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**Some cercosporoid hyphomycetes from Taiwan, including a new species of *Stenella* and new reports of *Distocercospora pachyderma* and *Phacellium paspali***

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A cercosporoid hyphomycete parasitic on *Itea parviflora* in Taiwan is described as the new species *Stenella iteae*. The grass-parasitic hyphomycete *Phacellium paspali* is for the first time reported for Asia from the new host plant *Setaria palmifolia*. *Distocercospora pachyderma* on *Dioscorea* sp. is for the first time reported for Taiwan. New details of the morphology of these fungi are given. *Pseudocercospora clematidis* is transferred to *Pseudocercospora*. Additional five species of cercosporoid fungi from our collections in Taiwan are listed.

**Key words:** anamorphic fungi, cercosporoid fungi, new combination, phytopathogenic fungi.

## **Introduction**

Plant pathogenic hyphomycetes with relationships to teleomorphic ascomycetes of the genus *Mycosphaerella* are generally named cercosporoid hyphomycetes. In many cases, however, connections with a teleomorph were not found, and morphologically-based generic concepts have often changed. Reviews and keys of the present stage of generic concepts in the cercosporoid hyphomycetes were provided by Braun (1998) and Crous and Braun (2003). Species of some genera of cercosporoid hyphomycetes are treated in this study. *Cercospora* is characterised by pigmented conidiophores producing mostly hyaline conidia from pigmented conidiogenous loci, *Distocercospora* is characterised by distoseptate conidia, and *Pseudocercospora* by pigmented conidiophores with unpigmented, unthickened conidiogenous loci. *Pseudocercospora* can be considered as the hyaline counterpart of *Pseudocercospora*. Species of *Phacellium* produce hyaline conidiophores in

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synnemata. *Stenella* differs from other pigmented cercosporoid hyphomycetes by verruculose hyphae.

Our recent studies on some plant pathogenic fungi in Taiwan resulted in additional new morphological and taxonomical findings (Kirschner *et al.*, 2002; Piepenbring, 2002). During collections of plant pathogenic fungi in Taiwan from 2001-2002 we also found several species of plant pathogenic hyphomycetes representing *Cercospora* and similar genera. The cercosporoid fungi from Taiwan were monographed by Hsieh and Goh (1990). Parts of this work were updated and incorporated in the monograph of *Pseudocercospora* by Guo *et al.* (1998). In this presentation, new findings on the host range, geographical distribution, morphology, and taxonomy of selected cercosporoid fungi are provided.

## Materials and methods

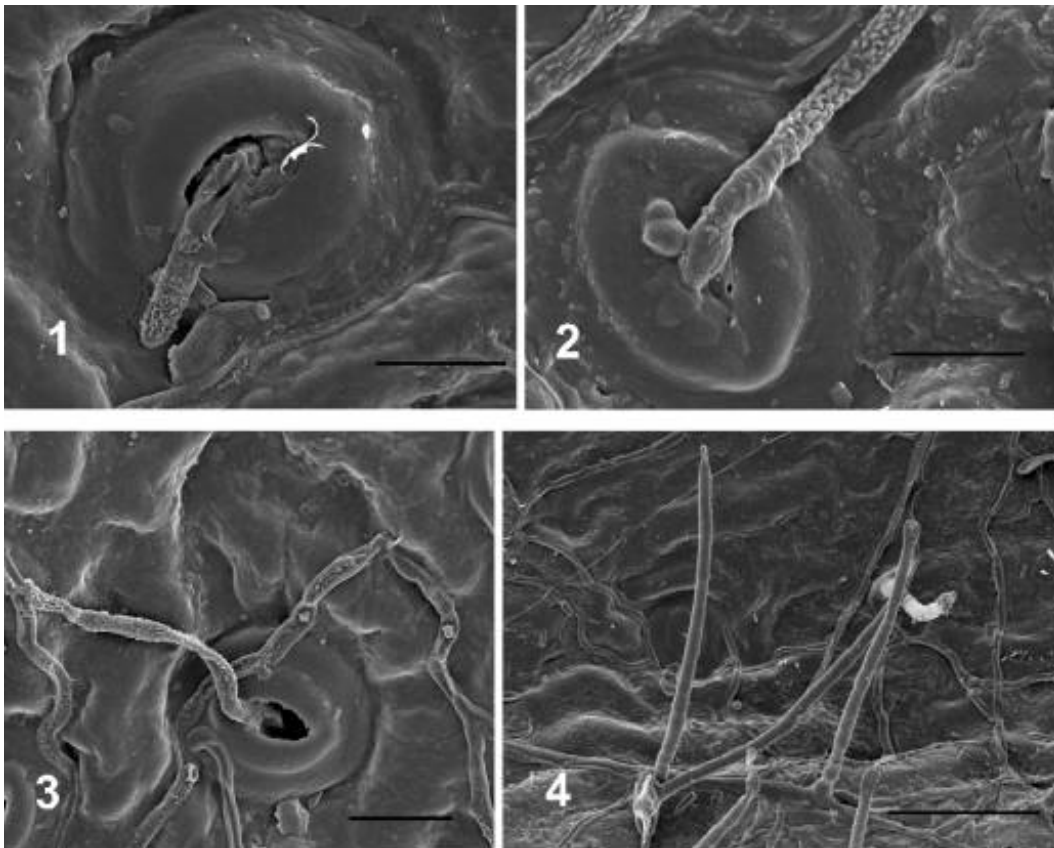
Plant pathogenic hyphomycetes were collected in different regions in Taiwan in 2001 and 2002, identified, and deposited in TNM. Conidia of three fungi identified as *Distocercospora pachyderma*, *Phacellium paspali*, and *Stenella iteae* were aseptically transferred to 2% (w/v) malt extract agar (MEA). Resulting cultures were deposited in the Centraalbureau voor Schimmelcultures (CBS). For morphological comparisons with our material from Taiwan, lectotype material of *P. paspali* was loaned from B and isotype material of *Pseudocercospora clematidis* from IMI.

Microscopic characteristics were observed using leaf sections made with a razor blade and culture material grown on MYP agar (Bandoni, 1972) or MEA at approx. 23°C. Material was mounted in 5% (w/v) KOH, with and without previous staining with 1% (w/v) aqueous phloxine solution. Statistical treatments of the measurements are indicated by extreme values given in brackets and mean value  $\pm$  1 SD. For scanning electron microscopy, air-dried material was directly mounted and sputtered with gold for 60 s. Photographs were made with a Hitachi S 4500 scanning electron microscope (SEM) and processed with Digital Image Processing System 2.5.

## Taxonomy

***Stenella iteae* R. Kirschner, sp. nov.** (Figs. 1-9)

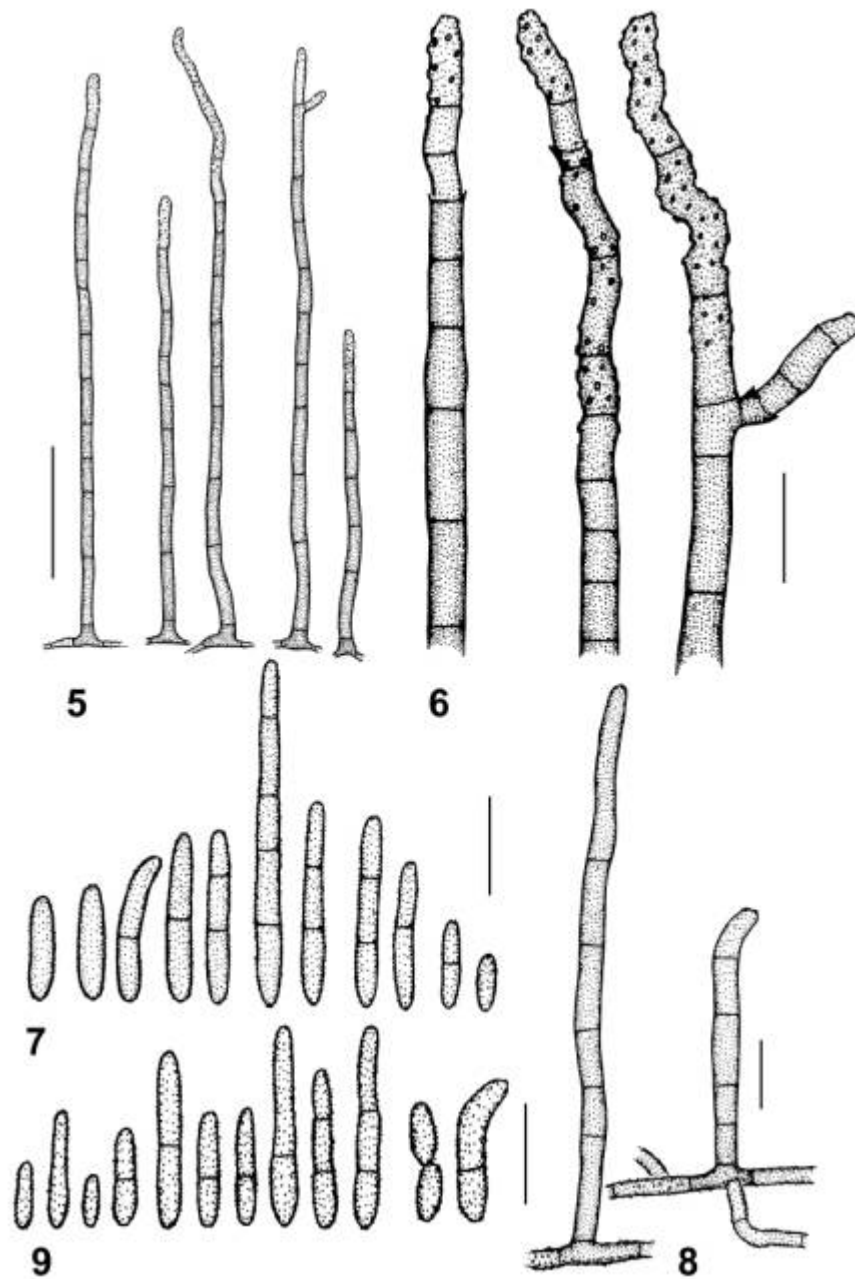
*Coloniae* hypophyllae, effusae. Stromata absentia. *Hyphae* externae per stomatibus apparentes, pallide brunneae, verrucosae, 1-3  $\mu$ m diam. *Conidiophora* ex hyphis externis oriunda, erecta, laevia, brunnea, non ramosa vel raro uno ramo laterali, (55-)85-175(-217)  $\times$  2.5-5  $\mu$ m. *Cellulae conidiogenae* terminales vel intercalares, (3-)4-13(-27)  $\mu$ m longae. *Cicatrices* conidiogenae numerosae, atrae, planae, 1  $\mu$ m diam. *Conidia* plerumque solitaria,



**Figs. 1-4.** *Stenella iteae* on *Itea parviflora* (from holotype). **1-3.** Stomata of the abaxial leaf surface of the host plant with emerging hyphae of *Stenella iteae*. **4.** Conidiophores arising from external hyphae. Bars: 1, 2 = 6  $\mu\text{m}$ ; 3 = 10  $\mu\text{m}$ ; 4 = 30  $\mu\text{m}$ .

raro in catenis, cylindrica, pallide brunnea, verrucosa, 0-2(-4)-septata, (6-)8-21(-36)  $\times$  1.5-3  $\mu\text{m}$ , hilo basali incrassato 1  $\mu\text{m}$  diam.

*Leaf spots* restricted to the lower leaf surface, reddish, round, minute, 0-40  $\mu\text{m}$  diam., aggregated to patches of up to 5 mm diam. *Stromata* absent. *External hyphae* arising through stomata (Figs. 1-3), pale brown, verrucose, 1-3  $\mu\text{m}$  diam. *Conidiophores* arising from external mycelium (Figs. 4, 5), erect, brown, smooth, mostly simple, rarely with a subapical lateral branch, distances between septa 4-27  $\mu\text{m}$ , (55-)85-175(-217)  $\times$  2.5-5  $\mu\text{m}$  (n = 30). *Conidiogenous scars* numerous, pigmented, flat, 1  $\mu\text{m}$  diam., on terminal and intercalary (3-)4-13(-27)  $\mu\text{m}$  long conidiogenous cells (Fig. 6). *Conidia* predominantly arising singly, rarely in chains, cylindrical, verrucose, pale brown, 0-2(-4)-septate, (6-)8-21(-36)  $\times$  1.5-3  $\mu\text{m}$  (n = 30), with pigmented, thickened basal hilum with 1  $\mu\text{m}$  diam. (Fig. 7).



**Figs. 5-9.** *Stenella iteae* (from holotype). **5-7.** From the natural habitat. **5.** Conidiophores. **6.** Apices of conidiophores with numerous scars. **7.** Conidia. **8, 9.** From culture (CBS 113094). **8.** Conidiophores. **9.** Conidia. Bars: 5 = 40  $\mu\text{m}$ ; 6-9 = 10  $\mu\text{m}$ .

*Culture (on MEA):* Colonies developing a radius of 6-7 mm in 21 days, dark brown on both surfaces, upper surface with a grey margin, densely velutinous. *Hyphae* subhyaline or pale brown, only the hyphal cell directly bearing a conidiophore darker, mostly verrucose, rarely with smooth parts, 1-3  $\mu\text{m}$  diam. *Conidiophores* brown, smooth, unbranched, 34-112  $\times$  3-4  $\mu\text{m}$  (Fig. 8). *Conidiogenous scars* few and inconspicuous, pigmented, on terminal conidiogenous cells. *Conidia* mostly single, rarely in short chains, pale brown, verrucose, basal scar inconspicuous, rarely distinctly pigmented, 0-2-septate, 6-22  $\times$  2-3  $\mu\text{m}$  (Fig. 9).

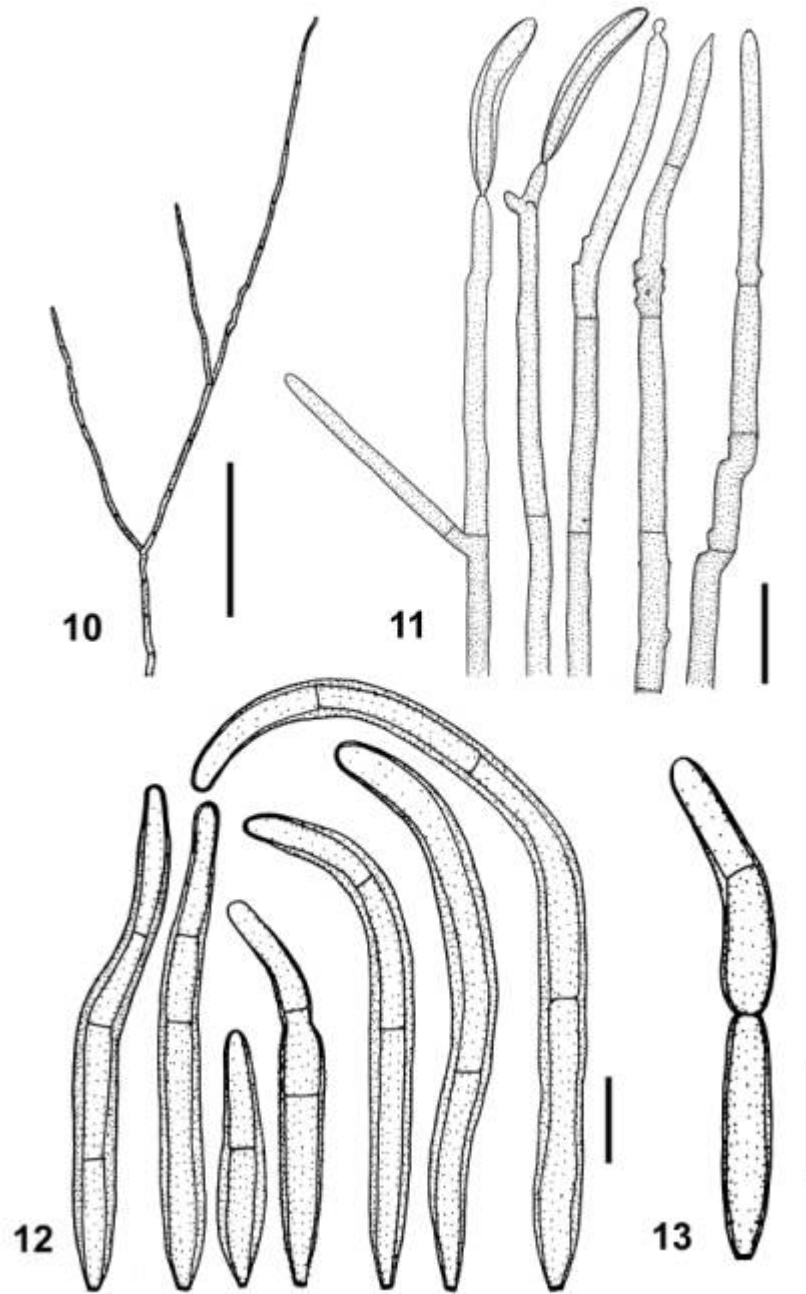
*Holotype:* TAIWAN, Pingtung, Nanrenshan, on leaves of *Itea parviflora* Hemsl. (*Iteaceae*), 2 June 2002, R. Kirschner and C.J. Chen 1279 (TNM).

*Living culture:* ex-type, CBS 113094.

*Notes:* Among cercosporoid hyphomycetes, only *Pseudocercospora iteae* (Sawada & Katsuki) Goh & Hsieh on *Itea oldhamii* Schneider was hitherto known from members of the *Iteaceae* (Hsieh and Goh, 1990). *Stenella iteae* is characterised by the absence of stromata, comparatively long conidiophores arising from the external mycelium and conidia mostly with 1-2 septa. Several other species with a similar combination of characteristics are, however, known from members of other plant families. In cercosporoid fungi, distinguishing of species is generally based on morphological differences between species parasitising members of the same plant family, mostly without considering morphologically similar species on hosts belonging to other plant families. Since this concept has hitherto not yet been verified and the family *Iteaceae* often was included in the *Saxifragaceae*, we extended our comparisons including species of *Stenella* on members of other families of the *Saxifragales*. The species of *Stenella* known from members of the plant families accepted within the *Saxifragales*, *S. mitellae* (Peck) U. Braun on species of *Heuchera*, *Mitella*, and *Tolmiea* (*Saxifragaceae s.s.*) and *S. sardoa* (Sacc.) U. Braun on *Paeonia* spp. (*Paeoniaceae*) differ from the new species by the presence of stromata and shorter conidiophores not exceeding 55  $\mu\text{m}$  (Braun, 1998). A species considered as plurivorous, *S. constricta* Mulder, differs by wider conidia (Mulder, 1982).

***Distocercospora pachyderma*** (Syd. & P. Syd.) Pons & B. Sutton, Mycological Papers 160: 78 (1988). (Figs. 10-13)

*Leaf spots* indistinct, consisting of a light yellowish discolouration with diffuse margin. *Stromata* not found. *External mycelium* absent. *Conidiophores* hypophyllous, emerging through stomata, fasciculate, irregularly branched, brown, becoming pale brown to the apex, smooth or rarely very finely verruculose, (224-)316-490(-624)  $\times$  3-5  $\mu\text{m}$  (n = 20; Fig. 10). *Conidiogenous cells* apical and intercalary, straight or geniculate (Fig. 11). *Conidiogenous*



**Figs. 10-13.** *Distocercospora pachyderma*. **10.** Conidiophore. **11.** Apices of conidiophores. **12.** Distoseptate conidia. **13.** Conidium breaking at the lower septum. Bars: 10 = 100; 11 = 20  $\mu\text{m}$ ; 12, 13 = 10  $\mu\text{m}$ .

*scars* 1  $\mu\text{m}$  diam., slightly protuberant (up to 1  $\mu\text{m}$ ) or appressed, pigmented and with slightly thickened wall. *Conidia* solitary (Fig. 12), rarely forming chains of two by breaking at a septum (Fig. 13), straight to strongly curved, pale brown, smooth or very finely verruculose, basally tapering to a truncate, pigmented and slightly thickened hilum, apically tapering to an obtuse end, 1-5-distoseptate, longitudinal cell walls thickened, (30-)46.5-94.5(-128)  $\mu\text{m}$  long and in the broadest part above the base (4.5-)5-6.5(-7)  $\mu\text{m}$  wide (n = 20).

*Culture*: Conidia germinated on the agar, but failed to produce growing mycelium.

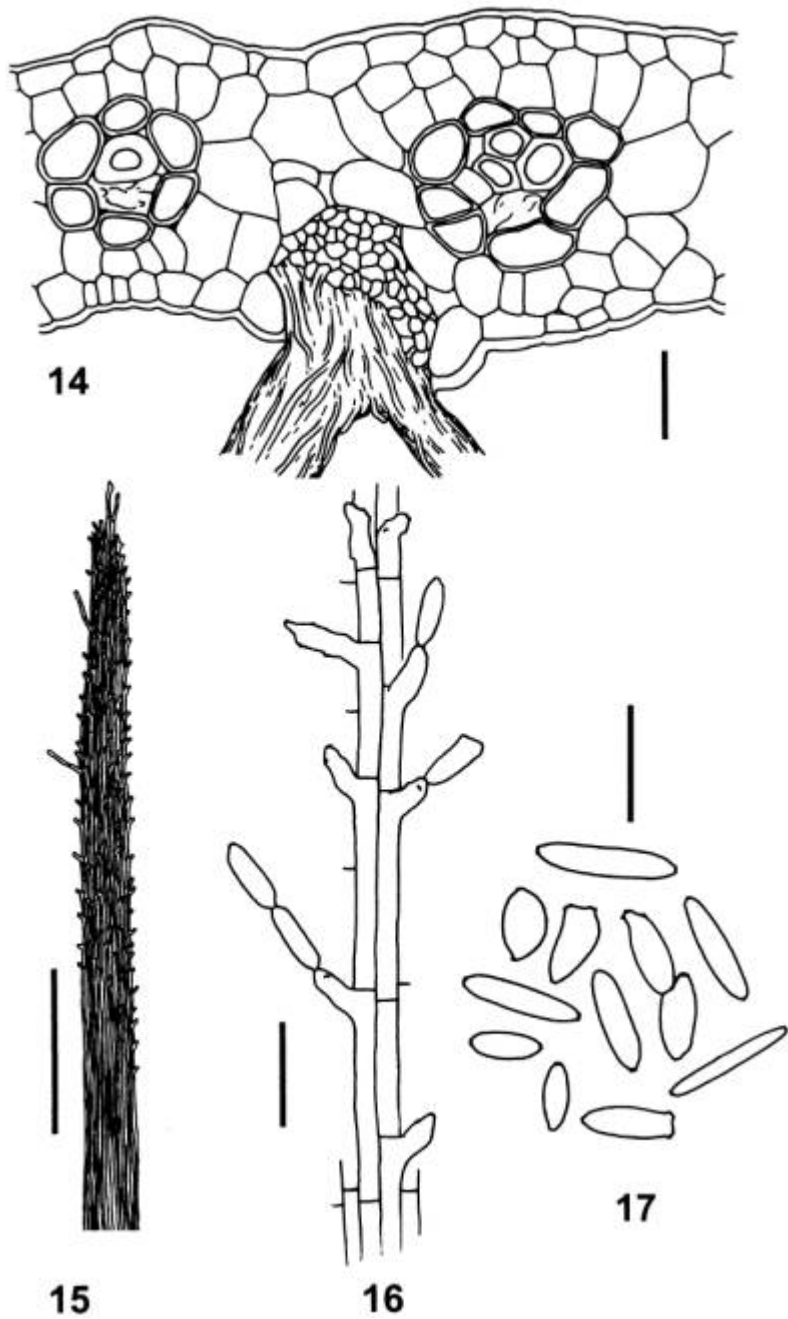
*Specimen examined*: TAIWAN, Taipei/Ilan, Fushan, ca. 550 m, on living leaves of *Dioscorea* sp. (*Dioscoreaceae*), 26 Aug. 2002, R. Kirschner, Y.M. Ju & D.C. Chang 1473 (TNM).

*Notes*: Though the maximum values of conidiophores and conidia of our specimen are considerably longer than those reported by Pons and Sutton (1988: conidiophores up to 450  $\mu\text{m}$ , conidia up to 82  $\mu\text{m}$ ), the species can be identified by its distoseptate conidia and its host belonging to the genus *Dioscorea*. A list of countries in Africa, Asia, and the Caribbean with reports of *D. pachyderma* was given by Pons and Sutton (1988), but without including Taiwan. The genus differs from other genera of cercosporoid fungi by its distoseptate conidia (Pons and Sutton, 1988). A further species parasitic on members of *Dioscorea* in South Africa, *D. africana* P.W. Crous & U. Braun, was added to the genus by Crous and Braun (1994) and differs by its shorter conidiophores.

***Phacellium paspali* (Syd.) U. Braun, Nova Hedwigia 56: 437 (1993).**

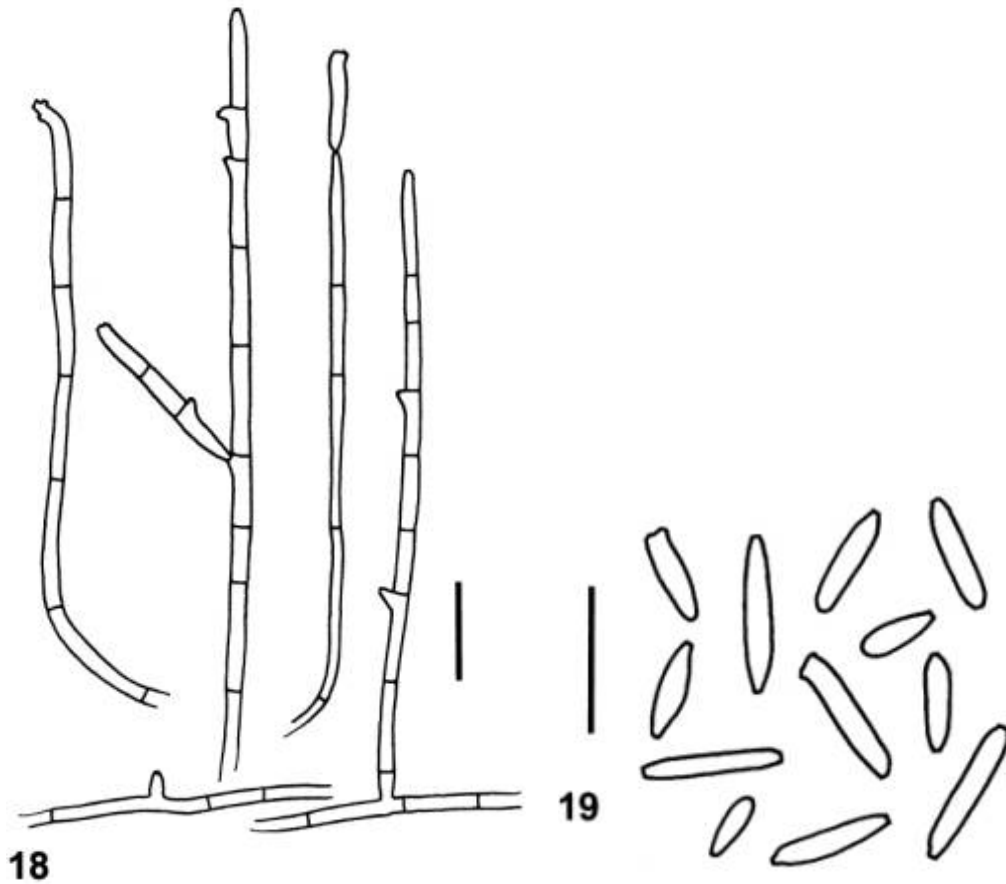
(Figs. 14-19)

*Leaf spots* amphigenous, spreading between longitudinal veins, green to pale brown, becoming surrounded by a brown margin. *Synnemata* hypophyllous, emerging through stomata and disrupting them, filling the substomatal chamber with a pseudoparenchymatic stroma, sometimes branched at the base (Fig. 14), hyaline or becoming slightly pigmented at the base, erect, 308-650  $\times$  17-30  $\mu\text{m}$ , formed by closely aggregated parallel conidiophores, cylindrical or apically narrowing, apex not or sparsely splaying out (Fig. 15). *Conidiogenous cells* apically and laterally arising from the synnemata, often covering the synnemata from closely above the base or from the lower third of the synnemata to the apex, intercalary and terminal in the conidiophores, hyaline, smooth, 9-15  $\times$  2-3.5  $\mu\text{m}$  (Fig. 16). *Fertile ends* of the conidiophores arising in an acute angle from the conidiophores, forming free, 3-20  $\mu\text{m}$  long ends, with slightly thickened and darkened scars. *Conidia* hyaline, catenate, aseptate, smooth, cylindrical, fusiform or ellipsoidal, (3.5-)5-11(-16)  $\times$  1.5-3.5



**Figs. 14-17.** *Phacellium paspali* on *Setaria palmifolia* (specimens from Taiwan). **14.** Section through a leaf of *S. palmifolia* with the base of a synnema in a substomatal chamber and the synnema branching into two stipes. **15.** Synnema separated from the leaf. **16.** Conidiophores. **17.** Conidia. Bars: 14 = 25  $\mu\text{m}$ ; 15 = 100  $\mu\text{m}$ ; 16, 17 = 10  $\mu\text{m}$ .





**Figs. 18, 19.** *Phacellium paspali*, from culture (CBS 113093). **18.** Mononematous conidiophores. **19.** Conidia. Bars = 10  $\mu\text{m}$ .

$\mu\text{m}$  ( $n = 30$ ), with a basal and 1-2 apical, slightly thickened and darkened scars (Fig. 17).

*Culture:* Colonies in culture on MEA and MYP growing slowly, reaching a diam. of 6.5 mm in 18 days on MEA. Colonies on MEA white by aerial mycelium in the centre, bright red at the margin, reverse dark brown with a bright red margin, on MYP white by aerial mycelium in the centre, violet-pinkish towards the margin on the upper side, reverse pink at the margin, dark violet in the centre. Aerial mycelium sparse, white. Hyphae hyaline, 1-2  $\mu\text{m}$  diam. Conidiophores mononematous, macronematous and micronematous, 3-220  $\times$  1-2  $\mu\text{m}$ , mostly shorter than 66  $\mu\text{m}$  (Fig. 18). Conidiogenous cells terminal and lateral, with 1-3 slightly thickened and darkened scars. Conidia hyaline, catenate, aseptate, smooth, cylindrical, fusiform or ellipsoidal, 4-16  $\times$  1-2  $\mu\text{m}$ , with a basal and 1-2 apical, slightly thickened and darkened scars (Fig. 19).

*Specimens examined:* ECUADOR, near Baños, Prov. Tungurahua, Hacienda San Antonio, on *Paspalum paniculatum* L. (*Poaceae*), 12 Jan. 1938, H. Sydow, F. exot. exs. 1247 (lectotype, B); TAIWAN, Kaohsiung, ChuYun Shanlin Dao, ca. 300 m, on living leaves of *Setaria palmifolia* (J. König) Stapf (*Poaceae*), 28 April 2001, R. Kirschner & C.J. Chen 819 (TNM); TAIWAN, Taichung, Tahsueshan, ca. 800 m, on living leaves of *Setaria palmifolia*, 13 April 2002, R. Kirschner & C.J. Chen 1144 (TNM).

*Living culture:* ex R. Kirschner & C.J. Chen 1144, CBS 113093.

*Notes:* *Phacellium paspali* was previously known only from *Paspalum paniculatum* L. in Ecuador (Braun, 1998). This grass was introduced from South America and naturalised in Taiwan (Hsu, 2000), but specimens of *Phacellium* were previously not found on this host in Taiwan. The synnemata of the specimens from Taiwan are considerably longer than those reported for the specimen from Ecuador (up to 250 µm; Braun, 1998). Investigating the synnemata of the lectotype of *P. paspali*, however, we found synnemata with a length of up to 495 µm. Furthermore, the genera *Setaria* and *Paspalum* belong to the same subfamily, *Panicoideae*, but their relationship within this subfamily is not clear and both genera could belong to two different clades (Giussani *et al.*, 2001). Because of the overlapping synnemata length, the similarity of the other morphological characteristics, and the close relationship between the two host plant species, we conclude that the specimens from Taiwan are conspecific with that from Ecuador.

Another cercosporoid fungus known from *Setaria palmifolia* is *Cercospora setariae* Atkinson which is known from Africa, Asia, North and South America (Hsieh and Goh, 1990). It distinctly differs from species of *Phacellium* by its pigmented divergent conidiophores and solitary multiseptate conidia (Hsieh and Goh, 1990).

Since *P. paspali* is now known to occur on *Setaria palmifolia* in Taiwan and on *Paspalum paniculatum* in Ecuador, it might be speculated which processes led to this situation. The host range of the fungus might be wider in Ecuador and other regions of the world, but *P. paspali* has rarely been collected. Or, on the other hand, *P. paspali* could have been introduced on its host *Paspalum paniculatum* from South America to Taiwan and jumped to *Setaria palmifolia*. In the context of coevolutionary events in speciation processes of plant parasitic fungi and their hosts, our findings show the necessity of more collections in the field and detailed morphological investigations which provide fundamental data of the distribution of these fungi and their host specificity.

In culture, the fungus exclusively forms a mononematous state and is similar to members of genera with hyaline, mononematous, denticulate conidiophores producing septate conidia in chains, like *Diploospora* and *Hormiactis*.

***Pseudocercospora clematidis* (Goh & W.H. Hsieh) R. Kirschner, **comb. nov.****

≡ *Pseudocercospora clematidis* Goh & W.H. Hsieh, Transactions of the Mycological Society of Taiwan R.O.C. 4: 6 (1989).

*Specimens examined:* TAIWAN, Taichung, Kukuan, on leaves of *Clematis gouriana* Roxb. (*Ranunculaceae*), 27 Oct. 1985, T.K. Goh (isotype IMI 312079); TAIWAN, Chiayi, Chungpu, below 100 m, on leaves of *Clematis* sp. (*Ranunculaceae*), 4 May 2001, R. Kirschner & C.J. Chen 834 (IMI 391785, TNM).

*Notes:* Stromata are absent or are not thicker than 15 µm. The absence of superficial mycelium according to Hsieh and Goh (1990) is confirmed. Stromata, conidiophores and conidia are hyaline in our specimen as well as the conidiophores and conidia seen in the isotype, which suggests a placement in *Pseudocercospora* Deighton.

Additional records of cercosporoid species already known for Taiwan and identified with the monograph by Hsieh and Goh (1990):

*Cercospora bidentis* Tharp

*Material examined:* TAIWAN, Kaohsiung, Shuanghsi, 'Tropical Forest', on leaves of *Bidens* sp. (*Asteraceae*), 23 Apr. 2001, R. Kirschner & C.J. Chen 814 (TNM).

*Cercospora cyperi* Sawada

*Material examined:* TAIWAN, Nantou, Hui Sun Lin Chang, ca. 700 m, on leaves of an unidentified species of the *Cyperaceae*, 21 July 2002, R. Kirschner & C.J. Chen 1388 (TNM).

*Pseudocercospora atromarginalis* (G.F. Atk.) Deighton

*Material examined:* TAIWAN, Nantou, Chushan ('Bamboo Mountain'), on leaves of *Solanum* sp. (*Solanaceae*), 11 May 2002, R. Kirschner & C.J. Chen 1217 (TNM).

*Pseudocercospora costina* (Syd. & P. Syd.) Deighton

*Material examined:* TAIWAN, Kaohsiung near Hulugu ('Gourd Gorge'), ca. 100-200 m, on leaves of *Costus* sp. (*Costaceae*), 11 Sept. 2002, R. Kirschner & C.J. Chen 1521 (TNM).

*Pseudocercospora cruenta* (Sacc.) Deighton

*Material examined:* TAIWAN, Changhua, below 100 m, on leaves of cultivated *Vigna* sp. (*Fabaceae*), 20 July 2002, R. Kirschner & C.J. Chen 1373 (TNM).

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