A revision of *Phyllachora* (Ascomycotina) on hosts in the angiosperm family *Asclepiadaceae*, including *P. gloriana* sp. nov. on *Tylophora benthamii* from Australia

## Ceridwen A. Pearce1\*, Paul Reddell2 and Kevin D. Hyde3

Pearce, C.A., Reddell, P. and Hyde, K.D. (1999). A revision of *Phyllachora* (Ascomycotina) on hosts in the angiosperm family *Asclepiadaceae*, including *P. gloriana* sp. nov. on *Tylophora benthamii* from Australia. Fungal Diversity 3: 123-138.

We have monographed species of *Phyllachora* occurring on the pantropical plant family *Asclepiadaceae* using traditional taxonomic methods. Eight *Phyllachora* species have been described previously from the *Asclepiadaceae*. We have found that five of these taxa are untenable. In addition, *P. gloriana* sp. nov. on *Tylophora benthamii*, north Queensland, Australia, is described. *Phyllachora gloriana* differs from other *Phyllachora* species occurring on the *Asclepiadaceae* in having larger ascospores surrounded by a distinct mucilaginous sheath, and larger asci with an apical apparatus. The accepted *Phyllachora* species from the *Asclepiadaceae* are described and illustrated with interference contrast micrographs.

Key words: foliicolous fungi, tar spots, taxonomy

## Introduction

The *Phyllachoraceae* are a diverse family of microfungi currently recognised as comprising 42 genera and more than 1100 species worldwide (Hawksworth *et al.*, 1995). Most members of this family are obligate biotrophs which produce black "tar spots" on leaves and stems of their host plants. Species of *Phyllachoraceae* have been recorded on a very wide range of host plant families (Cannon, 1997), with most taxa presumed to be host specific. In general they appear to be only minor plant pathogens (Cannon, 1991), although it has been postulated that the biotrophic nature of *Phyllachora* and their longevity on host plant leaves, may predispose, or provide a pathway for secondary infection by other, more severely pathogenic organisms (Cannon, 1991; Hock *et al.*, 1992; Parbery, 1996).

<sup>&</sup>lt;sup>1</sup> Australian Tropical Mycology Research Centre, PO Box 312, Kuranda, 4872, Queensland, Australia; \* e-mail: cpearce@tpgi.com.au

<sup>&</sup>lt;sup>2</sup> CSIRO Tropical Forest Research Centre, PO Box 780, Atherton, 4883, Queensland, Australia

<sup>&</sup>lt;sup>3</sup> Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong

During a survey of phyllachoraceous taxa in Australia (Pearce and Hyde, 1994, 1995a, b) we found a *Phyllachora* species on leaves and green stems of the rainforest vine *Tylophora benthamii* (*Asclepiadaceae*) in north east Queensland. This was the first record from the southern hemisphere of a *Phyllachora* on a member of the pantropical family *Asclepiadaceae*. In order to identify this species we obtained and examined the type specimens and/or descriptions of all eight *Phyllachora* species described previously from the *Asclepiadaceae*. During this assessment, it became evident that some of these taxa were of dubious value because they had been inadequately described and then transferred to *Phyllachora* without review, the type collections contained no fertile components, the host plant was in doubt, or the taxa were synonyms of previously described *Phyllachora* species. We subsequently made a more detailed re-appraisal of the type collections using key morphological features for the genus as defined by Parbery (1967) and Cannon (1991).

In this paper, we present the results of our re-appraisal of *Phyllachora* on the *Asclepiadaceae*, and describe the Australian collection as a new species, *P. gloriana* C.A. Pearce, P. Reddell and K.D. Hyde sp. nov.

### Materials and methods

Specimens of *Phyllachora* on leaves and green stems of the vine *Tylophora benthamii* were collected in Wongabel State Forest, on the Atherton Tablelands in north Queensland, and examined fresh and following drying. Type specimens of other *Phyllachora* species on the *Asclepiadaceae* were borrowed from herbaria ILL, LPS, PH, and S. The fruiting structures of all specimens were dissected *in situ*, under a drop of water. The ascomatal contents were allowed to swell, and the excess water was removed using filter paper. The contents were transferred using fine forceps to a drop of water on a glass slide and a coverslip was added. Squash mounts were made in water, Melzer's reagent and lactophenol cotton blue. Sections were made using a freezing cryotome. All photographs and measurements were made from material mounted in water, unless otherwise stated. Accepted taxa are arranged alphabetically by *Phyllachora* species. Differential interference and phase contrast optics were used in photographing the specimens.

# **Taxonomy**

After re-appraising the key morphological characters of the eight *Phyllachora* species described previously from the *Asclepiadaceae*, we have found only three legitimate taxa. In addition, we found that our collections of *Phyllachora* from *Tylophora benthamii* were sufficiently distinctive in their morphology to warrant recognition as a new species. On the basis of these studies

we have produced a revised key to Phyllachora on Asclepiadaceae which is presented below. Detailed taxonomic descriptions of the species accepted in the revision are also presented.

## Key to species of Phyllachora found on the host family Asclepiadaceae

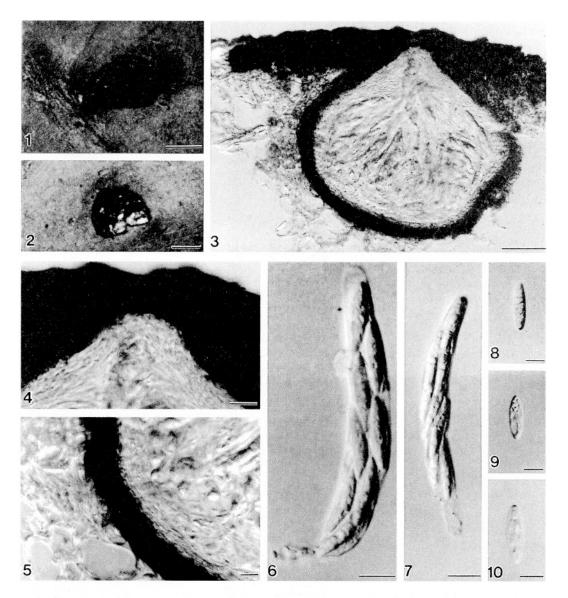
- Ascospores oval, 16-23 × 6-8.5 μm, with hyaline, bipolar, pad-like, appendages, up to 1.5 µm thick P. metastelmatis 1. Ascospores fusiform 2 2. 2. Ascospores 26-31  $\times$  4.5-6  $\mu m$ , fusiform to club-shaped, asci 88-114  $\times$  8-13  $\mu m$ , on
- Ascospores  $28-36 \times 5-8 \mu m$ , with a mucilaginous sheath up to 4  $\mu m$  thick, asci  $124-155 \times 10^{-2}$
- Phyllachora ajrekarii Syd. and P. Syd., Annales Mycologici 10: 408 (1912), as "ajrekari" (Figs. 1-10)
  - = Scolecodothis tylophorae J.S. Tendulkar, Ph. D. Thesis, Poona University (1971).
- = Phyllachora kanyakumariana V.B. Hosagoudar, Indian Phytopathology 48: 213-214 (1995).

Leaf spots: most prominent on the upper leaf surface,  $1.5-4 \times 1-3$  mm, black, shiny, roughly circular to elliptical, or irregular in shape, often developing along leaf veins, domed above the leaf surface with a flattened, granular central area, ostioles distinct as minute round pores in the apical surface, multiloculate, sometimes with a narrow, reddish-brown halo of discoloured host tissue (Figs. 1, 2). On the lower leaf surface, not as prominent, slightly smaller and less raised above the leaf surface.

Anamorph: not seen.

Andromorph: not seen.

Teleomorph: Ascomata occurring singly or often adjacent to other ascomata, immersed in the leaf tissue, often occupying 1/2 to full leaf thickness, 186-285 μm wide, 168-272 μm high, subglobose to oblate-sphaeroidal with a central, very shallow, wide, conical ostiolar canal, lined with numerous, fine, hyaline periphyses (Figs. 3, 4). Upper peridium clypeate, 30-50 µm thick, thickest adjacent to ostiolar canal, composed of a deeply melanized, brown-black. amorphous layer of host cuticle and epidermal cells, often merging with a lighter pigmented region of brownish, distorted parenchyma cells infiltrated with fungal hyphae, beneath the clypeus. Lower and lateral peridium composed of two layers; an outer region comprising several layers of dark brown, flattened, thin-walled



Figs. 1-10. *Phyllachora ajrekarii* (from holotype). 1, 2. Leaf spots. 3. Vertical section through immersed ascoma. 4. Vertical section through ostiolar canal. 5. Vertical section through lower lateral perdium. 6, 7. Asci. 8-10. Ascospores. Bars: 1, 2 = 1 mm,  $3 = 50 \mu \text{m}$ ,  $4-10 = 10 \mu \text{m}$ .

fungal cells,  $13-21~\mu m$  thick, which merge inwardly with several layers of hyaline, flattened, thin-walled fungal cells, ca. 5-8  $\mu m$  thick (Fig. 5). Lateral peridium fuses outwardly with an irregular, narrow region of distorted host parenchyma infiltrated by fungal cells. The basal peridium merges outwardly with either a narrow zone of infiltrated and distorted host parenchyma and

occasionally lower epidermal cells, or integrates directly with a lower clypeus, similar in construction to that above, but up to 52  $\mu$ m thick, and not as wide in the hosts lower epidermal layer. Asci and paraphyses arise from the basal wall. *Paraphyses* numerous, longer than asci, 2-4  $\mu$ m wide, with rounded apices, filiform, hyaline, guttulate, not constricted at septa, no branching seen. *Asci* 70-101  $\times$  9-13  $\mu$ m ( $\bar{x} = 89 \times 11 \mu$ m, n = 10), 8-spored, cylindrical, short pedicellate, thin-walled, unitunicate, tapering slightly to a rounded apex, no apical apparatus seen, non-reactive in Melzer's reagent (Figs. 6, 7). *Ascospores* arranged uni-or biseriately, usually oblique, often overlapping, 19-26  $\times$  4.5-6.5  $\mu$ m ( $\bar{x} = 23 \times 5 \mu$ m, n = 15), fusiform, one pole often more attenuated than the other, often slightly inequilateral, hyaline, guttulate, aseptate, no mucilaginous sheath seen (Figs. 8-10).

Host: Tylophora dalzellii Hook. f., T. subramanii Henry.

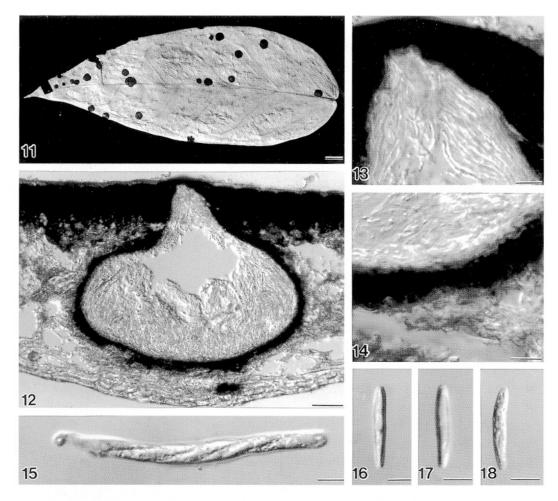
Known distribution: India.

Material examined: INDIA, Matheran, Bombay, on living leaves of Ceropegia hirsuta (= Tylophora dalzellii), 15 Mar. 1912 or 6 Nov. 1912, S.L. Ajrekar, Sydow Fungi exotici exsiccati 259, ex Herb. Sydow (S, designated as lectotype by Kamat, Seshadri and Pande, 1978).

Notes: The true identity of the host of *P. ajrekari* was clarified by Seshadri (1964) when he collected *Phyllachora* on *Tylophora dalzellii* from the type locality (Matheran) and Coorg, Mysore State. On critical comparison with HCIO material (No. 22679) and the lectotype, Kamat *et al.* (1978) concluded that the host of the holotype was *Tylophora dalzellii* and not *Ceropegia hirsuta* as originally identified by S.L. Ajrekar (in Sydow and Sydow, 1912). *Tylophora dalzellii* is found in Concan and Malabar peninsular of India (Hooker, 1885).

Kamat *et al.* (1978) described *P. ajrekari* with slightly larger fungal spots (up to 5 mm), slightly longer asci (up to 107  $\mu$ m), and ascospores (up to 28  $\mu$ m), and evanescent paraphyses. Since their description was based on examination of 7 specimens including the holotype, the increased size variation and evanescent paraphyses suggest that the material examined was slightly more mature.

Hosagoudar (1995) described *Phyllachora kanyakumariana* on *Tylophora subramanii* from Tamil Nadu, India. Although we requested the type collection from HCIO, we have received no reply from our application. Hosagoudar (1995) claimed *P. kanyakumariana* differed from *P. ajrekari* in forming yellow halos around the black tar spots on the leaf surface, cylindrical asci, persistent paraphyses, and lanceolate ascospores. In *P. ajrekari* the fungal spots often had a reddish-brown halo, cylindrical asci, persistent paraphyses, and fusiform ascospores, which could be termed lanceolate. The asci were slightly longer and narrower, but overlapped with *P. ajrekari*. The ascospores were also slightly wider, but also overlapped with *P. ajrekari*. The only other distinguishing character between *P. ajrekari* and *P. kanyakumariana* is the host species, *T.* 



Figs. 11-18. *Phyllachora dischidiae* (from holotype). 11. Leaf spots. 12. Vertical section through immersed ascoma. 13. Vertical section through ostiolar canal. 14. Vertical section through basal peridium. 15. Ascus. 16-18. Ascospores. Bars: 11 = 5 mm, 12 = 50  $\mu$ m, 13-18 = 10  $\mu$ m.

dalzelii and T. subramanii respectively. While host specificity in Phyllachora is an important character given that they are obligate biotrophs, host specificity to species level alone is insufficient to separate these taxa, in the absence of other defining features. It is possible that P. kanyakumariana could be classified as a subspecific taxon on the basis of the slight variations in ascospore and ascus measurements, and differing host species, however since type material was not made available of P. kanyakumariana, we must conclude material is lacking. We have therefore chosen to synonymise P. kanyakumariana with P. ajrekari.

Scolecodothis tylophorae Tendulkar is found on the same host and from the

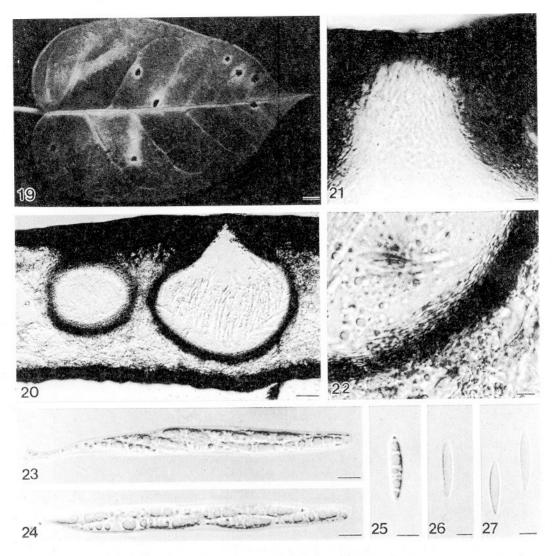
same area in India as P. ajrekari. Kamat et~al. (1978) examined the type, and found cylindrical to slightly fusoid phyllachoraceous ascospores 25-28  $\mu$ m in length, but no scolecosporic spores with a length of up to 64  $\mu$ m as reported by Tendulkar (1971). They therefore synonymised S. tylophorae with P. ajrekari.

2. *Phyllachora dischidiae* Syd. and P. Syd., Philippine Journal of Science, Sect C Botany 8: 277 (1913). (Figs. 11-18)

Leaf spots: similar on both sides of the leaf,  $1-3 \times 1-2.5$  mm, black, shiny, with a granular texture, roughly circular, moderately domed with a flattened apex, occurring singly, occasionally coalescing, sometimes elongated along leaf veins, ostioles distinct as a small raised pimple in the apical surface, with a minute, central, round pore, multilocular, no halo of discoloured host tissue (Fig. 11).

Anamorph: not seen.
Andromorph: not seen.

Teleomorph: Ascomata occurring singly or well separated from adjacent ascoma under a mutually continuous clypeus, often separated by the host's vascular tissue, immersed, often occupying 3/4 to full leaf thickness, 135-300 μm wide, 173-272 µm high, shape variable, subglobose to oblate-sphaeroidal, sometimes slightly irregular in shape due to close proximity of host vascular tissue, hyphae rarely infiltrating host vascular tissue, ostiolar canal usually central, occasionally slightly off-centre, narrow, conical, 50-65 µm wide, lined with numerous, fine, hyaline periphyses (Figs. 12, 13). Upper peridium clypeate, 30-70 µm thick, broadest adjacent to ostiolar canal, composed of a deeply melanized, brown-black, often amorphous layer of host cuticle, epidermal cells and fungal hyphae, often merging with a lighter pigmented region of brown distorted parenchyma cells, up to 30 µm, beneath the clypeus. Lower and lateral peridium composed of two layers; the outer region comprising several layers of dark brown, flattened, thin-walled fungal cells, 5-16 µm thick, which merge inwardly with several layers of hyaline, flattened, thin-walled fungal cells, ca. 5-10 µm thick (Fig. 14). The peridium fuses outwardly with an irregular region of fungal infiltrated, but distorted and flattened, host parenchyma, some infiltration also into the lower epidermal layer, which may or may not be clypeate, though usually not as well developed as the upper clypeus. Asci and paraphyses arise from the basal peridium, in an opaque gelatinous matrix. *Paraphyses* filiform, as long as asci, 2-3.5 µm wide, not constricted at the septa, no branching seen. Asci 88-114  $\times$  8-13 µm ( $\bar{x} = 104 \times 10$  µm, n = 10), 8-spored, cylindrical to narrowly cylindric-clavate, strongly conforming to the shape of ascospores, thin-walled, unitunicate, with a short stalk and a truncate apex, no apical apparatus seen, nonreactive in Melzer's Reagent (Fig. 15). Ascospores arranged uni to biseriately,



Figs. 19-27. *Phyllachora gloriana* (from holotype). 19. Leaf spots. 20. Vertical section through immersed ascomata. 21. Vertical section through ostiolar canal. 22. Vertical section through basal and lower lateral peridium. 23, 24. Asci. 25-27. Ascospores (26, 27 in lactophenol cotton blue). Bars: 19 = 2 mm,  $20 = 50 \mu\text{m}$ ,  $21-27 = 10 \mu\text{m}$ .

often overlapping,  $26-31 \times 4.5-6 \, \mu m$  ( $\overline{x} = 28 \times 5.5 \, \mu m$ , n = 15), fusiform to clubshaped with an attenuated base, sometimes slightly inaequilateral, hyaline, aseptate, no mucilaginous sheath seen (Figs. 16-18).

Host: Dischidia rosea Schlechter

Known distribution: Philippines.

Material examined: PHILIPPINES, Mt. Mariveles, Province of Baatan, Luzon, on living leaves of Dischidia rosea, Mar. 1911, E.D. Merrill No. 7623, Flora of the Philippines (S,

HOLOTYPE).

Notes: Our description compares closely with that of Sydow and Sydow (1913) and Theissen and Sydow (1915), although ascospores in the material we examined were slightly longer, 26-31 μm compared to 24-28 μm.

There are four species of *Dischidia* in Australia, *D. littoralis*, *D. major*, *D. nummularia* and *D. ovata*, and these species are also found in Papua New Guinea and possibly Melasia (Forster, 1996). Papuasia (Irian Jaya, Papua New Guinea and the Solomon Islands) has 28 species of *Dischidia* (Forster, 1994). It is possible that an intensive search of *Dischidia* in Australasia may extend the known range of *P. dischidiae*.

# 3. Phyllachora gloriana C.A. Pearce, P. Reddell and K.D. Hyde, sp. nov.

(Figs. 19-27)

Etymology: named in honour of Gloria Pearce for her help collecting this "glorious tar spot" on Tylophora benthamii.

Maculae 1-4 × 1-2 mm, circularia vel irregularia, nigrae et nitidae, elevatae, multiloculares, flavida halonata usque ad 2 mm lata. *Teleomorphosis*: Ascomata 230-370 μm diam., 290-400 μm alta, globosa vel subglobosa, immersa, clypeata. Ostiola centrala, paraphysatica. *Paraphyses* 2.5-3 μm diam., hyalinae, septatae, angustatae. Asci 124-155 × 10-16 μm, octospori, cylindrici vel cylindrico-clavati, unitunicati, longipedunculati, ad apicem truncati, apparatu apicali 4-4.5 μm diam., 2.5-3 μm alti praediti. Ascosporae 28-36 × 5-8 μm, uniseriatae vel biseriatae, unicellulares, hyalinae, fusiformes, tunico gelatinoso, usque ad 4 μm late praeditae.

*Holotype*: AUSTRALIA, Queensland, Wongabell State Forest, *ca.* 10 km south of Atherton, on Atherton to Ravenshoe road, (17°19'S, 145°30'E), on living leaves of *Tylophora benthamii*, 13 Apr. 1996, C.A. Pearce and G. Pearce, CP42 (BRIP 25668).

Leaf spots: on green stems and leaves, visible on both sides of the leaf surface, amphigenous, often more prominent on the upper leaf surface,  $1-4 \times 1-2$  mm, black, shiny, roughly circular to irregular in shape, sometimes elongated along leaf veins, erumpent above leaf surface, but of low relief, apex flattened, ostioles distinct as small round pores in the apex, multiloculate, often encircled by a region of yellow chlorotic leaf tissue, up to 2 mm wide (Fig. 19).

Anamorph: not seen.

Andromorph: not seen.

Teleomorph: Ascomata immersed in the host parenchyma tissue, sometimes occupying the full leaf thickness, 230-370 μm wide, 290-400 μm high, globose to subglobose, with a central, sometimes shallow, conical ostiolar canal, up to 140 μm wide, lined with fine, hyaline periphyses (Figs. 20, 21). Upper peridium clypeate, 10-20 μm thick in the vicinity of the ostiolar canal, increasing to ca. 25-120 μm thick at the outer edge of the ascoma, composed of a deeply melanised, amorphous layer of host epidermal cells and fungal hyphae, beneath the host cuticle. Lower and lateral peridium composed of an outer region of dark brown, flattened, thin-walled fungal cells, 15-23 μm thick, merging inwardly with

Table 1. Synopsis of *Phyllachora* species from the host family *Asclepiadaceae*.

| Phyllachora sp.  | Infected area       | Ascomata (µm)  | Asci (µm)            | Ascospores (µm)         |
|------------------|---------------------|----------------|----------------------|-------------------------|
| P. ajrekarii     | Foliicolous, 1.5-   | 186-285 diam.  | 70-101 × 9-13        | 19-26 × 4.5-6.5         |
|                  | $4 \times 1$ -3 mm, | 168-272 high   | cylindrical          | fusiform, one pole      |
|                  | ostioles distinct   |                |                      | often more attenuated   |
| P. dischidiae    | Foliicolous, 1-3    | 135-300 diam.  | $88-114 \times 8-13$ | $26-31 \times 4.5-6$    |
|                  | $\times$ 1-2.5 mm,  | 173-272 high   | cylindrical to       | fusiform, to club-      |
|                  | ostioles distinct   |                | narrow               | shaped, with an         |
|                  |                     |                | cylindric-           | attenuated base         |
|                  |                     |                | clavate              |                         |
| P. gloriana      | Caulicolous and     | 230-370 diam.  | 124-155 × 10-16      | $28-36 \times 5-8$      |
|                  | foliicolous, 1-4    | 290-400 high   | cylindrical to       | fusiform, often         |
|                  | $\times$ 1-2 mm,    |                | cylindric-           | inequilateral, in clear |
|                  | ostioles distinct,  |                | clavate, apical      | mucilaginous up to 4    |
|                  | halo of yellow      |                | apparatus 4-4.5      | thick                   |
|                  | chlorotic leaf      |                | × 2.5-3              |                         |
|                  | tissue, to 2 mm     |                |                      |                         |
| P. metastelmatis | Caulicolous and     | 272-350 diam., | 103-130 × 10-14      | $16-23 \times 6-8.5$    |
|                  | foliicolous, 1-6    | 155-390 high   | cylindrical to       | oval, with both poles   |
|                  | × 0.3-1 mm          |                | slightly             | attenuated, sometimes   |
|                  |                     |                | cylindric-           | ovoid, sometimes with   |
|                  |                     |                | clavate, no          | hyaline, bipolar, pad-  |
|                  |                     |                | apical apparatus     | like appendages, up to  |
|                  |                     |                | seen                 | 1.5 thick               |

several layers of hyaline, flattened, thin-walled fungal cells, ca. 8 µm thick. Peridium fuses outwardly with a narrow region of pale, yellow-brown, slightly melanized zone of compressed host parenchyma (Fig. 22). Asci and paraphyses arise from the inner basal and lower lateral walls. *Paraphyses* numerous, slightly longer than asci, up to 2.5-3 µm wide, tapering to rounded apices, hyaline, not constricted at the septa, no branching seen. *Asci* 124-155 × 10-16 µm ( $\bar{x}$  = 138 × 12 µm, n = 25), 8-spored, cylindrical to cylindric-clavate, long pedicellate, unitunicate, thin-walled at maturity, tapering slightly to a truncate apex, with an opaque apical discoid apparatus, 4-4.5 × 2.5-3 µm, non-reactive in Melzer's reagent (Figs. 23, 24). *Ascospores* arranged uni to biseriately, overlapping, 28-36 × 5-8 µm ( $\bar{x}$  = 32 × 6.5 µm, n = 50), fusiform, one pole often more acute than the other, often inequilateral, hyaline, smooth, aseptate, guttulate, often enveloped in a clear mucilaginous sheath up to 4 µm thick (Figs. 25-27).

Other material examined: AUSTRALIA, Queensland, 1.5 km along maintenance track from car park, Wongabel State Forest, ca. 10 km south of Atherton, on Atherton to Ravenshoe road (17°19'S, 145°30'E), on living leaves of *Tylophora benthamii*, 13 Apr. 1996, C.A. Pearce and G. Pearce, CP42 (BRIP 25668, HOLOTYPE); Queensland, 1 km along maintenance track from car park, Wongabel State Forest, ca. 10 km south of Atherton, on Atherton to Ravenshoe road

(17°19'S, 145°30'E), on living leaves of *Tylophora benthamii*, 24 Apr. 1996, C.A. Pearce and G. Pearce, CP39 (BRIP 25669); Queensland, 2 km along maintenance track from car park, Wongabel State Forest, *ca.* 10 km south of Atherton, on Atherton to Ravenshoe road (17°19'S, 145°30'E), on living leaves of *Tylophora benthamii*, Apr. 1996, C.A. Pearce and K.D. Hyde, CP 41 (BRIP 25670).

Host: Tylophora benthamii Tsiang. Known distribution: Australia.

Notes: Phyllachora gloriana is distinct morphologically from P. ajrekari having larger ascomata, and longer asci and ascospores (see Table 1). The asci of P. gloriana also have an apical apparatus and the ascospores have a hyaline mucilaginous sheath up to 4  $\mu$ m thick.

Phyllachora gloriana is also distinct in host species and geographical distribution. Its host, Tylophora benthamii, is endemic to tropical and subtropical areas of eastern and northern Australia (Forster, 1996). As such, P. gloriana is the first Phyllachora species to be described from the host family Asclepiadaceae in the southern hemisphere. Although presently known only from the midmontane tropical rainforest (complex nanophyll vine forest, sensu Tracey 1982) at Wongabel State Forest, near Atherton, this new species appears to be relatively common there.

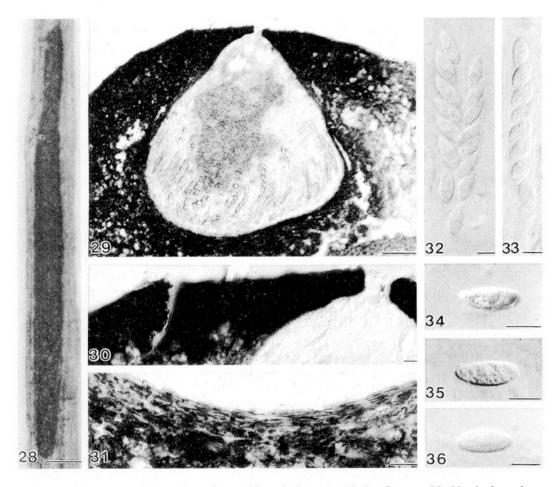
4. *Phyllachora metastelmatis* F. Stevens and Dalbey, Botanical Gazette 68: 57 (1919). (Figs. 28-36)

*Leaf spot*: on petiolate leaves and stems,  $1-6 \times 0.3-1$  mm, black, shiny, linear to elongate ellipsoidal, long axis parallel to leaf venation, slightly to moderately raised, surface slightly granular, ostiole indistinct, multiloculate. No halo of discoloured host tissue observed (Fig. 28).

Anamorph: not seen.

Andromorph: not seen.

Teleomorph: Ascomata immersed in host tissue, 272-350 μm diam., 155-390 μm high, suboblate to subglobose, with a wide conical ostiolar canal, no periphyses seen (Figs. 29, 30). Upper peridium clypeate, brown-black to black, often amorphous, composed of the host epidermis and cuticle, and brown intracellular fungal hyphae, sometimes extending into the host parenchyma, ca. 25-100 μm thick. Lower and lateral peridium composed of several layers of flattened, thin-walled fungal cells, usually hyaline, ca. 7-13 μm thick, sometimes becoming light brown at periphery, where it merges with a variable region of light-brown to brown, discoloured and compressed host parenchyma and fungal hyphae, extending up to 450 μm from lateral peridium (Figs. 29, 31). Asci and paraphyses arise from the basal wall. Paraphyses numerous, filiform, slightly longer than asci, up to 2.5 μm diam., hyaline, not constricted at septa, occasionally branching. Asci 103-130 × 10-14 μm ( $\bar{x}$  = 117 × 12 μm, n = 10), 8-



Figs. 28-36. *Phyllachora metastelmatis* (from holotype). 28. Leaf spots. 29. Vertical section through immersed ascoma. 30. Vertical section through ostiolar canal. 31. Vertical section through basal peridium. 32, 33. Asci. 34-36. Ascospores with bipolar, pad-like appendages. Bars: 28 = 0.5 mm, 29 = 50  $\mu$ m, 30-36 = 10  $\mu$ m.

spored, cylindrical, some slightly cylindric-clavate, unitunicate, medium pedicellate, apex rounded, no apical apparatus seen, non-reactive in Melzer's reagent (Figs. 32, 33). Ascospores usually arranged uniseriately and oblique, rarely biseriate,  $16\text{-}23 \times 6\text{-}8.5~\mu\text{m}$  ( $\bar{x}=18.5 \times 7~\mu\text{m}$ , n=10), oval, often with both poles attenuated, occassionally ovoid, sometimes inequilateral, hyaline, aseptate, some spores with hyaline, bipolar, pad-like appendages, up to 1.5  $\mu$ m thick (Figs. 34-36).

Host: Metastelma (= Cynanchum sp.)

Known distribution: Puerto Rico.

Material examined: PUERTO RICO, El alto de la Bandera, on stems of Metastelma sp., 16 July 1915, F.L. Stevens 8715 (ILL 8944, HOLOTYPE).

*Notes*: The ascospores of *P. metastelmatis* are oval, unlike the fusiform ascospores of the other asclepiadaceous *Phyllachora* species, and the polar mucilaginous pad-like appendages are unusual.

The original description by Stevens and Dalbey (1919) describes black, shiny stromata 1-2 mm wide, 5-15 mm long, partially encircling the stem, occupying all the tissues exterior to the wood. Locules are described as about 200 µm diam. and 120 µm high, asci 8-spored, ascospores 14 × 5 µm, cylindrical, and hyaline. A drawing included with the type illustrates spots on stems or petiolate leaves, and a cross section of ascomata, with no detail of ascospore shape. The host was identified as *Metastelma* sp. A later report by Stevenson (1975) gives the host as *Cynanchum* sp. from Puerto Rico. Mabberley (1987) lists *Metastelma* as a synonym of *Cynanchum*. The type packet contains what appears to be petiolate leaves and broken stems only. It would be impossible to confirm the host identity using this material. Future collections may clarify the identity of the host.

Cynanchum is a genus of over 200 species found worldwide (Forster, 1996). Forster (1996) suggested the genus was polyphyletic and currently under revision. To date, *P. metastematis* has only been found on *Cynanchum* in Puerto Rico. It was originally named *P. metastelmae*, however this was corrected in accordance with Rec. 60H.1 and articles 60 and 61 of the ICBN (Crane and Jones, 1997).

# Excluded and doubtful species

Phyllachora asclepiadis (Schwein.) Sacc., Sylloge Fungorum 2: 617 (1883).
 Synonyms: Dothidea asclepiadis Schwein., Transactions of the American Philosophical Society of Philadelphia 4: 234 (1832).

Material examined: USA, Pennsylvania, Bethlehem, on bark of Asclepias? or Asclepiadaceae, 1834, Schweinitz Herbarium Syn #1927 (PH, HOLOTYPE).

Notes: See under Phyllachora ornans.

6. *Phyllachora cinerascens* (Schwein.) Sacc., Sylloge Fungorum 2: 617 (1883).

Synonyms: Dothidea cinerascens Schwein., Transactions of the American Philosophical Society of Philadelphia 4: 234 (1832).

Material examined: USA, Pennsylvania, Bethlehem, on bark of Asclepias syriaca, 1834, Schweinitz Herbarium Syn #1928 (PH, HOLOTYPE).

Notes: See under Phyllachora ornans.

7. *Phyllachora ornans* (Schwein.) Sacc., Syllogue Fungorum, 2: 618 (1883). *Synonyms: Dothidea ornans* Schwein., Transactions of the American Philosophical Society of Philadelphia 4: 233 (1832).

Material examined: USA, Pennsylvania, Bethlehem, on leaves and bark of Asclepias incarnata, 1834, Schweinitz Herbarium Syn #1918 (PH, HOLOTYPE).

Notes: Saccardo (1883) transferred Dothidea asclepiadis, D. cinerascens and D. ornans to Phyllachora without explanation. His Latin descriptions mimic the original descriptions of Schweinitz (1832), and only offer macroscopic characters of the fungal spots. They described erumpent or immersed, ostiole-forming perithecia, but they did not describe any fertile components of the fungi. Theissen and Sydow (1915) included P. asclepiadis, P. cinerascens and P. ornans under the heading "Species Phyllachora delendae" and thus excluded the taxa from Phyllachora without an explanation. We have examined the type material of all three taxa. The specimens are on dead woody stems or bark, an unusual substrate for *Phyllachora*, species of which generally occur on leaves, or occasionally green stems or fruit. The spots are on the outer bark layer,  $0.2-0.75 \times 0.2-0.75$ mm diam., numerous, black, shiny, roughly circular to oblate, slightly domed, and often coalescing with maturity. They are usually scattered over grey, slightly raised stromatic regions of the bark surface. No fertile fungal structures were found. Phyllachora asclepiadis, P. cinerascens and P. ornans are therefore excluded from *Phyllachora* due to the inadequacy of their original descriptions, and their lack of phyllachoraceous fertile structures.

# 8. *Phyllachora trivialis* Speg., Boletín de la Academia Nacional de Ciéncias de Córdoba 11: 546 (1889).

Leaf spots: more prominent on one leaf surface, black, shiny, with a granular surface, roughly circular,  $1-1.6 \times 1-1.5$  mm, often developing on leaf veins, domed above the leaf surface, sometimes coalescing, multiloculate, ostioles visible as minute round pores in the apical surface, becoming dull and with a pale band of discoloured host tissue, ca. 2-3 mm wide.

Anamorph: not seen.

Andromorph: not seen.

*Teleomorph*: No phyllachoraceous reproductive parts found in the leaf spot examined. Some spores which are conidial and associated with a mycoparasite were found.

Host: ?Apocynaceae, ?Asclepiadaceae.

Known distribution: Brazil.

Material examined: BRAZIL, San Paulo, Aphiay, on leaves of Apocynaceae, 1881, J. Puigarri, no. 1564 (LPS, HOLOTYPE).

Notes: Phyllachora trivialis was originally described on the host "?Apocynaceae" from Brazil (Spegazzini, 1889). This is confirmed on the inner and outer wrapper of the holotype herbaria packet from LPS (No. 195). The leaf spots are typical of *Phyllachora*, but the fruiting body dissected contained no

fertile contents. A written note accompanying the specimen notes: loculi 250-300  $\mu m$  diam., asci 140-150  $\times$  10-12  $\mu m$ , and ascospores 16-20  $\times$  5-7  $\mu m$ . Our interpretation of the drawings is that the asci are cylindric-clavate, short pedicellate, 8-spored, with a truncate apex and no apical apparatus. The paraphyses are longer than the asci and filiform. Ascospores are fusiform to narrowly oval, aseptate, guttulate, and arranged uni-biseriately within the ascus.

Theissen and Sydow (1915) redescribed *P. trivialis* and gave the host as "Asclepiadaceae(?)" but noted that the identity of the matrix as Asclepiadaceae was doubtful, since Spegazzini recognised it as near to Jussiaea which is in the Onagraceae. According to Mabberley (1987), Jussiaea has been synonymised with Ludwigia in the Onagraceae. In an attempt to identify the host plant, the holotype was examined by two Australian botanists. From the fractions of leaves left in the holotype it was found to be an impossible task (B. Gray and B. Hyland, pers. comm.). Molecular studies may throw more light on the true host identity of P. trivialis. Phyllachora is considered to be a relatively host specific genus and therefore P. trivialis has been excluded from Phyllachora species described from the Asclepiadaceae in this study, until new collections on a known host can be found.

## Acknowledgements

We thank the Australian Biological Resources Study and The University of Hong Kong for funding this research. Laboratory space was provided by the Department of Primary Industries, Mareeba (1996), and the Tropical Forest Research Centre, CSIRO, Atherton (1997-1999). Gloria and Kyle Pearce are thanked for their assistance during field trips. A.Y.P Lee and Helen Leung are thanked for technical assistance. Specimens collected at Wongabel State Forest were taken under Collection Permit Number 954. Paul Cannon is thanked for the use of his *Phyllachoraceae* database, which greatly simplified the process of identifying records of phyllachoraceous taxa involved, and locating references. Ruth Huwer is gratefully acknowledged for her assistance with German translations. Thanks to the curators of herbaria LPS, PH, S, and ILL for the loan of fungal collections. The staff of QRS herbaria, B. Hyland, B. Gray, A. Ford and R. Ellick are thanked for their assistance in identifying the host of *Phyllachora gloriana*, and other valuable discussions regarding plant taxa involved. P. Forster (BRI) is also thanked for discussions regarding the distribution and inter-relationships of *Tylophora* spp.

### References

Cannon, P.F. (1991). A Revision of *Phyllachora* and some similar genera on the family Leguminosae. Mycological Papers 163: 1-302.

Cannon, P.F. (1997). Diversity of Phyllachoraceae with special reference to the tropics. In: *Biodiversity of tropical microfungi* (ed. K.D. Hyde). Hong Kong University Press, Hong Kong: 255-278.

Crane J.L., and Jones, A. (1997). An annotated catalogue of types of the University of Illinois mycological collections. Illinois Biological Monographs 58: 287.

Forster, P.I. (1994). A checklist of the Asclepiadaceae of Papuasia. Science in New Guinea 22:

- Forster, P.I. (1996). Asclepiadaceae. Flora of Australia 28: 197-307.
- Hawksworth, D.L., Kirk, P.M., Sutton, B.C. and Pegler, D.N. (1995). Ainsworth and Bisby's Dictionary of the Fungi. 8th edn. Cambridge University Press, CAB International, UK.
- Hock, J., Dittrich, U., Renfro, B.L. and Kranz, J. (1992). Sequential development of pathogens in the maize tarspot disease complex. Mycopathologia 117: 157-161.
- Hooker, J.D. (1885). The Flora of British India. Reeves and Co., London 4: 43-44.
- Hosagoudar, V.B. (1995). Two new *Phyllachora* species from Tamil Nadu. Indian Phytopathology 48: 213-214.
- Kamat, M.N., Seshadri, V.S. and Pande, A.A. (1978). A monographic study of the Indian species of *Phyllachora*. University of Agricultural Sciences, Hebbal, Bangalore, UAS Monograph Series 4: 9-10.
- Mabberly, D.J. (1987). The Plant Book: A portable dictionary of higher plants. Cambridge University Press, Cambridge, UK.
- Parbery, D.G. (1967). Studies on graminicolous species of *Phyllachora* Nke. in Fckl. V. A taxonomic monograph. Australian Journal of Botany 15: 271-375.
- Parbery, D.G. (1996). Trophism and ecology of fungi associated with plants. Biological Review of the Cambridge Philosophical Society 71: 474-521.
- Pearce, C.A. and Hyde, K.D. (1994). The genus *Phyllachora* from Australia. Observations on *Phyllachora grevillea* and two new taxa *P. velatispora* var. *velatispora* and *P. velatispora* var. *hillianae* on *Grevillea*. Mycological Research 98: 1402-1408.
- Pearce, C.A. and Hyde, K.D. (1995a). *Phyllachora* from Australia. Observations on *P. pseudostromatica*, *P. melaleuca* and a new species, *P. shivasii* from the host *Melaleuca*. Mycological Research 99: 1253-1260.
- Pearce, C.A. and Hyde, K.D. (1995b). *Phyllachora* from Australia. Observations on *P. grevilleae* and two new species: *P. victoriensis* and *P. hakeicola* from *Hakea*. Mycological Research 99: 1261-1267.
- Saccardo, P.A. (1883). Sylloge Fungorum. Vol. 2. Patavia.
- Schweinitz, L.D. von (1832). Synopsis fungorum in America boreali media degentium. Transactions of the American Philosophical Society of Philadelphia 4: 141-316.
- Seshadri, V.S. (1964). Studies in Indian Phyllachoraceae III. Journal University of Poona Science and Technology 22: 121-123.
- Spegazzini, C.L. (1889). Fungi Puiggariani. Pugillus I. Boletín de la Academia Nacional de Ciéncias de Córdoba 11: 381-622.
- Stevens, F.L. and Dalbey, N. (1919). Some *Phyllachoras* from Puerto Rico. Botanical Gazette 68: 54-59.
- Stevenson, J.A. (1975). The fungi of Puerto Rico and the American Virgin Islands. Contributions of Reed Herbarium 23: 1-743.
- Sydow, H. and Sydow, P. (1912). Novae fungorum species VIII. Annales Mycologici 10: 408.
- Sydow, H. and Sydow, P. (1913). Enumeration of Philippine fungi, with notes and descriptions of new species. Part I. Micromycetes. Philippine Journal of Science (Section C, Botany) 8: 265-285.
- Tendulkar, J.S. (1971). Ph. D. Thesis, University of Poona.
- Theissen, F. and Sydow, H. (1915). Die Dothideales. Annales Mycologici 13: 149-746.
- Tracey, J.G. (1982). The Vegetation of the Humid Tropical Region of North Queensland. CSIRO, Melbourne. Australia: 124.