CHAPTER 2
LITERATURE REVIEW

2.1 Cercosporoid

Cercosporoid fungi comprises one of the largest and more heterogeneous group of hypomycetes genera and were treated as an anamorphic genus linked to the teleomorph genus *Mycosphaerella* (*Capnodiales*, *Mycosphaerellaceae*; Stewart *et al*. 1999, Crous *et al*. 2000). The genus *Cercospora* contained over 1800 species names as proposed by Chupp (1954) and this number was increased to more than 3000 by Pollack (1987). Cercosporoid fungi are one of the most important groups of plant pathogenic fungi and are usually found in agricultural fields, associated with leaf and fruit spots, blights, fruit rot, necrotic lesions on flowers, fruits, bracts, seeds and pedicels (Chupp 1954, Deighton 1976, Silva & Pereira 2008) on a large number of plants such as cereals, vegetables, ornamentals, forest trees, grasses, etc. (Agrios, 2005). *Cercospora* fungi are distributed worldwide (Agrios 2005, To-Anun *et al*. 2011, Crous *et al*. 2012, Groenwald *et al*. 2012). They occur in a wide range of climates, cool temperate to tropical regions (Crous *et al*. 2012). They are saprobic, occasionally secondary invaders, but most commonly are destructive plant pathogens, causing considerable economic losses. Some speciesare also used as biological control agents of fungal plant pathogens and weeds (Morris & Crous 1994, Inglis *et al*. 2001, Tessman *et al*. 2001). Some species are highly host-specificity, but others which are morphologically have a wide host range, such as the *Cercospora apii* complex (Groenewald *et al*. 2005, 2006a).

Chupp (1954) proposed a broad concept of the genus *Cercospora* in his monograph, based only on limited morphological criteria: hila were thickened or not, and conidia were pigmented, and borne singly or in chains. Subsequent workers have used a different combination of characters such as conidiomatal structure (sporodochia, synnemata), 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) to divide the

Cercospora-complex into other genera, including *Cercosporella*, *Cercosporidium*, *Paracercospora*, *Pseudocercospora*, *Pseudocercosporella*, *Passalora*, *Stenella* and *Stigmina* (Deighton 1971, 1976, 1979, 1987, Braun 1995, 1998). Crous and Braun (2003) retreated and reexamined these names that related to *Cercospora s. lat*. based on these morphological characteristics comprising structure of conidiogenous loci (scars) and hila, as either thickened (or almost so, but slightly darkened or refractive) or unthickened as well as the presence or absence of pigmentation in conidiophores and conidia for the proposed taxonomic treatment of cercosporoid fungi.

The name *Cercospora*which is applied from the combination of Greek "*kerkok*" (tail) and "sporos" (seed), determines the filiform conidia of the fungus. The taxonomic situation is as follow (Kirk *et al*., 2008):

Domain: Eukaryota

Kingdom: Fungi

Subkingdom: Dikarya

Phylum: Ascomycota

Subphylum: Pezizomycotina

Class: Dothideomycetes

Subclass: Dothideomycetidae

Order: Capnodiales

Family: Mycosphaerellaceae

Genus: *Cercospora*

2.2 Host specificity

All plants can be attacked by at least one or more species of fungi. Many fungi exhibit host specificity depending on the particular host species or group of related species from with they derive nutrients (Zhou and Hyde, 2001). Many species of *Cercospora* have a high host-specificity because of specific environmental requirements (Groenewald*et al*., 2006). Host specificity and speciation of *Cercospora* has not been studied extensively. Lucas (1998) proposed the term “host specificity” to describe a relationship between hosts and fungi and mostly applied to plant pathogens. Plant pathogenic fungi are only able to infect certain living plants and show host specifity (Burnet, 2003). For example *Cercospora piaropi* occurs on *Pistia stratioltes* only (Jamenez and Lopez, 2001). *C. sojina* occurs on *Glycine max*, and *Cercospora coffeicola* on coffee (Nelson, 2008).

2.3 The morphological characteristics of Cercosporoid fungi

Deighton with his serial publications (1967, 1971, 1973, 1974, 1976, 1979 and 1983), Pons and Sutton (1988), Braun (1993), Braun and Melnik (1997) and other authors separated *Cercospora s lat*. into several smaller genera based on morphological characteristics. Combination of morphology and molecular analysis was proposed by Crous *et al.* (2000, 2001). Subsequently, Crous and Braun (2003) published the aggregation of the names in *Cercospora* and *Passalora*, and redetermined the morphological characteristics of *Cercospora s. lat*. based on morphology and molecular analysis results. The common description and illustration used to identify the cercosporoid fungi is as follows.

2.3.1 Symptoms on the Host Plants

Symptoms caused by cercosporoid fungi are variable, symptomless or almost so, but mostly forming conspicuous lesions. Leaf spots may be absent or present in every degree of distinctiveness from a faint discoloration on both the upper and lower leaf surface to definitely defined and distinct leaf spots with colored borders, eye-spot diseases or vein-limited lesions (Fig. 2.1 a-h). When the leaf spot is invisible, an effuse caespituli (or fruit bodies) of the fungus usually grows on the lower leaf surface. This fungal lesion may be so minute so as to require a hand lens to detect, or it may have any size from tiny lesion to entire leaf area. When the disease reaches a certain stage of severity, the leaf may curl, dry and drop from the plant. The more virulent species can cause almost complete defoliation.

Many cercosporoid fungi also affect the blossoms, fruits, pods, succulent petioles, and young stems. Frequently the dying portion dries and in shrinking tears away from the living leaf tissue, leaving a shot-hole effect. Punctiform to numerous spots may change the whole leaf to yellow or brown, after that it shrinks and dies. The shot-hole effect and defoliation are rarely mentioned in describing the symptoms of the cercosporoid fungi, because most herbarium specimens are pressed leaves so only the leaf symptoms as they show in freshly collected or herbarium material need here be taken into account.

Fig. 2.1 Various types of disease symptoms associated with cercosporoid

2.3.2 Caespituli (Fruit Bodies)

Caespituli or fruiting bodies of cercosporoid fungi, are defined as tufts of conidiophores as seen under a microscope or hand lens (fig. 2.2 a-d). The caespituli can grow on the upper leaf surface (epiphyllous), the underside of a leaf (hypophyllous), or on all sides (amphigenous); evenly distributed on the spot or aggregated along the margin of the spot. The caespituli often appear velvety, floccose, arachnoid, as effuse patches, punctiform (as minute black pustules), moldy; and the colors are variable from sooty, dark, grey, olivaceous to whitish.

Fig. 2.2 Appearance of caespituli (as tufts of conidiophores) of cercosporoid fungi

2.3.3 Conidiophores, Conidiogenous Cells, and Conidiogenesis

In the cercosporoid fungi, there are several species with tufts of mixed conidiophores. Some of them are one-celled, others are septate, consisting of two or more cells. This study uses a wider conidiophore concept: one-celled conidium-bearing structures can either be called conidiogenous cells or conidiophores, depending on the particular case. Conidiophores may be colorless (hyaline) or pigmented; the presence or absence of pigmentation is considered to be an important taxonomic feature.

Conidiogenous cells can be formed as part of an undifferentiated hypha, and they can form an unicellular conidiophore similar to the conidiogenous cell produced by several species of genus *Passalora*, or they can mostly form part of a multicellular conidiophore. In this case, they can be either terminal, intercalary, or pleurogenous (formed as lateral branchlets).

Conidial scars may be conspicuous by thickened walls with darkened coloration, refractive, bulging or protuberant (often papilla–shaped) (Fig. 2.3 a-e). The “darkened” and “refractive” characteristics are often difficult to distinguish, especially in minute and hardly or only slightly thickened scars. So both of them are often combined. “Hilum” is a term for a scar on a conidium at the point of former attachment to the conidiophore. Denticles are tooth-like projections supporting the young conidia (conidiogenous cells provided with denticles are defined to be denticulate). Scars and denticles are typically formed in a sympodial succession on conidiogenous cells.

Conidial development is called conidiogenesis that arises from the conidiogenous cell or conidiophore (Hennebert and Sutton, 1994). The cercosporoid fungi have holoblastic (monoblastic or polyblastic) conidiogenesis, but often sympodial proliferation, mostly schizolytic with single or conidia in chains occurs.

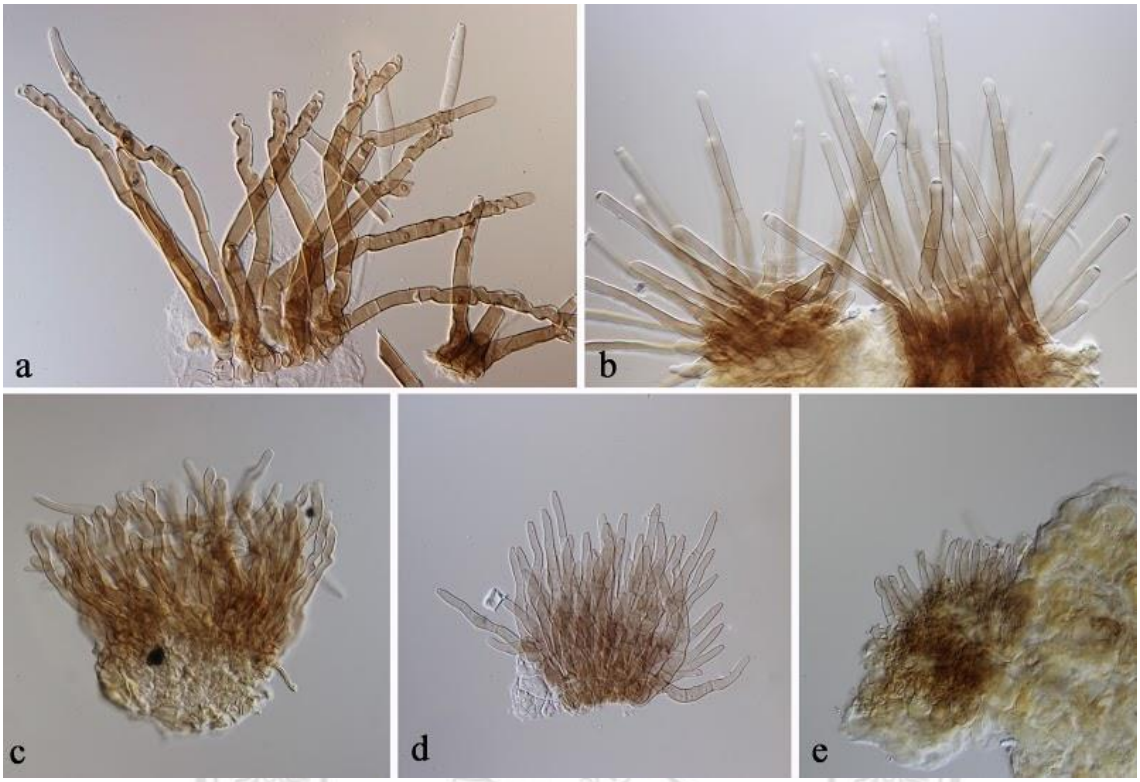

Fig. 2.3 Various types of conidiophores and conidiogenous cells of the cercosporoid fungi.a–b**.** Conidiophores and conidiogenous cellsof *Cercospora*. c–e**.** Conidiophores and conidiogenous cells of *Pseudocercospora*.

2.3.4 Conidia

The board concepts of conidia of the cercosporoid fungi are mostly related to the shape, septation, pigmentation, and surface (Fig. 2.4 a-e). They are often either straight to curved, with acicular, filiform, obclavate, ellipsoidal, or combinations of the shapes. There are two basic types of septation, euseptate (septa formed by all existing wall layers) and distoseptate/pseudoseptate (septa formed only by the innermost layer). Hyaline or pigmented structure (conidiophores, conidia etc.) is usually well separated in certain taxa (genera, species) of the cercosporoid fungi. The conidial surface of cercosporoid fungi mostly smooth, very rarely rough except the genus of *Stenella*.

However, some morphological characteristics have complicated identification; some species in this complex are very similar, and many intermediates cannot be placed into any of the taxa. So, species in the present study were compared based on their morphology, growth characteristics in culture, and molecular data, rendering it possible to initiate a comprehensive revision of the cercosporoid fungi.

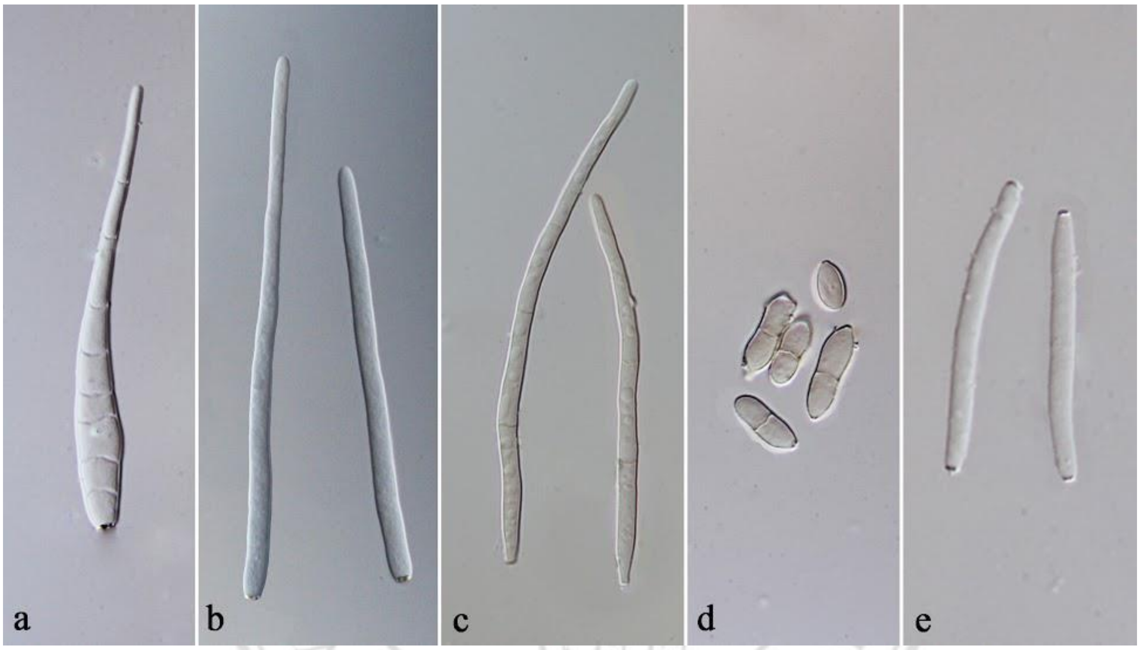

Fig. 2.4 Various types of conidia of the cercosporoid fungi found in this study. a–b. Conidia of genus *Cercospora*. c. Conidia of geuns *Pseudocercospora*. d. Conidia of other cercosporoid. e. Conidia of *Passalora*.

2.3.5 Culture characteristic

Colonies of cercosporoid fungi commonly are various shades of grey or olivaceous-grey on MEA, some are also red or form diffuse pigments in the agar (Fig. 2.5 a-f). Distinct differences between genus and species in colour and growth rate, temperature range for growth (Groenewald *et al*. 2005), feature of aerial mycelium and colony morphology (margin, colour, mycelium spreading or erumpent, chlamydospores, surface smooth or sectored, crystal formation; Crous 1998), and smooth or verrucose nature of creeping hyphae. Aerial hyphae can vary completely from those occurring on the agar surface in texture, pigmentation, width and constriction at septa.

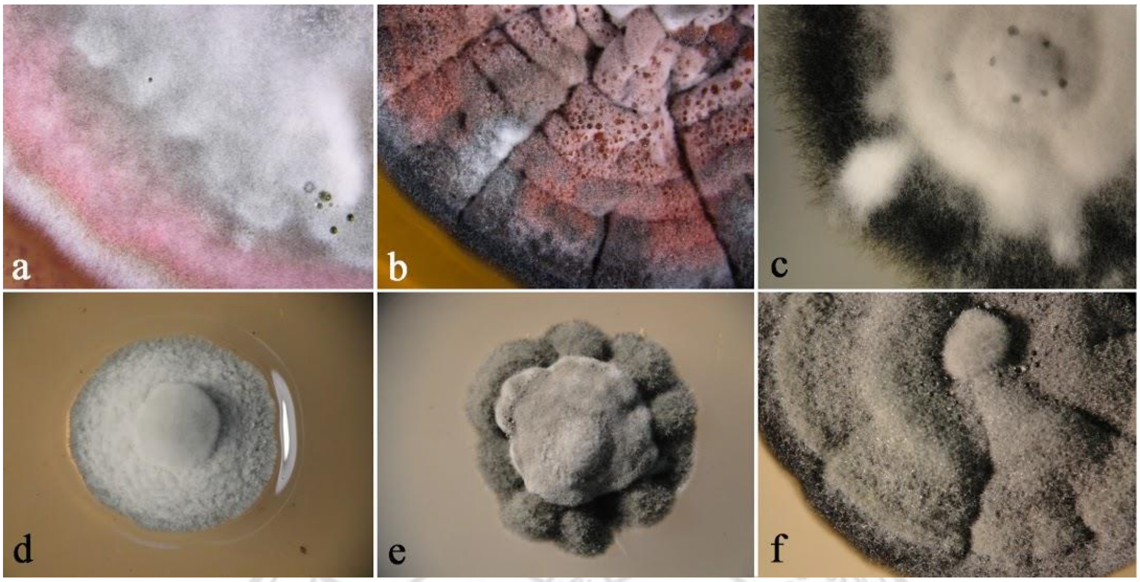

Fig 2.5 Cultures and microscopic features of the cercosporoid fungi at approximately 2 wk of growth on MEA. a–c. Colonies of genus *Cercospora*. d–f. Colonies of genus *Pseudocercospora* and *Pseudocercospora*-like.

2.4 Phylogenetic studies of the cercosporoid fungi

In the past, the identification of the cercosporoid fungi was mainly based on morphological differences alone. However, many morphologically similar species occur on the same host, making it typically very difficult or impossible to distinguish them. The first study by Stewart *et al.* (1999), which applied DNA phylogenetic analysis to species in the cercosporoid fungi, demonstrated the monophyletic nature of *Cercospora*, *Passalora*, and *Pseudocercospora* based on the ITS region of partial rDNA sequence analysis, and reaffirmed that *Ramulispora* Miura and *Mycocentrospora* Deighton are not related to *Mycosphaerella* teleomorph, and also reduced *Paracercospora* Deighton as a synonym of *Pseudocercospora*. Moreover, in several subsequent studies that have employed DNA sequence data from the Internal Transcribed Spacer (ITS) region of the rDNA operon for the cercosporoid fungi such as *Pseudocercospora* species, the DNA phylogenetic studies have shown that several other genera are congeneric with *Pseudocercospora* and thus *Cercostigmina*, *Paracercospora*, *Phaeoisariopsis* and *Pseudophaeoramularia* were reduced to synonymy with *Pseudocercospora* (Stewart *et al*. 1999, Crous *et al*. 2001, Braun & Hill 2002, Crous *et al*. 2006).

In recent research, molecular systematics has been incorporated and proved to be a useful method to gain an understanding of the phylogenetic relationships of species and genera and concepts at higher taxonomic levels of these fungi. However, because of the limited taxa studied and no other species with *Mycosphaerella* teleomorph were included in the analysis, it was unable to determine the phylogenetic relationship of the cercosporoid species to other anamorph genera. The The relatedness of genera of the cercosporoid fungi and the taxonomy and phylogeny of the *Mycosphaerella* teleomorph is also complicated (von Arx, 1983; Crous *et al*., 2000). With the large number of associated anamorphs, Crous and Wingfield (1996) found that *Mycosphaerella* was a polyphyletic assemblage of presumably monophyletic anamorph genera. Based on the analysis of a large number of anamorphs of *Mycosphaerella* using the ITS region of rDNA sequence it was also concluded that the genus *Mycosphaerella* was not monophyletic (Crous *et al*. 1999; Goodwin *et al*. 2001). The interesting results from Goodwin *et al*. (2001) are that *Cercospora s. str*. formed a highly supported monophyletic group to a relatively recent common ancestor that was able to, or acquired the ability to, produce cercosporin, a phytotoxic metabolite of polyketide origin (Daub & Ehrenshaft, 2000). The ability to produce cercosporin was suggested to have a single evolutionary origin Crous *et al*. (2007), found that the genus *Mycosphaerella* was shown to be polyphyletic using the Large Sub Unit (LSU) region of ribosomal DNA (28S rDNA). Crous*et al*. (2007) also distinguished the new family, the *Teratosphaeriaceae* from the *Mycosphaerellaceae* in the Order *Capnodiales* to accommodate many extreme-tolerant species.

However, 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* (Crous, 2009). Once multi-gene data were employed (Hunter *et al*., 2006; Schoch *et al*., 2006; Crous *et al*., 2007a, b; Arzanlou *et al*., 2007b; 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, also based on the phylogenetic studies several genera would have to be revised.

2.5 The anamorph of *Mycosphaerella*

Most anamorph genera, which include 30 genera, have been linked to *Mycosphaerella* as the hyphomycetes (Crous *et al*., 2000, 2001) and they have resulted from a reassessment of the cercosporoid complex (Braun, 1995, 1998; Crous and Braun, 2003). Many of them have been treated as synonyms, e.g. *Mycovellosiella* and *Phaeoramularia* were reduced to synonyms of *Passalora*; *Phaeoisariopsis*, *Paracercospora*, and *Cercostigmina* and *Stigmina*as synonyms of *Pseudocercospora* (Crous 2009). Several have been newly accepted as *Mycosphaerella*anamorphs e.g. *Batcheloromyces* (Taylor *et al*., 2003), *Zasmidium*, *Ramichloridium*, *Periconiella* (Arzanlou *et al*., 2007b) and *Verrucisporota* (Beilharz and Pascoe, 2002; Crous *et al*., 2009a) (Table 2.1).

Table 2.1 Anamorph genera linked to *Mycosphaerellaceae* (Crous 2009)

Genus	Conidiomata[1]	Synanamorph	Conidia					Mycelium[6]	Reference
			Proliferation[2]	Colour[3]	Conidial septation	Loci[4]	Arrangement[5]		
Batcheloromyces	S	*Catenulostroma*-like	P	P	0−3	I	S,C	I,E	Taylor. *et al*. (2003)
Cercospora	F	–	S	H (conidia) P (conidioph.)	0−multi	T,D,R	S	I	Crous and Braun (2003)
Cercosporella	F	–	S	H	multi	T,R	S	I	Braun (1995)
Dothistroma	A	–	P,S	H	1−5	I	S	I	Barnes *et al.* (2004)
Lecanosticta	A	–	P	P	0−multi	I	S	I	Suto and Ougi (1998)
Miuraea	F,Sol	–	S	H,P	muriform, multi	I	S	I,E	Von Arx (1983)
Passalora	F,S,Sol	–	S	P	0−multi	T,D,R	S,C	I,E	Crous and Braun (2003)
Periconiella	Sol	–	S	P	0−multi	T,D	C	I,E	Arzanlou *et al.* (2007b)
Phaeophleospora	P	–	P	P	0−multi	I	S	I	Crous *et al*. (1997)

Table 2.1 Anamorph genera linked to *Mycosphaerellaceae* (continued)

Genus	Conidiomata[1]	Synanamorph	Conidia					Mycelium[6]	Reference
			Proliferation[2]	Colour[3]	Conidial septation	Loci[4]	Arrangement[5]		
Phloeospora	A	−	S	H	multi	I	S	I	Sivanesan (1984)
Pseudocercospora	F,S,Sol,Syn	*Stigmina*	S	P	1−multi	I	S,C	I,E	Deighton (1976)
Pseudocercosporella	F,S,Sol	−	S	H	1−multi	I	S,C	I,E	Braun (1998)
Ramichloridium	Sol	−	S	P	0−1	D	S	I,E	Arzanlou *et al*. (2007b)
Ramularia	F,S,Sol	−	S	H	0−5	T,D,R	S,C	I,E	Braun (1998)
Ramulispora	S	Chlamydospores	S	H	0−multi	I	S	I,E	Crous *et al.* (2003a)
Septoria	P/A	−	S	H	1−multi	I	S	I	Von Arx (1983)
Sonderhenia	P	−	P	P	0−5	I	S	I	Swart and Walker (1988)
Trochophora	F,Sol	−	S	P	3	I	S	I,E	Zhao *et al.* (2007)

Table 2.1 Anamorph genera linked to *Mycosphaerella* (continued)

Genus	Conidiomata[1]	Synanamorph	Conidia					Mycelium[6]	Reference
			Proliferation[2]	Colour[3]	Conidial septation	Loci[4]	Arrangement[5]		
Verrucisporota	F,Sol	−	S	P	0−multi	T,D,R	S,C	I,E	Crous *et al*. (2009a)
Zasmidium	F,Sol	−	S	P	0−multi	T,D,R	S,C	I,E	Arzanlou *et al*. (2007b)

[1]Fasciculate (F), sporodochial (S), solitary (Sol), pycnidial (P), acervular (A), synnematous (Syn), phragmosporous (Ph), hyphae with endoconidia (En), multilocular (M).
[2]Sympodial (S), percurrent (P), monoblastic, determinate (M), phragmospores (Ph), endoconidia (En).
[3]Hyaline (H), pigmented (P).
[4]Thickened (T), darkened (D), refractive (R), protruding (P), inconspicuous (I).
[5]Solitary (S), chains (C).
[6]Internal (I), external (E).

2.6 Selected important cercosporoid genera

Cercospora resen.

Type species: *Cercospora penicillata* (Ces.) Fresen.

= *Cercospora depazeoides* (Desm.) Sacc.

Most of them are important plant pathogenic fungi from a wide host range, they are associated with leaf spot and can also be isolated from necrotic lesions of flowers, fruits and seeds (Agrios 2005). Species of genus *Cercospora* are characterized by their mycelium internal, rarely also external; hyphae colorless to pigmented, septate, branched, smooth to faintly rough-walled. Stromata absent to well developed, subhyaline to usually pigmented, substomatal to intraepidermal. Conidiophores solitary to fasciculate, arising from internal hyphae or stromata, emerging through stomata or erumpent, very rarely arising from superficial hyphae, erect, continuous to pluriseptate, subhyaline to pigmented, smooth to faintly rough-walled, thin to moderately thick-walled; conidiogenous cells integrated, terminal or intercalary or conidiophores reduce to conidiogenous cells, monoblastic to usually polyblastic, sympodial; conidiogenous loci (scars) conspicuous, thickened and darkened. Conidia solitary, scolecosporous, acicular, cylindrical-filiform, slightly obclavate, transversely euseptate, usually multiseptate, hyaline, rarely subhyaline, smooth or almost so, hilum conspicuously thickened and darkened (Crous and Braun 2003, Crous *et al*. 2000) (Figure 2.6–2.7).

Fig. 2.6 Overview of morphological structures of genus *Cercospora*. A. Fasciculate conidiophores situated on a stroma. B. Conidiophores reduced to uni-local conidiogenous cells. C. Conidiophores arising from a weakly developed stroma. D. Fasciculate conidiophore with flexuous conidiophores. E. Conidiophores arising from external mycelium. F. Thickened, darkened and somewhat refractive conidial loci (arrows). G. Conidiogenous cells with multi-local loci. H. Fascicle erumpent through stoma. I. Cylindrical conidium with obtuse apex. J. Filiformconidium. K, L. Acicular, undulate conidia with subobtusely rounded apices, and truncate bases. M–O. bclavate conidia with subobtusely rounded apices and obconically truncate bases. P. Subcylindicalconidium with long obconically truncate base (Groenewald *et al*. 2013).

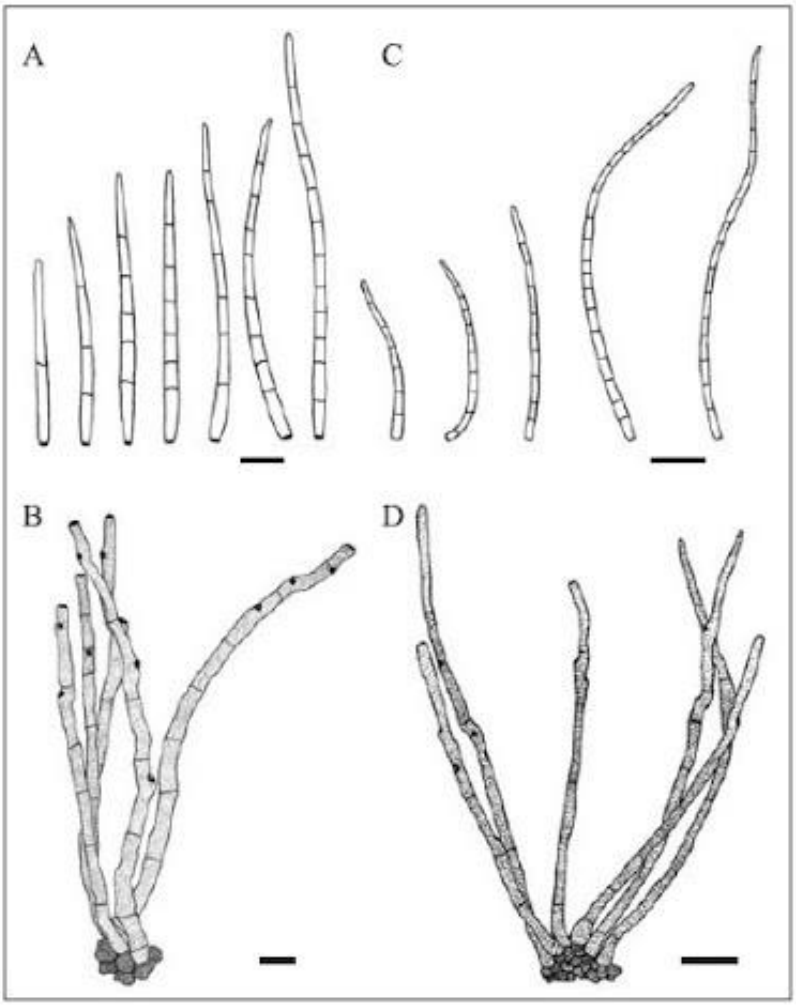

Fig. 2.7 A–B. Conidia and conidiophores of *Cercospora apii* on *Solanum glaucophyllum*. Scale bar = 10 µm. C–D. Conidia and conidiophores of *C. apii* on *Xanthium strumarium* (Rocha *et al*. 2007).

***Cercosporella* Sacc.**

Type species: *Cercosporella cana* (Sacc.) Sacc.

= *Cercosporella virgaureae* is independent species. It includes *C. cana* Sacc.

Cercosporella causes leaf spot. Species of genus *Cercosporella* are characterized by their mycelium internal; hyphae septate, branched, hyaline to faintly pigmented, rarely with external mycelium. Stromata none or well developed. Conidiophores fasciculate, emerging through stomata, erumpet through the cuticle, arising from stromata or internal hyphae, rarely solitary, arising from external hyphae, straight to curved, geniculate to geniculate-sinuous, continuous to septate, hyaline to faintly pigmented at the very basal portion; conidiogenous cells integrated, intercalary, terminal or conidiophores reduced to a single conidiogenous cell, polyblastic, sympodial, cicatrized; conidial scars conspicuous, thickened, often somewhat refractive, but not darkened, usually conspicuously convex. Conidia solitary, occasionally forming short chains of secondary conidia after being shed, hyaline or occasionally faintly greenish, subcylindric to obclavate, sometimes fusiform, uniseptate to pluriseptate; hilum slight thickened, not darkened, refractive, inconspicuous (Braun 1995, Kirschner 2009) (Figure 2.8).

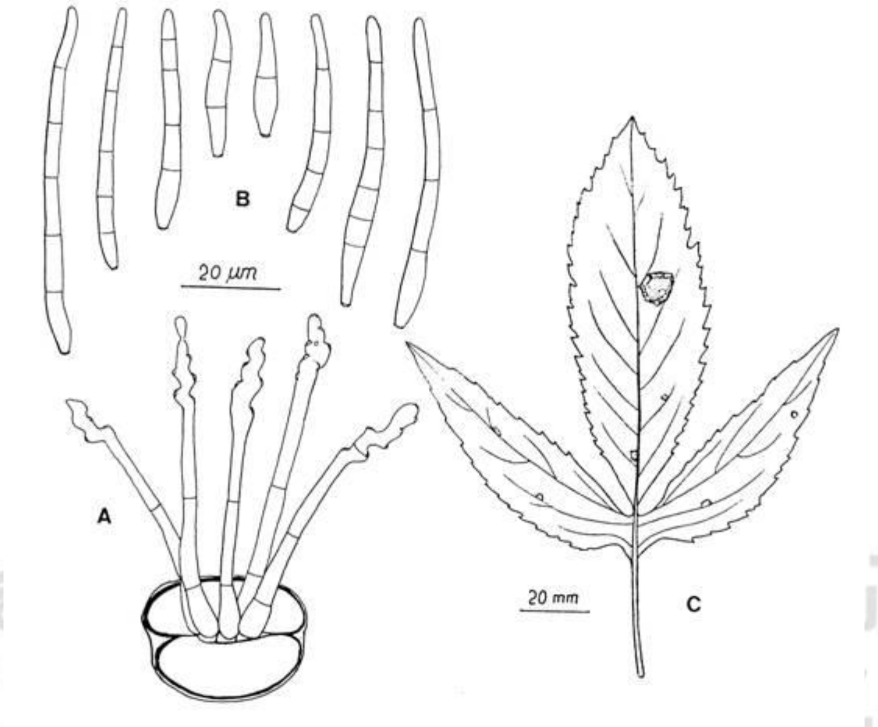

Fig. 2.8 *Cercosporella eupatorii*. A. Fascicle of conidiophores; B. Conidia; C. Leaf spots. (Hsieh and Goh *et al*. 1990).

***Pallidocercospora* Crous**

Type species: *Pallidocercospora heimii* (Crous) Crous 2013. *Pseudocercospora* p.p. [see Crous *et al*. (2013)] (Figure 2.9).

Morphologically indistinct from *Pseudocercospora* but distinguishable by phylogeny, group in different clades.Their only distinguishing formation of red crystals when grown in agar (on WA, SNA, PDA, MEA).

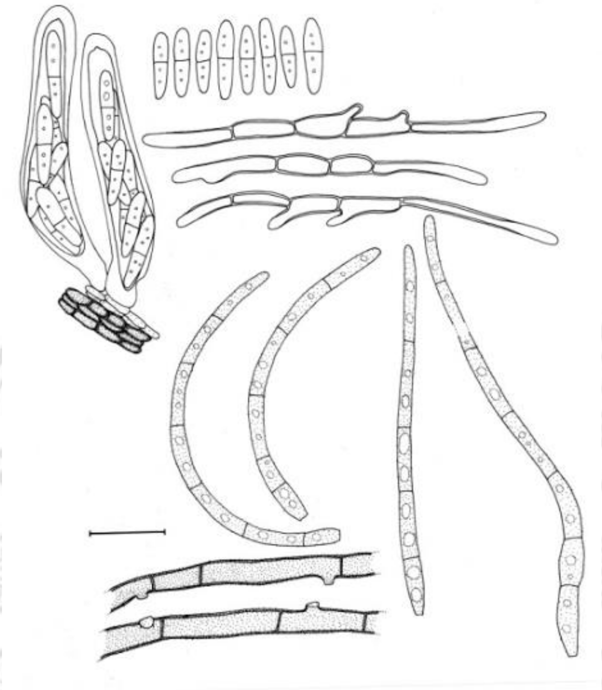

Fig. 2.9 *Pallidocercospora heimii* (CPC 1395).Asci, ascospores, germinating ascospores (after 24 h on malt extract agar), hyphae with conidiogenous loci, and conidia. Scale bar = 10 μm (Crous *et al*. 2013).

Paracercospora **Deighton**

Type species: *Paracercospora egenula*

In 2003, Crous and Braun reduced *Paracercospora* to synonymy with *Pseudocercospora* but *Paracercospora* was separated by molecular sequence analyses (Crous *et al*. 2013). The problem is the morphology of *Paracercospora* in its type is not clear enough and just only based on phylogenetic position to distinguish from *Pseudocercospora* (Braun et al. 2013), i.e. thickening along the rim of conidial scar and hila are the only main characters to distinguish between *Paracercospora* and *Pseudocercospora* (Figure 2.10).

Fig. 2.10 *Paracercospora egenula* (CPC 12537). A. Leaf spots on upper and lower leaf surface. B. Close-up of lesion. C–F. Fascicles with conidiogenous cells. G. Conidia. Scale bars = 10 µm (Crous *et al*. 2013).

***Passalora* emend. Crous & Braun (2003)**

Type species: *Passalora bacilligera* Fr. and Mont.

Passalora was the first genus published for cercosporoid hyphomycetes. Phytopathogenic and mostly causing leaf spots, sometimes almost indistinct, occasionally hyperparasitic, rarely saprobic. This group of fungi is characterized by primary mycelium internal, secondary mycelium lacking to well-develop, external, superficial; hyphae branched, septate, smooth, hyaline to pigmented. Stromata absent to well-developed, substomatal to intraepidermal, rarely deeply immersed, subhyaline to pigmented. Conidiophores arranged in loose to dense fascicles, sometimes in almost sporodocial or synnematous conidiomata, arising from internal or superficial hyphae or substomatal to intraepidermal stromata, emerging through stomata, erumpent through the cuticle or arising from creeping hyphae, terminal or as lateral branches, branched or unbranched, continuous to pluriseptate, subhyaline to pigmented, smooth to finely verruculose; Conidiogenous cells integrated, terminal or conidiophores reduced to a single conidiogenous cell, polyblastic, sympodial. Conidiogenous cells scars conspicuous, slightly thickened, somewhat darkened. Conidia solitary to catenate, in simple or branched chains, mostly non-scolecosporous, ellipsoid-ovoid, obclavate, broadly subcylindric to fusiform, pigmented, usually 0−4-septate, mostly broad, 4−15 µm, occasionally narrower, 3−5 µm, hilum conspicuous, thickened and darkened, more or less truncate (Crous & Braun 2003) (figure 2.11). This concept was also phylogenetically distinct in the first molecular analyses (Crous *et al*. 2000, 2001).

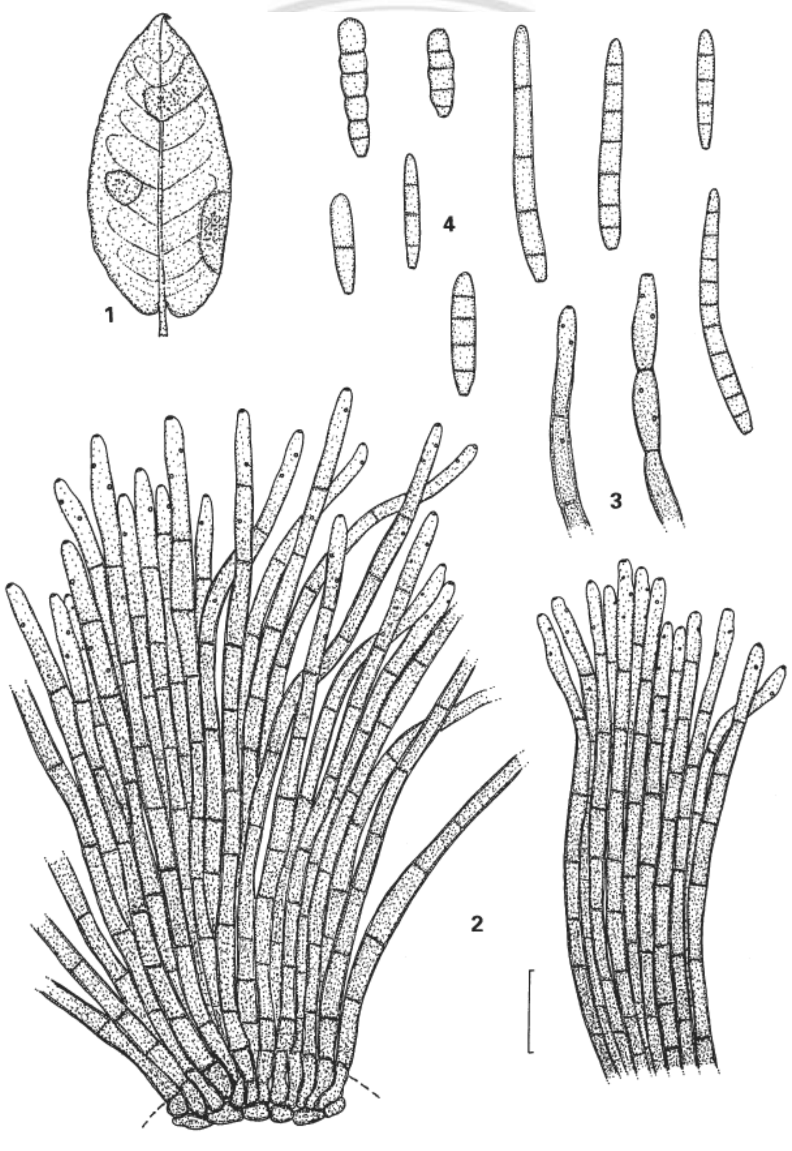

Fig. 2.11 *Passalora gochnatiicola* O.L. Pereira & U. Braun. 1. Leaf with symptoms (leaf spots). 2. Conidiomata. 3. Conidiophores with conidiogenous cells. 4. Conidia. Bar (2-4) = 20 µm, 1 (leaf) in original size (Pereira *et al*. 2006).

Pseudocercospora

Type species: *Pseudocercospora vitis* (Lév.) Speg.

Species of *Pseudocercospora* occur on a large number of plants,and are well recognized as plant pathogenic fungi, but also endophytic, and have been used as biocontrol agents of weeds (Den Breeÿen *et al*. 2006). The characteristics of genus *Pseudocercospora* are having mycelium internal and external, including smooth, septate, subhyaline to brown, branched hyphae. *Stroma* absent to well-developed. *Conidiophores* in vivo arranged in loose to dense fascicles, sometimes forming distinct synnemata or sporodochia, emerging through stomata or erumpent through the cuticle, often arising from substomatal or subcuticular to intraepidermal stromata, or occurring singly on superficial hyphae, short to long, septate or continuous, i.e. conidiophores may be reduced to conidiogenous cells, simple to branched and straight to geniculate-sinuous, pale to dark brown, smooth to finely verruculose. *Conidiogenous cells* integrated, terminal, occasionally intercalary, polyblastic, sympodial, or monoblastic, proliferating percurrently via inconspicuous or darkened, irregular annellations, at times denticulate, pale to dark brown; scars inconspicous, or only thickened along the rim, or flat, and slightly thickened and darkened, but never pronounced. *Conidia* solitary, rarely in simple chains, subhyaline, olivaceous, pale to dark brown, usually scolecosporous, i.e. obclavate–cylindrical, filiform, acicular, and transversely plurieuseptate, occasionally also with oblique to longitudinal septa, conidia rarely amero- to phragmosporous, short subcylindrical or ellipsoidal-ovoid, aseptate or only with few septa, apex subacute to obtuse, base obconically truncate to truncate, or bluntly rounded, with or without a minute marginal frill, straight to curved, rarely sigmoid, smooth to finely verruculose; hila usually unthickened, not darkened, at most somewhat refractive, occasionally slightly thickened along the rim, or rarely flat, and slightly thickened and darkened, but never pronounced (Crous *et al*. 2013) (Figures 2.12–2.13).

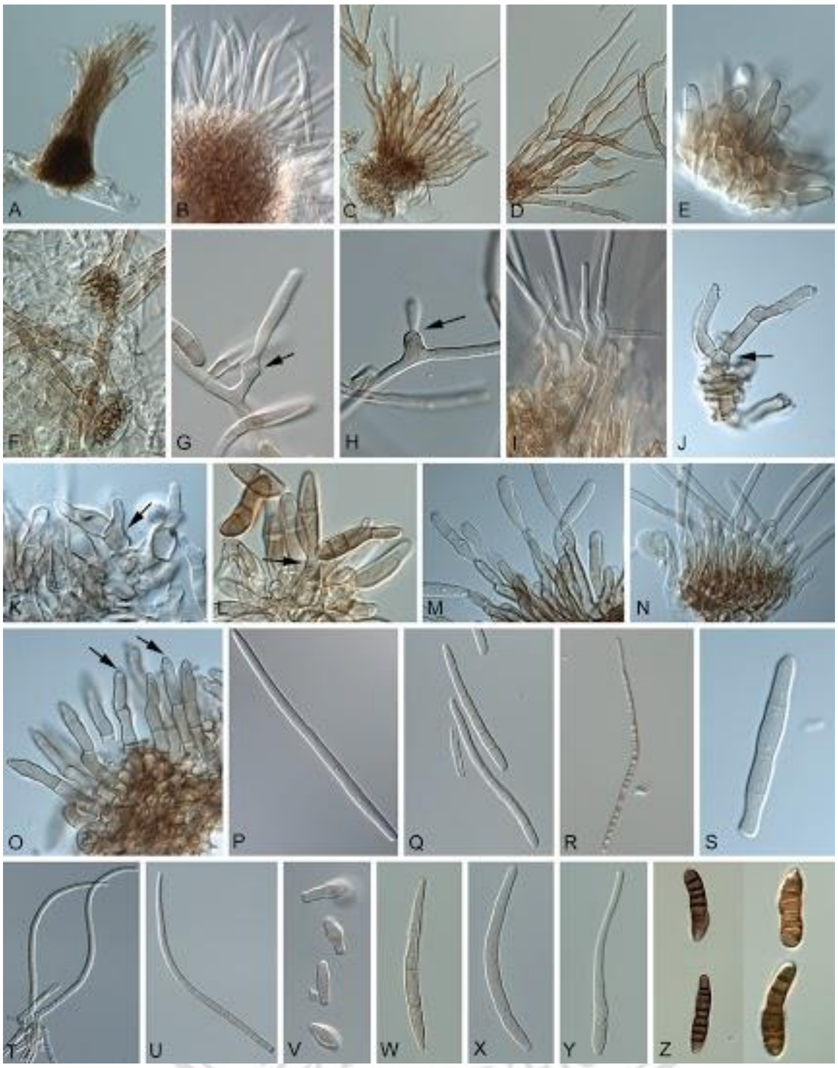

Fig. 2.12 Morphological structures of *Pseudocercospora* spp. A. Synnematous conidiophore. B. Densely aggregated fascicle of conidiophores with well-developed brown stroma. C, D. Loosely branched fascicles of conidiophores with moderate (C) and poorly (D) developed brown stroma. E. Fascicle reduced to conidiogenous cells. F. Conidiophore fascicles arising from stomata. G, H. Solitary conidiogenous cells on superficial hyphae. I. Geniculate conidiophore (arrow) with truncate apical locus. J, K. Conidiophores branched below (arrows). L. Conidiogenous cells with percurrent proliferations (arrows). M, N. Conidiophores with sympodial proliferation. O. Conidiophores with conidiogenous cells (note minutely thickened scars, arrows). P. Subcylindrical conidium with subacute apex and truncate base. Q. Conidia with constrictions at septa. R. Conidium with guttules. S. Cylindrical conidium with obtuse apex, and truncate base. T. Undulate conidia. U. Curved conidium. Aseptate to 1-septate

conidia. V. 1-septate conidia. W, X. Obclavate conidia with obconical base. Y. Obclavate conidium with short obconical base. Z. Dark brown, muriformly euseptate conidia (thick-walled, not distoseptate) (Crous *et al*. 2013).

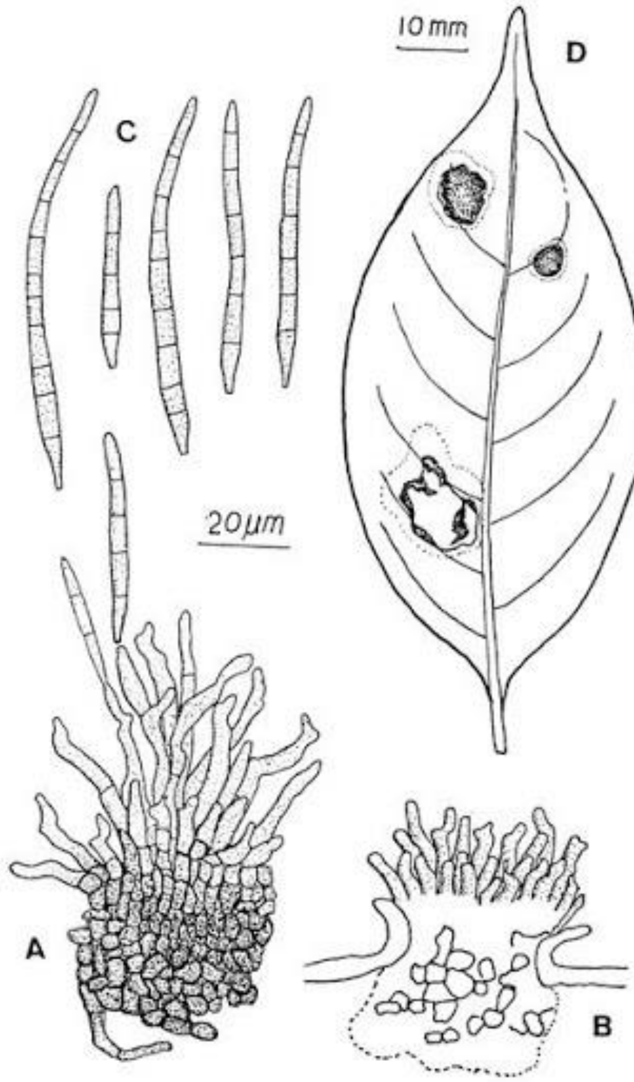

Fig. 2.13 *Pseudocercospora tabernaemontanae*. A. Fascicles of conidiophores on a stroma; B. An erumpent fascicle of fascicle; C. Conidia; D. Leaf spots (Deighton, 1976).

Pseudocercosporella

The genus *Pseudocercosporella* has been demonstrated to be polyphyletic within the family *Mycosphaerellaceae* (Frank *et al*. 2010, Crous *et al*. 2013). *Pseudocercosporella* is one of the colorless genera among the cercosporoid fungi. There are primary internal and secondary external myceliums, hyaline to pale brown, septate, branched, smooth. *Stroma* absent to well-developed, substomatal to intraepidermal, usually hyaline.*Conidiophores* solitary to fasciculate, emerging through stomata or erumpent through the cuticle, arising from inner hyphae or from stromata, sometimes formed as lateral branches of superficial hyphae, or aggregated in crustose to subglobose sporodochia, hyaline, rarely branched, straight and subcylindrical to geniculatesinuous, occasionally slightly pigmented at the base, conidiophore reduce to conidiogenous cells or septate. *Conidiogenous cells* sympodial, integrated, terminal, mono- to polyblastic, conidiogenous loci inconspicuous, unthickened and not darkened. *Conidia* solitary, rarely in chain, subcylindrical, filiform, somewhat obclavate, 1-multi-euseptate, thin-walled, obtuse to subacute at the apex, subtruncate at the base, hilum unthickened, not darkened and not refractive (adapted from Braun 1995) (Figure 2.14).

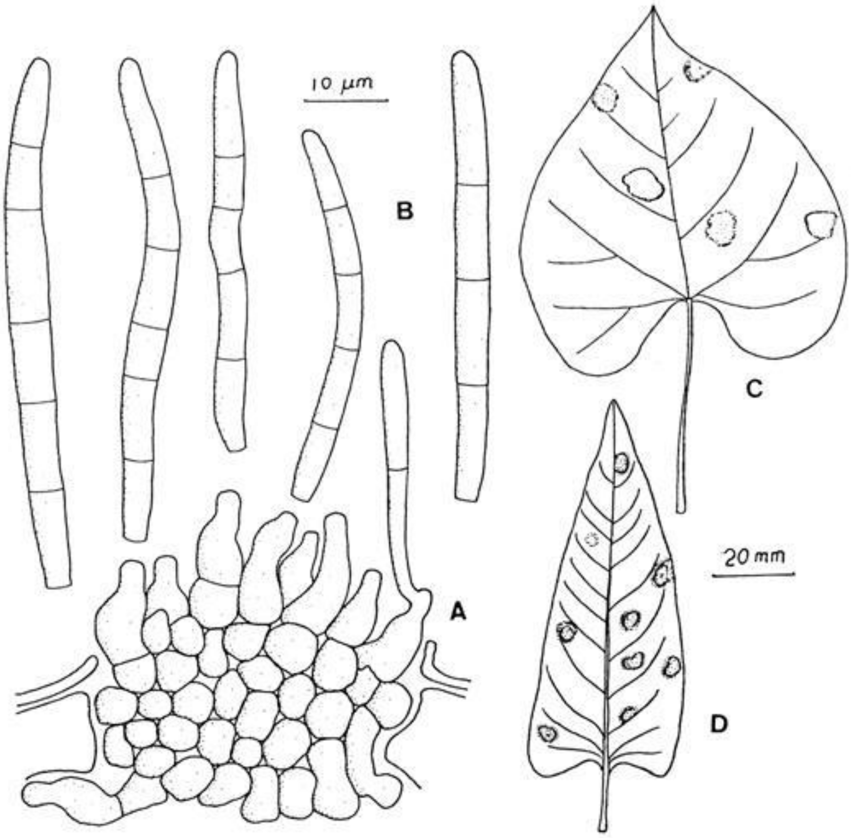

Fig. 2.14 *Pseudocercosporella ipomoeae*. A. Aggregation of short hyaline conidiophores on a sub-stromatal hyaline stroma; B. Conidia; C. Leaf spots on *Ipomoea acuminate*; D. Leaf spots on *Ipomoea aquatic* (Deighton, 1973).

Ramularia

The genus *Ramularia* was introduced by Unger (1833). *Ramularia* and allied genera are characterized by having hyaline conidiophores and conidia. Cercosporoid hyphomycetes that are colorless (*Cercosporella* Sacc., *Ramularia*, *Neoramularia* U. Braun, *Neoovularia* U. Braun, *Phacellium* Bonord., *Pseudocercosporella* Deighton) were described by Braun (1995a, 1998). *Ovularia* Sacc. (aseptate conidia) was reduced to synonymy with *Ramularia* sensu Saccardo (multiseptate conidia) (Hughes 1949, Sutton and Waller 1988, Braun 1998). *Ophiocladium* Cavara differs from this complex by its curled conidiophores, and conidia that have somewhat eccentrically positioned scars, however this genus was reduced to synonymy with *Ramularia* (Braun 1988a, Sutton and Waller 1988), and it was supported by molecular data presented by Crous *et al*. (2000). Conidium septation and unusual conidiophores shape with eccentric scars are thus characters to be considered at the species level. This group of fungi is characterized by internal primary mycelium and superficial-aerial secondary mycelium, internal mycelium often forming stroma-like hyphal aggregations septate, hyaline to subhyaline. Conidiophores straight to geniculate–sinuous, aseptate or septate, hyaline, rarely subhyaline at the base in species with subhyaline stromata, occasionally branched; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells with sympodial proliferation, polyblastic; conidiogenous loci conspicuous slightly thickened, darkened and refractive. Conidia solitary or in simple chains, sometimes in branched chains, short conidia (0–1-septate) mostly ellipsoid–ovoid to subglobose, longer conidia (mostly 0–4-septate) usually ellipsoid, cylindric, fusiform, rarely filiform, occasionally constricted at the septa, hyaline, thin-walled, smooth to verruculose, hila thickened, refractive, darkened (Figure 2.15).

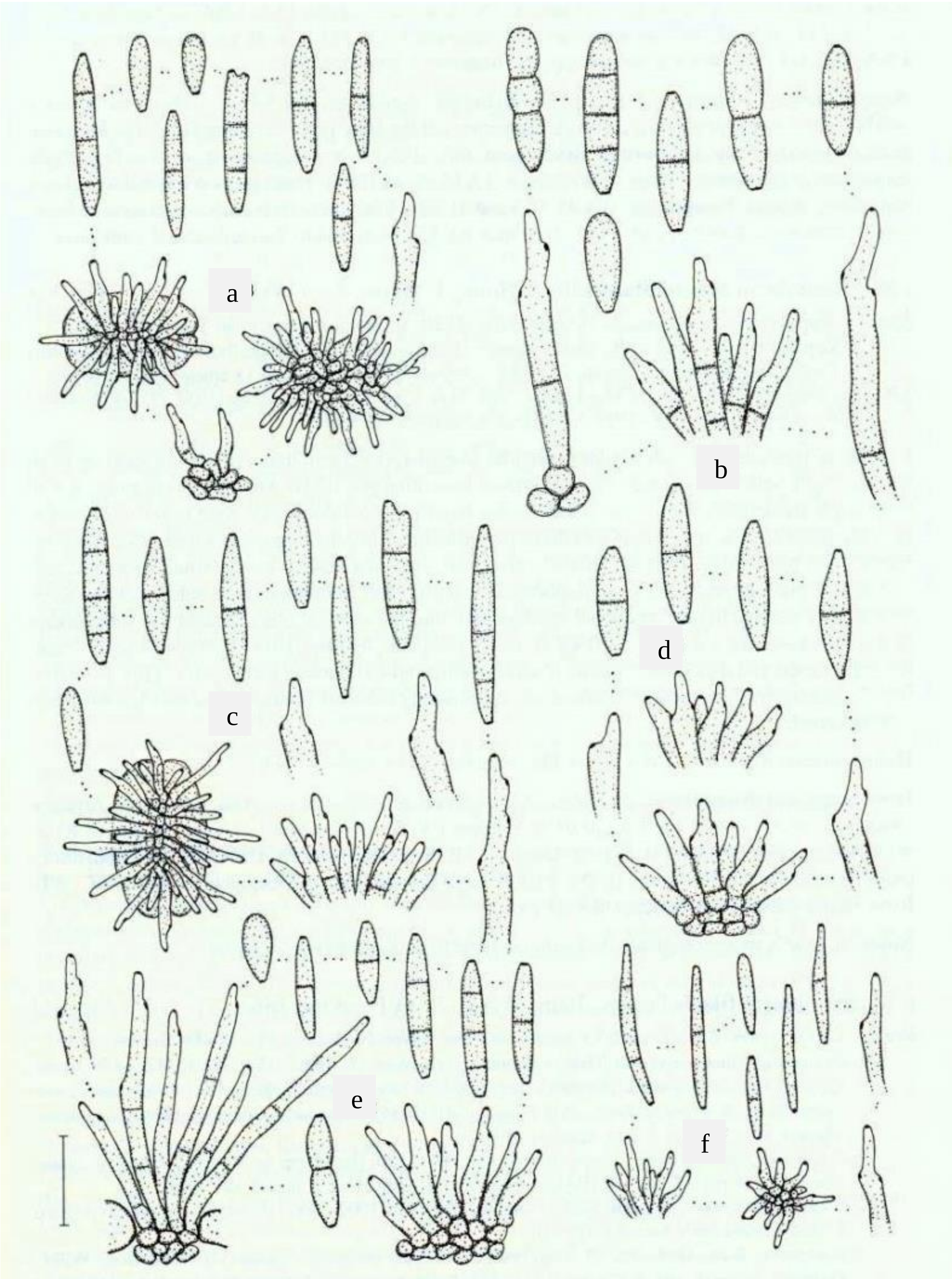

Fig. 2.15 a. *Ramularia serotina*; b. *R. bellunensis*; c. *R. asteris*; d. *R. concomitans*; e. *R. filaris*; f. *R. brunnea*.

Stenella* and *Zasmidium

Stenella was established by having or without superficial, verruculose hyphae with solitary to aggregated conidiophores, conidiogenous cells with conspicuous conidiogenous loci (thickened, darkened and refractive), and conidia formed singly or in chains, with thickened, darkened and refractive hila (Crous & Braun 2003) (Figures 2.16–2.17). Sexual morphs of *Stenella* have been linked to *Mycosphaerella* (Crous & Braun 2003, Crous *et al*. 2004, 2006). Subsequent phylogenetic studies have presented that *Mycosphaerella* is polyphyletic (Crous *et al*. 2007), and the type species of *Stenella* clusters in different families in the Capnodiales, i.e. the *Teratosphaeriaceae* (Crous et al. 2007a). Therefore the stenella-like anamorphs within the *Mycosphaerellaceae*, need to be represented to another genus. *Zasmidium* proved to be recent name for *Stenella*-like anamorph in the *Mycosphaerellaceae* (Arzanlou *et al*. 2007, Braun *et al*. 2010), due to the type species of this genus clusters in the *Mycosphaerellaceae* and its morphologically similar to *Stenella* (Arzanlou *et al.* 2008). The characteristics of these genus are identified by immersed mycelium as well as superficial, branched, septate, colourless or almost so to pigmented, thin-wall or somewhat thickened, immersed mycelium smooth to faintly verruculose, external mycelium distinctly verruculose to verrucose. *Stromata* lacking to well-developed, pigmented. *Conidiophores* solitary, arising from superficial hyphae, lateral, occasionally terminal, sometime fasciculate and arising from internal hyphae or stromata, semimacronematous to macronematous, cylindrical, filiform, subuliform, straight to strongly geniculate-sinuous, mostly unbranched, aseptate to multi-septate, subhyaline to pigmented, smooth to verruculose; conidiogenous cells integrated, terminal, occasionally intercalary, mostly polyblastic, sympodial, conidiogenous loci conspicuous, somewhat thickened and darkened-refractive, planate. *Conidia* solitary or in chain, in simple or branched acropetal chains, shape and size variable, ranging from amero- to scolecosporous, subhyaline to pigmented, smooth or almost so to usually distinctly verruculose, hila somewhat thickened and darkened-refractive, planate, conidial secession schizolytic (emend. Braun *et al.* 2010a) (Figure 2.18).

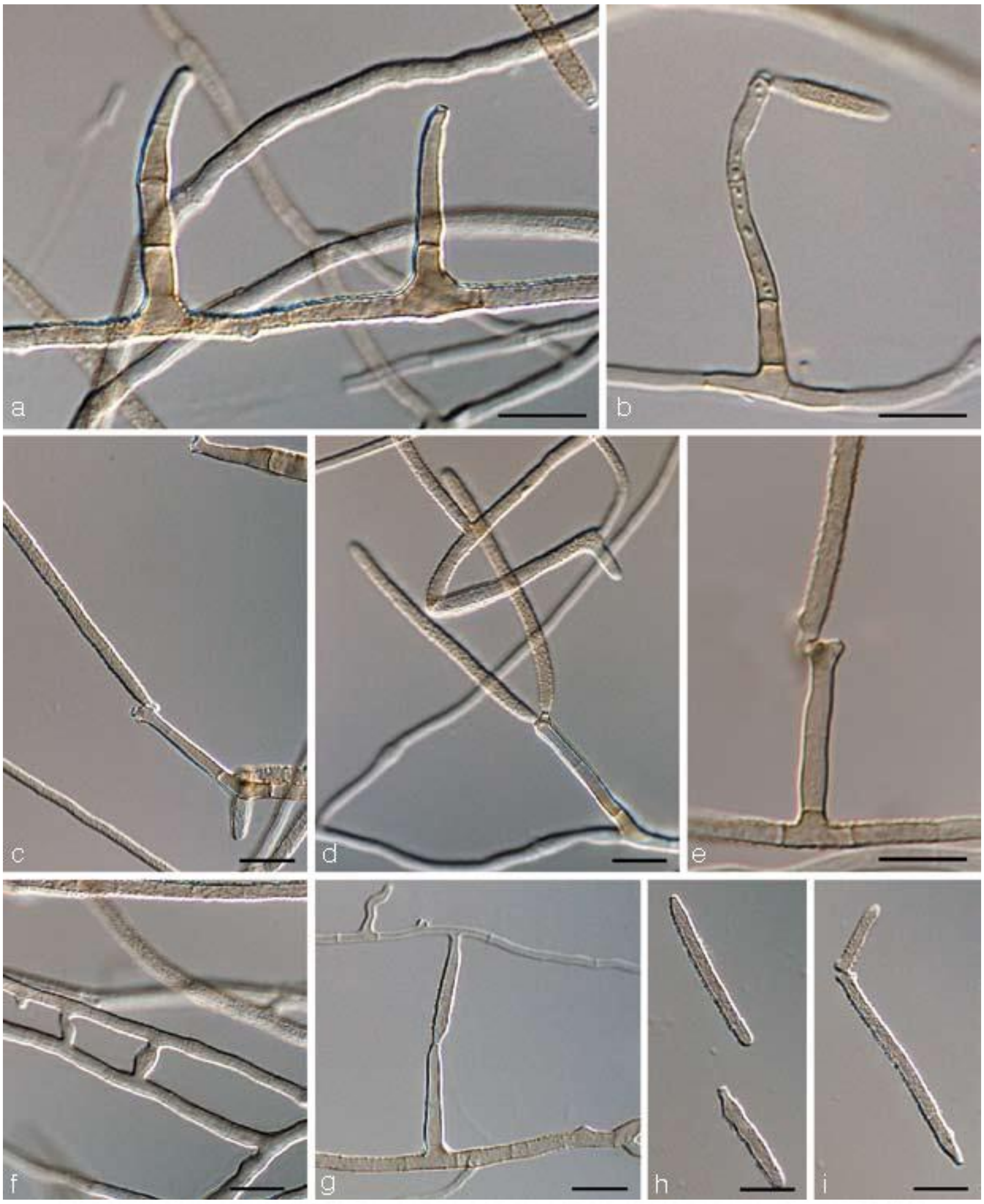

Fig. 2.16 *Stenella musicola* (CBS 122479).a–e. Conidiophores with sympodially proliferating conidiogenous cells and darkened, thickened loci; f–g. hyphal anastomoses; h–i. conidia. — Scale bar = 10 µm (Arzanlou *et al.* 2008).

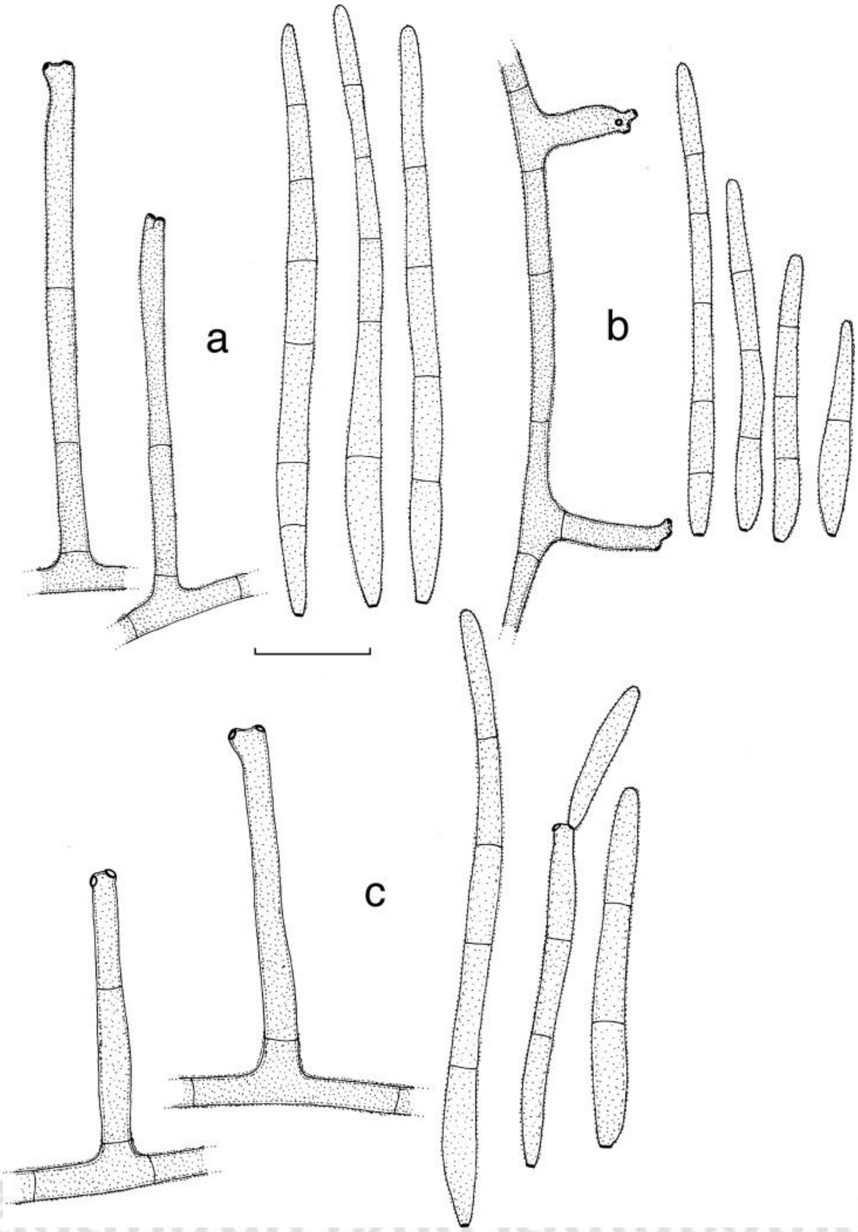

Fig. 2.17 a. *Stenella musae* (CBS 122477); b. *Stenella queenslandica* (CBS 122475); c. *Stenella musicola* (CBS 122479). — Scale bar = 10 µm (Arzanlou *et al.* 2008).

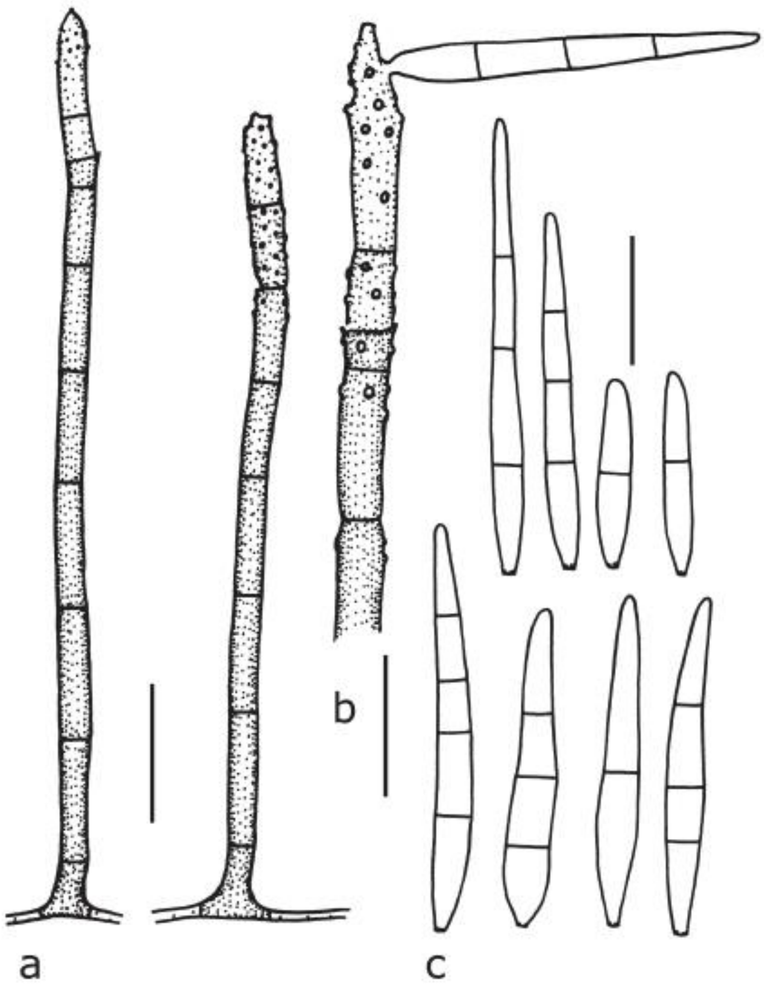

Fig. 2.18 *Zasmidium dicranopteridis* (from holotype, TNM). a. Conidiophores on external hyphae. b. Conidiophore apex with a percurrent extension, with intercalary and terminal conidiogenous cells and a conidium still attached. c. Conidia. Scale bars: a = 20μm, b, c = 10 μm (Kirschner & LIU, 2014).