

Phylogeny of *Cladonia uncialis* (*Cladoniaceae*, *Lecanoromycetes*) and its allies

Soili STENROOS, Raquel PINO-BODAS, Diana WECKMAN and Teuvo AHTI

Abstract: The species from *Cladonia* section *Unciales* are characterized by the absence of squamules and soredia on the corticate podetia and the presence of usnic acid. Different subspecies, varieties and forms have been distinguished in the type species *C. uncialis*. In this study, a molecular phylogeny of *Cladonia uncialis* and members of the traditionally recognized section *Unciales*, along with additional potentially allied species, was constructed. DNA sequences from three gene loci, namely ITS rDNA, IGS rDNA and β -tubulin, were analyzed using Maximum Parsimony, Maximum Likelihood, and Bayesian methods. Eleven species were analyzed for the first time using DNA sequence data. *Cladonia uncialis* subsp. *uncialis* and subsp. *biuncialis* were recognized as distinct taxa. The recognition of *C. pseudostellata* as a species was not supported by the analyses, but it represents a hypothamnolic acid chemotype, which is reported here as new to Europe (Scotland). The presence of subsp. *biuncialis* in North America (Newfoundland) was substantiated. The subsp. *uncialis* usually lacks squamatic acid, but in the eastern United States a morph referred to as subsp. *uncialis* does normally contain that acid. However, this morph did not attain taxonomic recognition based on phylogenetic analyses. All the other taxa formerly included in sect. *Unciales* turned out to belong to other groups of *Cladonia*, mainly *Amaurocraea*, *Borya*, *Divaricatae*, and *Perviae*. The formerly recognized genus *Cladina* (reindeer lichens) is non-monophyletic, consisting of three groups within *Cladonia*, making the concept *Cladina* even nomenclaturally useless. Alternative topology tests rejected the monophyly of *C. pseudostellata*, section *Unciales* and *Cladina*.

Key words: β -tubulin, *Cladina*, *Cladonia pseudostellata*, *Cladonia uncialis* subsp. *biuncialis*, IGS rDNA, infrageneric taxonomy, ITS rDNA, lichens

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Introduction

The formerly recognized *Cladonia* section *Unciales* (Delise) G. Merr. (e.g. Vainio 1887: 235; Ahti 2000; note the corrected author citation; the combination by Merrill 1908: 109 has been overlooked) included species typically defined by characters such as the soon evanescent primary thallus, the absence of podetial squamules and soredia, the presence of a more or less distinct cortex and the cortical substance usnic acid. This acid gives a visible yellowish tint to these species, depending on its concentration. There are over 40 *Cladonia* species in the world with these features (Ahti 2000).

The status of the section *Unciales* as a whole has been discussed by a few authors, including Nylander (1866), Vainio (1880, 1897), Mattick (1938, 1940, 1951), Dahl (1952) and Aasamaa (1961). Vainio (1897: 98) attempted a preliminary subdivision of the *Unciales* and distinguished three major lineages: *Cladonia divaricata*, *C. peltata*, and the rest of the group. Choisy (1928) claimed that, against common belief, *C. uncialis* (L.) F. H. Wigg. and *C. amaurocraea* were not closely related. In Mattick's (1951) final scheme, the *Unciales* were placed as a subsection under the section *Perviae*. Ahti (1973) recognized the so-called *C. boryi* group within the *Unciales*, including the species that develop needle crystals (presumably triterpenoids) on the tips of podetia. Finally, Stenroos *et al.* (2002) presented the first comprehensive phylogeny of the genus *Cladonia*, based on DNA sequences.

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Unciales was resolved as a non-monophyletic group, but recognized in the strict sense, many species being placed into other, provisionally recognized and informally named groups. The following species from the *Unciales* were transferred to other groups of *Cladonia*: *C. capitellata*, *C. peltastica* and *C. perforata* were placed in the Supergroup *Perviae*, and *C. subchordalis* in the Supergroup *Cladonia*. The Supergroup *Crustaceae* included most of the taxa of the former section *Unciales*, but they were placed in several Groups: Group *Amaurocraeae*, Group *Divaricatae*, and Group *Unciales*. Group *Unciales* was divided into two Subgroups: Subgroup *Borya*, which included the taxa that Ahti (1973) assembled in the *C. borya* group, and Subgroup *Unciales*, including our current target species *Cladonia uncialis*.

Cladonia uncialis itself is morphologically and chemically variable. Typically, its podetia are continuously corticate, yellowish, moderately branched, erect, brown-tipped and spine-like, with a well-developed and fairly smooth cortex (or sometimes with a slightly rough surface). These characters distinguish it from the reindeer lichens (former *Cladina*), which are its common associates in thick lichen mats on forest floors.

Vainio (1922) distinguished by morphology as many as six forms of *C. uncialis* in Finland. Kärenlampi (1964) and Kärenlampi & Pelkonen (1971) examined in detail its various morphological characters, such as the number of branches, the coverage of the podetial surface by algal cells, and the production of conidiomata and apothecia. As a result, two varieties of *C. uncialis* were distinguished, namely var. *uncialis* and var. *dicraea* (Ach.) Räsänen (author citation corrected here). The former variety was characterized by a polytomous branching pattern, a relatively low coverage of algal cells, and its common production of conidiomata and apothecia. The latter variety was described as mainly dichotomous, with a higher coverage of algal cells, and rare production of conidiomata and apothecia. Brodo & Ahti (1996) distinguished several morphotypes among *C. uncialis* s. lat. These morphs were based on branching patterns, surface structure, and chemical composition.

Today, the two morphotypes mentioned above are better known as subsp. *uncialis* and subsp. *biuncialis* (Hoffm.) M. Choisy (e.g. Ahti & Stenroos 2013). The subspecies concept was also suggested by Hawksworth (1973), who studied the variability of *C. uncialis* in Britain and introduced the combination *C. uncialis* subsp. *dicraea* (Ach.) D. Hawksw. Ahti (1978) noted that subsp. *biuncialis* is an older name at subspecies level. However, the taxonomic treatment of the subsp. *biuncialis* has varied. In addition to being distinguished as a subspecies, variety or form (originally as a species, *C. biuncialis* Hoffm.), it has often not been given any formal status. A chemotype of *C. uncialis* s. lat. containing hypothamnolic acid was found in Japan. Asahina described it as a different species, *C. pseudostellata*. However, Brodo & Ahti (1996) considered *C. pseudostellata* as synonymous with *C. uncialis* s. lat., since they did not find any correlation between the presence of hypothamnolic acid and morphology. To date, the taxonomic status of *C. uncialis* and its postulated segregates has not been resolved.

The goals of the present study therefore are: 1) to clarify the status of *Cladonia uncialis* s. lat. and test if its segregates can be distinguished and possibly warrant a species status, and 2) try to resolve the relationships of the members formerly referred to section *Unciales*.

Material and Methods

Taxon sampling

In the present study, 74 samples from 32 species of section *Unciales* (Ahti 2000) were included (Table 1). Our work emphasizes the circumscription of *Cladonia uncialis* s. lat. Therefore we included 14 specimens of *C. uncialis* subsp. *uncialis*, 11 specimens of *C. uncialis* subsp. *biuncialis*, and six specimens of *C. pseudostellata*. Specimens of other *Cladonia* groups, representing all the major clades outlined in the previous phylogeny of the genus (Stenroos *et al.* 2002), were included in this study: eight specimens of *Cladina*, three of *Cocciferae*, six of *Divaricatae*, three of *Mimiatatae*, three of *Perviae*, and five of *Cladonia* (Table 1). The taxa related to *Unciales* members (*sensu* Stenroos *et al.* 2002) were selected. *Cladonia furcata* was used to root the tree. The collections studied are deposited in the herbarium H (Botanical Museum, University of Helsinki).

TABLE 1. List of Cladonia specimens used in this study with voucher information and GenBank accession numbers.

Taxon	Voucher specimen	Code	GenBank accession numbers		
			ITS rDNA	IGS rDNA	β -tubulin
<i>C. ahtii</i> S. Stenroos	Brazil, Paraná, <i>Ahti & Eliasaro</i> 57582 (H)	AT727	AF453275	KR019426	AF458486
<i>C. albofuscescens</i> Vain.	Brazil, Minas Gerais, <i>Stenroos</i> 5019 (TUR)	SATU5	AF455242	KR019427	—
<i>C. albofuscescens</i> Vain.	Brazil, Minas Gerais, <i>Stenroos</i> 4946 (TUR)	SATU9	AF455241	KR019428	AF458548
<i>C. amaurocraea</i> (Flörke) Schaer.	Finland, <i>Stenroos</i> 5172 (TUR)	LK23	AF455245	KR019429	AF458549
<i>C. amaurocraea</i> (Flörke) Schaer.	Finland, <i>Stenroos</i> 5159 (TUR)	LK7	AF455244	KR019430	—
<i>C. amaurocraea</i> (Flörke) Schaer.	Greenland, Narsaq, <i>Högnabba</i> 871 (H)	DW16	KR019406	KR019501	KR019522
<i>C. arbuscula</i> (Wallr.) Flot.	Canada, Nova Scotia, <i>Ahti</i> 57095 (H)	LK36	AF458292	KR019431	AF458577
<i>C. arbuscula</i> (Wallr.) Flot.	USA, Alaska, Aleutian Islands, <i>Talbot & Schofield</i> s.n. (H)	DW27	KR019410	KR019505	KR019523
<i>C. atlantica</i> A. Evans	USA, Massachusetts, <i>Ahti</i> 56853 (H)	LK42	AF457884	KR019432	AF458561
<i>C. bahiana</i> Ahti	Brazil: Minas Gerais, <i>Splett</i> 968 (H)	AT715	AF456403	KR019433	—
<i>C. bahiana</i> Ahti	Brazil, Bahia, <i>Stocker</i> s.n. (H)	AT716	AF456402	KR019434	—
<i>C. borealis</i> S. Stenroos	Finland, <i>Stenroos</i> 5157 (TUR)	LK4	AF454434	KR019445	AF458504
<i>C. boryi</i> Tuck.	USA, New York, <i>Stenroos</i> 5742 (H)	DW6	KR019388	KR019446	—
<i>C. boryi</i> Tuck.	Canada, Newfoundland, <i>Ahti & Scott</i> 56962 (H)	LK50	AF457907	KR019447	—
<i>C. boryi</i> Tuck.	Canada, Nova Scotia, <i>Ahti</i> 57176 (H)	LK6	AF457906	KR019448	—
<i>C. candelabrum</i> (Bory) Nyl.	Madagascar, <i>Baranow & Szlachetko</i> (H)	CL106	KR019384	KR019449	KR019531
<i>C. candelabrum</i> (Bory) Nyl.	France, Réunion, <i>Boom</i> 40493 (H)	DW21	KR019385	KR019450	KR019532
<i>C. capitellata</i> (Hook. f. & Taylor) C. Bab.	New Zealand, <i>Stenroos</i> 6023 (H)	CL73	KR019386	KR019451	KR019533
<i>C. caroliniana</i> Tuck.	USA, South Carolina, <i>Ahti</i> 58186 (H)	AT636	AF456408	KR019452	AF458557
<i>C. cenotea</i> (Ach.) Schaer.	Finland, <i>Myllys</i> 213 (TUR)	LK61	AF457896	KR019453	AF458567
<i>C. consimilis</i> Vain.	Brazil, Minas Gerais, <i>Stenroos</i> 5050 (TUR)	SATU1	AF456405	KR019454	—
<i>C. consimilis</i> Vain.	Brazil, Minas Gerais, <i>Stenroos</i> 5048 (TUR)	SATU2	AF456400	KR019455	—
<i>C. delavayi</i> Abbayes	Bhutan, <i>Söchting</i> 8498 (H)	LK30	AF458304	KR019456	AF458582
<i>C. dilleniana</i> Flörke	Cuba, <i>Buck</i> 55790 (H)	CL312	—	KR019458	—
<i>C. dimorphoclada</i> Robbins	USA, South Carolina, <i>Ahti</i> 58180 (TUR)	AT635	AF456407	KR019459	AF458556
<i>C. divaricata</i> Nyl.	Brazil, Minas Gerais, <i>Stenroos</i> 4999 (TUR)	SATU10	AF457910	KR019460	AF458572
<i>C. fleigiae</i> Ahti & S. Stenroos	Brazil, Minas Gerais, <i>Stenroos</i> 5052a (TUR)	AT710	AF456401	—	AF458555
<i>C. furcata</i> (Huds.) Schrad.	USA, Georgia, <i>Ahti</i> 58283 (TUR)	AT638	AF455220	KR019461	AF458539
<i>C. kanewskii</i> Oxner	Russia, Kamchatka territory, <i>Himmelbrant</i> 02082011-1 (H)	CL244	KR019389	KR019462	KR019535
<i>C. kanewskii</i> Oxner	Russia, Kamchatka territory, <i>Himmelbrant</i> 31072011-1 (H)	CL245	KR019390	KR019463	KR019536
<i>C. kanewskii</i> Oxner	USA, Alaska, <i>Neillich & Hasselbach</i> 2160d (H)	LK52	AF456406	KR019464	—
<i>C. aff. kanewskii</i> Oxner	Russia, Buryatia, <i>Urbanavichene & Urbanavichus</i> s. n. (H)	DW22	KR019391	—	KR019537
<i>C. labradorica</i> Ahti & Brodo	Canada, Quebec, <i>Seppälä</i> E1 (H)	CL167	KR019394	KR019465	—
<i>C. lepidophora</i> Ahti & Kashiw.	Chile, Osorno, <i>Feuerer</i> 60297 (TUR)	AT507	AF454440	KR019466	AF458506
<i>C. lopezii</i> S. Stenroos	Brazil, Minas Gerais, <i>Stenroos</i> 5029 (TUR)	AT561	AF453279	KR019467	—
<i>C. medusina</i> (Bory) Nyl.	Madagascar, <i>Baranow & Szlachetko</i> s. n. (H)	CL161	KR019395	KR019468	KR019538

TABLE 1. *Continued*

Taxon	Voucher specimen	Code	GenBank accession numbers		
			ITS rDNA	IGS rDNA	β -tubulin
<i>C. medusina</i> (Bory) Nyl.	Madagascar, <i>Baranow & Szlachetko</i> s. n. (H)	CL162	KR019396	KR019469	KR019539
<i>C. minarum</i> Ahti	Brazil, Minas Gerais, <i>Stenroos</i> 4961 (TUR)	SATU7	AF457909	KR019470	—
<i>C. mitis</i> Sandst.	Finland, <i>Stenroos</i> 5209 (TUR)	AT651	AF457914	KR019471	AF458574
<i>C. mitis</i> Sandst.	Russia, Kamchatka territory, <i>Himmelbrant & Stepanchikova</i> s. n. (H)	FH336	KR019392	KR019472	KR019540
<i>C. nipponica</i> Asahina	USA, Alaska, Kodiak Island, <i>Dillman</i> 19 (H)	CL213	KR019397	KR019473	KR019541
<i>C. nipponica</i> Asahina	Russia, Primorskiy Kray, <i>Zhdanov</i> Sikh-3-3 (H)	FH338	KR019393	KR019474	—
<i>C. pachycladodes</i> Vain.	USA, Florida, <i>Rosentreter</i> 16470 (H)	DW18	KR019398	KR019475	KR019542
<i>C. pachyclados</i> (Vain.) Ahti	France, Réunion, <i>Boom</i> 40543 (H)	DW20	KR019399	KR019476	KR019543
<i>C. peltasta</i> Spreng.	France, Réunion, <i>Boom & Boom</i> 40397 (H)	CL104	KR019425	KR019477	—
<i>C. perforata</i> A. Evans	USA, Florida, 1993, <i>Yahr</i> 93/2 (US)	Yahr	AF457903	—	AF458570
<i>C. peziziformis</i> (With.) J. R. Laundon	USA, District of Columbia, <i>Stenroos</i> 5198 (TUR)	AT631	AF455221	KR019478	AF458540
<i>C. pleurota</i> (Flörke) Schaer.	Mexico, Veracruz, <i>Stenroos</i> 5143 (TUR)	AT587	AF454445	KR019479	AF458510
<i>C. pseudostellata</i> Asahina	Japan, Honshu, <i>Ohmura</i> 6000 (H)	FH328	KR019400	KR019480	KR019544
<i>C. pseudostellata</i> Asahina	USA, Alaska, Aleutian Islands, <i>Talbot & Schofield</i> TIG003-X-3 (H)	DW28	KR019411	KR019506	KR019547
<i>C. pseudostellata</i> Asahina	USA, Alaska, Aleutian Islands, <i>Talbot & Schofield</i> ADA122-X-01 (H)	DW5	KR019414	KR019509	KR019548
<i>C. pseudostellata</i> Asahina	USA, Alaska, Aleutian Islands, <i>Talbot & Schofield</i> KML.003-X-1 (H)	FH326	KR019421	KR019516	KR019553
<i>C. pseudostellata</i> Asahina	Great Britain, Scotland, <i>Stenroos</i> 6088 (H)	FH350	KR019423	KR019518	KR019555
<i>C. pseudostellata</i> Asahina	Great Britain, Scotland, <i>Stenroos</i> 6095 (H)	FH347	KR019383	KR019444	KR019530
<i>C. pycnoclada</i> (Pers.) Nyl.	Chile, Prov. Osorno, <i>Feuerer</i> 60257 (TUR)	AT509	AF458297	KR019482	AF458579
<i>C. rugulosa</i> Ahti	Guyana, <i>Stenroos</i> 4831 (TUR)	AT577	AF457894	KR019483	AF458565
<i>C. secundana</i> Nyl.	Guyana, <i>Stenroos</i> 4812b (TUR)	LK74	AF453274	KR019484	AF458485
<i>C. siamea</i> Abbayes	Indonesia, Papua, <i>Miettinen</i> 11620,3 (H)	CL77	—	KR019485	—
<i>C. solitaria</i> H. Marg.	USA, Hawaii, <i>Palmer et al.</i> 2677 (US)	2677	AF457905	—	—
<i>C. stenroosiae</i> Ahti	Brazil, Minas Gerais, <i>Stenroos</i> 5013 (TUR)	LK25	AF456409	KR019486	—
<i>C. stereoclada</i> Abbayes	Spain, Canary Islands, <i>Burgaz</i> s.n. (MACB 97913)	3STEREO	KR019556	KR019558	—
<i>C. stereoclada</i> Abbayes	Spain, Canary Islands, <i>Alanko</i> 128470a (H)	CL138	KR019557	KR019559	—
<i>C. steyermarkii</i> Ahti	Guyana, <i>Stenroos</i> 4916 (TUR)	LK89	AF456404	KR019487	—
<i>C. strepsilis</i> (Ach.) Grognot	Finland, <i>Stenroos</i> 5160 (TUR)	AT537	AF457880	KR019488	AF458559
<i>C. stygia</i> (Fr.) Ruoss	Finland, <i>Stenroos</i> 5200 (TUR)	AT647	AF458308	KR019489	AF458584
<i>C. subchordalis</i> A. Evans	Chile, Magallanes, <i>Feuerer</i> 60166 (TUR)	AT512	AF455175	KR019490	AF458524
<i>C. subchordalis</i> A. Evans	Chile, Osorno, <i>Feuerer</i> 60406 (TUR)	LK90	AF455174	KR019491	—
<i>C. subreticulata</i> Ahti	Brazil, Minas Gerais, <i>Stenroos</i> 5078 (TUR)	SATU3	AF457904	KR019492	AF458571
<i>C. subsetacea</i> Robbins ex A. Evans	USA, Florida, <i>Rosentreter</i> 16465 (H)	CL141	KR019402	KR019493	—
<i>C. subsetacea</i> Robbins ex A. Evans	USA, Florida, <i>Rosentreter</i> 16475 (H)	DW19	KR019403	KR019494	—
<i>C. substellata</i> Vain.	Brazil, Minas Gerais, <i>Stenroos</i> 4964 (TUR)	SATU21	AF457877	KR019495	AF458558

TABLE 1. *Continued*

Taxon	Voucher specimen	Code	GenBank accession numbers		
			ITS rDNA	IGS rDNA	β -tubulin
<i>C. subtenuis</i> (Abbeyes) Mattick	Canada, Nova Scotia, <i>Ahti</i> 57068 (H)	AT571	AF457911	KR019496	AF458573
<i>C. subulata</i> (L.) F. H. Wigg.	Germany, <i>Thell</i> 9932 (TUR)	LK64	AF455181	KR019497	AF458528
<i>C. terrae-novae</i> Ahti	Canada, Newfoundland, <i>Ahti</i> & <i>Scott</i> 56942(H)	AT572	—	KR019498	AF458580
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	Finland, <i>Haikonen</i> 26612 (H)	CL204	KR019404	KR019499	—
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	USA, New York, <i>Stenroos</i> 5743 (H)	DW2	KR019407	KR019502	—
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	Russia, Leningrad region, <i>Ahti</i> 68342 (H)	DW25	KR019408	KR019503	—
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	Russia, Kamchatka territory, <i>Himmelbrant</i> & <i>Stepanchikova</i> s.n. (H)	FH332	KR019401	KR019481	KR019545
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	Canada, Nova Scotia, <i>Ahti</i> 62683 (H)	DW26	KR019409	KR019504	—
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	USA, New York, <i>Stenroos</i> 5713 (H)	DW3	KR019412	KR019507	—
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	USA, Washington, <i>Droker</i> 24191208-1 (H)	DW4	KR019413	KR019508	—
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	USA, New York, <i>Stenroos</i> 5712 (H)	DW7	KR019415	KR019510	—
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	Canada, Newfoundland, <i>Stenroos</i> 5701 (H)	FH234	KR019417	KR019512	KR019550
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	Canada, Newfoundland, <i>Stenroos</i> 5702 (H)	FH235	KR019418	KR019513	KR019551
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	Canada, Quebec, <i>Stenroos</i> 5707 (H)	FH237	KR019419	KR019514	KR019552
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	Canada, Newfoundland, <i>Ahti</i> 67881 (H)	FH232	KR019387	KR019457	KR019534
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	USA, Washington, <i>Droker</i> 150208-1 (H)	FH324	KR019420	KR019515	—
<i>C. uncialis</i> (L.) F. H. Wigg. subsp. <i>uncialis</i>	USA, Alaska, Aleutian Islands, <i>Talbot</i> AML002-24 (H)	FH349	KR019422	KR019517	KR019554
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Greece, <i>Sipman</i> & <i>Raus</i> 58672 (H)	CL210	KR019374	KR019435	—
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	France, Haute-Savoie, <i>Ahti</i> 68190 (H)	DW1	KR019375	KR019436	—
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Finland, <i>Weckman</i> 2 (H)	DW10	KR019376	KR019437	—
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Finland, <i>Weckman</i> 3 (H)	DW11	KR019377	KR019438	KR019524
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Iceland, <i>Högnabba</i> 1345 (H)	DW14	KR019378	KR019439	KR019525
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Iceland, <i>Högnabba</i> 1391 (H)	DW15	KR019379	KR019440	KR019526
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Canada, Newfoundland, <i>Ahti et al.</i> 67978 (H)	FH233	KR019380	KR019441	KR019527
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Denmark, Faeroe Islands, <i>Våre</i> L1840 (H)	FH236	KR019381	KR019442	KR019528
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Finland, <i>Ahti</i> 68261a (H)	FH325	KR019382	KR019443	KR019529
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Iceland, <i>Högnabba</i> 1298 (H)	DW12	KR019405	KR019500	KR019546
<i>C. uncialis</i> subsp. <i>biuncialis</i> (Hoffm.) M. Choisy	Finland, <i>Weckman</i> 1 (H)	DW9	KR019416	KR019511	KR019549
<i>C. vareschii</i> Ahti	Guyana, <i>Stenroos</i> 4881 (TUR)	LK85	AF457878	KR019519	—
<i>C. variegata</i> Ahti	Guyana, <i>Stenroos</i> 4922 (TUR)	AT601	AF455240	KR019520	—
<i>C. zopfii</i> Vain.	Portugal, Trás-os-Montes, <i>Burgaz</i> s.n. (H)	FH344	KR019424	KR019521	—

Chemical study

The secondary metabolites of all the samples of *C. uncialis* subsp. *uncialis*, *C. uncialis* subsp. *bimucialis*, and *C. pseudostellata* were analyzed using thin-layer chromatography (TLC), in solvents A and B, according to White & James (1985).

DNA extraction, PCR and sequencing

DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen), according to the manufacturer's protocol. The extracted DNA was eluted in 160 µl of elution buffer included in the kit. Several potential loci (including mtSSU, *ef1a*, *mcm7*, ITS rDNA, IGS rDNA, β -tubulin and other unpublished markers) were tested in a subset of specimens in order to check the variation and the PCR success of each one. On the basis of the preliminary results, we decided to choose ITS rDNA, IGS rDNA and β -tubulin for the molecular study. The ITS rDNA region was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) or ITS1-LM (Myllys *et al.* 1999) and ITS2-KL (Lohtander *et al.* 1998); the IGS rDNA region using IGSf and IGSR (Wirth *et al.* 2008); and the β -tubulin gene using the primer pair Bt3-LM and Bt10-LM (Myllys *et al.* 2001). The amplification programmes were as follows: 95 °C for 5 min; 5 cycles of 30 s at 95 °C, 30 s at 58 °C, 60 s at 72 °C; 30 cycles of 30 s at 95 °C, 30 s at 56 °C, 60 s at 72 °C; 7 min at 72 °C for ITS rDNA; 95 °C for 5 min; 35 cycles of 30 s at 95 °C, 30 s at 54 °C, 60 s at 72 °C; 10 min at 72 °C for IGS rDNA; 95 °C for 5 min; 5 cycles of 30 s at 95 °C, 30 s at 55 or 56 °C, 60 s at 72 °C; 30 cycles of 30 s at 95 °C, 30 s at 52 or 54 °C, 60 s at 72 °C; 7 min at 72 °C for β -tubulin. PCR was carried out using Ready-To-Go PCR Beads (GE Healthcare), with 25 µl of final volume, 1 µl of each primer at 10 µM concentration, and 4 or 5 µl of DNA. PCR was performed in PTC-200 Thermal Cyclers (MJ Research) and Mastercycler ep gradient S (Eppendorf). PCR products were purified using illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). Sequencing reactions were carried out at MacroGen Inc. (<http://www.macrogen.com>).

Phylogenetic analyses

The alignments were assembled using MUSCLE (Robert 2004) for each locus separately. The ambiguously aligned regions were manually delimited (13 positions in ITS rDNA and four in IGS rDNA) and excluded from the analyses. Each locus was analyzed by maximum likelihood (ML), using RAxML 7.04 (Stamatakis 2006), assuming a GTRGAMMA model. The best ML trees were searched using every fifth bootstrap tree as a starting tree (100 independent searches). The nodal support was assessed with 500 bootstrap pseudoreplicates using the rapid bootstrap option. The β -tubulin locus was analyzed using two partition approaches: 1) considering it as a single subset, 2) considering the introns and each codon position from the coding regions as a different subset (in total eight subsets) and analyzed with the same model. The introns were delimited by comparing *Cladonia* sequences in

GenBank (AF458533 and AF458550) with our sequences. The topology of β -tubulin trees obtained with both approaches was the same and we decided to use the whole locus as a single subset in the combined analyses. The clades with bootstrap support >75% were examined to assess the congruence among the loci according to the method of Kauff & Lutzoni (2002, 2003). No strongly supported incongruence was detected among the loci, and the different datasets were therefore combined. In the combined matrix, only those samples were included for which the sequences of at least two loci were available, except for *C. nipponica* FH338, *C. siamea* CL77 and *C. solitaria* 2677. Only IGS rDNA or ITS rDNA sequences were obtained for these samples, but they were included because the species are rare and no fresh material was available. The combined dataset was analyzed by maximum parsimony (MP), ML and Bayesian inference.

The MP analysis was performed in PAUP ver. 4.0.b.10 (Swofford 2003) using heuristic searches with 1000 random taxon-addition replicates, and with TBR branch swapping and the MulTrees option. All the characters were treated as equally weighted and gaps were coded as missing data. The clade support was determined by bootstrap analysis, with 1000 replicates, using the heuristic option. The ML analyses were implemented using RAxML 7.04, considering a partition with three subsets: ITS rDNA, IGS rDNA, and β -tubulin, assuming a GTRGAMMA model, and using the same options as for single gene analyses. Bayesian analysis was carried out using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The best-fit evolutionary model for each locus was selected by MrModeltest 2.3 (Nylander 2004) under the AIC criterion. The three models are listed in Table 2. Two simultaneous runs with 10 000 000 generations, each starting with a random tree and employing four simultaneous chains, were executed. Every 1000th tree was saved into a file. The average standard deviation of split frequencies between the runs was below 0.01, indicating convergence of the chains. The first 1 000 000 generations (i.e., the first 1000 trees) were deleted as the 'burn-in' of the chain. Cumulative split frequency plot in AWTY (Nylander *et al.* 2008) was used to determine when the chains reached the stationary stage. The 50% majority-rule consensus tree was calculated using the 'sumt' command of MrBayes.

Tree topology test

The phylogenetic estimates of the concatenated dataset revealed that *C. pseudostellata* is not monophyletic. In order to dismiss the possibility that this result is an artefact of the phylogenetic analyses, we conducted a Shimodaira-Hasegawa test (SH; Shimodaira & Hasegawa 1999) and used the expected likelihood weight test (ELW; Strimmer & Rambaut 2002). RAxML 7.04 was used to estimate the maximum likelihood tree consistent with the alternative hypotheses (monophyly of *C. pseudostellata*, *Unciales* and *Cladina*). The topological constraints analyses enforced only the monophyly of the taxa under study (*C. pseudostellata*, *Unciales* taxa or

Cladonia taxa). The SH and ELW tests were run in TREE-PUZZLE 5.2 (Schmidt *et al.* 2002), using the GTR+I+G model and with four-category approximation to the gamma distribution for substitution rate among sites. The tests were estimated using 1000 replicates under the RELL method.

Genetic distances

The pairwise distances within and between *C. uncialis* subsp. *uncialis* and *C. uncialis* subsp. *biuncialis* were calculated in PAUP*, using the K2P model (Kimura 1980) for each locus separately (ITS rDNA, IGS rDNA and β -tubulin). We applied this model as it was used in Pino-Bodas *et al.* (2013). To compare the genetic variability between *C. uncialis* subsp. *uncialis* and *C. uncialis* subsp. *biuncialis*, the mean nucleotide difference within and between taxa was calculated in DnaSP 5.1 (Librado & Rozas 2009). In addition, the genetic similarity (GD) for each locus was calculated as $GD = 1 - (\text{genetic distance between sample pairs})$. Histograms of genetic distances and genetic similarity within and between taxa were created for each marker (Fig. 3).

Results

The combined dataset contained 102 sequences and 1819 characters. Table 2 summarizes the variation of the sequences. A total of 186 new sequences were generated for the study. The MP analysis yielded a total of 1000 equally parsimonious trees with 2058 steps, $CI = 0.5042$ and $RI = 0.7781$. The ML analysis yielded a tree with $\ln L$ (log likelihood) = -13871.90 , while the Bayesian analyses yielded a consensus tree with $\ln L = -13798.10$ (arithmetic mean). All three methods yielded trees with the same topology and only the 50% majority-rule tree from the Bayesian analysis is presented in Fig. 1. The provisional clade names mostly correspond to those presented in Stenroos *et al.* (2002).

The section *Unciales* is non-monophyletic, and most of the species appear in four groups,

viz. *Amaurocraeae*, *Borya*, *Divaricatae* and *Unciales*. Other species from section *Unciales* fall within *Perviae* (*C. candelabrum*, *C. capitellata*, *C. dillemiana*, *C. medusina*, *C. pachyclados*, *C. rugulosa*, *C. siamea* and *C. subsetacea*), and *Cladonia* (*C. subchordalis*); *C. delavayi* is closely related to *Impexae*. The SH and ELW tests rejected the monophyly of section *Unciales* with a P -value of <0.01 . Furthermore, the former genus *Cladonia* splits into three entities (*Cladinae*, *Impexae*, and *Tenues*), all of which are nested within the genus *Cladonia*. The ELW test rejected the monophyly of *Cladonia* ($P = 0.0464$) but it was not rejected by the SH test ($P = 0.3340$). *Cladonia uncialis* s. lat. appears monophyletic, and can be divided into two well-supported clades.

In all the phylogenetic analyses, *C. pseudostellata* was resolved as non-monophyletic (Fig. 1). The specimens of this taxon were clustered in the *Cladonia uncialis* s. lat. clade. The SH and ELW tests rejected the monophyly of *C. pseudostellata* with a P -value of <0.01 in both tests.

Cladonia uncialis s. lat. contains usnic acid as a major, constant substance. The presence of squamatic acid did not correlate with any of the clades obtained within *C. uncialis* or its subclades. This compound can be found in subsp. *uncialis* as well as in subsp. *biuncialis* (Fig. 2). The six specimens of *C. pseudostellata* contained usnic and hypothamnolic acids (Fig. 2).

The genetic distances and genetic similarity are shown in Table 3 and Fig. 3. In all the loci, *C. uncialis* subsp. *uncialis* had greater variation in the genetic distances and average nucleotide differences than *C. uncialis* subsp. *biuncialis*. The locus IGS rDNA showed the highest intra- and inter-clade distance values. Fig. 3 shows the similarity in *C. uncialis*

TABLE 2. Summary of variation in each locus studied in the *Cladonia uncialis* phylogeny: alignment length in number of bases (positions), number of variable characters, parsimony informative characters and evolutionary models selected by MrModeltest using AIC criterion.

Locus	Positions	Variable sites	Parsimony-informative	Model
β -tubulin	758	199	153	HKY + G
ITS rDNA	647	311	227	SYM + G
IGS rDNA	414	223	162	GTR + G



0-02

FIG. 1. Phylogeny of the section *Unciales* resulting from the Bayesian analysis of the combined dataset of ITS rDNA, IGS rDNA and β -tubulin. 50% consensus majority tree from the Bayesian analysis. The support values $\geq 70\%$ MP bootstrap, $\geq 70\%$ ML bootstrap, ≥ 0.95 posterior probability of Bayesian analysis appear on the branches. Branches supported in the three analyses (MP, ML & Bayesian) are indicated with thick lines. Grey rectangles show the species of the section *Unciales*. Black vertical bars indicate the provisional classification of the species according to Stenroos *et al.* (2002).

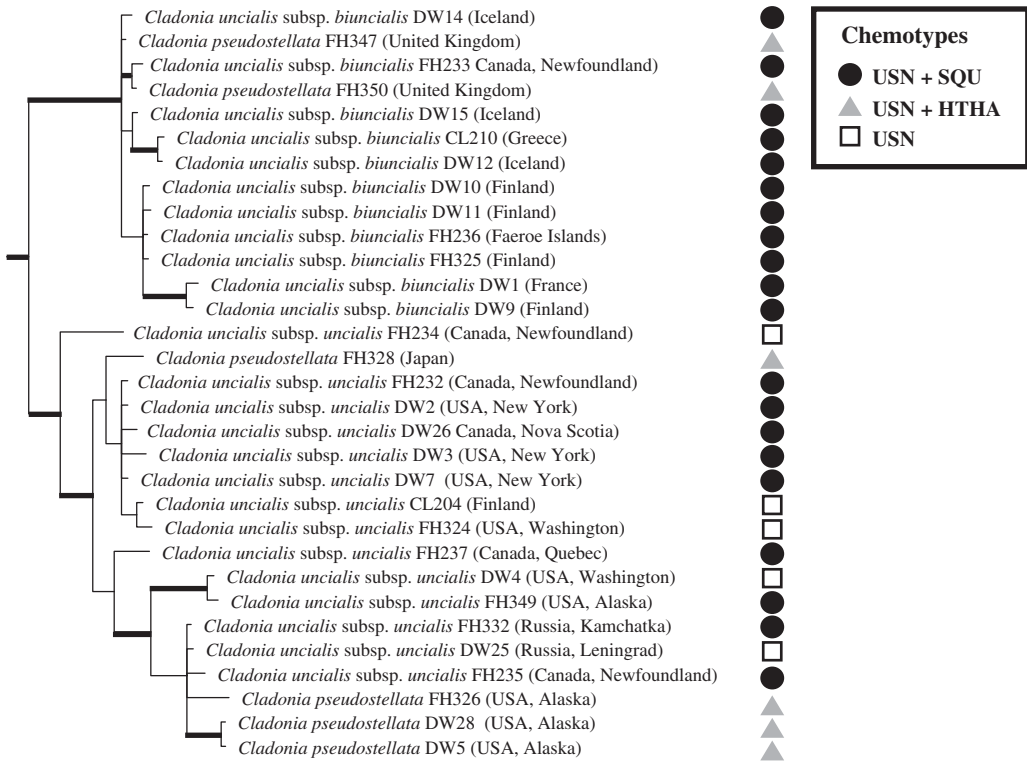


FIG. 2. The clade *Cladonia uncialis* s. lat. from the 50% consensus majority tree of the Bayesian analysis based on the concatenated dataset. The different chemotypes and the specimen localities are indicated for each specimen. HTHA = hypothamnolic acid, SQU = squamatic acid, USN = usnic acid.

subsp. *uncialis*, *C. uncialis* subsp. *biuncialis* and between them. In all cases, the similarity was very high (greater than 0.94). The lower values of similarity were obtained in IGS rDNA within *C. uncialis* subsp. *uncialis*, and between *C. uncialis* subsp. *uncialis* and *C. uncialis* subsp. *biuncialis*.

Discussion

Phylogenetic reconstructions have enormously changed the classification in several groups of lichenized fungi. Changes are still taking place, even in *Cladonia*, a conspicuous and fairly well studied genus (for example Ahti 2000; Stenroos *et al.* 2002; Burgaz & Ahti 2009; Ahti & Stenroos 2013). Stenroos *et al.* (2002) distinguished three informal Subdivisions within the genus *Cladonia*. These Subdivisions were further divided into

Supergroups, Groups and Subgroups. Some of their Supergroups essentially corresponded with former sections (such as *Cladonia*, *Cocciferae*, *Perviae*, etc.), but in many cases the formal sections were found to be non-monophyletic. Therefore, new groupings were distinguished, but no nomenclaturally accurate names were introduced for them. A new multigene analysis for the *Cladoniaceae* is currently under preparation, and while waiting for the results, we will not formalize any of the used group names here either.

Most *Cladonia* clades (Fig. 1) are well supported (with the exception of *Divaricatae* and *Amaurocraeae*); nonetheless the phylogenetic relationships among them remain unresolved in this study (Fig. 1). A greater number of taxa will be necessary to clarify this question; we confine ourselves here to discussing the taxa compositions.

TABLE 3. Genetic variability between and within *C. uncialis* ssp. *uncialis* and *C. uncialis* ssp. *biuncialis* for each locus.

Source of variation	Genetic variability								
	ITS rDNA			IGS rDNA			β -tubulin		
	GD	N	GS	GD	N	GS	GD	N	GS
Within <i>C. uncialis</i> ssp. <i>uncialis</i>	0.00–0.0181 (0.0091)	5.06	0.9818–1 (0.9908)	0.00–0.0532 (0.0224)	5.98	0.9467–1 (0.9775)	0.00–0.0158 (0.0059)	3.77	0.9841–1 (0.9945)
Within <i>C. uncialis</i> ssp. <i>biuncialis</i>	0.00–0.0162 (0.0076)	3.76	0.9837–1 (0.9923)	0.00–0.0073 (0.0027)	0.74	0.9926–1 (0.9972)	0.00–0.0031 (0.0015)	3.85	0.9968–1 (0.9984)
Between subsp. <i>uncialis</i> and <i>biuncialis</i>	0.0035–0.0217 (0.0149)	6.03	0.9782–0.9964 (0.9851)	0.0109–0.0493 (0.0244)	5.45	0.9506–0.9890 (0.9754)	0.0206–0.0287 (0.0266)	9.85	0.9712–0.9793 (0.9733)

GD = ranges of genetic distances; N = average nucleotide differences; GS = ranges of genetic similarities. The mean genetic distances are given in brackets.

According to our results, *Unciales* includes only *C. uncialis* s. lat. (*C. uncialis* is the type species of the formal section). The other species earlier assigned to *Unciales* are nested in other clades, primarily in *Borya*, *Divaricatae* and *Amaurocraeae*. In addition, quite a few species turned out to belong to *Perviae*, which includes taxa that have open branch axils and are typically without usnic acid. However, species such as *C. capitellata*, *C. dilleniana*, and *C. vareschii*, all with usnic acid, clearly belong to *Perviae*. In fact, Ahti (2000) already noted that some species from *Unciales* were morphologically similar to *Perviae* species but had a different chemical composition.

Borya is characterized by the production of needle-like 'steroid' crystals in the apical parts of the podetia (except for *C. solitaria*), and a fibrous skeletal tissue in the medulla (Ahti 1973). Altogether 11 species appear in the *Borya* clade, confirming the species composition of *Borya* presented by Ahti (2000). There might yet be an additional species in the group. Our *C. aff. kanewskii* DW22 collected from South Siberia, Russia, and which morphologically resembles *C. kanewskii*, is probably an undescribed species. However, it is a single specimen and its placement is not clear (only supported in the Bayesian analysis). Other than that, we believe that all of the potential *Borya* species of *Cladonia* have now been analyzed.

Divaricatae includes species with a spiny appearance. Usnic acid may be present or absent, without a correlation with morphological characters. We included eight species, the same as in Stenroos *et al.* (2002). *Divaricatae* appears basal to the clade consisting of the red-fruited *Cocciferae* and *Mimitatae*, as well as *Amaurocraeae* and *Perviae*. In the phylogenetic analysis presented by Stenroos *et al.* (2002), this group appeared closely related to *Borya*, *Unciales*, and *Cladoniae*. A few more species still await placement (*C. crassiuscula*, *C. ibitipocae*, *C. spinea*, and *C. sufflata*).

Amaurocraeae is a small, enigmatic group consisting only of *C. amaurocraea* and the now newly placed *C. peltasta*. The former species is elongated and slender, and the smoothly corticated podetia are often tipped

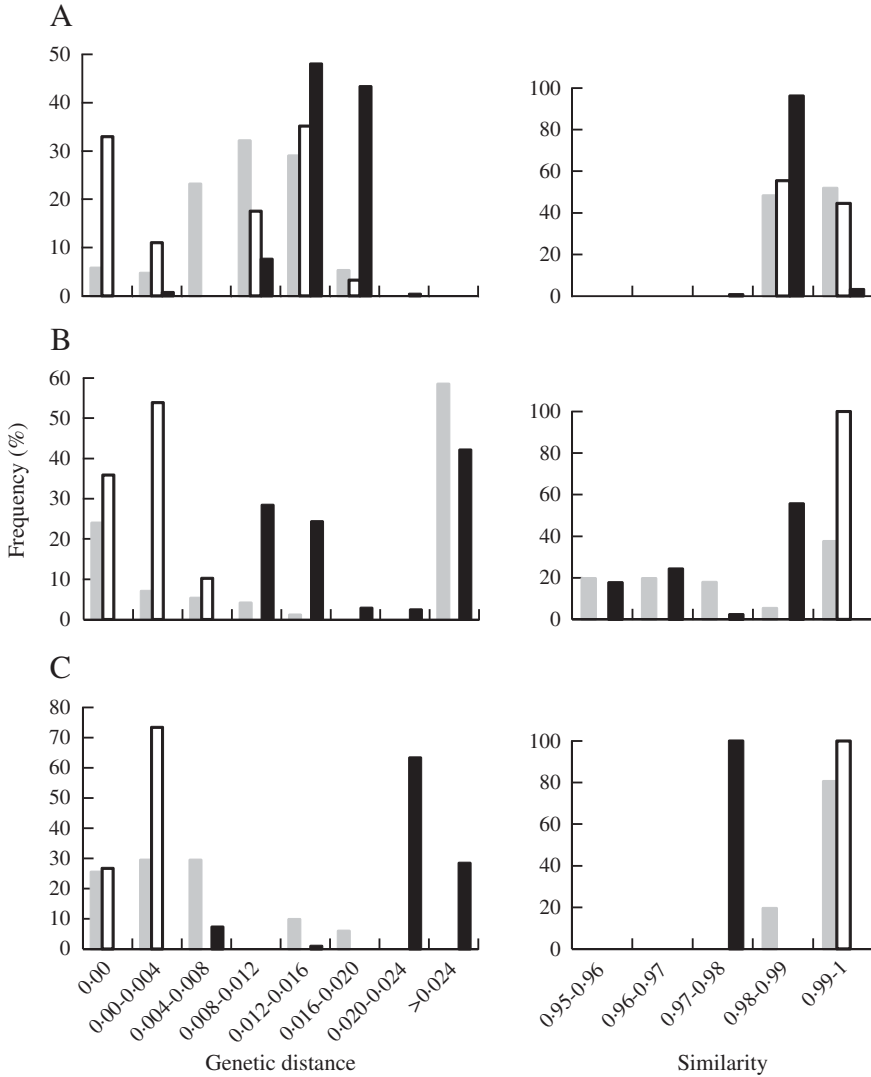


FIG. 3. Histograms illustrating variation in genetic distance and genetic similarity in *Cladonia uncialis* subsp. *uncialis* (■), *C. uncialis* subsp. *biuncialis* (□), and between the subspecies (■) A, ITS rDNA; B, IGS rDNA; C, β -tubulin.

with scyphi. *Cladonia peltasta* is also slender, but scyphi are absent and it produces ochraceous apothecia. Both species produce usnic and barbatic acid as secondary metabolites (Huovinen & Ahti 1986a). However, the relationship of *C. peltasta* with *C. amaurocraea* is not supported, therefore other relationships cannot be ruled out.

The following species have now been placed using phylogenetic analyses for the first time:

C. candelabrum, *C. dilleniana*, *C. medusina*, *C. pachyclados*, *C. siamea*, and *C. subsetacea* belong to *Perviae* in the present analysis; *C. peltasta* belongs to *Amaurocraeae*, and *C. kanewskii*, *C. labradorica* and *C. pachycladodes* belong to *Borya*.

The increase of taxa in future studies will clarify whether other species of the former and widely delimited section *Unciales* are phylogenetically closely related to

C. uncialis or belong to the other segregates of the section. The taxa not yet analyzed are: *C. bangii*, *C. chimantae*, *C. congesta*, *C. crassiuscula*, *C. glabra*, *C. hokkaidensis*, *C. ibitipocae*, *C. papuana*, *C. recticaulis*, *C. robusta*, *C. southlandica*, *C. spinea*, *C. sufflata* and *C. usambarensis*.

Taxonomic status of *Cladonia uncialis* s. lat.

The phylogenetic analyses based on three loci showed that *Cladonia uncialis* s. lat. is monophyletic. It is divided into two well-supported clades, which correlate with the two previously described subspecies, *C. uncialis* subsp. *uncialis* and *C. uncialis* subsp. *biuncialis*. In Stenroos *et al.* (2002), the two subspecies were non-monophyletic. However, the present study is based on an increased sampling of three loci, and therefore our results are more robust. Four specimens originally identified as *C. pseudostellata* (containing hypothamnolic acid, Fig. 2) were referred to *C. uncialis* subsp. *uncialis*, and two specimens to *C. uncialis* subsp. *biuncialis*. The monophyly of *C. pseudostellata* was also rejected by the SH and ELW tests. These results agree with the taxonomic proposal of Brodo & Ahti (1996) that *C. pseudostellata* is a chemotype of *C. uncialis*, although this suggestion has not been accepted by all lichenologists (e.g., Kurokawa & Kashiwadani 2006). This chemotype has now also been detected in Scotland, in subsp. *biuncialis*, and is a new record to Europe.

In the present work, we use genetic distances to discuss what the most appropriate taxonomic rank is for *C. uncialis* subsp. *uncialis* and *C. uncialis* subsp. *biuncialis*. In the genus *Cladonia*, the genetic distance variation ranges have been studied for 35 species that belong to the Supergroup *Cladonia* (*sensu* Stenroos *et al.* 2002; Pino-Bodas *et al.* 2013). The average values of the intraspecific genetic distances were 0.0094 for the ITS rDNA and 0.0289 for the IGS rDNA. Our results show that the average genetic distance for IGS rDNA between *C. uncialis* subsp. *uncialis* and *C. uncialis* subsp. *biuncialis* is lower than the average for *Cladonia*, which could be interpreted as a species including

two subspecies. In the ITS rDNA, the average value of the genetic distances between the two clades was higher than the average found by Pino-Bodas *et al.* (2013) in *Cladonia*. However, these authors found monophyletic species with higher values (e.g. *C. acuminata*, *C. cariosa*, and *C. rei*). The β -tubulin gene has not been used in previous barcoding studies with *Cladonia*, or other closely related genera of lichenized fungi, and therefore no genetic distance values are available for comparison. However, the three loci showed an overlapping of intra- and inter-clade genetic distances (Table 3, Fig. 3) and the genetic similarity among the taxa is very high. Therefore we consider that the subspecies status is the most appropriate one for our taxa. In addition, the genetic distances of ITS rDNA fell inside the intraspecific threshold (0.015–0.017) for the *Parmeliaceae* (Del-Prado *et al.* 2010).

The genetic divergence within the clade *C. uncialis* subsp. *uncialis* is higher than within the clade *C. uncialis* subsp. *biuncialis* for the three loci, particularly in IGS rDNA (Fig. 3, Table 3). This could indicate that the clade *C. uncialis* subsp. *uncialis* hides several infraspecific taxa. From a morphological viewpoint, this clade is much more variable than the clade *C. uncialis* subsp. *biuncialis* (Fig. 4). A wider sampling is necessary to test this hypothesis. Based on the observations by Ahti (2000) and J. Lendemer (pers. comm.) on the deviating populations of *C. uncialis* in the eastern United States (well-developed on Long Island, New York, for example; for description, see Hinds & Hinds 2007; Fig. 4F), we expected that the specimens from there would represent a distinct clade. However, though they clustered together, they were not resolved as a distinct group. Therefore, we are not recognizing a taxonomic entity there. These populations need special attention when additional loci are used in future analyses.

As to secondary chemistry, the yellow pigment usnic acid is constantly present in *C. uncialis*. Now that *C. pseudostellata* is included into the species, both subspecies may produce hypothamnolic acid, but as far as is known, only in some coastal areas such

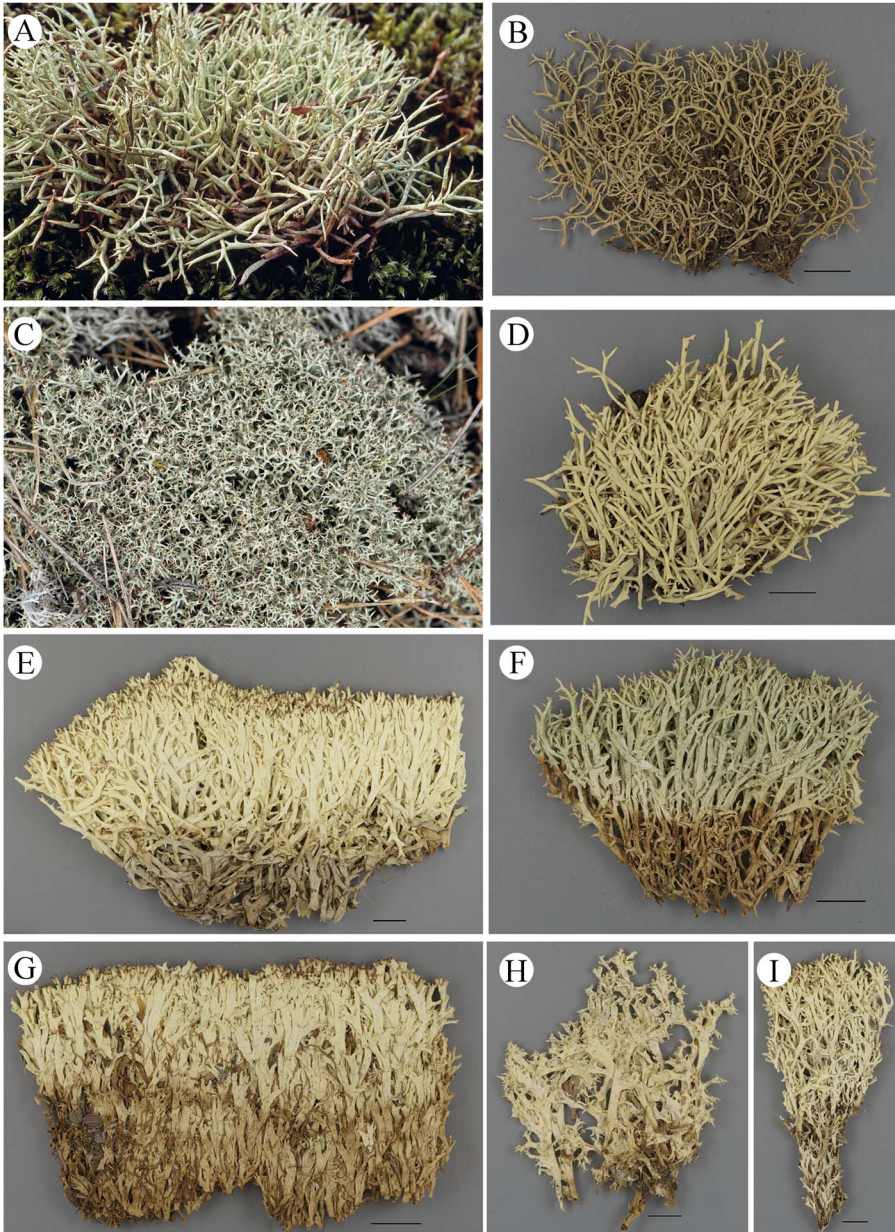


FIG. 4. Morphological variation of *Cladonia uncialis* s. lat. A, *C. uncialis* subsp. *biuncialis*, Finland (photo: V. Haikonen); B, *C. uncialis* subsp. *biuncialis*, C. N. Tavares VIII-51, Portugal; C, *C. uncialis* subsp. *uncialis*, Finland; D, *C. uncialis* subsp. *biuncialis*, T. Ahti 2603, Canada, Newfoundland; E, *C. uncialis* subsp. *uncialis*, T. Ahti 67881, Canada, Newfoundland; F, *C. uncialis* subsp. *uncialis*, R. C. Harris 56802, USA, New York; G, *C. uncialis* subsp. *uncialis*, G. W. Scotter 8097, Canada, Northwest Territories; H, *C. uncialis* subsp. *uncialis*, T. Ahti 39182, Canada, British Columbia; I, *C. pseudostellata*, S. Talbot & W. B. Schofield KML003-X-1, USA, Alaska. Scales = 1 cm. In colour online.

as Japan, Alaska (especially Aleutian Islands), and Scotland. In subsp. *uncialis*, only usnic acid is usually present (Leuckert *et al.* 1971; Carlin 1981; Huovinen & Ahti 1986a; Burgaz & Ahti 2009; Ahti & Stenroos 2013). However, squamatic acid is sometimes present as well, for example in Finland (Fig. 2). The somewhat deviating morph in eastern North America (Ahti 2000: 343), included here in subsp. *uncialis*, normally contains squamatic acid. On the other hand, subsp. *biuncialis* almost always produces squamatic acid. Very rarely, small amounts of barbatic acid are perhaps present (Ahti & Stenroos 2013), but the report might be based on confusion with *C. amaurocraea* which always contains barbatic acid, looks similar, and grows intermixed with *C. uncialis*.

There are obvious ecological differences between the two subspecies of *Cladonia uncialis*. In Europe, subsp. *biuncialis* is characteristic of oceanic, coastal conditions but extends far inland in the Iberian Peninsula (maps in Burgaz & Ahti 2009: 106) and Central Europe (Austria, Czech Republic, Germany, Poland, Slovakia, Switzerland, not reaching Russia beyond the Baltic Sea coast). Outside Europe it is known only from Newfoundland in eastern Canada (and the adjacent French possession St. Pierre & Miquelon), being absent from the Pacific coast. Subsp. *uncialis* is more continental, and is the only subspecies present within most of the wide, holarctic range of *C. uncialis* s. lat. (map in Litterski & Ahti 2004), although absent or rare right on the coast in Europe (e.g. absent from Iceland).

Although the two taxa are often easily identified morphologically, it is not always so. Kärenlampi & Pelkonen (1971) and Ahti & Stenroos (2013) pointed out that there are seemingly intermediate populations in areas where the subspecies meet, especially in southern Sweden and south-western Finland. Some authors (Coppins 1978; Burgaz & Ahti 2009) have paid attention to the inner surface of the podetial wall, which seems to be pulverulent in subsp. *biuncialis* and smooth in subsp. *uncialis*. The reliability of this character has not been tested.

Details of nomenclature and typification of the recognized subspecies

The treatment below includes a new typification and other nomenclatural notes.

Cladonia uncialis subsp. *biuncialis* (Hoffm.) M. Choisy

Bull. Mens. Soc. Linn. Lyon 20: 9 (Jan 1951).—*Cladonia biuncialis* Hoffm. *Deutschl. Fl.* 2: 116 (1796); type: [Germany?], hb. G. F. Hoffmann 8614 (MW-Hoffmann, neotype, designated by Ahti 1978: 9, as 'lectotype', corr. by Ahti 1993: 100).

Cladonia uncinata Hoffm., *Deutschl. Fl.* 2: 116 (1796); type: drawing in Dillenius, *Hist. Musc.* t. 16, fig. 21B (1742) (lectotype designated here by T. Ahti); sine loco, hb. Dillenius, *Hist. Musc.* No. 98.21B (OXF, epitype designated here by T. Ahti).

Baeomyces uncialis var. *dicraeus* Ach., *Methodus*: 353 (Jan–Apr 1803).—*Cladonia uncialis* var. *dicraea* (Ach.) Räsänen, *Meddeland. Soc. Fauna Fl. Fenn.* 46: 171 (1921) [not Kärenlampi & Pelkonen 1971: 55].—*Cladonia uncialis* subsp. *dicraea* (Ach.) D. Hawksw. in Heywood, *Taxonomy and Ecology*: 41 (1973); type: Sweden ('Suecia') (H-ACH 1625B = H 950273, lectotype, designated by Kärenlampi & Pelkonen 1971: 55).

Baeomyces aduncus Ach., *Methodus*: 353 (Jan–Apr 1803), nom. illeg. superfl. for *Cladonia uncinata* Hoffm.

Cladonia uncialis (L.) F. H. Wigg.

Fl. Holsat.: 90 (29 Mar 1780) subsp. *uncialis* *Lichen uncialis* L., *Sp. Pl.*: 1153 (1 Mai 1753); type (cons.): Sweden, Dalarna, Stora Kopparberg, Rotneby ('Rotneby prope urbem Fahlun Dalekarliae'), C. Stenhammar in Stenhammar, *Lich. Suec. Exs.*, ed. 2, No. 210 (UPS; isotypes H, MIN).

Cladonia pseudostellata Asahina, *J. Jap. Bot.* 18: 620 (10 Nov 1942); type: Japan, Hokkaido, Kamikawa Dist. (Prov. Ishikari), Mt. Daisetsu, 1937, Y. Asahina 37016 (TNS, lectotype designated by Ahti 1993: 91; isolectotype US-Evans).

Polyphyly of the old genus *Cladina*

It has already been shown many times that *Cladina* (reindeer lichens) do not warrant a generic status but should be included in *Cladonia* (Hyvönen *et al.* 1995; Stenroos *et al.* 1997, 2002; DePriest *et al.* 1999; Ahti & DePriest 2001). These lichens commonly appeared monophyletic within *Cladonia* (Stenroos *et al.* 2002), but non-monophyly was also demonstrated in some cases (DePriest *et al.* 1999, 2000). Interestingly, as

early as in Choisy (1928), *Cladina* was treated as non-monophyletic and three different lineages were distinguished. In the present analyses, *Cladina* is non-monophyletic (but the monophyly of *Cladina* was rejected only by the ELW test) and is divided into three lineages, referred to here as *Impexae*, *Tenues* and *Cladinae*. They all correspond to the former sections defined by Ahti (1984, under genus *Cladina*; see also Huovinen & Ahti 1986b and Ahti 2000). Guo & Kashiwadani (2004) obtained a similar result based on ITS rDNA. In their analysis, *C. uncialis* is placed as sister to either the *Tenues* or *Cladinae*, depending on the model used. In our analyses, the phylogenetic relationships between *Impexae*, *Tenues* and *Cladinae* remain unresolved (Fig. 1). The proper placing of these clades will therefore have to wait for a broader sampling.

As stated above, we are using the infra-generic group names informally for the time being. The lineages concur with chemical characters in addition to the branching types.

Impexae, represented here by *C. pycnoclada* and *C. terrae-novae*, are typically defined by richly branched, curly thalli and the presence of the depside perlatolic acid. The Himalayan *C. delavayi* is placed surprisingly close to *Impexae*. Although it was originally placed in *Unciales* (also in Huovinen & Ahti 1986a), it is not related to any current segregates of former *Unciales*. A similar result was obtained by Stenroos *et al.* (2002). This species is, however, different from other *Impexae* by containing 4-*O*-methylcryptochlorophaeic and cryptochlorophaeic acids, and having very slender, little branched podetia. In the present analyses, *C. delavayi* is positioned on a long branch separate from the rest of the *Impexae*, implying that it is substantially different from the others.

Tenues are represented here by *C. stygia* and *C. subtenuis*. A diagnostic character of *Tenues* was a red pigment in the conidiomata. The inclusion of *C. rangiferina* in this group (Stenroos *et al.* 2002) indicates that the red pigment is not diagnostic after all.

The third lineage of former *Cladinae* includes *C. arbuscula* and *C. mitis*, and is referred to here as *Cladinae*. Stenroos *et al.*

(2002) also showed *Cladinae* (*C. arbuscula* and allies) and *Tenues* (*C. subtenuis* and allies) as separate clades, although as sister to each other, and due to the overall topology the two clades were differently ranked.

Morphologically, *C. arbuscula* (*Cladinae*) as well as *C. stygia* and *C. rangiferina* (*Tenues*) are very similar in branch architecture, although they differ in chemistry and surface structure (Ahti & Stenroos 2013). The traditionally recognized characters, such as branching patterns, presence of cortex, and chemistries may be quite misleading, when related clades are compared. It appears that the former *Unciales* are spread across the sequence-based phylogenetic tree of the genus *Cladonia*.

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REFERENCES

- Aasamaa, H. (1961) Classification of the genus *Cladonia* Hill. *Eesti NSV Teaduste Akadeemia Jüures Asuva Loodusuurijate Seltsi Aastaraamat* **54**: 104–117. (In Russian).
- Ahti, T. (1973) Taxonomic notes on some species of *Cladonia*, subsect. *Unciales*. *Annales Botanici Fennici* **10**: 163–184.
- Ahti, T. (1978) Nomenclatural and taxonomic remarks on European species of *Cladonia*. *Annales Botanici Fennici* **15**: 7–14.
- Ahti, T. (1984) The status of *Cladina* as a genus segregated from *Cladonia*. *Nova Hedwigia* **79**: 25–61.
- Ahti, T. (1993) Names in current use in the *Cladoniaceae* (lichen-forming ascomycetes) in the ranks of genus to variety. In *Names in Current Use in the Families Trichomaceae, Cladoniaceae, Pinaceae, and Lemnaceae, Regnum Vegetabile Vol. 128* (W. Greuter, ed.): 58–106. Königstein: Koeltz Scientific Books.
- Ahti, T. (2000) *Cladoniaceae. Flora Neotropica Monograph* **78**: 1–362.
- Ahti, T. & DePriest, P. T. (2001) New combinations of *Cladina* epithets in *Cladonia* (Ascomycotina: *Cladoniaceae*). *Mycotaxon* **78**: 499–502.
- Ahti, T. & Stenroos, S. (2013) *Cladoniaceae*. In *Nordic Lichen Flora Vol. 5* (T. Ahti, S. Stenroos & R. Moberg, eds.): 1–117. Uppsala: Museum of Evolution, Uppsala University.

- Brodo, I. M. & Ahti, T. (1996) Lichens and lichenicolous fungi of the Queen Charlotte Islands, British Columbia, Canada. 2. The *Cladoniaceae*. *Canadian Journal of Botany* **74**: 1147–1180.
- Burgaz, A. R. & Ahti, T. (2009) *Cladoniaceae*. *Flora Ibérica Liqueológica* **4**: 1–111.
- Carlin, G. (1981) De svenska bägarlavarna (*Cladonia* undersläktet *Cladonia*). *Svensk Botanisk Tidskrift* **75**: 361–396.
- Choisy, M. (1928) Sur le phylétisme des Ascomycètes du genre *Cladonia* (Lichens). *Bulletin de la Société Mycologique de France* **43**: 267–271.
- Coppins, B. J. (1978) H. M. Livens lichen collection at Bolton Museum: notes on some interesting specimens. *Naturalist* **103**: 105–107.
- Dahl, E. (1952) On the use of lichen chemistry in lichen systematics. *Revue Bryologique et Lichénologique* **21**: 119–134.
- Del-Prado, R., Cubas, P., Lumbsch, H. T., Divakar, P. K., Blanco, O., Amo de Paz, G., Molina, M. C. & Crespo, A. (2010) Genetic distances within and among species in monophyletic lineages of *Parmeliaceae* (Ascomycota) as a tool for taxon delimitation. *Molecular Phylogenetics and Evolution* **56**: 125–133.
- DePriest, P. T., Piercey-Normore, M., Sikaroodi, M., Kärkkäinen, K. & Oksanen, I. (1999) Phylogenetic analyses of *Cladonia* and *Cladina* (lichen-forming Ascomycota). In *Abstracts of XVI International Botanical Congress, 1–7 August, 1999, St. Louis, Missouri, USA*, p. 325.
- DePriest, P. T., Piercey-Normore, M., Sikaroodi, M., Kärkkäinen, K., Oksanen, I., Yahr, R. & Ahti, T. (2000) Phylogenetic relationships among sections of *Cladonia* and *Cladina*. In *Abstracts of the 4th International Lichenological Symposium, 3–8 September, 2000, Barcelona, Spain*, p. 14.
- Gardes, M. & Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.
- Guo, S. & Kashiwadani, H. (2004) Recent study on the phylogeny of the genus *Cladonia* (s. lat.) with the emphasis on the integrative biology. *National Science Museum Monographs, Tokyo* **24**: 207–225.
- Hawksworth, D. L. (1973) Ecological factors and species delimitation in the lichens. In *Taxonomy and Ecology. Systematics Association Special Volume No. 5* (V. H. Heywood, ed.): 31–69. London & New York: Academic Press.
- Hinds, J. W. & Hinds, P. L. (2007) The macrolichens of New England. *Memoirs of the New York Botanical Garden* **96**: 1–584.
- Huelsenbeck, J. P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Huovinen, K. & Ahti, T. (1986a) The composition and contents of aromatic lichen substances in *Cladonia*, section *Unciales*. *Annales Botanici Fennici* **23**: 173–188.
- Huovinen, K. & Ahti, T. (1986b) The composition and contents of aromatic lichen substances in the genus *Cladina*. *Annales Botanici Fennici* **23**: 93–106.
- Hyvönen, J., Ahti, T., Stenroos, S. & Gowan, S. (1995) The genus *Cladina* and the section *Unciales* of the genus *Cladonia* (*Cladoniaceae*, lichenized Ascomycotina), a preliminary phylogenetic analysis. *Journal of the Hattori Botanical Laboratory* **78**: 243–253.
- Kärenlampi, L. (1964) Preliminary notes on the variability of *Cladonia uncialis* (L.) Wigg. in Eastern Fennoscandia. *Annales Botanici Fennici* **1**: 220–223.
- Kärenlampi, L. & Pelkonen, M. (1971) Studies on the morphological variation of the lichen *Cladonia uncialis*. *Reports from the Kevo Subarctic Research Station* **7**: 47–56.
- Kauff, F. & Lutzoni, F. (2002) Phylogeny of the *Gyalectales* and *Ostropales* (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. *Molecular Phylogenetics and Evolution* **25**: 138–156.
- Kauff, F. & Lutzoni, F. (2003) *Compat.py – a program to detect topological conflict between supported clades in phylogenetic trees*. Available at <http://www.lutzonilab.net/downloads/index.shtml>.
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111–120.
- Kurokawa, S. & Kashiwadani, H. (2006) Checklist of Japanese lichens and allied fungi. *National Science Museum Monographs* **33**: 1–157.
- Leuckert, C., Bärman, U. & Schug, G. (1971) Chemische Flechtenanalysen II. *Herzogia* **5**: 465–475.
- Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.
- Litterski, B. & Ahti, T. (2004) World distribution of selected European *Cladonia* species. *Symbolae Botanicae Upsalienses* **34**: 205–236.
- Lohtander, K., Myllys, L., Sundin, R., Källersjö, M. & Tehler, A. (1998) The species pair concept in the lichen *Dendrographa leucophaea* (*Arthomiales*): analyses based on ITS sequences. *Bryologist* **101**: 404–411.
- Mattick, F. (1938) Systembildung und Phylogenie der Gattung *Cladonia*. *Botanisches Zentralblatt* **58B**: 215–234.
- Mattick, F. (1940) Übersicht der Flechtengattung *Cladonia* in neuer systematischer Anordnung. *Repertorium Specierum Novarum Regni Vegetabilis* **49**: 140–168.
- Mattick, F. (1951) Alte und neue Probleme der Lichenologie. *Berichte der Deutschen Botanischen Gesellschaft* **64**: 94–107.
- Merrill, G. K. (1908) Lichen notes no. 7: Yukon lichens. *Bryologist* **11**: 105–113.
- Myllys, L., Lohtander, K., Källersjö, M. & Tehler, A. (1999) Sequence insertions and ITS data provide congruent information on *Rocella canariensis* and *R. tuberculata* (*Arthomiales*, *Euascomycetes*) phylogeny. *Molecular Phylogenetics and Evolution* **12**: 295–309.
- Myllys, L., Lohtander, K. & Tehler, A. (2001) β -tubulin, ITS and group I intron challenge the species pair concept in *Physcia aipolia* and *P. caesia*. *Mycologia* **93**: 335–343.
- Nylander, J. A. A. (2004) *MrModeltest v2*. Evolutionary Biology Centre, Uppsala University, Uppsala,

- Sweden. Computer program distributed by the author, available from <http://www.abc.se/~nylander/>.
- Nylander, J. A. A., Wilgenbusch, J. C., Warren, D. L. & Swofford, D. L. (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24**: 581–583.
- Nylander, W. (1866) Lichenes Lapponiae orientalis. *Notiser ur Sällskapet pro Fauna et Flora Fennica Förhandlingar* **5**: 101–192. (preprint).
- Pino-Bodas, R., Martín, M. P., Burgaz, A. R. & Lumbsch, H. T. (2013) Species delimitation in *Cladonia* (Ascomycota): a challenge to the DNA barcoding philosophy. *Molecular Ecology Resource* **13**: 1058–1068.
- Robert, C. E. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acid Research* **32**: 1792–1797.
- Schmidt, H. A., Strimmer, K., Vingron, M. & von Haeseler, A. (2002) TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18**: 502–504.
- Shimodaira, H. & Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114–1116.
- Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.
- Stenroos, S., Ahti, T. & Hyvönen, J. (1997) Phylogenetic analysis of the genera *Cladonia* and *Cladina* (Cladoniaceae, lichenized Ascomycota). *Plant Systematics and Evolution* **207**: 43–58.
- Stenroos, S., Hyvönen, J., Myllys, L., Thell, A. & Ahti, T. (2002) Phylogeny of the genus *Cladonia* s. lat. (Cladoniaceae, Ascomycetes) inferred from molecular, morphological, and chemical data. *Cladistics* **18**: 237–278.
- Strimmer, K. & Rambaut, A. (2002) Inferring confidence sets of possibly misspecified gene trees. *Proceedings of the Royal Society of London, Series B* **269**: 137–142.
- Swofford, D. L. (2003) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4.0b10. Sunderland, Massachusetts: Sinauer Associates.
- Vainio, E. A. (1880) *Tutkimus Cladoniain Phylogenetilliestä Kehityksestä*. Helsinki: Frenckell & Poika.
- Vainio, E. A. (1887) Monographia Cladoniarum universalis. 1. *Acta Societatis pro Fauna et Flora Fennica* **4**: 1–509.
- Vainio, E. A. (1897) Monographia Cladoniarum universalis. 3. *Acta Societatis pro Fauna et Flora Fennica* **14**: 1–268.
- Vainio, E. A. (1922) Lichenographia Fennica II. Baeomyceae et Lecideales. *Acta Societatis pro Fauna et Flora Fennica* **53**: 1–341.
- White, F. J. & James, P. W. (1985) A new guide to microchemical techniques for the identification of lichen substances. *Bulletin of the British Lichen Society* **57** (Suppl): 1–41.
- White, T. J., Bruns, T., Lee, S. B. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a Guide to Methods and Application* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. San Diego: Academic Press.
- Wirth, N., Printzen, C. & Lumbsch, H. T. (2008) The delimitation of Antarctic and bipolar species of neuropogonoid *Usnea* (Ascomycota, Lecanorales): a cohesion approach of species recognition for the *Usnea perpusilla* complex. *Mycological Research* **112**: 472–484.