
Short Communication

The Antarctic lichen *Cetraria subscutata* is a synonym of *Nephromopsis chlorophylla*

The *Parmeliaceae* is a highly diverse family among the lichenized Ascomycota, both in terms of species number, with more than 2500 species distributed worldwide (Thell *et al.* 2012; Jaklitsch *et al.* 2016), and morphological and anatomical variation (Blanco *et al.* 2006; Crespo *et al.* 2007). It also has a complex taxonomy which dates back to the time of Acharius and has been studied by some of the most well-known lichenologists of the 20th century (Thell *et al.* 2012). Generic concepts and delimitation have often undergone dramatic changes (Crespo *et al.* 2007; Nelsen *et al.* 2011) but in recent years molecular phylogenetics have helped to establish a more natural classification (Thell *et al.* 2009; Crespo *et al.* 2011; Miadlikowska *et al.* 2014; Divakar *et al.* 2017). Within the informal group of “cetrarioid” species, the most recent classification includes only two genera, *Cetraria* and *Nephromopsis* (Divakar *et al.* 2017).

However, as pointed out by Randlane *et al.* (2013), there are still some poorly known species in this group with an unresolved taxonomy. One such case is the Antarctic species *Cetraria subscutata* D. C. Linds., described from collections made during the 1964 British Antarctic Surveys in the Argentine Islands Archipelago, west Antarctic Peninsula (Lindsay 1973). It was included in *Cetraria* Ach., the most important genus within the “cetrarioid group” at that time, mainly due to the similarity in external morphology and its chemistry, despite the lack of apothecia. Lindsay compared it with two other *Cetraria* species, namely *C. chlorophylla* (Wild.) Vain. (now in *Nephromopsis* Müll. Arg.), from which it was distinguished by the lack of soredia, and with

C. antarctica Zahlbr. [= *Pseudocyphellaria glabra* (Hook f. & Taylor) C. W. Dodge] (Lindsay 1973). Later, Øvstedal & Lewis-Smith (2001) re-examined Lindsay’s species as part of their Antarctic Flora and kept it in *Cetraria*, although with some hesitation, stating that it would probably be better placed under *Tuckermannopsis* (a genus resurrected by Lai (1980), now included in *Nephromopsis*) but the observation of sexual structures was needed for confirmation. So far, the species has been collected in Antarctica only four times; all these sterile specimens were studied by Lindsay (1973) and by Øvstedal & Lewis-Smith (2001). As a result, it has never been included in any of the recent molecular studies and is currently considered as an enigmatic species of unknown taxonomic position (Randlane *et al.* 2013).

During the 2014 Argentine Antarctic Survey, we identified some sterile specimens as “*C. subscutata*” and these were used for molecular phylogenetic studies. The results confirmed that the species belongs to *Nephromopsis* and is conspecific with *N. chlorophylla* (Willd.) Divakar *et al.* However, the specimens from Antarctica show some differences with collections from other regions, which are discussed below.

The specimens of “*Cetraria subscutata*” were collected by the authors (AP and JMR) on the Danco Coast, west of the Antarctic Peninsula near Argentine Primavera Station, c. 1000 km from its original collection site (Fig. 2A). The specimens were deposited in BCRU and COR herbaria. Morphological and anatomical observations were performed following standardized methods as described in Passo *et al.* (2004). Secondary metabolites of the material were studied with thin-layer chromatography (Orange *et al.* 2001). Digital images of the type specimens of “*C. subscutata*” provided by AAS

and O herbaria were compared with the material collected from Antarctica.

Total genomic DNA was extracted using the commercially available Ultraclean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, USA). The internal transcribed spacer regions (ITS, including 5.8S) of nuclear ribosomal DNA were amplified with the following primers: the fungal specific ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). Amplicons generated by PCR were sequenced by the INTA Castelar (Argentina) sequencing service. For a more detailed description of the methodology, see Messuti *et al.* (2016).

A matrix was constructed with 16 sequences representing the type species of main genera of the *Nephromopsis* clade and a small number of sequences from the *Cetraria* clade, *sensu* Thell *et al.* (2012). Although the most recent delimitation of the *Parmeliaceae* accepts only two genera in the “cetrarioid core”, names of species were left as they are found in GenBank, on the figure and in the text, whenever making a specific reference to the older name. After preliminary analysis a few additional sequences of the formal genus *Tuckermannopsis* were also incorporated. The species *Melanelia hepaticum* (Ach.) A. Thell and *Melanelia stygia* (L.) Essl. were used as outgroup. All sequences were downloaded from GenBank. The final matrix consisted of 17 sequences, including that from “*Cetraria subscutata*” (Table 1).

Sequences were aligned and trimmed using MAFFT multiple sequence alignment software v.7 (Kato & Standley 2013). The phylogenetic reconstruction was carried out using the IQ-Tree web server for maximum likelihood reconstruction criterion (Trifinopoulos *et al.* 2016) with the ModelFinder software for search of the best model of character evolution (Kalyaanamoorthy *et al.* 2017). Branch support was attained through ultrafast bootstrap with 1000 replicates (Minh *et al.* 2013). Genetic distances were calculated using MEGA software (Kumar *et al.* 2008).

The resulting alignment contained 17 sequences and 805 bps, with 571 constant sites (70.9% of all sites). The resulting consensus tree from the ML inference (Fig. 1) is in general agreement with those found using multigene datasets (Nelsen *et al.* 2011; Miadlikowska *et al.* 2014; Divakar *et al.* 2017). Minor differences may be explained by the low resolution of the ITS at generic level, as well as the low support of some branches. However, two main clades are observed, namely the *Cetraria* clade and the *Nephromopsis* clade. The position of *C. subalpina* Imshaug, included in the analysis because it was cited as similar to *C. subscutata* (Lindsay 1973), agrees with its uncertain affinity reported by Thell *et al.* (2009). The ITS sequence of “*C. subscutata*” falls within *Nephromopsis* with strong support. It is almost identical with the two sequences from *Nephromopsis chlorophylla* (shown as *Tuckermannopsis chlorophylla* in Fig. 1, one from Europe and the other from South America), as shown in the distance matrix (Table 2).

These results suggest that the species is not related to *Cetraria* but rather belongs to *Nephromopsis*, and that it is conspecific with *N. chlorophylla*. The morphological, anatomical and chemical examinations also suggest the conspecificity with *N. chlorophylla*, despite some particular variations. Therefore, we

TABLE 1. Collection data and sequences with GenBank Accession numbers used in the phylogenetic analysis. New sequences are in bold.

Species	Country	Collection/herbaria	GenBank Accession number
“ <i>Cetraria subscutata</i> ”	Antarctica	<i>Passo & Rodriguez</i> (BCRU)	MG195572
<i>Ahtiana sphaerospora</i>	Canada	<i>Thell & Veer</i> BC-9672 (LD)	AF141859
<i>Allocetraria stracheyi</i>	China	<i>Obermayer</i> (GZU)	AF404130
<i>Arctocetraria andrejevii</i>	USA	<i>Zhurbenko</i> 1189 (UPS)	DQ980001
<i>Cetraria islandica</i> ssp. <i>islandica</i>	Finland	<i>Thell</i> FIN-9925 (TUR)	AF228299
<i>Cetrellopsis rhytidocarpa</i>	Philippines	<i>Bawingan</i> CL0582	DQ980008
<i>Masonhalea richardsonii</i>	Canada	<i>Westberg</i> 1246 (LD)	AF254634
<i>Melanelia stygia</i>	Finland	<i>Haikonen</i> 20365	AY611097
<i>M. hepaticum</i>	Iceland	LA31861	KY963376
<i>Nephromopsis stracheyi</i>	Bhutan	<i>Søchting</i> 8095 (TUR)	AF451785
<i>Tuckermannella weberi</i>	USA	<i>Westberg</i> 547 (LD)	AF451792
<i>Tuckermannopsis americana</i>	Canada	TG-961350 (UBC)	AF072233
<i>T. chlorophylla</i> 1	Sweden	<i>Wedin</i> 6995 (UPS)	DQ980025
<i>T. chlorophylla</i> 2	Chile	Unknown	AF255620
<i>T. ciliaris</i>	USA	Unknown	FJ005090
<i>T. orbata</i>	USA	Unknown	DQ004572
<i>T. subalpina</i>	Canada	BC-9606 (LD)	AF072237

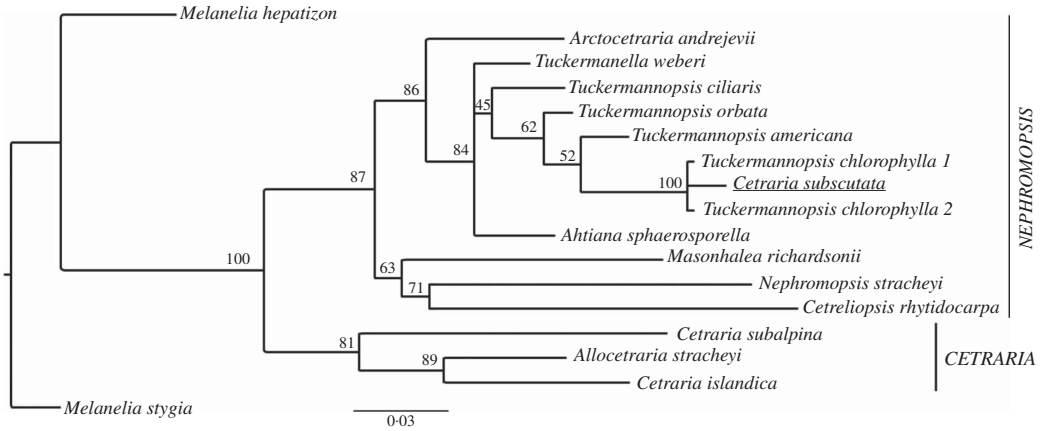


FIG. 1. Phylogenetic position of “*C. subscutata*”. Maximum likelihood consensus tree based on ITS data with ultrafast bootstrap support on branch nodes. Names of species are shown as they are found in GenBank, their current classification is indicated on the bars.

TABLE 2. Genetic distances of ITS sequences of selected species of *Cetraria* and *Tuckermannopsis* closely related to *C. subscutata*.

	“ <i>C. subscutata</i> ”	<i>C. islandica</i>	<i>T. ciliaris</i>	<i>T. chlorophylla</i> 1	<i>C. subalpina</i>	<i>T. orbata</i>	<i>T. americana</i>
“ <i>C. subscutata</i> ”							
<i>C. islandica</i>	0.127						
<i>T. ciliaris</i>	0.053	0.106					
<i>T. chlorophylla</i> 1	0.007	0.119	0.045				
<i>C. subalpina</i>	0.119	0.107	0.109	0.111			
<i>T. orbata</i>	0.038	0.114	0.032	0.031	0.103		
<i>T. americana</i>	0.050	0.114	0.036	0.043	0.103	0.016	
<i>T. chlorophylla</i> 2	0.009	0.121	0.048	0.002	0.113	0.034	0.045

conclude that *C. subscutata* should be regarded as a synonym of *N. chlorophylla*. A brief description and notes on the taxonomy and distribution are included here.

***Nephromopsis chlorophylla* (Willd.) Divakar, Crespo & Lumbsch**

Fungal Biology **84**: 112 (2017).—*Lichen chlorophyllus* Willd., in Humboldt, *Flor. Friburg. Spec.*: 20 (1793); type: “*In cortice Pyni sylvestris beym Vorwerk Hals copiose*” (B-Willd, *non vidi*).

Cetraria subscutata D. C. Linds., *Bull. Brit. Antarct. Surv.* **36**: 107 (1973); type: Antarctica, Antarctic Peninsula, Argentine Islands, Galindez Island, on rock surfaces in *Usnea* community, 30 m, 21 April 1964, R. W. M. Corner 514 (AAS—holotype, *non vidi*; O!—isotype).

(Fig. 2B–D)

Thallus foliose to subfruticose, suberect, lobes divided and irregularly branched, broadly to weakly canaliculated; upper surface pale brown to olive-brown (brown to dark brown in Antarctic specimens), glossy and wrinkled; margins with small scattered white to greyish soredia (moderate to profusely sorediate in Antarctic specimens), soredia also laminal in older parts of the thallus and becoming corticated (Fig. 2D); lower surface pale brown to almost whitish, wrinkled with a few dispersed and short rhizines.

Apothecia rare (absent in Antarctic specimens).

Pycnidia uncommon, marginal or laminal, globose with a short stalk, black to black-brown, scattered; *conidia* bifusiform.

Chemistry. Protolichesterinic acid.

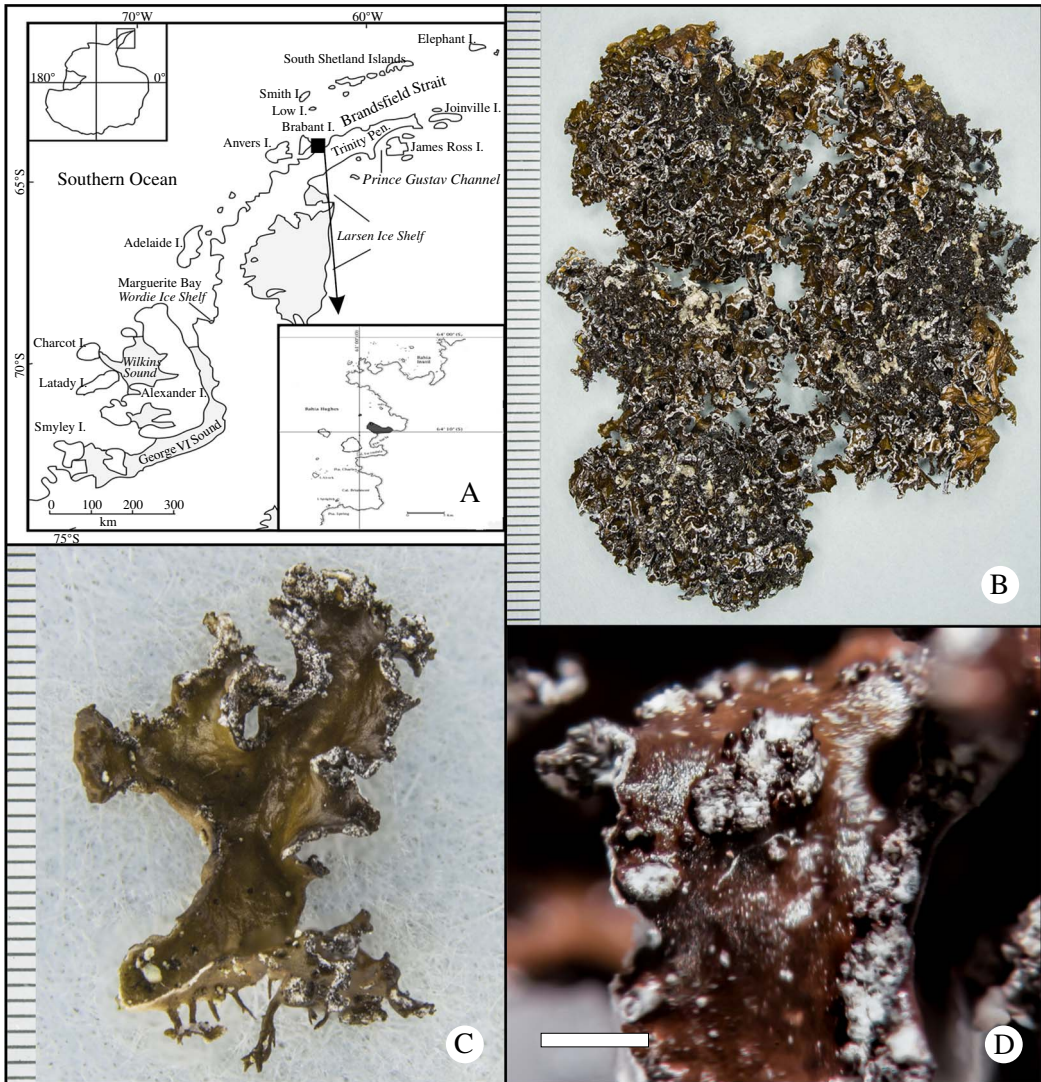


FIG. 2. "*Cetraria subscutata*" (*Nephromopsis chlorophylla*) (BCRU 04596). A, location of Primavera Station on the Antarctic Peninsula; B, habitus; C, thallus margin with abundant soredia; D, detail of corticate soredia. Scales: B & C = 1 mm; D = 0.5 mm. In colour online.

For a more detailed description (as *T. chlorophylla*) see Brodo *et al.* (2001), Kärnefelt & Thell (2001) and Gilbert (2009), and in the original description under *C. subscutata* (Lindsay 1973).

When Lindsay (1973) described *C. subscutata*, *Cetraria* was the only cetrarioid genus in the *Parmeliaceae*. The lack of sexual structures on the Antarctic specimens, as

well as the limited information about the species, prevented a better placement in the subsequent treatments of the group. However, recent studies on the family suggest that the shape of conidia is a more important character than apothecial structures (Thell *et al.* 2004, 2012). Bifusiform conidia are characteristic of the *Nephromopsis* clade, while the *Cetraria* clade includes species with

single swelling conidia. This supports the new proposed delimitation of the cetrarioid core (Divakar *et al.* 2017). The observation of conidia in our Antarctic collections strengthens the molecular results reported here, and shows that Lindsay's species belongs to *Nephromopsis*.

Both Lindsay (1973) and Øvstedal & Lewis-Smith (2001) discussed the similarity between *C. subscutata* and the widespread *N. chlorophylla* but considered them as separate species. Lindsay (1973) commented on the absence of soredia in his species as the main difference and, later, Øvstedal & Lewis-Smith (2001) mentioned the presence of marginal pseudocyphellae in the Antarctic collections as the key difference but, contrary to Lindsay, stated that soredia were present. However, Øvstedal & Lewis-Smith (2001) focused more on its generic position than on the difference from *T. chlorophylla*. Our examination of the high-resolution images of the isotype (RWMC 514, O!), as well as some of the collections revised by Lindsay (RWMC 559, RILS 951, AAS!), confirmed the presence of marginal soredia, although poorly developed. Furthermore, our Antarctic material is copiously sorediate, not only at the margins but also occasionally on the lamina, becoming pseudoisidiate in older parts of the thallus (Fig. 2D). Adler & Calvelo (1993) and Calvelo (1994) also found laminal and corticate soredia on specimens of *N. chlorophylla* (as *C. chlorophylla*) from subantarctic *Nothofagus* forest and regarded this as a variation from the typical form. In the isotype (O, E. Timdal, pers. comm.) and in our fresh material, it was not possible to observe any pseudocyphellae. One explanation of why Øvstedal & Lewis-Smith (2001) mentioned these structures in *C. subscutata* is that they interpreted immature soralia as pseudocyphellae.

Nephromopsis chlorophylla is a common and widespread species in both the Southern and Northern Hemispheres, mainly in cool-temperate forests (Adler & Calvelo 1993; Kärnefelt & Thell 2001). In the Maritime Antarctic region, the species has a very restricted distribution, confined to the mid-west Antarctic Peninsula. Although this region

is frequently visited by many international lichenological expeditions, this peculiar distribution may be the reason that the species is poorly collected or overlooked. There are only four published records of *N. chlorophylla* as "*C. subscutata*" (e.g. with traceable collections deposited in official herbaria) so far: RWMC 514 (AAS and O!-types), RWMC 559 (AAS!), RILS 951 (AAS!) and RILS 2579 (?hb.) (Øvstedal & Lewis-Smith 2001).

Nephromopsis chlorophylla usually grows on bark, even in extreme conditions as in Tierra del Fuego, southernmost South America (Adler & Calvelo 1993). In Antarctic territories, where woody vegetation is absent, it is found over rock and between mosses. Severe and extreme conditions seem to trigger the morphological variation and substratum preferences discussed here. Wide morphological plasticity has been described previously from related groups under similar conditions, for example in *Cetraria* s. str. in the Arctic region (Kärnefelt 1979).

Specimens examined. As *C. chlorophylla*. **Argentina:** Río Negro: Cerro Chalhuaco, 1350 m, on *Nothofagus pumilio*, 13 v 1992, S. Calvelo (BCRU 00292). Tierra del Fuego e Islas del Atlántico Sur: Tolhuin, near Lago Fagnano, on *N. antarctica*, xi 1993, S. Calvelo & M. T. Adler (BCRU 00409).

As *C. subscutata*. **Antarctica:** Antarctic Peninsula: Danco Coast, Punta Cierva, 64°09'18.5" S, 60°57'09.1" W, on rock near sea shore, 11 ii 2014, A. Passo & J. M. Rodríguez (BCRU 05496), J. M. Rodríguez & A. Passo (CORD); Graham Coast, Argentine Islands, Uruguay Island, on *Andreaea* sp. and rock, 1967, R. Smith 951 (AAS); Skua Island, crags overlooking Skua Creek, on exposed rock surface, no snow or ice, alt. 20 ft., 1964, R. W. M. Corner 559 (AAS).

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