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Chaenotheca meridionalis, a new calicioid lichen from Australasia

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Abstract

Chaenotheca meridionalis Tibell & Kantvilas is described as new, based on morphological characters and ITS-based phylogenetic data. Maximum Likelihood analysis was run on 62 taxa and four *C. meridionalis* sequences received maximum Ultrafast bootstrap support within the strongly supported 'chrysocephala' group. *Chaenotheca meridionalis* differs from its close relative, *C. chlorella*, by its taller apothecia, 1.04–2.04 mm high, versus 0.71–0.94 mm in *C. chlorella,* and wider ascospores, 3.25–4.75 µm versus 2.89–3.81 µm in *C. chlorella*. It inhabits decorticated wood in temperate forests and is widely distributed in Australasia.

Key words: biodiversity, Coniocybomycetes, lichenised fungi, new species, molecular taxonomy

Introduction

Chaenotheca (Th.Fr.) Th.Fr. is a genus of calicioid lichens, classified in the Coniocybomycetes by Prieto & Wedin (2013). It was revised by Tibell (1980) and has since been included in numerous regional revisions of calicioid lichens (Tibell 1981, 1982, 1999, 2001; Tibell & Ryan 2004), including those of Australasia (Tibell 1987). In many areas of the world, certain species of *Chaenotheca* have been found in habitats with long forest continuity (Tibell 1992; Selva 1996; Maloof 2016; Kantvilas & Jarman 2012) and, as a result, several have been included in Red Lists of various countries. However, many taxonomic problems remain in the genus and additional species continue to be described, even from relatively well-explored areas. Examples include *C. balsamconensis* J.L.Allen & McMullin (Allen & McMullin 2015) [= *Chaenotricha obscura* (G. Merr.) Suija, McMullin & P. Lohmus] and *C. longispora* Reese Næsborg & Tibell (Reese Naesborg *et al.* 2019) from North America, and *C. biesboschii* Tibell & van der Pluijm (Tibell *et al.* 2019) from Europe. A rich diversity of species, some of which are undescribed, has also been discovered in the Himalayas (L.T., unpublished results), and further undescribed species are known from Australia and New Zealand. In this paper, the majority of the material from Australasia that was previously identified as *C. carthusiae* (Harm.) Lettau [= *C. chlorella* (Ach.) Müll.Arg.)] by

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Tibell (1987) is shown to belong to a new species, here described as *C. meridionalis*.

Material and methods

Material

Material was collected from many parts of New Zealand and Australia (mostly by LT, originally identified as C. *carthusiae* and lodged in UPS; see Tibell 1987) and, more recently, from Tasmania by GK (lodged in HO). Occasional collections from other sources were also studied. For comparisons, the following specimens of *C. chlorella* (all in UPS) were used: *Tibell 16867* (Uppland, Sweden), *Tibell 17106* (Jämtland, Sweden), *Tibell 21053* (Uppland, Sweden) and *Tibell 17889* (Uppland, Sweden). For establishing a detailed phylogeny, 60 ITS sequences of 24 species of *Chaenotheca* and *Coniocybe* from 16 countries in Africa, Asia, Australasia, Europe, and North and South America (Table 1) were used.

Methods

Mature apothecia were sectioned longitudinally with a freezing microtome, using a 5% solution of gum arabic as the embedding medium. Forty mature ascospores were randomly selected and measured. Statistical estimates are given in the following format: A-B (X= ; sd= ; n= ; N=), where A is X minus one sd, B is X plus one sd, X is the arithmetic mean, sd is the standard deviation, n is the number of observations per collection (40) and N is the number of collections measured (4).

DNA extraction and sequencing

Material for DNA extraction was obtained either from fresh material or from cultures 10–15 years old, stored in a refrigerator at +4°. The cultures were obtained from fresh material after plating ascospores on MYE medium. The culture material was immersed in liquid nitrogen and crushed with a small pestle. For recently collected material, carefully selected apothecia were used for the isolation. The DNeasy Plant Mini Kit (Qiagen) was used for isolating total DNA following the instructions of the manufacturer. Diluted (1:10) or undiluted DNA was used for PCR amplifications with AccuPower® PCR PreMix (Bioneer) adding 3 µl of DNA extraction, 1.5 µl of each primer (10 µM) and water to a total volume of 20 µl.

Primers used were for the nucITS were ITS1f (Gardes & Bruns 1993) and ITS 4 (White *et al.* 1990). Thermal cycling parameters were: initial denaturation for 4 min at 95°C, followed by 35 cycles of 1 min at 94°C, 1 min at 54°C and 45 secs at 72°C, and final elongation for 5 mins at 72°C. Amplification products were visualized on 0.5% agarose gels stained with GelRed. The product was cleaned with Illustra™ExoProStar (Thermo Fisher Scientific) following the protocol of the manufacturer. The DNA amplification product was sequenced in both directions by using the above mentioned primers also as sequencing primers (5 µM). Sequencing, automated reaction clean up and visualization were carried out as described by Macrogen Inc. (www.macrogen.com).

Alignments and phylogenetic analyses

Newly generated ITS sequences of *Chaenotheca* were aligned together with a wide selection of *Chaenotheca* and *Coniocybe* sequences downloaded from GenBank (Table 1) by MAFFT v.7 (Kuraku *et al*. 2013; Katoh *et al.* 2019) on an online server (http://mafft.cbrc.jp/alignment/server/). An alignment was created utilizing the L-INS-i iterative refinement method and using the default settings (gap opening penalty = 1.53 and offset value = 0.0). The alignment was manually edited using AliView (Larsson 2014). The alignment comprises 62 sequences with 575 columns (351 distinct patterns, 251 parsimony-informative, 55 singleton sites and 269 constant sites), representing *Chaenotheca* and *Coniocybe* species; as outgroup, two sequences of *Sclerophora farinacea* (Chevall.) Chevall. were chosen based on the phylogeny presented by Tibell *et al.* (2019).

Maximum-Likelihood (ML) Phylogenetic relationships were inferred using the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/; Trifinopoulos *et al*. 2016). Prior to the ML analysis, model selection was performed on the same server, with no partition of ITS data; for all three Information Criteria (Akaike, Corrected Akaike and Bayesian) the same best-fit model SYM+I+G4 was identified. ML tree reconstruction (Nguyen *et al.* 2015) was combined with branch support using ultrafast bootstrap (UFBoot) with 2000 replicates implemented in IQ-TREE (Hoang *et al*. 2017), where a UFBoot ≥95% was considered as strong support. Additional support metrics (SH-aLRT and aBayes) were also calculated, although they were well in line with UFBoot values, and thus not shown.

Phylogenetic trees were visualized using FigTree

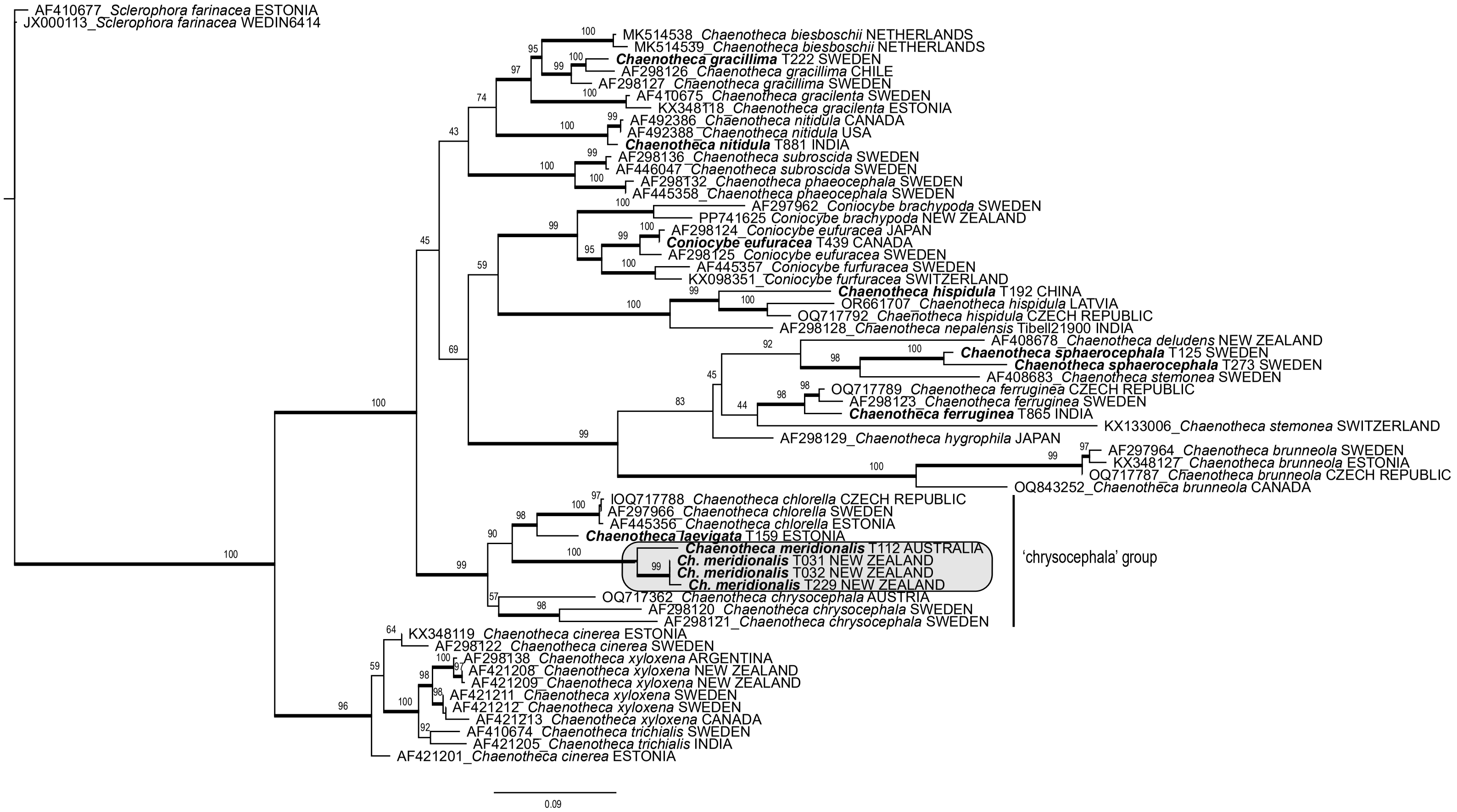

Figure 1. ITS-based phylogeny of 62 sequences (Table 1) representing 21 species of *Chaenotheca* and three of *Coniocybe*, rooted with two sequences of *Sclerophora farinacea*, serving an ML analysis. The support value associated with each internal branch corresponds to UFBoot, where branches in bold indicate a high support (UFBoot ≥95%). GenBank numbers are followed with the name of the species and the country of specimen origin. Species name in bold show the new sequences used in this study. Four *Chaenotheca meridionalis* sequences are encompassed in the shaded box, while the 'chrysocephala' group is indicated by vertical line.

v1.4.3 (Rambaut 2012). Inkscape v. 1.4.2. (https://inkscape.org/), a free software and open-source vector graphics editor, was used for artwork.

Results

Analyses were based on a total of 62 ITS sequences, representing 21 species of *Chaenotheca* and three species of *Coniocybe* (Table 1), with two sequences of *Sclerophora farinacea* as outgroup (Fig. 1). Four sequences of *Chaenotheca meridionalis*, three from New Zealand and one from Australia, form a strongly supported clade (UFBoot=100%) in the phylogeny (Fig. 1), and are part of a clade (UFBoot=99%) that was referred to as the 'chrysocephala' group in Tibell *et al*. (2019).

Taxonomy

Chaenotheca meridionalis* Tibell & Kantvilas, *sp. nov.

MycoBank no.: MB 863509

Similar to *Chaenotheca chlorella* (Ach) Müll.Arg., with which it shares a yellow pruina and a *Stichococcus s.lat.* (henceforth referred to as just '*Stichococcus*') photobiont, but differing by the taller apothecia, 1.04–2.04 mm high, and the ascospores being 4.03–6.03 x 3.25–4.75 µm (wider than in *C. chlorella*).

***Type*: AUSTRALIA:** Tasmania, along Lyell Highway, *c.* 3 km north of Wyatinah, 42°22'S, 146°30'E, on lignum of fire-scarred *Eucalyptus regnans* at the edge of an *Atherosperma*-dominated gully, 400 m elevation., 25 Oct. 1998, *G. Kantvilas 213/98* (holo: HO 328962; iso: UPS).

Thallus immersed to superficial, pale glaucous green, developing small, lumpy, ecorticate patches with a minutely roughened surface. *Photobiont Stichococcus*. *Apothecia* 1.04–2.04 mm high (X=1.54 mm; sd=0.50 mm, N=4, n=40), with slender, flexuose stalks; *stalks* 0.037–0.073 mm diam. (X=0.055 mm; sd=0.018 mm, N=4, n=40), black, epruinose or with a citrine yellow pruina in the upper part; *capitulum* 0.14–0.29 mm diam. (X=0.21 mm; sd=0.08 mm, N=4, n=40), lenticular to spherical, with a well-developed excipulum; *excipulum* short, collar-shaped, consisting of periclinally arranged, intertwined hyphae, covered on the lower side by a yellow pruina; *paraphyses* branched, < 1 µm diam.; *asci* single, cylindrical, 14.7–18.5 (X= 16.6 µm, sd= 1.9 µm, N=4, n=40) x 1.8–2.4 (X=2.13 µm, sd=0.31 µm, N=4, n=40), well-stalked and arising from croziers; *ascospores* medium brown, 4.03–6.03 µm (X= 5.03 µm, sd= 1.00 µm, N=4, n=40 µm) long and 3.25–4.75 µm wide (X=4.00 µm; sd=0.75 µm, N=4, n=40), ornamented with coarse, irregular cracks. *Secondary chemistry*: the yellow pruina consists of vulpinic acid. Fig. 2.

Etymology

The specific epithet, meaning 'southern', alludes to the geographic distribution of the new species.

Habitat and distribution

The new species occurs in dry, sheltered recesses on the lignum of large, dead trees in wet forests, a habitat favoured by many calicioid lichens in Australia (e.g. see Tibell 1987, Kantvilas & Jarman 2004) as well as in the Northern Hemisphere (Tibell 1992). Although herbarium specimens suggest that the preferred host is *Eucalyptus*, *Chaenotheca meridionalis* has also been collected from dead *Leptospermum*, dead *Phyllocladus*, the dead, woody basidiocarp of a bracket fungus attached to an old *Nothofagus cunninghamii,* and old, dead, dry leaves of *Richea pandanifolia* that are retained as a collar on the living plant. Thus, its apparent predilection for *Eucalyptus* is more a case of this tree providing the physically largest host and having lignum that is stable and long-lived. In New Zealand, it mainly occurs on lignum of *Nothofagus* and podocarps. The distribution of the species is illustrated by Tibell (1987) under the name *C. carthusiae*, and its habitat ecology is also discussed there (p. 82). The habitat of the related *C. chlorella* is generally similar, although this species is more often found on the bark of living trees.

Specimens examined

The restudying of the material cited by Tibell (1987) as *C. carthusiae* revealed that most specimens belong to *C. meridionalis*. Thus, in the list below, these specimens are referred to solely by their collection number and only additional records are cited in full. *Chaenotheca meridionalis* has so far been recorded from 25 localities presented in the list below.

AUSTRALIA: Australian Capital Territory: Tidbinbilla Nature Reserve, 29 km SSW of Cotter Dam, Red Hill Trail, 35°29'S 148°52'E., 900m, 1981, *L. Tibell*

11900 (UPS); also *L. Tibell 11877, 11945* (UPS). **New South Wales:** Lowden Forest Park, 14 km ENE of Majors Creek, 35°31′S, 149°36′E, *c.* 900 m, 1981, *L. Tibell 11968* (UPS); also *L. Tibell 12006, 12309* (UPS). **Victoria:** Healesville, Corranderrk Sandways, 1974, *R. Filson 15009* (UPS); Errinundra National Park, 13 km SE of Bendoc, 37°14′S 148°58′E, 860 m, 1990, *M. Wedin 3346, 3348* (UPS); near Caveat, junction of Molesworth and Highlands roads, 37°07′S 145°29′E, 860 m, 1981. *H. Streiman* (UPS); also *L. Tibell 11728a*, *11736*, *11764*, *11774*, *11798*, *11821*, *11830* (UPS). **Tasmania:** Anthony Road, 41˚50′S 145˚38′E, 1991, *G. Kantvilas 247/91* (UPS); Mt. Field National Park, 8.5 km N of Maydena, outlet of Lake Fenton, 42°40′S 146°37′E, *c.* 1000 m, 1981, *L. Tibell 11141* (UPS); Fern Tree, Bower Track, 42°55′S 147°16′E, 450 m, 1981, *R.D. Seppelt 11420* (HO); Myrtle Forest, Collinsvale, 42°52′S 146°35′E, 1981, *G. Kantvilas 128/81 & L. Tibell* (HO); Yarlington Tier, 42°32′S 147°18′E, 620 m, 1987, *G. Kantvilas 99/87* (HO); Warra Creek, 43°05′S 146°43′E, 175 m, 1996, *G. Kantvilas s.n.* (HO); Hartz Mountain Road, 43°12′S 146°46′E, 560 m., 1998, *G. Kantvilas 199/90* (HO, UPS); near Mossy Marsh Canal, 3.5 km SW of Tarraleah, 42°18′S 146°24′E, 650 m, 1998, *G. Kantvilas 211/98* (HO, UPS); 2 km SW Renison Bell, along forestry road to Argent Dam, 1992, *Wedin 4298* (UPS); also *L. Tibell 10974*, *10995*, *11057*, *11096*, *11120*, *11180*, *11365*, *11371*; *R. Filson 15009* (all UPS). **Western Australia:** Beedelup National Park, 15 km W of Pemberton, at Beedelup Falls, 34°25′S 115°52′E., 120m, 1981, *L. Tibell 14004, 14012* (UPS); Porongurup National Park, 21 km ESE of Mt Barker, 34°41′S 117°53′E., 520m,

Table 1. ITS sequences used in the phylogenetic analysis, together with their GenBank Accession numbers. New sequences in bold.

	Species	Country	Isolation	Voucher	GB number
1	*Chaenotheca biesboschii*	Netherlands	L378	A. v. d. Pluijm 3130	MK514538
2	*Chaenotheca biesboschii*	Netherlands	L380	A. v. d. Pluijm 3244	MK514539
3	*Chaenotheca brunneola*	Sweden	T056	Tibell 22202 (UPS)	AF297964
4	*Chaenotheca brunneola*	Canada		CCDB-33187-G08	OQ843252
5	*Chaenotheca brunneola*	Czech Republic		PRA-Vondrak24672	OQ717787
6	*Chaenotheca brunneola*	Estonia	T056	TU<EST>:76415	KX348127
7	*Chaenotheca chlorella*	Czech Republic		PRA-Vondrak24769	OQ717788
8	*Chaenotheca chlorella*	Estonia	T161	Tibell 22372 (UPS)	AF445356
9	*Chaenotheca chlorella*	Sweden	T080	Tibell 22187 (UPS)	AF297966
10	*Chaenotheca chrysocephala*	Austria		PRA-Vondrak 26008	OQ717362
11	*Chaenotheca chrysocephala*	Sweden	T057	Tibell 22162	AF298120
12	*Chaenotheca chrysocephala*	Sweden	T085	Tibell 21799 (UPS)	AF298121
13	*Chaenotheca cinerea*	Estonia		TU<EST>:39194	KX348119
14	*Chaenotheca cinerea*	Estonia	T163	Tibell 22374 (UPS)	AF421201
15	*Chaenotheca cinerea*	Sweden	T089	Jonsson& Nordin s.n.	AF298122
16	*Chaenotheca deludens*	New Zealand	T106	T. 16575; UPSC2454	AF408678
17	*Chaenotheca ferruginea*	Czech Republic		PRA-Vondrak24394	OQ717789
18	*Chaenotheca ferruginea*	Sweden	T099	Tibell 22276 (UPS)	AF298123
19	***Chaenotheca ferruginea***	India	T865	Tibell 23334 (UPS)	**PZ491430**
20	*Chaenotheca gracilenta*	Sweden	T055	T. 22197 (UPS)	AF410675
21	*Chaenotheca gracilenta*	Estonia		TU<EST>:30149	KX348118
22	*Chaenotheca gracillima*	Chile	T008	Tibell 17943 (UPS)	AF298126
23	***Chaenotheca gracillima***	New Zealand	T222	T. 16575; UPSC2300	**PZ491431**
24	*Chaenotheca gracillima*	Sweden	T037	Tibell 17052 (UPS)	AF298127
25	*Chaenotheca nepalensis*	India	T093	Tibell 21900 (UPS)	AF298128
26	***Chaenotheca hispidula***	China	T192	Titov 6691B	**PZ491432**
27	*Chaenotheca hispidula*	Latvia	CH361	TUF051093	OR661707
28	*Chaenotheca hispidula*	Czech Republic		PRA-Vondrak24330	OQ717792
29	*Chaenotheca hygrophila*	Japan	T024	Thor 15612	AF298129
30	***Chaenotheca laevigata***	Estonia	T159	Tibell 22368	**PZ491433**

1983, *L. Tibell 14154* (UPS); Walpole National Park, 8 km NE of Walpole, Tingle Drive, 34°58'S 116°48'E., 760m, 1983, *L. Tibell 14055* (UPS). **NEW ZEALAND: Canterbury:** Awa Awa Reserve, 11.3 km NW of Methven, 43°34'S 171°32'E, 530 m, 1980, *L. Tibell 9892* (UPS); also *L. Tibell 9051*, *9056*, *9129b*, *9178*, *9523*, *9634*, *9755*, *9892*, *10024*, *10944* (all UPS). **North Auckland:** Waivera Hill Scenic Reserve, 4.5 km NNE of Orewa, 36°33'S 174°43'E, *c.* 60 m, 1986, *L. Tibell 16700* (UPS); *L. Tibell 13391*, *14895*, *16709* (all UPS). **Gisborne:** Taupo-Napier Rd, near Tarawera, 39°02'S 176°32'E, 1982, *J. Bartlett 18367* (UPS); 1.8 km SE of Waikaremoana, along Ngamoko Track, 38°46'S 117°10'E, 1050 m, 1992, *L. Tibell 19421* (UPS); also *L. Tibell13009*, *19408* (UPS). **Nelson:** Nelson Lakes National Park, Lake Rotoiti, 1.5 km SSE of St. Arnaud, along St. Arnaud Track, *L. Tibell 19061* (UPS); also *L. Tibell 13677*, *13769* (UPS). **Otago:** *L. Tibell 10205*, *10289*, *10581* (UPS). **South Auckland:** Wairare Falls Scenic Reserve, Kaimai Range, 1 km NE of Matamata, 300 m, 1992, *M. Wedin 3973* (UPS); near Tarawea 39°02'S 176°32'E, *c.* 550 m, 1982, *J. Bartlett 18767* (UPS). **Southland:** *L. Tibell 10373*, *10387*, *10391*, *10501* (all UPS). **Taranaki:** Tongariro National Park, along Ohakune Mountain Road, 3 km NE of Ohakune, *1990*, *L. Tibell 18972* (UPS).

Discussion

Our phylogeny recovered the groups variously identified and nick-named by Tibell *et al.* (2019). The morphology of *Chaenotheca meridionalis*, initially reported and illustrated from Australia and New

31	***Chaenotheca meridionalis***	Australia	T112	Kantvilas 213/98	**PZ491434**
32	***Chaenotheca meridionalis***	New Zealand	T031	Tibell 16700; UPSC2196; CBS 100902	**PZ491435**
33	***Chaenotheca meridionalis***	New Zealand	T032	Tibell 16700; UPSC2073; CBS 113821	**PZ491436**
34	***Chaenotheca meridionalis***	New Zealand	T229	T. 18972	**PZ491437**
35	*Chaenotheca nitidula*	Canada	T182	Koffman 222 (UPS)	AF492386
36	***Chaenotheca nitidula***	India	T881	Tibell 23357 (UPS)	**PZ491438**
37	*Chaenotheca nitidula*	USA	T290	T. 21490	AF492388
38	*Chaenotheca phaeocephala*	Sweden	T001	Hermansson 8290 (UPS)	AF445358
39	*Chaenotheca phaeocephala*	Sweden	T010	Tibell 16885 (UPS)	AF298132
40	***Chaenotheca sphaerocephala***	Sweden	T125	Herm. 4/6/00 (UPS)	**PZ491439**
41	***Chaenotheca sphaerocephala***	Sweden	T273	Koffman 010823 (UPS)	**PZ491440**
42	*Chaenotheca stemonea*	Sweden	T115	Tibell 22191 (UPS)	AF408683
43	*Chaenotheca stemonea*	Switzerland		LIFU098-16 (versC)	KX133006
44	*Chaenotheca subroscida*	Sweden	T050	Tibell 22150	AF298136
45	*Chaenotheca subroscida*	Sweden	T064	Tibell 22167	AF446047
46	*Chaenotheca trichialis*	Sweden	T130	T. 22355	AF410674
47	*Chaenotheca trichialis*	India	T151	Tibell 22090 (UPS)	AF421205
48	*Chaenotheca xyloxena*	Argentina	T011	Tibell 18431	AF298138
49	*Chaenotheca xyloxena*	Sweden	T131	Tibell 22361 (UPS)	AF421211
50	*Chaenotheca xyloxena*	Sweden	T181	Tibell 22329 (UPS)	AF421212
51	*Chaenotheca xyloxena*	Canada	T188	Selva 7753 (UMFK)	AF421213
52	*Chaenotheca xyloxena*	New Zealand	T006	Tibell 16673 (UPS)	AF421208
53	*Chaenotheca xyloxena*	New Zealand	T028	Tibell 16605 (UPS)	AF421209
54	*Coniocybe brachypoda*	Sweden	T030	Tibell 17062	AF297962
55	*Coniocybe brachypoda*	New Zealand	T027	Tibell 16627	PP741625
56	*Coniocybe eufuracea*	Japan	T036	Thor 15698	AF298124 as *'furfuracea'*
57	*Coniocybe eufuracea*	Sweden	T081	Wedin 6366 (UPS)	AF298125
58	***Coniocybe eufuracea***	Canada	T439	Koffman 387 (UPS)	**PZ491441**
59	*Coniocybe furfuracea*	Sweden	T155	Tibell 22364	AF445357
60	*Coniocybe furfuracea*	Switzerland		WSL:DF252	KX098351
61	*Sclerophora farinacea*	unknown		Wedin 6414 (UPS)	JX000113
62	*Sclerophora farinacea*	Estonia		Tibell 22373 (UPS)	AF410677

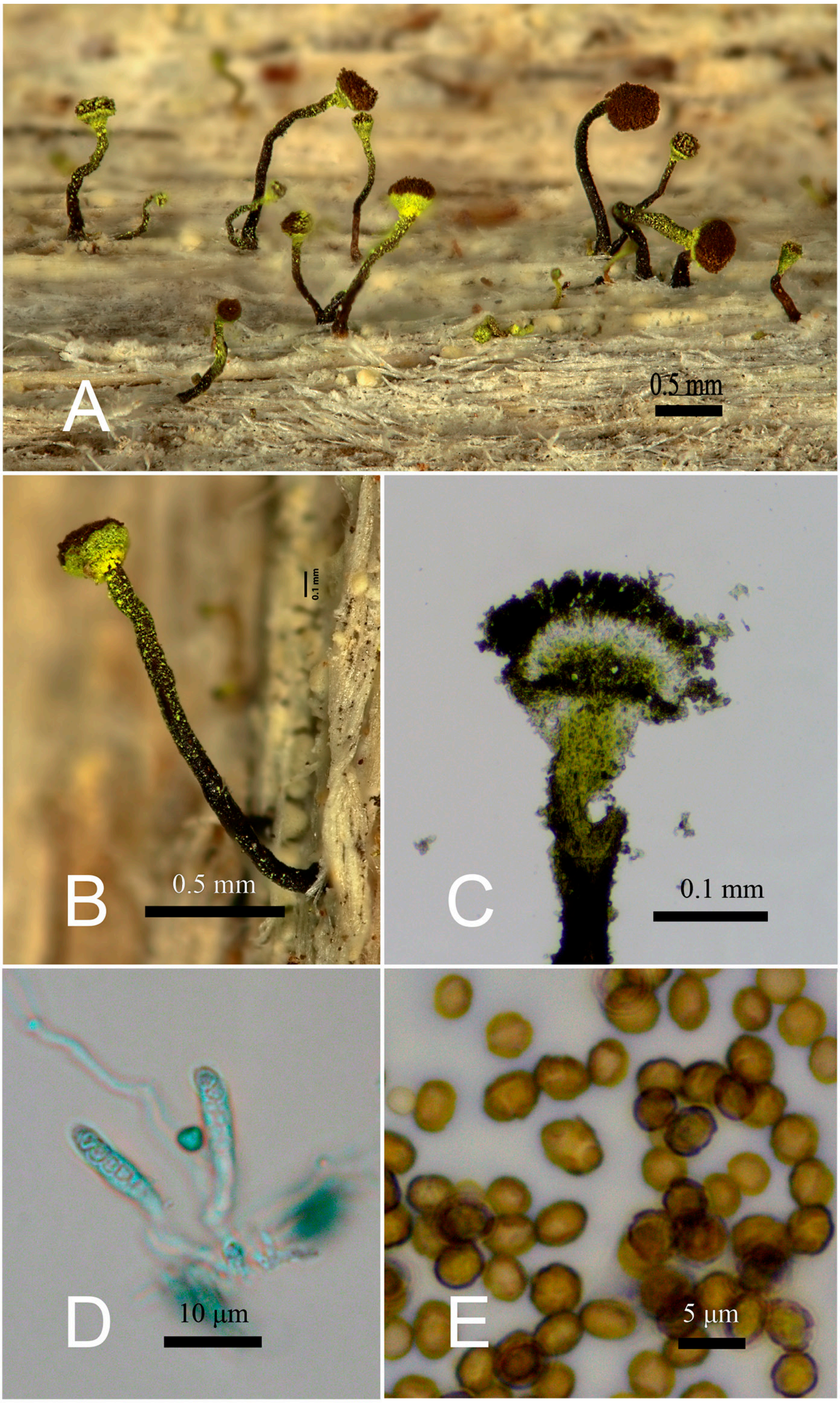

Figure 2. *Chaenotheca meridionalis*. A-B: ascomata, habit; C: ascomata, longitudinal section; D: asci; E: ascospores.

Zealand under the name *C. carthusiae* by Tibell (1987), indicates that it belongs to the '*chrysocephala*' group. Its placement there was confirmed by our ITS-based phylogenetic analyses (Fig. 1) of a wide representation of the infrageneric variation in *Chaenotheca*. The monophyly of *C. meridionalis* is strongly supported (Fig. 1) and it forms the sistergroup to a clade comprising *C. chlorella* and *C. laevigata* Nádv. However, in pairwise comparisons, the analysis also reveals considerable differences between the New Zealand collections and the type from Tasmania (23–27 bps). This might indicate that *C. meridionalis* as described here includes two closely related but morphologically very similar species, but to further elucidate the relationships between these two putative subclades, a much wider sampling of molecular sequences is needed, and that is beyond the aim and resources of this paper. *Chaenotheca meridionalis* is in the ITS region characterized by 'signature sequences' (Tibell 2006); that is, sequences unique to *C. meridionalis* as compared to the 59 sequences representing 21 other species of *Chaenotheca* (Table 1): CATTATCGAGTGCGGGTCCTCTG (ITS1); GGGGCGTGCCCCC (ITS1); TGCTGTCTGAGCAAA (ITS1), and GGACTCCCGTC (ITS2).

The new species displays the salient morphological features of this group, such as having stalked, yellow-pruinose apothecia with a brown mazaedium on a crustose thallus, and is morphologically most similar to *C. chlorella* in that both species have a yellow pruina. However, they differ in *C. meridionalis* having taller apothecia, 1.04–2.04 mm, with flexuous stalks (see Tibell 1987: Fig. 10, and our Figs. 2 A-B) versus 0.71–0.94 mm in *C. chlorella*, and wider ascospores, 3.25–4.75 µm versus 2.89–3.81 µm in *C. chlorella*. In *C. chlorella*, the stalks are thus comparatively short and straight (illustrated in Tibell 1999: 83). For a comparison of apothecium and spore sizes, the following estimates were obtained from four specimens of *C. chlorella* (see 'Material' above): apothecium height = 0.71–0.94 mm (X=0.82 mm; sd=0.12, N=4, n=40); ascospore length: 4.4–6.3 µm (X=5.34 µm; sd=1.00 µm, N=4, n=40); ascospore width: 2.89–3.81 µm (X=3.35 µm; sd=0.46 µm, N=4, n=40); ascospore length/width ratio = 1.59 (compared to 1.26 for *C. meridionalis*). There is also a difference in thallus morphology with *C chlorella* having a verrucose to subsquamulose thallus with a distinct cortex, whereas in *C meridionalis*, the thallus is immersed or develops in small, lumpy patches with a minutely roughened, ecorticate surface.

Several additional, calicioid taxa in the Australasian lichen biota also have yellow-pruinose apothecial stalks: *C. chrysocephala* (Ach.) Th.Fr. differs by having a conspicuous thallus of coarse, bright yellow granules; *C. hispidula* (Ach.) Zahlbr. differs by having a *Trentepohlia* photobiont and apothecial stalks to 1 mm tall. There is also *Coniocybe confusa* (Tibell) Tibell & Temu, which has a farinose to granular, greenish yellow thallus and apothecia that lack a well-developed excipulum.

Numerous taxonomic problems in *Chaenotheca* remain, and a detailed investigation of its taxonomy is underway (by LT and ST).

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