

# Five species of *Candelaria* and *Candelariella* (Ascomycota, Candelariales) new to Switzerland

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## Abstract

*Candelaria pacifica*, *Candelariella antennaria*, *C. boleana*, *C. granuliformis* and *C. xanthostigmoides* are reported from Switzerland for the first time. *Candelariella xanthostigmoides* is also new to Europe. *Candelariella aggregata*, *C. efflorescens*, *C. subdeflexa* and *C. viae-lactea* are confirmed to occur in Switzerland. *Candelariella antennaria* is also reported new to Austria. Brief notes on their identification, ecology and distribution in Switzerland are given.

## Key words

*Candelariaceae*, *Candelariomycetidae*, Europe, lichens, lichenized ascomycetes

## Introduction

*Candelariella* is a well-known and commonly occurring genus, growing on many types of substrates, particularly in exposed and nitrogen-enriched localities. The species of the genus are a prominent component of the lichen flora on e.g., road-side trees, limestone rocks and in alpine, terricolous habitats. However, the knowledge of the distribution and ecology of individual species is still poor for many species. It appears that few lichenologists collect and study *Candelariella*, possibly because of the presumed difficulties in correctly identifying the species. In Switzerland, a large number of recent collections of *Candelariella* exists due to field work done in the framework of the Red List of threatened and rare epiphytic and terricolous lichens in Switzerland (Scheidegger and Clerc 2002). Part of this study led to the first terricolous lichen inventory in Switzerland where all vegetated landscapes were explored between 1996 and 1997 (Vust 2011). A detailed revision

of the herbarium material filed under *Candelariella* and *Candelaria* at the Conservatoire et Jardin botaniques de la Ville de Genève (G), revealed six new species to Switzerland not mentioned in the recent checklist of the Swiss lichens (Clerc and Truong 2010). One of these, *C. aggregata*, was recently reported for Switzerland (Spinelli 2011) and the remaining species are reported here with brief descriptions of important characteristics of the species together with notes on their ecology and distribution in Switzerland. One of the species, *C. antennaria* is also reported new to Austria. We want to emphasize that many species can be readily identified. Collecting and studying them is a rewarding effort as there are many interesting discoveries to be made in this neglected group of lichens.

## Notes on the species

***Candelaria pacifica* M. Westb. & Arup. *Bibl. Lich.* 106: 352. 2011.**

Mycobank: MB 541817

[http://species-id.net/wiki/Candelaria\\_pacifica](http://species-id.net/wiki/Candelaria_pacifica)

**Holotype.** U.S.A. California: San Luis Obispo Co., Along Shell Creek Rd 0.3 miles N of junction to State Route 58. 35°28'N, 120°20'W. Alt. c. 400 m. 26 July 1998, *M. Westberg* 953 (LD!, isotype S!).

New to Switzerland. This species was recently formally described and reported from Europe as well as North and South America (Westberg and Arup 2011). Compared to *C. concolor* (Dicks.) Stein, *C. pacifica* is characterized by an arachnoid appearance of the lower surface of the lobes due to the lack of a cortex (see also photographs in Bomble 2012). It also has 8-spored asci (polyspored in *C. concolor*) but it is rarely fertile in Europe. *Candelaria pacifica* is widespread in Europe and common at least in the north-western parts, e.g., in the southern half of Scandinavia (Westberg and Arup 2010) but its frequency and ecology is not well known outside Scandinavia. In Switzerland it is apparently much less common than *C. concolor* and we have so far only seen a few specimens from Graubünden, Jura and Valais. *Candelaria concolor* on the other hand is common in Switzerland and also noticeably spreading, possibly due to nitrogen pollution.

**Specimens examined.** Graubünden: Scuol, alt. 1990 m, 22 Sept 1998, *Roth* (G 00298405); Zernez, alt. 1550 m. 20 June 1997, *Vust* (G 00298079); Jura: Le Noirmont, Le Creux-des-Biches, alt. 1020 m., 8 Aug 1996, *Groner* (G 00298072); Valais: Zeneggen, Eich, 1010–1030 m, 19 Nov 2010, *Clerc* (G 00057817).

***Candelariella aggregata* M. Westb. *Bryologist* 110: 393. 2007.**

Mycobank: MB 529546

[http://species-id.net/wiki/Candelariella\\_aggregata](http://species-id.net/wiki/Candelariella_aggregata)

**Holotype.** U.S.A. Colorado: Larimer Co., Trail Ridge, 0.5 mi SE of Ranger Station, NW of Tombstone Ridge, 11500–11700 ft alt., 30 June 1962, *R. A. Anderson* 2229 (COLO!, isotype BRY!).

*Candelariella aggregata* was recently reported from Europe for the first time from the Murmansk Region in Russia (Urbanavichus and Urbanavichene 2008) and also from Switzerland (Spinelli 2011). It is a terricolous species growing in arctic-alpine areas as well as in dry, steppe-like habitats in North America, Asia and Europe (Westberg 2007b, Westberg and Sohrabi manuscript). It is recognized by its yellow granular to areolate thallus, numerous and often crowded apothecia with a thin margin (Fig. 1). The asci are 8-spored with narrowly ellipsoid spores  $(14-15-18(-21) \times 5.0-6.0 \mu\text{m})$ . Compared to e.g., *C. aurella* (Hoffm.) Zahlbr it also has a proper exciple that does not form a distinct stipe below the hymenium and the structure of the exciple is paraplechtenchymatous with thin cell-walls (Westberg 2007b). *Candelariella aggregata* appears to be common in the continental parts of Switzerland in steppe-like habitats at low altitudes or on well lit calcareous walls on south-facing slopes at higher altitudes.

There is a large variation in thallus morphology, apothecia and in spore size in 8-spored terricolous material in Switzerland. Possibly several species are involved and this group is clearly in need of revision. The name *C. unilocularis* (Elenkin) Nimis has been used for a terricolous species with a well-developed thallus and long spores but this name is a synonym of *C. aurella* (Khodosovtsev 2005, Westberg and Sohrabi in press). Material of the long-spored species from the Swiss Alps will be described in a forthcoming paper (Otte and Westberg, in prep.).

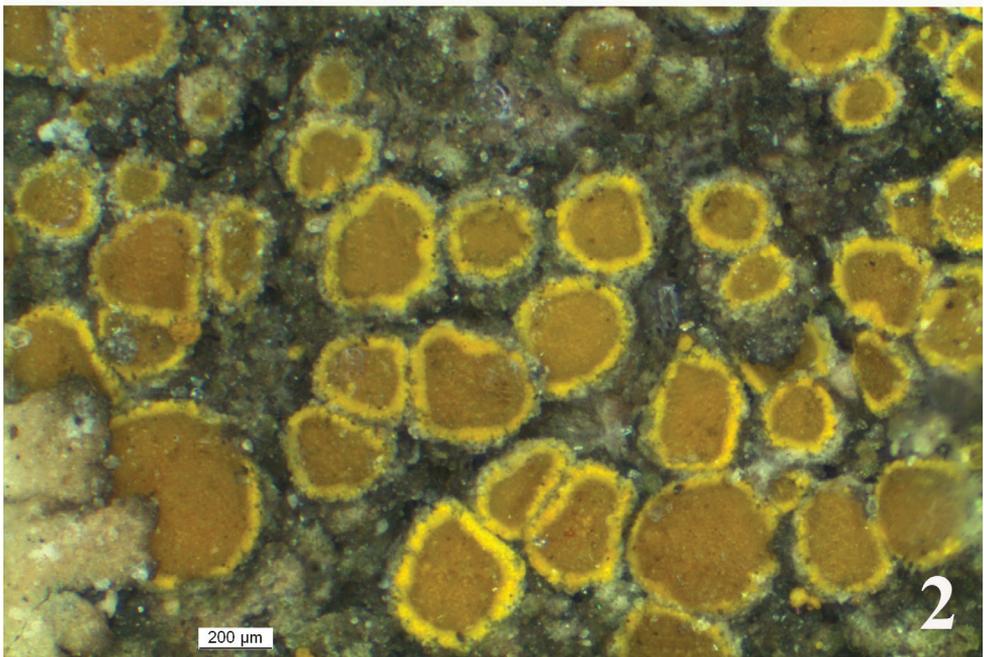
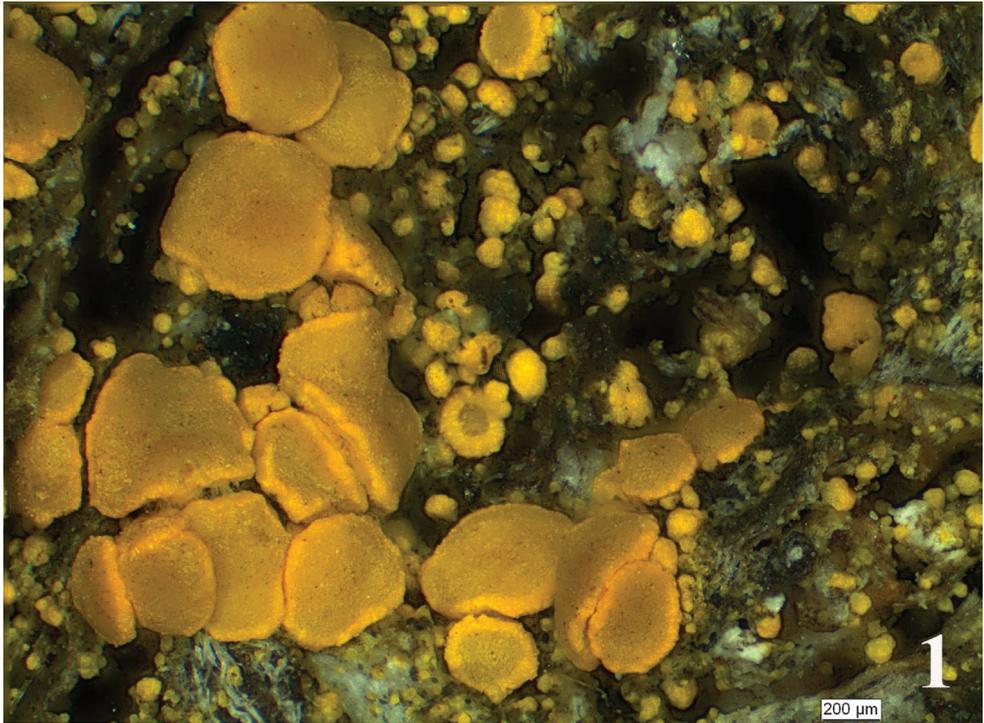
**Specimens examined.** Graubünden: Ardez, alt. 1491 m, 11 Oct 2007, *Vust* (G 00298392, G 00298400); sous l'église de Feldis, alt. 1470 m, 6 June 1999, *Clerc* (G 00298388); Fetan, Mot da l'Hom, alt 2380 m, 24 July 1956, *Frey* (G 00298391); National Park, Piz Pisoc, 29 July 1934, *Frey* (G 00298390); Tarasp, alt. 1440 m, 10 Aug 1995, *Vust* (G 00298398); Tarasp, alt. 1420 m, 12 June 1998, *Vust* (G 00298389); Valais: Bagnes, LaLy, alt. 2350 m, 24 July 2008, *Vust* (G 00298397, 00298399); Charrat, alt. 518 m, 2 Oct 2007, *Vust* (G 00298394); Guttet/Leuk, alt. 1000 m, 18 Oct 1997, *Vust* (G 00298403); Loèche, alt. 855 m, 7 Aug 1997, *Vust* (G 00298395); Mauvoisin, alt. 2740 m, 15 Aug 1998, *Vust* (G 00298413); Mazembroz, alt. 600 m, 28 April 1999, *Vust* (G 00298402); Rarogne, Heidnischbiel, alt. 759 m, 16 Oct 2007, *Vust* (G 00122115); Saillon, colline du château, alt. 504 m, 20 Sept 2007, *Vust* (G 00298396); Saillon, W part of the Saillon hill, alt. c. 500 m, 19 Nov 2010, *Westberg 10-178, 10-182* (S F177773, F177825); Vex, les Crêtes, alt. 1083 m, 5 Aug 1996, *Vust* (G 00298404); Zeneggen, alt. 1315 m, 15 Oct 2007, *Vust* (G 00298393, G 00298401); Zeneggen, Eich, 1010-1030 m, 19 Nov 2010, *Westberg 10-193* (S F178469).

***Candelariella antennaria* Räsänen. Anales Soc. Ci. Argent. 128: 137. 1939.**

Mycobank: MB 365342

[http://species-id.net/wiki/Candelariella\\_antennaria](http://species-id.net/wiki/Candelariella_antennaria)

**Holotype.** ARGENTINA, Mendozae: Depto. Las Heras, pr. Quebrada de la Meina la Atala, 2 July 1937, *A. Ruiz Leal* (H!).



**Figure 1, 2.** 1 *Candelariella aggregata* – granular thallus and crowded apothecia with thin margins  
2 *Candelariella antennaria* – grey thallus, composed of scattered to contiguous areoles or indistinct and with lecanorine, flattened apothecia

New to Switzerland and Austria. *Candelariella antennaria* is a corticolous or lignicolous species characterized by a grey thallus and 8-spored asci. The Swiss specimens were earlier identified as *C. viae-lacteeae*. This species also has a grey thallus but it is uniformly composed of spherical granules, whereas the thallus in *C. antennaria* is contiguous or with scattered, convex areoles or indistinct but never distinctly granular (Fig. 2). *Candelariella antennaria* was first reported from Europe from Crete by Vondrak et al. (2008). There are several specimens from Switzerland, all from the continental valleys in Valais and Graubünden. In addition we have seen one specimen from Tirol in Austria. *Candelariella antennaria* probably has a circumpolar distribution in continental, dry regions but the name possibly represents a complex of species.

**Specimens examined.** Austria. Tirol: Wipptal bei Steinach am Brenner, alt. 1100 m. 10 Sept. 1973, *Wunder* (M 0140870); Switzerland. Graubünden: Brusio, Casat, am Poschiavino, alt. 661 m, 30 Aug 1995, *Groner* (G 00298383); Sent, alt. 1490 m, 20 June 1995, *Frei* (G 00298382); Valais: Eggerberg, alt. 900 m, 30 Sept 1997, *Frei* (G 00298386); Naters, alt. 675 m, 22 July 1997, *Frei* (G 00298385); Ried-Brig, alt. 890 m, 22 July 1997, *Frei* (G 00298387); Sion, Préjeux, alt. 490 m, 23 Aug 1995, *Keller* (G 00298384); Viège, alt. 650 m, 22 Sept 1997, *Frei* (G 00298364); Pfynwald, Preissen, alt. 600 m, 19 Nov 2010, *Westberg 10-199* (S F178476).

***Candelariella boleana* Etayo, Palice & T.Sprib. Nova Hedwigia 89: 546. 2009.**

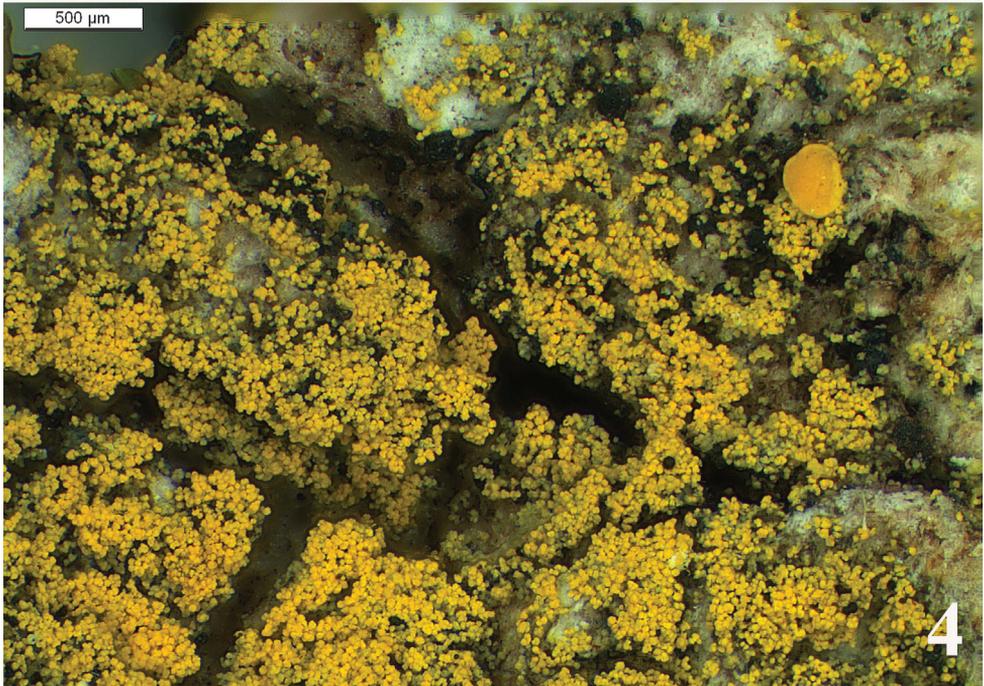
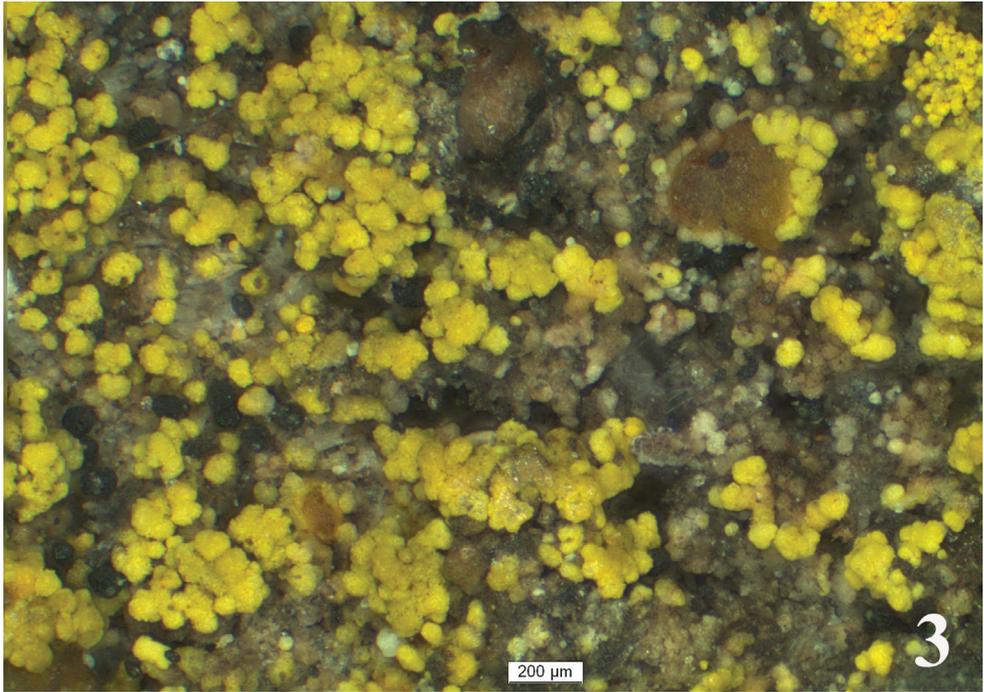
Mycobank: MB 513429

[http://species-id.net/wiki/Candelariella\\_boleana](http://species-id.net/wiki/Candelariella_boleana)

**Holotype.** SPAIN, Huesca, Central Pyrenees, Biescas, valle de Asieso, Barranco de Sta. Elena, 30TYN2126, 1100 m. 5 Aug 1993, *J. Etayo 13812* & *A. Gómez-Bolea* (BCC-Lich., isotype herb. Etayo).

New to Switzerland. This newly described species is similar to *C. xanthostigma* (Ach.) Lettau but is easily identified by its spherical spores (ellipsoid in *C. xanthostigma*). Its distribution is little known and it has hitherto been reported from Greece, Slovakia and Spain (Etayo et al. 2009). All Swiss specimens were collected on deciduous trees in the lowest part of the montane belt between 700 and 1000 m. Only one specimen was collected in the framework of the Red List project and was identified as *C. xanthostigma*. The small granular, corticolous species is a difficult group in *Candelariella*. When sterile it is often not possible to identify the different species. The thallus in *C. boleana* is granular or becoming somewhat areolate with deeply incised areoles, up to c. 0.15 mm wide (Fig. 3). The Swiss specimens also showed aggregates of smaller granules (20–30 µm wide) here and there which could be interpreted as either groups of young granules or blastidiate soredia formed by disintegration of mature areoles. We do not know whether this is a characteristic feature of *C. boleana*.

**Specimens examined.** Bern: Frienisbergwald bei Bern - Meikirch, alt. 820 m, 15 June 1960, *Frey* (G 00298408); Fribourg: kleiner Wald zwischen Vaulruz und Sem-sales, westlich Les Ponts d'Amont, alt. 880 m, 1 July 1969, *Frey* (G 00122116); Nid-



**Figure 3, 4.3** *Candelariella boleana* – granular thallus becoming somewhat areolate with deeply incised areoles, or showing aggregates of smaller granules here and there **4** *Candelariella efflorescens* – granular thallus dissolved into soredia, appearing as a confluent sorediate crust

wald: Emmetten, Alt Berg, alt. 985 m, 25 July 1995, *Frei* (G 00298380); Zug: Sihltal bei Unterschwand, alt. 660 m, 1964, *Erb* (G 00298381).

***Candelariella efflorescens* R.C.Harris & W.R.Buck. Michigan Bot. 17: 155. 1978.**

Mycobank: MB 341677

[http://species-id.net/wiki/Candelariella\\_efflorescens](http://species-id.net/wiki/Candelariella_efflorescens)

**Holotype.** U.S.A. Michigan: Mackinac Co.: Edge of swamp across highway from Island Point State Forest campground (Hog Island State Forest Campground). 5 Oct 1974, *W. R. Buck* (MICH, isotype H!).

We can here confirm the presence of *C. efflorescens* (Fig. 4) in Switzerland by reporting six fertile specimens with c. 30-spored asci. They were collected mainly in the montane belt on deciduous and coniferous trees, mostly close to the ground. The three specimens collected in the framework of the Red List project were identified as *C. xanthostigma*. As most of the specimens of the small, sorediate *Candelariella* species are sterile, it is usually not possible to separate between *C. efflorescens* and *C. xanthostigmoides* (see under this species) and sterile specimens are referred by us to *C. efflorescens* agg.

**Specimens examined.** Bern: Lauterbrunnen, Stechelberg, alt. 1000 m, 23 Oct 1996, *Keller* (G 00298407); Graubünden: bei Strada unterhalb der Brücke, alt. 1070 m, 7 Aug 1962, *Frey* (G 00298406); Malix, alt. 1700 m, 27 May 1999, *Dietrich* (G 00057815); Seewis im Praetigau, alt. 1240 m, 22 Sept 1995, *Groner* (G 0057816); Valais: Pfywald, Preissen, alt. 600 m, 19 Nov 2010 *Westberg 10-200* (S F178475); Zeneggen, Eich, alt. 1010-1030 m, 19 Nov 2010, *Westberg 10-188* (S F178464).

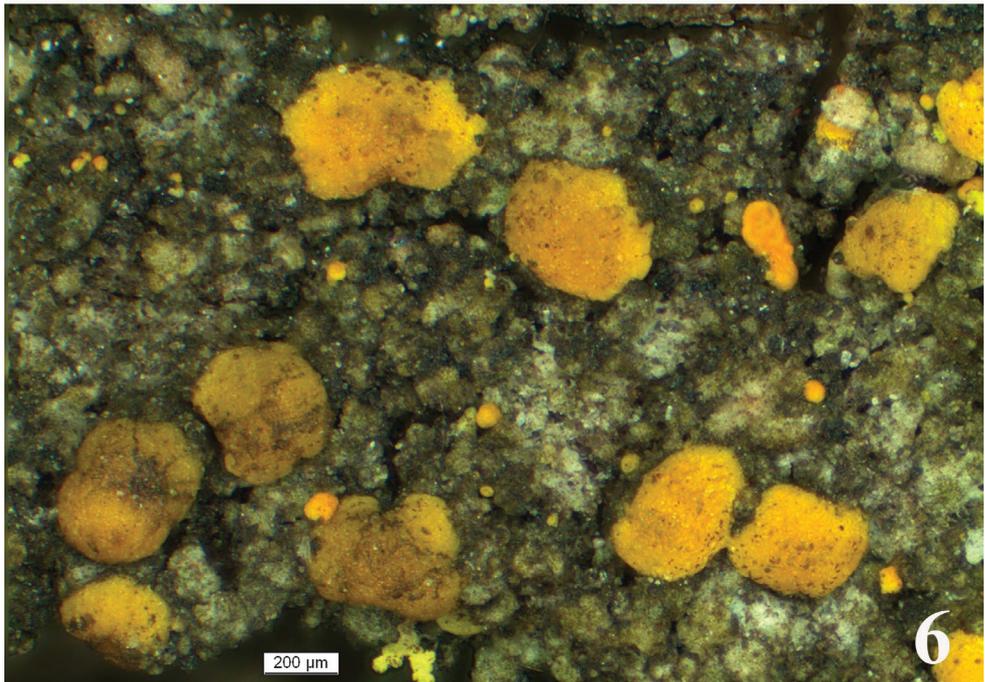
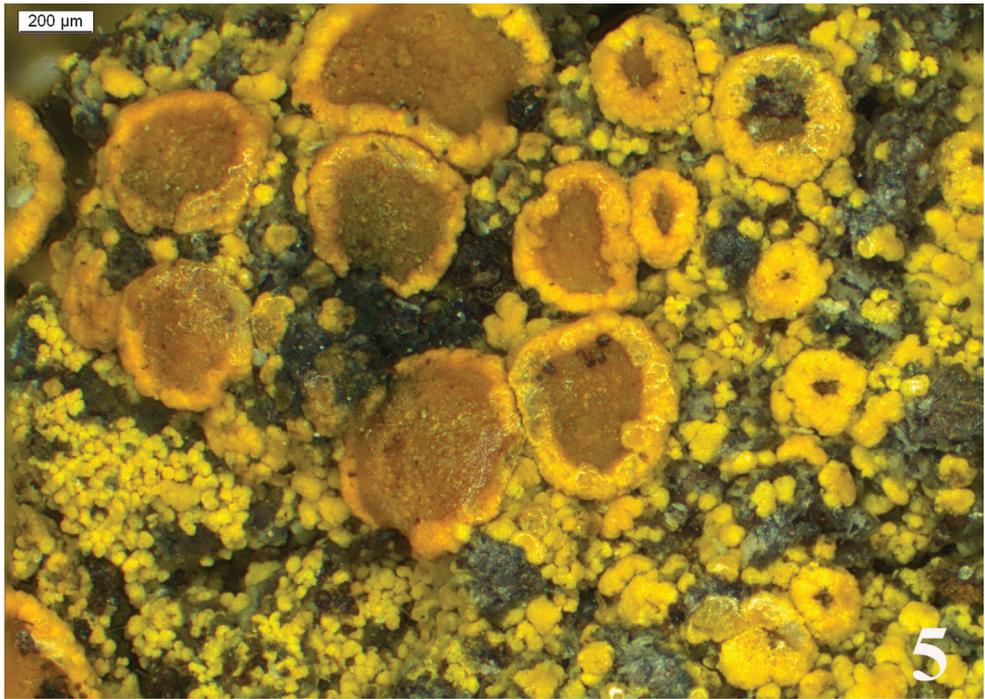
***Candelariella granuliformis* M.Westb. Bryologist 114: 328. 2011.**

Mycobank: MB 519356

[http://species-id.net/wiki/Candelariella\\_granuliformis](http://species-id.net/wiki/Candelariella_granuliformis)

**Holotype.** CANADA. Nunavut: Kitikmeot. Victoria Island, Wollaston Peninsula, Falaïse Bay. Lat/long: 69:28N 114:42W. Alt.: 100 m. 24 July 1999, *Jan-Eric Mattsson 5209* (UPS L-105166!).

New to Switzerland. This is a recently described arctic-alpine species reported from North America and northern Scandinavia (Westberg et al. 2011). This is possibly a circumpolar species and it is not surprising that it has now been found in Switzerland. The thallus is composed of small granules (Fig. 5) that soon disintegrate into blastidia (35–80 µm diam.) and the asci are polyspored. It is reminiscent of the corticolous species *C. xanthostigma* but the substrate and the disintegrating areoles separate it from this species. Of the other terricolous species *Candelariella aggregata* has 8-spored asci and *C. vitellina* has a much larger thallus of minute, effigurate areoles-subsquamules. The Swiss specimens were all collected in the continental parts of the country and were left unidentified until this study.



**Figure 5, 6.** **5** *Candelariella granuliformis* – lecanorine apothecia on a granular thallus with small granules soon disintegrating into blastidia **6** *Candelariella subdeflexa* –inconspicuous, grey thallus and biatorine apothecia

**Specimens examined.** Graubünden: Scarl, Tamangur, alt. 2100, 24 July 1934, *Frey* (G 00298409); Valais, Cabane des Dix, alt. 2850 m. 14 Aug 1998, *Vust* (G 00298081); Vallon de Réchy, alt. 2370 m, 15 Aug 1996, *Vust* (G 00298080).

***Candelariella subdeflexa* (Nyl.) Lettau. Hedwigia 52: 196. 1912.**

Mycobank: MB 381926

[http://species-id.net/wiki/Candelariella\\_subdeflexa](http://species-id.net/wiki/Candelariella_subdeflexa)

**Basionym.** *Lecanora subdeflexa* Nyl., Flora 62: 355. 1879.

**Lectotype.** FRANCE. [locality uncertain] 1871, *Weddell* (H-NYL 29173! designated by Westberg 2007a: 369).

*Candelariella subdeflexa* was first reported from Switzerland by Lettau (1956) but remained overlooked until it was collected several times within the framework of the Red List project. The species was described in detail in Westberg (2007a) based on North American material. It is unusual, but not unique, for a *Candelariella* in having biatorine apothecia (Fig. 6). North American populations typically have a thallus composed of grey, shiny squamules. The material from Europe and North Africa, seen during this study has an inconspicuous grey thallus that is indistinct, granular or composed of scattered, grey, narrow and incised squamules. This is a rare species but easily overlooked as the apothecia are small, mostly c. 0.2–0.4 mm wide. It is known in Europe from Austria, France, Germany, Italy, Spain and Switzerland. The distribution within Switzerland is distinctly southern.

**Specimens examined.** Bern: Unterseen, Unteres Stadtfeld, alt. 560 m, 2 April 1997, *Keller* (G 00298366); Graubünden: Brusio, Scala, alt. 920 m, 30 Aug 1995, *Groner* (G 00298411); Luzern: Escholzmatt, Irmibodenweidli, alt. 1120 m, 29 July 1996, *Frei* (G 00298365); Tessin: Malvaglia, Cregua, alt. 1233 m, 26 Sept 1995, *Keller* (G 00298368); Valais: Ausserbinn, Weng, alt 1270 m, 17 Sept 1996, *Frei* (G 00298366); Eggenberg, alt. 900 m, 30 Sept 1997, *Frei* (G 00298412); Ried-Brig, Schallberg, alt. 1400 m, 1 Sept 1998, *Frei* (G 00298367); Viège, alt. 650 m, 22 Sept 1997, *Frei* (G 00298364).

***Candelariella viae-lacteeae* G.Thor & V.Wirth. Stuttgarter Beitr. Naturk., A, 445: 2. 1990.**

Mycobank: MB 354941

[http://species-id.net/wiki/Candelariella\\_viae-lacteeae](http://species-id.net/wiki/Candelariella_viae-lacteeae)

**Holotype.** HUNGARY, Bács-Kiskun Prov.: Kecskemét area, Fülöphaza (20 km W of Kecskemét), alt. c. 150 m. 1987, *G. Thor 7015* (S!, isotypes STU, VBI).

*Candelariella viae-lacteeae* is characterized by its grey thallus, uniformly composed of sphaerical granules (Thor and Wirth 1990). Previous collections of this species from Switzerland have turned out to belong to other species, mostly *C. antennaria*, another

species with a grey thallus. However, a recent find of *C. viae-lacteeae* was made on mosses on a stem base of *Fraxinus excelsior*.

**Specimens examined.** Solothurn: Messen, alt. 500 m., 15 June 1998, *Zimmermann* (herb. E. Zimmerman).

***Candelariella xanthostigmoides* (Müll.Arg.) R.W.Rogers. *Muelleria* 5: 32. 1982.**

Mycobank: MB 108739

[http://species-id.net/wiki/Candelariella\\_xanthostigmoides](http://species-id.net/wiki/Candelariella_xanthostigmoides)

**Basionym.** *Lecanora xanthostigmoides* Müll.Arg., *Flora* 65: 484. 1882.

**Lectotype.** AUSTRALIA. New South Wales: Parramatta, *W. Woolls* (G! [G00290997], designated by Rogers 1982: 32).

New to Europe. This species is morphologically identical to *C. efflorescens* and can only be separated when fertile on account of its 8-spored asci compared to the c. 30-spored asci in *C. efflorescens* (Lendemer and Westberg 2010). The majority of the small, sorediate specimens in *Candelariella* are sterile and the distribution and frequency of the two species is thus poorly known. We found three fertile specimens with 8-spored asci which correspond well in all characters to *C. xanthostigmoides*. These specimens were mostly collected in humid places on *Salix* sp., *Betula* sp. and *Alnus* sp. The two specimens collected in the framework of the Red List project were identified as *Candelariella reflexa* (Nyl.) Lettau, another 8-spored, sorediate species. The latter species is however morphologically distinct also when sterile (see Lendemer and Westberg 2010). Earlier, the name *C. sorediosa* Poelt & Reddi had been used occasionally (e.g., Poelt and Vězda 1977) for similar specimens from Europe. We prefer to use the older name *C. xanthostigmoides* as the distinction between these two species is not clear and the type of *C. sorediosa* is poor (see also Lendemer and Westberg 2010).

**Specimens examined.** Basel-Land: Arisdorf, alt. 435 m, 4 Aug 1998, *Frei* (G 00057814); Jura: Le Noirmont, alt. 985 m, 8. Aug 1996, *Groner* (G 00057813); Schwytz, Rothenturm-Sattel, alt. 910-960 m, 30 Sept 1969, *Frey* (G 00122119).

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# *Boletopsis nothofagi* sp. nov. associated with *Nothofagus* in the Southern Hemisphere

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## Abstract

*Boletopsis nothofagi* sp. nov., an ectomycorrhizal taxon is described from *Nothofagus* forests in New Zealand. A comparison of available molecular ITS sequences, and morphological data was carried out to confirm the novelty of the taxon. This is the first report of the genus in the Southern Hemisphere.

## Key words

Bankeraceae, *Boletopsis nothofagi*, *Nothofagus*, New Zealand

## Introduction

A collection of a *Boletopsis* species was made in 2009 during the annual foray of the Fungal Network of New Zealand (FUNNZ), at the Orongorongo Valley in the Rimutaka Forest Park east of Wellington, North Island, New Zealand. The material was initially thought to be sterile but subsequent examination showed a few spores with the characteristic telephoroid morphology of *Boletopsis*. A subsequent collection in 2010 from South Island and re-collection of material at the North Island site provided more fertile material and is the basis for this description of a new species. This appears to be the first record of the genus in the Southern Hemisphere.

*Boletopsis* is a genus of ectomycorrhizal, stipitate, poroid fungi phylogenetically related to the hydroid (toothed) genera *Phellodon*, *Hydnellum*, *Bankera* and *Sarcodon* and placed in the Bankeraceae (Kirk et al. 2008). Four species are currently recognised in the subgenus *Boletopsis* (Stalpers 1993; Watling and Milne 2006); *B. leucomelaena*

(Pers.) Fayod, the type of the genus, *B. grisea* (Peck) Bond. & Sing., *B. smithii* K. Harrison and *B. perplexa* Watling & J. Milne. *Boletopsis leucomelaena* and *B. grisea* are widely distributed across North America, Europe and Asia, but are nowhere common (Gilbertson 1986). *Boletopsis leucomelaena* is usually associated with *Picea*, whereas *B. grisea* and *B. perplexa* are associated with *Pinus*, but there are records of these species associated with hardwoods. The ectomycorrhizal association of *B. smithii* is unknown. Recent molecular data supports the existence of additional taxa in North America (Watling and Milne 2008). *Boletopsis grisea* has also been reported from oak dominated cloud forest in Costa Rica and this represents the most southerly record of *Boletopsis* subgenus *Boletopsis* to date (Mata and Ryvardeen 2007). Two other species, *B. atrata* Ryvardeen and *B. subcitrina* Corner are pleuropodal, without inflated hyphae, and possess spores with small warts or spines. These species were segregated into *Boletopsis* subgenus *Boletopsina* (Stalpers, 1993). *Boletopsis subcitrina* subsequently formed the basis of a new genus *Corneroporus* (Hattori, 2001) and it seems likely this group is more distantly related to *B. leucomelaena* than the remaining four accepted species.

## Methods

DNA was extracted from dried herbarium material of the collection PDD96007 using REDExtract-N-Amp Plant PCR Kits (Sigma, USA). The tissue was ground in extraction buffer with a plastic pestle in the Eppendorf tube, then DNA extraction and PCR were carried out following the manufacturer's instructions. The extract was sequenced for the rRNA loci ITS1+5.8+ITS2 and LSU following the methods of (Johnston and Park 2005). Primers were ITS1F and ITS4 for the ITS region and LR0R and LR6 for LSU (Gardes and Bruns 1993). The chromatographs were assembled using Geneious (Drummond et al. 2011). Existing sequences were downloaded from GenBank for *Boletopsis* species, related New Zealand material and selected sequences of members of the Bankeraceae from other parts of the world (Table 1). Few LSU sequences are available for *Boletopsis*. This study focussed on ITS sequences resulting from an analysis of the relationships between *B. grisea*, *B. leucomelaena* and *B. perplexa* (Watling and Milne 2008).

Data exchange between applications was facilitated using Alter (Glez-Peña et al. 2010). Sequence alignment was carried using MAFFT (Katoh et al. 2002) within Geneious using the G-INS-i algorithm. Gblocks (Castersana 2000) was used to eliminate poorly aligned segments, with the number of contiguous conserved positions set to 8, the minimum block set to 10, and allowed gap positions to half, resulting in an alignment of 493 bases. The alignment was analysed by Jmodeltest (Posada 2008). A best-fit model of nucleotide substitution of GTR+G was proposed by jmodeltest. Phylogenetic analyses were performed MrBayes v3.2 (Huelsenbeck and Ronquist 2001) using the recommended model with two sets of four chains, one cold and three heated, with a chain temperature of 0.2. A sequence of *Piptoporus betulinus* was selected as an outgroup. All prior probabilities were left on default values. The model was run with a sampling fre-

**Table 1.** Sequences considered in the analysis.

GenBank Accession Numbers	Taxon	Collection	Country	Host	Notes
AF438567	<i>Boletopsis leucomelaena</i>		Korea	?	Without ITS2
DQ408766	<i>Boletopsis</i> sp.	Holden 150627	UK	<i>Pinus sylvestris</i>	= <i>B. perplexa</i> in Watling and Milne 2008
DQ408767	<i>Boletopsis</i> sp.	Wat. 28788	UK	<i>Pinus sylvestris</i>	= <i>B. perplexa</i> in Watling and Milne 2008, ≡Holden 077897
DQ408768	<i>Boletopsis grisea</i>	Kytovuori 94-1247	Finland	<i>Pinus sylvestris</i>	≡Rec227607 see Watling and Milne 2008
DQ408769	<i>Boletopsis grisea</i>	Niemela 6166	Finland	<i>Pinus sylvestris</i>	≡Rec227608 see Watling and Milne 2008
DQ408770	<i>Boletopsis grisea</i>	Palmen Aug01	Finland	<i>Pinus sylvestris</i>	Partial ITS1. ≡Rec227609 see Watling and Milne 2008, duplicated as EF457900 <i>B. sp.</i>
DQ408771	<i>Boletopsis leucomelaena</i>	Niemela 7749	Finland	<i>Picea</i> sp.	
DQ484064	<i>Boletopsis leucomelaena</i>	PBM 2678/ AFTOL 1527	USA		
EF457898	<i>Boletopsis</i> sp.	Rec227649	USA	?	
EF457899	<i>Boletopsis grisea</i>	Rec227658	USA	<i>Tsuga canadensis</i>	
EF457900	<i>Boletopsis</i> sp.	Rec227652	USA	?	Short sequence. Duplicate reference to Rec227609 as <i>B. grisea</i> see Watling and Milne 2008,
EF457901	<i>Boletopsis grisea</i>	Rec227653	USA	?	=Rec227652? see Watling and Milne 2008
EF457902	<i>Boletopsis grisea</i>	Rec227659	USA	<i>Pinus sylvestris</i>	=Rec227653? see Watling and Milne 2008
EF457903	<i>Boletopsis grisea</i>	Rec227656	USA	<i>Pinus contorta</i>	
EU622325	<i>Boletopsis</i> sp.	SL23	UK?	?	
FJ845401	<i>Boletopsis subsquamosa</i>	SMI350	USA?	?	= <i>B. grisea</i> see Watling and Milne 2008
AY569026	<i>Hydnellum cumulatum</i>	SEW 69	USA	<i>Tsuga</i>	
GU222291	<i>Phellodon sinclairii</i>	PDD89028	NZ	<i>Nothofagus</i>	
GU222318	<i>Phellodon</i> sp.	PDD89898	NZ	<i>Nothofagus fusca</i>	
GU222316	<i>Phellodon</i> sp.	PDD89880	NZ	<i>Nothofagus</i>	
GQ267479	<i>Pseudotomentella</i>	K98C35T239	NZ	<i>Pinus radiata</i>	
GQ267480	<i>Pseudotomentella tristis</i>	K91C38T363	NZ	<i>Pinus radiata</i>	

GenBank Accession Numbers	Taxon	Collection	Country	Host	Notes
FJ845438	<i>Sarcodon imbricatus</i>	SMI347	Canada	<i>Pinus?</i>	
GQ267491	<i>Tomentella</i> sp.	K80C37T323	NZ	<i>Pinus radiata</i>	
HQ533015	<i>Tomentellopsis</i> sp.	PDD95789	NZ	<i>Nothofagus solandri</i>	<i>T.</i> cf. <i>zygodesmoides</i> , with pale brown spores
JQ417193	<i>Boletopsis nothofagi</i>	PD96007	NZ	<i>Nothofagus fusca</i>	South Island, St Arnaud

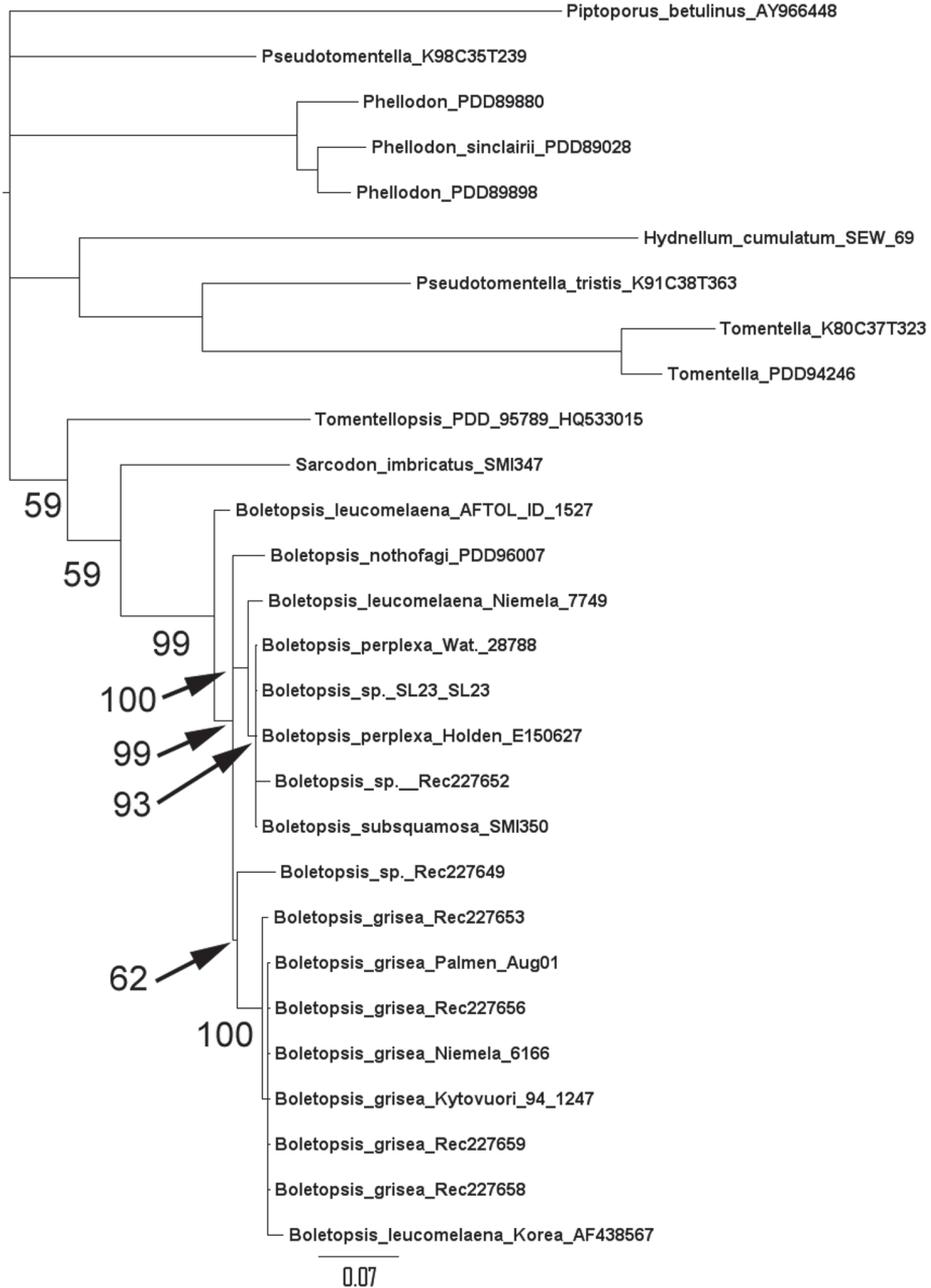
quency of 500 until the split-deviation frequency had fallen below 0.01, ca 1.2 million iterations. The results were examined to ensure good Metropolis coupling of chains and convergence statistics using Tracer (Rambaut and Drummond 2009). The first 25% of trees were removed in constructing a 50% majority rule consensus phylogram.

## Results

Figure 1 shows the results of the phylogenetic analysis. The species concepts *B. grisea* and *B. perplexa* are supported. *Boletopsis* sp. (SL23), *B. subsquamosa* (SMI350), and *Boletopsis* sp. (Rec227652) are also referable to *B. perplexa*. The latter collections confirm the presence of this taxon in North America, as suggested by (Watling and Milne 2008). A consensus concept of *B. leucomelaena* is less well supported by these preliminary data with the collections appearing separately in the analysis. It is possible the current use of the name *B. leucomelaena* represents multiple cryptic taxa; it is reported as occurring in widely separate geographic regions and with differing ectomycorrhizal hosts. A similar situation was recently demonstrated in the case of European species of related *Hydnellum* and *Phellodon* (Ainsworth et al. 2010). *Boletopsis nothofagi* is clearly supported as a new taxon differing in 22 sites relative to *B. leucomelaena* (AFTOL, DQ484064) and 18 sites relative to *B. leucomelaena* (Niemela, DQ408771).

## Discussion

The presence of *B. nothofagi* in New Zealand beech forests appears to have been overlooked despite a long history of the study of similar fungi in New Zealand (Cunningham 1958) (Maas Geesteranus 1971). This suggests *B. nothofagi* is a relatively rare (or rarely fruiting) indigenous member of the New Zealand ectomycorrhizal beech forest mycota. In addition no records of *Boletopsis* have been traced for any localities of naturally occurring *Nothofagus* forests in Australia, New Caledonia, New Guinea or South America. An alternative explanation for the presence of *B. nothofagi* in New Zealand is



**Figure 1.** Bayesian consensus phylogram, showing posterior probability, scale = substitutions per site

as a recent introduction of a species that usually has a different ectomycorrhizal host. Such introductions into New Zealand beech forests have occurred at least once in the case of *Amanita muscaria* (Johnston et al. 2008).

The absence of previous records of this recognisable species combined with the wide separation of the two currently known sites indicate that *Boletopsis nothofagi* is most likely a rare indigenous member of the New Zealand beech forest mycota. Its conservation status in New Zealand requires further investigation considering the status of other members of the family elsewhere in the world. For example many hydroid members of the family are threatened in Europe due to a variety of causes (Arnolds 2010). Many of these species are listed on European national red-data lists of fungi (Dahlberg and Mueller 2011). *Boletopsis grisea* is currently designated as threatened on five national lists and is subject to a number of management plans (Anon 1998–2011).

## Taxonomic treatment

### *Boletopsis nothofagi* J.A. Cooper & P. Leonard, sp. nov.

Registration Identifier: IndexFungorum IF550039

[http://species-id.net/wiki/Boletopsis\\_nothofagi](http://species-id.net/wiki/Boletopsis_nothofagi)

Holotype: PDD96007

**Description.** Basidiomes fasciculate, occasionally solitary, centrally stipitate, tough and fibrous. Pileus more or less convex, 10–80 mm diameter × 5–20 mm high, becoming undulate and edge somewhat incurved when young, smooth to finely fibrillose, grey [1D1, 1E1] (Kornerup and Wanscher 1989), weakly nigrescent when bruised and eventually becoming black. Stipe stuffed, clavate or cylindrical, 20–60 mm in length × 10–25 mm diameter, narrowing slightly at base and apex, smooth, dry, concolorous with pileus and darkening where bruised. Hymenial layer white 1–2 mm deep, bruising tan, pores angular, 2–3 per mm, drying pinkish tan, sometimes with lacerate edges. Pore layer extending slightly down the stipe and clearly delineated. Smell of dried material weakly of fenugreek, taste slightly acidic. Pileus immediately black in KOH, pigment leaching olivaceous black into white absorbent paper. Spore print not obtained.

Hyphal system monomitic. Pileus with a differentiated pileipellis consisting of a cutis, hyphae to 2 µm diameter, with brown plasmatic pigment, hyphal surface covered in small amorphous granular material becoming dark green in KOH and dispersing into medium. Subcutis with inflated gloeoplerous-like hyphae, thin-walled, to 6 µm diameter, clamped. Basidia pleurobasidial, cylindrical to clavate 5–10 × 20–30 µm, 4-spored, with basal clamp. Pores with fringe of slender clavate cystidia-like elements to 80 × 4 µm. Spores very pale tan, thin-walled, not dextrinoid or amyloid, non-cyanophilous, flat-topped tuberculate, with a narrowed waist. Spores dimensions are of a bounding rectangle encompassing maximum length and width of each spore. Length  $\mu=5.3$  µm,  $\sigma=0.5$ , width  $\mu=4.1$  µm,  $\sigma=0.5$ , Q  $\mu=1.35$ ,  $\sigma=0.2$  (combined statistics of measurement of 4, 13, 26, 20 spores from three fruiting bodies of the three collections).

**Distribution.** North and South Islands of New Zealand

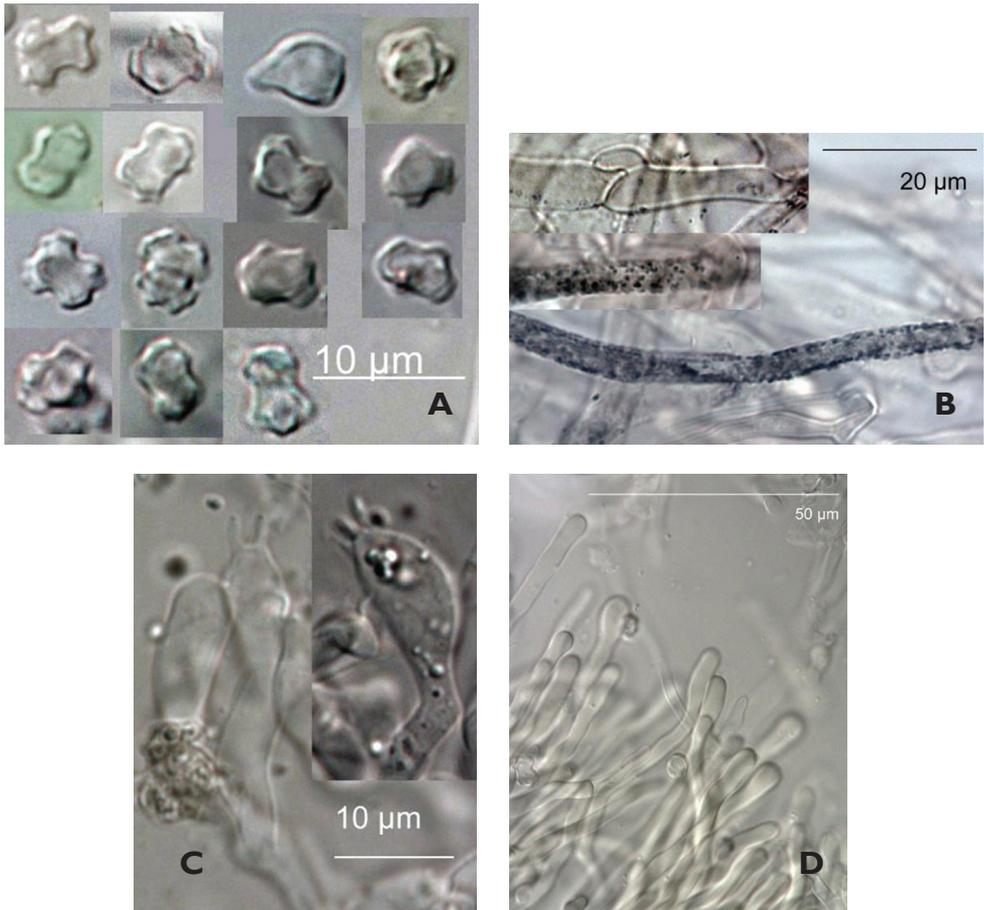
**Ecology.** ectomycorrhizal in southern beech (*Nothofagus*) forests and so far found only in association with *Nothofagus fusca*.



**Figure 2.** Basidiomes in natural habitat (PDD96007).



**Figure 3.** Basidiomes (PDD96007).



**Figure 4.** Microscopic Details. **A** Spores in Melzers (PDD96012) **B** Cap hyphae showing clamps and granules in KOH (PDD96012) **C** Basidia in KOH (PDD96012) **D** Cystidia-like elements in KOH (PDD96012)

**Etymology.** *nothofagi* for its ectomycorrhizal association with *Nothofagus*.

**Conservation status.** Although there are no data on the stability of the population size or historical changes in distribution of this species, it is likely to be naturally uncommon according to the New Zealand Threat Classification System (Townsend et al. 2008).

**Holotype.** NEW ZEALAND, North Island, Rimutaka Forest Park (under *Nothofagus fusca*), NZMG: 2671550E, 5982715N, 2<sup>nd</sup> May 201, D. Batchelor & P. Leonard, PDD96007 (PL3511)

**Other specimens examined.** New Zealand, North Island, Rimutaka Forest Park (under *Nothofagus fusca*), NZMG: 2671550E, 5982715N, 15<sup>th</sup> May 2009, T. Lebel, PDD95529 (JAC11078). West Bay, St Arnaud (*Nothofagus fusca*), South Island, NZMG: 1586280E, 5372097N, 11<sup>th</sup> May 2010, S. Kerr & P. Leonard, PDD96012.

**Discussion.** *Boletopsis nothofagi* differs from described species in the more elongate spores with a narrow central waist, granular extra-cellular material becoming green in KOH and habitat in *Nothofagus* forests (Niemala and Saarenoska 1989; Harrison 1975; Watling and Milne 2006).

## Acknowledgements

Thanks go to Teresa Lebel who discovered the original collection and made extensive notes of PDD95529, and to Shirley Kerr who discovered the second collection. Dukchul Park of Landcare Research carried out DNA extraction and sequence generation. This work was supported by the New Zealand Ministry of Science and Innovation.

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# Lectera, a new genus of the *Plectosphaerellaceae* for the legume pathogen *Volutella colletotrichoides*

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## Abstract

*Volutella colletotrichoides* is shown to belong to the *Plectosphaerellaceae* rather than the *Hypocreales* where other species of that genus reside. The new genus *Lectera* is described for *V. colletotrichoides*, and for a further, previously undescribed species with slightly longer conidia and differences in rDNA ITS and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sequences. *Lectera* species are found to be soil-dwelling organisms associated particularly with species of *Fabaceae*, and *L. colletotrichoides* is demonstrated to be a pathogen of *Cicer arietinum* and other grain legumes.

## Key words

Systematics, plant pathogenic fungi, phylogeny, *Cicer arietinum*

## Introduction

*Volutella colletotrichoides* was first described from diseased alfalfa and other forage legumes collected from Iowa, USA (Chilton 1954), evident as pink acervular dark-setose fruit bodies erumpent from stems and leaves. It was compared with four other species of *Volutella* known to be associated with legumes, and distinguished by its acervular setae that are dark rather than hyaline, and by the size of its conidia. The dark setae and

relatively large conidia invited comparison with *Colletotrichum* and led to choice of the specific epithet. A collection with especially numerous setae was given varietal status as *V. colletotrichoides* var. *setosa*.

The pathogenicity of *Volutella colletotrichoides* was demonstrated by observations of naturally infected plants in the glasshouse and in the field, and also by infection experiments (Chilton 1954). The original samples derived from stem cuttings of *Medicago sativa* (alfalfa), *Trifolium pratense* (red clover) and *Lotus corniculatus* (bird's foot trefoil), with *Medicago* plants proving the most vulnerable to infection, and were reproduced in plant samples transferred to Lincoln, Nebraska. Periodic observations over the following two years showed sparse and erratic occurrence of the pathogen, suggesting that the fungus is not a virulent pathogen, at least in those local conditions. Chilton found that inoculations on mature alfalfa and red clover plants produced petiole girdling and girdling of the stem tips, causing a blighted appearance in severely infected plants. Infection of mature stem tissues produced elliptical sunken lesions to a limited extent. No evidence of systemic invasion of the plant was found although stem infections gradually enlarged to involve much of the plant under high moisture conditions.

Host range experiments (Chilton 1954) provided evidence that *Trifolium hybridum* and *Medicago falcata* were susceptible to infection, in addition to the three plant species on which the original observations were made. In contrast, *Melilotus alba* and *Glycine max* were not infected under similar experimental conditions. As the plants originally infected were of foreign origin (Chilton did not specify from where) and the occurrence of the pathogen was highly localized, it seems likely that *Volutella colletotrichoides* was a pathogen introduced into the USA that did not become established, rather than a native species. There are no further records of the species from the USA, and no material in the national fungus collection **BPI** (Farr and Rossman 2011).

Published reports of *Volutella colletotrichoides* from other regions are sparse. It was reported from *Glycine max* in Ethiopia by Mengistu and Sinclair (1979), from the same host (as *G. ussuriensis*) in Democratic Republic of the Congo by Lenné (1990), and on *Senna sophera* from India (Lenné 1990). Eken et al. (2002) showed the fungus to be present on alfalfa in Turkey. A sequence from an unlocalised sample on *Viola* sp. was submitted to GenBank (<http://www.ncbi.nlm.nih.gov/nuccore/AJ301962.1>) by Hagedorn (unpubl., 2002), and cultures from alfalfa from Turkey (reported above) and South Africa are stored in the CBS culture collection (<http://www.cbs.knaw.nl/>). The plant associates given above do not necessarily imply a biological relationship.

In the winter of 1988, a field survey was carried out for diseases of chickpea (*Cicer arietinum*) in the vicinity of Salheia, at that time a newly reclaimed region between the Nile Delta and the Suez Canal (Ismaelia) in northern Egypt. There, blighted chickpea plants were observed and collected, with the causal organism initially assumed to be the common chickpea pathogen *Ascochyta rabiei*. The macroscopic visible symptoms were circular or elongated dark brown to black anthracnose-like lesions approximately one centimeter long on the lower stem parts in addition to partly chlorotic leaves. The

causal organism was isolated and sent to the Danish Government Institute of Seed Pathology for Developing Countries (DGISP), and designated as DGISP 271. Later the diagnostic studies were transferred to The Danish Veterinary and Agricultural University. Here the organism was registered as CP 2035. After an extended period of analysis using both morphological and molecular methods with multiple collaborators, the affinities of CP 2035 have finally been confirmed to be with *V. colletotrichoides*.

As Chilton (1954) had observed, we found that *Volutella colletotrichoides* differed in a number of morphological characteristics from other *Volutella* species. As molecular analysis has confirmed this separation, we therefore describe the new genus *Lectera* for *V. colletotrichoides* and a similar, closely related species.

## Materials and methods

Isolates were retrieved from storage in the CABI Genetic Resources Collection (see Table 1). For morphological analysis, strains were grown at 25°C for 7 d on PCA and PDA media (Smith and Onions 1994). Observations were made using microscope preparations in water and lactic acid, with measurements made from slides mounted in lactic acid.

Infection studies were carried out, using chickpea cultivar Family 88 as well as certain other species of *Fabaceae* as hosts: *Vicia faba*, *Glycine max*, *Pisum sativum*, *Vigna unguiculata*, and *Phaseolus vulgaris*. The inoculum was produced as above. Stems and leaves were inoculated without prior wounding using a spore suspension produced by flooding four week old cultures with 10 ml sterile distilled water. The conidial concentration was adjusted to  $2 \times 10^5$  ml<sup>-1</sup> and finally one drop of Tween 20 (as detergent) was added. Symptoms were observed two weeks after inoculation.

For the molecular analysis, strains were grown on malt extract (MADW) agar plates (Smith and Onions 1994) at 28°C for 5–7 days. The isolates were subcultured onto fresh MADW plates and incubated at 28°C for 7–10 days prior to DNA extraction.

Total genomic DNA was obtained from a small loopful (1 µl) of each strain using a proprietary complex DNA release solution (microLYSIS®-PLUS; Microzone Ltd, UK) in accordance with manufacturer's instructions. The thermal cycler lysis profile was: 15 min at 65°C, 2 min at 96°C, 4 min at 65°C, 1 min at 96°C, 1 min at 65°C, 30 s at 96°C and hold at 20°C.

Partial ribosomal RNA gene clusters (part of 18S small subunit RNA gene, internal transcribed spacer 1 (ITS1), 5.8S ribosomal RNA gene, internal transcribed spacer 2 (ITS2), part of 28S large subunit ribosomal RNA gene) were amplified by polymerase chain reaction (PCR) using primer set TW81 (fwd): 5'-GTTTCCGTAGGTGAACCTGC-3' & AB28 (rev): 5'-ATATGCTTAAGTTCAGCGGGT-3' (Curran et al. 1994; Sigma Genosys, UK). Part of the glyceraldehyde 3-phosphate dehydrogenase gene was amplified using the primers GDF: 5'-GCCGTCAACGACCCCTTCATTGA-3' & GDR: 5'-GGGTGGAGTCGTACTIONTGGAGCATGT-3' (Prihastuti et al.

**Table 1.** Newly sequenced strains in this study

Reference no.	Species	Host/substratum, geographical origin	ITS	GAPDH
IMI 185380	<i>Acremonium nepalense</i>	<i>Pinus</i> sp. soil, Nepal	JQ647430	
IMI 337226	<i>Bionectria coronata</i>	<i>Serjania</i> sp., Venezuela	JQ700569	
IMI 46339	<i>Gibellulopsis nigrescens</i>	Soil, UK	JQ647441	JQ724142
IMI 118380	<i>Gibellulopsis nigrescens</i>	<i>Beta vulgaris</i> , Canada	JQ647440	JQ724134
IMI 265740	<i>Lectera colletotrichoides</i>	Soil, Brazil	JQ647449	JQ724136
IMI 303685	<i>Lectera colletotrichoides</i>	<i>Capsicum annuum</i> , Morocco	JQ647450	JQ724140
IMI 332702	<i>Lectera colletotrichoides</i>	<i>Cicer arietinum</i> , Egypt	JQ647428	
IMI 366179	<i>Lectera colletotrichoides</i>	<i>Phaseolus vulgaris</i> , Ethiopia	JQ693168	
IMI 368065	<i>Lectera colletotrichoides</i>	<i>Xanthium spinosum</i> , Argentina	JQ647451	JQ724141
IMI 181698	<i>Lectera longa</i>	<i>Triticum</i> sp., Australia	JQ647448	JQ724135
IMI 312228	<i>Plectosphaerella cucumerina</i>	<i>Daucus carota</i> , Denmark	JQ647434	JQ724139
IMI 49467b	<i>Pseudonectria buxi</i>	<i>Buxus sempervirens</i> , UK	JQ693165	
IMI 61338	<i>Pseudonectria buxi</i>	<i>Buxus</i> sp., UK	JQ647445	
IMI 311153	<i>Pseudonectria buxi</i>	<i>Buxus sempervirens</i> , France	JQ693164	
IMI 322095	<i>Verticillium dahliae</i>	<i>Alectryon conaceum</i> , UK	JQ647437	JQ724138
IMI 364520	<i>Verticillium dahliae</i>	<i>Prunus persica</i> , Yugoslavia	JQ647438	JQ724137
IMI 62131	<i>Verticillium nonalfalfae</i>	<i>Lycopersicon esculentum</i> , UK	JQ647435	JQ724134
IMI 67913	<i>Volutella ciliata</i>	<i>Pisum sativum</i> , UK	JQ647447	
IMI 74420	<i>Volutella ciliata</i>	unknown host, Germany	JQ693167	
IMI 341382	<i>Volutella ciliata</i>	<i>Sclerotinia sclerotiorum</i> , locality unknown	JQ693166	
IMI 341383	<i>Volutella ciliata</i>	<i>Sclerotinia sclerotiorum</i> , locality unknown	JQ647446	
IMI 332191	<i>Volutella consors</i>	unknown source, India	JQ693163	
IMI 92688	<i>Volutella lini</i>	unknown host, India	JQ647452	
IMI 224502	<i>Volutella lini</i>	unknown host, India	JQ693169	
IMI 136704	<i>Volutella ramkumarii</i>	Submerged petioles, Papua New Guinea	JQ647453	

2009; Sigma Genosys, UK). PCR was undertaken in a ThermoHybaid PCR Express thermal cycler (Thermo-Hybaid, UK) using a reaction mix containing 3 pmoles of each primer, 1  $\mu$ l of template DNA solution and 10  $\mu$ l of MegaMix-Royal (Microzone Ltd, UK) containing optimised mixture of *Taq* polymerase, anti-*Taq* polymerase monoclonal antibodies in 2  $\times$  Reaction Buffer (6 mM MgCl<sub>2</sub>) with 400  $\mu$ M dNTPs made up to a final volume of 20  $\mu$ l with sterilised ultrapure H<sub>2</sub>O. Amplification conditions were: 95°C for 5 min followed by 30 cycles of 30 s at 95°C, 30 s at 50°C, 45 s at 72°C, followed by 5 min at 72°C and hold at 10°C.

Aliquots (4  $\mu$ l) of amplification products were assessed for quality by gel electrophoresis using 1.5 % Seakem LE agarose (BMA, UK) for 2 h at 5V cm<sup>-1</sup> in half-

strength Tris-Borate-EDTA buffer (*i.e.*, 0.5 × TBE buffer; 45 mM Tris; 45 mM Boric acid; 1.25 mM EDTA, pH 7.5, see Sambrook et al. 1989) containing 5 µl of SafeView Nucleic Acid Stain (NBS Biologicals Ltd, UK) per 100 ml of buffer. Gel images were captured by using the U:Genius gel documentation system (Syngene, UK) and stored as TIFF bitmaps for later use.

Remaining unused PCR products were purified with the microCLEAN PCR Purification Kit (Microzone Ltd, UK) following the manufacturer's instructions. The purified PCR products were utilised in sequencing reactions undertaken in a Primus 96 plus thermal cycler (MWG-BIOTECH AG, Germany) by using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) with primer TW81 (as above). Sequencing conditions were: 96°C for 1 min followed by 25 cycles of 20 s at 96°C, 10 s at 50°C, 4 min at 60°C (ramp rate: 1°C s<sup>-1</sup>). Excess unincorporated BigDye was removed with DyeEx 2.0 affinity columns (Qiagen Ltd., UK) according to the manufacturer's instructions and the sequencing reaction products were suspended in HiDi Formamide (Applied Biosystems, UK). These products were separated on a capillary array 3130 Genetic Analyser (Applied Biosystems, UK).

Sequence trace files were first assessed for quality using Sequencing Analysis Software v5.2 Patch 2 (Applied Biosystems, UK) and then checked manually using the software package Chromas v2.23 (Technelysium, Australia) and exported as text files. Sequences were deposited in GenBank and Accession numbers obtained (see Table 1).

Sequences of studied species were aligned using the default parameters of Clustal W (Thompson et al. 1994), then optimised manually using the CLUSTALW plug-in of MEGA5 (Tamura et al. 2011). Phylogenetic inferences were made from Neighbour Joining trees constructed from Maximum Likelihood in Mega5. Branch support was estimated by bootstrap analysis (1000 replicates). Accession numbers of rDNA ITS and GAPDH sequences derived from the public databases are cited on the phylogenetic trees.

## Results

### Pathogenicity

Besides *Cicer arietinum* (from which the tester strain CP2035 (IMI 332702) was isolated), the following leguminous plants were found to function as hosts after experimental inoculation: *Glycine max*, *Phaseolus vulgaris*, *Pisum sativum*, *Vicia faba* and *Vigna unguiculata*. The main symptoms were evident as brown to black lesions on the lower stem parts, within which acervuli develop. Leaves also showed similar symptoms in all species, in addition to chlorosis. The symptoms were very similar to those caused by *Ascochyta rabiei*, a well-known and commonly occurring pathogen causing ascochyta blight on chickpea.

## Taxonomy

### ***Lectera* P.F. Cannon, gen. nov.**

<http://species-id.net/wiki/Lectera>

Mycobank: MB 550041

**Etymology.** Named after Dr. Hannibal Lecter (Harris 1988), another aggressive organism with a liking for fava beans (*Vicia faba*).

Conidiomata intermediate between sporodochia and acervuli, erumpent through host tissues and without a clear upper wall, hemispherical to ± globose, pink or flesh-coloured, accompanied by dark brown septate tapering, primarily marginal, setae. Conidia formed from hardly modified conidiogenous cells, hyaline, aseptate, slightly curved, smooth, fusiform with pointed ends. Appressoria formed after conidial germination, dark brown, round to ovate with smooth margins.

**Type species.** *Lectera colletotrichoides* (Chilton) P.F. Cannon

### ***Lectera colletotrichoides* (Chilton) P.F. Cannon, comb. nov.**

Mycobank: MB 550042

*Volutella colletotrichoides* Chilton, *Mycologia* 46: 801 (1954), basionym.

*Volutella colletotrichoides* var. *setosa* Chilton, 46: 801 (1954).

**Description.** Conidiomata formed at the apex of a short peg-like vegetative structure that is erumpent through host tissues, 80–350 µm diam, cushion-shaped to almost globose, with a compact palisade of conidiogenous cells usually surrounded by setae. Setae variably produced, with some conidiomata dark grey in coloration due to copious setae while others are pale pink or flesh-coloured with few or no setae; 50–130 µm in length, 3–6 µm in diam at the base, gradually tapering, golden to dark brown, smooth or sparsely verrucose, 2- to 3-septate, the apex acute. Conidiogenous cells 15–32 × 3–5 µm, cylindrical or slightly tapering with the apex rounded, proliferating percurrently with inconspicuous periclinal thickening and sometimes a minute collarete. Conidia inoculated onto *Medicago sativa* stem (6.5-) 7–10 (-11.5) [mean 8.35 µm, sd 0.73, n = 140] × 2.5–3 (-3.5) µm [mean 2.67 µm, sd 0.24, n = 140], mean length/width ratio 3.14: 1, cylindrical to cylindrical-fusiform or navicular, the ends acute, slightly usually inaequilateral with one longitudinal face ± flat, hyaline, aseptate, smooth-walled, without a gelatinous sheath or appendages. Cultures on PCA and PDA at 25°C under alternating daylight/near UV growing moderately slowly, reaching 25–30 mm after 7 d, bright orange with a waxy appearance, the central part becoming brownish after 14 d, aerial mycelium poorly developed. Conidiomata absent or poorly developed, with setae fewer, narrower, shorter and less pigmented than in colonies on host tissue, and conidiogenous cells often formed singly at the apex of vegetative hyphae. Conidia 6.5–9 (-10.5) [mean 7.41 µm, sd 0.68, n = 100] × 2–3 µm [mean 2.43 µm, sd 0.22, n

= 100], mean length/width ratio 3.07: 1, similar in appearance to those formed after inoculation on *Medicago sativa* stems. Appressoria 4.5–8 × 4–6.5 µm, circular to ovate with entire margins, dark brown. Sclerotia not observed.

**Typification. USA:** Iowa, Ames, on stems of *Medicago sativa*, Oct. 1954, J. Chilton (ISC 217496, **lectotype, here designated**; K(M) 176269, **isolectotype** of *Volutella colletotrichoides*). **USA:** Iowa, Ames, on stems of *Medicago sativa*, Oct. 1954, J. Chilton (ISC 217482, **lectotype, here designated**; K(M) 176270, **isolectotype** of *Volutella colletotrichoides* var. *setosa*). **Other authentic material:** same collection data, ISC 217488, ISC 217497 and ISC 217498 – syntypes of *V. colletotrichoides*; same collection data, ISC 217483 and ISC 217484 – syntypes of *V. colletotrichoides* var. *setosa*.

**Host species.** Associated with *Asteraceae* (*Xanthium spinosum*), *Fabaceae* (*Cicer arietinum*, *Glycine max*, *Lotus corniculatus* [Chilton, 1954], *Medicago falcata* [Chilton, 1954], *M. sativa*, *Phaseolus vulgaris*, *Pisum sativum*, *Senna sophora*, *Trifolium hybridum* [Chilton, 1954], *T. pratense* [Chilton, 1954], *T. subterraneum*, *Vicia faba* and *Vigna unguiculata*), *Lamiaceae* (*Tectona grandis*), *Poaceae* (*Agrostis stolonifera*, *Hordeum vulgare*, *Triticum* sp. and *Zea mays*), *Solanaceae* (*Capsicum annuum*) and *Violaceae* (*Viola* sp.).

**Distribution.** Africa (Democratic Republic of the Congo, Egypt, Ethiopia, Morocco, Nigeria, South Africa [<http://www.cbs.knaw.nl/>], Zimbabwe [unpublished IMI record without voucher material]). Asia (India, Kuwait [unpublished IMI record without voucher material], Turkey [Eken et al. 2002]). Australasia (Australia, New Zealand). Europe (United Kingdom). North America (USA: Iowa, presumed introduced and now eradicated). South America (Argentina, Brazil).

**Interactions.** Strains of *Lectera colletotrichoides* have been demonstrated to be pathogenic towards a range of *Fabaceae* species, but they are also commonly found associated with plants from other families and isolated from soil and plant litter. It also grows well in standard agar culture. It therefore can be presumed to exist (and probably grow actively) as a saprobe, and it is possible that the non-legume isolates originate from soils used to grow legumes in rotation.

**Conservation assessment.** The species as currently circumscribed has not been reported since 2002 but is very widely distributed, is associated with a wide range of plant taxa and apparently can exist as a saprobe in soil and leaf litter without a direct plant association. It does not appear to be an economically important pathogen except perhaps in limited circumstances. However, as there is a high risk of confusing disease symptoms of *Lectera colletotrichoides* with those caused by *Ascochyta rabiei*, the economic impact of *Lectera colletotrichoides* may be underestimated. It may be sensitive to agricultural pesticides, but is unlikely to face major threats from specific eradication measures. Its conservation status (Dahlberg and Mueller 2011) must be considered as Data Deficient, but is probably of Least Concern.

**Specimens examined. Africa: DRC:** Mulungu, causing leaf spot of *Glycine soja*, comm. 28 Mar. 1978, *D.J. Allen* (IMI 226829a). **Egypt:** Ismailia, Salheia, isol. ex *Cicer arietinum*, 1988, *M. Askar* CP2035 (IMI 332702). **Ethiopia:** unlocalized, isol. ex seed of *Phaseolus vulgaris*, undated, *s. coll.* (IMI 366179). **Morocco,** unlocalized, isol. ex *Capsicum annuum*, Feb. 1986, *Hasmi* (IMI 303685). **Nigeria:** unlocalized, isol. ex

*Vigna sinensis*, comm. 22 May 1972, R. Williams 4150 (IMI 166385); same data, R. Williams 4152 (IMI 166394). **Asia: India:** Rajupur, Mirzapur, on and isol. ex *Senna sophora*, Apr. 1982, S.N.P. Chaurasia SNPC-5 (IMI 269185); Jabalpur, isol. ex leaf litter of *Tectona grandis*, comm. 5 Feb. 1986, Jamaluddin (IMI 265505). **Australasia:** **Australia:** New South Wales, unlocalized, isol. ex *Agrostis stolonifera*, comm. 26 Jan. 1983, G. Schultz (IMI 275263); Western Australia: Esperance, isol. ex *Trifolium subterraneum*, comm. 13 Jul. 1972, R.F. Doepel (IMI 167533); **New Zealand:** Wellington, isol. ex *Hordeum vulgare*, comm. 11 Apr. 1980, L. Chong 18P14 (IMI 247318). **Europe: United Kingdom:** Kent, nr Ashford, Wye College, comm. 24 Oct. 1979, D.W. Parry 6 (IMI 242382); same locality, isol. ex *Zea mays*, comm. 24 Oct. 1979, D.W. Parry 11 (IMI 242387). **South America: Argentina:** Córdoba, San Francisco, Monte Cristo, isol. ex stem of *Xanthium spinosum*, 1995, s. coll. (IMI 368065). **Brazil:** unlocalized, isol. ex soil, comm. 18 Feb. 1982, J. Diehl & E. Reis 46/81 (IMI 265740).

***Lectera longa* P.F. Cannon, sp. nov.**

[http://species-id.net/wiki/Lectera\\_longa](http://species-id.net/wiki/Lectera_longa)

Mycobank: MB 550043

**Description.** Differs from *Lectera colletotrichoides* by its longer conidia (7.8–10 × 2–2.5 µm; mean 8.87 µm, sd 0.56, n=20) with a mean length/breadth ratio of 3.98: 1, with three short insertions and a single substitution in the ITS sequence.

**Typification: Australia.** Western Australia: Nedlands, isol. ex *Triticum* sp., 25 Jan. 1974, K. Sivasithamparam 530 (IMI 181698) – holotype of *Lectera longa* (dried specimen) and associated living culture.

**Host species.** Associated with *Triticum* sp. (*Poaceae*).

**Distribution.** Only definitely known from the type locality.

**Interactions.** No details are known; the data associated with the type do not indicate whether the fungus was thought to be pathogenic.

**Conservation assessment.** The species is only known from a single collection made in 1974, though a strain isolated from *Viola* sp. and identified as *Volutella colletotrichoides* with an identical ITS sequence (AJ301962) was deposited in the BBLF culture collection as BBA 71246. Its geographical origin is unknown, and the status of the living culture is uncertain. Its conservation status (Dahlberg and Mueller 2011) must be considered as Data Deficient, but is potentially Critically Endangered.

## Discussion

The genus *Volutella* is poorly researched and no modern monograph is available, but some of the more well-known species, including the type *V. ciliata*, were included in a recent phylogenetic study of *Fusarium*-like fungi (Gräfenhan et al. 2011). This work demonstrated that *Volutella* (as represented by its type) occupies a distinct clade within

the *Nectriaceae*, with *Chaetopsina* as sister group and *Pseudonectria buxi* (the anamorph of which was at one time ascribed to *Volutella*) also related. *Volutella* in its currently understood sense is clearly polyphyletic (Seifert et al. 2011), with many of its constituent species referable to other genera.

*Lectera* has brightly coloured sporodochia surrounded by brown setae (those in true *Volutella* species are hyaline). Other sporodochial genera with these characteristics include *Kutilakesa*, *Sarcopodium* and *Actinostilbe* (Seifert et al. 2011). The taxonomic affinities of the type of *Kutilakesa* are currently unclear (no sequences are available), but the only other species in that genus has a *Nectriella* teleomorph (Alfieri and Samuels 1979) and is therefore likely to belong to the *Hypocreales*. The type of *Sarcopodium* belongs to the *Lanatonectria* clade (*Nectriaceae*, *Hypocreales*) according to Summerbell et al. (2011). Both *Kutilakesa* and *Sarcopodium* have flexuous verruculose setae with rounded apices, in contrast to *Lectera*, where they are straight, hardly ornamented and tapering to an acute tip. On one occasion, a teleomorphic fungus with thin-walled asci and aseptate ascospores was observed on old wilted plant material infected by *L. colletotrichoides*, but the observation could not be repeated and we are not confident that the two fungi are genetically connected.

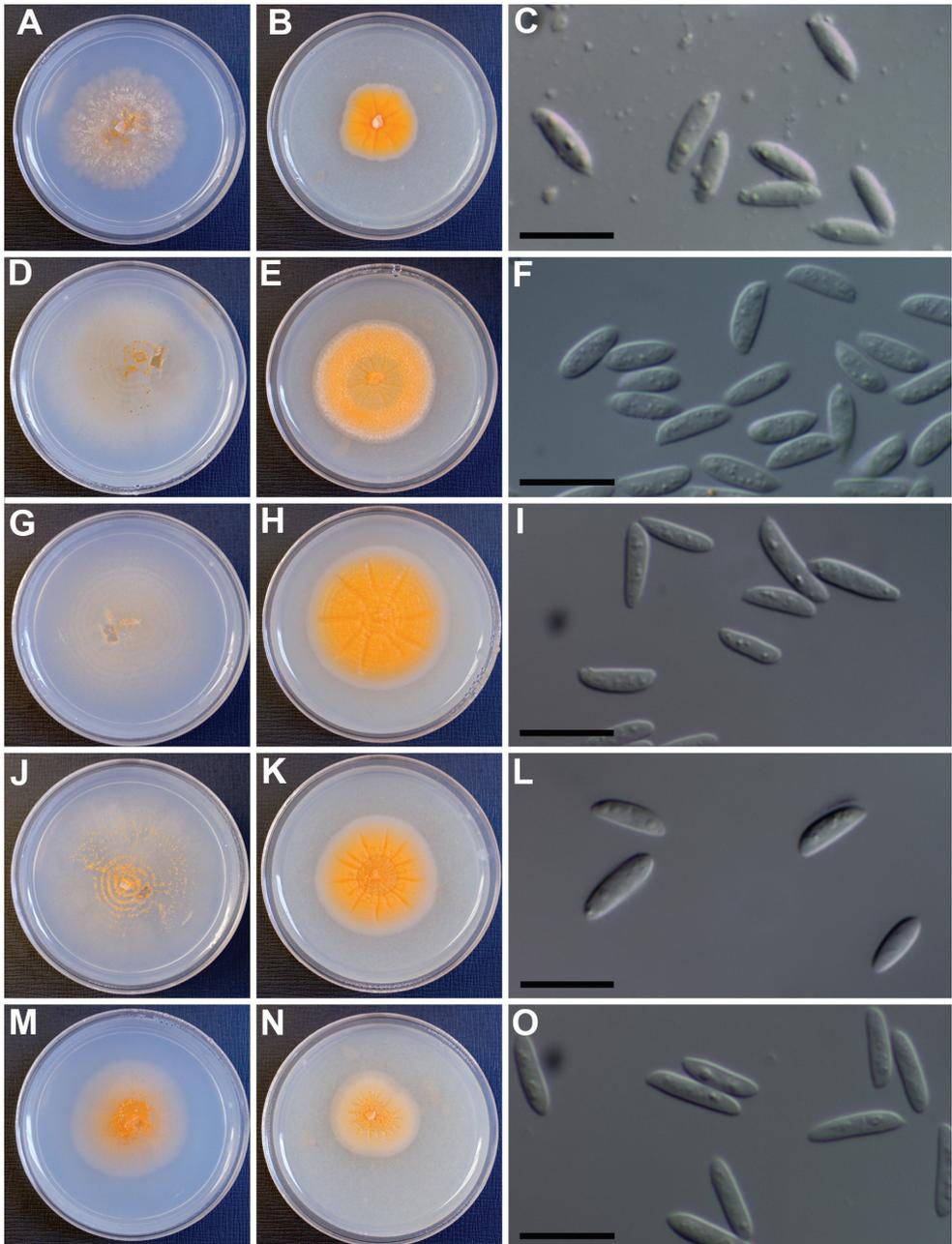
One other species of *Volutella* has orange sporodochia surrounded by brown tapering setae, in common with *Lectera colletotrichoides*, *V. melaloma* Berk. & Br. which was described from dead leaves of *Carex* in the UK (Berkeley and Broome 1850). It is possible that this species is congeneric with *L. colletotrichoides*, but it differs in a number of important characters: the conidiogenous cells are thicker-walled and do not taper, the setae are longer, more closely septate and smooth-walled, and the conidia are significantly larger and more tapered, measuring  $15\text{--}18 \times 3.5\text{--}4.5 \mu\text{m}$ . No recently collected material is available for sequencing.

The ITS sequences generated in this study strongly suggest that *Lectera* has affinities with the *Plectosphaerellaceae*, and may be a sister group to *Verticillium* (Fig. 1), although this relationship only receives weak bootstrap support. That systematic relationship was previously noted incidentally by Réblová and Seifert (2004), while researching into the phylogeny of *Conioscypha* and its relatives. Many fungi in these groups are wide-spectrum soil-borne plant pathogens, in common with *Lectera*. The *Plectosphaerellaceae* includes a series of *Acremonium*-like fungi as well as *Plectosphaerella*, *Verticillium*, *Gibellulopsis* and *Musicillium* (Zare et al. 2004, 2007). *Verticillium* has recently been monographed using multigene phylogenetic data (Inderbitzin et al. 2011), but its generic position was not assessed. Based on LSU and SSU rDNA data, Réblová et al. (2011) considered the *Plectosphaerellaceae* to be well-defined, and a sister group with the *Glomerellales* to the *Microascales*, an order containing important plant pathogens such as *Ceratocystis*.

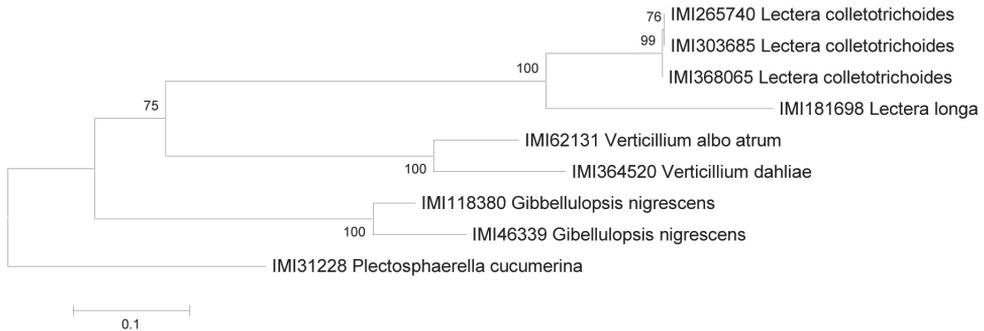
*Lectera colletotrichoides* contains two distinct ITS phylotypes, IMI 265740 differing from the other strains sequenced by only a single base pair. The ITS sequence of *L. longa* differs from *L. colletotrichoides* by three short insertions and a single substitution, and this combined with differences in conidial length and length/width ratio justifies its separation. The distinction between the two species is supported by GAPDH sequences also (see fig. 3). The gene fragment sequenced here is a ~200 bp intron in the glyceraldehyde 3-phosphate dehydrogenase gene, used for phylogenetic studies of



Figure 1. ML ITS phylogram showing the phylogenetic position of *Lectera* species.



**Figure 2.** *Lectera* species. **A–L** *L. colletotrichoides* **A–C** IMI 166394 **D–F**, IMI 265740 **G–I** IMI 303685 **J–L** IMI 368065 **M–O** *L. longa* (IMI 181698) **A, D, G, J, M** colonies on PCA agar after 7 d **B, E, H, K, N** colonies on PDA agar after 7 d **C, F, I, L, O**: conidia mounted in lactic acid: bar = 10  $\mu$ m.



**Figure 3.** ML tree of GAPDH sequences, of a subset of the strains used for the ITS sequence set.

*Colletotrichum* by Damm et al. (2009) and Guerber et al. (2003), not the same fragment used for *Verticillium* by Inderbitzin et al. (2011).

The wide distribution of *Lectera colletotrichoides* and its association with agricultural ecosystems make it difficult to assess its geographical origin, although its apparent preference for dry-land legumes might indicate an evolutionary history centred on the Near East. However, judging from environmental sequencing studies, it (or a closely related species) does seem to be present in soils in SE USA (Wu et al. 1997, Jackson 2010), suggesting that its distribution in that country is not as restricted as indicated by Chilton (1954).

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# Two new species of *Lecanora sensu stricto* (Lecanoraceae, Ascomycota) from east Africa

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## Abstract

The new sorediate species *Lecanora kenyana* from Mount Kenya and *L. orientoafricana* from the Rift Valley in Kenya are described. *L. kenyana* has red-brown apothecia with a constricted base, a *melacarpella*-type amphithecium, *pulicaris*-type epihymenium, a hyaline hypothecium, and contains usnic acid as major constituent. *L. orientoafricana* is characterized by having a dark hypothecium, *pulicaris*-type amphithecium, *chlarotera*-epihymenium, and contains atranorin and gangaleoidin. A phylogenetic analysis using maximum likelihood and a Bayesian approach based on DNA sequence data of mtSSU and ITS rDNA support that both new species belong to *Lecanora sensu stricto* and cluster with species containing usnic acid or having a dark hypothecium, respectively.

## Key words

Kenya, Lecanorales, new species, taxonomy, tropical lichens

## Introduction

*Lecanora* is the major genus of Lecanoraceae (Lumbsch and Huhndorf 2010) and includes crustose (incl. placodioid) lichens with hyaline, usually non-septate ascospores, *Lecanora*-type asci and mostly lecanorine apothecia. The morphological and chemical diversity is large in this heterogeneous genus and molecular data have supported that the genus as currently circumscribed is not monophyletic (Arup and Grube 1998; 2000; Grube et al. 2004; Lumbsch 2002). The core group of *Lecanora sensu stricto* is characterized by the presence of calcium oxalate crystals in the amphithecium, filiform conidia, and the presence of atranorin and/or usnic acid. This agrees with an extend-

ed circumscription of the *Lecanora subfusca* group to include taxa containing usnic acid and a dark hypothecium (Guderley 1999; Lumbsch 1995; Lumbsch et al. 1995; Lumbsch et al. 1996; Lumbsch et al. 2003; Papong et al. in press). African species of *Lecanora* sensu stricto are poorly known but our recent studies resulted in the description of a new species and new records of *Lecanora* species for Kenya (Kirika et al. in press; Lumbsch et al. 2011). Among the collections from the Mount Kenya area and the Rift Valley we found two corticolous species, one sorediate taxon with usnic acid and a morphologically somewhat similar species with a dark hypothecium. The two taxa do not agree with known species (Lumbsch 1995; Lumbsch et al. 1995; Lumbsch et al. 1996; Papong et al. 2011; Papong and Lumbsch 2011) and consequently are described as new. To confirm the placement of the new species in *Lecanora* sensu stricto, we also generated DNA sequence data of the internal transcribed spacer region (ITS) and partial sequences of the small subunit of the mitochondrial ribosomal DNA (mtSSU) and performed a phylogenetic analysis with sequences available in Genbank.

## Materials and methods

### Taxon sampling and molecular methods

The study is based on material deposited in EA and F and DNA sequences downloaded from Genbank. Sequences of five *Ramboldia* spp. were included as outgroup since the genus has been shown previously to be related to *Lecanora* (Kalb et al. 2008). Sequence data of the two new species were assembled with sequences of the mitochondrial small subunit (mtSSU) and nuclear ITS rDNA downloaded from Genbank (Table 1). Sample preparation, DNA isolation, PCR and direct sequencing were performed as described previously (Mangold et al. 2008; Wirtz et al. 2012). Primers for amplification were: mr SSU1 (Zoller et al. 1999) and MSU7 (Zhou and Stanosz 2001) for mtSSU and ITS1F and ITS4 (Gardes and Bruns 1993) for ITS rDNA. Sequence fragments obtained were assembled with SeqMan 4.03 (DNASTAR) and manually adjusted.

**Table 1.** Species and specimens used in the present study. Newly obtained sequenced in bold.

Species	Collection data	mtSSU acc. no.	ITS acc. no
<i>Lecanora achroa</i>		JQ782663	JN943714
<i>Lecanora</i> aff. <i>achroa</i>		JQ782662	JQ782708
<i>Lecanora allophana</i>		AY567710	AF070031
<i>Lecanora argentata</i>		JQ782664	JQ782704
<i>Lecanora argopholis</i>		DQ787358	JQ782705
<i>Lecanora austrotropica</i>		JQ782665	JQ782706
<i>Lecanora californica</i>		JQ782668	JQ782707
<i>Lecanora campestris</i>		DQ787362	AF159930
<i>Lecanora elatinoides</i>		JQ782669	JQ782709
<i>Lecanora flavopallida</i> 1		JQ782673	JN943723

Species	Collection data	mtSSU acc. no.	ITS acc. no
<i>Lecanora flavopallida</i> 2		JQ782674	JN943724
<i>Lecanora flavoviridis</i>		JQ782675	JQ782711
<i>Lecanora gangaleoides</i>		JQ782676	JQ782712
<i>Lecanora helva</i> 1		JQ782679	JQ782716
<i>Lecanora helva</i> 2		JQ782680	JQ782715
<i>Lecanora</i> aff. <i>helva</i>		JQ782677	JQ782713
<i>Lecanora</i> aff. <i>helva</i>		JQ782678	JQ782714
<i>Lecanora hybocarpa</i>		DQ912273	DQ782849
<i>Lecanora kenyana</i>	Kenya, Kirika 1179 (F)	<b>JQ900616</b>	<b>JQ900618</b>
<i>Lecanora leproplaca</i> 1		JQ782683	JQ782718
<i>Lecanora leproplaca</i> 2		JQ782684	JQ782719
<i>Lecanora leprosa</i> 1		JQ782682	JQ782721
<i>Lecanora leprosa</i> 2		JQ782685	JQ782720
<i>Lecanora orientoafricana</i>	Kenya, Kirika 2205 (F)	<b>JQ900617</b>	<b>JQ900619</b>
<i>Lecanora pacifica</i>		JQ782686	JQ782722
<i>Lecanora paramerae</i>		EF105418	EF105413
<i>Lecanora phaecardia</i> 1		JQ782687	JQ782724
<i>Lecanora phaecardia</i> 2		JQ782688	JQ782723
<i>Lecanora plumosa</i> 1		JQ782689	JQ782725
<i>Lecanora plumosa</i> 2		JQ782690	JQ782726
<i>Lecanora pseudogangaleoides</i> ssp. <i>verdonii</i>		JQ782691	JQ782727
<i>Lecanora queenslandica</i>		JQ782692	JQ782728
<i>Lecanora</i> sp.C		JQ782693	JQ782729
<i>Lecanora</i> sp.E (sorediate)		JQ782694	JQ782730
<i>Lecanora subimmersens</i> 1		JQ782696	JQ782732
<i>Lecanora subimmersens</i> 2		JQ782695	JQ782731
<i>Lecanora subimmersa</i>		JQ782697	JQ782733
<i>Lecanora toroyensis</i>		JQ782698	JQ782734
<i>Lecanora tropica</i>		JQ782699	JN943720
<i>Lecanora ulrikii</i>		JQ782700	-
<i>Lecanora vainioi</i> 1		JQ782702	JN943716
<i>Lecanora vainioi</i> 2		JQ782701	JN943717
<i>Lecanora wilsonii</i>		JQ782703	-
<i>Ramboldia arandensis</i>		EU075527	EU075541
<i>Ramboldia brunneocarpa</i>		EU075528	EU075542
<i>Ramboldia laeta</i>		EU075530	EU075544
<i>Ramboldia petraeoides</i>		EU075531	EU075545
<i>Ramboldia stuartii</i>		EU075535	EU075549

## Sequence alignments and phylogenetic analysis

Alignments were done using Clustal W (Thompson et al. 1994). Ambiguously aligned regions were removed manually. The single locus and concatenated alignments were analyzed by maximum likelihood (ML) and a Bayesian approach (B/MCMC). To test for potential conflict, ML bootstrap analyses were performed on the individual data sets, and 75% bootstrap consensus trees were examined for conflict (Lutzoni et al. 2004). Maximum likelihood analyses were performed using the program GARLI (Zwickl 2006), employing

the general time reversible model of nucleotide substitution (Rodriguez et al. 1990), including estimation of invariant sites, and assuming a discrete gamma distribution with six rate categories. Bootstrapping (Felsenstein 1985) was performed based on 2000 replicates. The B/MCMC analysis was conducted on the concatenated data set using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), with the same substitution model as in the ML analysis. The dataset was portioned into the two parts (mtSSU, ITS) and each partition was allowed to have own parameters (Nylander et al. 2004). A run with 20,000,000 generations, starting with a random tree and employing 4 simultaneous chains, was executed. Every 100<sup>th</sup> tree was saved into a file. The first 500,000 generations (i.e. the first 5000 trees) were deleted as the “burn in” of the chain. We used AWTY (Nylander et al. 2007) to compare splits frequencies in the different runs and to plot cumulative split frequencies to ensure that equilibrium was reached. Of the remaining trees a majority rule consensus tree with average branch lengths was calculated using the sumt option of MrBayes. Posterior probabilities were obtained for each clade. Only clades that received bootstrap support equal or above 70% under ML and posterior probabilities  $\geq 0.95$  were considered as strongly supported. Phylogenetic trees were visualized using the program Treeview (Page 1996).

## Anatomical and chemical studies

Anatomical studies were conducted using standard light microscopy on hand-cut sections mounted in water. Secondary lichen substances were identified by high performance thin-layer chromatography (HPTLC) according to the standard methods (Arup et al. 1993).

## Data resources

The data underpinning the analyses reported in this paper are deposited in the Dryad Data Repository at doi: 10.10.5061/dryad.b1068.

## Results and discussion

### The Species

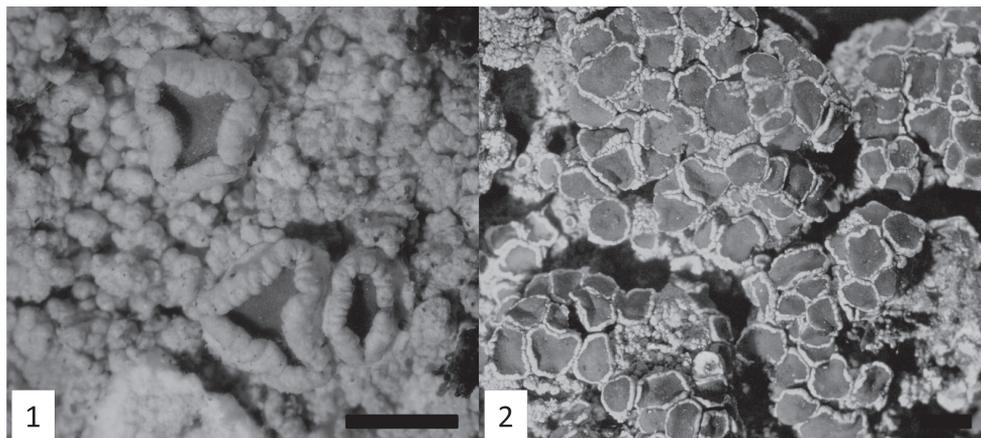
#### ***Lecanora kenyana* Kirika & Lumbsch, sp. nov.**

Mycobank no. MB800051

[http://species-id.net/wiki/Lecanora\\_kenyana](http://species-id.net/wiki/Lecanora_kenyana)

Figure 1

**Type.** Kenya, Eastern Prov., Mount Kenya National Park, Chogoria Track, close to Chogoria Gate, open *Juniperus-Podocarpus* woodland, 0°09'S, 37°26'E, 2960m alt., 27.01.2010, on *Juniperus*, *P. Kirika* 1179, *G. Mugambi* & *H. T. Lumbsch* (holotype EA, isotype F).



**Figure 1, 2.** Morphology of the new *Lecanora* species. **1** *L. kenyana*, isotype (F). **2** *L. orientoaficana*, isotype (F). Scale bars = 1 mm

**Description.** Thallus crustose, verrucose to verruculose, thin to thick, glossy, whitish to greenish; margin indistinct; prothallus not visible; sorediate. Soralia roundish, concave, 0.5–1.2 mm diam., remaining distinct or coalescing, with granular soredia, yellowish green to yellowish gray. Apothecia sessile, strictly constricted at base, 0.6–2.0 mm diam., lecanorine; disc red-brown, shiny, plane, epruinose; margin concolourous with thallus, prominent, thick, smooth, entire to verruculose, flexuose. Amphithecium cortex uniform, gelatinous, interspersed with crystals, hyaline, 25–45  $\mu\text{m}$  thick, with hyphae growing out basally. Amphithecium with small and large crystals (= *melacarpella*-type). Hypothecium hyaline, 25–30  $\mu\text{m}$  high, parathecium hyaline, with yellowish crystals, 5–7  $\mu\text{m}$  thick. Hymenium hyaline, 55–70  $\mu\text{m}$  high, clear. Epihymenium red-brown, 10–12  $\mu\text{m}$  thick, with numerous, small crystals; pigmentation and crystals dissolving in K (= *pulicaris*-type). Paraphyses sparingly branched, apically slightly swollen, hyaline. Asci clavate, 50–60  $\times$  10–14  $\mu\text{m}$ , 8-spored. Ascospores ellipsoid to narrowly ellipsoid, 12–17  $\times$  4.5–6.5  $\mu\text{m}$ . Pycnidia not seen.

**Chemistry.** Thallus and apothecial margin K<sup>+</sup> yellow, C<sup>-</sup>, KC<sup>-</sup>, containing atranorin (minor), and usnic acid (major).

**Etymology.** The new species is named after the country Kenya where the new species has been found.

**Notes.** *Lecanora kenyana* is characterized by relatively large, red-brown apothecia with a constricted base, a *melacarpella*-type amphithecium, *pulicaris*-type epihymenium, the presence of usnic acid as major constituent, and the presence of soralia. There are only few sorediate *Lecanora* sensu stricto species with usnic acid, including *L. brodoana*, *L. elatinooides*, *L. floridula*, *L. jamesii*, *L. mobergiana*, and *L. transvaalensis* (Brodo and Elix 1993; Lumbsch and Elix 1998; Lumbsch et al. 1995; Lumbsch and Nash 1995). The saxicolous *L. brodoana* and *L. mobergiana* differ in having an egranulose epihymenium among other characters, whereas *L. elatinooides* (containing pannarin) and *L. jamesii* (containing 2-*O*-methylsulphurellin) are readily distinguished by their alter-

native chemistry and smaller ascospores. Similar species include *L. floridula* described from Florida (USA) and *L. transvaalensis* from South Africa. The former species can be distinguished by having smaller apothecia (up to 1 mm), thinner and poorly developed amphithecial cortex, a *chlarotera*-type epihymenium, smaller ascospores, and the presence of unidentified triterpenes (Lumbsch et al. 1995). *Lecanora transvaalensis* differs from *L. kenyana* in having smaller apothecia (up to 0.9 mm), a thinner and poorly developed amphithecial cortex, and smaller, broadly ellipsoid ascospores ( $9.5\text{--}11.5 \times 7.0\text{--}8.5 \mu\text{m}$ ). Further, it contains unidentified terpenoids (Lumbsch et al. 1995).

**Ecology and distribution.** At present this species is known from bark of juniper trees in open habitats at altitudes above 2800m in forests dominated by *Hagenia* and *Podocarpus*. Associated lichens included *Heterodermia leucomelos*, *Lecanora caesiorubella*, *Leptogium laceroides*, *Lobaria pulmonaria*, *Pannaria fulvescens*, *Physcia albata*, *Pseudocyphellaria aurata*, *P. crocata*, *Varicellaria velata*, and several *Usnea* spp.

**Additional specimen examined.** Kenya: Eastern Prov., Mt. Kenya National Park, Sirimon route, ca. 3km for KWS gate towards Old Moses Camp,  $00^{\circ}00'N$ ,  $37^{\circ}15'E$ , mature montane forest with *Podocarpus*, *Olea*, *Hagenia* and *Arundinaria alpina*, 2870m, on bark, 7.10.2010, *P. Kirika 2051*, *G. Mugambi*, *G. Gatere* and *M. Mutembei* (EA).

***Lecanora orientoaficana* Kirika & Lumbsch, sp. nov.**

Mycobank no. MB800052

[http://species-id.net/wiki/Lecanora\\_orientoaficana](http://species-id.net/wiki/Lecanora_orientoaficana)

Figure 2

**Type.** Kenya, Rift Valley Prov., Cherangani Hills, Kerer forest, degraded montane forest, 3240m, on bark, 25.07.2011, *P. Kirika 2205* (EA, holotype, F-isotype).

**Description.** Thallus crustose, verrucose to verruculose, thin to thick, glossy, whitish to greenish grey; margin indistinct; prothallus not visible; sorediate. Soralia roundish, 0.3–1.0 mm diam., with granulose soredia, light pale greenish white to grayish green. Apothecia sessile, constricted at base, 0.4–1.4 mm diam., lecanorine; disc light red-brown to brown, matt, plane or concave, sparsely grayish pruinose; margin concolourous with thallus, prominent, thick, smooth, verruculose. Amphithecial cortex uniform, gelatinous, interspersed with crystals, hyaline, 20–30  $\mu\text{m}$  thick. Amphithecium with large crystals (=pulicaris-type). Hypothecium red-brown to yellowish brown, 30–40  $\mu\text{m}$  high, parathecium hyaline, lacking crystals, 5–7  $\mu\text{m}$  thick. Hymenium hyaline, 70–85  $\mu\text{m}$  high, clear. Epihymenium red-brown, 10–12  $\mu\text{m}$  thick, with coarse crystals; pigmentation and crystals dissolving in K (=chlarotera-type). Paraphyses sparingly branched, apically slightly swollen, hyaline. Asci clavate, 50–60  $\times$  10–12  $\mu\text{m}$ , 8-spored. Ascospores ellipsoid to broadly ellipsoid, 12.5–15.5  $\times$  6.0–8.5  $\mu\text{m}$ . Pycnidia not seen.

**Chemistry.** Thallus and apothecial margin K<sup>+</sup> yellow, C<sup>-</sup>, KC<sup>-</sup>, containing atranorin and gangaleoidin.

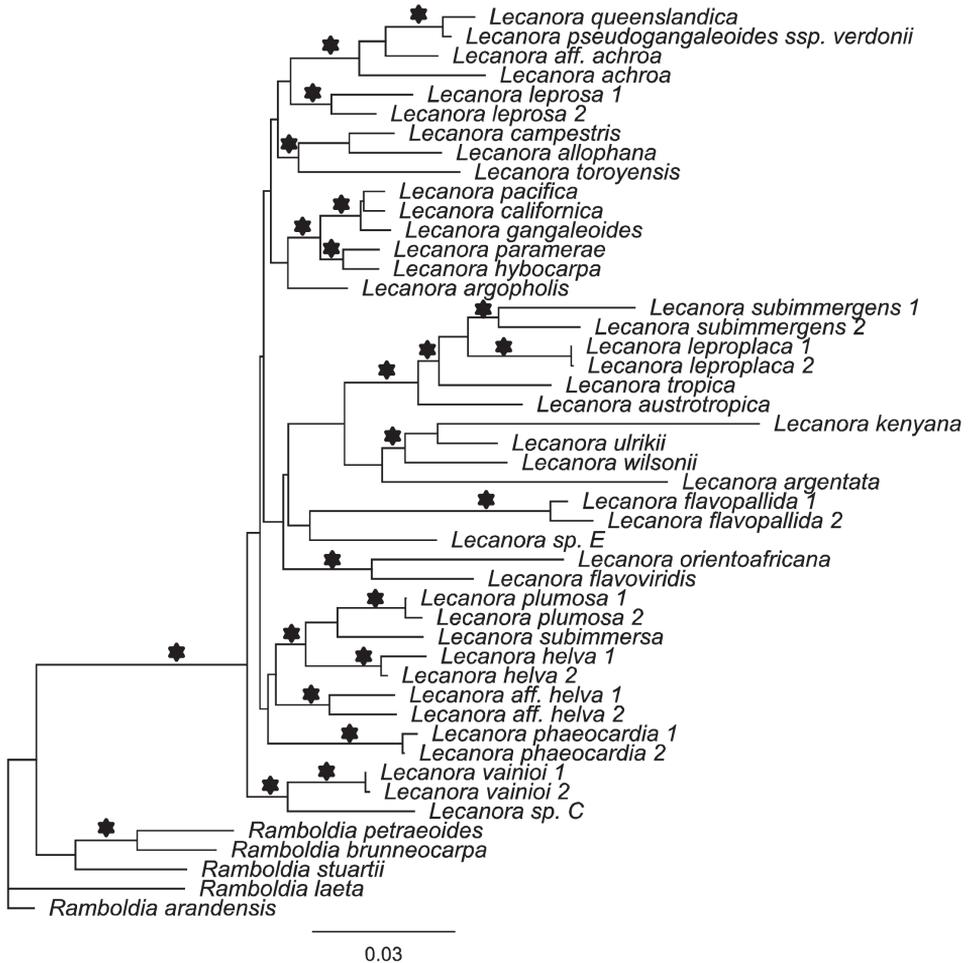
**Etymology.** The new species is named after the area East Africa where it has been collected.

**Notes.** *Lecanora orientoaficana* is characterized by the presence of granular soredia, sparsely pruinose, brown apothecia, a *pulicaris*-type amphithecium, *chlarotera*-type epihymenium, dark hypothecium, broadly ellipsoid ascospores, and the presence of atranorin and gangaleoidin. Soredia are rare among *Lecanora* sensu stricto species with a dark hypothecium. Some specimens of *L. coronulans* are sorediate, but this species is readily distinguished by epruinose apothecial discs, an egranulose epihymenium, and the presence of protoconstipatic acid and zeorin and major constituents in addition to atranorin (Lumbsch et al. 1996). Similar esorediate species include *L. egranulosa* and *L. phaeocardia*. The latter differs from *L. orientoaficana* in having epruinose apothecial discs, a thinner amphithecial cortex, and alternative chemistry. *Lecanora egranulosa* is readily distinguished by darker, epruinose apothecial discs, an indistinct, thin amphithecial cortex, small crystals in the epihymenium, shorter ascospores, and the presence of zeorin (Lumbsch et al. 1996).

**Ecology and distribution.** This new species is currently only known from the type locality in the Rift Valley province of Kenya, where it was found growing on bark in a degraded montane forest dominated by *Podocarpus falcatus*, *Rapanea melanophloes* and *Faurea saligna* at an altitude of 3240m. Associated species included *Sphaerophorus melanocarpus*, *Pannaria* cf. *rubiginosa*, and *Ramalina* spp.

### Phylogenetic study

Four new sequences were generated for this study and aligned with sequences downloaded from Genbank (Table 1). The single gene locus did not show any conflicts and hence the concatenated data set was analyzed. Our combined data set included 820 unambiguously aligned positions, 174 of which were constant. The ML tree had a likelihood value of  $-3718.083$  and in the B/MCMC analysis of the combined data set, the likelihood parameters in the sample had the following mean (Variance):  $\text{LnL} = -3794.172$  (0.21). The ML tree and the tree from the B/MCMC tree sampling were almost identical with no differences in well-supported clades. Thus, only the ML tree is shown here (Fig. 3). In our analysis, species of the genus *Lecanora* form a strongly supported monophyletic group as in a previously published study (Papong et al. in press). Since Papong et al. (in press) discussed the relationships of the different *Lecanora* groups in detail, these discussions are not reiterated here, but we focus only on the relationships of the two newly described species here. *Lecanora kenyana* clusters strongly supported with *L. ulrikii* and *L. wilsonii*, two species which also contain usnic acid (Lumbsch et al. 1995; Papong et al. 2011; Papong et al. 2012; Papong and Lumbsch 2011). *Lecanora orientoaficana* is sister to *L. flavoviridis*, which also has a dark hypothecium (Lumbsch et al. 1996; Papong et al. in press). The molecular data support the placement of the new species in *Lecanora* sensu stricto. However, given the few sequences available from tropical *Lecanora* in Genbank, the molecular data cannot be used to confirm that the species have indeed not been described previously. We conclude that they are new based on our database of known *Lecanora* species and our examinations of type material of *Lecanora* spp. over more than 24 years.



**Figure 3.** Phylogenetic placement of the two new *Lecanora* species as inferred from a concatenated alignment of mtSSU and ITS DNA sequences. This is the optimal tree under maximum likelihood. Branches in bold received likelihood bootstrap support values above 70%, and posterior probabilities equal or above 0.95

## Acknowledgements

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