Two new pathogenic ascomycetes in *Guignardia* and *Rosenscheldiella* on New Zealand's pygmy mistletoes (*Korthalsella*: *Viscaceae*)

A. Sultan[1], P.R. Johnston[2*], D. Park[2] and A.W. Robertson[1]

[1]Ecology Group, Institute of Natural Resources, Massey University, Palmerston North, New Zealand; [2]Landcare Research, Private Bag 92170, Auckland 1142, New Zealand

*Correspondence: Peter Johnston, johnstonp@landcareresearch.co.nz

Abstract: Two new pathogens, *Guignardia korthalsellae* and *Rosenscheldiella korthalsellae,* are described from New Zealand's pygmy mistletoes (*Korthalsella*, *Viscaceae*). Both form ascomata on living phylloclades with minimal disruption of the tissue. Fungal hyphae within the phylloclade are primarily intercellular. *Guignardia korthalsellae* disrupts a limited number of epidermal cells immediately around the erumpent ascoma, while the ascomata of *Rosenscheldiella korthalsellae* develop externally on small patches of stromatic tissue that form above stomatal cavities. *Rosenscheldiella* is applied in a purely morphological sense. LSU sequences show that *R. korthalsellae* as well as another New Zealand species, *Rosenscheldiella brachyglottidis*, are members of the *Mycosphaerellaceae sensu stricto*. Genetically, *Rosenscheldiella,* in the sense we are using it, is polyphyletic; LSU and ITS sequences place the two New Zealand species in different clades within the *Mycosphaerellaceae. Rosenscheldiella* is retained for these fungi until generic relationships within the family are resolved. Whether or not the type species of *Rosenscheldiella, R. styracis,* is also a member of the *Mycosphaerellaceae* is not known, but it has a similar morphology and relationship to its host as the two New Zealand species.

Key words: ITS, LSU, *Mycosphaerellaceae, Phaeocryptopus*, phylogeny.

Taxonomic novelties: *Guignardia korthalsellae* A. Sultan, P.R. Johnst., D.C. Park & A.W. Robertson, sp. nov.; *Rosenscheldiella korthalsellae* A. Sultan, P.R. Johnst., D.C. Park & A.W. Robertson, sp. nov.

INTRODUCTION

The pygmy mistletoes of New Zealand belong to the genus *Korthalsella* in the family *Viscaceae*. Species of *Korthalsella* are leafless, aerial hemiparasites, having terete or flattened internodes with minute, unisexual flowers borne on the tip of internodes in the axils of rudimentary leaves or on specialised inflorescence branches. *Korthalsella* has an unusual, scattered, and discontinuous distribution with high levels of species and sectional diversity in Malesia extending from Hawaii, the Marquesas and Henderson Island in the east, to Ethiopia and Madagascar in the west, and from Japan in the north, to Australia and New Zealand in the south (Barlow 1983, Molvray 1997, Burrows 1996). Barlow (1997) estimated that there may be as many as 25 species. Molvray (1997) reduced the number of species to eight; however, her classification is not generally accepted and was not adopted by Barlow (1997) or Wagner *et al*. (1999) in monographs of the floras of Malesia and Hawaii respectively.

New Zealand is home to three pygmy mistletoe species, *Korthalsella salicornioides*, *K. clavata,* and *K. lindsayi*, although there is debate about the taxonomic status of the latter two as separate species (see Danser 1940, Molvray 1997, Molvray *et al.* 1999). *Korthalsella salicornioides* mainly occurs on *Leptospermum scoparium* and *Kunzea* spp. (*Myrtaceae*) and has also been recorded on the introduced *Erica lusitanica* and *E. vagans* (*Ericaceae*, Bannister 1989). *Korthalsella clavata* is known on *Aristotelia fruticosa (Eleocarpaceae), Coprosma propinqua, C. wallii (Rubiaceae),* and *Discaria toumatou (Rhamnaceae).* While the main host for *K. lindsayi* is *Melicope simplex,* it also occurs on *Coprosma* spp., *Lophomyrtus obcordata (Myrtaceae)*, and *Myrsine divaricata*

(Myrsinaceae). *Korthalsella salicornioides* occurs throughout the North and South Islands and on Stewart Island. *Korthalsella clavata* and *K. lindsayi* occur throughout the South Island and the southern half of the North Island. *Korthalsella salicornioides* may have an even broader range. Barlow (1996) reports it from New Caledonia; Molvray (1997) includes *K. madagascarica* from Madagascar as a synonym of *K. salicornioides*. *K. salicornioides* is classified as "at risk-sparse" in the threatened and uncommon plants list for New Zealand (de Lange *et al*., 2004) and *K. clavata* is regarded as a regionally threatened plant in Wellington Conservancy (Anonymous 2001).

The only fungi reported previously from *Korthalsella* have been from Hawaii, specifically *Cucurbitaria obducens* (as *Teichospora obducens*), *Echidnodes visci* (Petrak 1953), *Meliola visci* (Stevens 1925), and *Pleospora* sp. (Kliejunas *et al.* 1979).

This paper describes two new stem parasites on *Korthalsella* spp. from New Zealand. The phylogenetic position of *Rosenscheldiella korthalsellae* sp. nov. and another New Zealand species, *R. brachyglottidis*, is determined on the basis of ITS and LSU sequences. The genus was placed in the *Venturiaceae* by Kirk *et al*. (2008), although Sivanesan & Shivas (2002) referred it to the *Mycosphaerellaceae* in a paper in which they described *R. dysoxyli*, a species with erumpent ascomata morphologically reminiscent of typical *Mycosphaerella* spp.

MATERIALS AND METHODS

Morphological studies

Specimens were examined from dried collections; asci and ascospores are described from squash mounts following rehydration in water or 3 % KOH. Ascomata and conidiomata were sectioned at a thickness of about 10 μm using a freezing microtome and sections were mounted in lactic acid for light microscopy. All collections have been deposited in the New Zealand Fungal and Plant Disease Herbarium (PDD).

Molecular analyses

Guignardia
DNA was extracted using REDExtract-N-Amp Plant PCR Kits (Sigma, USA) from small pieces of tissue taken from within three individual fruiting bodies from three different infected plants stored as dried herbarium specimens, following the removal of the upper surface of the fruiting body. The tissue was ground in extraction buffer with a plastic pestle in the Eppendorf tube, then DNA extraction and PCR were carried out following the manufacturer's instructions. ITS sequences were obtained separately from each extract following the methods of Johnston & Park (2005) using ITS1F and ITS4 amplification primers (White *et al.* 1990, Gardes & Bruns 1993). Using ClustalW (Larkin *et al.* 2007) our newly generated ITS sequences were aligned with sequences deposited in GenBank from taxa representing the genetic diversity of *Phyllosticta* as reported by Okane *et al.* (2003) and Rodrigues *et al.* (2004) (Table 1). *Botryosphaeria dothidea* was selected as the outgroup following Crous *et al.* (2006b) who showed *Phyllosticta sensu stricto* to be monophyletic and have a sister relationship with *Botryosphaeria*. Taxa in more distantly related clades of the *Botryosphaeriaceae* could not be reliably aligned. The 599-bp-long alignment has been deposited in TreeBase. A 70-bp segment near the start of the alignment could not be reliably aligned and was excluded from the analyses as was the 5.8S part of the alignment, because this was not available for all of the sequences deposited in GenBank, leaving 425 characters in the analyses. Phylogenetic analyses were performed using Bayesian maximum liklelihood in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) and a heuristic maximum likelihood analysis in PAUP v. 4.01b (Swofford 2002) with the GTR+I+G model, selected using the AIC method in MrModelTest v. 2.3 (Posada & Crandall 1998, Posada & Buckley 2004). The Bayesian analysis was run with two chains for 10 M generations, trees sampled every 1 000 generations with a burn-in of 10 %. Bayesian posterior probabilities were obtained from 50 % majority rule consensus trees. The PAUP ML analysis used addition sequence random and TBR branch swapping with 100 replicates to avoid local optima. A bootstrap analysis used the ML tree as a starting tree; each of the 100 bootstrap samples run with a single replicate.

Rosenscheldiella
DNA was extracted from dried herbarium specimens using the same methods as for *Guignardia*. Tissue was extracted separately from seven individual pseudothecia taken from several different plants collected from two separate sites for *Rosenscheldiella korthalsellae* and from individual pseudothecia taken from three different leaves from a fresh collection of *R. brachyglottidis* (PDD 94939). ITS sequences were generated using the same primers

as *Guignardia* and LSU using LROR and LR5 (Bunyard *et al.* 1994, Vilgalys & Hester 1990). Our newly generated ITS and LSU sequences were aligned with sequences deposited in GenBank from taxa representing the genetic diversity of *Mycosphaerellaceae* as reported in recent papers (*e.g.* Arzanlou *et al.* 2008, Crous *et al.* 2006a, 2007) as well as the specimens that formed the closest matches for *R. brachyglottidis* and *R. korthalsellae* in a GenBank BLAST search. In all cases the fungi included in the analysis had both ITS and LSU sequences available from the same voucher specimen, and the vouchers are available through public collections (Table 2). *Davidiella tassiana* was chosen as the outgroup following Crous *et al.* (2007) who showed the *Davidiellaceae* to be basal within the *Capnodiales* to *Mycosphaerellaceae* plus *Teratosphaeriaceae*. The alignment has been deposited in TreeBase. Several short segments within the ITS could not be reliably aligned and these were excluded from subsequent analyses, leaving 1384 characters. Phylogenetic analyses followed the procedure described for *Guignardia* using the GTR+I+G model as selected by MrModelTest.

RESULTS AND DISCUSSION

Phylogenetic analyses

Guignardia
Identical sequences were obtained from all three fruiting bodies of *Guignardia korthalsellae;* one has been deposited as GenBank FJ655899. Phylogenetic analysis shows that *G. korthalsellae* groups most closely with the *Vitaceae*-associated *G. bidwellii*, the *Eugenia*-associated *P. eugeniae*, and the *Muehlenbeckia*-associated *P. beaumarisii* (Fig. 1).

Rosenscheldiella
For both of the species of *Rosenscheldiella* sequenced, all samples from each of the species had matching DNA sequences, making it unlikely that a contaminating fungus had been sequenced.

Based on these sequences *R. brachyglottidis* and *R. korthalsellae* are members of the *Mycosphaerellaceae sensu* Crous *et al.* (2007, 2009). Micromorphologically these two species are similar to *Mycosphaerella*, with fissitunicate, fasciculate asci, hamathecial elements lacking or poorly developed, and ascospores 1-septate, slightly constricted at septum, upper cell slightly wider than the lower. However, the anatomy of the ascomata and their relationship to the host tissue is unusual for the *Mycosphaerellaceae*. In both *R. brachyglottidis* and *R. korthalsellae,* as well as *R. styracis*, the type species of the genus, groups of pseudothecia develop externally to the host leaf or phylloclade on small pads of stromatic tissue that develop superficially from hyphae growing through the stomata. Within the leaf, the substomatal cavity is tightly packed with hyphae, but otherwise the hyphae are confined to leaf tissue in the immediate vicinity of the fruiting body and are always intercellular. Unusual for *Mycosphaerellaceae*, the host leaves show little or no symptoms beyond the presence of the fruiting bodies. This relationship to the host leaf with development of pseudothecia superficially on small pads of stromatic tissue growing from stomata is the same as has been described for *Phaeocryptopus gaeumannii*, a pathogen of Douglas fir (Stone *et al.* 2008). Like *Rosenscheldiella*, *Phaeocryptopus* is a member of the *Mycosphaerellaceae* (Winton *et al.* 2007).

Although *Rosenscheldiella brachyglottidis, R. korthalsellae*, and *Phaeocryptopus gaeumannii* share a similar morphology,

Table 1. Isolates included in the phylogenetic analyses of *Guignardia*.

Fungus[1]	Isolate voucher number[2]	GenBank accession number	Host and geographic origin of voucher
Guignardia aesculi	CBS 756.70	AB095504	*Aesculus hippocastanum*, Netherlands
Guignardia bidwellii	IFO 9466	AB095509	*Parthenocissus tricuspidata*, Japan
Guignardia citricarpa	IMI 304799	AY042917	*Citrus aurantiacum*, India
	CBS 102374	FJ824767	*Citrus aurantiacum*, Brazil
Guignardia gaultheriae	CBS 447.70	AB095506	*Gaultheria humifusa*, Netherlands
Guignardia korthalsellae	PDD 94884	FJ655899*	*Korthalsella lindsayi*, New Zealand
Guignardia mangiferae	IFO 33119	AB041233	*Rhododendron* sp., Japan
	ETH 02038	AY277711	*Anarcardium giganteum*, Brazil
Guignardia philoprina	CBS 174.77	AB095507	*Cryptomeria japonica*, Netherlands
	CBS 447.68	AF312014	*Taxus baccata*, USA
Guignardia vaccinii	CBS 126.22	AB095508	*Oxycoccus macrocarpos*, Netherlands
Phyllosticta beaumarisii	CBS 535.87	AY042927	*Muehlenbeckia adpressa*, Australia
Phyllosticta eugeniae	CBS 445.82	AY042925	*Eugenia aromatica*, Indonesia
Phyllosticta hypoglossi	CBS 434.92	AY042923	*Ruscus aculeatus*, Italy
Phyllosticta owaniana	CBS 776.97	AF312011	*Brabejum stellatifolium*, South Africa
Phyllosticta podocarpi	CBS 111647	AF312013	*Podocarpus lanceolata*, South Africa
Phyllosticta pyrolae	–	AF312010	*Pyrola* sp., USA
	IFO 32652	AB041242	*Erica carnea*, Japan
Phyllosticta spinarum	IMI 070028	AY042907 and AY042908	Japan
	CBS 292.90	AF312009	*Chamaecyparis pisifera*, France
Phyllosticta telopeae	DAR 60749	AY042909 and AY042910	*Telopea speciosissima*, Australia

*Sequences newly generated for this study.

[1]Names used are those cited in GenBank.

[2]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAR, Plant Pathology Herbarium, Orange, Australia; IFO: Inst. for Fermentation Culture Collection, Osaka, Japan; IMI: International Mycological Institute, CABI, United Kingdom; PDD: The New Zealand Fungal Herbarium, Landcare Research, Auckland, New Zealand; ETH: ETH Culture Collection, Swiss Federal Institute of Technology, Zurich, Switzerland.

Table 2. Isolates included in the phylogenetic analyses of *Rosenscheldiella*.

Fungus[1]	Isolate voucher number[2]	GenBank accession number (ITS, LSU)	Host and geographic origin of voucher
Cercospora apii	CBS 536.71	AY752133, AY152629	-
Cercospora beticola	CBS 116456	AY840527, DQ678091	*Beta vulgaris*, Italy
Davidiella tassiana	STE-U 5101	AY251078, AY342092	CCA-treated Douglas-fir pole, USA
Mycosphaerella aurantia	CBS 110500	AY725531, DQ246256	*Eucalyptus globulus*, Australia
Mycosphaerella buckinghamiae	CBS 112175	EU707856, EU707856	*Buckinghamia* sp., Australia
Mycosphaerella colombiensis	CMW 11255	DQ239993, DQ204745	*Eucalyptus* sp., Colombia
Mycosphaerella communis	CBS 110976	AY725537, DQ246261	*Eucalyptus* sp., South Africa
Mycosphaerella fori	CMW 9096	DQ267581, DQ204749	*Eucalyptus grandis*, South Africa
Mycosphaerella graminicola	CBS 100335	EU019297, EU019297	*Triticum aestivum*, The Netherlands
Mycosphaerella grandis	CMW 8554	DQ267584, DQ246240	*Eucalyptus globulus*, Chile
Mycosphaerella heimii	CPC 15429	EU882122, EU882141	*Eucalyptus* sp., Thailand
Mycosphaerella lateralis	CBS 111169	AY725550, DQ246260	*Eucalyptus globulus*, Zambia
Mycosphaerella pini	ATCC 28973	EF114684, EF114697	*Pinus ponderosa*, USA
Mycosphaerella punctata	CBS 113315	EU167582, EU167582	*Syzygium cordatum*, South Africa
Mycosphaerella walkeri	CMW 20333	DQ267593, DQ267574	*Eucalyptus globulus*, Chile
Phaeocryptopus gaeumannii	CBS 267.37	EF114685, EF114698	*Pseudotsuga menziesii*, Germany
Pseudocercospora natalensis	CBS 111069	DQ303077, DQ267576	*Eucalyptus nitens*, South Africa
Pseudocercospora paraguayensis	CBS 111286	DQ267602, DQ204764	*Eucalyptus nitens*, Brasil
Pseudocercospora vitis	CPC 11595	DQ073923, DQ073923	*Vitis vinifera*, South Africa
Readeriella novae-zelandiae	CBS 114357	DQ267603, DQ246239	*Eucalyptus botryoides*, New Zealand
Rosenscheldiella brachyglottidis	PDD 94939	GQ355335*, GQ355334*	*Brachyglottis repanda*, New Zealand
Rosenscheldiella korthalsellae	PDD 94885	GQ355332*, GQ355333*	*Korthalsella lindsayi*, New Zealand
Teratosphaeria mexicana	CBS 110502	AY725558, DQ246237	*Eucalyptus globulus*, Australia
Teratosphaeria nubilosa	CBS 116005	AY725572, EU019304	*Eucalyptus globulus*, Australia

*Sequences newly generated for this study.

[1]Names used are those cited in GenBank.

[2]ATCC: American Type Culture Collection, Virginia, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; CMW: Culture collection of Mike Wingfield, housed at FABI, Pretoria, South Africa; PDD: The New Zealand Fungal Herbarium, Landcare Research, Auckland, New Zealand; STE-U: Culture collection of Stellenbosch University, South Africa.

they are phylogenetically distinct within the *Mycosphaerellaceae*. Of the taxa sampled in this study, *R. korthalsellae* forms a sister relationship with *Mycosphaerella aurantii, M. buckinghamiae* and *M. pini, P. gaeumannii* forms a sister relationship with *Mycosphaerella heimii*, and *R. brachyglottidis* forms no close relationship with other sampled species (Fig. 2). The distinctive biology and morphology shown by these three species has evolved several times within the *Mycosphaerellaceae*. These fungi are retained in *Rosenscheldiella* and *Phaeocryptopus* for the time being, awaiting resolution of generic relationships within the family.
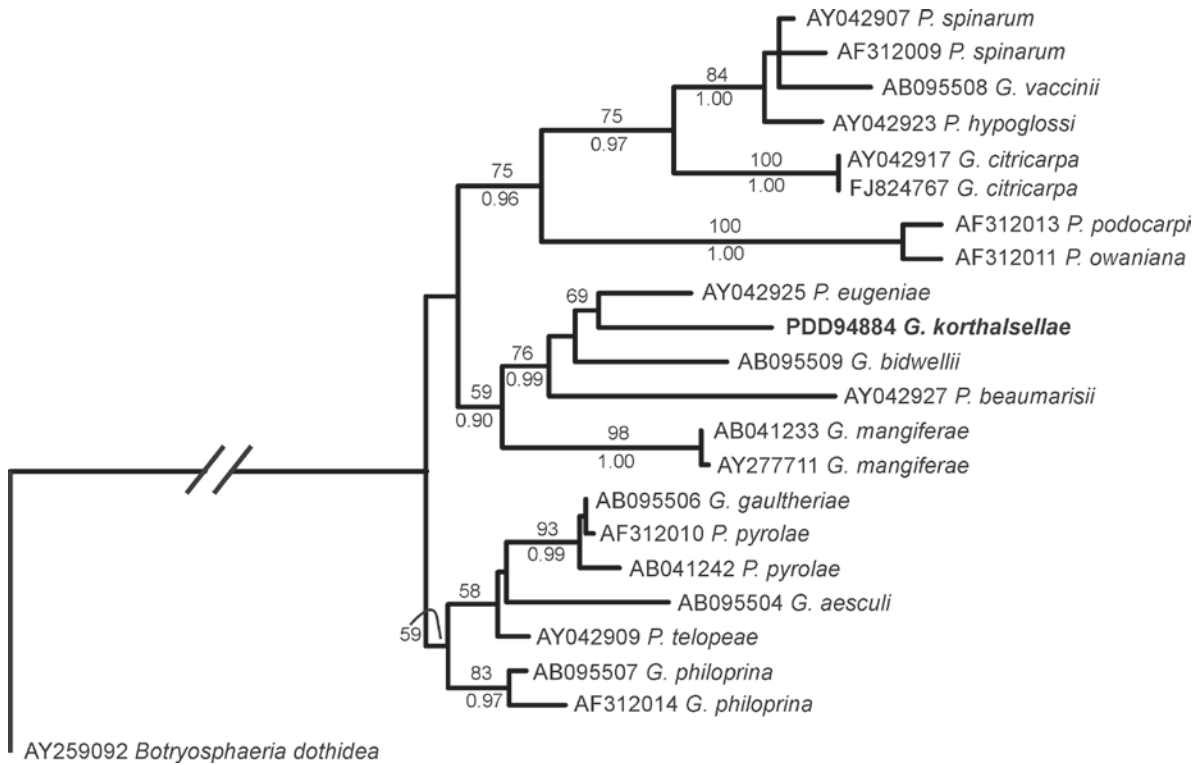
Fig. 1. Maximum likelihood tree from the PAUP analysis (Ln = – 2273.33147) based on ITS sequences from *Guignardia korthalsellae* (PDD 94884, GenBank FJ655899) and GenBank data from other species of *Guignardia* and *Phyllosticta*, (Table 1) representing the genetic diversity accepted for these fungi by Okane *et al.* (2003) and Rodrigues *et al.* (2004). Bootstrap values shown above the branches where greater then 50 % and Bayesian posterior probabilities below the branches where 0.90 or above. Tree rooted with *Botryosphaeria dothidea* as outgroup.
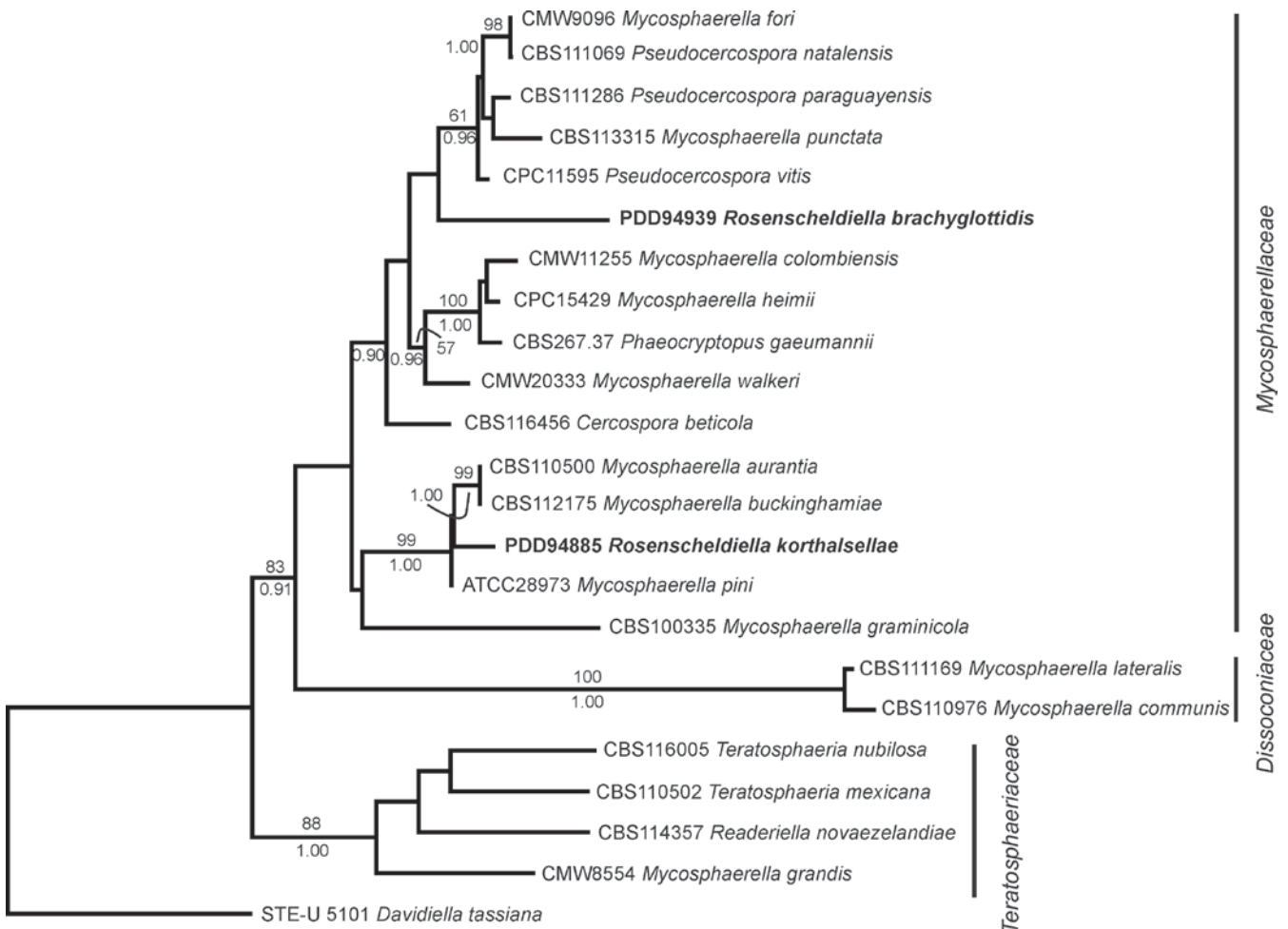
Fig. 2. Maximum likelihood tree from the PAUP analysis (Ln = – 5475.66915) based on LSU and ITS sequences from *Rosenscheldiella brachyglottidis* and *R. korthalsellae*, together with GenBank data from related *Mycosphaerella* species (Table 2). Bootstrap values shown above the branches where greater than 50 % and Bayesian posterior probabilities below the branches where 0.90 or above. Tree rooted with *Davidiella tassiana* as outgroup.
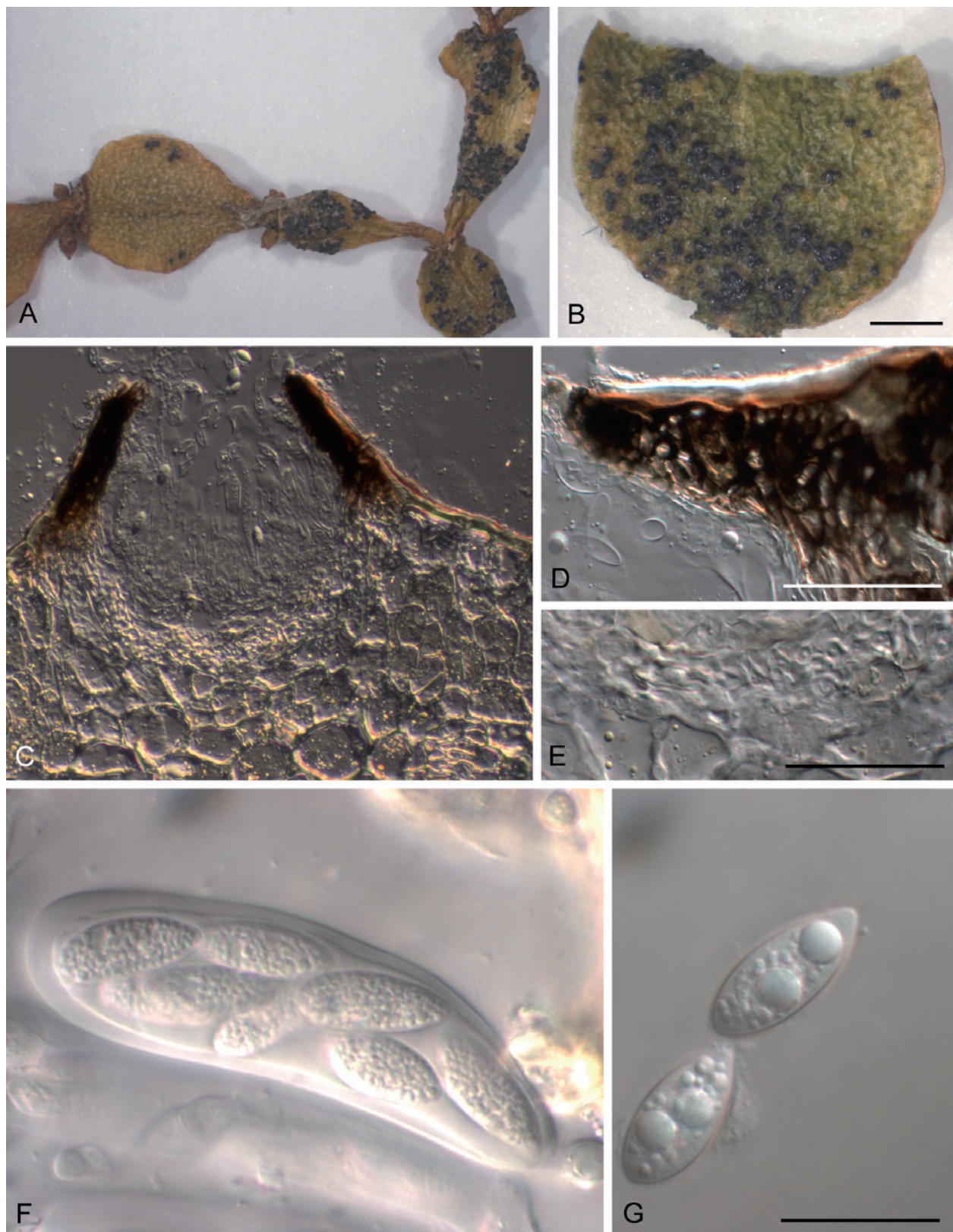
Fig. 3. *Guignardia korthalsellae* A. Infected plant. B. Detail of infected internode showing gregarious fruiting bodies. C. Ascoma in vertical section. D. Detail of dark, upper part of wall of ascoma near ostiole. E. Detail of lower, pale part of wall of ascoma. F. Ascus and developing ascospores. G. Released ascospores. A, F = PDD 65953, B–E, G = PDD 94922. Scale bars: A–B = 1 mm, C = 50 µm, D–G = 20 µm.

TAXONOMY

Guignardia korthalsellae A. Sultan, P.R. Johnst., D.C. Park & A.W. Robertson, **sp. nov**. MycoBank MB514115. Figs 3, 4.

Etymology: *korthalsellae* refers to the genus of the host plant.

Ab *G. bidwellii* ascosporis 19–27.5 × 8–11 µm, habitanti *Korthalsella lindsayi* differens.

Holotypus: **New Zealand**, Wanganui, vic. Palmerston North, Coles Bush, living internodes of *Korthalsella lindsayi*, 22 Nov. 2008, A. Sultan, PDD 94922.
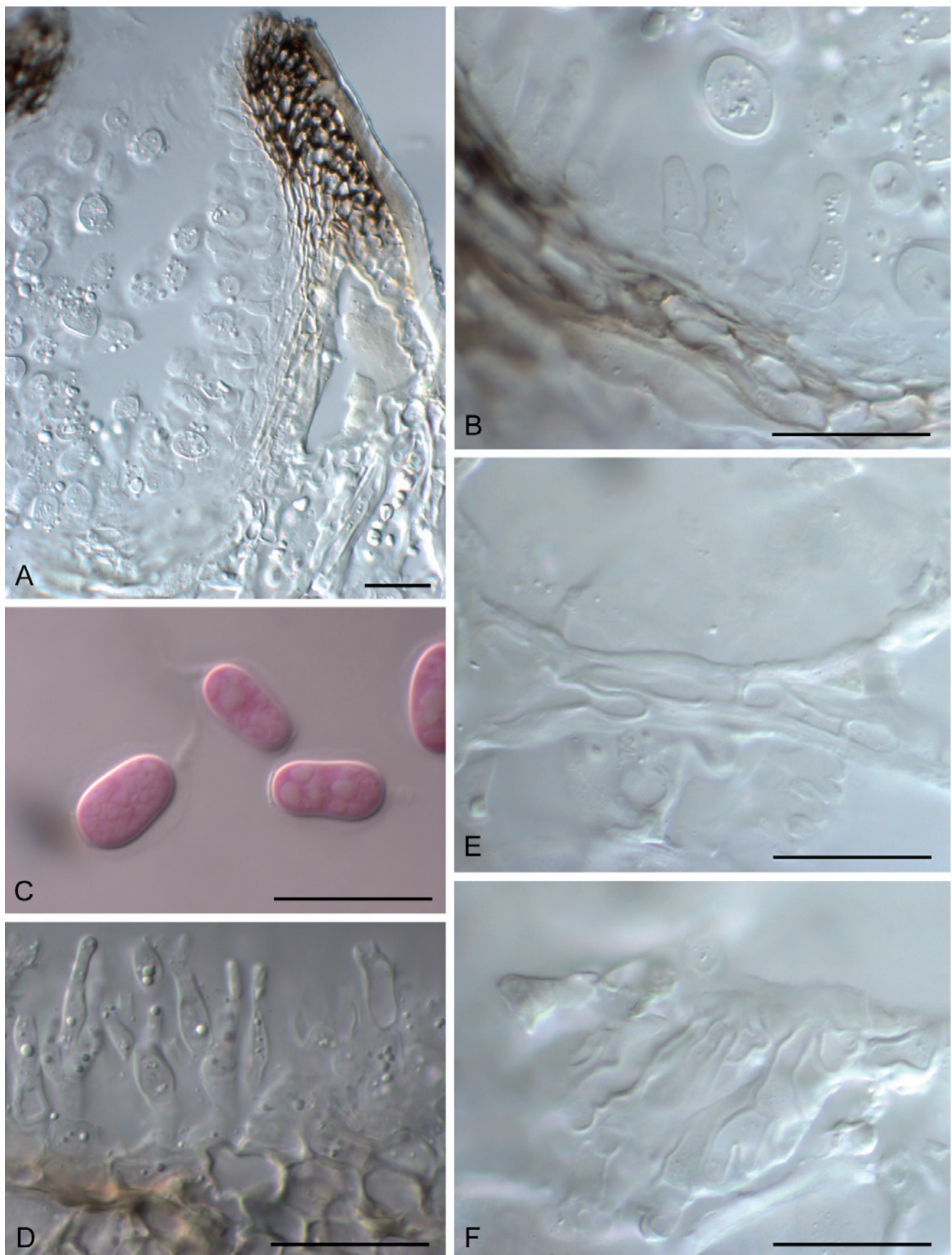
Fig. 4. *Guignardia korthalsellae* A. Conidioma in vertical section. B. Wall of conidioma with conidiogenous cells. C. Conidia. D. Conidiogenous cells and conidia of spermatial *Leptodothiorella* state. E. Fungal hyphae within phylloclade between hypodermal cells. F. Fungal hyphae within phylloclade, showing broad, plate-like layer of hyphae between hypodermal cells. A, B, E, F = PDD 65953; C, D = PDD 94922. Scale bars: A = 50 µm, B–F = 20 µm.

Ascomata and conidiomata 0.15–0.40 mm diam, black, globose, erumpent, with a single, round, apical, nonpapillate ostiole, solitary or often coalescing, gregarious, developing on flattened, leaf-like internodes of living plants, rarely associated with obvious chlorotic or necrotic symptoms, indistinct chlorotic halos sometimes seen on heavily infected leaves. Fruiting bodies develop within epidermal

layer, breaking down 3–4 epidermal cells, with immediately adjacent hypodermal cells pushed aside. Otherwise, host tissue disrupted to a minimal extent, fungal hyphae within plant restricted to plate-like layers of hyaline, thin-walled hyphae developing between 2–3 layers of host hypodermal cells and between cuticle and epidermal cells adjacent to fruiting bodies. *Ascomatal and conidiomatal wall* 20–25 μm thick, comprising 4–5 layers of short-cylindric cells 3–5 μm diam, with cell walls slightly thickened, hyaline in lower part, darkened in upper part, innermost layers of cells narrower, thinner-walled; cells adjacent to ostiole angular to globose with walls thick and dark. Groups of fruiting bodies may be entirely ascomatal, entirely conidiomatal, or have a mixture of both forms. Spermatial conidiomata sometimes also present. *Asci* clavate, bitunicate, 65–100 × 18–20 μm, attenuated at base to a short stipe, 8-spored. *Ascospores* ellipsoid, 19–27.5 × 8–11 μm, hyaline, 0-septate, no gelatinous sheaths or caps observed. Pseudoparaphysoid-like elements broad-cylindric, thin-walled, absent in mature ascomata.

Conidiogenous cells lining entire inner layer of conidiomata, solitary, cylindrical to lageniform, 6–14 × 4.5–5 μm, wall not thickened at single, apical conidiogenous locus. *Conidia* 13–15 × 7–9 μm, ellipsoid to clavoid, apex broadly rounded, base truncate, 0-septate, hyaline, surrounded by a thin gelatinous sheath, with a gelatinous, tapering apical appendage (4–)6–9(–20) μm long.

Spermatial state Leptodothiorella with conidiogenous cells cylindric, 7.5–10 × 3–5 μm, wall thickened at single, apical conidiogenous locus, in groups of 2–3 on a single, short-cylindric basal cell. *Microconidia* 5.5–7 × 2 μm, straight, dumbbell-shaped, 0-septate, hyaline.

Habitat: On living internodes of *Korthalsella lindsayi*. Ascomata more common in summer, conidiomata in winter and spring. A macroscopically similar fungus was observed on *K. clavata*, but no collections were made and its identity was not confirmed.

Distribution: New Zealand.

Additional specimens examined. **New Zealand**, Wanganui, vic. Palmerston North, Coles Bush, living internodes of *Korthalsella lindsayi*, Oct. 2008, A. Sultan, PDD 94884; Wanganui, vic. Palmerston North, Coles Bush, living internodes of *K. lindsayi*, 23 Dec. 2008, A. Sultan, PDD 95152; Bay of Plenty, Paengaroa Scenic Reserve, living internodes of *K. lindsayi*, Oct./Nov. 2008, A. Sultan, PDD 94900; Mid Canterbury, Christchurch, Riccarton Bush, on living internodes of *K. lindsayi*, 11 Apr. 1996, R.C. Close, PDD 65953.

Notes: This fungus is probably common on *Korthalsella lindsayi* throughout its range. No *Guignardia* or *Phyllosticta* spp. have been previously reported from *Korthalsella*. *Phyllosticta phoradendri* reported on *Phoradendron,* another member of the *Viscaceae* from California (Bonar 1942), was not accepted in the genus by van der Aa & Vanev (2002); these authors considered it to be probably an *Asteromella*-like species. De Lange (1997) reported a *Phyllosticta* sp. on the loranthaceous mistletoe *Ileostylus micranthus* in New Zealand. We could find no literature report or voucher specimen to support this record and suspect it may have been a miscitation of the host of PDD 65953, *K. lindsayi*, the only mistletoe-associated specimen of *Phyllosticta* available in 1997.

Guignardia and its anamorph *Phyllosticta* is monophyletic within the *Botryosphaeriaceae* (Crous *et al.* 2006b). Within *Guignardia*, *G. korthalsellae* is genetically distinct from the geographically widespread, biologically unspecialised *G. mangiferae* and from all other *Guignardia* spp. represented in recent phylogenetic studies (Fig. 1). However, many species have no molecular data available, and acceptance of the species described here as new is based in part on its host preference. Although recent studies (*e.g.* Rodrigues *et al.* 2004) have shown that some *Phyllosticta* species isolated as symptomless endophytes may have a broad host range, the biological relationship between these fungi and the hosts from which they have been isolated is poorly understood. In a study based on herbarium specimens, van der Aa (1973) considered most *Phyllosticta* species to be host specialised pathogens. *Guignardia korthalsellae* develops within living host tissue but causes minimal damage. This apparently highly developed biological relationship supports the likelihood of host specialisation in this case.

Rosenscheldiella brachyglottidis G.F. Laundon & Sivan. in Laundon, New Zealand J. Bot. 9: 619. 1972 [1971]. Fig. 5.

Ascomata develop within dense tomentum of hairs on underside of leaves, no visible symptoms on upper surface. Ascomata with one or a small number of black-walled pseudothecia, up to about 0.1 mm diam, held on a stroma-like structure comprising a small group of hyaline to pale brown, globose to angular cells that arise from hyphae growing through leaf stomata. *Ascomata* generally develop in gregarious groups of 10–20, forming patches up to about 5 mm diam. Hyaline, thin-walled fungal hyphae ramify amongst leaf tomentum adjacent to ascomata. Internally, host substomatal cavity packed with hyaline, thin-walled fungal hyphae, extensive plates of hyphae between host cells close to substomatal cavity. *Hamathecium* lacking. *Asci* fissitunicate, subsaccate with a small basal foot, 8-spored. *Ascospores* 16–22 × 5–6 μm, ellipsoid, 1-septate, slightly constricted at median septum, hyaline.

Specimens examined: **New Zealand**, Wellington, Levin, Waiopehu Reserve, on *Brachyglottis repanda*, 27 Dec. 1969, G.F. Laundon, PDD 50728, holotype; Wellington, Levin, Waiopehu Reserve, on *B. repanda*, 5 Apr. 1969, G.F. Laundon, PDD 50727; Auckland, Waitakere Ranges, roadside near Rose Hellaby House, on *B. repanda*, 8 Dec. 2008, P.R. Johnston & E.M. Gibellini, PDD 94939.

Rosenscheldiella korthalsellae A. Sultan, P.R. Johnst., D.C. Park & A.W. Robertson, **sp. nov.** MycoBank MB514116, Fig. 6.

Etymology: *korthalsellae* refers to genus of the host plant.

Ab *R. styracis* ascosporis 21.5–27 × 4.5–6 μm, habitanti *Korthalsella lindsayi* differens.

Holotypus: **New Zealand**, Rangitikei, vic. Taihape, Paengaroa Scenic Reserve, on living internodes of *Korthalsella lindsayi*, 3 Nov. 2008, A. Sultan, PDD 94885.

Ascomata develop superficially, with one to several globose, dark-walled pseudothecia forming on small pads of reddish stromatic tissue above stomata in host phylloclade, stroma forming from hyphae that emerge through stomata. Infected areas of host with large numbers of stromatic pads and their associated pseudothecia. Basal stroma comprising more or less globose cells 5–7 μm diam, with walls thin, pale, encrusted with small, reddish crystals. Irregular, short strands of hyphae, 3–5 μm diam with reddish contents, radiating away from stromata across host surface. *Stromata* arise from hyphae that extend through phylloclade stomata from substomatal cavity packed with hyaline, thin-walled fungal cells. Hyphae within phylloclade otherwise sparse and intercellular, confined to immediate area around ascomata. *Pseudothecia* initially reddish, darker with age, 100–150 μm diam, in vertical section wall
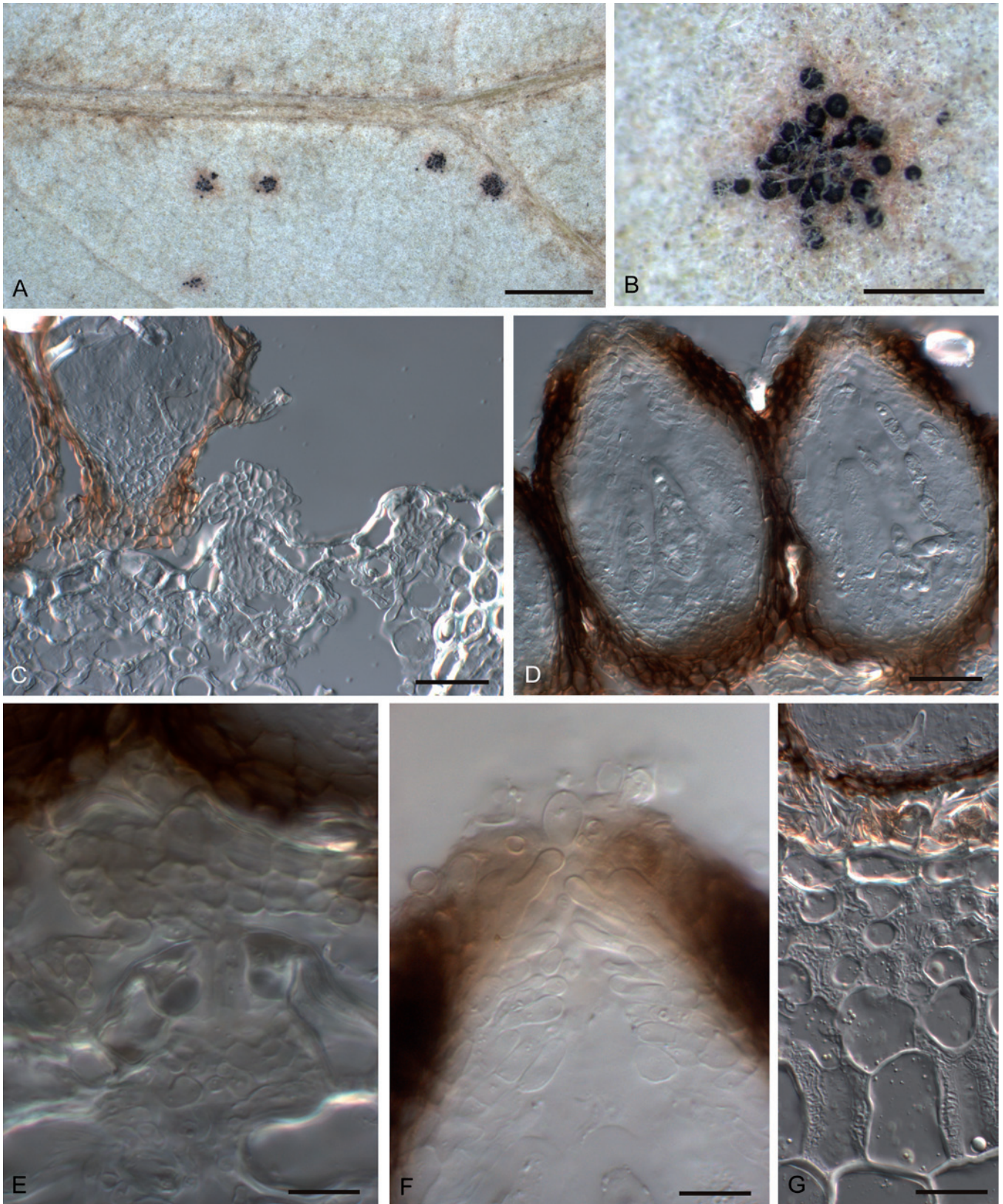
Fig. 5. *Rosenscheldiella brachyglottidis* A. Infections on underside of leaf, several patches of gregarious pseudothecia. B. Single infection, pseudothecia associated with reddish patch amongst leaf hairs on underside of leaf. C. Pseudothecia in vertical section, fungal hyphae arising from several adjacent stomata, guard cells of stomata indicated by asterisks, and forming poorly differentiated stromatic base on which pseudothecia are held. D. Pseudothecia in vertical section. E. Detail of base of ascoma in vertical section, fungal hyphae emerging from substomatal cavity, guard cells of stoma indicated by asterisks, and forming stromatic pad on leaf surface. F. Detail of ostiole in vertical section. G. Extensive network of intercellular hyphae within host leaf; base of pseudothecia visible amongst leaf hairs above intact epidermal layer of leaf. A, B = PDD 94939; C–G = PDD 50727. Scale bars: A = 5 mm; B = 1 mm; C, D, G = 20 μm; E, F = 10 μm.

15–20 μm thick, comprising 4–5 layers of short-cylindric cells, towards inside of wall cells narrower and hyaline, towards outside wider, walls encrusted with dark brown material. *Ostiole* apical, round, non-papillate, surrounded by a few short-cylindric, outwardly projecting cells. *Hamathecium* of short-cylindric to globose cells arranged amongst asci more or less in loose columns. *Asci* 55–70 × 12–14 μm, fissitunicate, cylindric to clavate with a short, foot-like base, 8-spored, overlapping 2–3 seriate. *Ascospores* 21.5–27 × 4.5–6 μm, cylindric, straight, hyaline, 1-septate, upper cell slightly wider than lower, slightly constricted at septum.
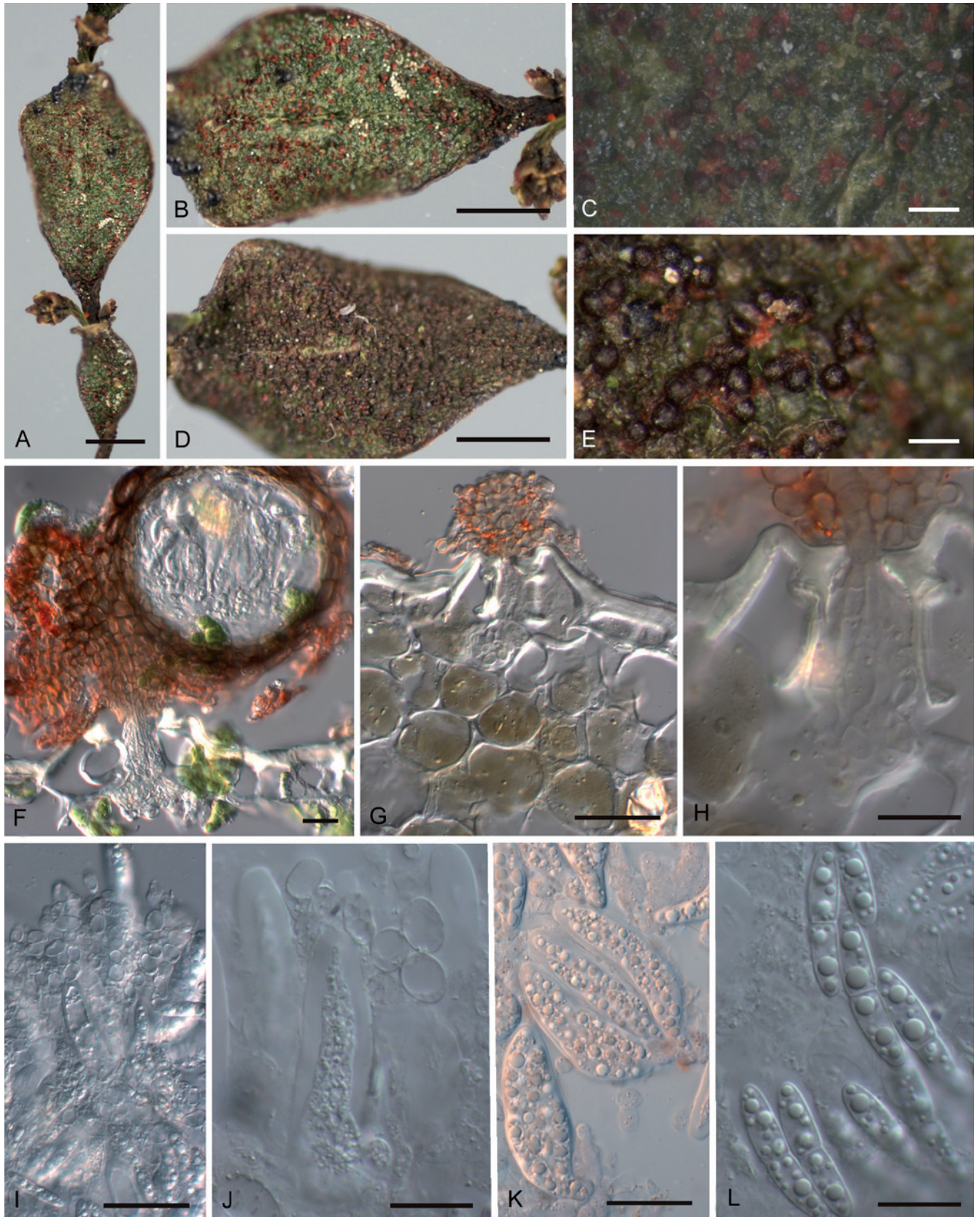
Fig. 6. *Rosenscheldiella korthalsellae* A. Infected internodes. B. Detail showing immature, reddish ascomata. C. Detail of B. D. Infected internode densely covered with mature, blackish ascomata. E. Detail of D. F. Ascoma in vertical section, pseudothecium on pad of stromatic tissue developing above stoma. G. Pad of stromatic tissue above stoma, fungal hyphae packing substomatal cavity but otherwise sparse within the internode. H. Detail of G. I. Hymenium, squash mount showing loose, more or less globose cells of hamathecial tissue. J. Detail of hamathecial cells. K. Asci. L. Ascospores. PDD 94885. Scale bars: A, B, D = 2 mm; C, E = 0.5 mm; F = 100 µm; G, I, K = 20 µm; H, J, L = 10 µm.

Habitat: On living internodes of all three *Korthalsella* spp. in New Zealand.

Distribution: New Zealand, probably common throughout the range of its host species.

Other specimens examined. **New Zealand**, Mackenzie, Lake Ohau, on *Korthalsella clavata*, Jan. 2009, A.W. Robertson, PDD 95153; Mid Canterbury, Banks Peninsula, Price's Valley, on *K. lindsayi*, 2 Sep. 1995, J.E. Braggins, PDD 65042; Mid Canterbury, Castle Hill, on *K. clavata*, 17 Jan. 2008, A. Sultan & A.W. Robertson, PDD 95150; South Canterbury, Peel Forest, on *K. lindsayi*, 22 June 1995, B.P.J. Molloy, PDD 35039; Taupo, vic. Motuoapa, on *K. salicornioides*, 3 Apr. 2008, A.
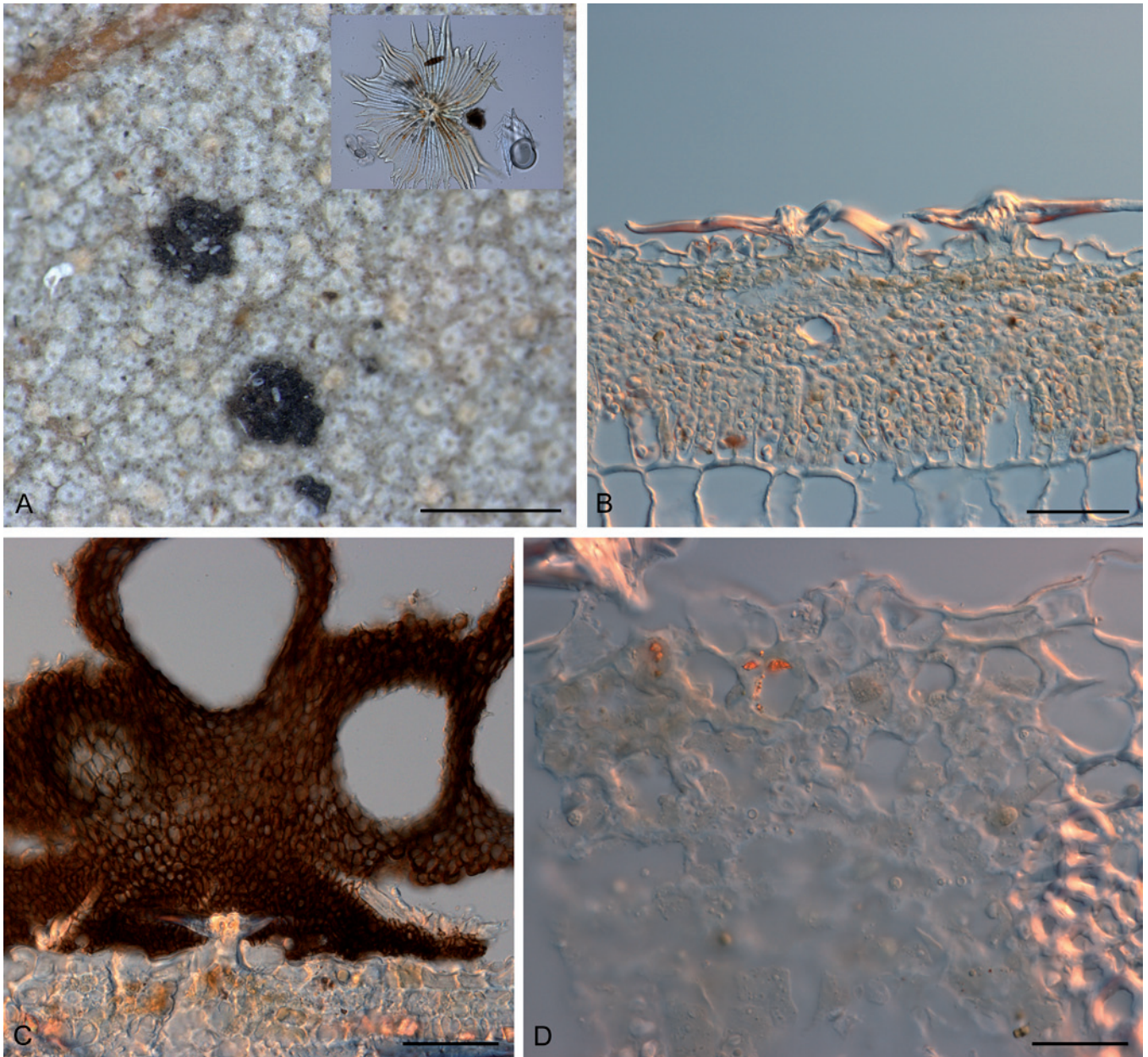
Fig. 7. *Rosenscheldiella styracis* A. Ascomata, comprising groups of superficial pseudothecia amongst leaf hairs on underside of host leaf, detail of leaf hair inset. B. Uninfected host leaf in vertical section showing leaf hairs. C. Ascoma in vertical section, several pseudothecia held on extensive basal stroma, superficial amongst leaf hairs. D. Intercellular fungal hyphae within leaf immediately below ascoma. PDD 38182. Scale bars: A = 1 mm; B, C = 50 μm; D = 10 μm.

Sultan, PDD 95151; Wanganui, vic. Palmerston North, Coles Bush, living internodes of *K. lindsayi*, 22 Nov. 2008, S. Amir, PDD 94923.

Notes: The only species of *Rosenscheldiella* previously reported from a viscaceous mistletoe is *R. phoradendri* known from El Salvador on *Phoradendron robustissimum*. Based on the published description (Jenkins & Limber 1952), *R. phoradendri* has larger ascospores, 35–48 × 13–16 μm, than our new species.

Rosenscheldiella styracis (Henn.) Theiss. & Syd., Ann. Mycol. 13: 645. 1915. Fig. 7.

Ascomata develop on lower surface of leaf, comprising 10–30 globose, dark-walled pseudothecia in confluent groups up to 0.7 mm across. *Pseudothecia* develop on extensive stromatic pads of globose cells with thick, dark walls that form amongst thick-walled, multi-lobed hairs on lower surface of leaves. Stromatic pads appear to arise from hyphae growing through stomata, but this not clearly seen. Within leaf, fungal hyphae confined to area immediately adjacent to ascomata. *Hamathecium* lacking. *Asci* fissitunicate, cylindric, about 110 × 20 μm, 8-spored. *Ascospores* 32.5–36 × 7–7.5 μm, cylindric, tapering slightly to rounded ends, 1 median septum, slightly constricted at septum, hyaline.

Specimen examined: **Uruguay**, Dept. Treinta y Tres, Tacuari, on *Styrax leprosus*, Nov. 1933, W.G. Herter, Reliquiae Petrakianae 105, PDD 38182. Although not the type, this specimen is considered authentic.

ACKNOWLEDGEMENTS

REFERENCES

Aa HA van der (1973). Studies in *Phyllosticta* I. *Studies in Mycology* **5**: 1–110.

Aa HA van der, Vanev S (2002). *A revision of the species described in Phyllosticta.* CBS, Utrecht.

Anonymous (2001). *Mistletoes in Wellington Conservancy: current status and management requirements.* Department of Conservation, Wellington Conservancy.

Arzanlou M, Groenewald JZ, Fullerton RA, Abeln ECA, Carlier J, *et al.* (2008). Multiple gene genealogies and phenotypic characters differentiate several novel species of *Mycosphaerella* and related anamorphs on banana. *Persoonia* **20**: 19–37.

Bannister P (1989). Nitrogen concentration and mimicry in some New Zealand mistletoes. *Oecologia* **79**: 128–132.

Barlow BA (1983). A revision of the *Viscaceae* of Australia. *Brunonia* **6**: 25–57.

Barlow BA (1996). *Viscaceae. Flore de la Nouvelle-Calédonie et Dépendances. Muséum National d'Histoire Naturelle, Paris* **20**: 92–99.

Barlow BA (1997). *Viscaceae. Flora Malesiana* **13**: 403–442.

Bonar L (1942). Studies of some Californian fungi II. *Mycologia* **34**: 180–192.

Bunyard BA, Nicholson MS, Royse DJ (1994). A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. *Mycologia* **86**: 762–772.

Burrows C (1996). Dispersal of *Korthalsella* seeds. *Canterbury Botanical Society Journal* **31**: 69–70.

Crous PW, Wingfield MJ, Mansilla JP, Alfenas AC, Groenwald JZ (2006a). Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs on *Eucalyptus* II. *Studies in Mycology* **55**: 99–131.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, *et al.* (2006b). Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* **55**: 235–253.

Crous PW, Braun U, Groenwald JZ (2007). *Mycosphaerella* is polyphyletic. *Studies in Mycology* **58**: 1–32.

Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, *et al.* (2009). Phylogenetic lineages in the *Capnodiales*. *Studies in Mycology* **64**: 17–47.

Danser BH (1940). A supplement to the revision of the genus *Korthalsella*. *Bulletin du Jardin Botanique Buitenzorg* **16**: 329–342.

Gardes M, Bruns TD (1993). ITS primers with enhanced specificity for basidiomycetes — application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.

Jenkins AE, Limber DP (1952). Two new ascomycetes on *Phoradendron*. *Mycologia* **44**: 557–560.

Johnston PR, Park D (2005). *Chlorociboria* (*Fungi*, *Helotiales*) in New Zealand. *New Zealand Journal of Botany* **43**: 679–719.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Ainsworth and Bisby's Dictionary of the Fungi. 10th Ed. CAB International: Wallingford, UK.

Kliejunas JT, Scharpf RF, Smith RS (1979). *Teichospora obducens* and a *Pleospora* species on *Korthalsella* in Hawaii. *Plant Disease Reporter* **63**: 1060–1062.

Lange PJ de (1997). Decline of New Zealand loranthaceous mistletoes – a review of non-possum (*Trichosurus vulpecula*) threats. In: *New Zealand's Loranthaceous mistletoes* (de Lange PJ, Norton DA, eds). Proceedings of a workshop hosted by Threatened Species Unit, Cass, 17–20 July 1995. Department of Conservation, Wellington, New Zealand: 155–163.

Lange PJ de, Norton DA, Heenan PB, Courtney SP, Molloy BPJ, *et al.* (2004). Threatened and uncommon plants of New Zealand. *New Zealand Journal of Botany* **42**: 45–76.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, *et al.* (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947–2948.

Molvray M (1997). A synopsis of *Korthalsella* (*Viscaceae*). *Novon* **7**: 268–273.

Molvray M, Kores PJ, Chase MW (1999). Phylogenetic relationships within *Korthalsella* (*Viscaceae*) based on nuclear ITS and plastid trnL-F sequence data. *American Journal of Botany* **86**: 249–260.

Okane I, Lumyong S, Nakagiri A, Ito T (2003). Extensive host range of an endophytic fungus, *Guignardia endophyllicola* (anamorph: *Phyllosticta capitalensis*). *Mycoscience* **44**: 353–363.

Petrak F (1953). Beitrage zur pilzflora von Hawaii. *Sydowia* **7**: 381–409.

Posada D, Buckley TR (2004). Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**: 793–808.

Posada D, Crandall KA (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Rodrigues KF, Sieber TN, Grünig CR, Holdenrieder O (2004). Characterization of *Guignardia mangiferae* isolated from tropical plants based on morphology, ISSR-PCR amplifications and ITS1-5.8S-ITS2 sequences. *Mycological Research* **108**: 45–52.

Sivanesan A, Shivas RG (2002). New species of foliicolous *Loculaoascomycetes* on *Dysoxylum*, *Melaleuca* and *Syzygium* from Queensland, Australia. *Fungal Diversity* **11**: 151–158.

Stevens FL (1925). Hawaiian Fungi. *Bishop Museum Bulletin* **19**: 1–189.

Stone JK, Capitano BR, Kerrigan JL (2008). The histopathology of *Phaeocryptopus gaeumannii* on douglas-fir needles. *Mycologia* **100**: 431–444.

Swofford DL (2002). *PAUP*. Phylogenetic analysis using parsimony (*and other methods).* Version 4. Sunderland, Massachusetts, Sinauer Associates.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

Wagner WL, Herbst DR, Sohmer SH (1999). *Manual of the flowering plants of Hawai'i.* Revised edition. Bishop Museum Special Publication 97. University of Hawaii Press, Honolulu.

White TJ, Bruns T, Lee S, Taylor JW (1990). Amplification of direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: A guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ eds). Academic Press, San Diego: 315–322.

Winton LM, Stone JK, Hansen EM, Shoemaker RA (2007). The systematic position of *Phaeocryptopus gaeumannii*. *Mycologia* **99**: 240–252.