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Unusual new *Chaetosphaeria* species from New Zealand: intrafamilial diversity and elucidations of the Chaetosphaeriaceae – Lasiosphaeriaceae relationship (Sordariomycetes, Ascomycotina)

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Abstract *Chaetosphaeria albida*, *C. bombycina*, and *C. metallicans* are described and compared with other *Chaetosphaeria* taxa using morphological and molecular methods. The fresh ascomata of *C. albida* are almost white, translucent, and aereolate; they are papillate with a distinctive 4-layered peridium, and the ascospores are scolecosporous, multiseptate, and hyaline. *C. bombycina* is similar, but the fresh ascomata are light fawn-grey with a reflective silken appearance, non-papillate, and the similar peridium is 3-layered. *C. metallicans* has ascomata which are blue-black, shiny and metallic when fresh; the thick peridium is heavily melanised, and the ascospores are straight to allantoid, 3-septate, and hyaline. The scolecosporous ascospores of *C. albida* and *C. bombycina* would have traditionally referred these taxa to *Lasiosphaeria*. However, like *C. metallicans*, they lack a peridial tomentum, and have asci with light-refractive, non-amyloid apical rings, without a sub-apical globule. Despite the major differences in spore shape and ascomal wall structure, analyses of the LSU and ITS regions of ribosomal DNA suggest that genetically all three fall within *Chaetosphaeria*, near to *C. raciborskii*, and in a sister clade to the type species *C. innumera*. The placement of these species considerably expands current morphological conceptions of *Chaetosphaeria*, particularly in terms of ascomal wall appearance and structure, and confirms the existence of a scolecosporous group within the genus. In the search for morphological characters which mimic genetic relationships, this study further elucidates the relationship between the Chaetosphaeriaceae and the Lasiosphaeriaceae.

Keywords *Chaetosphaeria*; Chaetosphaeriaceae; Lasiosphaeriaceae; Sordariales; LSU; ITS; systematics; New Zealand

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INTRODUCTION

Consecutive autumn collecting trips to the Oparara Basin, near Karamea, on the South Island's west coast in 2004 and Ohakune in the central North Island in 2005 yielded several new pyrenomycetous ascomycetes from well-decayed wood. Both areas are mixed beech-podocarp forest which has been selectively logged in the past. Dominant tree species in the Oparara Basin are *Nothofagus menziesii* (silver beech), *N. fusca* (red beech), and *Dacrydium cupressinum* (rimu), with silver beech being the probable substrate for these collections. Three beech species are present in Ohakune, silver beech, red beech, and *N. solandri* (black beech), with silver or black beech being the probable substrate. Initial morphological examination revealed taxa with clustered, superficial ascomata with differences in coloration and surface texture but similarities in ascus apex structure. All collections had light refractive apical annuli but lacked sub-apical globules, and all collections had hyaline, septate ascospores.

Current knowledge suggested that they belonged within the Chaetosphaeriaceae, probably in *Chaetosphaeria*. Phylogenetic analysis was necessary to confirm their placement.

Chaetosphaeria Tul. & C.Tul. is cosmopolitan in distribution but better known in temperate zones (Réblová et al. 1999). These saprobic pyrenomycetes are common on decaying wood, typically having superficial, globose to subglobose, setose or otherwise, black ascomata, and hyaline ascospores with 1–3 transverse septa, and are frequently associated with conspicuous dematiaceous anamorphs (Réblová et al. 1999). Although several taxonomists (Barr 1990; Eriksson & Hawksworth 1993) placed *Chaetosphaeria* within the Lasiosphaeriaceae, it was later given its own family based on morphological grounds (Réblová et al. 1999). Recently, the Chaetosphaeriaceae was given its own order, Chaetosphaeriales, based on molecular data (Huhndorf et al. 2004). Extensive discussion of morphological distinctions between the Chaetosphaeriaceae and the Lasiosphaeriaceae can be found in Réblová et al. (1999) and Réblová (1999a). Réblová (2000) and Réblová & Winka (2000) redefined *Chaetosphaeria* sens. str. and divided the genus into four (and then five) "natural groups" based on morphological, cultural, and molecular studies. In Réblová's (1999a, p. 391) opinion, *Chaetosphaeria* sens. str. "remains a large aberrant taxon" whose species are rather "uniform in anatomy and form of perithecia, hamathecium, asci and ascospores" (Réblová et al. 1999, p. 63).

Miller & Huhndorf's (2004a) work with the Lasiosphaeriaceae and higher order taxonomy of the Sordariales led them to observe that certain scolecosporous* taxa formerly placed in *Lasiosphaeria* Ces. & de Not. belong within *Chaetosphaeria* genetically. Subsequently, Huhndorf & Fernández (2005) showed the existence of an extensive scolecosporous clade within *Chaetosphaeria*, centred around *Chaetosphaeria raciborskii* (Penz. & Sacc.) F.A.Fernández & Huhndorf. *C. raciborskii* is located in a sister clade to the type species *C. innumera*, but these and most other *Chaetosphaeria* taxa are within a larger clade which has 100% bootstrap support in some analyses (Huhndorf & Fernández 2005). Several recent papers have attempted to correlate morphology and genetics within the Sordariales (e.g., Huhndorf et al. 2001; Miller & Huhndorf 2004b, 2005), but among the sister family Chaetosphaeriaceae, this appears to be increasingly challenging. Many new taxa are still being described. Recent work in the Americas resulted in the description of 10 new *Chaetosphaeria* taxa, two new closely related taxa including a new genus within the family, and a key to 23 *Chaetosphaeria* taxa in the Americas (Fernández & Huhndorf 2005). Recent work in New Zealand resulted in the description of four new *Chaetosphaeria* taxa and a key to 47 *Chaetosphaeria* taxa worldwide (Réblová 2004). Most recently, a revision of north temperate and neotropical taxa within *Chaetosphaeria* and allied genera concluded that *Chaetosphaeria* is "a morphologically complex genus" comprised of "a distinct monophyletic group ... with diverse morphologies" in combination with "a highly divergent group of paraphyletic, mostly poorly supported species (that includes the type species) with relatively uniform teleomorphs" (Fernández et al. 2006, p. 126). The inclusion here of additional diverse new taxa further widens the morphological concept of a genus that, with the discoveries of modern genetics, is increasingly interesting.

A main aim of this study was to ascertain whether peridial characters could be correlated with genetic relationships in the Chaetosphaeriaceae, particularly in the placement of these new taxa.

MATERIALS AND METHODS

Microscopy and imaging

Photographs of fresh ascomata were taken on the day of collection. Fig. 1, 2, 19 were taken with a Fuji Finepix S3100 digital camera pointed down one eyepiece of a Leica Wild MZ8 dissecting microscope. Fig. 12, 13 were taken with a Nikon Coolpix E995 digital camera mounted on an Olympus SZX7 dissecting microscope. Fig. 3, 9, 14, 20 were taken using a Nikon Coolpix 995 digital camera mounted on a Leica Wild MZ8 dissecting microscope and using Ulead Photo Explorer 8.0 (2001–2002 Ulead Systems Inc.) for image capture. Open source software called CombineZ (Alan Hadley) was used to stack a series of photographs taken at different focal distances into a single image with enhanced depth of focus (Fig. 9, 12, 13, 20). Ascomata were

*A scolecospore is defined by Hawksworth et al. (1995) as a spore with a length/width ratio > 15:1. In practice, the term tends to be used more loosely, and is interchangeable with "long-spored". However, with one exception (*C. lapaziana*), the taxa discussed as scolecosporous here fit the definition for at least part of their size range.

squash-mounted, initially in water, then in Shear's Mounting Media, Aniline blue, and Meltzer's Reagent. Measurements were made of material in water. At least 30 ascospores were measured for each collection, from a minimum of two ascomata. Ascomata were sectioned at 20–30 µm for light microscopy (following Miller 2003) using a Leitz 1310 freezing microtome, or sectioned at 3–4 µm using wax embedding. Sections were mounted in Shear's Mounting Media. Images of microscopic morphological structures were captured using a Leica DC 300 digital camera mounted on a Leica DMRE compound microscope using Leica IM50 Image Manager for image capture. Air-dried ascomata of *C. metallicans,* which had not been coated, and ascospores coated with platinum were examined using a JEOL 6700F Field Emission Scanning Electron Microscope (JEOL, Japan), with a Gatan Alto 2500 cryo stage (Gatan, UK). The photographic plates were produced electronically using Adobe Photoshop 5.0 (Adobe Systems Incorporated, Mountain View, California). All type specimens are deposited in PDD.

Phylogenetic analyses

Total genomic DNA was extracted from exsiccata using c. 40 ascomata per sample. Samples were ground in CTAB buffer at room temperature, incubated for 10 min, and then extracted twice with equal volumes of chloroform. The DNA was precipitated using 2 volumes of 95% ethanol and 1/10 volume 3*M* sodium acetate, pH 4.8. The DNA was washed in 70% ethanol and resuspended in TE buffer, pH 8.

A portion of the large subunit (LSU) region of nuclear 28S ribosomal DNA was amplified from 1 µl of extracted DNA using the primers LROR and LR5 (Vilgalys & Hester 1990; Rehner & Samuels 1995). The entire internal transcribed spacer (ITS) region of nuclear ribosomal DNA was amplified from 1 µl of extracted DNA using the primers PN3 and PN10 (Viaud et al. 2000). The polymerase chain reactions (PCR) for both LSU and ITS were prepared in 50 µl reaction volumes, which contained 25 µl Amplitaq Gold PCR Master Mix (Applied Biosystems, New Jersey, USA), 1 µl of each primer at a concentration of 30 pmol/µl, and 22 µl water. The PCR amplification protocol for LSU was: initial denaturation at 95° for 5 mins, followed by 30 cycles of 94° for 30 s, 47° for 30 s, and 72° for 1 min, with a final extension of 72° for 10 mins. The PCR amplification protocol for ITS was: 95° for 5 mins, 30 cycles of 95° for 60 s, 50° for 90 s, 72° for 60 s, followed by a final extension of 72° for 10 mins. The PCR products were visualised in 1.5 % (wt/vol) agarose gels, stained with ethidium bromide and photographed under UV transillumination. PCR products were purified using spin column purification (QIAquick PCR Purification Kit, QIAGEN Pty Ltd., Australia), but with a final centrifugation of 5 min. DNA concentration was measured using a spectrophotometer (Nanodrop).

Sequencing of the LSU region from purified PCR products was only partially successful. Therefore, these regions were cloned and re-sequenced. Cloning was conducted following the protocol described in the Invitrogen TOPO TA Cloning manual for the One Shot Chemical Transformation method. Plasmid DNA was isolated by the Modified Alkaline Lysis/PEG Method (Applied Biosystems 2005). The purified PCR products (ITS) were sequenced using the original amplification primers, and the plasmid DNA (containing cloned LSU) was sequenced using the primers M13f and M13r on an ABI3730 Genetic Analyser (Applied Biosystems Inc.) following the manufacturer's instructions. Sequencing was done by the Allan Wilson Centre for Molecular Ecology and Evolution at the Albany Campus of Massey University, New Zealand.

Electropherograms were assembled and edited using AutoAssembler v 1.3.0 (Applied Biosystems Inc.). Sequences were aligned using ClustalX v 1.83 (Thompson et al. 1997) followed by manual adjustment using Se-Al 2.0a11 (Rambaut 2002). Due to differences in taxon sampling, ITS and LSU datasets were incongruent and were therefore analysed separately. All phylogenetic analyses were performed using PAUP* v 4.0b10 (Swofford 2002). In the LSU analysis, gaps were treated as missing data, uninformative characters were excluded, and maximum parsimony heuristic searches (100 replicates) with stepwise random addition were used to find the most parsimonious trees. A bootstrap analysis consisting of 500 replicates with one random addition per replicate (and no more than 5 trees saved in each replicate) was performed to determine branch support. Maximum parsimony analysis was conducted as above on the ITS dataset using 10 000 replicate heuristic searches with stepwise random addition of taxa. A bootstrap analysis consisting of 10 000 replicates with one random stepwise addition of taxa per replicate was performed. A maximum likelihood analysis was also conducted on the ITS dataset using the most parsimonious tree as a starting tree. Maximum likelihood parameters were estimated using the likelihood ratio test as implemented in MODELTEST v 3.06 (Posada & Crandall 1998).

MODELTEST determined that the best model of sequence evolution for the ITS dataset was the general time reversible model (Rodríguez et al. 1990) with a proportion of invariable sites (0.410) and a gamma distribution shape parameter (0.787) (GTR + I + G). Phylogenetic trees were prepared for publication using Adobe Illustrator 10 (Adobe Systems Inc.).

TAXONOMY

Descriptions of the type species, *C. innumera*, which falls within Réblová's (2000) group 3, can be found in Gams & Holubová-Jechová (1976) and Booth (1957). Species descriptions for the group 4 (Réblová 2000) taxa used in our phylogenies are to be found as follows: *C. abietis*, Gams & Holubová-Jechová (1976); *C. capitata*, *C. chlorotunicata*, *C. conirostris*, *C. lignomollis*, and *C. spinosa*, Fernández & Huhndorf (2005); *C. raciborskii*, Carroll & Munk (1964), Huhndorf & Fernández (2005); *C. caesariata* (as *Umbrinosphaeria*), Réblová (1999b). Brief descriptions within a key are given for *C. acutata*, *C. chalaroides*, *C. cubensis*, *C. cylindrospora*, *C. decastyla*, *C. fennica*, and *C. fusiformis* in Réblová (2004).

Chaetosphaeria albida T.J.Atk., A.N.Mill. & Hunhdorf, sp. nov. Fig. 1–11

Ascomata albida, interdum pauxillum pallide ferruginata, translucida, aereolata, glabra, ovoidea usque late obpyriforma, 0.3–0.4(–0.5) mm diametro, 0.5–0.75 mm alta, superficialia, dispersa usque gregaria, ad papillata, cum ostiolo nigro. In statu sicco saepe collapsa lateralibus, furfuracea, pseudo-lanata. Paries ascomatis superficialis textura epidermoidea, in sectione longitudinali 50–75 µm crassus, quadristriatus. Asci unitunicati, 220–260 × 16–20 µm, longe stipitati, pars sporifer 110–50 µm, octospori, multiseriati, annulo apice refractivo, non amyloideo, sine globulo sub-apice. Ascosporae scolecosporae, rectae vel curvatae, (47)60–80 × 5–7 µm, multiseptatae, hyalinae, laeves, vagina gelatinosa.

HOLOTYPE: New Zealand, South Island, Nelson, Karamea, Oparara Basin, a few metres from Mirror Tarn, on decorticated, rather decayed, 4 cm wide branch of unknown substrate, probably beech (*Nothofagus*), *T. Atkinson TJA728*, 16 May 2004, PDD 92537.

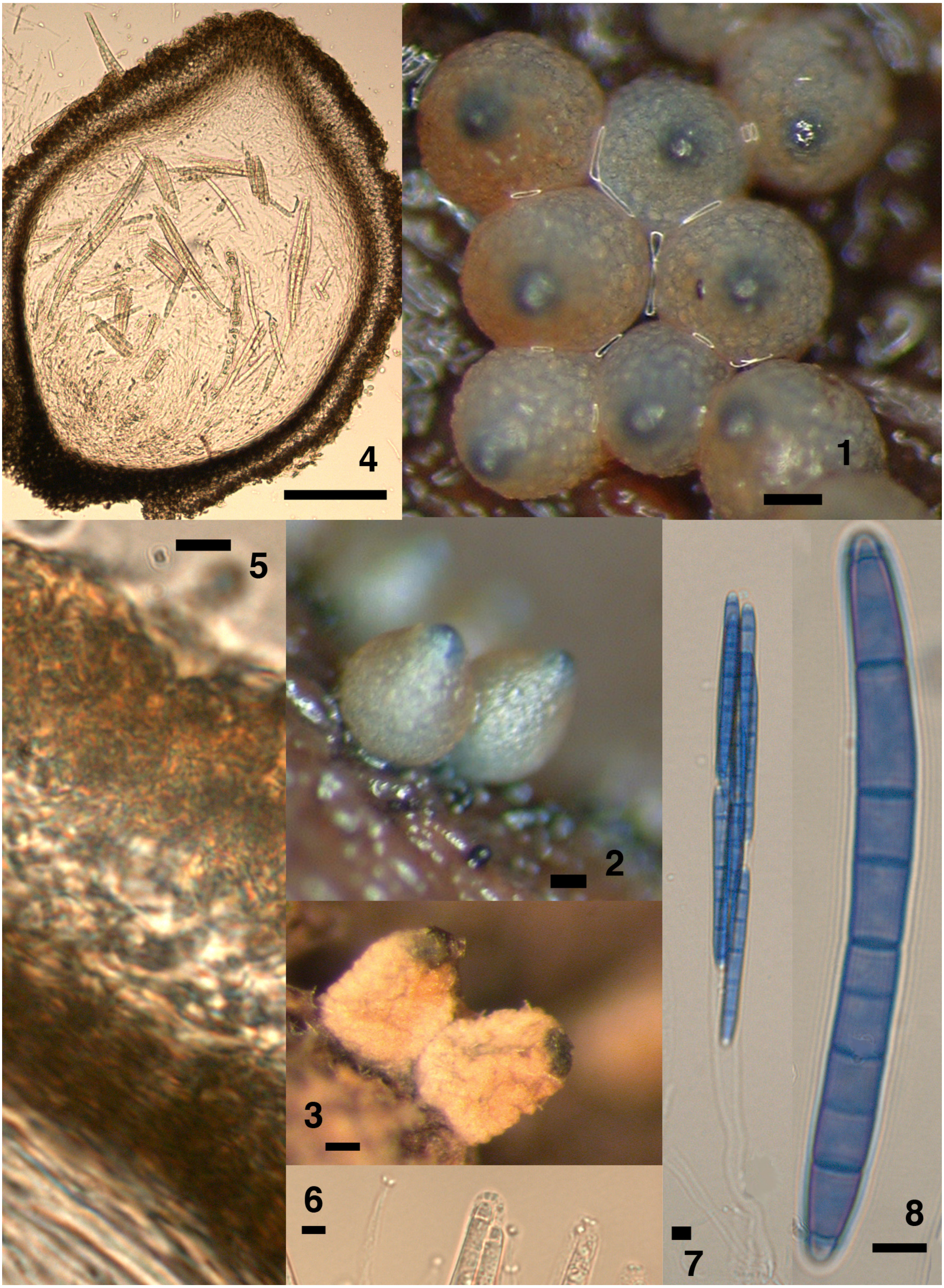
DESCRIPTION: Ascomata whitish to light brown, glabrous, ovoid to broadly obpyriform, 0.3–0.4(–0.5) mm diam., 0.5–0.75 mm high, superficial, scattered to gregarious, with black ostiole. Surface of fresh ascomata often aereolate, somewhat translucent or pearly. With age dull cream or light yellow-brown or ginger-brown, scurfy, scaly, or almost woolly, often collapsing laterally and shrivelling to around 0.25–0.3 mm in diameter, (0.25–)0.5–0.7 mm high. Ascomatal wall of textura epidermoidea in surface view; in longitudinal section 50–75 µm thick, 4-layered; outer layer 15–20 µm thick, composed of 2–4 cell layers of yellow-brown pseudoparenchymatous cells; second layer 10–15 µm thick, composed of 2–4 cell layers of nearly hyaline pseudoparenchymatous cells; third layer 10–15 µm thick, composed of 5–7 cell layers of brown to dark brown slightly flattened cells; inner layer 5–20 µm thick, thickest at apex, composed of 3–7 cell layers of elongate to flattened hyaline cells. Ascomatal apex short papillate, with periphyses. Paraphyses not easily visible, possibly deliquescent. In Meltzer's Reagent they appear sparse, hyaline, 5–10 µm wide, of variable length, some much longer than asci, unbranched, probably septate. Asci unitunicate, persistent, cylindrical, 220–260 × 16–20 µm; spore-bearing part 110–150 µm long, long-stipitate, stipe 100–120 µm long, tapering to 4 µm wide near base, apex rounded, with non-amyloid, light-refractive ring 2 × 3–4 µm, without sub-apical globule; with 8 multiseriate ascospores. Ascospores scolecosporous, straight to gently curved, (47–)60–80 × 5–7 µm, with rounded ends, becoming (5–)7(–12)-septate, sometimes slightly consticted at septa, remaining hyaline, smooth, with gelatinous sheath. Ascospores dextrinoid. Ascospores stain blue in aniline blue, except for apex and base which remain hyaline; ascomatal centrum remains hyaline.

ETYMOLOGY: Whitish, referring to the surface of the fresh ascomata.

HABITAT: Decorticated, well decayed wood, probably *Nothofagus*, in mixed native *Nothofagus*, podocarp, broadleaf forest.

ANAMORPH: Unknown.

Fig. 1–8 *Chaetosphaeria albida* (*TJA728*). **Fig. 1**, Fresh ascomata. Note aereolate appearance. Photograph: A. N. Miller. **Fig. 2**, Fresh ascomata (side view). Photograph: A. N. Miller. **Fig. 3**, Air-dried, collapsed ascomata. Note almost woolly appearance. **Fig. 4**, 30 µm freezing microtome longitudinal section of ascoma. **Fig. 5**, Peridium, 50–75 µm in thickness, 4-layered, from 30 µm longitudinal section. **Fig. 6**, Light-refractive apical rings of asci. **Fig. 7**, Ascus, 220–260 × 16–20 µm (aniline blue). **Fig. 8**, Ascospore, (47–)60–80 × 5–7 µm (aniline blue). Note ends do not stain. Scale bars: 1–4 = 0.1 mm; 5–8 = 5 µm.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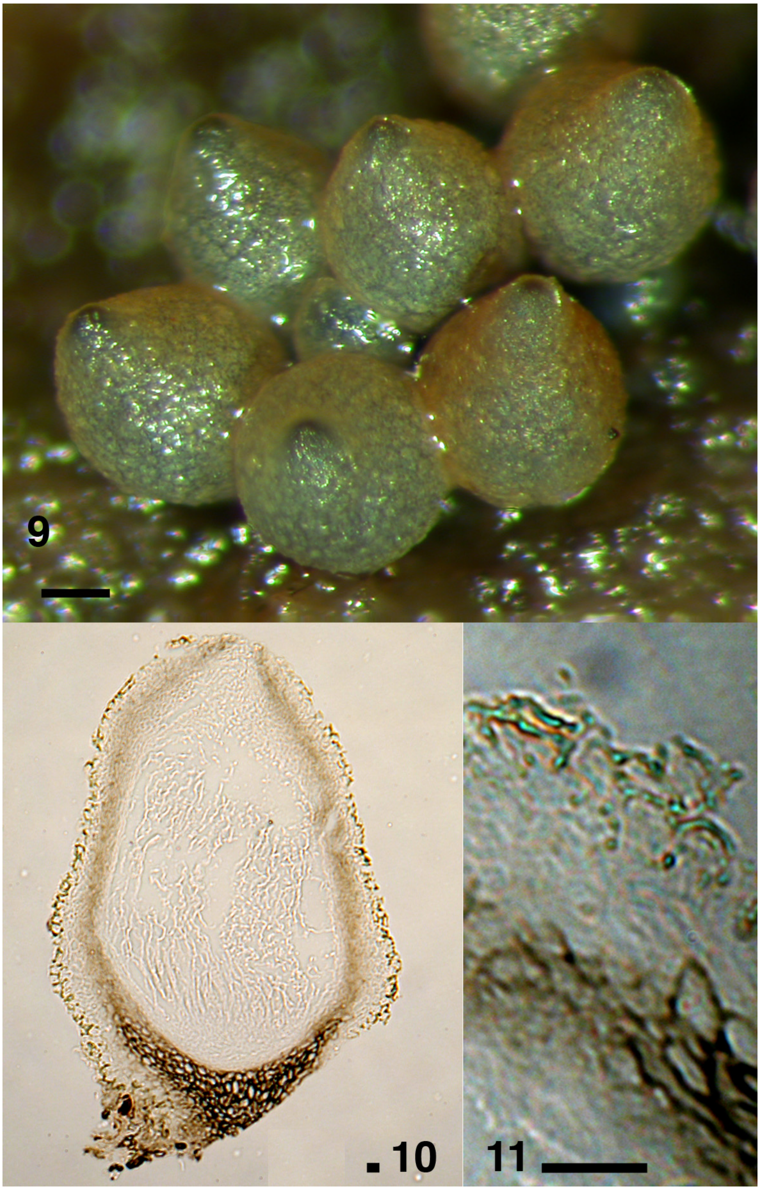


Fig. 9–11 *Chaetosphaeria albida*. **Fig. 9**, Fresh ascomata (*TJA953*). **Fig. 10**, 3–4 µm wax embedded, longitudinal section of ascoma (*TJA955*). Note ostiole. **Fig. 11**, Close-up of wall layers of same. Note light-reflective properties of golden, outer cells. Scale bars: 9 = 0.1 mm); 10, 11 = 10 µm.

KNOWN DISTRIBUTION: North and South Islands, and Codfish Island, New Zealand.

ADDITIONAL SPECIMENS EXAMINED: NORTH ISLAND: AUCKLAND: Waitakere Ranges, Home Track, on *Metrosideros robusta*, *S. J. Hughes*, 16 Sep 1963, PDD 39504; Hunua Ranges, Mangatangi Dam, Walkman Track, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA570*, 19 May 2003, PDD 92542; Walkman Track, on *Nothofagus* sp., *T. Atkinson TJA470*, 19 May 2003, PDD 92543. SOUTH ISLAND: WESTLAND: Haast, ?Cascade Forest, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA106*, 7 May 2002, PDD 92540; Cascade Forest, on decorticated, well-decayed wood of unknown substrate, probably beech (*Nothofagus* sp.), *S. Whitton & T. Atkinson TJA114*, 7 May 2002, PDD 92547; Haast, Okuru, Hapuku Estuary Walk, climbing *Metrosideros* sp., *A. Bell TJA110*, 9 May 2002, PDD 92541. NELSON: Karamea, Oparara Basin, near Honeycomb Cave/ Box Canyon carpark, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA953*, 12 May 2006, PDD 92548; near Honeycomb Cave/ Box Canyon carpark, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA955*, 12 May 2006, PDD 92549; Oparara Basin, near Mirror Tarn, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA956*, 12 May 2006, PDD 92550. CODFISH ISLAND: North-East Bay Track, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA771*, 10 Mar 2004, PDD 92544; Loop Track (between Eric and Miro), on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA813*, 12 Mar 2004, PDD 92545; Wounded Knee Track, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA770*, 13 Mar 2004, PDD 92546.

Chaetosphaeria bombycina T.J.Atk., A.N.Mill. & Huhndorf, sp. nov. Fig. 12–18

Ascomata hinnulea usque cinerea, margaritacea, bombycina, glabra, subglobosa usque late ovoidea, c. 0.3–0.4(–0.5) mm diametro, c. 0.5–0.6 mm alta, superficialia, dispersa usque gregaria, non-papillata, cum ostiolo griseo. In statu sicco collapsa lateralibus et verticalibus, furfuracea, tristis, sordida. Paries ascomatis superficialis textura epidermoidea, in sectione longitudinali 30–50 µm crassus, tristriatus. Asci unitunicati, 215–270 × 11–14 µm, longe stipitati, pars sporifer 70–135 µm, octospori, multiseriati, annulo apice refractivo, non amyloideo, sine globulo sub-apice. Ascosporae scolecosporae, rectae vel curvatae, 62–88 × 4.5–6 µm, multiseptatae, hyalinae, laeves, vagina gelatinosa.

HOLOTYPE: New Zealand, North Island, Ohakune, Jubilee Park, around 100 m along the track that enters the park from Burns St, on decorticated, rather decayed branch of unknown substrate c. 10 cm wide and 25 cm long, probably *Nothofagus*, *T. Atkinson TJA837*, 8 Apr 2005, PDD 92538.

DESCRIPTION: Ascomata fawn to light grey, glabrous, subglobose to broadly ovoid, 0.3–0.4(–0.5) mm diam., 0.5–0.6 mm high, superficial, scattered to gregarious. Surface of fresh ascomata pearly or silky, with an inconspicuous grey ostiole. With age becoming grey, or yellow-grey, scurfy, collapsing and shrivelling to around 0.25–0.4(–0.5) mm diam., 0.25–0.4(–0.5) mm high. Ascomatal wall of textura epidermoidea in surface view; in longitudinal section 30–50 µm thick, 3-layered; outer layer 10–20 µm thick, composed of 3–5 cell layers of light yellow-brown pseudoparenchymatous cells; middle layer 10–15 µm thick, composed of 5–8 cell layers of brown to dark brown predominantly flattened cells; inner layer 2–15 µm thick, composed of 3–6 cell layers of elongate to flattened hyaline cells, layers thickest at apex. Ascomatal apex with periphyses. Paraphyses sparse, septate, 4–5 µm wide, as long as the asci. Asci unitunicate, persistent, cylindrical, 215–270 × 11–14 µm; spore-bearing part 70–135 µm long, long-stipitate, stipe 80–120 µm long, tapering to 4 µm wide near base, apex rounded, with non-amyloid, light-refractive ring 2 × 3–4 µm, without sub-apical globule; with 8 multiseriate ascospores. Ascospores scolecosporous, straight to gently curved, 62–88 × 4.5–6 µm, with rounded ends, (7–)11(–13)-septate, septa often unequally spaced, not constricted at septa, remaining hyaline, smooth, with gelatinous sheath. Ascospores dextrinoid. Ascospores stain blue in aniline blue, except for apex and base which remain hyaline; ascomatal centrum remains hyaline.

ETYMOLOGY: Silken, referring to the surface of the fresh ascomata.

HABITAT: Decorticated, well-decayed wood, in mixed native forest including three *Nothofagus* species: *N. menziesii* (silver beech), *N. solandri* (black beech), *N. fusca* (red beech). Co-occurring on substrate with *C. metallicans* and a *C. raciborskii*-like species.

ANAMORPH: Unknown.

KNOWN DISTRIBUTION: Ohakune, central North Island, New Zealand.

Chaetosphaeria metallicans T.J.Atk., A.N.Mill. & Huhndorf, sp. nov. Fig. 19–28

Ascomata nigra, glabra, subglobosa usque late ellipsoida, 0.3–0.5 mm diametro, 0.5–0.75 mm alta, superficialia, gregaria, non-papillata usque ad papillata. In statu vivo, nitida, metallicans. In statu sicco ellipsoida, tristia, atroschistacea, coriacea, sporis accumulatus ex ostiolo nigro. Paries ascomatis superficialis textura globosa, in sectione longitudinali (40–)55–90(–130) µm crassus, striatus indistinctus. Asci unitunicati, 160–180 × 10–15 µm, octospori, biseriati, truncati, annulo apice refractivo, non amyloideo, sine globulo sub-apice. Ascosporae rectae usque allantoidae, 20–27 × (4.5–)6(–7) µm, tandem 3-septatae, hyalinae, laeves, guttulatae, vagina gelatinosa.

HOLOTYPE: New Zealand, South Island, Nelson, Karamea, Oparara Basin, near carpark for Honeycomb Caves, on decorticated, very decayed, 2.5 cm fragment of unknown substrate, probably beech (*Nothofagus*), *T. Atkinson TJA736*, 16 May 2004, PDD 92539.

DESCRIPTION: Ascomata dark grey to black, glabrous, subglobose to broadly ovoid, 0.3–0.5 mm diam., 0.5–0.75 mm high, superficial, gregarious, non-papillate or slightly papillate. Surface of fresh ascomata shiny, smooth, metallic grey to black. With age becoming ellipsoidal, dull, dark grey, coriaceous, usually with a darker ostiole. Ascomatal wall of textura angularis in surface view, very dark brown to olive brown; in longitudinal section (40–)55–90(–130) µm thick (Karamea collection 70–90(–130) µm, Ohakune collection (40–)55–75 µm), indistinctly layered; composed of 8–15 cell layers of dark brown pseudoparenchymatous cells; inner 3–5 cell layers of more elongate cells. Ascomatal surface covered with a layer of yellow-green crystalline pruina around 2–10 µm in width. Ascomatal apex with periphyses, ostiole around 30 µm diam. Paraphyses common, filliform, 1–2 µm wide, as long as the asci, hyaline. Asci unitunicate, persistent, cylindrical, 160–180 × 10–15 µm, short stipitate, stipe <50 µm in length; apex truncate, with non-amyloid, light-refractive ring 2 × 4 µm, without sub-apical globule; with 8 more-or-less biseriate ascospores. Ascospores straight to allantoid, 20–27 × (4.5–)6(–7) µm*, subacute at each end, becoming 3-septate, sometimes slightly constricted at median septum, remaining hyaline, smooth, filled with 1 µm diam. guttules, gelatinous sheath.

ETYMOLOGY: Metallic, referring to the shiny surface of the fresh ascomata.

HABITAT: Decorticated, well-decayed wood, under a *Nothofagus menziesii* (silver beech) tree, in mixed native *Nothofagus*, podocarp, broadleaf forest.

ANAMORPH: Unknown.

KNOWN DISTRIBUTION: North and South Islands, New Zealand.

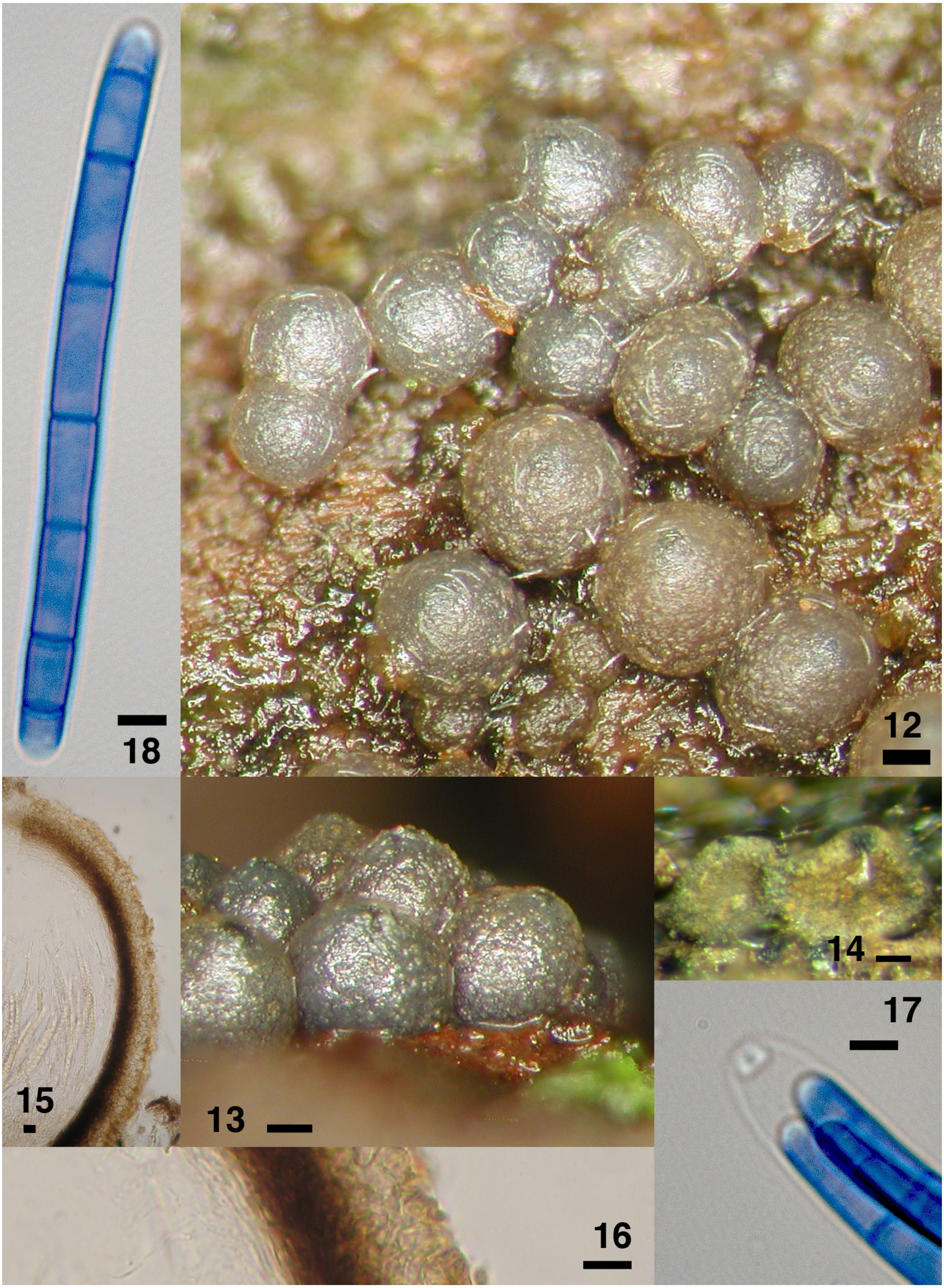
*The original Oparara Basin collection (TJA736) has ascospores which are startlingly uniform in length – no variation was observed from 24 µm among 30 ascospores measured, and many others examined by eye for obvious differences (see Table 1).

ADDITIONAL SPECIMENS EXAMINED: NORTH ISLAND: AUCKLAND: Waitakere Ranges, Karekare/Piha road, Huia Dam Track, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA872*, 17 May 2003, PDD 92552; Hunua Ranges, Mangatangi Dam, Workman Track, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA473*, 19 May 2003, PDD 92553; Workman Track, on decorticated, well-decayed wood of unknown substrate, *T Atkinson TJA474*, 19 May 2003, PDD 92554; Workman Track, on decorticated, well-decayed wood of unknown substrate, *T Atkinson TJA896*, 19 May 2003, PDD 92555. WELLINGTON: Ohakune, Jubilee Park, on decorticated, well-decayed wood of unknown substrate, probably beech (*Nothofagus* sp.), *T. Atkinson TJA836*, 8 Apr 2005, PDD 92557. SOUTH ISLAND: ?Stewart Island or Westland (Haast), on decorticated, well-decayed wood of unknown substrate, ?*S. Whitton TJA949*, May 2002, PDD 92551. NELSON: Karamea, Oparara Basin, near Mirror Tarn, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA734*, 16 May 2004, PDD 92556; Oparara Basin, near Honeycomb Cave/Box Canyon carpark, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA967*, 11 May 2006, PDD 92558; near Honeycomb Cave/Box Canyon carpark, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA961*, 12 May 2006, PDD 92560; Oparara Basin, Moria Gate Track, on decorticated, well-decayed wood of unknown substrate, *T. Atkinson TJA971*, 11 May 2006, PDD 92559.

MORPHOLOGICAL RELATIONSHIPS

The Karamea and Ohakune collections of all three taxa are compared in Table 1. Two of the taxa, *C. albida* and *C. bombycina*, have light coloured ascomata and scolecosporous, hyaline ascospores (Fig. 1–18). Although similar to each other, *C. albida* (Fig. 1, 2) has whitish, sometimes aereolate, translucent, distinctly papillate ascomata, while *C. bombycina* (Fig. 12, 13) has pearly, fawn-grey, distinctly globose ascomata. In thin section *C. albida* has a more melanised outer wall (Fig. 4, 5, 15, 16).

Fig. 12–18 *Chaetosphaeria bombycina* (*TJA837*). **Fig. 12**, Fresh ascomata. Note long spores/asci visible on ascomatal surface. Photograph: Jerry Cooper (Landcare Research). **Fig. 13**, Fresh ascomata (side view). Photograph: Jerry Cooper. **Fig. 14**, Air-dried, collapsed ascomata. **Fig. 15**, 30 µm freezing microtome longitudinal section of ascoma; section broken at ostiole. **Fig. 16**, Peridium, 30–50 µm thick, 3-layered, from 30 µm longitudinal section. **Fig. 17**, Light-refractive apical ring of ascus (aniline blue). **Fig. 18**, Ascospore, (47–)60–80 × 5–7 µm (aniline blue). Note ends do not stain. Scale bars: 12–14 = 0.1 mm; 15, 16 = 10 µm; 17, 18 = 5 µm. ➤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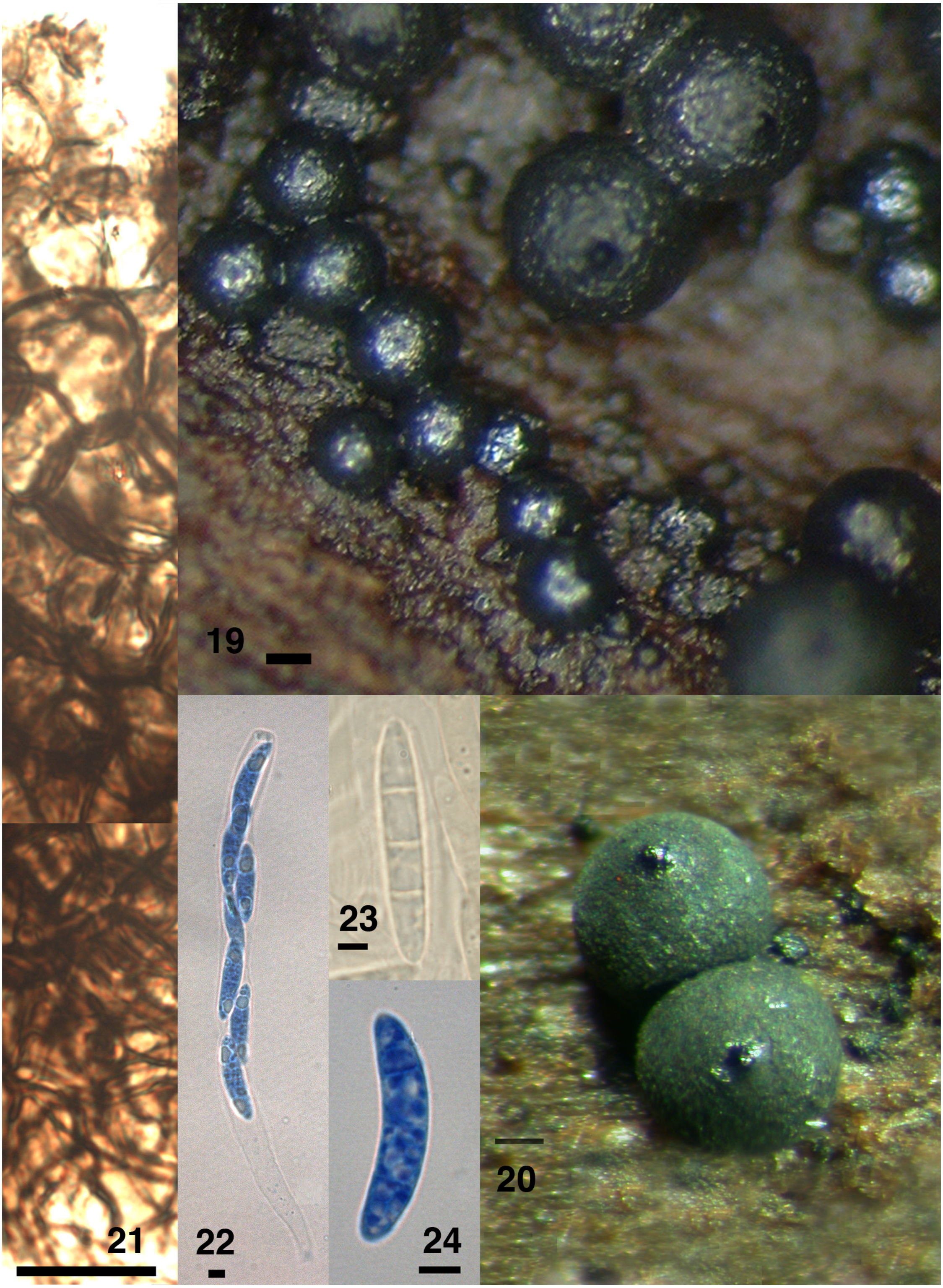
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Table 1 Comparison of *C. albida* (Karamea) and *C. bombycina* (Ohakune), and of two collections of *C. metallicans* (Karamea and Ohakune). Major differences between the members of each pair are in bold.

Collection		PDD 92537 Karamea	PDD 92538 Ohakune	PDD 92539 Karamea	PDD 92557 Ohakune
Species		*C. albida*	*C. bombycina*	*C. metallicans*	*C. metallicans*
Ascomal appearance	fresh	**almost white, translucent, aereolate,** (some other collections darker and not as aereolate), **distinctly papillate**	**light fawn-grey, not aereolate, not papillate**	black, shining like silver	not observed
	air-dried	**very light ginger-brown, darker around ostiole, scurfy, scaly, or woolly, many collapsed laterally**	**grey, or yellow grey, slightly darker around ostiole, scurfy, collapsed vertically and/or laterally**	dark grey, dull, not papillate	mid-grey, slightly glossy, slightly papillate
	re-wet	**very light ginger-brown**	**mid-dark grey, dull**	black	black
Ostiole	fresh/air dried/re-wet	**black, obvious**	**grey, barely visible**	black, shiny	black, shiny
Ascomal size (mm)	fresh	**0.3–0.4(–0.5) diam., 0.5–0.75 high**	**c. 0.3–0.4(–0.5) diam., c. 0.5–0.6 high**	0.3–0.5 diam., 0.5–0.75 high	0.3–0.5 diam., 0.5–0.75 high
	air-dried	**0.25–0.3 diam., (0.25–)0.5–0.7 high**	**0.25–0.4(–0.5) diam., 0.25–0.4 (–0.5) high**	similar to fresh	similar to fresh
Peridium	thickness	**50–75 µm**	**30–50 µm**	**70–90 (–130) µm**	**(40–) 55–75 µm**
	no. of layers	**4**	**3**	indistinct, ?2 layers	indistinct, ?2 layers
	features	**middle and outer layers strongly melanised**	**only middle layer strongly melanised**	large cells, heavily melanised, yellow-green crystalline pruina on outer surface	cells melanised, yellow-green crystalline pruina on outer surface
Ascospore	size (µm)	(47–)60–80 × 5–7	62–88 × 4.5–6	24 × (5–)6(–7) no variabilty in length	20–27 × 4.5–5.5 more variable in length
	septations	(5–)7(–12)	(7–)11(–13)	0–3	0–3
ITS sequences		identical to each other		one base pair different from each other (A/G)	

◄ **Fig. 19–24** *Chaetosphaeria metallicans*. **Fig. 19**, Fresh ascomata (*TJA736*). Note shiny, metallic appearance, particularly of young ascomata. **Fig. 20**, Fresh ascomata with surface dried under microscope light, except for damp ostioles; ascomata dull but still somewhat metallic (*TJA961*). **Fig. 21**, Peridium, (40–)55–100(–130) µm thick, 2- or 3-layered, from 30 µm freezing microtome section (*TJA736*). **Fig. 22**, Ascus, 160–180 × 10–15 µm (aniline blue) (*TJA736*). **Fig. 23**, Ascospore showing septations (*TJA736*). **Fig. 24**, Ascospore, 24 × (5–)6(–7) µm (aniline blue) (*TJA736*). Scale bars: 19, 20 = 0.1 mm: 21–24 = 5 µm.

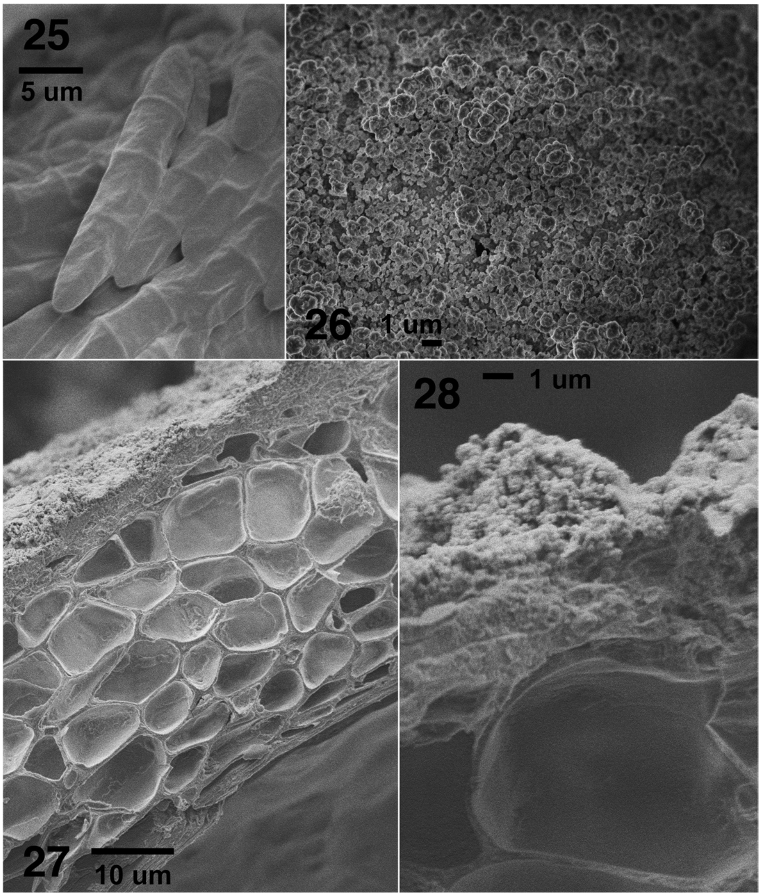

Fig. 25–28 Scanning electron micrographs of *Chaetosphaeria metallicans* (*TJA736*). **Fig. 25**, Ascospores with 3 septa clearly visible. **Fig. 26**, Outer ascomatal surface showing covering of non-cellular, crystalline pruina. **Fig. 27**, Section through peridium showing covering of pruina. **Fig. 28**, Outer cells of peridium showing covering of pruina. Scale bars: 25 = 5 µm; 26, 28 = 1 µm; 27 = 10 µm.

Its ascomata air-dry to a very light ginger-brown, retaining the distinct black ostiole (Fig. 3), while the smaller ascomata of the pearly-grey *C. bombycina* dry to dull grey or yellow-grey, with an indistinct grey ostiole (Fig. 14). *C. bombycina* so far remains unique to Ohakune. Further collection and examination of previously collected specimens revealed that *C. albida* is not uncommon throughout New Zealand and seems to exhibit variable ascomatal colour between and within collections. The fresh ascomata of collections made in 2006 from the Oparara Basin were less aereolate and more pearly, varying in colour from light ginger-brown (Fig. 9) to light grey, sometimes within the same collection. Upon air-drying, some specimens are whitish, while others, such as *TJA813* from Codfish Island, are distinctly ginger-brown with prominent black ostioles. A collection morphologically inseparable from the latter was made in the Waitakere Ranges, West Auckland, by S. J. Hughes in 1963 (PDD 39504). The third taxon described here, *C. metallicans*, was collected from both Karamea and Ohakune, and has shiny, black, metallic ascomata and short, 3-septate, hyaline ascospores (Fig. 19–28). The main difference among collections of this species is in the thickness of the peridium. It has also been found in several other New Zealand localities.

Ascus apex and ascospore morphology

All three new taxa described here possess light-refractive apical rings and lack sub-apical globules (Fig. 6, 17, 22). The 3-septate ascospores of *C. metallicans* are of the kind that are considered typical of *Chaetosphaeria* (hyaline, with 1–3 transverse septa and "narrowly to broadly ellipsoidal with narrow or broadly rounded ends". Réblová et al. 1999, p. 61) (Fig. 23, 24). Scolecosporous taxa were placed in *Chaetosphaeria* based on LSU, β-tubulin, and ITS sampling (Miller & Huhndorf 2004a; Huhndorf & Fernández 2005). The ascospores of *C. albida* and

C. bombycina are of this type (Fig. 8, 18). Morphological comparison of all known scolecosporous taxa within *Chaetosphaeria* is summarised in Table 2.

Peridium morphology

The peridia of *C. albida* and *C. bombycina* are quite unlike any previously known *Chaetosphaeria* taxa, both in general appearance and in thin section. The whitish *C. albida* has a 4-layered peridium with an outer melanised layer (Fig. 4, 5), while that of the pearly grey *C. bombycina* is more convincingly 3-layered, with the outer layer of melanisation lacking or only weakly present (Fig. 15, 16).

Much information would have been lost about the three new taxa if fresh ascomata had not been not observed, described, and photographed prior to drying. When fresh, the peridia of all three taxa have unusual optical effects. *C. albida* appears translucent, almost transparent (Fig. 1), despite its outer melanised peridium. Many of these ascomata are delicately tinged with light brown. The aereolations or white "scales" which are apparent under the dissecting microscope are not visible in thin-section or squash mount. These ascomata can also reflect light (Fig. 2). This white, aereolate form has also been collected from Haast and south-east Auckland. However, topotypic collections from 2006 from the Oparara Basin have optical qualities more similar to *C. bombycina* from Ohakune. While still distinctly papillate, their colour ranges from whitish to golden brown between and within collections (Fig. 9). Wax embedded, thin sections of one of these later collections show a golden lustre in the melanised walls of the outer cells (Fig. 10, 11).

Examination of the dried specimen (PDD 39504) collected by S. J. Hughes and identified as *Lasiosphaeria depilata* by Rossman (1977) suggests that it is more correctly considered *C. albida*. Rossman would not have seen this collection in its fresh state; when fresh it probably looked very similar to the collection shown in Fig. 9. Dried, the ginger-brown ascomata fit within Fuckel's (1873) original circumscription of the ascomata of *L. depilata* as "fusco-nigris", and the ascospore size and number of septations are in agreement also. Rossman (1977, p. 374) described *L. depilata* as having a "leathery" texture, and being covered with "loose, globose cells". The dried ascomata of *C. albida*, including the specimen Rossman examined, appear to be covered by loose, globose cells, described as a scurfy, scaly, or woolly texture herein. However, in squash mount or thin section the outer layers of these specimens are fine textura epidermoidea which somehow shrivels to give a misleading appearance.

The ascomata of *C. bombycina* reflect light (Fig. 12, 13); although lacking an outer melanised

Table 2 Scolecosporous taxa within *Chaetosphaeria.* Those marked ** are included in our LSU tree (or for *C. bombycina*, our ITS tree), and fall within Révlová's group 4, genetically. Ascospores are hyaline unless otherwise stated.

Species	Ascospore size (µm) length	Ascospore size (µm) width	Septations at maturity	Reference
***C. albida* sp. nov.**	(47–)60–80	5–7	7	this paper
***C. capitata* Sivan. & H.S.Chang**	48–100	3–5	7–10	Fernández & Huhndorf 2005
	subhyaline to light brown			
***C. bombycina* sp. nov.**	62–88	4.5–6	(7–)11(–13)	this paper
C. ellisii (Barr) Huhndorf & F.A.Fernández	(40–)50–75 (–80)	3–4.5	7	Huhndorf & Fernández 2005
C. lapaziana (Carroll & Munk) F.A.Fernández & Huhndorf	40–50 or (45–)50–100 (–120)	5–6 or (3–)4.5–6(–7)	7	Carroll & Munk 1964; Fernández & Huhndorf 2005; Huhndorf & Fernández 2005
C. panamensis Huhndorf & F.A.Fernández	65–75	3–4	7	Huhndorf & Fernández 2005
***C. raciborskii* (Penz. & Sacc.) F.A.Fernández & Huhndorf**	(50–)60–100 (–150)	3–3.75(–4.5)	7	Huhndorf & Fernández 2005
C. rubicunda Huhndorf & F.A.Fernández	80–100	3.5–4.2	7	Huhndorf & Fernández 2005
***C. spinosa* F.A.Fernández & Huhndorf**	68–78	2–3 filiform	non-septate	Fernández & Huhndorf 2005

layer they are darker than those of *C. albida*. It too has outer cells approximating textura epidermoidea in squash mount, and while the ascomata of *C. albida* have a woolly appearance when dry (Fig. 3), the smaller dried ascomata of *C. bombycina* are dull grey and camouflaged on the substrate almost to the point of being invisible (Fig. 14).

When freshly collected and wet the metallic-looking ascomata of *C. metallicans* can appear gelatinous and shine like silver (Fig. 19). In squash mount and thin-section, yellow-green crystalline pruina are apparent on the outer peridial surface. Under scanning electron microscopy the surface is distinctively patterned with a lumpy granular appearance (Fig. 26). Peridial cross-section under SEM clearly shows an outer layer of pruina that is 2–10 μm wide (Fig. 27, 28). In its fresh state this layer is probably responsible for the metallic lustre of the ascomata. The fresh ascomata dry quickly, in air or under a dissecting microscope light, to a dull dark-grey, but retain a metallic appearance, usually with a darker ostiole (Fig. 20).

Two other pruina-covered taxa are present in the genus. The ascomata of *Chaetosphaeria capitata* have crystalline yellow pruina on their surface and particularly the apices of the ascomatal setae (Fernández & Huhndorf 2005), seen in both the fresh and dried state. The *C. raciborskii*-type ascomata of *C. rubicunda* have unprecedented "red surface crystals not dissolving in water, 3% KOH or lactophenol", making the fresh specimens bright reddish purple in colour except for the black ostiole (Huhndorf & Fernández 2005).

There are other differences between typical *Chaetosphaeria* ascomata and those of the new taxa. The peridia of all the taxa described here are considerably thicker than the 15–25 μm which has been thought characteristic of the genus (Gams & Holubová-Jechová 1976; Réblová et al. 1999; Réblová 2000). Fernández & Huhndorf (2005) constructed a specific epithet, *crassiparies*, for their new *Tainosphaeria* species (Chaetosphaeriaceae) on the basis of its "relatively thick ascomal wall" of 22–33 μm. The melanised peridium of *C. metallicans* (Fig. 21) is twice or three times this thickness when similarly measured in thin section: (40–)55–90 (–130) μm. Even the delicate ascomatal walls of the light coloured *C. albida* and *C. bombycina* are between 30 and 75 μm in thickness. The other scolecosporous *Chaetosphaeria* taxa have ascomatal walls in this range, generally 40–100 μm in thickness (Huhndorf & Fernández 2005).

The ascomata of all three new species are larger in size than is typical of *Chaetosphaeria* taxa; usually c. 0.1–0.3 mm diam. (Fernández & Huhndorf 2005). Those of *C. metallicans* and *C. albida* are up to 0.5 mm diam. and 0.75 mm high. Those of *C. bombycina* are slightly smaller. However, none is as large as those of the "very large" *C. lapaziana*, previously placed in *Lasiosphaeria*, which are (0.4–) 0.5–0.95 mm in diameter (Huhndorf & Fernández 2005).

Phylogenetic analysis

Amplification of the LSU region following cloning produced sequences that were approximately 900 base pairs in length. Final alignment of the LSU dataset included 79 sequences, representing 53 taxa. Amplification of the ITS region produced sequences which were approximately 550 base pairs in length. Initial alignment of the ITS dataset included 37 taxa (data not shown), similar in taxonomic diversity to the LSU dataset. Since the variability of the ITS region makes accurate alignment of this wide a range of taxa extremely difficult, it was decided to focus only on the clade which included Réblová's (2000) group 4 taxa and our new species. Final alignment of the ITS dataset, therefore, included 14 sequences, representing 9 taxa, one of which (*C. raciborskii*) was found to be polyphyletic. Parsimony analysis of the LSU dataset which contained 287 parsimony informative characters, generated 16 equally parsimonious trees. A strict consensus tree is shown in Fig. 29. Maximum likelihood analysis generated one tree with high bootstrap support on most branches (Fig. 30).

The LSU phylogeny shows that *C. albida* and *C. metallicans* both fall within *Chaetosphaeria*, and more specifically within Réblová's (2000) group 4 (or group 2B following Réblová & Winka 2000); this group has 83% bootstrap support. The LSU dataset also indicates that *C. albida* and *C. metallicans* fall within a subclade of group 4, although the separation of the two subclades has low bootstrap support. The subclade comprises *C. raciborskii*, *C. chalaroides*, *C. fennica*, *C. fusiformis*, *C. cholorotunicata*, *C. capitata*, *C. spinosa*, *C. lignomollis*, and *C. caesariata*. Réblová (2000) placed *C. chalaroides* within group 3, presumably on the basis of its anamorph, but these analyses suggest that this species is within group 4.

Bootstrap analysis of the LSU dataset shows a high level of support for the sister relationship between *C. albida* and *C. raciborskii*, but the placement

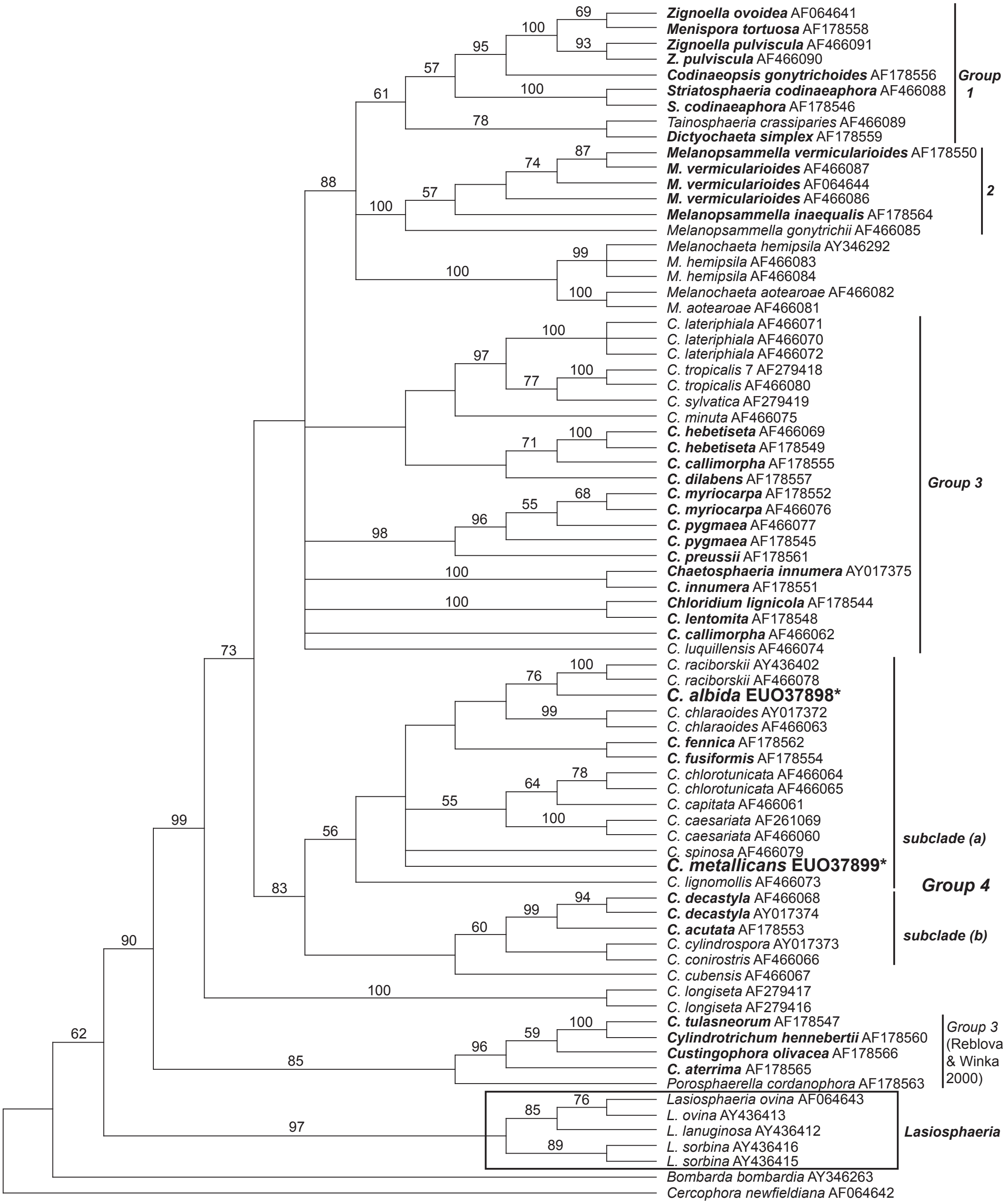

Fig. 29 Strict consensus of 16 equally parsimonious trees from analysis of the LSU dataset. Tree length = 1269, consistency index = 0.344, retention index = 0.732. Bootstrap values (%) are shown above branches. Réblová's (2000) four groups are indicated, and also a fifth group (called group 3) following Réblová & Winka (2000). Taxa with accession numbers marked * are new to Genbank. Taxa in bold within each group are those identified by Réblová as belonging to that group. Sequences of *C. albida* (PDD 92537) and *C. metallicans* (PDD 92539) are from types collected at Karamea.

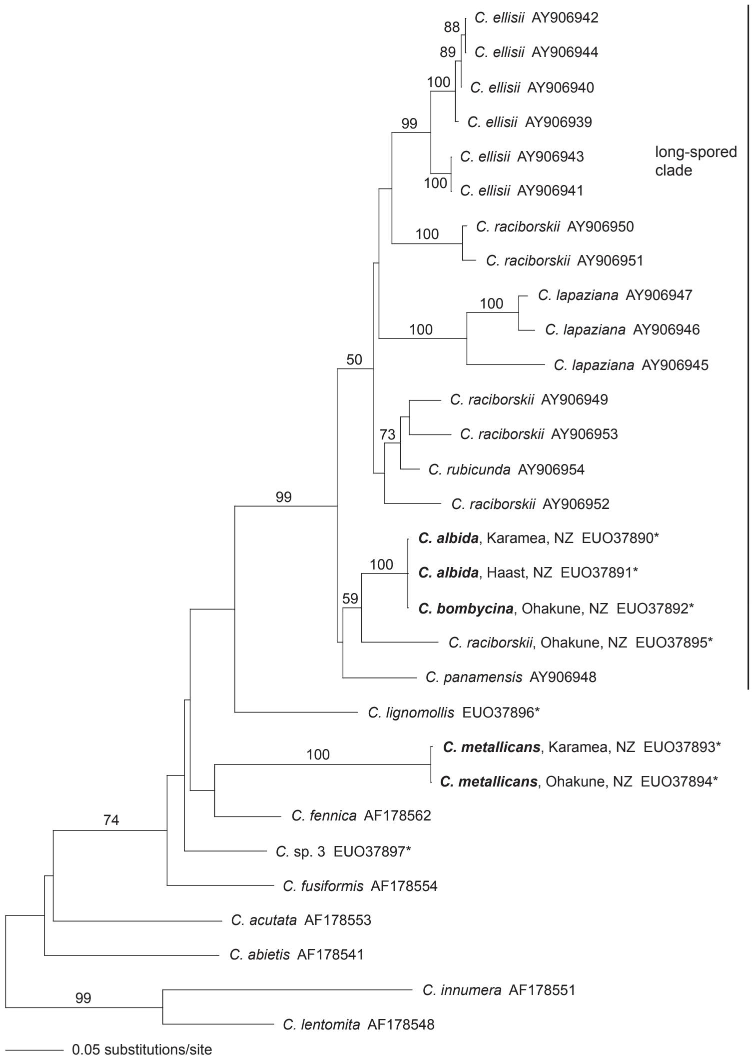

Fig. 30 Phylogram of the maximum likelihood tree generated with the best-fit model from analysis of a subset of the ITS dataset: taxa which group with Reblova's (2000) group 4. Outgroup taxa are from Reblova's group 3. The likelihood score of the best tree found = 4267.83. Bootstrap values (%) are shown above branches. Taxa with accession numbers marked * are new to Genbank. Note that the 7 scolecosporous taxa, several of which are polyphyletic, occur together in a clade with 99% bootstrap support.

of *C. metallicans* within this group is unresolved. The clade of tomentose *Lasiosphaeria* species is well supported and is independent of the Chaetosphaeriaceae, occurring next to the polyphyletic outgroup comprised of two other members of the Lasiosphaeriaceae.

The relationships within group 4 shown by the ITS tree are similar to those suggested by the LSU analysis. The maximum likelihood tree of the group 4 subset of the ITS dataset shows that all scolecosporous taxa known to date within *Chaetosphaeria* occur within group 4 and group together with 99% bootstrap support (Fig. 30). *C. albida* and *C. bombycina* appear to be more closely related to a New Zealand collection identified as *C. raciborskii* on the basis of its morphology, than this collection is to those identified as *C. raciborskii* from other parts of the world.

DISCUSSION

Although *C. albida* and *C. bombycina* are morphologically very different (Fig. 1, 12), they share identical ITS sequences. It would be interesting to compare the intergenic spacer (IGS) region of these taxa as this is thought to contain the greatest amount of sequence variation within the nuclear ribosomal DNA (Vilgalys 2007). These taxa may differ only in their expression of genes. It is considered, however, that their significantly different morphology merits independent nomenclatural recognition. Morphologically distinguishable taxa which share identical ITS sequences are found within other ascomycete groups such as the pathogenic pyrenomycete genus *Mycosphaerella*, for which it is necessary to sequence several loci to obtain species resolution (Crous et al. 2004; Hunter et al. 2006). Similarly, the Xylariaceae includes morphologically distinguishable pyrenomycetes with highly similar ITS (M. Stadler pers. comm., 2007).

Conversely, the Oparara and Ohakune collections of *C. metallicans* are one base pair different (A/G) from each other in their ITS region, but so morphologically similar that they are considered a single species. Although the Oparara collection is less papillate than is typical, the main morphological difference between these collections is the thickness of the peridium. In their study of *C. raciborskii* and its scolecosporous sisters, Huhndorf & Fernández (2005, p. 17) found considerable variation in wall thickness between species, and "similar variation in thickness among collections of the same species."

These results confirm that the affinities of some scolecosporous taxa have been missed in the absence of genetic sampling. The first indication was given by the transfer of *C. raciborskii* to *Chaetosphaeria* (Miller & Huhndorf 2004a), which considerably widened the concept of the genus in terms of ascospore shape and ascomal wall structure, and preluded the placement of further scolecosporous taxa within *Chaetosphaeria* (Huhndorf & Fernández 2005; Fernández & Huhndorf 2005). The addition of the scolecosporous *C. albida* and *C. bombycina* and the short-spored *C. metallicans*, with their contrasting ascomatal walls, creates more convoluted puzzles for those seeking correlations between morphology and genetics.

In general, the phylogenetic relationships shown by our results closely match those of Réblová (2000), Réblová & Winka (2000), Miller & Huhndorf (2004a), Huhndorf & Fernández (2005), and Fernández et al. (2006). Réblová (2000) redefined *Chaetosphaeria* sens. str. and divided it into four "natural groups", based on morphological, cultural, and molecular studies. Genetically, *C. albida*, *C. bombycina*, and *C. metallicans* all fall into Réblová's group 4 (later 2B), the *Kylindria*-group. The anamorph genera Réblová (2000) included within group 4 are: *Cylindrotrichum* Bonord. pro parte; *Kylindria* DiCosmo, Berch & W.B.Kendr.; *Xenokylindria* DiCosmo, Berch & W.B.Kendr.; and *Chloridium* Link section *Chloridium* pro parte. In contrast, known anamorphs for the scolecosporous clade within group 4 appear to be consistently *Craspedodidymum*-like, with the occasional *Chloridium* synanamorph (Huhndorf & Fernández 2005). Unfortunately, anamorphs are unknown for the new species described here.

Knowledge of the anamorph currently helps distinguish those *Chaetosphaeria* species with morphologically similar or indistinguishable teleomorphs (Réblová et al. 1999; Réblová 2004), although in the opinion of Fernández et al. (2006) the large number of morphospecies within some anamorph taxa such as *Dictyochaeta* Speg. poses "enormous challenges" to teleomorph delimitation. Anamorph and teleomorph are only sometimes collected together. Culturing, particularly of scolecosporous *Chaetosphaeria* taxa, appears difficult, and sometimes results in "altered or aberrant" anamorph morphologies (Fernández et al. (2006:121). As the opportunity to culture recurs with every fresh collection, in time these gaps may be filled. The frequency of synanamorphs within the genus (Réblová 1999a) makes genetic confirmation advisable.

Ascospore morphology

In this study ITS sequences from scolecosporous *C. raciborskii*-type taxa (*C. ellisii*, *C. lapaziana*, *C. panamaensis*, *C. raciborskii*, *C. rubicunda*) (Huhndorf & Fernández 2005) were compared with the new species *C. albida*, *C. bombycina*, and *C. metallicans* and other short-spored *Chaetosphaeria* taxa (full ITS data not shown). It is clear from both LSU and ITS analyses that all scolecosporous taxa known to date within *Chaetosphaeria* occur in a unique clade within Réblová's (2000) group 4. Our ITS phylogeny of group 4 shows that this scolecosporous clade has 99% bootstrap support (Fig. 30).

Réblová (2000, p. 156) characterised group 4 teleomorphs as having "long-fusiform to cylindrical, 3–6 septate, hyaline, non-fragmenting ascospores, generally up to 46 μm long". However, the scolecosporous taxa have ascospores which are all somewhere between 50 and 100 μm in length. Furthermore, our LSU phylogeny shows that in addition to the scolecosporous taxa, two taxa within group 4 possess versicoloured ascospores (middle cells brown, end cells hyaline); *C. chlorotunicata* and *C. caesariata*. As these taxa all fall within subclade (a) of group 4 (Fig. 29) ascospore characteristics within this subclade are intriguingly heterogenous (Table 3).

In their circumscription of the Chaetosphaeriaceae, Réblová et al. (1999) included seven genera: *Ascocodinaea* Samuels, Cand. & Magni; *Chaeto-*

sphaeria Tul. & C.Tul.; *Melanochaeta* E.Müll., Harr & Sulmont; *Melanopsammella* Höhn.; *Porosphaerella* E.Müll. & Samuels; *Porosphaerellopsis* Samuels & E.Müll.; and *Striatosphaeria* Samuels & E.Müll. Recently, an eighth genus, *Tainosphaeria* F.A.Fernández & Huhndorf was created within the family (Fernández & Huhndorf 2005). These genera have been delineated on the basis of differing ascospore morphology and/or anamorph type. *Striatosphaeria* taxa have 1-septate, ridged ascospores with a germ pore in the median septum (Réblová et al. 1999; Samuels & Müller 1978), and the sole *Tainosphaeria* taxon has fusiform, hyaline, mostly 3-septate ascospores (Fernández & Huhndorf 2005). Both of these genera are in a clade with members of Réblová's group 1. *Melanopsammella* taxa have had a convoluted taxonomic history as a result of their 1-septate ascospores which disarticulate into part-spores (Réblová et al. 1999), as do the ascospores of *C. dilabens* and *C. preussii* (within group 3) (Réblová 2000). Both *Melanopsammella inaequalis* and *M. vermicularioides* were transferred from other taxa to *Chaetosphaeria* (Gams & Holublová-Jechová 1976). Réblová et al. (1999) reinstated *Melanopsammella*, reviving Höhnel's (1920) genus for *M. inaequalis*, and transferring *vermicularioides* to it. However, Réblová (2000) and Réblová & Winka (2000) reverted to *Chaetosphaeria* for both species, based on genetic analysis, and identified them as within group 2 (or 1B) of *Chaetosphaeria*. The two *Melanochaeta* species (with versicoloured ascospores similar to *C. chlorotunicata* and *C. caesariata* in group 4), form a

Table 3 Ascospore types of taxa which group with Réblová's (2000) group 4 within *Chaetosphaeria* sens. str. Names in bold were included in group 4 by Réblová due to shared anamorph characteristics (*C. chalaroides* was placed in group 3). Ascospores hyaline unless otherwise stated.

Taxon	Sub-clade	Ascospore size (μm)	Septations at maturity	Notable features	Reference
C. abietis	?b	27–36.5(–41) × (2.5–)3–4	**3**		Réblová 2004
C. acutata	b	(28–)30.5–38(–44) × 3–4(–5)	**3**		Réblová 2004
C. albida sp. nov.	a	(47–)60–80 × 5–7	7	scolecosporous	this paper
C. bombycina sp. nov.	b	62–88 × 4.5–6	(7–)11(–13)	scolecosporous	this paper
C. caesariata	a	(38–)40–47.5(–55) × (6–)7–8.5	7	versicoloured	Réblová 1999b
C. capitata	a	75–100 × 3–4 48–100 × 3–5	7–10	scolecosporous, subhyaline to light brown	Réblová 2004 Fernández & Huhndorf 2005
C. chalaroides	a	9–17 × 3–4	1		Réblová 2004
C. chlorotunicata	a	27–62 × 6–9.5	7 (–9)	versicoloured	Fernández & Huhndorf 2005
C. conirostris	b	35.5–48.5 × 5.5–7.5	1 (–3)		Fernández & Huhndorf 2005
C. cubensis	b	12–18(–20) × 2.5–3.5	> 1		Réblová 2004
C. cylindrospora	b	25–32 × 4–5	6–7		Réblová 2004
C. decastyla	b	(28–)30–42(–46) × 3–4	**3–5**		Réblová 2004
C. fennica	a	(34.5–)36.5–42(–43) × (3.5–)4(–4.5)	**3**		Réblová 2004
C. fusiformis	a	(34.5–)39–53.5(–62) × 2.5–3(–4)	**3**		Réblová 2004
C. lignomollis	a	24–33 × 4.7–6	7		Fernández & Huhndorf 2005
C. metallicans sp. nov.	a	24 × (5–)6(–7)	3		this paper
C. raciborskii	a	(50–)60–100(–150) × 3–3.75(–4.5)	7	scolecosporous	Huhndorf & Fernández 2005
C. spinosa	a	68–76 × 2–3	0	scolecosporous, filiform	Fernández & Huhndorf 2005

sister clade to Réblová's (2000) groups 1 and 2. Both species were formerly placed in *Chaetosphaeria*.

All of these genera are represented in the LSU tree (Fig. 29) and most fall within a single clade that corresponds to the "sister lineage" of Fernández et al. (2006, p. 125), and groups 1A and 1B (*Melanochaeta* not sampled) of Réblová & Winka (2000). This group of taxa is consistently well supported but in this analysis their separation from *Chaetosphaeria* is not well resolved. From the present analysis it appears that these taxa which have been excluded from *Chaetosphaeria* on the basis of ascospore morphology and/or anamorph type might be considered to fall within the genus on genetic grounds. Whether it is taxonomically useful to retain unique generic names remains a subjective question. A period of informal accretion of species back into *Chaetosphaeria* may be necessary while genetic phylogenies develop, before the question can be adequately considered.

Ascocodinaea, *Porosphaerella*, and *Porosphaerellopsis* are maintained as distant from the Chaetosphaeriaceae as was determined by Réblová & Winka (2000) and Huhndorf et al. (2004), despite occurring together in a clade at the base of our LSU tree. These other analyses have clearly shown that while they have some morphologically similar characterisitics these taxa are not closely related to *Chaetosphaeria*.

Ascus apex morphology

Our observations concur with those of Réblová (2000) in that a plasmatic globule characteristic of the Lasiosphaeriaceae is never present in *Chaetosphaeria* taxa. In revising "subfamily Lasiosphaerioideae" and delineating *Lasiosphaeria ovina* as the type for the genus, Lundqvist (1972) cautiously stated that a "thickened apical ring" is "rarely lacking", and that a sub-apical globule is "usually" present in the subfamily. The latter has been clarified by genetic analysis; a few species (*L. sorbina*, for example), while clearly falling within *Lasiosphaeria* genetically, lack a sub-apical globule (Miller & Huhndorf 2004b). Thus, while its absence may occasionally be a source of confusion, its presence reliably indicates the Lasiosphaeriaceae.

Peridium morphology

The addition of the three new taxa described here to a core group within *Chaetosphaeria* challenges traditional assumptions about wall morphology within the genus. The exceedingly thick-walled *C. metallicans* necessitates expansion of the traditional view. However, the real challenge comes from *C. albida* and *C. bombycina* which group among the scolecosporous members of group 4. The founding member of this group, *C. raciborskii*, is known to be polyphyletic, and the inclusion of a New Zealand *C. raciborskii*-like specimen from Ohakune, increases this polyphyly. However, the *C. raciborskii*-type peridium, with its distinctive, globose outer cells (Huhndorf & Fernández 2005) and, usually, setae which originate from inner wall layers, is shared by all the taxa within the scolecosporous clade except for *C. albida* and *C. bombycina*. Prior to the inclusion of *C. albida* and *C. bombycina*, the *C. raciborskii*-type peridium appeared to be a synapomorphy correlated with the possession of scolecospores. Similarly, all other taxa within the scolecosporous clade and within the wider group 4 and, to our knowledge, all the taxa within the whole Chaetosphaeriaceae, have dark ascomata, except for surface vestiture in *Melanochaeta* (light-coloured), and *C. rubicunda* (red crystals). Thus, New Zealand's light-coloured *C. albida* and *C. bombycina* are currently unique on a world scale and have shown us that the possession of scolecospores does not indicate peridial type.

Peridial diversity seems to be characteristic of these taxa at familial, generic, specific, and subspecific levels. The diversity of fresh ascomatal appearance within *C. albida*, even collections from the same locality, suggests that specific environmental conditions may influence peridial development. That later topotypic collections of *C. albida* resemble *C. bombycina* in optical properties and are not distinctly aereolate is disconcerting. Whether *C. albida* and *C. bombycina* are best designated as a syntypic series rather than separate species, only further collection can reveal. At this time, their significantly different ascomatal shape and size is considered support for their separate status.

Collections of *C. albida* with the darkest ginger-brown ascomata, such as *TJA813* and PDD 39504, have not been sequenced; but it seems unlikely that they are significantly different genetically as a similar amount of variation has been observed within collections (*TJA956*), and even the very different ascomatal forms of *C. albida* and *C. bombycina* cannot be separated genetically.

CONCLUSION

Striking patterns of divergence and convergence characterise the morphology of *Chaetosphaeria* and its relatives. Ascospores, traditionally regarded as arbiters of relationship, seem to have become almost incidental at generic level. Ascomatal wall morphology, recently thought destined to help clarify the situation, appears equally variant in parts of the genus. Indeed, the variance shown by wall morphology, even between taxa which share the same or similar ITS sequences, suggests that walls conceal a particularly alluring evolutionary story.

Sequencing additional parts of the genome may give greater confidence to phylogenetic trees. However, Miller & Huhndorf (2004b) found only slight differences between trees produced independently from 4 different regions of the *Lasiosphaeria* genome (ITS, LSU, β-tubulin, and RPB2 genes). While the relationships shown by our ITS analyses reflect those of our LSU dataset, by being more variable they give greater resolution at a lower taxonomic level. Our analyses agree on the placement of *C. albida*, *C. bombycina*, and *C. metallicans* within a subclade of Réblová's (2000) group 4. Currently this subclade seems determined to drastically alter conceptions of *Chaetosphaeria* in several ways, particularly with the appearance of a large scolecosporous group as well as some light-coloured ascomata; no characters remain as morphological indicators of genetic relationship at the generic level. Detailed morphological understanding is not superseded by genetic research, but increases in importance when the results of the latter are morphologically convoluted phylogenies. In future, *Chaetosphaeria* sens. str. could be redefined to include only those taxa which group with the type, *C. innumera*, but Fernández et al. (2006, p. 129) advise against this at the present time, due to "the lack of a well-supported clade" surrounding it. The predominantly scolecosporous subclade may deserve a unique name, but the short-spored taxa within it cannot be separated from other *Chaetosphaeria* taxa on the basis of present morphological knowledge. Fernández et al. (2006) commented that some of the short-spored *Chaetosphaeria* taxa "hardly provide enough morphological variation to define a species, let alone to define a genus".

The traditional classification within *Chaetosphaeria* was based primarily on temperate, northern hemisphere taxa. With the exception of the North American *C. ellisii*, known taxa within the scolecosporous clade are tropical or from the Southern Hemisphere. It was initially intriguing that New Zealand is the only temperate collection site of *C. raciborskii*, generally considered "probably tropical worldwide" (Huhndorf & Fernández 2005). The recently discovered polyphyly of the tropical *C. raciborskii* caused speculation as to whether the New Zealand representative could be even more genetically divergent. This has proved to be the case, and the New Zealand *C. raciborskii* sequenced for the ITS analysis is more closely affined with *C. albida* and *C. bombycina* than to the Costa Rican sisters which share its name. When and if sequences were to become available, a similar comparison could be made between the *Melanochaeta aotearoae* sequences from Central American collections (used in this study) and the type of the species, which was collected in New Zealand (Hughes 1966). Other New Zealand *Chaetosphaeria* taxa (Hughes 1965; Hughes & Kendrick 1968; Réblová 2004) await sequencing and comparison with their relatives worldwide. *Chaetosphaeria albida*, *C. bombycina*, and *C. metallicans* may be endemic and indicative of the high level of microfungal endemism in New Zealand, but in the absence of wider Australasian data this cannot be concluded. The genetic variability within *C. metallicans* or the morphological variability within *C. albida* may indicate species complexes. Further collection on these relatively isolated islands seems likely to reveal complexes and additional sister species. Whether these help to convolute or explain the morphological picture remains to be seen.

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