigs or stems, associated with dieback or cankers. Many species occur as symptomless endophytes, and are only observed to sporulate on plant debris.

Teleomorph characters: *Ascomata* of different species frequently vary in size between the larger- and smaller-spored species. *Ascomatal* distribution (upper or lower leaf surface), and aggregation (dense, sparse) and association with stromatic tissue, are very characteristic features among different taxa. Dimensions of the *ascomatal* wall cells tend to vary little among small- or large-spored species. However, some taxa have characteristically thick walls, consisting of more layers than the general 3–4 cell layers observed in common species. *Periphyses* are commonly present, lining ostiolar canals, and their level of development varies among taxa. *Asci* are aparaphysate, bitunicate, sessile, and formed in a fascicle, vary in shape from obovoid to narrowly or broadly ellipsoidal, or narrowly ellipsoidal to cylindrical (Fig. 2). *Ascospores* are mostly hyaline (*Mycosphaerella* s.str.), or slightly olivaceous in some taxa. They are usually bi- to triseriate in *asci* of large-spored species, or multiseriate in those with small-spored taxa. *Ascospores* can either be straight, curved, or frequently both curved and straight. They vary from being strongly guttulate to non-guttulate, thin- to thick-walled, and prominently, slightly or not constricted at the septum. *Ascospores* are mostly medianly 1-septate, but in some species the basal cell is slightly longer than the apical cell. The widest point in the *ascospore* can either be at the median septum, in the middle of the apical cell, or closer to the apex. The apical cell can also be asymmetrical. *Ascospores* vary in shape from narrowly ellipsoidal, fusoid-ellipsoidal, or obovoid. They taper from the middle toward both ends, or more prominently from the tip or middle of the upper cell toward the base.

Ascospore germination: *Ascospore* germination patterns represent a valuable feature to help distinguish morphologically similar species (Crous et al. 1993a, b, Carnegie & Keane 1994, Crous & Alfenas 1995, Crous & Wingfield 1996, Crous 1998). Crous et al. (1991) studied *ascospore* germination by letting spores shoot from leaf lesions onto 2 % malt extract plates. *Ascospores* were usually ejected within 24 h, enabling germination patterns to be determined the following day. If left too long, *ascospores* from some faster growing species become totally distorted, clouding their germination patterns. For some species, germination is most characteristic at the very onset, whereas others tend to form lateral branches 24–48 h after they have been shot onto the agar surface (Crous & Wingfield 1996). These germination patterns have been found to be stable and reproducible, even when spores produced *in vitro* are germinated on agar. However, the patterns change when spores germinate in water or on different media, or are left for inordinately long after *ascospore* discharge. Standardisation to ensure reproducibility is, therefore, essential, and the time from discharge to observation must be carefully monitored. In studying the species occurring on *Eucalyptus*, Crous (1998) observed 14 different germination patterns (Fig. 3). *Ascospores* tend to become slightly swollen, or completely distorted, with one to several germ tubes emerging, growing at various angles to the long axis of the spore, remaining hyaline, or turning brown upon germination.

Colony growth in culture: Colony characteristics and *ascospore* morphology are generally consistent *in vitro*. *Mycosphaerella*

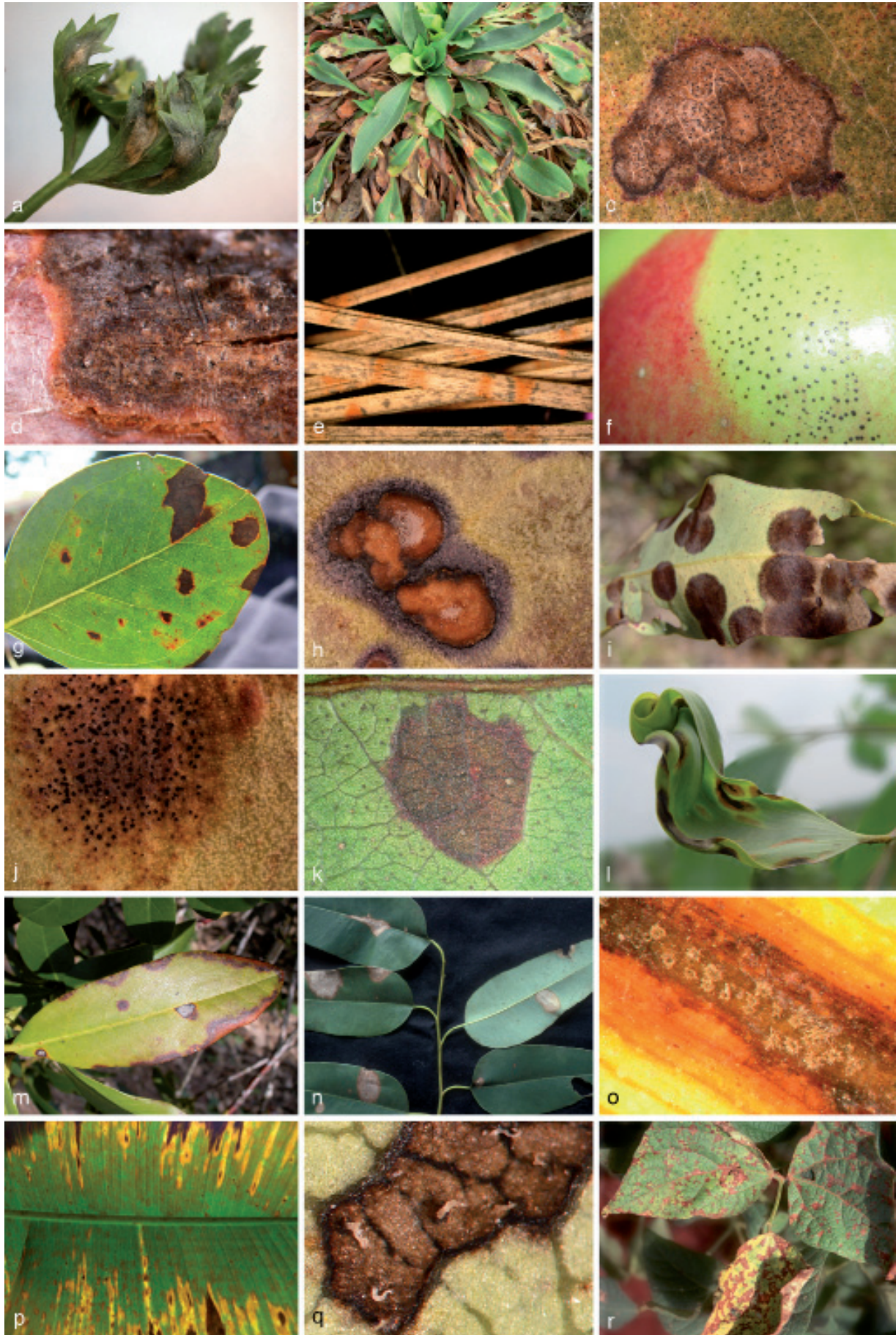


Fig. 1 Disease symptoms associated with species of the *Mycosphaerella* complex. a. *Cercospora apii* leaf spots on *Apium graveolens* (M. Groenewald); b. *Cercospora beticola* spots on *Goniolimon tataricum* (S.G. Bobev); c. *M. sumatrensis* spot on *Eucalyptus*; d. *Teratosphaeria gauchensis* canker on *Eucalyptus* (M-N. Cortinas); e. red band needle blight of *Pinus* caused by *Dothistroma pini*; f. *Schizothyrium pomi* causing flyspeck of apple (J. Batzer); *T. verrucosa* spots on *Eucalyptus* (M.J. Wingfield); h. *Pseudocercospora lilacis* spot on *Ligustrum*; i. *T. fimbriata* spots on *Eucalyptus*; j. *Batcheloromyces protea* spot on *Protea*; k. *M. sphaerulinae* spot on *Eucalyptus*; l. *Passalora perplexa* blight of *Acacia* (M.J. Wingfield); m. *Mycosphaerella handelii* spot on *Rhododendron*; n. *Teratosphaeria parkii* spots on *Eucalyptus*; o. *Mycosphaerella fijensis* spot on *Musa*; p. *Mycosphaerella* spots on *Musa*; q. leaf spot with conidial cirri of *Septoria provencialis* on *Eucalyptus*; *Pseudocercospora griseola* causing angular leaf spot of *Phaseolus* (M.M. Liebenberg).



Fig. 2 Teleomorphs. a. Ascomata of *M. gracilis*; b. squashed ascoma of a *Teratosphaeria* sp. on *Eucalyptus*; c. asci as arranged inside ascoma of *M. acaciigena*; d. asci of *Mycosphaerella* sp. occurring with *Cercospora acaciae-mangii*; e. asci of *M. gracilis*; f. asci of *T. toledana*; g. asci of *Teratosphaeria* sp. on *Eucalyptus*; h. asci of *T. pseudocryptica*; i. Ascus of *M. cussonia*; j. asci of *Davidiella tassiana*; k. ascus of *T. jonkershoekensis*; l. ascospores of *M. longibasalis*. — Scale bars: a = 80; b, d–h = 40; c, i–l = 10 μ m.

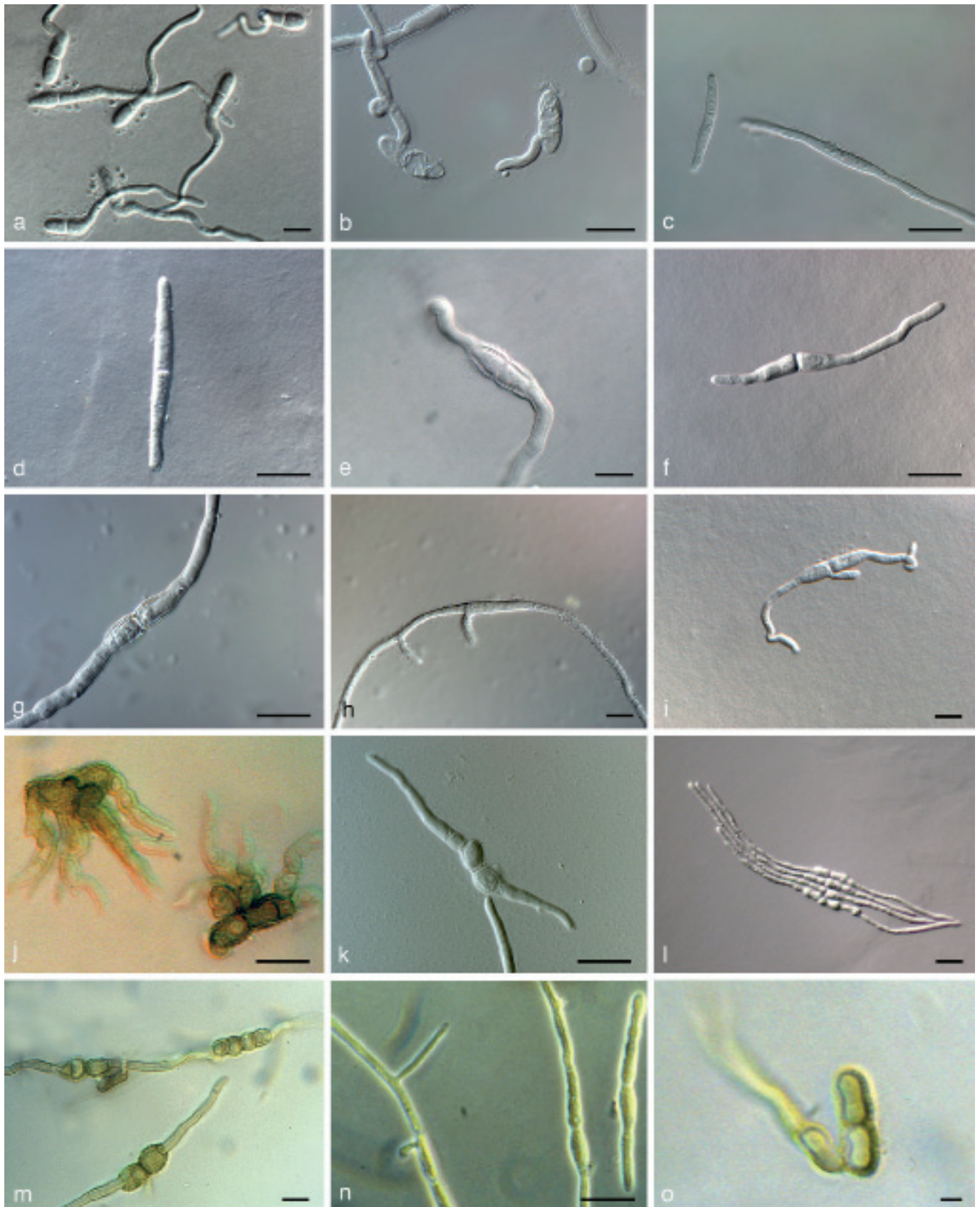


Fig. 3 Ascospore germination patterns sensu Crous (1998). a. *Teratosphaeria cryptica* (Type A); b. *Mycosphaerella mozambica* (Type A); c. *M. gracilis* (Type B); d. *M. cussonia* (Type B); e. *Davidiella tassiana* (Type C); f. *T. alistairii* (Type C); g. *T. jonkershoekensis* (Type G); h. *M. elaeocarpi* (Type H); i. *M. graminicola* (Type D); j. *T. suberosa* (Type E); k. *M. parkiiifinis* (Type F); l. *T. nubilosa* (Type F); m. *T. africana* (Type G); n. *M. colombiensis* (Type J); o. *T. parva* (Type N). — Scale bars = 10 μ m.



Fig. 4 Species of the *Mycosphaerella* complex in culture. a. *Teratosphaeria molleriana* on oatmeal agar (OA); b. *Stenella eucalypti* on malt extract agar (MEA); c. *Readeriella brunneotengens* on MEA; d. *Cibiessia minutispora* on OA; e. *Cercospora* sp. on MEA; f. *Cercospora ipomoeae* on OA; g. *Phaeothecoidea proteae* on OA; h. *Cibiessia dimorphospora* on PDA; i. *Teratosphaeria majorizuluensis* on MEA; j. *Teratosphaeria dendritica* on OA; k. *Phaeophleospora stonei* on OA; l. *Mycosphaerella heimii* on water agar (note crystals); m. *Ramularia* sp. on MEA; n. *Readeriella eucalypti* on OA; o. *Septoria* sp. on OA; p. *Teratosphaeria* sp. on OA; q. *Teratosphaeria alistairii* on potato-dextrose agar (note crystals); r. *Septoria proteae* on OA.

spp. commonly have colonies that are various shades of grey or olivaceous-grey on MEA, though taxa with cream to brown or red colonies also exist, and some form diffuse pigments in the agar (Fig. 4). Distinct differences exist between species in growth rate, temperature requirements for growth (Groenewald et al. 2005), presence of aerial mycelium and colony morphology (margins, colour, mycelium spreading or erumpent, chlamydo-spores, surface smooth or sectored, crystal formation; Crous 1998), formation of spermatogonia and (syn)anamorphs (Crous et al. 2007a, c), and smooth or verrucose nature of creeping hyphae. Aerial hyphae can vary completely from those occurring on the agar surface in texture, pigmentation, width, constriction at septa, etc.

Anamorphic *Mycosphaerella* in the post-Chupp era

Asteromella is now commonly accepted as the spermatial state that occurs with species of *Mycosphaerella* when studied in culture, or on host material (Crous & Wingfield 1996), and it is possible that spermatial states have also been described as anamorphs in genera such as *Ascochyta*, *Asteroma* and *Phoma*. Most attention to date has been directed towards the hyphomycetous anamorphs of *Mycosphaerella*. Crous et al. (2000, 2001) listed 30 anamorph genera which had been linked to *Mycosphaerella* (Fig. 5, 6). Since then, many have been reduced to synonymy, namely *Uwebraunia* and *Dissoconium* (Crous et al. 2004a), *Paracercospora* and *Pseudocercospora* (Stewart et al. 1999), *Cercostigmia*, *Stigmia*, *Phaeoisariopsis* and *Pseudocercospora* (Crous et al. 2004b, 2006b, Braun & Crous 2006), *Ovularia*, *Ophiocladium* and *Ramularia* (Sutton & Waller 1988, Braun 1998, Crous et al. 2000, 2001). Several anamorph genera have been newly introduced to accommodate *Mycosphaerella* anamorphs, newly linked to *Mycosphaerella*, or newly introduced to accommodate anamorphs formerly linked to *Mycosphaerella* (Table 1). These include *Lecanosticta*, *Lecanostictopsis* (Sutton & Crous 1997, Verkley & Priest 2000), *Xenostigmia* (Crous 1998, Crous & Corlett 1998), *Metulocladosporiella* (Crous et al. 2006c), *Cladoriella* (Crous et al. 2006d), *Helgardia* (Crous et al. 2003a), *Batcheloromyces* (Taylor et al. 2003), *Cibiessiae*, *Phaeothecoidea*, (Crous et al. 2007c), *Pseudotaeniolina*, *Devriesia*, *Capnobotryella*, *Hortaea*, *Readeriella*, *Staninwardia*, *Penidiella* (Summerell et al. 2006, Crous et al. 2007a, c), *Rachicladosporium*, *Toxicocladosporium*, *Verrucocladosporium*, *Ochrocladosporium*, *Rhizocladosporium*, *Graphiopsis* (= *Dichocladosporium*) (Schubert et al. 2007a, Braun et al. 2008), *Zasmidium*, *Ramichloridium*, *Periconiella* (Arzanlou et al. 2007b), *Dothistroma* (Barnes et al. 2004, Groenewald et al. 2007), *Parapericoniella*, *Digitopodium* (Heuchert et al. 2005), *Trochophora*, *Verrucisporota* (Beilharz & Pascoe 2002, Crous et al. 2009a), *Baudoinia* (Scott et al. 2007), *Ramulispora* (Crous et al. 2003a), and *Colletogloeum* (Crous et al., unpubl. data).

Considerably fewer genera of coelomycetes have been linked to *Mycosphaerella*. Although the *Septoria* – *Phloeospora* – *Stagonospora* complex has not yet been resolved (Verkley & Priest 2000), other coelomycetous genera associated with the *Mycosphaerella* complex include *Lecanosticta* (Sutton & Crous 1997, Verkley & Priest 2000), *Phaeophleospora* (Crous et al. 1997, Crous 1998), *Colletogloeopsis* (Crous & Wingfield 1997), *Kirramyces* (Walker et al. 1992, Andjic et al. 2007), *Clypeispora* (Ramaley 1991), *Sonderhenia* (Park & Keane 1984, Swart & Walker 1988), *Readeriella*, *Staninwardia* and *Nothostrasseria* (Summerell et al. 2006, Crous et al. 2007a).

Most hyphomycetous genera linked to *Mycosphaerella* have

traditionally been dealt with as part of the cercosporoid complex (Table 1) (Braun, 1995, 1998, Crous & Braun 2003). These anamorph genera have been separated into more 'natural' or recognisable units based on features such as the presence or absence of superficial mycelium, and its texture. Conidiophore characteristics include arrangement, branching, pigmentation, conidiogenous cell placement, proliferation, scar type and conidial formation, shape, septation, wall texture and pigmentation. In most cases cercosporoid fungi have been treated as asexual fungi, and teleomorphs have been confirmed for only a few species. As is the case with their *Mycosphaerella* teleomorphs, cercosporoid fungi are associated with leaf spots, but can also cause necrotic lesions on flowers, fruits, bracts, seeds and pedicels of numerous hosts in most climatic regions. Furthermore, other than important pathogens of major agricultural crops, cercosporoid fungi are also known to be hyperparasitic to other plant pathogenic fungi (Shin & Kim 2001), and are also employed as biocontrol agents of alien weeds (Morris & Crous 1994, Den Breeÿen et al. 2006).

Chupp (1954) proposed a broad concept for the genus *Cercospora*, simply recording if hila were thickened or not, and if conidia were pigmented, single or in chains. As very little was known about the sexual states and relationships of cercosporoid fungi, Chupp chose a more practical approach by retaining all these taxa in *Cercospora*. Subsequent workers such as Deighton (1973, 1976, 1979, 1987, 1990) and Braun (1995, 1998) divided the *Cercospora*-complex into smaller, more morphologically similar units based on a combination of characters including conidiomatal structure (sporodochia, synnemata, etc.), mycelium (presence or absence of superficial mycelium and texture thereof), conidiophores (arrangement, branching, pigmentation and ornamentation), conidiogenous cells (placement, proliferation and scar type) and conidia (formation, shape, septation, ornamentation, pigmentation and catenulation).

The abandonment of the 'Chupp concept' has resulted in close to 50 genera being recognised in this complex (Braun 1995, Crous & Braun 2003). One of the reasons for this was the strict interpretation of the numerous conidiogenous events as defined by Sutton and Hennebert (1994), as well as the additional characters discussed above. Several anamorph genera have been found to have species with conidiomata varying from mononematous, scattered conidiophores to sporodochia with a basal stroma, or from pycnidia to sporodochia and synnemata. Based on similar observations Sutton (1980) and Nag Raj (1993) saw the need to abandon the distinction between hyphomycetes and coelomycetes, as acervuli were frequently found to form a continuum with more stromatic, sporodochial forms. If this plasticity is taken into consideration when examining the 23 anamorph genera accepted by Crous et al. (2000), many appear superfluous. However, recent phylogenetic studies have shown that many of the current generic concepts are represented as paraphyletic clades within some families in the *Capnodiales* (e.g. *Mycosphaerellaceae* or *Teratosphaeriaceae*) (Arzanlou et al. 2007b, Crous et al. 2007a), suggesting that some of these anamorph concepts still represent more than one genus. In other families in the order, such as *Schizothyriaceae* (Batzer et al. 2008) and *Davidiellaceae* (Crous et al. 2007b, Schubert et al. 2007a, b), this appears not to be the case, and the teleomorph is thus far linked to a single anamorph.

Characters such as the presence or absence of superficial mycelium, the formation of stromata, conidiomatal structure (conidiophores solitary, fasciculate to synnematos, sporodochia to pycnidia and acervuli), conidial shape, size and septation (even eusepta vs. distosepta), as well as solitary vs. catenate conidia,

saprobic, hyperparasitic and phytopathogenic habit, were rejected as single characters at the generic level by Crous & Braun (2003). These recent findings suggest, however, that all these characters again need to be re-evaluated in light of novel DNA data.

From these studies it was shown that most of these cercosporoid genera (with the possible exception of *Cercospora* and *Ramularia*), evolved more than once in the *Mycosphaerellaceae*. The majority of the 'anamorph genera' linked to *Mycosphaerella* in the broad sense, therefore, represent several phylogenetic units, e.g. *Pseudocercospora*, *Passalora*, *Septoria* and *Stenella*. To reduce the number of novel anamorph genera being introduced, Crous et al. (2007a) accepted the concept of paraphyletic anamorph genera within a specific family. This approach, however, has not been widely accepted, which means that many more genera will be introduced as *Mycosphaerella* is further separated into natural units. Teleomorph, as well as anamorph characters will have to be re-evaluated. The characters used by Crous & Braun (2003) to delineate anamorph genera still apply, namely the structure of conidiogenous loci (scars) and hila, and the presence or absence of pigmentation in conidiophores and conidia. In cases where genera are paraphyletic, however, these characters require further refinement.

DNA phylogeny of *Mycosphaerella* species complexes on different hosts

Although the *Mycosphaerella* complex accommodated several thousand species, very few are known from culture. Largely due to the lack of cultures, the first DNA phylogeny paper on *Mycosphaerella* was that published by Stewart et al. (1999). Based on ITS phylogenetic data, subsequent workers (Crous et al. 1999, Goodwin et al. 2001) concluded that *Mycosphaerella* was monophyletic. This research was continued by Crous et al. (2000, 2001), wherein the anamorph concepts were re-evaluated, and based on the limited number of species available, most genera were shown to represent well-defined clades within *Mycosphaerella*. Once multi-gene data were employed (Hunter et al. 2006, Schoch et al. 2006, Arzanlou et al. 2007b, Crous et al. 2007a, b, Batzer et al. 2008), *Mycosphaerella* was shown to be polyphyletic, and the well-defined anamorph genera were shown to have evolved in several clades, within and outside the order, suggesting that in many cases the generic circumscriptions would have to be revised.

DNA phylogenetic techniques further revealed, that for all hosts investigated, there were a surprisingly high number of novel species. This was true for example on *Citrus* (Pretorius et al. 2003), *Acacia* (Crous et al. 2004b), *Chromolaena* (Den Breejën et al. 2006), *Eucalyptus* (Crous et al., 2004a, 2006e, 2007c, Cheewangkoon et al. 2008), *Zea mays* (Crous et al., 2006a), *Encephalartos* (Crous et al. 2008b), *Proteaceae* (Crous et al. 2008a), and *Musa* (Arzanlou et al. 2008), to name but a few. From these various studies, the same pattern emerged, namely that many morphologically similar species occur on the same host, and that based on morphology alone, it is typically very difficult or impossible to distinguish them. What this in turn implies for species numbers, is that in coming years there will be a significant expansion in the number of novel taxa described, and that the *Mycosphaerella* complex accommodates far more species than the 10 000 taxa described to date.

Host specificity in *Mycosphaerella*

A significant problem pertaining to the taxonomy of *Mycosphaerella* is the degree of host specificity of the various species. Most species are still defined based on host, and they are assumed to be host-specific or restricted at least to a family of phanerogamic plants (Chupp 1954, Corlett 1991, Braun 1995). However, the tenability of many species may be called into question because some taxa, including *M. punctiformis*, the type species of *Mycosphaerella*, have been shown to be non-host specific (Verkley et al. 2004).

Although many may be host specific, some *Mycosphaerella* species are able to colonise different and even unrelated hosts. In some cases this appears to be due to the endophytic nature of these fungi (Crous 1998, Verkley et al. 2004), while in others species appear to actively undergo host shifts in the process of locating their ideal hosts (Crous et al., 2004b; Crous & Groenewald, 2005). Crous et al. (2008a) reported that many host-specific necrotrophic pathogenic species of *Mycosphaerella* and *Teratosphaeria* appeared to also exhibit a facultative saprobic behaviour. It was concluded, therefore, that the definitions of 'necrotroph' or 'saprobe' do not clearly define all species of *Mycosphaerella* and *Teratosphaeria*, as some have obviously retained the ability to also grow on dead tissue when they lose the connection to their real host.

In many instances, species of *Mycosphaerella* with wide host ranges are morphologically indistinguishable, such as those in the *Cercospora apii* complex (Groenewald et al. 2005). In the genus *Cercospora*, however, several species are known that are highly host-specific, and thus there appears to be no general rule regarding this ecological trait (Groenewald et al. 2006a). The fact that many species can co-occur in the same lesion or leaf spot, the so called 'co-occurrence phenomenon' (Crous et al. 2009b), adds a new level of complexity to the isolation of these fungi, suggesting that only those strains that are fertile in culture, can be confirmed as representing the fungus studied on host material.

The majority of the plant pathogenic species of *Mycosphaerella* are thought to be host-specific (Goodwin et al. 2001, Crous & Groenewald 2005, Groenewald et al. 2006a, Stukenbrock et al. 2007), such as *M. fijiensis*, *M. musicola* and *M. eumusae* on banana (Arzanlou et al. 2008) and *M. graminicola* on wheat (Stukenbrock et al. 2009). In contrast, Crous et al. (2009b) reported several species to occur on multiple hosts, namely: *M. communis* (on *Eucalyptus* in South Africa, Spain, New Zealand, *Musa* in Trinidad, *Protea magnifica* in Australia), *M. konae* (*Leucospermum* in Hawaii, *Eucalyptus* in Thailand) (Crous et al. 2007c), *M. marksii* (*Eucalyptus*, Australia, Bolivia, China, Ecuador, Ethiopia, Papua New Guinea, New Zealand, South Africa, Spain, Tanzania, Uruguay, *Leucadendron* on the Madeira Islands, and *Musa* in Mozambique) (Arzanlou et al. 2008), *T. associata* (*Eucalyptus* and *Protea* in Australia) (Summerell et al. 2006, Crous et al. 2007c), *T. parva* (*Eucalyptus* in Australia, Chile, Ethiopia, Portugal, South Africa, Spain, and *Protea* in South Africa), *T. nubilosa* (*Eucalyptus* in Australia, New Zealand, Europe, South America, and *Acacia* in Thailand (Crous & Groenewald 2005, Hunter et al. 2008), and *M. citri* (*Musa* in Florida, *Acacia* in Thailand, and *Eucalyptus* in Vietnam, and *Aeglopsis*, *Citrus*, *Fortunella*, *Murraya*, and *Poncirus* in North and South America, as well as Asia (Pretorius et al. 2003, Crous et al. 2004a, b, Crous & Groenewald 2005, Burgess et al. 2007).

The genetics of host-specificity of well-known pathogens such as *M. graminicola* has been studied extensively. For example, Banke et al. (2004) demonstrated that this species infects only



Fig. 5 Anamorphs associated with the *Mycosphaerella* complex. a–d. Fascicles of *Cercospora zeina*; e. Conidiophore giving rise to conidium of *Cercospora* sp.; f. conidium of *Cercospora* sp.; g. macro and microconidia of *Dissoconium dekkeri* anamorph of *M. lateralis*; h. hyphae with endoconidia of *Phaeothecoidea eucalypti*; i. conidia of *Phaeophleospora eugeniae*; j. conidia of *Batcheloromyces leucadendri*; k. conidia of *Ciblicia dimorphospora*; l. conidiophore of *Cladosporium sphaerospermum*; m. conidia of *Sonderhermia eucalypticola* anamorph of *M. walkeri*; n. conidiophores of *Pseudocercospora punctata* anamorph of *M. syzygii*; o. conidiophores of *Lecanostictopsis syzygii*; p. conidia of *Nothotrasseria dendritica* anamorph of *T. dendritica*; *Passalora* sp. sporulating in culture; r. pigmented conidia of *Passalora* sp. with thickened hila. — Scale bars = 10 µm, except d = 40 µm.

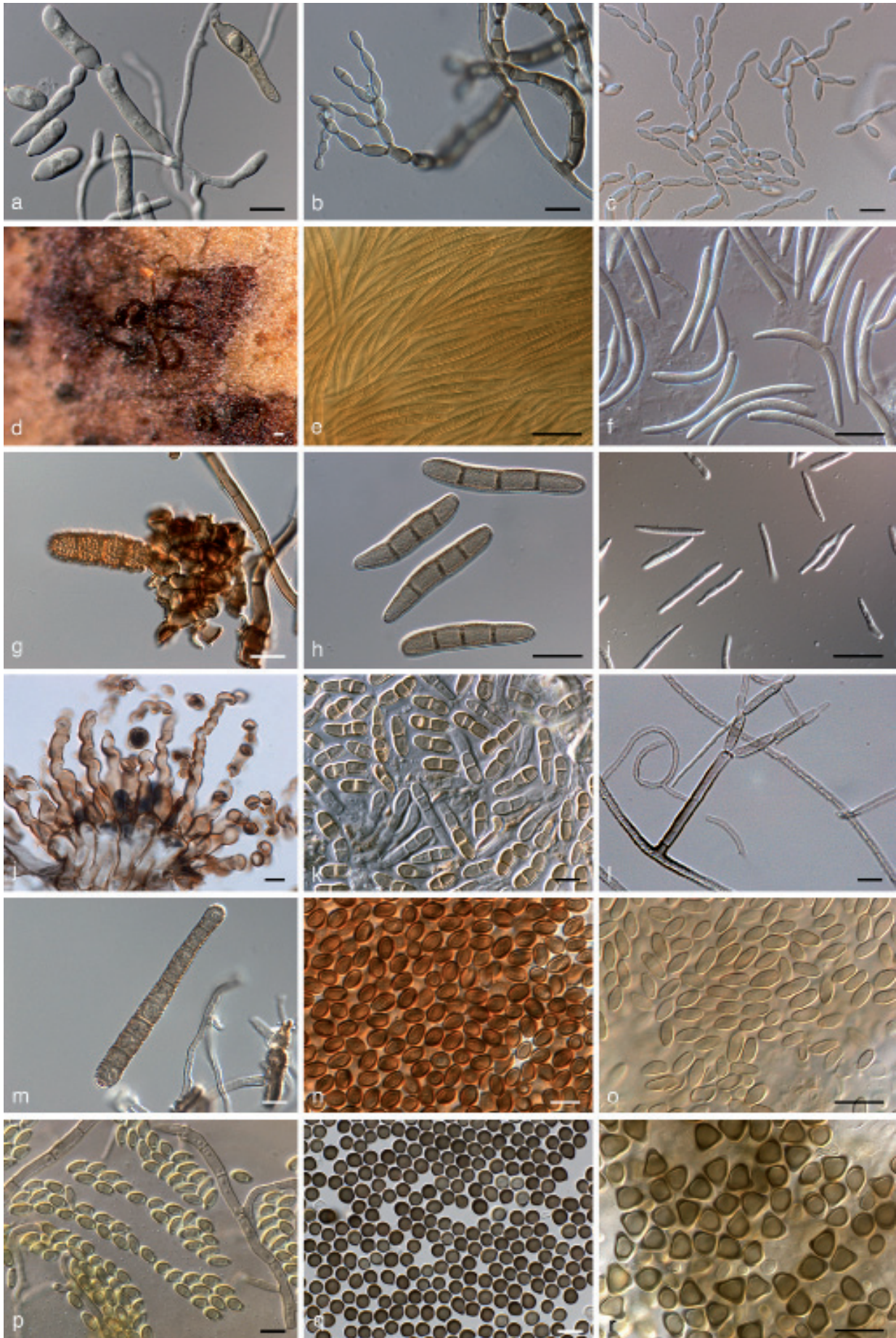


Fig. 6 Anamorphs associated with the *Mycosphaerella* complex. a. Conidia of *Passalora fulva*; b. conidiophore of *Penidiella* anamorph of *Teratosphaeria encephalarti*; c. conidia of *Ramularia eucalypti*; d. exuding cirrus of *Kirramyces* anamorph of *T. suttonii*; e. conidia of *Kirramyces destructans*; f. conidia of *K. eucalypti*; g. conidium of *Stigmia eucalypti*; h. conidia of *Sonderhenia eucalyptorum* anamorph of *M. swartii*; i. conidia of *Septoria eucalyptorum*; j. conidiophore of *Polythrincium trifolii*, anamorph of *Cymadothea trifolii*; k. conidia of *Staninwardia suttonii*; l. conidiophore of *Stenella* sp.; m. conidium of *Verrucisporota grevilleae*; n. conidia of *T. verrucosa*; o. conidia of *T. gauchensis*; p. conidia of *Readeriella readeriellophora*; q. conidia of *R. eucalypti*; r. conidia of *R. mirabilis*. — Scale bars = 10 µm, except d = 40 µm.

Table 1 Anamorph genera linked to *Mycosphaerellaceae* (M) and *Teratosphaeriaceae* (T)¹.

Genus	Conidiomata ²	Synanamorph	Proliferation ³	Colour ⁴	Conidia				Reference
					Conidial septation	Loci ⁵	Arrangement ⁶	Mycelium ⁷	
<i>Batcheloromyces</i> (M)	S	catenulostroma-like	P	P	0–3	I	S,C	I,E	Taylor et al. 2003
<i>Baudoinia</i> (T)	Ph	–	Ph	P	0–1	I	C	E	Scott et al. 2007
<i>Capnobotryella</i> (T)	Ph	Endoconidia	Ph	P	0–1	I	C	E	Sugiyama & Amano 1987
<i>Catenulostroma</i> (T)	A,S,F	–	Ph	P	0-multi	I	S,C	I,E	Crous et al. 2007a
<i>Cercospora</i> (M)	F	–	S	H (conidia)	0-multi	T,D,R	S	I	Crous & Braun 2003
<i>Cercospora</i> (M)	F	–	S	P (conidioph.) H	multi	T,R	S	I	Braun 1995
<i>Cibiessiae</i> (T)	Ph	readeriella-like	Ph	P	1–3	I	C	I,E	Crous et al. 2007c
<i>Clypeispora</i> (?)	P	–	M	H	0	I	S	I	Ramaley 1991
<i>Colletogloeopsis</i> (T)	A/P	–	P,S	P	0–1	I	S	I,E	Crous & Wingfield 1997
<i>Davisoniella</i> (T)	M	coelomycete	P	P	0	I	S	I	Crous et al. 2006e
<i>Devriesia</i> (T)	Sol	Chlamydo-spores	S	P	0–3	T,D	C	E	Seifert et al. 2004
<i>Dothistroma</i> (M)	A	–	P,S	H	1–5	I	S	I	Barnes et al. 2004
<i>Hortaea</i> (T)	Sol	–	P,M	H (conidia) P (hyphae)	0–2	I	S	I,E	Bonifaz et al. 2008, Plemenitaš et al. 2008
<i>Kirramyces</i> (T)	P	pseudocercospora-like	P,S	P	0-multi	I	S	I	Andjic et al. 2007
<i>Lecanosticta</i> (M)	A	–	P	P	0-multi	I	S	I	Suto & Ougi 1998
<i>Miuraea</i> (M)	F,Sol	–	S	H,P	muriform, multi	I	S	I,E	von Arx 1983
<i>Nothostrasseria</i> (T)	P	–	M	P	0	I	S	I	Crous et al. 2007c
<i>Passalora</i> (M)	F,S,Sol	–	S	P	0-multi	T,D,R	S,C	I,E	Crous & Braun 2003
<i>Penidiella</i> (T)	Sol,F,Syn	–	S	P	0–1	I or T,D	C	I,E	Crous et al. 2007a

Table 1 (continued) Anamorph genera linked to *Mycosphaerellaceae* (M) and *Teratosphaeriaceae* (T)¹.

Genus	Conidiomata ²	Synanamorph	Proliferation ³	Colour ⁴	Conidia				Reference
					Conidial septation	Locis ⁵	Arrangement ⁶	Mycelium ⁷	
<i>Periconiella</i> (M)	Sol	–	S	P	0-multi	T,D	C	I,E	Arzanlou et al. 2007b
<i>Phaeophleospora</i> (M)	P	–	P	P	0-multi	I	S	I	Crous et al. 1997
<i>Phloeospora</i> (M)	A	–	S	H	multi	I	S	I	Sivanesan 1984
<i>Phaeothecoidea</i> (T)	En	–	En	P	0–2	I	S	I,E	Crous et al. 2007c
<i>Pseudocercospora</i> (M)	F,S,Sol,Syn	<i>Stigmia</i>	S	P	1-multi	I	S,C	I,E	Deighton 1976
<i>Pseudocercosporella</i> (M)	F,S,Sol	–	S	H	1-multi	I	S,C	I,E	Braun 1998
<i>Pseudotaeniolina</i> (T)	Sol	–	Ph	P	0–2	I	C	I,E	Crane & Schoknecht 1986
<i>Ramichloridium</i> (M)	Sol	–	S	P	0–1	D	S	I,E	Arzanlou et al. 2007b
<i>Ramularia</i> (M)	F,S,Sol	–	S	H	0–5	T,D,R	S,C	I,E	Braun 1998
<i>Ramulispora</i> (M)	S	Chlamydo-spores	S	H	0-multi	I	S	I,E	Crous et al. 2003
<i>Readeriella</i> (T)	P	–	P,M	P	0	I	S	I	Crous et al. 2007a
<i>Septoria</i> (M)	P/A	–	S	H	1-multi	I	S	I	Von Arx 1983
<i>Sonderhenia</i> (M)	P	–	P	P	0–5	I	S	I	Swart & Walker 1988
<i>Staninwardia</i> (T)	A	–	P	P	1–2	I	C	I	Summerbell et al. 2006
<i>Stenella</i> (T)	F,Sol	scytalidium-like	S	P	0-multi	T,D,R	S,C	I,E	Sivanesan 1984
<i>Trochophora</i> (M)	F,Sol	–	S	P	3	I	S	I,E	Zhao et al. 2007
<i>Verrucisporota</i> (M)	F,Sol	–	S	P	0-multi	T,D,R	S,C	I,E	Crous et al. 2009a
<i>Zasmidium</i> (M)	F,Sol	–	S	P	0-multi	T,D,R	S,C	I,E	Arzanlou et al. 2007b

¹*Asteromella* spermatial states have also been described in *Ascochyta*, *Asteroma*, *Phyllosticta* and *Phoma*. Excluded genera are *Dissoconium* (= *Uwebraunia*), *Cladosporium* (*Davidiellaceae*), *Thezogonia* (*Helotiales*), *Xenostigmia* (*Pleosporales*). *Mycovellosiella* and *Phaeoramularia* are treated as synonyms of *Passalora*; *Phaeoisariopsis*, *Paracercospora* and *Stigmia* as synonyms of *Pseudocercospora*.

²Fasciculate (F), sporodochial (S), solitary (Sol), pycnidial (P), acervular (A), synnematosus (Syn), phragmosporous (Ph), hyphae with endoconidia (En), multilocular (M).

³Sympodial (S), percurrent (P), monoblastic, determinate (M), phragmospores (Ph), endoconidia (En).

⁴Hyaline (H), pigmented (P).

⁵Thickened (T), darkened (D), refractive (R), protruding (P), inconspicuous (I).

⁶Solitary (S), chains (C).

⁷Internal (I), external (E).

bread wheat and durum wheat. Of interest, however, is the fact that based on certain genes, durum wheat isolates of *Mycosphaerella* clearly separate from bread wheat isolates (Groenewald & Crous, unpubl. data), suggesting that at some stage, these were two distinct species infecting these hosts. Among the *Mycosphaerella* species infecting *Eucalyptus*, some species such as *Teratosphaeria cryptica* (syn. *M. cryptica*) have a broad host range and cause disease on 38 species across the *Eucalyptus* sub-genera *Monocalyptus* and *Symphyomyrtus*, while *T. nubilosa* shows a more narrow host range, infecting only 12 *Eucalyptus* species and a few hybrids within the subgenus *Symphyomyrtus* (Park et al. 2000, Maxwell et al. 2005, Hunter et al. 2008).

To successfully manage and control plant disease epidemics, a thorough understanding of the genetic variation and epidemiology of the causal agent(s) is required. Because *Mycosphaerella* species are morphologically similar, are not necessarily host specific, and several species could co-occur in the same lesion, fungal identification and the choice of subsequent control regimes is not always straight forward. PCR-based techniques have in recent years contributed greatly to disease diagnosis and detection, and have also successfully been employed in the early detection of *Mycosphaerella* infections (Waalwijk et al. 2004, Lievens et al. 2005, Arzanlou et al. 2007a).

Sex in *Mycosphaerella*

Ascomycetes with both a sexual and asexual reproductive cycle are haploid for the majority of their lifecycle (Heitman 2006). During the short phase of sexual reproduction, they become dikaryotic, and diploid. Sexual reproduction in fungi involves meiosis, which is preceded by the fusion of two cells (plasmogamy), followed by fusion of the two parental nuclei (karyogamy). Sexual reproduction together with mutation, recombination and natural selection are major forces that drive evolution (Heitman 2006, Zhan et al. 2009). It is generally accepted that asexual reproduction generates genetically identical clones, though the role of the parasexual cycle should not be underestimated, as anastomosis between different mycelial types will again influence the genetic makeup of eventual progeny. Conidia can result from fragmentation of hyphal cells (frequently observed in aerial mycelium of *Mycosphaerella* species), or via the production of conidia in naked (hyphomycete) or enclosed (coelomycete) fruiting bodies. Some species of *Mycosphaerella* form several anamorphs (synanamorphs), including hyphomycetes and coelomycetes (Crous et al. 2007a), which enables them to better utilise changing environmental conditions, ensuring optimal spore production and dispersal. Detailed studies on sexual reproduction in fungi may provide better insights into genetic regulation and evolution of closely related taxa (Turgeon 1998, McDonald & Linde 2002, Conde-Ferraez et al. 2007).

In the absence of selection pressure, asexual reproduction dominates populations. Changes in the availability of food resources, environmental conditions and other selection pressures favour a shift towards the sexual reproduction cycle (Heitman 2006, Zhan et al. 2007). In fungi like *Neurospora crassa*, individuals are hermaphrodites, producing both male and female reproductive structures. Sexual exchange of genetic material relies on the existence of simple cell recognition mechanisms that stimulate out-crossing. The term 'mating type' defines sexually compatible individuals. Heterothallism (self-sterility), occurs between two fungal strains with a compatible mating system. In contrast, homothallism (self-fertility) is where a single isolate can complete a successful

sexual cycle. Pseudohomothallism or secondary homothallism occurs in some ascomycetes such as *Neurospora tetrasperma*, *Podospora anserina* and *Gelasinospora tetrasperma* (Merino et al. 1996), where self-fertile ascospores carry nuclei of both mating types.

In fungi sexual development is controlled by mating type loci, which contain a number of genes which occupy a continuous region on a chromosome (Debuchy & Turgeon 2006). In ascomycetes, sexual development is controlled by a single mating type locus (*MAT*). This mating type locus contains one of two forms of dissimilar sequences occupying the same chromosomal position, termed 'idiomorph' in fungal species with a heterothallic mating strategy (Metzenberg & Glass 1990). Complementary idiomorph isolates are referred to as *MAT1-1* and *MAT1-2* mating strains (Turgeon & Yoder 2000).

Although *Mycosphaerella* contains several thousand species, the mating behaviour of most species has not been resolved. Although some species have been observed to be either homo- or heterothallic, pseudohomothallism has not yet been reported for any *Mycosphaerella* species. By continuing the research done on the heterothallic mating system of *M. graminicola*, the mating behaviour of several apparently asexual species of *Cercospora* has been clarified (Groenewald et al. 2006b). Much attention was also devoted to the elucidation of the mating systems active in the *Mycosphaerella* spp. occurring on banana (Conde-Ferraez et al. 2007, Arzanlou et al., in prep.). Using the same approach our knowledge for other *Mycosphaerella* species such as *Passalora fulva* (Stergiopoulos et al. 2007), *Dothistroma septosporum* and *Dothistroma pini* (Groenewald et al. 2007), and *Septoria passerinii* (Ware et al. 2007) has also been extended. From these data it is clear that sex is active in several apparently asexual species of *Mycosphaerella*, and in species where the teleomorph is seldom observed. Furthermore, a study of the genes and ORFs involved in these mating type loci suggest that this is an area of research that will be rewarding to pursue more in depth, and this will also be one of the main focus areas of my research in the coming years. This research will also link to the activities surrounding the whole genome sequences of *M. graminicola* and *M. fijiensis* that are now becoming available for study (<http://genome.jgi-psf.org/Mycf11/Mycf11.home.html>).

How do we deal with the poly- and paraphyletic nature of *Mycosphaerella* and its anamorphs?

Early phylogenetic trees treating *Mycosphaerella* were based on ITS DNA sequence data, and these suggested that the genus is monophyletic (Crous et al. 1999, 2000, 2001, Stewart et al. 1999, Goodwin et al. 2001). However, once additional loci were included in later analyses, it was shown that *Mycosphaerella* is polyphyletic (Hunter et al. 2006, Crous et al. 2007a). This complex has in recent years been separated into *Davidiella* species with *Cladosporium* anamorphs (*Davidiellaceae*) (Braun et al. 2003, Crous et al. 2007b, Schubert et al. 2007a, b, Zalar et al. 2007, Dugan et al. 2008), *Schizothyrium* species with *Zygophiala* anamorphs (*Schizothyriaceae*) (Batzer et al. 2008), *Teratosphaeria* species with many anamorphs (*Teratosphaeriaceae*) (Crous et al. 2007a, c), and *Mycosphaerella* species, also with numerous anamorph genera (*Mycosphaerellaceae*) (Crous & Braun 2003), all belonging to the *Capnodiales* in the *Dothideomycetes* (Schoch et al. 2006). Although *Davidiella* (*Cladosporium*) and *Schizothyrium* (*Zygophiala*) have a clear one to one relationship with anamorph genera, this is

far from true for *Mycosphaerella* and *Teratosphaeria*, where the teleomorph morphology is relatively conserved throughout the two families. Here the same anamorph morphology has evolved in different clades, and in some cases also outside the families (Crous et al., unpubl. data).

The option of accepting anamorph genera as paraphyletic concepts within the family and order (Arzanlou et al. 2007b, Crous et al. 2007a), has not been widely accepted by the scientific community (see Cortinas et al. 2006, Andjic et al. 2007, Crous et al. 2007a, 2009b). This suggests that new generic names need to be provided for distinct lineages, and novel morphological characters be identified to distinguish them. In order to halt the unnecessary proliferation of generic names, it would thus be preferable to not continue with dual nomenclature, i.e. to use a single generic name per unambiguous phylogenetic lineage. What this would imply, is that in several clades, where anamorph generic names are already available, preference will have to be given to anamorph names to try and achieve a more natural classification among the genera in these families. The greatest challenge, however, is to obtain a workable system, where morphological data can still be used to separate genera in what is presently seen as *Mycosphaerella* s.lat., as *Mycosphaerella* s.str. needs to be confined to those taxa with *Ramularia* anamorphs.

The name *Mycosphaerella* has been confused in the past, and used widely for numerous genera not congeneric with the type species, *M. punctiformis*. If a single generic name is to be used for this '*Mycosphaerella*' clade, the older generic name, *Ramularia* (1833) may be preferable to *Mycosphaerella* (1884); thus *Ramularia endophylla*, not *M. punctiformis*, though both names would remain available, unless this change is implemented via some formal modification of the International Code of Botanical Nomenclature, giving preference to older generic names, irrespective of their sex.

Conclusions

The genus *Mycosphaerella* is commonly known as the largest genus of *Ascomycetes*, containing over 10 000 taxa if anamorph states are included. This assumption has been shown to be false, as *Mycosphaerella* is now known to be para- and polyphyletic. Furthermore, *Mycosphaerella* s.str has been shown to be confined to taxa with *Ramularia* anamorphs, representing approximately 1000 species. In spite of this narrower circumscription of the genus, major taxonomic challenges remain unresolved. The teleomorph has been shown to be morphologically conserved throughout the family, while minute differences in anamorphs are indicative of different genera, complicated by the phenomenon of synanamorph states that commonly develop in culture, as well as on host material. Although much attention has in recent years been focused on hosts in the *Myrtaceae* and *Proteaceae*, a few preliminary studies on other hosts have indicated that most host plants have a rich representation of underscribed species in the *Mycosphaerella* complex. This aspect is further complicated by the fact that many of these taxa can co-occur on the same lesion, and have the ability to colonise non-host tissue, in an attempt to locate their ideal host to which they are pathogenic. Preliminary studies on their mating types and sexual behaviour have also indicated that some species are having cryptic sex, and that the teleomorph is present, though seldom or not yet observed. These findings are also relevant for the import and export of agricultural and forestry produce, as for some species either one or both mating types have been introduced to different continents, suggesting that quarantine regulations also need to focus below the species level on clones and mating types.

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Articles included for DSc dissertation

Over the past 20 years I have published 114 papers and two books dealing with the genus *Mycosphaerella* and its associated anamorphs. The papers included in this DSc represents a selection of papers published from 2003 onwards. In 2003 the paper by Braun et al. (2003) separating *Davidiella* (*Cladosporium*) from the *Mycosphaerella* complex was published, representing the onset of a new approach to the taxonomy of this group of organisms. All selected papers deal with species of the *Mycosphaerella*-complex known from culture, and are supported by molecular phylogenetic data. Initially these studies were largely based on phylogenies of the ITS rDNA region to resolve taxa occurring on specific plant hosts. In certain anamorph groups, however, the ITS domain has provided insufficient resolution to enable me distinguish all taxa, and a multi-gene approach needed to be employed. Other papers incorporate sequence data of the LSU and SSU genes to enable the various generic issues within the *Mycosphaerella* complex to be addressed. In recent years I have also developed a focus on the sexual behaviour of *Mycosphaerella*, and the first papers dealing with sex in some prominent sexual and asexual members of the *Mycosphaerella* complex have thus also been included.

1. Arzanlou M, Groenewald JZ, Gams W, Braun U, Shin H-D, Crous PW. 2007. Phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied genera. *Studies in Mycology* 58: 57–93. [own contribution 40%]
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