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Chlorociboria (Fungi, Helotiales) in New Zealand

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Abstract Fifteen species of Chlorociboria are reported for New Zealand, including 13 new species and one new subspecies. All occur on decorticated wood and all are associated with blue-green staining of that wood. Some species are consistently associated with soft, rotten wood, while others are consistently associated with fallen wood that remains hard and appears more or less intact. The range of morphological variation accepted in the genus is broader than has previously been described, especially with respect to ascospore size and shape. Two of the species have filiform ascospores that coil on release from the asci. Species are distinguished on the basis of variation in ascospore size and shape, the presence or absence of either smooth-walled or rough-walled tomentum hyphae, and on macroscopic appearance. The validity of the generic and species concepts was tested using rDNA ITS sequences. Of species previously reported for New Zealand, only C. argentinensis is accepted.

Keywords fungi; discomycete; Helotiales; *Chlorociboria*; New Zealand; phylogeny; ITS

INTRODUCTION

Tunbridge Wells, a town in southern England, became famous during the nineteenth centry for finely inlaid woodware—Tunbridge Ware. Many different kinds of naturally coloured woods were used in the craft, including oak wood stained blue-green by

the discomycete Chlorociboria aeruginascens (= Chlorosplenium aeruginascens). The blue-green staining of the wood inhabited by this fungus makes Chlorociboria one of the most distinctive and easilv recognised discomvcetes on the forest floor. The genus has a worldwide distribution. At some sites in New Zealand, especially in Nothofagus forests at high altitudes, such blue-green stained wood can be very common. In other forest types, although these fungi are present, they are patchy in distribution and seen much less frequently. Chlorociboria species are associated with guite recently fallen wood with the bark still attached, decorticated but hard wood with few signs of decay, through to wood that has become soft and spongy, presumably as the result of earlier infection by white-rot basidiomycetes. Individual species appear to be consistently associated with wood at a particular state of decay. The blue-green staining associated with Chlorociboria may extend for several metres through a fallen log or branch.

Dixon (1974, 1975) monographed the genera Chlorosplenium, Chlorociboria, and Chlorencoelia. He discussed past confusion surrounding use of the name Chlorosplenium, which had been applied uncritically to a wide range of discomvcetes with apothecia with greenish pigmentation. He provided a key that included 13 other genera he considered had been confused with Chlorosplenium, Chlorociboria, or Chlorencoelia in the past. Features characteristic of Chlorociboria include stipitate, blue-green pigmented apothecia developing on wood stained blue-green, an ectal excipulum comprising textura intricata, textura angularis, or textura globosa, and cells of the ectal excipulum with gelatinised walls but not embedded in gel. The outer layers of the ectal excipulum are oriented at a high angle to the receptacle surface, this feature probably being the basis on which Nannfeldt (1932) and Seaver (1936) considered these fungi to be closely related to Ciboria.

Dixon (1975) accepted four species in *Chlorociboria*, with one divided into two geographically distinct subspecies. He reported *C. aeruginascens* as cosmopolitan in distribution, *C. aeruginosa* as

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widely distributed in temperate and tropical North and South America with a few collections in Asia and Russia, *C. aeruginascens* subsp. *brasiliensis* from tropical America, *C. omnivirens* from Tasmania and Japan, and *C. argentinensis* from Argentina. A species treated as *Claussenomyces salviicolor* by Dixon (1974) was transfered to *Chlorociboria* by Ouellette & Korf (1979). It is known from a few collections from temperate North America and Central America (Dixon 1974).

Chlorociboria species recorded from New Zealand have been *C. aeruginascens* (Dennis 1961, as *Chlorosplenium aeruginascens*; McKenzie et al. 2000, 2002), *C. aeruginosa* (Dennis 1958, 1961, as *Chlorosplenium aeruginosum*; McKenzie et al. 2000), *C. argentinensis* (Gamundí & Romero 1998; McKenzie et al. 2000), and *C. omnivirens* (Cooke 1879, as *Chlorosplenium omnivirens*; McKenzie et al. 2000). Of these species, only *C. argentinensis* is accepted for New Zealand in this paper.

Three other Chlorosplenium species have been reported from New Zealand, C. flavesens, C. rugipes, and C. chrysotrichum. Chlorosplenium flavovirens is an unpublished name given to New Zealand material by Massee (Dennis 1961). Dennis considered the Massee specimen to match the South American species Helotium magellanicum Dennis (a nom. nov. for Chlorosplenium fuegianum Speg.). However, Dixon (1974) considered the Massee specimen to represent an unpublished Cyathicula sp. Chlorosplenium rugipes (Peck) Korf (first reported for New Zealand by Dennis (1961)) is a synonym of Chlorencoelia torta (Schwein.) J.R.Dixon (Dixon 1974). Chlorosplenium chrysotrichum (Berk.) Dennis, based on a New Zealand type specimen, occurs throughout the country on Nothofagus. Its orange apothecia are erumpent through the bark of recently dead twigs, the excipular tissue is gelatinous, and the receptacle is ornamented with short, indistinct, hair-like elements with thin, roughened walls. The apothecia turn bright red in KOH, and reddish or yellowish pigments are released into KOH (specimens examined: PDD 1213, 19031, 20841, 26356). Dennis (1961) noted for this species that the "... systematic position still presents some difficulty". Dixon (1974) agreed, and although not accepting it as a good species of Chlorosplenium, did not provide a recombination. Verkley (2004) has recombined this species as Dicephalospora chrysotrichum (Berk.) Verkley.

The present paper reports 15 species of *Chlorociboria* for New Zealand, including 13 new species and 1 new subspecies. All occur on decorticated wood and all are associated with blue-green staining of that wood. The range of morphological variation accepted here in the genus is broader than has previously been described, especially with respect to ascospore size and shape. The validity of this generic concept was tested using rDNA ITS sequences.

The diversity of *Chlorociboria* in New Zealand suggests a possible Asian/Australasian centre of diversity for the genus. Despite being a widely collected and well-known group of fungi, there are only two species known from the Northern Hemisphere (Dixon 1974). The work of Gamundí & Romero (1998) suggests that it also lacks diversity in temperate South America. The discomycete biota of New Zealand shares many species with Australia, and it is likely that targeted collecting of this genus in Australia will also reveal a high species diversity.

METHODS

Macroscopic features were described from freshly collected specimens and microscopic features from dried material rehydrated in 3% KOH. Amyloid reactions were tested using Melzer's reagent after pre-treatment with 3% KOH. Apothecia were sectioned at about 10 µm thickness using a freezing microtome, and the sections were mounted in lactic acid. Colour codes in brackets are from Kornerup & Wanscher (1963). Cultures were obtained from some specimens using the methods of Johnston (1998). All cultures remained sterile, and brief descriptions of them are provided only when useful as a feature to distinguish species. Appearance in culture may vary widely when retreived from storage under liquid nitrogen, compared with the appearance of freshly isolated cultures. Each culture has been deposited in ICMP (International Collection of Microorganisms from Plants, Landcare Research, Auckland), the ICMP number being cited along with the PDD (New Zealand Fungal Herbarium) herbarium number of the collection from which they were derived.

For those collections from which DNA sequences were obtained, individual apothecia were excised from herbarium specimens or mycelium scraped from the surface of agar cultures and ground with liquid nitrogen in a mortar and pestle. Genomic DNA was isolated using the DNeasy Plant mini kit (Qiagen, USA); the final DNA solution was concentrated using Speed Vac SPC111V (ThermoSavant, USA). The ITS region was amplified using the primers ITS1 and ITS4 following the protocol of White et al. (1990) with FastStart Taq enzyme (Roche, USA) on a GeneAmp PCR system 9700 (Applied Biosystems, USA). PCR conditions were: 1 cycle at 95°C for 4 min; 40 cycles at 94°C for 1 min; 45°C for 1 min; 72°C for 1 min; and a final cycle at 72 °C for 7 min. Sequencing reactions were performed with ABI PRISM BigDye Terminator Ready Reaction Kit V 3.1 (Applied Biosystmes, USA) and run on an Applied Biosystems ABI prism 310 Genetic Analyzer. Sequencing reactions were run in both directions, and the raw sequence data were compared and edited using Sequencer v3.1.

ITS sequences were compared with representative isolates from the Sclerotiniaceae study of Holst-Jensen et al. (1997) together with a *Neofabraea* isolate from de Jong et al. (2001), a *Lachnum* isolate from Cantrell & Hanlin (1997), an isolate identified as *Hymenoscyphus fructigenus* (the type species of *Hymenoscyphus*) from Zhang & Zhuang (2004), and unpublished ITS sequences from New Zealand specimens of *Lanzia* and *Torrendiella*. Sequences were aligned using Clustal W (Thompson et al. 1994). The alignment has been deposited in TreeBase. Phylogenetic analyses were performed with PAUP* 4.0b10 (Swofford 2002) using a neighbor-joining analysis with Jukes-Cantor distances, and parsimony. Support for branches was tested using 1000 bootstrap replicates. Between sequence similarity was assessed using the PairDiff command in PAUP*. Genbank numbers for ITS sequences generated as part of this study are listed in Table 1.

Species	Voucher specimen or culture	GenBank accession number
Chlorociboria aeruginascens	PDD 77803	AY755358
Chlorociboria aeruginascens	PDD 77804	AY755359
Chlorociboria aeruginascens	PDD 76435	AY755349
Chlorociboria aeruginascens ssp. australis	PDD 74099	AY755350
Chlorociboria aeruginascens ssp. australis	PDD 74101	AY755351
Chlorociboria aeruginascens ssp. australis	PDD 81763	AY947345
Chlorociboria aeruginosa	PDD 81292	AY755360
Chlorociboria albohymenia	PDD 70089	AY755347
Chlorociboria argentinensis	PDD 77445	AY755337
Chlorociboria argentinensis	PDD 77447	AY755338
Chlorociboria awakinoana	PDD 74077	AY755339
Chlorociboria awakinoana	PDD 71672	AY755340
Chlorociboria campbellensis	PDD 74019	AY755357
Chlorociboria clavula	PDD 73914	AY755346
Chlorociboria duriligna	PDD 81278	AY755341
Chlorociboria halonata	PDD 71675	AY755354
Chlorociboria halonata	PDD 71610	AY755355
Chlorociboria halonata	PDD 73933	AY755356
Chlorociboria macrospora	PDD 73994	AY755343
Chlorociboria pardalota	PDD 71611	AY755353
Chlorociboria poutoensis	ICMP 15618 (culture ex PDD 60009	AY755352
Chlorociboria procera	PDD 74093	AY755345
Chlorociboria spathulata	PDD 77695	AY755342
Chlorociboria spathulata	PDD 70084	AY755344
Chlorociboria spiralis	PDD 77771	AY755348
Lanzia allantospora	ICMP 15649 (culture ex PDD 60137	AY755334
Lanzia griseliniae	ICMP 15650 (culture ex PDD 64240	AY755333
Torrendiella eucalypti	ICMP 15651 (culture ex PDD 77802	AY755335
Torrendiella madsenii	ICMP 15648 (culture ex PDD 58572	AY755336

 Table 1
 Genbank accession numbers for ITS sequences generated as part of this study.

TAXONOMIC TREATMENT

Chlorociboria Seaver ex C.S.Ramamurthi, Korf & L.R.Batra

Apothecia develop on decorticated wood, or erumpent through bark still attached to fallen branches, associated with blue-green staining of the substrate. Apothecia solitary or arising in groups from a basal stromatic mass. Apothecia short-stipitate to stipitate, glabrous or felted in appearance, receptacle sometimes with whitish bloom. Receptacle and hymenium of most species blue-green to dark blue-green when fresh, dark blue-green to black when dry, some species with whitish or yellowish hymenium, and sometimes with pale receptacle with scattered dark blue-green flecks. Base of stipe often darker than receptacle, sometimes coarsely roughened with appressed, scale-like elements. Medullary excipulum comprising usually non-gelatinous, hyaline textura intricata oriented more or less parallel to surface of the receptacle. Ectal excipulum comprising textura intricata or textura prismatica, oriented at a high angle to the surface of the receptacle, cell walls hyaline, thickened and gelatinous. Walls of the outermost lavers of ectal excipular cells often encrusted with dark green material. End cells of excipular elements may be free and hair-like, the walls of these cells being either roughened or smooth. These hair-like elements are referred to as the "tomentum hyphae", following Korf (1958) and Dixon (1975). In some species, non-gelatinous, appressed hyphae partially cover the surface of the receptacle. Paraphyses are typically undifferentiated at the apex and about the same length as the asci. Asci with thickened apex and amyloid pore, 8-spored. Ascospores variable in shape, occasionally surrounded by a gelatinous sheath, and sometimes budding to produce ascoconidia within the ascus.

NOTES: The genus as accepted here shows betweenspecies variation in the shape of the cells of the ectal excipulum, varying from long and thin to short and broad. When the cells are long and thin, the tissue of the ectal excipulum has the appearance of textura intricata; when they are short and broad it looks like textura prismatica to textura angularis. In all cases the cells of the ectal excipulum are oriented at a high angle to the surface of the receptacle, and have thickened, gelatinous walls. The gelatinisation is more obvious in those species with cells of a narrow diameter.

Each of the New Zealand *Chlorociboria* species can be distinguished on the basis of variation in ascospore size and shape, the presence or absence of the tomentum hyphae, which may be either smoothwalled or rough-walled, and macroscopic appearance. The ITS-based molecular comparison (see Molecular Results and Analyses below) confirmed the validity of the species morphological concepts, with narrow limits in relation to both ascospore size and shape, and macroscopic appearance of apothecia.

Chlorociboria omnivirens (Berk.) J.R.Dixon and C. aeruginosa (Oeder) Seaver ex C.S.Ramamurthi, Korf & L.R.Batra, previously reported from New Zealand, are not accepted as occurring in New Zealand. The fragmentary type specimen of C. omnivirens (Tasmania, on wood, Archer, K (M)110245) was examined. The apothecia appear to be quite large and robust with a well-developed stipe. The ascospores are larger than reported by Dixon (1975), $(18-)19-21(-25) \times (4.5-)5-5.5(-6) \mu m$ (mean 20.1) \times 5.2 µm, n = 27), cylindric, tapering slightly to broadly rounded ends, flattened on one side, sometimes slightly curved. When compared with the New Zealand species of Chlorociboria, the ascospore size and shape of C. omnivirens is most similar to C. macrospora (described below as new). However, C. macrospora has a well-developed layer of rough-walled tomentum hyphae; C. omnivirens lacks tomentum hyphae.

Chlorociboria aeruginosa was described by Dixon (1975) as having rough-walled tomentum hyphae, and ascospores $(8-)9-14(-15) \times 2-4 \mu m$. Three of the species described below as new, Chlorociboria campbellensis, C. duriligna, and C. poutoensis, are similar in ascospore size and in having rough-walled tomentum hyphae. All three of the New Zealand species are associated with blue-green staining confined to the surface of quite intact wood, compared with C. aeruginosa, associated with soft, rotten wood with extensive internal blue-green staining. C. poutoensis also differs in having a very pale hymenium, while C. campbellensis has ascospores more or less acute to the ends, compared with the ascospores of C. aeruginosa which have broadly rounded ends. ITS sequences confirm the distinctness of these species (see Molecular results and analyses below). See also notes under C. duriligna.

The New Zealand Chlorociboria species form two groups according to their pattern of colonisation of their associated substrate, and the physical structure of that wood. Five species (C. aeruginascens subsp. australis, C. argentinensis, C. clavula, C. pardalota, and C. spathulata) are consistently associated with soft wood that is extensively stained blue-green internally. Typically, if the surface of the infected wood has not been physically damaged, the blue-green staining is not visible externally. The soft texture of the wood suggests it has been degraded by white-rot fungi. Whether the *Chlorociboria* are active white-rot fungi, or whether they preferentially invade wood that has previously been degraded by white-rot fungi, is not known.

The other New Zealand species are consistently associated with decorticated wood that is hard, most with the blue-green staining confined to the uppermost 2–4 mm of the substrate (*Chlorociboria albohymenia*, *C. awaikoana*, *C. campbellensis*, *C. colubrosa*, *C. duriligna*, *C. poutoensis*, *C. procera*, and *C. spiralis*). If the colonised wood has been tunnelled by insects, then the blue-green staining typically extends along the inner surface of the insect tunnels. *C. halonata* and some collections of *C. macrospora* are similarly associated with hard wood, but as well as blue-green staining across the surface, they are associated with narrow lines of blue-green staining deep within the wood, these appearing to be associated with vessels running through the wood.

Dixon (1975) noted that the blue-green pigment produced by these fungi has been characterised as Xylindein. Because this compound is insoluable in water, Dixon presumed the blue-green pigment within the wood permeates directly from active hyphae. If this is the case, the between-species differences in the pattern of blue-green staining suggest a difference in the extent to which these fungi colonise the substrate, as well as the kind of substrate preferred. Assuming these fungi are not active white-rotters, the substrate preference may be an illustration of the effect that species succession can have on fungal colonisation of wood.

At some sites in New Zealand *Chlorociboria*colonised wood is very common. Such sites typically also have a high species diversity, several species of *Chlorociboria* occuring in close proximity. Examples include the length of track within 500 m of the carpark at Lake Hauroko (*C. argentinensis*, *C.* spathulata, and C. halonata), Canaan Downs in the vicinity of the track to Harwood's Hole (C. aeruginascens subsp. australis, C. argentinensis, C. macrospora, C. pardalota, and C. spathulata), within 500 m of the start of the access road to the Steuart Russell Awakino Beech Reserve (C. awakinoana, C. halonata, and C. pardalota), and the forests near the Kiko Road entrance to the Kaimanawa Forest Park (C. aeruginascens subsp. australis, C. awakinoana, C. clavulis, C. halonata, and C. macrospora). All these localities are in Nothofagus forest.

Two-thirds of the collections examined were from *Nothofagus* forest. Five species were found only in *Nothofagus* forest (*Chlorociboria argentinensis*, *C. colubrosa*, *C. duriligna*, *C. macrospora*, *C. spathulata*, and *C. spiralis*), with *C. albohymenia* (3 of the 13 collections examined) and *C. halonata* (5 of the 15 collections examined) less commonly found there. Only two species were never found in *Nothofagus* forest (*C. campbellensis* and *C. poutoensis*). *Chlorociboria aeruginascens* subsp. *australis* showed no preference for forest type.

Not all species of wood-inhabiting discomycetes associated with greening of the substrate are Chlorociboria. Two collections (PDD 70096, Pelorus Bridge, Marlborough Sounds, and PDD 74220, Urewera National Park) of a species with a blue-green, stipitate disc developing on very dark blue-green to black wood superficially resemble Chlorociboria. However, the receptacle of this species is covered with long (up to 135 um), straight hairs with thick, smooth walls; the ectal excipulum comprises gelatinous textura intricata oriented more or less parallel with the surface of the receptacle; and the ascospores are small $(5-6 \times 2.5-3 \mu m)$, broad elliptic to ovate, hyaline, with a coarsely warted wall. Although clearly a member of the Helotiales, its taxonomic position within the order is unknown. ITS sequences exclude it from Chlorociboria (Genbank accession number AY755361).

Key to species of Chlorociboria known from New Zealand

1	Ascospores more than 30 µm long, filiform to clavate
	Ascospores less than 30 µm long, ellipsoidal to fusiform
2	Ascospores tapering to base, not coiling on release from asci C. clavula
	Ascospores not tapering to base, coiling on release from asci
3	Hymenium and receptacle blue-green when dry, undifferentiated at margin of the cup; receptacle smooth, outer excipular elements smooth-walled and appressed
4	Ascospores elliptic to fusiform in shape, tapering to more or less acute ends
	Ascospores cylindric in shape, more or less uniform in width, with broadly rounded ends

5	Ascospores $10-14 \times 2.5(-3) \ \mu m$
	Ascospores $16-25 \times 2.5-3(-3.5) \ \mu m$
6	Apothecia with long, narrow stipe; tomentum hyphae lackingC. procera
	Apothecia short-stipitate; tomentum hyphae lacking, or present
7	Hymenium white when fresh, pale yellow to pale blue-green when dry; ascospores asymmetrical,
	surrounded by a gelatinous sheath
	Hymenium yellowish, blue-green to dark blue-green when fresh, translucent yellow, dark blue-green
	to black when dry; ascospores more or less symmetrical, not surrounded by a gelatinous sheath 8
8	Ascospores 1.5(-2) µm wide, mostly less than 10 µm long
	Ascospores more than 2 µm wide, more than 10 µm long
9	Apothecia with whitish bloom, often several arising from common stromatic base; tomentum hyphae
	smooth-walled, coiling
	Apothecia lacking whitish bloom, solitary, not arising from stromatic base; tomentum hyphae absent
	C. argentinensis
10	Tomentum hyphae rough-walled
	Tomentum hyphae lacking or smooth-walled
11	Ascospores more than 20 µm long
	Ascospores 9–17 µm long
12	Ascospores $(12-)13.5-15(-17) \times 3-4 \mu m$; hymenium bright yellow when dry
	Ascospores $(9-)10.5-11.5(-15) \times 2-2.5(-3)$ µm; hymenium translucent blue-green when dry
	C. duriligna
13	Often with several apothecia arising from a common stromatic basal mass; hymenium pale greenish
	when fresh, yellow when dry; receptacle when mature pale yellow with small, dark flecks; stipe taper-
	ing to base, with coarse vertical ridges
	Apothecia solitary, not arising from a stromatic mass; hymenium dark blue-green when fresh, black
	when dry; receptacle when mature dark blue-green; stipe more or less equal in diameter, not ridged
14	Ascospores slightly wider towards one end, $11.5-15 \times 3-4 \mu m$; ectal excipulum covered with incomplete
	layer of coarsely encrusted hyphae, ends of these hyphae not swollen; tomentum hyphae lacking
	C. awakinoana

Ascospores equal in shape toward both ends, $13.5-17.5 \times 4-5$ µm; ectal excipulum covered with a more or less complete layer of darkened, smooth-walled hyphae, ends of these hyphae typically swollen clavate to fusoid; tomentum hyphae may be lacking, or if present, smooth-walled, coiling C. spathulata

Chlorociboria aeruginascens subsp. australis P.R.Johnst., subsp. nov.

Fig. 1, 2

DIAGNOSIS: Apothecia in ligno molli aerugineo. Apothecia 4-10 mm diam.; receptaculum pruinato. Excipulum ectale "textura intricata". Hyphae tomentosae spirales, parietibus laevibus. Asci 40-55(-75) $\times 4-5 \,\mu\text{m}$. Ascosporae (5–)6–8(–10) $\times 1.5(-2) \,\mu\text{m}$, cylindricae, leviter curvae.

HOLOTYPUS (here designated): New Zealand: Taupo: Rangitoto Station, Mangatutu, White Marker Track, on wood, P. R. Johnston D1609 & S. R. Whitton, 5 May 2001, PDD 74101 (culture from type specimen, ICMP 15642).

ETYMOLOGY: Australis refers to the Southern Hemisphere distribution of this genetically distinct subspecies.

DESCRIPTION: Apothecia develop on decorticated wood or bark of fallen branches (wood soft, rotten, extensively stained blue-green internally, although surface may be unstained), several apothecia arising from single black basal stroma. Apothecia 4-10 mm diam., stipitate, stipe often slightly eccentric; disc variable in colour when dry, black, or with broad blue-green (25A7, 25B7) or yellow (2B8) patches, with a translucent quality to the surface, receptacle blue-green (25B7-25B8), typically with a well-developed white bloom; stipe tapering to base, concolorous with receptacle to black. Ectal excipulum 50-65 µm thick, textura intricata, elements oriented at high angle to receptacle surface, comprising hyphae 2-3 µm diam. with walls slightly thickened, hyaline, gelatinous, in outer layers encrusted with dark green material. Medullary excipulum textura



Fig. 1 *Chlorociboria aeruginascens* subsp. *australis* (A, PDD 74101; B, C, PDD 74092; D, E, PDD 44379). A, apothecia on wood (from dried herbarium specimen), arrows indicate apothecia; **B**, side of apothecium, vertical section; **C**, detail of B, gelatinous ectal excipulum to right, coiling, smooth tomentum hyphae to left; **D**, tomentum hyphae; **E**, ascospores. Scale bars: A = 1 mm; B-E = 10 µm.

intricata, comprising hyphae 2–3 µm diam. with walls thin, hyaline, nongelatinous. Tomentum hyphae macroscopically indistinct, 7–15 × 1.5–2.5 µm, coiling, walls thin, smooth, hyaline. Subhymenium textura intricata, comprising hyphae 1.5–2.5 µm diam. with walls hyaline, thin, nongelatinous. Paraphyses 1.5–2 µm diam., apex undifferentiated, about same length as asci. Asci 40–55(–75) × 4–5 µm, cy-lindric, tapering slightly to rounded apex or tapering to subtruncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate or biseriate, spores filling upper 25–35(–50) µm of ascus. Ascospores (5–)6–8(–10) × 1.5(–2) µm,

Fig. 2 Chlorociboria aeruginascens subsp. australis (PDD 74095). A, asci; B, ascospores; C, tomentum hyphae; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \mu m$.



(mean $7.2 \times 1.6 \ \mu\text{m}, n = 40$), cylindric, ends rounded, equal in shape to each end, curved, 0-septate, wall hyaline.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: AUCKLAND ISLANDS: between Terror Cove and Dea's Cove, on fallen wood, P. R. Johnston AK67, E. H. C. McKenzie & E. Edwards, 22 Mar 2000, PDD 81763; Enderby Island, Sandy Bay, on Metrosideros umbellata fallen wood, P. R. Johnston AK68, 21 Mar 2000, PDD 81764, BAY OF PLENTY: Mt Te Aroha, on beech wood, B. S. Parris, 9 Jan 1971, PDD 28776. BULLER: near Murchison, between Murchison & Maruia Saddle, on Nothofagus sp., G. J. Samuels 82-139, A. P. Hawthorne, P. R. Johnston & R. H. Petersen, 11 May 1982, PDD 44350; road between Murchison & Maruia Saddle, on Nothofagus sp., G. J. Samuels 82-196, 12 May 1982, PDD 44390; Reefton, Staircase Creek, on dead wood, S. D. Baker, 26 Nov 1952, PDD 11728. CAMPBELL ISLAND: fenceline to western cliffs from Tucker Stream mouth, on Dracophyllum longifolium, rotten wood, P. R. Johnston CAM71.2, E. H. C. McKenzie, R. Leschen, M. Bullians & E. Edwards, 9 Mar 2000, PDD 74020. CHATHAM ISLANDS: Taiko Camp, on wood, P. R. Johnston C264 & E. H. C. McKenzie, 2 Apr 1993, PDD 62680, ICMP 15619. GISBORNE: Urewera National Park, Lake Waikaremoana, Waikareiti Track, on decorticated wood, G. J. Samuels 81-334, A. P. Hawthorne, P. R. Johnston, E. Horak & R. H. Petersen, 24 May 1981, PDD 49581; Urewera National Park, Ngamoko Track, on Nothofagus sp. decort. wood, P. R. Johnston, 10 May 2001, PDD 73911; vic. Ruatahuna, Papatotara Ranges, south side of main road, on Pseudopanax sp. wood, P. R. Johnston (D1635) & S. R. Whitton, 7 May 2001, PDD 73929, ICMP 15646; vic. Ruatahuna, Papatotara Range, south side of main road, on wood, P. R. Johnston D1637 & S. R. Whitton, 7 May 2001, PDD 74092, ICMP 15647; vic. Ruatahuna, Papatotara Ra., south side of main road, on Pseudopanax sp. wood, P. R. Johnston D1636 & S. R. Whitton, 7 May 2001, PDD 74096. MID CANTERBURY: Craigieburn Forest Park, Lyndon Saddle Track, on Nothofagus solandri var. cliffortioides fallen log. P. W. Clinton, P. K. Buchanan & R. B. Allen, 21 May 1996, PDD 70495. NELSON: Abel Tasman National Park, Canaan Downs, track to Harwood's Hole, on Nothofagus menziesii, G. J. Samuels 82-162, A. P. Hawthorne, P. R. Johnston & R. H. Petersen, 15 May 1982, PDD 43172; Abel Tasman National Park, near Harwood's Hole, start of Rameka Track, on fallen wood Metrosideros umbellata, P. R. Johnston D1846, 14 May 2004, PDD 80549;

Nelson Lakes National Park, Lake Rotoiti, St Arnaud Track, on Nothofagus sp., G. J. Samuels 82-172, A. P. Hawthorne, P. R. Johnston & R. H. Petersen, 13 May 1982, PDD 44371. NORTHLAND: Waipoua State Forest, between Forest H.Q. and Yakas Track, on Agathis australis, G. J. Samuels 82-207 & P. R. Johnston, 30 May 1982, PDD 44397; Waipoua Forest, near Yakas tree, on fallen wood, P. R. Johnston & H. Burdsall, 16 Apr 2004, PDD 81270; Waipoua Forest, Yakas Track between Yakas tree and northern entrance, on ?Beilschmiedia sp. fallen wood, P. R. Johnston & H. Burdsall, 16 Apr 2004, PDD 81271. OTAGO LAKES: Upper Hollvford, on Weinmannia racemosa, J. M. Dingley, Jan 1950, PDD 13969. SOUTH CANTERBURY: Peel Forest, Dennistoun Bush Walk, on wood, dead, decorticated, J. A. Cooper 8453, 26 May 2002, PDD 77468. STEWART ISLAND: Pryces Peak, near summit, on decort fallen wood, P. R. Johnston D1679, R. A. B. Leschen & S. R. Whitton, 26 Apr 2002, PDD 77439; Glory Cove, southern end Ocean Beach Track, on Metrosideros umbellata fallen wood, P. R. Johnston D1714, R. A. B. Leschen & S. R. Whitton, 30 Apr 2002, PDD 77443; Doughboy Bay, track across headland to the west, on fallen wood, P. R. Johnston D1699, R. A. B. Leschen & S. R. Whitton, 29 Apr 2002, PDD 77442; Doughboy Bay, track across headland to the west, on fallen wood, P. R. Johnston D1697, R. A. B. Leschen & S. R. Whitton, 29 Apr 2002, PDD 77441; track to Pryces Peak, on Weinmannia racemosa, fallen wood, P. R. Johnston D1687, R. A. B. Leschen & S. R. Whitton, 26 Apr 2002, PDD 77440; Ulva Island, track between West End and Landing, on fallen wood, P. R. Johnston D1669, R. A. B. Leschen & S. R. Whitton, 24 Apr 2002, PDD 77438. TAUPO: vic. Kiko Rd, Tiraki Rd, on Nothofagus sp. wood, P. R. Johnston D1576 & S. R. Whitton, 3 May 2001, PDD 74065, ICMP 15632; Kaimanawa Ranges, Mohaka River, on Nothofagus fusca, J. M. Dingley, 31 May 1953, PDD 12193; vic. Kiko Rd, near corner Mangatera Rd, on wood, P. R. Johnston D1601 & S. R. Whitton, 4 May 2001, PDD 74095, ICMP 15638; Kaimanawa Ranges, on Nothofagus cliffortioides, J. M. Dingley, Apr 1955, PDD 14362; Horopito, J. M. Dinglev, 8 Mar 1963, PDD 20809; National Park, Ohakune Mountain Road, 3500 ft, on Pseudopanax colensoi, J. M. Dingley, 7 Mar 1963, PDD 20812; Pureora, Link Road, Mt Pureora, summit track, on bark of fallen tree, P. R. Johnston D1353, 17 Apr 1998, PDD 68626; vic. Kiko Rd, Tiraki Rd, on Nothofagus sp. wood, P. R. Johnston D1570 & S. R. Whitton, 3 May 2001, PDD 74091; vic. Kiko Rd, Tiraki Rd,

on Nothofagus sp. wood, P. R. Johnston D1574 & S. R. Whitton, 3 May 2001, PDD 74058, ICMP 15630; vic. Kiko Rd, near corner Mangatera Rd, on wood, P. R. Johnston D1604 & S. R. Whitton, 4 May 2001, PDD 74100, ICMP 15641; vic. Kiko Rd, near corner Mangatera Rd, on wood, P. R. Johnston D1602 & S. R. Whitton, 4 May 2001, PDD 73913; vic. Kiko Rd, near corner Mangatera Rd, on wood, P. R. Johnston D1603 & S. R. Whitton, 4 May 2001, PDD 73912, ICMP 15640; vic. Kiko Rd, near corner Mangatera Rd, on Nothofagus sp. wood, P. R. Johnston D1596 & S. R. Whitton, 4 May 2001, PDD 74097, ICMP 15636; Pureora Forest Park, Select Loop Road, R. Freeston & A. Freeston, 16 Apr 1998, PDD 70082; Pureora Forest Park, Link Road, track to Mt Pureora summit, P. R. Johnston D1350, 17 Apr 1998, PDD 70087; vic. Kiko Rd. Tiraki Rd, on Nothofagus sp. wood, P. R. Johnston D1573 & S. R. Whitton, 3 May 2001, PDD 74099. WAIKATO: Waitomo, on rotten wood, Hastings, 15 Apr 1960, PDD 19301. WESTLAND: Weheka, on Metrosideros robusta, J. M. Dingley, Apr 1955, PDD 14352.

C. aeruginascens. USA: MICHIGAN: Interstate 75, Cheboygan County, 10 km north of Indian River, on rotten wood, *P. R. Johnston*, 27 Aug 2002, PDD 76435, ICMP 14692. RHODE ISLAND: *Zheng Wang WZ RI5*, 14 Oct 2002, PDD 77803. CHINA: Beijing, Donglingshan Mountains, *Zheng Wang JXD15*, 5 Sep 2002, PDD 77804.

NOTES: Chlorociboria aeruginascens subsp. australis is macroscopically distinctive amongst the New Zealand species, with a well-developed white bloom on the receptacle, and typically with several, often eccentrically stipitate, apothecia arising from a common black, cushion-like stromatic base.

Chlorociboria aeruginascens subsp. australis is morphologically indistinguishable from the Northern Hemisphere C. aeruginascens subsp. aeruginascens. However, ITS sequences are distinct (see Molecular results and analyses, below), and the new subspecies is established on this basis. A subspecific taxon is proposed because for many purposes it may remain appropriate to continue to refer to these fungi at the specific level. To define them at the subspecific level requires knowledge about DNA sequences, at present available for collections from only a few parts of the world. Dixon (1975) recognised another subspecies of C. aeruginascens known from tropical America, C. aeruginascens subsp. brasiliensis (Berk. & Cooke) J.R.Dixon, on the basis of small differences in ascus and ascospore size.

The asci and ascospores of *C. aeruginascens* and *C. aeruginascens* subsp. *australis* are indistinguishable from those of *C. argentinensis*. *Chlorociboria argentinensis* lacks the tomentum hyphae of *C. aeruginascens*, this difference being the only reliable way to distinguish these species. However, this difference has to be treated with care, as Dixon (1975) noted, as in some collections the tomentum hyphae of *C. aeruginascens* may be very poorly developed. *Chlorociboria argentinensis* typically has solitary apothecia, while in most collections of *C. aeruginascens*, groups of apothecia arise from a common stromatic base.

Chlorociboria aeruginascens subsp. australis occurs on wood that is soft, with the appearance of having been rotted by white-rot fungi. The surface of the infected wood typically has no blue-green staining visible, while inside the soft wood is extensively stained blue-green. This stain becomes externally visible only on older pieces of wood, where the surface layers have been weathered away. The fungus remains viable, apothecia continuing to develop, on these heavily weathered pieces of wood. Often, apothecia are found on wood with bark still loosely attached. In these cases apothecia are erumpent through the unstained bark.

See also notes under Chlorociboria argentinensis.

Chlorociboria albohymenia P.R.Johnst., sp. nov Fig. 3, 4

DIAGNOSIS: Apothecia in ligno duro aerugineo vel atroveneto. Apothecia 0.6-1.5 mm diam.; hymenium album, luteum ubi siccum. Excipulum ectale "textura angularis" vel "textura prismatica". Hyphae tomentosae cylindricae vel brevicylindricae, parietibus verrucatis. Asci $85-105 \times 9.5-12$ µm. Ascosporae $(11.5-)14-16.5(-18.5) \times 4-5(-5.5)$ µm, ellipticifusiformis, complanatae in latere uno.

HOLOTYPUS (here designated): New Zealand: Taupo: Pureora Forest, Link Track, on decorticated wood, *P. R. Johnston D1419 & B. M. Spooner*, 24 Aug 1999, PDD 70882 (culture from type ICMP 15624).

ETYMOLOGY: From albus = white and hymenium referring to the distinctive white hymenium of this species when fresh.

DESCRIPTION: Apothecia develop on blue-green to very dark blue-green stained decorticated wood or bark of fallen branches, often gregarious (infected wood hard, blue-green stain restricted to top few mm of wood). Apothecia 0.6–1.5 mm diam., substipitate or short stipitate; disc white when fresh,



Fig. 3 *Chlorociboria albohymenia* (A, PDD 77459; B–D, PDD 70089; E, G, PDD 50141; F, PDD 58448). **A**, apothecia on wood (from dried herbarium specimen), arrows indicate apothecia; **B**, side of apothecium, vertical section; **C**, **D**, detail of B, medullary excipulum, ectal excipulum, and rough-walled tomentum hyphae; **E**, tomentum hyphae, squash mount; **F**, **G**, ascospores and ascus. Scale bars: A = 1 mm; B-G = 10 µm.

sometimes with greenish tinge in patches, whitish to pale, bright yellow when dry, receptacle pale blue-green, slightly darker when dry, glabrous; stipe $0.1-0.3 \times 0.2-0.5$ mm, darker than receptacle. Ectal excipulum 30 µm thick, textura angularis or textura prismatica, elements oriented at high angle to receptacle surface, comprising short cylindric cells 4.5-8 µm diam. with walls thick, hyaline, refractive. Medullary excipulum textura intricata, comprising hyphae 1-1.5 µm diam. with walls thin, hyaline, nongelatinous. Tomentum hyphae macroscopically indistinct, $5-17.5 \times 3-4.5 \mu m$, cylindric or shortcylindric with swollen apex, to more or less globose, 1–3-septate, walls thin, roughened, greenish. Subhymenium textura intricata, comprising hyphae 1–1.5 μm diam. with walls hyaline, thin, nongelatinous. Paraphyses 1.5–2 μm diam., apex undifferentiated, extending up to 10 μm beyond asci. Asci 85–105 × 9.5–12 μm , cylindric or subclavate, tapering to subtruncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate, spores filling upper 60–70 μ m of ascus. Ascospores (11.5–)14–16.5(–18.5) × 4–5(–5.5) μ m (mean 15.3 × 4.7 μ m, n = 35), asymmetrical, in side view flattened one side, wider towards apex, tapering to narrowly rounded ends, often more acute to apex, at some orientations appearing more or less sigmoid, 0-septate, wall hyaline, thin, smooth; surrounded by gelatinous sheath 1–1.5 μ m thick.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: BULLER: Rahu Saddle, Duffy's Creek Track, on Nothofagus wood, P. R. Johnston D635, 23 Mar 1991, PDD 58448. NELSON: Abel Tasman National Park, track to Harwood's Hole, on decorticated Nothofagus wood, P. R. Johnston D1845, 14 May 2004, PDD 81293, STEWART ISLAND: Garden Mound Tr. near summit, on decorticated wood, P. R. Johnston D1720, 1 May 2002, PDD 77459; Glory Cove, north end of Ocean Beach Track, on Metrosideros umbellata decorticated wood, P. R. Johnston D1717, S. R. Whitton & R. A. B. Leschen, 30 Apr 2002, PDD 77458; Glory Cove, north end of Ocean Beach track, on decorticated wood, P. R. Johnston D1715, S. R. Whitton & R. A. B. Leschen, 30 Apr 2002, PDD 77457; Ryans Creek Track, on decorticated wood, P. R. Johnston D1672, S. R. Whitton & R. A. B. Leschen, 25 Apr 2002, PDD 77456. TARANAKI: Egmont National Park, track from Stratford to Dawson Falls, blackened wood, G. J. Samuels, P. R. Johnston & R. H. Petersen, 24 Apr 1983, PDD 50143. TAUPO: Pureora Forest, Mt Pureora, Link Track, on decorticated wood, P. R. Johnston & B. M. Spooner, 24 Aug 1999, PDD 70882; Pureora Forest Park, Link Road, track to Mt Pureora summit, on decorticated wood, P. R. Johnston D1354, 17 Apr 1998, PDD 70089; Tongariro National Park, Turoa skifield, on Dracophyllum sp. dead wood, P. R. Johnston SB135 & S. L. Stephenson, 4 Dec 2000, PDD 81716. WESTLAND: Haast, Cascades Rd, near end of road, on bark of fallen wood, P. R. Johnston D1744, 7 May 2002, PDD 77460; Westland National Park, Franz Josef, track to Lake Wombat, on wood, G. J. Samuels & R. H. Petersen, 10 Apr 1983, PDD 50141; Saltwater State Forest, on fallen wood Weinmannia racemosa, G. J. Samuels, May 1974, PDD 49842.

NOTES: Chlorociboria albohymenia is distinctive both macoscopically and microscopically. The hymenium of fresh apothecia is white (sometimes with scattered greenish patches, possibly bruised following damage) and the receptacle is a bright blue-green colour. The apothecia are typically quite small (less than 1.5 mm diam.) and short-stipitate to sessile. The excipular hairs are typically short



Fig. 4 Chlorociboria albohymenia (A, B, PDD 70882; C, PDD 70089). A, asci; B, ascospores; C, tomentum hyphae; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \mu m$.

and broad, swollen at the apex, sometimes with the appearance of a more or less free, globose cell. The ascospores are distinctly asymmetric, flattened on one side, wider towards one end, from some angles sigmoid in appearance, and surrounded by a gelatinous sheath.

Chlorociboria poutoensis is similar macroscopically, but differs in ascospore size and shape. See notes under this species.

The apothecia of *C. albohymenia* are found usually in large numbers and always on blue-green stained wood. In some collections the staining is very dark, almost black in appearance when fresh.



Fig. 5 *Chlorociboria argentinensis* (A, PDD 77452; B, PDD 77694; C–E, PDD 77446; F, PDD 15988). A, apothecia on wood (from dried herbarium specimen); B, detail of A, showing scale-like roughening on receptacle and stipe; C, side of apothecium, vertical section; D, detail of C showing gelatinous ectal excipulum and free hypha-like elements at margin; E, detail of C showing section through one of the scale-like roughenings; F, encrusted hyphae on surface of receptacle, scalp section. Scale bars: A, B = 1 mm; C–F = 10 μ m.

The wood on which this species is found is typically hard, with the blue-green staining restricted to the uppermost 5 mm of the wood. In some collections *C. albohymenia* develops on bark attached to recently fallen wood, and in these collections the blue-green staining is again restricted to the upper layers of the bark. The staining may extend for 20 cm or more along an infected branch.

Chlorociboria argentinensis J.R.Dixon, Mycotaxon 1, 214 (1975) Fig. 5, 6

DESCRIPTION: Apothecia develop on decorticated wood (stained blue-green (25B7), infected wood,

soft, extensively stained internally, although surface may be unstained). Apothecia 5–10 mm diam., margin wavy, often inrolling over hymenium when dry, short stipitate or stipitate, stipe often eccentric, thinfleshed; disc blue-green to dark blue-green when fresh, variable in colour when dry, black, or with broad blue-green (25A7, 25B7) or yellow (2B8) patches, with a translucent quality to the surface, receptacle when fresh blue-green to dark blue-green, when dry pale blue-green (25A5–25A7) with small dark flecks, to very dark blue-green (25E7–25E8) to black, sometimes lacking blueish tinge (26E7), glabrous; stipe 10–30 × 4–8 mm, cylindric, dark green to blue-green, black towards base, coarsely roughened, sometimes also with vertical ridges. Ectal excipulum 65 µm thick, textura intricata, elements oriented at high angle to receptacle surface, comprising hyphae 4-6 µm diam. with walls thick, hyaline, gelatinous, encrusted patchily with bluegreen pigment; exciple overlaid with incomplete, 1cell-wide layer of hyphae 2-2.5 µm diam. with walls thin, hyaline, encrusted with green pigment, end cells of hyphal elements more or less free, swollen, fusoid, 4-5.5 µm diam. Medullary excipulum a loose textura intricata, comprising hyphae 3-4.5 µm diam. with walls thin, hyaline, nongelatinous. Paraphyses 1.5-2 µm diam., apex undifferentiated, about same length as asci. Asci $45-65 \times 3.5-4.5 \mu m$, cylindric or subclavate, tapering slightly to rounded apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate or biseriate, spores filling upper 25-30 µm of ascus. Ascospores 5.5-9.5 \times 1–2 µm (mean 7.0 \times 1.5 µm, n = 38), cylindric, ends rounded, curved, 0-septate, wall hyaline.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: BULLER: Lake Rotoiti, on Nothofagus fusca, S. D. Brook & P. J. Brook, Apr 1956, PDD 15987; Reefton, Staircase Creek, on dead wood, S. D. Baker, 26 Nov 1952, PDD 11727; Lewis Pass, Nina River Track, on decort. wood, P. R. Johnston D502, 11 May 1990, PDD 57728; Lewis Pass summit, track to treeline, on Nothofagus sp. rotten wood, P. R. Johnston & P. F. Cannon, 7 Feb 2003, PDD 77699; Lake Rotoiti, on rotten wood, P. J. Brook, Apr 1956, PDD 15988; Maruia, Lake Stream Falls, on Nothofagus fusca rotten wood, J. A. Cooper 8633, 25 May 2003, PDD 77698. FIORDLAND: Fiordland National Park, Borland Lodge Nature Trail, on Nothofagus sp., P. R. Johnston D661, 19 Mar 1991, PDD 58570; Kepler Track, vic. Te Anau control gate, on Nothofagus sp. wood, P. R. Johnston D497, 17 May 1990, PDD 57543; Fiordland National Park, Kepler Track, vic. Te Anau control gate, on Nothofagus sp., P. R. Johnston D780, 2 Mar 1992, PDD 59975, ICMP 15617; Boyle Lodge, nature trail, on Nothofagus sp. wood, P. R. Johnston D1471-2, 9 May 2000, PDD 77446; Kepler Tr, control gates, on Nothofagus sp. fallen rotten wood, P. R. Johnston D1484, R. E. Beever, S. R. Pennycook, R. Leschen & T. Lebel, 10 May 2000, PDD 77447; Te Anau, Kepler Tr., Shallow Bay, near Moturau Hut, on Nothofagus sp. decort, rotten wood, T. Atkinson, 12 Mar 2003, PDD 78363; Lake Hauroko, lakeshore near car park, to the west, on Nothofagus sp. decort rotten wood, P. R. Johnston D1727 & S. R. Whitton, 3 May 2002, PDD 77448; Lake Hauroko, lakeshore near car park.



Fig. 6 Chlorociboria argentinensis (A, B, PDD 49836; C, PDD 75828). A, asci; B, ascospores; C, encrusted hyphae on surface of receptacle, sometimes with fusoid end-cell; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \mu m$.

to the west, on Nothofagus sp. decort rotten wood, P. R. Johnston D1729 & S. R. Whitton, 3 May 2002, PDD 77449; Lake Hauroko, lakeshore near car park, to the west, on Nothofagus sp. decort rotten wood, P. R. Johnston D1728 & S. R. Whitton, 3 May 2002, PDD 77450; Lake Hauroko, lakeshore near car park, to the west, on Nothofagus sp. decort rotten wood, P. R. Johnston D1733 & S. R. Whitton, 3 May 2002, PDD 77451; Boyle Lodge, nature trail, on Nothofagus sp. wood, P. R. Johnston D1471, 9 May 2000, PDD 77445; Te Anau, near Mt Prospect, on Nothofagus sp. decorticated, rotten wood, T. Atkinson, 9 Mar 2003, PDD 78362. MARLBOROUGH SOUNDS: Mt Somers Track, about half way to treeline, on Nothofagus sp., P. R. Johnston D1319, 10 May 1997, PDD 70101. NELSON: Canaan Road Track, P. Leonard 21202, 15 Feb 2002, PDD 75828; Abel Tasman N.P., Canaan, on log, P. Leonard 53403, 10

Fig. 7 *Chlorociboria awakinoana* (A, PDD 71674; B, C, PDD 74077; D, E, PDD 71672). **A**, apothecia on wood (from dried herbarium specimen); **B**, side of apothecium showing medullary excipulum and ectal excipulum, vertical section; **C**, detail of B through a clump of encrusted hyphae; **D**, encrusted hyphae on surface of receptacle, scalp section; **E**, ascospores. Scale bars: A = 1 mm; B-E = 10 µm.

Apr 2003, PDD 77728; Abel Tasman National Park, track to Harwood's Hole, on rotten wood Nothofagus sp., P. R. Johnston D1840, 14 May 2004, PDD 80553; Arthur Range, Graham Valley Rd, track from Flora carpark to Mt Arthur Hut, on Nothofagus sp. wood, P. R. Johnston D993, 6 May 1994, PDD 77444, ICMP 15620; Cobb Resevoir, vic. Mytton Hut, on wood, P. R. Johnston D979, 5 May 1994, PDD 77452. NORTH CANTERBURY: Mt Thomas Forest, Richardson Track, on Nothofagus sp., P. R. Johnston D679, 15 Mar 1991, PDD 58574, ICMP 15616; Foley Track, eastern side of Lewis Pass, on Nothofagus sp. wood, P. R. Johnston & P. F. Cannon, 7 Feb 2003, PDD 77700; near Lewis Pass summit, top of Cannibal Gorge Tr, on decort. wood, P. R. Johnston D1789 & P. F. Cannon, 6 Feb 2003, PDD 77694. TAUPO: Horopito, Mangaturuturu Stream, on Nothofagus solandri var. cliffortioides

rotten wood, J. M. Dingley, Mar 1948, PDD 10750; Tongariro National Park, Ohakune Mt Rd, Blyth Tr, on Nothofagus sp. rotten wood, T. Atkinson, 5 May 2003, PDD 78364. WESTLAND: Paparoa Forest, vic. Moonlight Creek, on decorticated wood, G. J. Samuels, 9 May 1974, PDD 49836.

NOTES: Macroscopically, the hymenial surface of *C. argentinensis* when dry is sometimes similar to that of *C. aeruginascens* subsp. *australis* and *C. campbellensis* (characteristically patchy, bluegreen to yellow to grey, with a translucent quality), although in all three species the dry hymenium is often black. The three species are also similar in having thin-fleshed apothecia, the edges of which curl up to partially cover the hymenium when dry. *Chlorociboria argentinensis* differs from the other two species in lacking a white bloom on the receptacle. Its apothecia are typically solitary, while those of *C. aeruginascens* are usually grouped, with several arising from a common stromatic base.

Ascospore size and shape of *C. argentinensis* closely matches *C. aeruginascens*, as does the gelatinous textura intricata of the ectal excipulum. These two species can be distinguished microscopically only by the lack of tomentum hyphae in *C. argentinensis*. This feature needs to be used with care, as Dixon (1975) noted that some collections of *C. aeruginascens* have a very poorly developed tomentum.

In New Zealand *C. argentinensis* is always associated with very rotten, soft wood, which is extensively stained blue-green. The staining may sometimes not extend to the surface of the infected piece of wood. In these cases, the presence of the fungus is indicated only by the erumpent apothecia, until the wood is broken open to reveal the extensive internal blue-green staining.

Chlorociboria awakinoana P.R.Johnst., sp. nov. Fig. 7, 8

DIAGNOSIS: Apothecia in ligno duro aerugineo. Apothecia 1–3(–5) mm diam.; hymenium nigrum ubi siccum. Excipulum ectale "textura intricata". Hyphae tomentosae nullae. Asci 75–95 × 6.5–8 μ m. Ascosporae (11–)13–14.5(–15.5) × (3–)3.5–4 μ m, cylindricae, leviter curvae, complanatae in latere uno, lattissimae versus extremum unum.

HOLOTYPUS (here designated): New Zealand: Waikato: vic. Awakino, Steuart Russell Awakino Beech Reserve, on decorticated wood *Nothofagus truncata*, *P. R. Johnston D1554*, 11 Jul 2000, PDD 71674 (culture from type ICMP 15628).

ETYMOLOGY: Awakinoana refers to Awakino, the locality from which the type specimen was collected.

DESCRIPTION: Apothecia develop on blue-green stained, decorticated wood (infected wood hard, blue-green stain restricted to top few mm of wood). Apothecia 1-3(-5) mm diam., stipitate; disc dark blue-green when fresh, black when dry, receptacle blue-green to dark blue-green, macroscopically glabrous; stipe $0.5-1 \times 0.4-0.7$ mm, stipe cylindric with coarse vertical ridges, dark blue-green, black near base, glabrous. Ectal excipulum 20-30 µm thick, textura intricata, elements oriented at high angle to receptacle surface, comprising hyphae 2.5-3 µm diam. with walls thick, hyaline, gelatinous; exciple overlaid with incomplete, 1-cell-wide layer of hyphae 2-4.5 µm diam. with walls thick, hyaline, encrusted thickly with dark green material, sometimes clumping to form layer several cells thick. Medul-



Fig. 8 *Chlorociboria awakinoana* (PDD 74077). **A**, asci; **B**, ascospores; **C**, distribution of New Zealand collections examined. Scale bar: A, $B = 20 \mu m$.

lary excipulum textura intricata, comprising hyphae with walls slightly thick, hyaline, refractive. Subhymenium textura intricata, hyphae with walls hyaline, thin, nongelatinous. Paraphyses $1.5-2.5 \mu m$ diam., apex undifferentiated, about same length as asci.



Fig. 9 Chlorociboria omnivirens (Type, K). A, ectal excipulum, squash mount; B, ascospores. Scale bars = 10 µm.

Asci 75–95 × 6.5–8 µm, cylindric or subclavate, tapering slightly to truncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate or biseriate, spores filling upper 65–80 µm of ascus, basal stalk short. Ascospores (11–)13–14.5(–15.5) × (3–)3.5–4 µm (mean 13.5 × 3.5 µm, n = 33), oblong-elliptic or cylindric, ends rounded, flat one side, slightly curved in side view, widest point slightly toward the apex, 0-septate, wall hyaline, thin.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: BULLER: between Murchison and Springs Junction, Shenandoah Rd, on fallen wood Nothofagus sp., P. R. Johnston D1838, 15 May 2004, PDD 80502. TAUPO: vic. Kiko Rd, Tiraki Rd, on Nothofagus sp. wood, P. R. Johnston D 1575 & S. R. Whitton, 3 May 2001, PDD 74077, ICMP 15631; Taumarunui, on Podocarpus dacrydioides, M. Peacock, 11 Jul 1971, PDD 29197. WAIKATO: vic. Awakino, Steuart Russell Awakino Beech Reserve, on Nothofagus truncata decort. wood, P. R. Johnston D1556, 11 Jul 2000, PDD 71673, ICMP 15629; vic. Awakino, Steuart Russell Awakino Beech Reserve, on Nothofagus truncata decort. wood, P. R. Johnston D1549, 11 Jul 2000, PDD 71672.

NOTES: Chlorociboria awakinoana has ascospores of similar size and shape to *C. pardalota* and *C. spathulata*. Chlorociboria pardalota is macroscopically distinctive: when dry the hymenium is yellow and the receptacle of the mature apothecium is pale yellow with a few scattered dark flecks. The other two species have a black hymenium when dry and a dark blue-green receptacle.

Chlorociboria awakinoana and C. spathulata are difficult to distinguish morphologically. Some

collections of *C. spathulata* have smooth-walled, coiling tomentum hyphae, but these are often difficult to see and are not always present. *Chlorociboria spathulata* has slightly larger ascospores of a slightly different shape (13.5–17.5 × 4–5 μ m and uniform in shape to both ends), and the hyphae covering the outside of the ectal excipulum form a more complete layer, are smooth-walled, and are swollen at the apices. Despite the somewhat cryptic morphological differences between these species, molecular data clearly distinguish them (see Molecular results and analyses, below).

Chlorociboria omnivirens (Berk.) Cooke, originally described from Tasmania, was described by Dixon (1975) as having ascospores of a similar size to C. awakinoana, as well as lacking tomentum hyphae. As there are many inoperculate discomycete species shared between south-eastern Australia and New Zealand, a comparison between C. omnivirens and C. awakinoana is warranted. The type specimen of C. omnivirens (Tasmania, on wood, coll. Archer, K (M)110245!) is fragmentary, although the apothecia appear to have been quite large and robust with a well-developed stipe. I found the ascospores to be larger than reported by Dixon (1975), $(18-)19-21(-25) \times (4.5-)5-5.5(-6) \mu m$ (mean 20.1) \times 5.2 µm, n = 27), cylindric, tapering slightly to broadly rounded ends, flattened on one side, sometimes slightly curved (Fig. 9). They are also larger than C. awakinoana. When compared with all the New Zealand Chlorociboria species, the ascospore size and shape of C. omnivirens is most similar to C. macrospora. However, C. macrospora has a well-developed layer of rough-walled tomentum hyphae.



Fig. 10 *Chlorociboria campbellensis* (PDD 74109). **A**, apothecia on wood (from dried herbarium specimen); **B**, ectal excipulum, squash mount; **C**, rough-walled tomentum hyphae, squash mount; **D**, ascospores. Scale bars: A = 1 mm; B-D = 10 µm.

All collections of *C. awakinoana* are on decorticated but hard, largely intact wood. The blue-green staining is confined to the outermost 2–3 mm of the wood.

Chlorociboria campbellensis P.R.Johnst., sp. nov. Fig. 10, 11

DIAGNOSIS: Ab C. aeruginascenti ascosporis fusiformibus, hyphis tomentosis parietibus verrucatis differens.

HOLOTYPUS (here designated): New Zealand: Campbell Island: along fenceline from western cliffs to Tucker Stream mouth, on *Dracophyllum longifolium* fallen twig, *P. R. Johnston CAM* 71.1, *E. H. C. Mc-Kenzie, R. A. B. Leschen, M. Bullians & E. Edwards*, 9 Mar 2000, PDD 74019.

ETYMOLOGY: Campbellensis refers to Campbell Island, the known distribution of this species.

Fig. 11 Chlorociboria campbellensis (PDD 74019). A, asci; B, ascospores; C, tomentum hyphae; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \ \mu m$.





Fig. 12 *Chlorociboria clavula* (A, PDD 73914; B–D, PDD 73928; E, PDD 77467). **A**, apothecia on wood (from dried herbarium specimen); **B**, side of apothecium showing medullary excipulum, ectal excipulum, and tomentum hyphae, vertical section; **C**, detail of B showing ectal excipulum and tomentum hyphae; **D**, ectal excipulum in stipe, vertical section; **E**, septate ascospores. Scale bars: A = 1 mm; B-E = 10 µm.

DESCRIPTION: Apothecia develop on bark of fallen branches (wood rotten, blue-green stained, with bark still attached), erumpent. Apothecia 1.2-2 mm diam., short stipitate, thin-fleshed; disc translucent yellow (1A4), blue-green (26C8) or dark grey in irregular patches, receptacle pale blue-green (25B7) with whitish bloom; stipe $0.3-0.5 \times 0.2$ mm, stipe short and narrow cylindric, concolorous with receptacle. Tomentum hyphae macroscopically indistinct, $4.5-7.5 \times 2.5-3 \mu m$, short-cylindric to more or less globose, septate (0-3), walls thin, roughened, hyaline. Paraphyses 1.5-2 µm diam., apex undifferentiated, about same length as asci. Asci 60–70 \times 5-7 µm, cylindric or subclavate, tapering to rounded apex or tapering to subtruncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate or biseriate, spores filling upper 50-60 µm of ascus. Ascospores (10-)10.5-12.5 $(-13.5) \times 2.5(-3) \mu m$ (mean $11.6 \times 2.5 \mu m$, n = 28), fusoid, ends more or less acute, tapering more or less evenly to each end, widest point at centre or slightly towards one end, 0-septate, wall hyaline, thin.

NOTES: Known from a single specimen, macroscopically this species resembles *C. aeruginascens* in hymenium colour, the receptacle with a distinct white bloom, and the disc thin-fleshed with the margin somewhat wavy in appearance and inrolled over the hymenium when dry. It differs from *C. aeruginascens* by its tomentum hyphae being roughwalled and by ascospore shape. The two species also differ in appearance in culture: *C. campbellensis* has strong orange pigments diffusing into the agar, while in culture *C. aeruginascens* develops bluegreen pigments.

No apothecia were sectioned, because only a single, small collection of this species is known.

From squash mounts, the ectal excipulum appears to comprise highly gelatinous textura intricata.

The apothecia of *C. campbellensis* are erumpent through bark still firmly attached to the wood on which they are developing. There is a 3-5 mm deep zone of blue-green pigmentation in the upper layers of the wood immediately below the bark.

Chlorociboria campbellensis closely matches the description of *C. aeruginosa* provided by Dixon (1975). *C. aeruginosa* differs in ascospore shape, having spores broadly rounded at each end. See also notes under *C. duriligna*. ITS sequences confirm the distinctness of *C. campbellensis* at the species level (see Molecular results and analyses, below).

Chlorociboria clavula P.R.Johnst., sp. nov. Fig. 12, 13

DIAGNOSIS: Apothecia in ligno molli aerugineo. Apothecia 1–2 mm diam.; hymenium album, luteum ubi siccum. Excipulum ectale "textura angularis" vel "textura prismatica". Hyphae tomentosae cylindricae, parietibus verrucatis. Asci 90–120 × 8–10 μ m. Ascosporae (31.5–)41–50(–52) × (1.5–)2 μ m, filiformes vel clavatae, 0–3-septatae.

HOLOTYPUS (here designated): New Zealand: Taupo: vic. Kiko Rd, near corner Hingapo & Tiraki Rd, on wood (not *Nothofagus*), *P. R. Johnston D1595* & *S. R. Whitton*, 4 May 2001, PDD 73928 (culture from type ICMP 15635).

ETYMOLOGY: Clavula = small club, referring to the club-shaped ascospores (epithet as noun in apposition).

DESCRIPTION: Apothecia develop on decorticated wood associated with blue-green (25B6) staining (infected wood soft, extensively stained internally, although surface may be unstained). Apothecia 1-2 mm diam., subsessile or short stipitate; disc whitish when fresh, translucent vellowish (1B5) to dark grey when dry, receptacle blue-green (25B8), usually with distinct whitish bloom; stipe 0.1-0.3 × 0.3-0.4 mm, short, dark blue-green to black. Ectal excipulum 40-50 µm thick, textura angularis or textura prismatica, elements oriented at high angle to receptacle surface, comprising short cylindric cells or angular cells 4.5-10 µm diam. with walls thick, hyaline, gelatinous; toward base of stipe ectal excipulum comprising textura intricata, with hyphae 3-4 µm diam. embedded in hyaline gel. Medullary excipulum of more or less parallel rows comprising hyphae $2-3 \mu m$ diam. with walls thin, hyaline, gelatinous, gel greenish (especially in stipe). Tomentum hyphae macroscopically indistinct,



Fig. 13 *Chlorociboria clavula* (A, C, E, PDD 73914; B, PDD 73928; D, PDD 73919). **A**, asci; **B**, globose ascoconidia within ascus; **C**, released ascospores; **D**, septate ascospores; **E**, tomentum hyphae; **F**, distribution of New Zealand collections examined. Scale bar: A–E = 20 μm.

20–30 × 5–8 µm, cylindric with rounded apex, wall coarsely roughened, hyaline. Subhymenium textura intricata, hyphae with walls hyaline, thin, nongelatinous. Paraphyses 2 µm diam., apex undifferentiated, about same length as asci. Asci 90–120 × 8–10 µm, cylindric or subclavate, tapering to subtruncate apex, wall slightly thickened at apex, apical pore amyloid; 8-spored, spores filling upper 50–90 µm of ascus. Ascospores (31.5–)41–50(–52) × (1.5–)2 µm (mean 45.2 × 2.0 µm, n = 14), clavate or filiform, straight, apex rounded, tapering gradually to more or less



Fig. 14 *Chlorociboria colubrosa* (PDD 44404). **A**, apothecia on wood (from dried herbarium specimen); **B**, apothecium, vertical section; **C**, side of apothecium showing ectal excipulum, vertical section; **D**, detail of C showing gelatinous ectal excipulum and nongelatinous hyphae on surface; **E**, nongelatinous hyphae with fusoid end cells on surface of receptacle, scalp section. Scale bars: A = 1 mm; $B = 50 \text{ \mum}$; C-E = 10 µm.

acute base, 0-3-septate, wall hyaline. Ascoconidia sometimes present, globose, $1.5-2.5 \mu m$ diam.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: SOUTH CANTERBURY: Sharplin Falls, on dead wood, J. A. Cooper 8451, 25 May 2002, PDD 77467. TAUPO: Rangitoto Station, Ranginui Summit, on Coprosma foetidissima fallen twigs, P. R. Johnston D1611 & S. R. Whitton, 6 May 2001, PDD 73932; vic. Kiko Rd, near corner Hingapo & Tiraki Rd, on wood (not Nothofagus), P. R. Johnston D1593 & S. R. Whitton, 4 May 2001, PDD 73919, ICMP 15633; vic. Kiko Rd, near corner Hingapo & Tiraki Rd, on wood (not Nothofagus), P. R. Johnston

D1594 & S. R. Whitton, 4 May 2001, PDD 73914, ICMP 15634, 15626.

NOTES: All collections of *C. clavula* have small, solitary short-stipitate apothecia, often with a yellowish hymenium when dry. Microscopically they are distinctive with their long-cylindric to filiform, clavate ascospores. In culture the fungus is very slow growing (less than 20 mm diam. after 3 weeks), with intense blue-green pigment produced on malt extract agar.

All collections examined are on wood that is partly decomposed and soft. Although associated with extensive blue-green staining within the wood, the blue-green staining is rarely visible on the surface of the wood.

Chlorociboria colubrosa P.R.Johnst., sp. nov. Fig. 14, 15

DIAGNOSIS: Apothecia in ligno duro aerugineo. Apothecia 0.6–0.8 mm diam., hymenium nigrum ubi siccum. Excipulum ectale "textura porrecta". Hyphae tomentosae nullae. Asci (100–)130–145 × 6.5– 7.5μ m. Ascosporae 45–55(–60) × 2–2.5 μ m, filiformes, spirales.

HOLOTYPUS (here designated): New Zealand: Gisborne: Urewera National Park, Lake Waikaremoana, near Aniwaniwa, on *Nothofagus* sp., *G. J. Samuels* 82-218 & C. E. Samuels, 25 May 1982, PDD 44404.

ETYMOLOGY: Colubrosa = serpentine or winding, referring to the ascospore shape.

DESCRIPTION: Apothecia develop on decorticated wood stained blue-green (25D8-25F8) (infected wood hard, blue-green stain restricted to top few mm of wood). Apothecia 0.6-0.8 mm diam., stipitate or long stipitate; disc blue-green (25B7), black when dry, receptacle concolorous with disc, dark bluegreen when dry, glabrous; stipe $5-25 \times 0.2-0.3$ mm, cylindric or increasing slightly to base, becoming blackened, sometimes coarsely roughened near base. Ectal excipulum 70-80 µm thick, textura porrecta, elements oriented at low angle to receptacle surface (in the inner half) or at high angle to receptacle surface (in the outer half), comprising narrow cylindric cells or cylindric cells 5-8 µm diam., hyaline, gelatinous (embedded in hyaline gelatinous matrix); exciple overlaid with incomplete, 1-cell-wide layer of hyphae 3-5 µm diam. with walls thin, hyaline, sometimes encrusted with dark blue-green material, end cells of hyphal elements more or less free, swollen, fusoid, 5-6 µm diam. Medullary excipulum with parallel rows of hyphae 3-5 µm diam. with walls thin, hyaline, nongelatinous or refractive. Paraphyses 1.5 µm diam., apex slightly swollen to 3 µm diam., clavate or fusoid, extending 10-15 µm beyond asci, embedded in common gelatinous matrix. Asci (100-)130-145 × 6.5-7.5 µm, cylindric or subclavate, tapering slightly to rounded apex, wall slightly thickened at apex, apical pore amyloid; 8spored, spores filling upper 65-80 µm of ascus, basal stalk long. Ascospores $45-55(-60) \times 2-2.5 \ \mu m$, filiform, ends rounded, coiling on release, 0-septate, wall hvaline.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEA-LAND: BULLER: Rahu Saddle, on Nothofagus sp.



Fig. 15 *Chlorociboria colubrosa* (A–C, PDD 81294; D, PDD 44404). A, asci; B, apex of paraphyses, slightly swollen and sometimes embedded in gel; C, ascospores (PDD 81294); D, ascospores (PDD 44404); E, distribution of New Zealand collections examined. Scale bar: A–D = 20 μ m.



Fig. 16 *Chlorociboria duriligna* (A, PDD 60009; B–D, PDD 81282; E, PDD 44369; F, PDD 81281). A, apothecia on wood (from dried herbarium specimen); **B**, apothecium, vertical section; **C**, side of apothecium, vertical section; **D**, detail of C showing ectal excipulum and tomentum hyphae; **E**, tomentum hyphae, scalp section; **F**, ascospores. Scale bars: A = 1 mm; $B = 50 \text{ \mum}$; $C-F = 10 \text{ \mum}$.

decorticated wood, *P. R. Johnston D1834*, 15 May 2004, PDD 80577; Rahu Saddle, on *Nothofagus* sp. decorticated wood, *P. R. Johnston D1835*, 15 May 2004, PDD 81294.

NOTES: Macroscopically, *Chlorociboria colubrosa* is characterised by a long, robust stipe. The hyphae meandering across the surface of the receptacle are smooth-walled, with the end cells typically slightly swollen, often fusoid. See notes under *Chlorociboria spiralis* for differences between *C. colubrosa* and *C. spiralis*, another species with coiling ascospores.

The wood on which the specimens were found is decorticated and hard. The blue-green discoloration is extensive across the surface of the wood, but confined to the uppermost 2-3 mm of the wood.

Chlorociboria duriligna P.R.Johnst., sp. nov. Fig. 16, 17

DIAGNOSIS: Apothecia in ligno duro aerugineo. Apothecia 1.5–3 mm diam.; hymenium pallidum ubi siccum. Excipulum ectale "textura angularis" vel "textura prismatica". Hyphae tomentosae cylindricae vel spirales, parietibus verrucatis. Asci 75–85 × 5.5–7 µm. Ascosporae (9–)10.5–11.5(–15) × (1.5–)2–2.5(–3) µm, cylindricae, interdum leviter curvae, complanatae in latere uno, lattissimae versus extremum unum. HOLOTYPUS (here designated): New Zealand: Nelson: Spooner Saddle, reserve to north of main road, on decorticated wood, *P. R. Johnston D1812*, 13 May 2004, PDD 81278.

ETYMOLOGY: From durus = hard and lignum = wood, referring to the hard wood with which this species is associated, in contrast to the soft, rotten wood with which the morphologically similar *C. aeruginosa* is associated.

DESCRIPTION: Apothecia develop on decorticated wood (infected wood hard, blue-green stain restricted to top few mm of wood). Apothecia 3 mm diam. when fresh, 1.5-2.5 mm diam. when dry, stipitate; disc pale to dark blue-green when fresh, when dry off-white to translucent blue-green (25A3-25B6), receptacle slightly darker than hymenium, glabrous; stipe $0.3-0.6 \times 0.3-0.4$ mm, stipe cylindric, concolorous with receptacle, often darker at base. Ectal excipulum 40-50 µm thick, textura angularis or textura prismatica, elements oriented more or less perpendicular to receptacle surface, comprising short cylindric, angular or globose cells 6-10 µm diam. with walls thick, hyaline, refractive, outer rows encrusted with dark green material. Medullary excipulum textura intricata, comprising hyphae 2-2.5 µm diam. with walls thin, hyaline, nongelatinous. Tomentum hyphae macroscopically indistinct, $10-20 \times 3.5-5 \,\mu\text{m}$, cylindric with rounded apex, often coiling, walls thin, roughened. Subhymenium 30 µm thick. Subhymenium textura intricata, comprising hyphae 1.5-3 µm diam. with walls hyaline, slightly thickened, gelatinous. Paraphyses 1.5 µm diam., apex undifferentiated, about same length as asci. Asci 75-85 × 5.5-7 µm, cylindric or subclavate, tapering to subtruncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate or biseriate, spores filling upper 45-60 µm of ascus. Ascospores (9-)10.5-11.5(-15) \times (1.5–)2–2.5(–3) µm (mean 11.0 \times 2.2 µm, n = 51), oblong-elliptic or cylindric, ends rounded, tapering slightly to each end, widest point slightly toward one end, flat one side, straight or slightly curved, 0-septate, wall hyaline.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: BULLER: Lewis Pass, vic. Boyle Lodge, Nina River Track, on decorticated wood, *P. R. Johnston D518*, 11 May 1990, PDD 57558; Nelson Lakes National Park, Lake Rotoiti, St Arnaud Track, on decorticated wood, *G. J. Samuels 82-168*, *A. P. Hawthorne*, *P. R. Johnston & R. H. Petersen*, 13 May 1982, PDD 44369. NELSON: Spooner Saddle, reserve to north of main road, on decorticated wood, *P. R. Johnston D1813*, 13 May 2004, PDD 81279; Spooner Saddle,



Fig. 17 Chlorociboria duriligna (PDD 81281). A, asci; B, ascospores; C, tomentum hyphae; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \mu m$.

reserve to north of main road, on decorticated wood, *P. R. Johnston D1814*, 13 May 2004, PDD 81280; Spooner Saddle, reserve to north of main road, on decorticated wood, *P. R. Johnston D1815*, 13 May 2004, PDD 81281; Spooner Saddle, reserve to north of main road, on decorticated wood, *P. R. Johnston D1818*, 13 May 2004, PDD 81282.

Chlorociboria aeruginosa. USA: MAINE: White Mountains National Park, *A. Wilson AWW146*, 27 Sep 2003, PDD 81292. IOWA: Decorah, on rotten wood, *E. W. Holway*, PDD, Ellis North American Fungi No. 987.

NOTES: Chlorociboria duriligna is morphologically very similar to C. aeruginosa, a species not accepted for New Zealand in this paper. Chlorociboria aeruginosa differs in having ascospores a little wider (most spores $2.5-3 \mu m$ wide) and in being associated with



Fig. 18 *Chlorociboria halonata* (A, PDD 64635; B, D, E, PDD 73933; C, PDD 77464; F, PDD 74098). A, apothecia on wood (from dried herbarium specimen); **B**, side of apothecium showing medullary excipulum and ectal excipulum, vertical section; **C**, tomentum hyphae, squash mount; **D**, detail of B showing ectal excipulum and tomentum hyphae; **E**, detail of B, showing a group of encrusted hyphae; **F**, ascospores. Scale bars: A = 1 mm; B-F = 10 µm.

soft, rotten wood, which is extensively stained bluegreen. *Chlorociboria duriligna* is consistently associated with decorticated wood which has retained its hardness and with the blue-green stain confined to the upper 2–3 mm of wood. Although these morphological and ecological differences appear to be slight, the ITS sequences of the two species are quite distinct, differing at 11% of the sites (see Molecular results and analyses, below).

In culture, growth is slow, 10–15 mm diam. after 4 weeks. On CMD the felt-like aerial mycelium was bright blue-green, otherwise no green pigments were seen. On OA and PDA reddish pigments were formed in reverse, and a reddish yellow pigment diffused into the agar surrounding the colonies on PDA.

Chlorociboria halonata P.R.Johnst., sp. nov Fig. 18, 19

DIAGNOSIS: Apothecia in ligno duro aerugineo vel atroveneto; 1.5–3 mm diam.; margine pruinato. Excipulum ectale "textura angularis" vel "textura prismatica". Hyphae tomentosae spirales, parietibus verrucatis. Asci 70–100 × 7.5–8.5 μ m. Ascosporae (16–)18.5–22(–24.5) × 2.5–3(–3.5) μ m, ellipticifusiformis, complanatae in latere uno. HOLOTYPUS (here designated): New Zealand: Southland: Catlins, 5 km north of Papatowai, Table Hill Reserve, on *Nothofagus* sp. decort. wood, *P. R. Johnston D1196*, 9 May 1995, PDD 64635 (culture from type ICMP 15622).

ETYMOLOGY: From halonatus = surrounded by an outer circle, referring to the white, halo-like appearance of the tomentum hyphae at the edge of the apothecia.

DESCRIPTION: Apothecia develop on decorticated wood or bark of fallen branches, stained blue-green to black (infected wood hard, blue-green stain mostly restricted to top few mm of wood, but in some cases restricted, internal blue-green staining associated with the wood vessels). Apothecia 1.5-3 mm diam., short stipitate or stipitate; disc blue-green when fresh, variable when dry, translucent vellowish (1A4-1B4) to dark grey to black, receptacle dark blue-green (25B8-25D8) or black, if black then with paler zone near edge of cup, edge of cup itself with whitish bloom; stipe $0.5-0.7 \times 0.15-0.2$ mm, cylindric or tapering to base, dark blue-green to black near base. Ectal excipulum 50-65 µm thick, textura angularis or textura prismatica, elements oriented at high angle to receptacle surface, comprising broad cylindric cells 4-7.5 µm diam. with walls slightly thickened, hyaline, refractive, thickly encrusted with dark green material. Medullary excipulum textura intricata, comprising hyphae 2-3 µm diam. with walls thin, hyaline, nongelatinous, encrusted patchily with dark green material. Tomentum hyphae macroscopically indistinct, $10-25 \times 2-3 \mu m$, coiling, walls thin, roughened, hyaline. Subhymenium textura intricata, comprising hyphae 1.5-2 µm diam. with walls hyaline, thin, nongelatinous. Paraphyses 1.5 µm diam., apex undifferentiated or slightly swollen to 1.5-2.5 µm diam., about same length as asci. Asci 70–100 \times 7.5–8.5 µm, cylindric or subclavate, tapering slightly to rounded or subtruncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, biseriate, spores filling upper 45-60 µm of ascus. Ascospores (16-)18.5- $22(-24.5) \times 2.5 - 3(-3.5) \mu m$ (mean $20.2 \times 2.9 \mu m$, n = 34), fusiform, ends more or less acute, flat one side, straight to slightly curved, tapering evenly to more or less acute ends, 0-septate, wall hyaline, thin, smooth. Ascoconidia sometimes present, globose, 2-2.5 µm diam.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: BAY OF PLENTY: vic. Ruatahuna, near Te Waiiti, Tuhoe Tuawhenua Trust forest, on bark of fallen branch, *P. R. Johnston*, 14 Oct 2003, PDD 78031.



Fig. 19 Chlorociboria halonata (PDD 71675). A, ascus; B, ascospores; C, tomentum hyphae; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \mu m$.

FIORDLAND: Lake Hauroko, lakeshore to the west of the car park, on *Nothofagus* sp. decort wood, *P. R. Johnston D1734 & S. R. Whitton*, 3 May 2002, PDD 77463. SOUTHLAND: vic. Tuatapere, toward Bluecliffs Beach Rd, on decort wood, *P. R. Johnston D1739 & S. R. Whitton*, 4 May 2002, PDD 77464. STEWART ISLAND: Ulva I., track between West End and Landing, on decorticated wood, *P. R.*



Fig. 20 *Chlorociboria macrospora* (A, PDD 73994; B–F, PDD 73935). **A**, apothecia on wood (from dried herbarium specimen), arrows indicate apothecia; **B**, side of apothecium showing medullary excipulum and ectal excipulum, vertical section; **C**, detail of B, medullary excipulum; **E**, detail of B, ectal excipulum; **E**, tomentum hyphae, squash mount; **F**, ascospores. Scale bars: A = 1 mm; B–F = 10 µm.

Johnston D1665, R. A. B. Leschen & S. R. Whitton, 24 Apr 2002, PDD 77462, TAUPO: Rangitoto Station, Ranginui Summit, on Coprosma foetidissima fallen twigs, P. R. Johnston D1626 & S. R. Whitton, 6 May 2001, PDD 74098; Rangitoto Station, Ranginui Summit, on Coprosma foetidissima fallen twigs, P. R. Johnston D1625 & S. R. Whitton, 6 May 2001, PDD 73984; Rangitoto Station, Ranginui Summit, on Coprosma foetidissima fallen twigs, P. R. Johnston D1624 & S. R. Whitton, 6 May 2001, PDD 73981; Rangitoto Station, Ranginui Summit, on Coprosma foetidissima fallen twigs, P. R. Johnston D1612 & S. R. Whitton, 6 May 2001, PDD 73934, ICMP 15645; Rangitoto Station, Ranginui Summit, on Coprosma foetidissima, P. R. Johnston D1610 & S. R. Whitton, 6 May 2001, PDD 73933; Rangitoto Station, Zig Zag Track, on decort. wood, P. R. Johnston D1530, 25 May 2000, PDD 71610, ICMP 15625; vic. Kiko Rd, near corner Mangatera Rd, on Nothofagus sp. wood, P. R. Johnston D1600 & S. R. Whitton, 4 May 2001, PDD 73930, ICMP 15637; Tongariro National Park, Ohakune Mountain Road, Mangawhero River campground, beside stream, on Coriaria arborea fallen wood, P. R. Johnston D1360, R. E. Beever & E. H. C. McKenzie, 20 May 1998, PDD 70091, WAIKATO: vic. Awakino. Steuart Russell Awakino Beech Reserve, on Nothofagus truncata decort. wood, P. R. Johnston D1553, 11 Jul 2000, PDD 71675, ICMP 15627. WELLINGTON: Kaitoke Regional Park, on Coriaria arborea fallen twigs, P. R. Johnston D1290, 7 May 1997, PDD 77461, ICMP 15623; York Bay, on rotten wood, E. J. Butler, 27 Jul 1923, PDD 1209. NOTES: Macroscopically C. halonata varies with respect to the length of the stipe. Some collections have more or less sessile apothecia, while in others the stipe is quite prominent. Whenever a stipe

is present, it is narrow. In many collections the

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tomentum hyphae form a distinctive, white, halo-like bloom around the edge of the cup. Microscopically this species is distinguished by its copious roughwalled tomentum hyphae and fusiform ascospores, which are acute to each end.

In most collections *C. halonata* is associated with hard, decorticated wood. The surface of the wood is typically extensively stained dark blue-green to black. The surface staining is usually confined to the uppermost 1-2 mm of wood. Internally the wood is also extensively stained, but the staining is restricted to discrete zones and plates, often associated with vessels running through the wood. In several collections the wood has been extensively tunneled by insect larvae. In one of these collections, where the detritus left by the larvae was still packing the tunnels, the larvae appeared to have avoided tunneling through the parts of the wood that had been stained by the fungus.

Chlorociboria macrospora P.R.Johnst., sp. nov. Fig. 20, 21

DIAGNOSIS: Apothecia in ligno duro aerugineo. Apothecia 0.8–2 mm diam.; hymenium luteum ubi siccum; receptaculum pruinato. Excipulum ectale "textura angularis" vel "textura prismatica". Hyphae tomentosae cylindricae vel spirales, parietibus verrucatis. Asci (100–)120–140 × 11–13 µm. Ascosporae (21.5–)24–26(–27) × (4.5–)5–5.5(–6) µm, oblongo-ellipticae, leviter curvae, complanatae in latere uno.

HOLOTYPUS (here designated): New Zealand: Gisborne: vic. Ruatahuna, main road to Papatotara Ridge, on *Nothofagus* sp. wood, *P. R. Johnston D1641 & S. R. Whitton*, 7 May 2001, PDD 73994.

ETYMOLOGY: Macrospora refers to the size of the ascospores, the largest for all known species of *Chlorociboria*.

DESCRIPTION: Apothecia develop on decorticated wood, associated with blue-green staining (infected wood hard, blue-green stain mostly restricted to top few mm of wood). Apothecia 0.8-2 mm diam., stipitate; disc when dry dull yellow (2B8–3B8), surface often pruinose, receptacle when fresh dark bluegreen, when dry blue-green (25B7–25D7), often with a whitish bloom; stipe $0.3-0.8 \times 0.3-0.5$ mm, short and broad cylindric, often coarsely roughened and warted. Ectal excipulum 50 µm thick, textura angularis or textura prismatica, elements oriented at high angle to receptacle surface, comprising cylindric or angular cells 4.5-6.5 µm diam. with walls slightly thickened, hyaline, refractive or gelatinous,



Fig. 21 *Chlorociboria macrospora* (A, PDD 73935). A, ascus; B, ascospores; C, tomentum hyphae; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \ \mu m$.

outermost 2–3 rows encrusted with dark green material. Medullary excipulum textura intricata oriented more or less parallel with receptacle surface, comprising hyphae 2–3 μ m diam., walls hyaline, gelatinous. Tomentum hyphae macroscopically indistinct,



Fig. 22 Chlorociboria pardalota (A, PDD 71612; B–F, H, PDD 71611; G, PDD 45488). **A**, apothecium on wood (from dried herbarium specimen); **B**, side of apothecium, vertical section; **C**, detail of B, showing ectal excipulum and clumps of encrusted hyphae; **D**, detail of C; **E**, outer ectal excipulum and tomentum hyphae, vertical section; **F**, medullary excipulum, vertical section; **G**, encrusted hyphae on surface of receptacle, squash mount; **H**, ascospores. Scale bars: A = 1 mm; B = 50 µm; C–H = 10 µm.

 $6.5-15 \times 2.5-5.5 \,\mu\text{m}$, cylindric, loosely coiling, walls thin, roughened, hyaline. Subhymenium textura intricata, comprising hyphae 2-3 µm diam. with walls hyaline, slightly thickened, gelatinous. Paraphyses 2-2.5 µm diam., apex slightly swollen, 3-3.5 µm diam., extending 10-15 µm beyond asci. Asci (100–)120–140 \times 11–13 μ m, cylindric or subclavate, tapering to subtruncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate or biseriate, spores filling upper 100-115 µm of ascus. Ascospores (21.5-)24-26(-27) × (4.5–)5–5.5(–6) μ m (mean 24.6 × 5.3 μ m, n = 42), oblong-elliptic, ends rounded, flattened one side in side view, curved, tapering more or less evenly to both rounded ends, or one end slightly more broadly rounded than the other, 0-septate, wall hyaline.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: NELSON: Abel Tasman National Park, Canaan Downs Rd, on decorticated wood Nothofagus sp., P. R. Johnston D1826, 14 May 2004, PDD 80550. TAUPO: vic. Kiko Rd, Tiraki Rd, on Nothofagus sp. wood, P. R. Johnston D1590 & S. R. Whitton, 3 May 2001, PDD 73961; vic. Kiko Rd, Tiraki Rd, on Nothofagus sp. wood, P. R. Johnston D1572 & S. R. Whitton, 3 May 2001, PDD 73947; vic. Kiko Rd, Tiraki Rd, on Nothofagus sp. wood, P. R. Johnston D1571 & S. R. Whitton, 3 May 2001, PDD 73935.

NOTES: Large ascospores and coarsely roughened tomentum hyphae distinguish *Chlorociboria mac-rospora* from other species. Some collections of *C. halonata* have spores of similar length, but they are narrower and acute to the ends.

All collections of *C. macrospora* have been found on wood that is decorticated but still hard and largely intact. The surface of the wood is either blue-green in colour or dark grey to blackish. When dark grey to blackish, there is a blue-green zone immediately below the surface. The blue-green staining is largely confined to the upper 3–4 mm of the wood, but in some collections it also extends as narrow blue-green zones through the wood. In one collection, the staining is also present along the sides of tunnels made through the wood by insect larvae.

Chlorociboria pardalota P.R. Johnst., sp. nov. Fig. 22, 23

DIAGNOSIS: Apothecia in ligno molli aerugineo. Apothecia 2–4.5 mm diam.; hymenium luteum ubi siccum. Excipulum ectale "textura prismatica" vel "textura intricata". Hyphae tomentosae spirales, parietibus laevibus; interdum nullae. Asci 90–105 $(-120) \times 8-9 \ \mu$ m. Ascosporae (11-)13.5-15.5



Fig. 23 Chlorociboria pardalota (PDD 71611). A, ascus; B, ascospores; C, tomentum hyphae; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \mu m$.

 $(-17.5) \times (3-)4-4.5 \ \mu\text{m}$, cylindricae vel ellipticae, complanatae in latere uno.

HOLOTYPUS (here designated): New Zealand: Waikato: vic. Awakino, Steuart Russell Awakino Beech Reserve, on *Nothofagus truncata* decorticated wood, *P. R. Johnston D1542*, 25 May 2000, PDD 71612.

ETYMOLOGY: From the Greek pardalotos, spotted like a leopard, referring to the spotted patterning of the receptacle.



Fig. 24 Chlorociboria poutoensis (PDD 60009). **A**, apothecia on wood (from dried herbarium specimen); **B**, side of apothecium showing medullary excipulum, ectal excipulum, and tomentum hyphae, vertical section; **C**, detail of B showing ectal excipulum and tomentum hyphae; **D**, tomentum hyphae, squash mount; **E**, ascospores. Scale bars: A = 1 mm; B-E = 10 µm.

DESCRIPTION: Apothecia develop on decorticated wood, associated with blue-green staining (infected wood soft, staining restricted to narrow plates deep within wood), arising from basal stroma in groups of up to 4 apothecia. Apothecia 2–4.5 mm diam., stipitate; disc very pale blue-green when fresh, bright mustard yellow (4B7) when dry, receptacle when mature usually pale yellow with scattered, small, dark blue-green flecks, darker near edge of cup, darker when immature, glabrous; stipe $0.6-2 \times 0.5-1.5$ mm, tapering to base or cylindric, dark blue-green to black, coarsely roughened, with deep, vertical, ridge-like folds. Ectal excipulum $60-75 \mu$ m thick, textura prismatica or textura intricata, elements oriented more or less perpendicular to receptacle surface, comprising hyphae $4.5-6.5 \,\mu\text{m}$ diam. with walls thick (about $1.5 \,\mu\text{m}$), hyaline, refractive (with poorly developed intercellular gelatinous layer), outermost layer encrusted in scattered patches with dark green material; exciple overlaid with incomplete, 1-cell-wide layer of hyphae $4-5 \,\mu\text{m}$ diam. with walls thin, hyaline, encrusted with dark green material. Medullary excipulum textura intricata, comprising hyphae $4-5 \,\mu\text{m}$ diam. with walls thin, hyaline, scattered, sometimes lacking, $8-12 \times 2-3 \,\mu\text{m}$, cylindric, coiling, walls thin, smooth, hyaline. Subhymenium textura intricata, comprising hyphae $2-2.5 \,\mu\text{m}$ diam. with walls hyaline, gelatinous. Paraphyses 1.5 μm diam.

apex undifferentiated, about same length as asci. Asci 90–105(–120) × 8–9 μ m, subclavate, tapering slightly to rounded apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate, spores filling upper 70–95 μ m of ascus, basal stalk short. Ascospores (11–)13.5–15.5(–17.5) × (3–)4–4.5 μ m (mean 14.6 × 4.1 μ m, *n* = 44), cylindric, ends rounded, in side view flattened one side, not curved, widest point slightly toward one end, tapering slightly to both rounded ends, 0-septate, wall hyaline. Ascoconidia seen occasionally, shortcylindric, about 5.5 × 2 μ m.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: AUCKLAND: Swanson, on Leptospermum sp. decaying wood, G. J. Samuels GJS 81-84, P. R. Johnston & E. Horak, 6 May 1981, PDD 45488. NELSON: Abel Tasman National Park, near Harwood's Hole, Rameka Track, on Nothofagus sp. decort. wood, P. R. Johnston D1847, 14 May 2004, PDD 81272. TAUPO: Kaimanawa Forest Park, Clement Mill Rd, on fallen wood Nothofagus menziesii, K. Hosaka, 22 May 2004, PDD 80328. WAIKATO: vic. Awakino, Steuart Russell Awakino Beech Reserve, on Nothofagus truncata decort. wood, P. R. Johnston D1541, 25 May 2000, PDD 71611; near Te Awamutu, Mt Pironga, on decorticated wood, G. J. Samuels & C. E. Samuels, 27 Mar 1982, PDD 44276.

NOTES: *Chlorociboria pardalota* is macroscopically distinctive when mature, with a pale hymenium that dries yellow, and the sides of the receptacle pale yellow with scattered, small, dark blue-green patches. The stipe is very dark and coarsely roughened. Before becoming fully expanded the immature apothecia are uniformly dark blue-green. Typically several apothecia arise from a single basal stromatic mass.

Microscopically *C. pardalota* is similar to *C. awakinoana* and *C. spathulata* with all three species having ascospores of similar size and shape. *Chlorociboria awakinoana* and *C. spathulata* both have solitary apothecia with a dark blue-green hymenium (black when dry), and a dark blue-green receptacle. See notes under *C. awakinoana* on differences between *C. awakinoana* and *C. spathulata*.

All collections of *C. pardalota* have been on soft, decomposing wood. Although the associated bluegreen staining extends deep into the wood, it appears to be confined to narrow plates within the wood.

Chlorociboria poutoensis P.R. Johnst., sp. nov.

Fig. 24, 25

DIAGNOSIS: Apothecia in ligno duro aerugineo. Apothecia 0.8–1.5 mm diam.; hymenium album, luteum



Fig. 25 *Chlorociboria poutoensis* (A, B, D, PDD 60009; C, PDD 59170). A, ascus; B, ascospores; C, ascospores; D, tomentum hyphae; E, distribution of New Zealand collections examined. Scale bar: $A-D = 20 \mu m$.

ubi siccum. Excipulum ectale "textura prismatica". Hyphae tomentosae spirales, parietibus verrucatis. Asci 95–120 × 8–9 µm. Ascosporae (12–)13.5– $15(-17) \times (3-)3.5-4$ µm, oblongo-ellipticae, leviter curvae, complanatae in latere uno, lattissimae versus extremum unum.

HOLOTYPUS (here designated): New Zealand: Northland: North Kaipara Heads, vic. Pouto, Tapu Bush, on decorticated wood, *P. R. Johnston D724*, 22 May 1991, PDD 59170.

ETYMOLOGY: Poutoensis refers to Pouto, the locality from which the type specimen was collected.



Fig. 26 *Chlorociboria procera* (A–F, PDD 77453; G, PDD 70094). A, apothecium on wood (from dried herbarium specimen); **B**, stipe, showing roughly warted surface; **C**, side of receptacle showing medullary excipulum and ectal excipulum, vertical section; **D**, detail of C showing medullary excipulum and ectal excipulum; **E**, detail of C showing medullary excipulum and ectal excipulum; **E**, detail of C showing ectal excipulum and outer layer of encrusted hyphae; **F**, encrusted hyphae on surface of receptacle, squash mount. **G**, ascospores. Scale bars: A, B = 1 mm; C–G = 10 μ m.

DESCRIPTION: Apothecia develop on decorticated wood stained blue-green (infected wood hard, blue-green stain restricted to top few mm of wood). Apothecia 0.8–1.5 mm diam. when dry, short stipitate; disc

white to slightly greenish when fresh, bright yellow (2A5–3A5) when dry, receptacle dark blue-green (25D7–8), glabrous; stipe $0.3-0.4 \times 0.3-0.4$ mm when dry, short, more or less concolorous with

receptacle near top, black towards base. Ectal excipulum 35-45 µm thick, textura prismatica, elements oriented more or less perpendicular to receptacle surface, comprising short cylindric cells 3-7 µm diam., with walls slightly thickened, hyaline, refractive, encrusted irregularly, with dark green material. Medullary excipulum textura intricata, comprising hyphae 2.5 µm diam. with walls thin, hyaline, nongelatinous. Tomentum hyphae macroscopically indistinct, $10-25 \times 2-3 \mu m$, coiling, walls thick, roughened. Subhymenium 10-15 µm thick, textura intricata comprising hyphae 1.5-2 µm diam. with walls hyaline, thin, nongelatinous. Paraphyses 1-2 µm diam., apex undifferentiated or slightly swollen, about same length as asci. Asci 95–120 \times 8–9 µm, cylindric or subclavate, tapering slightly to subtruncate apex, wall slightly thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate, spores filling upper 70-80 µm of ascus. Ascospores $(12-)13.5-15(-17) \times (3-)3.5-4 \mu m$ (mean 14.3 \times 3.7 μ m, n = 36), oblong-elliptic, ends rounded, flat one side, slightly curved in side view, tapering to rounded ends, widest point toward one end, 0-septate, wall hyaline.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: AUCKLAND: Waitakere Ranges, Spraggs Bush, on decorticated wood, *P. R. Johnston D788*, 17 Mar 1992, PDD 60009, ICMP 15618; Waitakere Ranges, Parau Dam, on *Leptospermum* sp. decorticated wood, *P. R. Johnston D789*, 17 Mar 1992, PDD 60014.

NOTES: Macroscopically *Chlorociboria poutoensis* is similar to *C. albohymenia*, both having a hymenium more or less white when fresh and bright yellow when dry. Both species also have rough-walled to-mentum hyphae, although those of *C. albohymenia* are short-cylindric to globose compared with the longer, coiling tomentum hyphae of *C. poutoensis*. The two species differ in ascospore size and shape. See also notes under *C. albohymenia*.

Chlorociboria poutoensis has been consistently found in association with hard, decorticated wood, the blue-green pigmentation confined to the uppermost 2–3 mm of wood.

Chlorociboria procera P.R.Johnst., sp. nov.

Fig. 26, 27

DIAGNOSIS: Apothecia in ligno duro aerugineo. Apothecia 0.5–4 mm diam., stipete longo, verrucato; hymenium nigrum ubi siccum. Excipulum ectale "textura angularis" vel "textura prismatica". Hyphae tomentosae nullae. Asci $60-85 \times 5-6.5 \mu m$. Ascosporae (12–)14–15.5(–17) × 2–2.5(–3) μm ,



elliptici-fusiformis, complanatae in latere uno, leviter curvae.

HOLOTYPUS (here designated): New Zealand: Westland: vic. Haast, Jackson Bay Rd, about 5 km past Cascades turnoff, on decorticated wood and bark of fallen branch *Nothofagus* sp., *A. Oliver*, 8 May 2002, PDD 77453.

ETYMOLOGY: Procera = tall and slender, referring to the long, narrow stipe.

DESCRIPTION: Apothecia develop on decorticated, blue-green stained wood or erumpent through bark of fallen branches (infected wood hard, blue-green stain restricted to top few mm of wood). Apothecia 0.5–4 mm diam., long stipitate; disc pale to dark



blue-green, black when dry, receptacle dark bluegreen, glabrous; stipe $1-4 \times 0.2-0.3$ mm, long and thin cylindric, dark blue-green to black near base, with roughly warted appearance, as though with groups of appressed hairs. Ectal excipulum 30 µm thick, textura angularis or textura prismatica, elements oriented at high angle to receptacle surface, comprising broad cylindric cells or angular cells 5-10 µm diam. with walls slightly thickened, hyaline, gelatinous; exciple overlaid with incomplete, 1-cellwide layer of hyphae 4-5 µm diam. with walls thin, hyaline, encrusted finely with dark green material. Medullary excipulum comprising more or less parallel rows of long cylindric cells 2.5-4 µm diam. with walls thin, hyaline, nongelatinous, encrusted finely and irregularly with dark material. Subhymenium textura intricata, comprising hyphae 1.5-2.5 µm diam. with walls hyaline, thin, nongelatinous. Paraphyses 2 µm diam., apex undifferentiated, about same length as asci. Asci $60-85 \times 5-6.5 \mu m$, cylindric or subclavate, tapering slightly to subtruncate apex, wall slightly thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate or biseriate, spores filling upper 45-50 µm of ascus. basal stalk short. Ascospores (12-)14-15.5(-17) × $2-2.5(-3) \mu m$ (mean 14.6 × 2.4 μm , n = 44), fusoid, ends more or less acute, flat one side, straight to often curved, tapering more or less uniformly to each end, 0-septate, wall hyaline, thin. Ascoconidia rarely seen, globose, 2-3 µm diam.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: BULLER: Rahu Saddle, on Nothofagus sp. decorticated wood, P. R. Johnston D1833, 15 May 2004, PDD 80576. GISBORNE: Urewera National Park, Lake Waikaremoana, Waikareiti Track, on wood, G. J. Samuels GJS 81-313, A. P. Hawthorne, P. R. Johnston, E. Horak & R. H. Petersen, 24 May 1981, PDD 49565; Urewera National Park, Lake Waikaremoana, near Aniwaniwa, G. J. Samuels GJS 82-181 & C. E. Samuels, 23 May 1982, PDD 44379; Urewera National Park Headquarters, on dead wood, P. R. Johnston & E. Horak, 27 May 1981, PDD 40878; Urewera National Park, Manuoha Track, P. R. Johnston, 8 May 2001, PDD 74284; Urewera National Park, Manuoha Track, on decort. wood, P. R. Johnston D1650, 8 May 2001, PDD 74257; Urewera National Park, track to Lake Waikareiti, on Nothofagus sp. decorticated wood, R. E. Beever 2419, 20 May 2004, PDD 81148; vic. Ruatahuna, Papatotara Ra., south side of main road, on fallen wood, P. R. Johnston D1640, S. R. Whitton, 7 May 2001, PDD 74093. MARLBOROUGH SOUNDS: Mt Somers Track, on fallen wood Nothofagus *menziesii*, *P. R. Johnston D1315*, 10 May 1997, PDD 70094. TAUPO: Kaimanawa Forest Park, Clements Mill Road, on *Nothofagus* sp. decort. wood, *P. R. Johnston D262*, 6 May 1987, PDD 48770. WESTLAND: vic. Haast, Roaring Billy Track, on decort. wood, *P. R. Johnston*, 9 May 2002, PDD 75783; vic. Haast, Jackson Bay Rd, 5 km past Cascades turnoff, on decort wood, *S. R. Whitton*, 7 May 2002, PDD 77454; vic. Haast, Jackson Bay Rd, 5 km past Cascades turnoff, on decort wood, *J. Frohlich*, 7 May 2002, PDD 77455; vic. Haast, near end of Cascades Rd, on decort. wood, *P. R. Johnston D1748*, 7 May 2002, PDD 75710.

NOTES: Chlorociboria procera is macroscopically distinctive, with a long, thin stipe, which is characteristically roughened with coarse, scale-like elements. The apothecia lack tomentum hyphae, the outer layer of the receptacle comprising nongelatinous hyphal elements with walls encrusted with dark green material. All other species that lack tomentum hyphae have ascospores broadly rounded at each end; *C. procera* has ascospores more or less acute to each end.

Apothecia develop singly on blue-green stained bark and decorticated wood. All collections are on twigs, or on larger pieces of wood that have been extensively tunnelled by insects. The wood is always hard, and the blue-green staining is restricted to a thin (up to 0.2 mm) layer. For most collections the blue-green staining is confined to the surface layer of the exposed wood or bark; however, in PDD 75783 the blue-green staining is in a 0.2-mm-wide layer beneath the surface of the wood, forming a zone-line like pattern within the wood.

Chlorociboria spathulata P.R.Johnst., sp. nov.

Fig. 28, 29 DIAGNOSIS: Apothecia in ligno molli aerugineo. Apothecia 0.7–2.5 mm diam.; hymenium nigrum ubi siccum. Excipulum ectale "textura globosa" vel "textura prismatica". Hyphae tomentosae spirales, parietibus laevibus; interdum nullae. Asci 95–120(–130) × 8.5–10 μ m. Ascosporae (13.5–)14.5–16.5(–17.5) × (4–)4.5–5 μ m, cylindricae vel oblongo-ellipticae,

HOLOTYPUS (here designated): New Zealand: Nelson: Abel Tasman National Park, track to Harwood's Hole, on *Nothofagus* sp. decorticated wood, *P. R. Johnston D1842, H. Burdsall*, 14 May 2004, PDD 80552.

leviter curvae, complanatae in latere uno.

ETYMOLOGY: Spathulata = spathulate, referring to the shape of the ends of the hyphae on the surface of the receptacle.



Fig. 28 *Chlorociboria spathulata* (A, PDD 77446; B–E, G, PDD 77695; F, PDD 77699). **A**, apothecia on wood (from dried herbarium specimen); **B**, side of receptacle, vertical section; **C**, detail of B showing medullary excipulum and ectal excipulum; **D**, detail of C; **E**, hyphae with swollen apices on surface of receptacle, squash mount; **F**, tomentum hyphae, squash mount; **G**, ascospores. Scale bars: A = 1 mm; B = 50 µm; C-G = 10 µm.

DESCRIPTION: Apothecia develop on soft, rotten decorticated wood, extensively blue-green stained internally, although the surface may be unstained. Apothecia 0.7–2.5 mm diam., stipitate; disc bluegreen when fresh, black when dry, receptacle dark blue-green, glabrous; stipe $0.3-0.8 \times 0.3-0.7$ mm, cylindric, dark blue-green to black. Ectal excipulum 35–45 μ m thick, textura globosa or textura prismatica, elements oriented at high angle to receptacle surface, comprising short cylindric cells or globose cells 5–6.5 μ m diam. (inner layers with cells up to 15 μ m long, toward the outside more or less globose) with walls thick, hyaline, gelatinous; exciple overlaid with more or less complete



Fig. 29 Chlorociboria spathulata (A, B, PDD 70084; C, PDD 77469). A, ascus; B, ascospores; C, tomentum hyphae; D, distribution of New Zealand collections examined. Scale bar: $A-C = 20 \mu m$.

1-cell-wide layer of hyphae $2.5-3 \mu m$ diam. with walls thin, pale brown, swollen, fusoid or swollen, clavate, ends of hyphae $4.5-6 \mu m$ diam. Medullary excipulum more or less parallel, loosely tangled, comprising hyphae $3-3.5 \mu m$ diam. with walls thin, hyaline, nongelatinous. Tomentum hyphae

macroscopically indistinct (may be lacking in some collections), $15-20 \times 2-3 \mu m$, coiling, walls thin, smooth, hyaline. Subhymenium textura intricata, comprising hyphae 2-3 µm diam. with walls hyaline, thin, nongelatinous. Paraphyses 1.5-2 µm diam., apex undifferentiated or slightly swollen, about same length as asci. Asci $95-120(-130) \times$ 8.5-10 µm, subclavate, tapering slightly to subtruncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, uniseriate or biseriate, spores filling upper 70-80 µm of ascus. Ascospores $(13.5-)14.5-16.5(-17.5) \times (4-)4.5-$ 5 μ m (mean 15.6 × 4.5 μ m, n = 38), oblong-elliptic or cylindric, ends rounded, in side view flattened one side, slightly curved, tapering slightly to both broadly rounded ends, uniform in shape to both ends, 0septate, wall hvaline.

ADDITIONAL SPECIMENS EXAMINED: NEW ZEALAND: FIORDLAND: Lake Hauroko, lake shore near car park, on Nothofagus sp. decorticated wood, P. R. Johnston D1725 & S. R. Whitton, 3 May 2002, PDD 77695; lake shore near car park, on Nothofagus sp. decorticated wood, P. R. Johnston D1736 & S. R. Whitton, 3 May 2002, PDD 77466; lake shore near car park, on Nothofagus sp. decorticated wood, P. R. Johnston D1732 & S. R. Whitton, 3 May 2002, PDD 77465. MID CANTERBURY: Kowai Bush, on Nothofagus solandri var. cliffortioides dead decorticated wood, J. A. Cooper 8459, 26 May 2002, PDD 77469. vic. Springfield, Kowai Bush Reserve, on Nothofagus solandri, P. R. Johnston D1165, 5 May 1995, PDD 64919, ICMP 15621. NELSON: Abel Tasman National Park, Takaka Hill, Harwood's Hole, on Nothofagus sp. decorticated wood, P. R. Johnston & E. M. Gibellini, 28 May 1989, PDD 70123; Abel Tasman National Park, Canaan Road, on Nothofagus sp. decorticated wood, P. R. Johnston D1822, 14 May 2004, PDD 80574. TAUPO: Tongariro National Park, Ohakune Mountain Road, Mangawheroa River campground, beside stream, on decortictaed partly rotten wood, P. R. Johnston D1365, R. E. Beever & E. H. C. McKenzie, 20 May 1998, PDD 70084.

NOTES: Chlorociboria spathulata is macroscopically and microscopically very similar to C. awakinoana. The two species are distinguished by small differences in ascospore size and shape and in the shape of the outer excipular hyphae (see notes under C. awakinoana).

Chlorociboria spathulata has been found typically on partly rotten, soft, decorticated wood. In most cases there is little blue-green pigment visible on the surface of the wood, although internally the wood is extensively coloured.



Fig. 30 *Chlorociboria spiralis* (PDD 77771). **A**, apothecia on wood (from dried herbarium specimen); **B**, apothecium, vertical section; **C**, side of receptacle showing medullary excipulum, ectal excipulum, and tomentum hyphae; **D**, ectal excipulum and tomentum hyphae, vertical section; **E**, ectal excipulum, vertical section; **F**, ectal excipulum and tomentum hyphae, vertical section; **E**, ascospores. Scale bars: A = 1 mm; B = 50 µm; C-G = 10 µm.

Chlorociboria spiralis P.R. Johnst., sp. nov.

Fig. 30, 31 DIAGNOSIS: Apothecia in ligno duro atroveneto. Apothecia 0.3–0.7; hymenium croceum ubi siccum. Excipulum ectale angustum, cellulis globosis vel angularibus. Hyphae tomentosae cylindricae, parietibus verrucatis. Asci $95-105 \times 7.5-8.5 \mu$ m. Ascosporae $50-60 \times 1.5-2 \mu$ m, filiformes, spirales. HOLOTYPUS (here designated): New Zealand: Buller: Rahu Saddle, vic. Newcombe Creek, on decorticated wood *Nothofagus* sp., *P. R. Johnston D1762* & *E. M. Gibellini*, 20 Sep 2002, PDD 77771.

ETYMOLOGY: Spiralis = coiled or spiralled, referring to the ascospore shape.

DESCRIPTION: Apothecia develop on decorticated wood stained dark blue-green (25C8-25F8) (infected wood hard, blue-green stain restricted to top few mm of wood). Apothecia 0.3-0.7 mm diam., narrow stipitate; disc when dry orange brown (6B7), receptacle variable, due either to age or to exposure to the enviorment, receptacle either of similar colour to hymenium, overlaid with often dense off-white (4A2), matted, hair-like elements, or the hair-like elements are thickly encrusted with dark green material (25D7-25D8) with surface of receptacle hidden, in this case the hair-like elements around margin of disc retain their whitish colour, glabrous or scurfy; stipe $0.4-0.6 \times 0.1-0.15$ mm, narrow cylindric, when dry concolorous with receptacle, usually black near the base. Ectal excipulum 20-30 µm thick, comprising a poorly developed, 3-4 cells wide layer, elements mostly oriented at low angle to receptacle surface, but end cells of elements short, more or less globose, and these with higher orientation, excipular cells short-cylindric, angular or globose, 8-12 µm diam. with walls slightly thickened, hyaline, refractive, outer 1-3 rows of cells encrusted thickly with dark green material. Medullary excipulum more or less parallel to loosely tangled, comprising hyphae 2-3 µm diam. with walls thin, hyaline, nongelatinous. Tomentum hyphae macroscopically indistinct, $15-45 \times 3-4 \mu m$, cylindric with rounded apex, occasionally septate, walls thin, roughened, hyaline, in places encrusted with dark green material. Subhymenium textura intricata, comprising hyphae 2-3 µm diam. with walls hyaline, thin, nongelatinous. Paraphyses 1.5 µm diam., apex undifferentiated or slightly swollen, 2-3 µm diam., about same length as asci. Asci 95-105 × 7.5-8.5 µm, cylindric or subclavate, tapering slightly to truncate apex, wall thickened at apex, apical pore amyloid; 8-spored, spores overlapping, basal stalk short. Ascospores $50-60 \times 1.5-2 \mu m$, filiform, ends rounded, coiling on release, 0(-1)-septate, wall hyaline.

NOTES: Chlorociboria spiralis is known from a single collection. The macroscopic appearance is distinctive with the margin of the cup having a slightly hairy, yellowish margin. There are two New Zealand species, C. spiralis and C. colubrosa, with filiform, spiralling ascospores. They can be distinguished macroscopically. C. spiralis has small, short and



Fig. 31 *Chlorociboria spiralis* (PDD 77771). **A**, ascus; **B**, ascospores; **C**, tomentum hyphae; **D**, distribution of New Zealand collections examined. Scale bar: A–C = 20 μm.

narrow stipitate apothecia, an orange-brown hymenium when dry, and a receptacle covered with densely matted hair-like elements, these hairs often thickly encrusted with dark green material. *Chlorociboria colubrosa* has apothecia with a long, broad stipe, blue-green receptacle and hymenium, and a smooth receptacle surface. *Chlorociboria colubrosa* lacks the rough-walled tomentum hyphae characteristic of *C. spiralis*.

Chlorociboria spiralis is morphologically somewhat atypical of Chlorociboria as accepted in this paper. The elements comprising the ectal excipu-



Fig. 32 Tree from the neighbour-joining analysis based on ITS sequences. The tree is rooted using *Hymenoscyphus fructigenum* as the outgroup. Bootstrap values are given where above 50%. Sequences labelled with Genbank accession numbers (starting with Z, U, AF, AJ) were obtained from Genbank; sequences labelled with numbers starting PDD and ICMP were generated as part of this study. The PDD numbers refer to the voucher specimen (in the New Zealand Fungal Herbarium, Landcare Research, Auckland) from which the sequences were obtained. The ICMP numbers are voucher cultures derived from ascospores isolated from PDD specimens.

lum are mostly arranged more or less parallel with the surface of the receptacle. This may reflect the physical limits placed on cell arrangement in the relatively poorly developed excipular layer of this species. DNA sequence data show that *C. spiralis* is a *Chlorociboria*.

Chlorociboria spiralis is known from a single collection. This was on decorticated but hard wood, with the blue-green staining confined to the uppermost 0.5-1 mm of the wood.

MOLECULAR RESULTS AND ANALYSES

Parsimony and neighbor-joining (Fig. 32) analyses both place *Chlorociboria* as monophyletic within the range of Helotiales with which it was compared. The six shortest parsimony trees closely match the topography of the neighbor-joining tree.

Excluded from the analysis was a very variable part of the sequence, positions 83-136 of the 537 bp alignment. This region was not alignable across all the species, but within Chlorociboria there were groups of species within which this region was sufficiently similar to be alignable within each group. These groups were congruent with well-supported clades within Fig. 32. They included C. aeruginascens, C. aeruginascens subsp. australis, and C. argentinensis; and C. aeruginosa, C. poutoensis, C. pardalota, C. macrospora, C. spathulata, and C. awakinoana. The other Chlorociboria species. although more divergent within the insert, were similar to the second of these groups. DNA was not successfully extracted from any of the C. colubrosa specimens available.

Where more than one specimen was sequenced from a single species, sequences were identical between collections. The level of genetic divergence within the ITS region between the Chlorociboria species was high, up to 17% between C. aeruginascens and C. campbellensis. This contrasts with a maximum ITS divergence amongst a morphologically and biologically divergent set of 17 Torrendiella spp. from New Zealand, Australia, and South America of 5.4% (P. R. Johnston unpubl. data). From data provided for the families Sclerotiniaceae and Rutstroemiaceae by Holst-Jensen et al. (1997), the within-Chlorociboria level of divergence is closer to that found for within-family divergence for these other discomycete taxa, 11% and 16%, respectively. Despite this level of divergence, Chlorociboria remains monophyletic amongst the taxa analysed, and the various clades within *Chlorociboria* exhibit no obvious morphological features that could sensibly be used to divide the genus.

Within *Chlorociboria* there is little correlation between the degree of morphological novelty and genetic diversity. The most genetically distinct species, *C. campbellensis*, is morphologically more similar to the well-known Northern Hemisphere species *C. aeruginosa* and *C. aeruginascens* than to the genetically less distinct *C. spiralis*, a species with filiform, coiling ascospores, a feature completely out of place for *Chlorociboria* as known previously.

Dixon (1974) placed Chlorociboria within the family Helotiaceae (as Leotiaceae). Earlier authors debated its familial relationships. White (1941) suggested it might belong in the Sclerotiniaceae (as Ciborioideae sensu Nannfeldt 1932). Korf (1958) agreed, noting the Rutstroemia-like character of the formation of ascoconidia. Dixon (1974), while placing the genus in the Helotiaceae, noted that the rough-walled tomentum hyphae appear to be similar to the rough-walled hairs in some Hyaloscyphaceae. The molecular study of Holst-Jensen et al. (1997) excluded Chlorociboria from the Sclerotiniaceae or their segregate family Rutstroemiaceae. The superficial sequencing study presented here aimed simply to address the question of the monophyly of Chlorociboria and the validity of the morphological species limits being used, although it does support the results of Holst-Jensen et al. (1997). Molecular data will eventually be used to define family-level taxa within the Helotiales, but as yet there is an inadequate level of taxon sampling to suggest a relationship between Chlorociboria and other genera within the Helotiales.

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